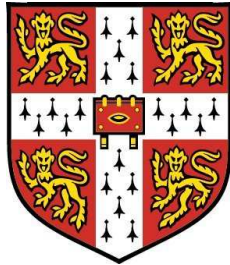


Mechanics of Biomimetic Materials for Tissue Engineering of the Intervertebral Disc



Daniel G. T. Strange

Department of Engineering

Christ's College, University of Cambridge

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Abstract

Tissue engineering offers a paradigm shift in the treatment of back pain. Engineered intervertebral discs could replace degenerated tissue and overcome the limitations of current treatments that disrupt the biomechanics of the spine. New materials, which exhibit sophisticated mechanical responses, are needed to provide templates for tissue regeneration. These behaviours include time-dependent deformation—facilitating shock absorption and nutrient transfer—and strong material anisotropy and tensile-compressive nonlinearities—providing flexibility in controlled directions. In this work, frameworks for the design of materials with controllable structure-property relationships are developed. The time-dependent mechanical properties of composites of agar, alginate and gelatin hydrogels are investigated. It is shown that the time-dependent responses of the composites can be tuned over a wide range. It is then demonstrated that materials mimicking the fibre-reinforced nature of natural tissues can be developed by infiltrating thick electrospun fibre networks with alginate. These fibre-reinforced hydrogels have tensile and compressive properties that can be separately altered. To better understand the mechanical behaviour of these hydrogel-based materials, improved methods for characterising poroelastic and poroviscoelastic time-dependent material properties using indentation are developed. It is shown that poroviscoelastic relaxation is the product of separate poroelastic and viscoelastic relaxation responses. The techniques developed here provide a methodology to rapidly characterise the properties of time-dependent materials and to create materials with complex structure-property relationships similar to those found in natural tissues; they present a framework for biomimetic materials design. The work in this thesis can be used to inform the design of clinically relevant tissue engineering treatments and help the quarter of a million people each year who undergo spinal surgery to reduce back pain.

Declaration

I hereby declare that thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. It contains 57 000 words and 70 figures.

Cambridge, 7th December 2012

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Contents

Nomenclature	xviii
1 Introduction and literature review	1
1.1 Introduction	2
1.1.1 Back pain and the intervertebral disc	2
1.1.2 Current repair strategies	3
1.1.3 Tissue engineering	6
1.1.4 Biomimetic scaffolds	7
1.2 The intervertebral disc	8
1.2.1 The nucleus pulposus	10
1.2.2 The annulus fibrosus	14
1.2.3 The cartilaginous endplate	17
1.2.4 Future investigations	17
1.3 Biomimetic scaffolds	19
1.3.1 Materials	19
1.3.2 Processing	23
1.3.3 Properties	26
1.3.4 Performance	31
1.4 Summary and thesis outline	35
2 Material characterisation methods and analytical techniques	39
2.1 Introduction	40
2.2 Material models	40
2.2.1 Elasticity	40
2.2.2 Viscoelasticity	42

2.2.3	Poroelasticity	46
2.3	Mechanical testing	52
2.3.1	Uniaxial tension and unconfined compression	52
2.3.2	Confined compression	54
2.3.3	Indentation	54
2.4	Analysis	59
2.4.1	Viscoelasticity	59
2.4.2	Poroelasticity	62
2.5	Composite models	67
2.5.1	Elastic behaviour	67
2.5.2	Models of porosity	70
2.5.3	Viscoelastic composites	70
2.6	Summary	70
3	Composite hydrogels	73
3.1	Introduction	74
3.2	Materials and methods	76
3.2.1	Materials	76
3.2.2	Swelling	77
3.2.3	Mechanical testing	77
3.3	Theory	78
3.3.1	Viscoelastic analysis	78
3.3.2	Poroelastic analysis	80
3.3.3	Composite models	82
3.4	Results	83
3.4.1	Swelling	83
3.4.2	Viscoelastic results	85
3.4.3	Poroelastic results	90
3.5	Discussion	93
3.5.1	Flexible design space	93
3.5.2	Characterisation techniques	96
3.5.3	Comparison to native tissue properties	100
3.6	Conclusions	102

CONTENTS

4	Electrospun fibre-reinforced hydrogels	103
4.1	Introduction	104
4.2	Methods	105
4.2.1	Electrospinning apparatus	105
4.2.2	Sample preparation	107
4.2.3	Characterisation	108
4.3	Results	111
4.3.1	Formation of polycaprolactone structures and composites	111
4.3.2	SEM and FTIR spectroscopy	113
4.3.3	Mechanical characterisation	115
4.4	Discussion	118
4.4.1	Elastic properties	120
4.4.2	Failure	122
4.4.3	Time-dependent properties	124
4.4.4	Comparison to native tissue properties	125
4.4.5	Future investigations	126
4.5	Conclusions	127
5	Indentation of poroelastic hydrogels	129
5.1	Introduction	130
5.2	Theory	134
5.2.1	Poroelastic	134
5.2.2	Elastic	135
5.3	Finite element model setup	138
5.3.1	Model geometry and mesh	139
5.3.2	Material properties	139
5.3.3	Deformation and contact	140
5.3.4	Boundary conditions	141
5.3.5	Time-stepping and integration	143
5.3.6	Scripting	144
5.3.7	Model validation and mesh optimisation	145
5.4	Results	147
5.4.1	Simulations	147

5.4.2	Non-dimensional master-curves	148
5.5	Analysis and curve fitting	150
5.5.1	Curve fitting method	152
5.5.2	Ramp correction factor expressions	153
5.6	Validation	154
5.6.1	Validation with forward finite element models	155
5.6.2	Experimental validation	163
5.7	Discussion	168
5.8	Conclusions	171
6	Poroviscoelastic characterisation of hydrogels	173
6.1	Introduction	174
6.2	Separable poroviscoelastic load-relaxation	175
6.2.1	Theory	175
6.2.2	Comparison to forward finite element methods	179
6.3	Identification of poroviscoelastic parameters	184
6.3.1	Theory	186
6.3.2	Method	187
6.3.3	Identification results	187
6.4	Experimental characterisation of agar	191
6.4.1	Experimental methods	193
6.4.2	Results	194
6.4.3	Discussion of agar experiments	198
6.5	Discussion	200
6.6	Conclusions	203
7	Conclusions and future directions	205
7.1	Conclusions	206
7.2	Future directions	211
7.2.1	Performance of an intervertebral disc scaffold	211
7.2.2	Mechanical behaviour of hydrogels and soft tissues	214
	Bibliography	217

List of Figures

1.1	Model of a segment of the lumbar spine, showing vertebrae, IVDs and spinal cord.	3
1.2	(a) Spinal fusion using an interbody device and (b) A total disc replacement (ProDisc-C).	4
1.3	The tissue engineering approach: components needed to engineer an IVD.	6
1.4	Diagram of the IVD showing the three key regions of the disc and illustrating the laminate structure of the annulus fibrosus.	9
1.5	Schematic showing the hierarchical structure of the IVD with corresponding approximate length-scales.	9
1.6	(a) Experimental set-up of confined compression and (b) schematic displacement-time curve of healthy NP showing creep.	12
1.7	(a) Electron micrograph of AF showing collagen fibres that are initially “crimped” under small tensile strains but straighten under greater strain. (b) Schematic tensile stress-strain curve of a single laminate AF specimen.	15
1.8	A summary of structure-property relationships that are present in the IVD.	18
1.9	Chemical structures of four polymers: (a) polycaprolactone, (b) polyglycolic acid, (c) agarose and (d) alginate.	20
1.10	Schematic of a hydrogel.	21
1.11	SEM images of (a) lamellar and (b) isotropic porous silk scaffolds prepared using a freeze-drying process.	23

LIST OF FIGURES

1.12	Diagram demonstrating the principle of electrospinning: (a) A random mesh of nanofibres is collected on a grounded plate. (b) Aligned nanofibres are collected on a rotating mandrel.	25
1.13	Anisotropic tensile modulus of electrospun PCL fibre mats.	28
1.14	Polarised light micrograph of Picrosirius Red-stained section of tissue engineered annulus fibrosus cultured for 6 weeks with mesenchymal stem cells.	32
2.1	Schematic of elastic material illustrating: (a) the six components of stress at any point (as $\sigma_{12} = \sigma_{21}$, $\sigma_{13} = \sigma_{31}$ and $\sigma_{23} = \sigma_{32}$) and (b) the uniaxial deformation and (c) shear deformation of the elastic material.	41
2.2	(a) Stress-strain responses of a viscoelastic material subjected to different strain-rates. (b) Idealised creep response of a viscoelastic material subjected to a sudden applied stress. (c) Relaxation response of a viscoelastic material subjected to a sudden applied strain.	43
2.3	The standard linear solid model.	45
2.4	Schematic depicting the response of a poroelastic material to a step-load.	47
2.5	Schematic showing various microstructures and their theoretical permeabilities, including: (b) a thin-walled pipe, (c) a cylindrical porous network of thin-walled pipes and (d) Jackson & James (1986)'s model for random fibrous networks.	50
2.6	Diagram depicting the four mechanical test methods that will be used to characterise the materials in this work.	52
2.7	Schematic diagram of spherical indentation of a bulk half-space showing the tip radius, R , contact radius, a , indenter displacement, h and contact depth, h_c	55
2.8	Principle stress and strain fields under a Hertzian indenter	56
2.9	Schematic of a ramp-hold profile showing both the strain-time loading (a) and the resultant material stress-time (b) curve.	60

LIST OF FIGURES

2.10	Theoretical microstructures corresponding to various composite bounds: (a) Voigt, (b) Reuss, (c) upper Hashin-Shtrikman, (d) lower Hashin-Shtrikman, (e) 2-D Christensen and Waals.	68
2.11	Composite bounds on the elastic modulus plotted for a material with $E_2 = 100$ MPa and $E_1 = 10, 1$ or 0.1 MPa; $\nu_1 = \nu_2 = 0.5$. . .	69
3.1	Hydrogel swelling, as quantified as percent weight gain, for (a,b) gelatin-agar composite gels after 24 hours swelling and for (c) gelatin-alginate gels after 48 hours crosslinking and swelling. . . .	84
3.2	Instantaneous (G_0 , hollow markers) and equilibrium (G_∞ , solid markers) shear moduli as a function of gel concentration (w/w) for (a) agar and (b) gelatin gels as measured by microindentation testing. The equilibrium moduli increase with concentration, while the viscoelastic ratio (G_∞/G_0) values for each gel are constant (c, d).	85
3.3	Instantaneous (G_0) shear modulus, equilibrium (G_∞) shear modulus and viscoelastic ratio (G_∞/G_0) values plotted as a function of gelatin concentration for composite gels in a (a-c) 30%Gel-1%Agar system and (d-f) 7.5%Gel-3%Agar system.	87
3.4	(a) Instantaneous (G_0) shear modulus, (b) equilibrium (G_∞) shear modulus and (c) viscoelastic ratio (G_∞/G_0) values plotted as a function of gelatin concentration for composite gels in a gelatin-alginate system in which the alginate component is or is not cross-linked.	88
3.5	Typical load-displacement curves measured through indentation (a-c) and unconfined compression (d-f) for two homogeneous gels and one composite gel ($V_f = 0.5$).	89
3.6	(a) Shear modulus, G , and (b) intrinsic permeability, k , determined using poroelastic analysis of agar, alginate and gelatin gels plotted as a function of their concentration pre-swelling.	90

LIST OF FIGURES

3.7	Poroelastic parameters identified from spherical microindentation tests on composite gels in a (a-c) 30%Gel-1%Agar system and (d-f) 7.5%Gel-3%Agar system showing the shear modulus, G , drained Poisson's ratio, ν and intrinsic permeability, k	92
3.8	(a) Composite gel viscoelastic ratio (G_∞/G_0) results (figure 3.3) re-plotted as a function of the gelatin solids fraction (S_f). (b) Composite gel viscoelastic ratio (G_∞/G_0) plotted against equilibrium shear modulus (G_∞).	94
3.9	Illustration of a mechanism by which hydrogels could have negative Poisson's ratios.	98
3.10	A plot of identified κ vs ν for constant ratio of consolidation coefficient to equilibrium reduced modulus determined from equation 3.16.	99
4.1	Electrospinner (a) set up in a horizontal configuration to produce thin fibre membranes and (b) set up in a vertical configuration in order to spin fibres into a fluid bath.	106
4.2	Images showing: (a) a PCL fibre structure fabricated in preliminary experiments, where the ethanol flow rate was controlled by hand and (b) a PCL fibre structure fabricated using the current method, where the ethanol flow rate was controlled with a syringe pump.	111
4.3	Photograph of PCL-5%Alg composite.	112
4.4	SEM micrographs of a PCL electrospun fibre structure, showing: (a) a top-down view and (b) a cross-section of the structure. . . .	112
4.5	SEM micrographs of dehydrated PCL-Alg composites.	113
4.6	Side by side comparison of SEM micrographs of (a) a PCL electrospun fibre structure and (b) a PCL-5%Alg composite (1000 $\times$ magnification).	113
4.7	FTIR spectra of constituent phases found in the PCL-3%Alg composite system: (a) water, (b) 3% alginate and (c) PCL.	114

LIST OF FIGURES

4.8	FTIR spectra of PCL fibres (a) infiltrated with water and (b) infiltrated with 3% alginate, compared with the spectra of their primary constituent.	115
4.9	Representative tensile stress-strain curves of (a) PCL fibres, (b) 3% alginate and (c) PCL-3%Alg composite.	116
4.10	Representative indentation load-displacement curves of (a) PCL fibres, (b) 3% alginate and (c) PCL-3%Alg composite.	117
4.11	Viscoelastic moduli of single phase materials and composites tested in indentation.	118
4.12	(a) Elastic modulus, (b) strength and strain-to-failure of single phase materials and composites tested in tension.	119
4.13	Images of single phase materials and composites undergoing tensile testing, at the point of failure.	120
4.14	Composite bounds calculated for two PCL-Alg composites from tensile elastic moduli.	122
4.15	Three-dimensional section of fluorescent PCL fibre structure imaged with confocal microscopy.	126
5.1	Non-dimensional load plotted against non-dimensional time for a spherical indenter under an applied step displacement (calculated using the expression of Hu et al., 2010).	133
5.2	(a) Geometry of spherical contact. (b) Normalised indentation load as a function of indentation geometry (a/R) calculated using the Hertz, Hay and Wolff, and Sneddon expressions, for a material with $\nu = 0.2$	136
5.3	(a) Diagram illustrating the biased mesh used for the half-space model (not to scale). (b) Boundary conditions applied to half-space model.	138
5.4	Pore pressure (a-c) and fluid velocity (d-f) in model during a ramp of 10 s with the user-defined impermeable indenter boundary condition.	142

5.5	(a) The simulation load, P normalised by the load predicted by the Hay and Wolf expression (P_{HW}) plotted against the number of nodes in contact with the indenter, for two sizes of the fine-mesh region, F . (b) P/P_{HW} plotted against the half-space height and width size.	146
5.6	(a) The full master-curve database for non-dimensional ramp times ranging from 10^{-2} to 10^2 . (b) A series of master-curves with varying drained Poisson's ratios.	149
5.7	Comparison of the master-curve generated in this work for a very short ramp time ($t_{\text{R}}^* = 0.01$), with the step displacement solutions of Hu et al. (2010) and Hu et al. (2011).	149
5.8	A series of master-curves generated from the FE model plotted with (a) a curve fit of a series of stretched exponential with a ramp correction factor, (b) the Hu et al. (2011) solution modified with a ramp correction factor.	150
5.9	Error in identified poroelastic properties (e.g. G_{id}/G) obtained by fitting load-relaxation data from forward FE simulations with four separate expressions for non-dimensional load-time.	158
5.10	Error in identified poroelastic properties over the range of $0.1 \leq t_{\text{R}}^* \leq 3$ for simulations with a long non-dimensional hold time ($t_{\text{H}}^* = 100t_{\text{R}}^*$), determined for four expressions describing non-dimensional load-relaxation.	159
5.11	The effect of the initial permeability guess on the error in identified poroelastic properties for forward FE simulations with $t_{\text{R}}^* = 1$, $t_{\text{H}}^* = 100$	160
5.12	Error (e.g. G_{id}/G) in identified poroelastic parameters from forward FE models with properties described in table 5.4.	162
5.13	Experimental load-relaxation curves from nanoindentation of cartilage, compared with fits obtained with Ramp RCF equation and the Hu et al. (2011) equation for step displacement indentation. .	166

LIST OF FIGURES

5.14	Stress-relaxation of a material with a stretched exponential relaxation function, with varying β , generated by numerically solving the Boltzmann hereditary integral and by calculating the response using a ramp correction factor approach.	170
6.1	Schematic of ramp-hold displacement-controlled unconfined compression experiment, where the load-relaxation of a PVE material is expressed in terms of an effective shear stiffness, $G_{\text{eff}}(t)$, and effective Poisson's ratio, $\nu_{\text{eff}}(t)$	176
6.2	The load-relaxation response of a PVE material calculated using equation 6.9 for (a) unconfined compression, (b) indentation and (c) confined compression.	182
6.3	The load-relaxation response of a PVE material calculated using equation 6.9 for unconfined compression using (a) a constant consolidation coefficient, c and (b) an effective consolidation coefficient, $c_{\text{eff}}(t)$, compared with load-relaxation curves produced using FE simulations.	183
6.4	The load-relaxation response of a material in confined compression with 0.1 strain applied over a 1000 s ramp time.	184
6.5	PVE parameter identification of multi-scale forward FE indentation.	188
6.6	Average load-relaxation curves obtained from spherical indentation of agar hydrogels with three different indenter radii. Normalised load is plotted against real-time (a) and time normalised by contact radius (b).	195
6.7	Experimental data from multi-scale indentation experiments, compared with fits of the data obtained by simultaneously fitting the experimental data with a semi-analytical expression for PVE load-relaxation.	196
6.8	Experimental data from unconfined compression experiments on agar hydrogels showing both the change in load (a) and Poisson's ratio (b) with time.	197
6.9	Schematic of a mechanism for a tensile-compressive nonlinearity in a physically crosslinked hydrogel.	200

6.10 PVE relaxation calculated using equation 6.9 compared with FE
simulations of a PVE material with a constant bulk relaxation
function for situations where (a) viscoelastic and poroelastic re-
laxation occur currently and (b) where poroelastic relaxation com-
pletes well before viscoelastic relaxation. 202

List of Tables

1.1	Approximate elastic modulus of the IVD and several commonly used biomaterials.	5
1.2	Composition of the NP, AF, and CEP. Dry weight refers to the total weight of the tissue excluding the water phase.	10
1.3	Compressive mechanical properties of the NP.	13
1.4	Elastic mechanical properties of (a) polymer scaffolds and (b) hydrogels for IVD tissue engineering.	27
1.5	Materials used for the generation of total IVD scaffolds, containing separate NP- and AF-like regions.	33
2.1	Common relationships between elastic constants.	42
2.2	Summary of the advantages (+) and disadvantages (−) of various mechanical characterisation techniques.	72
3.1	Comparison between unconfined compression and indentation tests for two homogeneous gels and one composite gel.	89
3.2	Comparison between poroelastic properties determined for unconfined compression and indentation tests for two homogeneous gels and one composite gel.	93
4.1	Elastic and viscoelastic moduli of single phase materials measured using tension and indentation testing.	116
5.1	The non-dimensional ramp time calculated for a variety of indentation experiments.	132
5.2	Material parameters of the base poroelastic and elastic models.	140

LIST OF TABLES

5.3	(a) Parameters determined from fitting three stretched exponentials with ramp correction factors to master-curves with $0.01 \leq t_R^* \leq 5$. (b) Parameters determined from fitting two stretched exponentials with ramp correction factors to a master-curve with $t_R^* = 0.014$	154
5.4	Experimental (a,b) and material parameters (c) used for forward FE models of poroelastic relaxation by three different materials using three different indenter modes.	155
5.5	Polyacrylamide poroelastic parameters identified by fitting experimental polyacrylamide data with the Ramp RCF expression and the Hu et al., 2011 expression for a step displacement.	164
5.6	Cartilage poroelastic parameters identified by fitting experimental cartilage nanoindentation load-relaxation data with the Ramp RCF expression (table 5.3a) and the Hu et al., 2011 expression for a step displacement.	167
6.1	Equilibrium, viscoelastic and load-relaxation expressions used in equation 6.9 to determine PVE load-relaxation.	181
6.2	PVE parameter identification of multi-scale forward FE indentation.	189

Nomenclature

Upper Case Symbols

A	Area
C_i	Relaxation function coefficient
D_i	Creep function coefficient
E	Elastic modulus
$E(t)$	Uniaxial relaxation function
E_0	Instantaneous (zero-time) elastic modulus
E_∞	Equilibrium (infinite-time) elastic modulus
E_{eff}	Effective elastic modulus (i.e stress divided by strain)
E_{linear}	Elastic modulus of linear region of stress strain curve
$E_{R\infty}$	Equilibrium (infinite-time) reduced modulus
E_R	Reduced modulus
E_{toe}	Elastic modulus of toe region of stress strain curve
G	Shear modulus
$G(t)$	Shear relaxation function
G^*	Complex shear modulus

G_0	Instantaneous (zero-time) shear modulus
G_∞	Equilibrium (infinite-time) shear modulus
$G_{\text{eff}}(t)$	Effective shear modulus
H_A	Aggregate modulus
$J_E(t)$	Uniaxial creep function
$J_G(t)$	Shear creep function
K	Bulk modulus
$K(t)$	Bulk relaxation function
L	Length or height
L_D	Drainage length
P	Load
P^*	Poroelastic non-dimensional load
P_0	Instantaneous (undrained) load
P_∞	Equilibrium (drained) load
$P_{\text{PE}}(t)$	Poroelastic load-relaxation
$P_{\text{VE}}(t)$	Viscoelastic load-relaxation
R	Indenter radius
RCF	Ramp correction factor
S_f	Gelatin solids fraction
V_f	Volume fraction
W	Weight
Q	Volumetric flow rate

Nomenclature

Lower Case Symbols

a	Contact radius of indenter
c	Poroelectric consolidation coefficient
g_i	Normalised shear relaxation function coefficient
h	Displacement or extension
$\dot{h}$	Displacement rate
$c_{\text{eff}}(t)$	Effective poroelectric consolidation coefficient
h^*	Poroelectric non-dimensional displacement
h_0	Instantaneous (undrained) displacement
h_∞	Equilibrium (drained) displacement
k	Intrinsic permeability
p	Pressure
r	Radius
t	Time
t^*	Poroelectric non-dimensional time
t_H	Hold time
t_H^*	Poroelectric non-dimensional hold time
t_R	Ramp time
t_R^*	Poroelectric non-dimensional ramp time

Greek Symbols

α	Effective stress coefficient
$\dot{\epsilon}$	Strain rate

ϵ	Strain
γ	Hay and Wolff correction factor
κ	Hydraulic permeability
μ	Dynamic viscosity
ν	Poisson's ratio (drained)
$\nu_{\text{eff}}(t)$	Effective Poisson's ratio
ν_{u}	Undrained Poisson's ratio
ϕ	Porosity
ρ	Density
σ	Stress
τ_i	Viscoelastic time constant
ζ	Increment of fluid content

Abbreviations

AF	Annulus fibrosus
AFM	Atomic force microscopy
CEP	Cartilaginous endplate
ECM	Extracellular matrix
FE	Finite element
FTIR	Fourier transform infrared spectroscopy
GAG	Glycosaminoglycan
IVD	Intervertebral disc
NP	Nucleus pulposus

Nomenclature

PBS	Phosphate buffered saline
PCL	Polycaprolactone
PEG	Polyethylene glycol
PGA	Polyglycolic acid
PLA	Polylactic acid
PLGA	Poly(L-lactide-co-glycolide)
PTFE	Polytetrafluoroethylene
PVA	Poly(vinyl alcohol)
SEM	Scanning electron microscopy
UHMWPE	Ultra-high molecular weight polyethylene

Miscellaneous

0.22 ± 0.2	Mean $\pm$ standard deviation
$\mathcal{H}(t)$	Heavyside step function
$\tan \delta$	The loss tangent
E_{∞}/E_0	Viscoelastic ratio between equilibrium and instantaneous moduli
Gel-Agar	Composite of gelatin and agar
Gel-Alg	Composite of gelatin and alginate
PCL-Alg	Composite of polycaprolactone and alginate
Ramp RCF	Load-relaxation expression for poroelastic indentation
Step RCF	Load-relaxation expression for poroelastic indentation
w/w	Weight per weight

Chapter 1

Introduction and literature review

In this chapter, the role of the intervertebral disc in chronic back pain is discussed, along with current surgical treatments for back pain. The tissue engineering approach is introduced with a focus on the materials that are used as scaffolds for the regeneration of intervertebral disc tissue. An overview of the structure-property-performance relationships present in the intervertebral disc is presented, followed by a discussion of how these relationships can be mimicked for the purposes of tissue engineering. Significant recent advances are discussed as well as areas where substantial further work remains. The chapter concludes with an outline of the work presented in this thesis.

1.1 Introduction

A fundamental shift is occurring in back pain research. Rather than solely trying to treat the symptoms of back pain, researchers are now seeking to regenerate degenerated intervertebral disc (IVD) tissue and restore normal function to the affected area. Tissue engineering is an emerging field that aims to produce a functional tissue replacement by encouraging the body to form new tissue and heal itself. New materials are needed to act as scaffolds for the tissue during the regenerative process, providing a template for new tissue growth. These materials must provide initial mechanical support and promote cell growth and differentiation, in order to encourage the growth of tissue that restores lost disc function.

1.1.1 Back pain and the intervertebral disc

Every year, more than 300,000 people in the US alone receive surgery to relieve back pain (Katz, 1995). While the processes that lead to back pain are complex and the precise anatomical cause often cannot be identified, back pain is frequently linked to degeneration of the IVD (Atlas & Nardin, 2003). The IVD is a cartilaginous tissue which separates the bony spinal vertebrae, permits a limited range of motion and protects the spine from shock loads (figure 1.1). The IVD lacks a significant blood supply and relies on diffusion and convection of nutrients from the surrounding vertebrae and periphery. As a result of ageing, disruption of the nutrition supply, genetic predisposition and loading history, IVDs often undergo progressive structural failure (Adams & Roughley, 2006). By the time an individual is 50 years old, the majority of the IVDs in their lower back will show signs of degeneration (Sehgal & Fortin, 2000). Degenerated discs are more compliant, less viscous and less able to resist compressive shocks and distribute loads evenly to the adjacent vertebrae (Adams et al., 1996b; Keller et al., 1987; Sehgal & Fortin, 2000). The majority of degenerated discs do not cause long-term debilitating pain, but a minority will through direct mechanical impingement of the spinal cord or aberrant biochemical processes, leading to a severely degraded quality of life (Manchikanti et al., 2001).

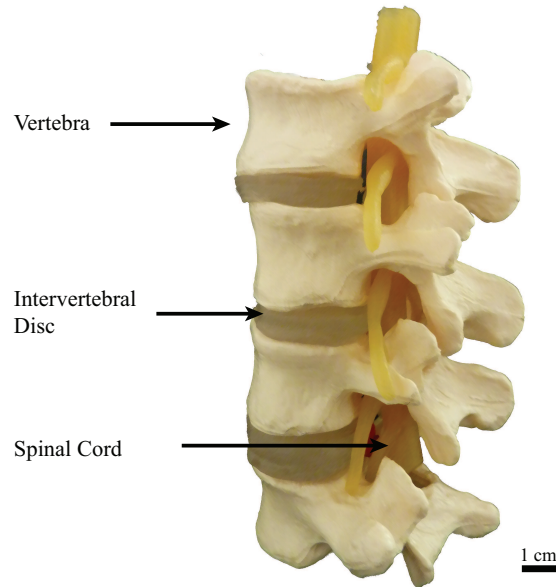


Figure 1.1: Model of a segment of the lumbar spine, showing vertebrae, IVDs and spinal cord. Ligaments are not shown. The reader is referred to Ebraheim et al. (2004) for further information on the anatomy of the spine.

1.1.2 Current repair strategies

There are several surgical options aimed at replacing or removing the IVD if more conservative treatments fail to relieve pain. Nearly all of these treatments disrupt the natural biomechanics of the spine. Lumbar fusion—the current gold standard treatment—entails fusing two vertebrae together with a bone graft or stiff biocompatible material in order to eliminate motion in the intervertebral space and the pain that results from this motion (figure 1.2a; Bertagnoli et al., 2005; Lehmann et al., 1987). Despite its frequent use, the surgery often fails to alleviate pain and can accelerate the degeneration of adjacent discs as it substantially changes the biomechanics of the spine (Mirza & Deyo, 2007; Nerurkar et al., 2010b).

Total disc replacement involves replacing the degenerated disc with an artificial structure that has an articulating metal-polyethylene interface similar to that found in other total joint replacements (figure 1.2b). In principle, the technique promises to improve upon the outcomes of spinal fusion by preserving motion within the spinal segment. Yet, in the original study that led to FDA approval

1.1 Introduction

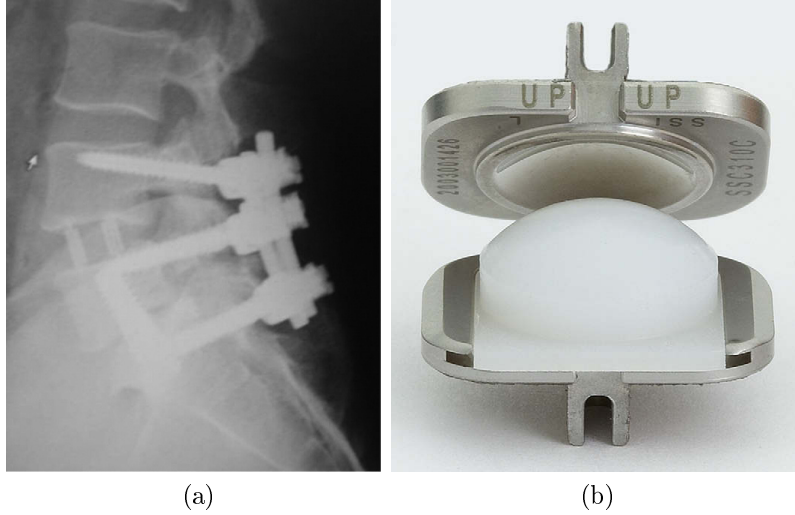


Figure 1.2: Two methods of surgically treating pain arising from degenerative disc disease: (a) Spinal fusion using an interbody device. From Francke (2008) with permission from Elsevier. (b) A total disc replacement (ProDisc-C). From Murrey et al. (2009) with permission from Elsevier.

of the Charité artificial disc, nearly 40% of patients had 5° or less motion at the implantation site after surgery, which even the study authors acknowledged does not improve upon the FDA criteria for fusion (Mirza, 2005).

There is a fundamental mismatch between the types and stiffnesses of materials that are used and the IVD tissue that they replace (table 1.1). Current biocompatible materials such as ultra-high molecular weight polyethylene (UHMWPE) and metal alloys are designed to be long-lasting and bio-inert—that is, to cause as little biological response from the body as possible. They are mechanically robust and have relatively large wear resistance compared with other materials but they lack the regenerative capacity of native tissue; the implant may fail over time as wear particles accumulate or as the implant subsides into the adjacent vertebrae due to the substantial modulus mismatch (Kurtz et al., 2007). The spinal segment can undergo 10 000 compression cycles with peak loads between 2–10 times body weight within a two week period (Johannessen et al., 2004). Unlike patients who receive other total joint replacements, those who undergo spinal surgery are generally younger—usually of middle age (Santos et al., 2004). Spinal implants need to have comparatively longer lifetimes if revision

1.1 Introduction

Class	Material	Elastic Modulus (MPa)	References
Biological tissues	Intervertebral disc	3–10 [†] [‡]	Nerurkar et al., 2010b
	Nucleus pulposus	0.005–0.044 [†]	Cloyd et al., 2007; Séguin et al., 2004
	Annulus fibrosus	18–45 [‡] [*]	Nerurkar et al., 2010b
	Cortical bone	17,000	El-Ghannam & Ducheyne, 2005
Metals	Cobalt-chromium alloy	220,000	El-Ghannam & Ducheyne, 2005
	Titanium	110,000	El-Ghannam & Ducheyne, 2005
	Stainless steel	200,000	El-Ghannam & Ducheyne, 2005
Ceramics	Alumina	362,700	El-Ghannam & Ducheyne, 2005
	Hydroxyapatite	120,000	Ratner et al., 2004
Polymers	Polyethylene [‡]	600–1,800	Ratner et al., 2004
	Poly(lactic acid)	1200–3000 [‡]	Engelberg & Kohn, 1991
	Poly(ϵ -caprolactone)	400 [‡]	Engelberg & Kohn, 1991
Hydrogels	Polyvinyl alcohol	0.001–1.3 [†]	Wang & Campbell, 2009
	Alginate	0.005–0.1 [†]	Thomas et al., 2003

[†] Compressive modulus. [‡] Tensile modulus.

[‡] The elastic modulus was measured assuming the intervertebral disc is a homogeneous material.

^{*} The elastic modulus is reported for the circumferential direction under large strains

[‡] High density polyethylene and ultrahigh molecular weight polyethylene

Table 1.1: Approximate elastic modulus of the IVD and several commonly used biomaterials. The modulus values listed for the biological tissues are approximate and only valid in certain conditions. For a more comprehensive set of mechanical benchmarks, the reader is referred to Nerurkar et al. (2010b).

1.1 Introduction

surgery is to be avoided. There is still a need to develop an implant that restores the functional biomechanics of the IVD and can withstand the repetitive high stresses experienced by the disc *in vivo* over the lifetime of a patient.

1.1.3 Tissue engineering

Tissue engineering can potentially tackle the underlying cause of disc degeneration whilst maintaining the biomechanics of the spine. The classic tissue engineering approach is to seed a scaffold with cells and encourage extracellular matrix (ECM) production with chemical signalling molecules known as growth factors (figure 1.3; Oyen, 2008b). There is also a growing realisation that it may be necessary to apply some sort of mechanical stimulation, either in the lab (*in vitro*) or after implantation into the human or animal (*in vivo*), in order to encourage cells to create mechanically functional structures similar to their native tissues (Hutton et al., 1999; Neidlinger-Wilke et al., 2006; Reza & Nicoll, 2008; Setton & Chen, 2004; Walsh & Lotz, 2004). The seeded scaffold is then implanted into the body; it erodes over time leaving space for new tissue to grow into. The scaffold provides initial structural support and acts as a three-dimensional matrix that encourages cellular function (Oyen, 2008b).

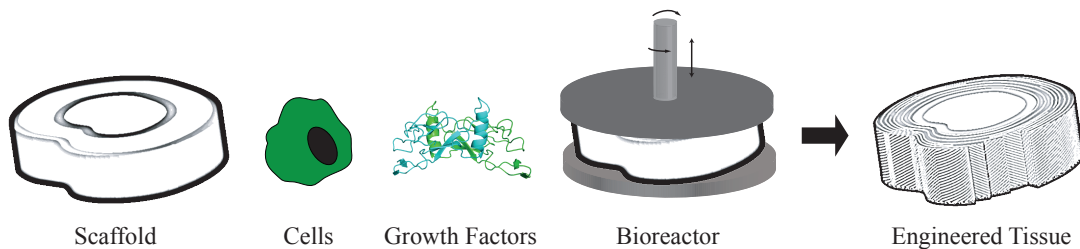


Figure 1.3: The tissue engineering approach: components needed to engineer an IVD.

Regardless of the tissue that a scaffold is designed to regenerate, the materials that are to be used for it must meet a number of common requirements. Scaffolds must be biocompatible; any material that is implanted into the body must not cause any unintended detrimental responses in the body. New materials that have not previously been used in medical devices face significant regulatory barriers

before they can be used in any implant, as adequate testing must be performed to ensure their lack of toxicity (Ratner et al., 2004).

In addition, scaffolds must act as templates for new tissue growth. They must have some degree of porosity to allow for the diffusion of nutrients and provide ample space for cells to grow. Many scaffolds are designed to make way for new tissue ingrowth by eroding or degrading *in vivo*. The biodegradability of the scaffold can be matched with the rate of tissue in-growth (weeks to years). As a result, metals are largely excluded from tissue engineering efforts. Many polymers degrade *in vivo* through the hydrolysis of their covalent backbone. Polymer degradation is a complex subject and modifications in structure and processing of the polymer can have a significant effect on the long-term success of the scaffold. The reader is referred to Ratner et al. (2004) for an introduction to the subject. In general, synthetic biodegradable polymers allow finer tuning of biodegradability rates, while natural polymers can take advantage of cell-mediated degradation processes (Tibbitt & Anseth, 2009).

1.1.4 Biomimetic scaffolds

In addition to all of the above requirements, researchers are increasingly designing scaffold materials that more closely replicate the structure and function of the IVD. The motivations behind using biomimetic materials—materials that actively mimic the behaviour of native tissue—as scaffolds are two-fold. Firstly, there are many similarities between tissue engineering pathways and wound healing or developmental processes *in vivo*. Researchers hope to mimic features of native tissue that direct and control cells, facilitating cell proliferation, cell differentiation and tissue formation (Ma, 2008). Secondly, the IVD is a complex tissue that is well adapted to its loading conditions. By mimicking the structure of the IVD it should be possible to reproduce some of its properties and create a functional and long-lasting scaffold. A prolonged *in vitro* culture process can also potentially be avoided by designing a scaffold with mechanical properties that are comparable to the IVD. Furthermore, by mimicking the structure-property relationships of the IVD, an implanted scaffold will transmit mechanical cues to

1.2 The intervertebral disc

regenerative cells that are similar to those that the cells experience *in vivo* during development and wound healing (Adams & Dolan, 2005; van der Meulen & Huiskes, 2002).

However, prior to creating a biomimetic material, it is necessary to first understand what functions are important to mimic and what properties, structural or compositional, are necessary to match or exceed the native disc's performance. Without understanding the disc's properties and function, it is impossible to benchmark the success of these tissue engineering techniques in restoring function to the spinal segment. In the following section, the mechanisms through which the IVD achieves its remarkable performance will be reviewed.

1.2 The intervertebral disc

The IVD is a remarkable tissue that can support substantial compressive loads, while at the same time remaining relatively flexible to bending motions. A healthy IVD uniformly distributes load to the surrounding vertebrae, even when an eccentric compressive load is applied, removing stress concentrations that could damage the vertebral bodies (Horst & Brinckmann, 1981). Although only 60% of body mass is located above the L3 vertebra (one of the vertebra in the lower back) when standing straight, if the spine is curved 20° forward the joint must support a force equivalent to over 250% of the body's weight. The majority of this force is transferred through the IVD that lies between the L3 and L4 vertebrae (Niosi & Oxland, 2004). The disc also reduces the effect of shock loading to the brittle vertebrae through the viscoelastic dissipation of energy. Although the vertebrae are much stiffer than the IVD, they invariably fail first in pure compression due to brittle fracture (Skrzypiec et al., 2007).

Unlike conventional engineering materials, the disc has a hierarchical structure with characteristic dimensions ranging from the nanoscale to the macroscale (figure 1.4; figure 1.5). The centre of the disc, the nucleus pulposus (NP), is an amorphous gel that incorporates a large bound water content and bulges outward under compressive loading. The NP is surrounded by a thick fibrocartilage, the annulus fibrosus (AF), which constrains the NP and carries significant hoop stress through a cross-ply collagen nanofibre network. Finally, the disc is covered by

1.2 The intervertebral disc

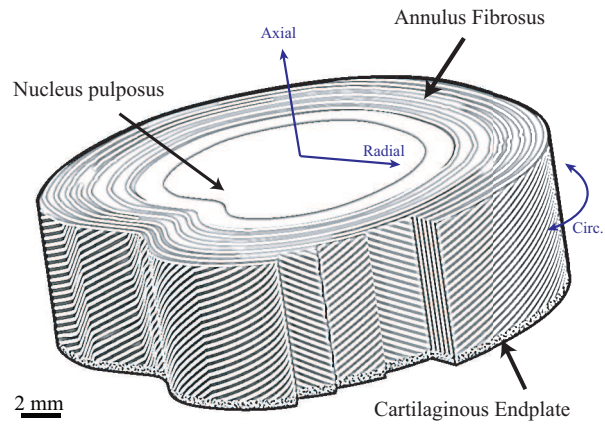


Figure 1.4: Diagram of the IVD showing the three key regions of the disc and illustrating the laminate structure of the annulus fibrosus.

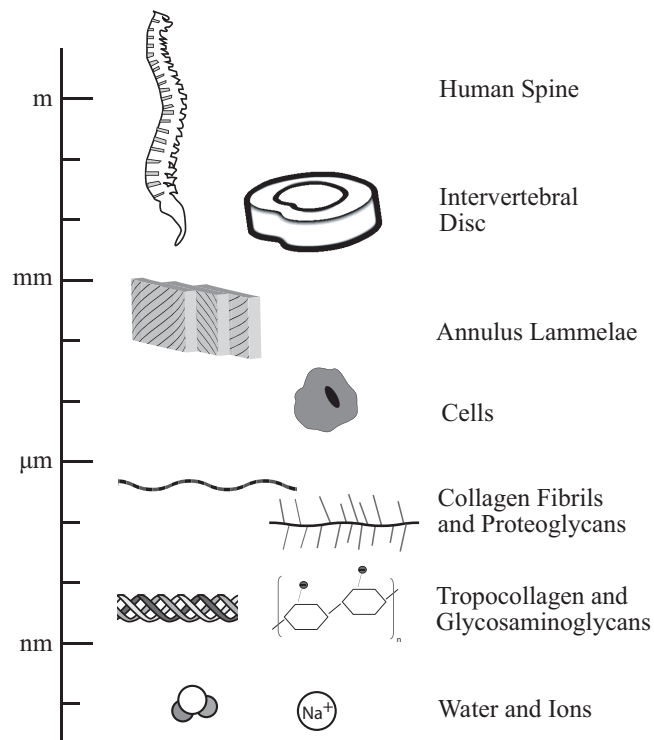


Figure 1.5: Schematic showing the hierarchical structure of the IVD with corresponding approximate length-scales.

1.2 The intervertebral disc

a cartilaginous endplate (CEP) that acts as the interface between the disc and vertebrae. Although the local material properties of the disc vary a great deal, the different regions are still composed of the same basic components: collagen, proteoglycans and water (table 1.2).

	Outer Annulus Fibrosus	Inner Annulus Fibrosus	Nucleus Pulposus	Endplate
Water (per wet weight)	65-75%	75-80%	75-90%	60%
Collagen (per dry weight)	75-90%	40-75%	25%	55-80%
Proteoglycans (per dry weight)	10%	20-35%	20-60%	10%
Other proteins (per dry weight)	5-15%	5-40%	15-55%	-

Table 1.2: Composition of the NP, AF, and CEP. Dry weight refers to the total weight of the tissue excluding the water phase. The data are compiled from a number of different experiments, and hence the total percentages do not add up to 100%. Adapted from Stokes & Iatridis (2005). Data taken from Antoniou et al. (1996), Roberts et al. (1989) and Setton et al. (1993).

1.2.1 The nucleus pulposus

Structure and composition

The NP forms the centre of the IVD. It is a gelatinous tissue that distributes hydrostatic loads to the rest of the disc. The NP displays behaviour characteristic of both a fluid and a solid and is largely composed of water—up to 90% (Iatridis et al., 1996; Sehgal & Fortin, 2000). The remainder of the tissue consists of proteoglycans (20–60% of the dry weight), collagen (15–25% of the dry weight) and other non-collagenous proteins including elastin (Iatridis et al., 1996; Stokes & Iatridis, 2005).

Properties

The structure and composition of the NP gives rise to its unique mechanical properties. Proteoglycans are proteins that have covalently bonded polysaccharides known as glycosaminoglycans (GAGs) bonded to them. Many GAGs contain sulphate groups and are highly negatively charged and strongly hydrophilic. They swell with large amounts of water, creating an osmotic pressure that can oppose compressive forces (Ventre et al., 2009). As the NP is compressed, proteoglycans are pushed closer together and the osmotic pressure increases. This change in osmotic pressure with dilation results in an increase in the effective bulk modulus of the tissue (Ateshian et al., 2004). The contribution of this osmotic stiffness to the apparent modulus of the NP measured in confined conditions (the aggregate modulus, H_A) has been estimated *in vitro* to be approximately 97% (Johannessen & Elliott, 2005). In the absence of external forces, the osmotic swelling pressure is resisted by a randomly aligned tensile network of type II collagen fibres which comprise approximately 25% of the dry weight of the NP (table 1.2).

The change in osmotic pressure with dilation can also contribute to an increase in the effective Poisson's ratio, ν of the tissue (Ateshian et al., 2004). The effective Poisson's ratio of the NP has been estimated to be nearly equal to 0.5, i.e. it is nearly incompressible (Cloyd et al., 2007; Stokes & Iatridis, 2005). For an isotropic material:

$$H_A = \frac{E(1 - \nu)}{(1 + \nu)(1 - 2\nu)}. \quad (1.1)$$

The aggregate modulus, H_A is approximately 10–100 times greater than the unconfined modulus, E (table 1.3).

The NP exhibits significant time-dependent behaviour, resulting from the flow of water through the ECM, so much so that the height of a standing human will on average decrease by nearly 5 mm over a period of 5 minutes, following a period of prolonged horizontal rest (Magnusson et al., 1990). When a compressive load is applied to the NP, at first the NP is stiff as the bulk of the load is supported by the incompressible fluid phase. As time progresses, fluid flows out of the tissue and some of the load is redistributed through the solid phase so the tissue creeps. Once the load is removed, water swells back into the tissue and the tissue

1.2 The intervertebral disc

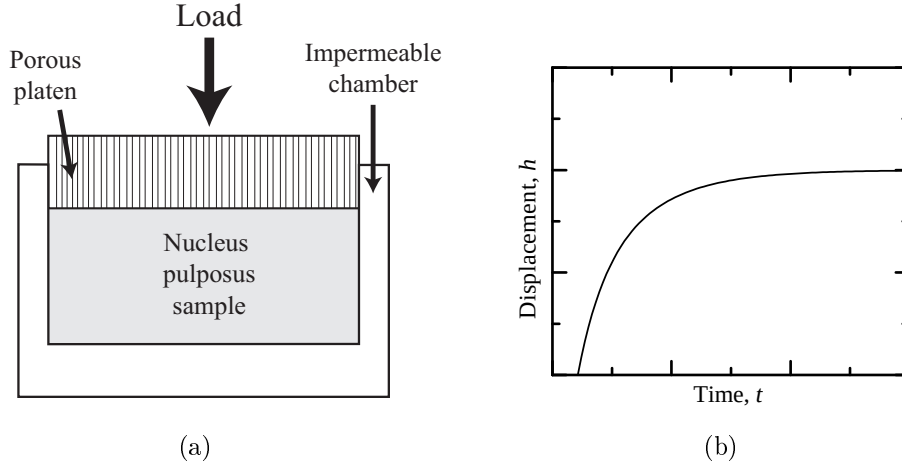


Figure 1.6: The confined compression experiment can be used to evaluate the aggregate modulus and poroelastic properties of the NP: (a) Experimental set-up and (b) schematic displacement-time curve of healthy NP showing creep. Adapted from Johannessen & Elliott (2005).

height recovers. The hydraulic permeability, κ of the NP has been evaluated *in vitro* directly through permeability measurements (Heneghan & Riches, 2008), and indirectly through confined compression experiments (figure 1.6; table 1.3; Johannessen & Elliott, 2005; Périé et al., 2005). The permeability is directly related to the intrinsic pore size of the material. Articular cartilage in the knee, which has a similar permeability to the NP, has an estimated pore size of 5.6 nm (Mow et al., 1984).

Performance

The NP has a very small elastic modulus; when loaded in pure shear it is very compliant, providing flexibility to the overall IVD joint. However, *in vivo*, the NP is semi-confined by the AF radially and the CEP and vertebrae axially. When compressive axial loading is applied to the NP *in vivo*, it is unable to significantly bulge outwards and substantial hydrostatic pressures can be generated, which evenly distribute eccentric loads to the surrounding tissues. The NP pressure *in vivo* ranges from approximately 0.1 MPa to 2 MPa depending on the loading

1.2 The intervertebral disc

Material Parameter	Value	References
Equilibrium Young's Modulus, E	5.4 – 44 kPa	Cloyd et al., 2007; Séguin et al., 2004
Drained Poisson's Ratio, $\nu^\dagger$	~ 0.5	Cloyd et al., 2007
Aggregate Modulus, H_A	0.31 – 1.01 MPa	Johannessen & Elliott, 2005; Périé et al., 2005
Hydraulic Permeability, $\kappa^\ddagger$	$0.67 - 1.8 \times 10^{-15} \text{ m}^4(\text{Ns})^{-1}$	Heneghan & Riches, 2008; Périé et al., 2005
Intrinsic Permeability, $k^\S$	$0.67 - 1.8 \times 10^{-18} \text{ m}^2$	Heneghan & Riches, 2008; Périé et al., 2005

$\dagger$ There has only been one experimental measure of the Poisson's ratio of the nucleus pulposus. The value measured (0.62) suggests that the nucleus pulposus is anisotropic. As the isotropic limit of 0.5 was within one standard deviation of the measured value, the authors of the experiment suggested that 0.5 would still be a reasonable value to use for model purposes.

$\ddagger$ The nucleus pulposus is known to exhibit strain dependent permeability, where the permeability decreases with increasing compressive strain. The value given here is thought to represent the permeability under zero strain, although measurements have differed in assuming zero strain from a free swelling nucleus, or a nucleus that swelled to the deformation imposed *in vivo*.

$\S$ The intrinsic permeability was calculated from the hydraulic permeability assuming a viscosity of 0.001 Nsm^{-2}

Table 1.3: Compressive mechanical properties of the NP.

history of the disc (Schultz et al., 1982; Wilke et al., 1999). Hence the IVD joint is much stiffer under pure compressive loading. The near incompressibility of the NP is crucial to its performance, perhaps more than the elastic modulus itself (Joshi et al., 2009; Strange et al., 2010).

The time-dependent behaviour exhibited by the NP may protect it from sudden shock loads; in the articular cartilage of the knee it has been observed that over 90% of a step compressive load is initially carried by the interstitial fluid in the cartilage, protecting the collagen-proteoglycan matrix from excessive shear stress (Ateshian & Mow, 2005). Furthermore, this fluid flow potentially increases the transport of large nutrient molecules in and out of the disc. Ferguson et al. (2004) used a poroelastic mass-diffusion model of the IVD to show that its swelling behaviour may increase the solute transport of large molecules (40 kDa) by up to 100% over diffusion alone during recovery, and up to 30% for a whole 24 hour cycle.

1.2 The intervertebral disc

1.2.2 The annulus fibrosus

Structure and composition

The AF is a thick fibrocartilage that surrounds the NP and constrains it. The AF is primarily loaded in tension in the circumferential direction of the IVD—it carries the hoop stress generated by the NP—but the inner AF also supports some compressive load. The AF has a much more complex structure than the NP and consists of approximately 20 concentric overlapping lamellae. The laminates are approximately 0.1–0.2 mm thick and consist primarily of aligned collagen nanofibres (75–90% dry weight in the outer AF) embedded in a ground substance (matrix) consisting of proteoglycans and water (Antoniou et al., 1996; Marchand & Ahmed, 1990). The biochemical composition changes radially and the outer AF has a greater collagen content than the inner AF, while the inner AF has a larger proteoglycan and water content (Best et al., 1994). The type of collagen also transitions from the outer AF to the NP. The outer AF consists primarily of thick type I collagen fibres while the transition zone near the NP is composed primarily of fine type II collagen fibres (Sehgal & Fortin, 2000). The angle of the fibres alternates with each successive ply from (approximately) $+30^\circ$ to -30° relative to the circumferential direction (the principal loading direction), although this angle changes from the outer AF to the inner AF from an average of 28° to an average of 43° close to the NP (Cassidy et al., 1989). While the structure of the AF is often approximated as an angle-ply laminate ring, Marchand & Ahmed (1990) found that approximately 40% of the layers in any 20° circumferential arc were interrupted, involving sharp discontinuities in the laminate.

Properties

The fibrous nature of the AF gives rise to a complex nonlinear material response in tension often characterised by a “toe-region” modulus, E_{toe} and a linear-region modulus, E_{linear} . When the AF is loaded in tension the tissue is more compliant at small strains (the toe region) but becomes stiffer as the strain increases (the linear region; figure 1.7). This nonlinear behaviour is thought to occur as a result of the following phenomena: the collagen fibres of the AF are “crimped” (undulating) in an unloaded state (Cassidy et al., 1989). Straightening of these

1.2 The intervertebral disc

fibres occurs when load is applied to the tissue. Fibres that are not orientated directly along the line of the load are also realigned and recruited. Furthermore, this realignment is resisted as a result of shear interactions between the fibres and ground substance (Guerin & Elliott, 2007). Hence, the transition region of the stress-strain curve is a result of the progressive straightening and reorientation of the fibres (Wren & Carter, 1998).

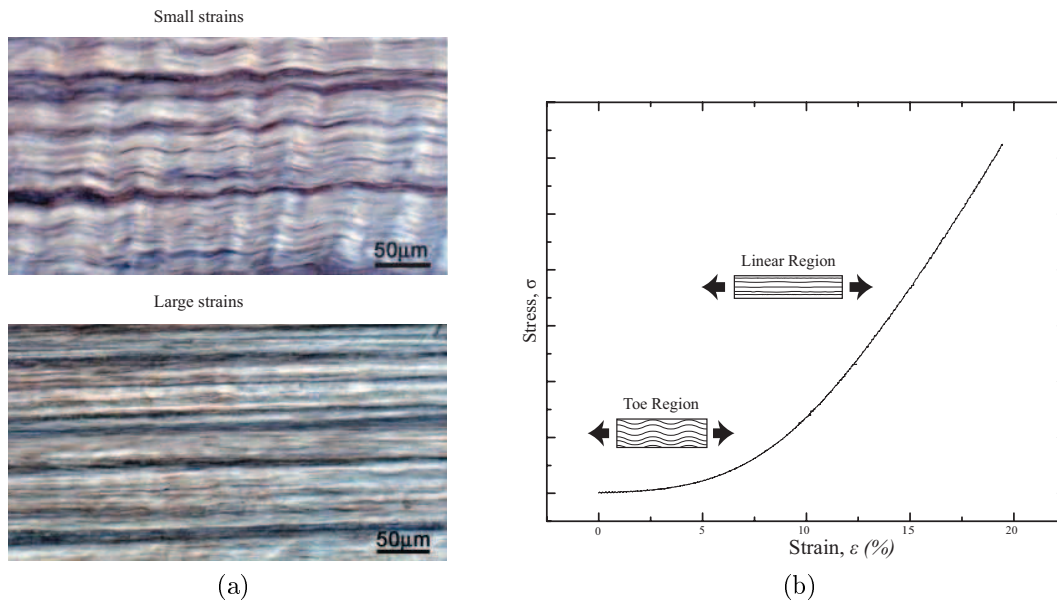


Figure 1.7: (a) Electron micrograph of AF showing collagen fibres that are initially “crimped” under small tensile strains but straighten under greater strain. From Pezowicz et al. (2005) with permission from John Wiley and Sons. (b) Schematic tensile stress-strain curve of a single laminate AF specimen.

As a result of its fibrous nature the AF is highly anisotropic with a tensile modulus that is greatest along the fibre direction. Skaggs et al. (1994) and Holzapfel et al. (2005) performed uniaxial testing on single AF laminates and determined that E_{linear} along the fibre direction ranged from 30 to 140 MPa. The modulus of the laminates measured perpendicular to the fibre direction is only on the order of 0.22 ± 0.2 MPa (mean $\pm$ standard deviation; Holzapfel et al., 2005). Multilaminate samples containing alternate $\pm 30^\circ$ laminates also demonstrate anisotropy. The circumferential modulus of multilaminate AF samples is approximately 17.4 ± 14.3 MPa, while the axial tensile moduli is an order of magnitude lower ($0.8 \pm$

1.2 The intervertebral disc

0.9 MPa; Elliott & Setton, 2001). Although these modulus values are much less than that of synthetic polymers or for that matter a single collagen fibre ($E \sim 1$ GPa; Gautieri et al., 2011), they are many orders of magnitude greater than the modulus of the NP.

In contrast to its tensile properties, the compressive behaviour of the AF does not substantially depend on orientation. The collagen fibres do not provide significant resistance to compression and instead the load is supported by the poroelastic ground substance (Iatridis et al., 1998; Klisch & Lotz, 2000). The inner AF undergoes more compressive loading than the outer AF and as such compressive moduli and permeabilities vary radially through the tissue, although the effect of these functionally graded properties is still not fully understood (Williams et al., 2007). These changes in composition and structure lead to a variation in tensile mechanical properties as well as changes in compressive behaviour. Ebara et al. (1996) performed quasi-static tensile tests on human multilaminate AF fibrous samples and found that the outer AF samples were much stiffer than the inner samples.

Performance

The AF resists the hydrostatic pressure generated in the NP and constrains it. The collagen fibres in the AF are aligned near the optimum cross-ply fibre angle for an infinitely long pressure vessel (35.3° relative to the circumferential direction) predicted by netting analysis and are similarly aligned to carbon fibres used in composite pressure vessels (Evans & Gibson, 2002). Furthermore, the AF has a functionally graded stiffness and is more compliant towards the centre. This potentially has significant physiologic relevance: a uniform stiffness in a thick-walled structure like the AF would lead to a stress concentration at the inner wall; a gradient of stiffness allows for a uniform stress state throughout the structure (Skaggs et al., 1994). In addition, the inner AF laminates have a larger strain-to-failure than the outer AF (potentially due to its lower stiffness) and can dissipate more energy (Holzapfel et al., 2005; Skaggs et al., 1994). The AF also provides some stability to the spinal segment; the stiffness of the AF is relatively small during physiological motion, but the resistance to deformation increases as the strains are large, preventing excessive movements under small forces.

1.2.3 The cartilaginous endplate

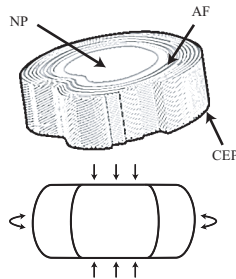
The CEP is the least studied part of the IVD. It consists of a very thin layer (approximately 0.6 mm thick) of hyaline cartilage that separates the adjacent vertebral body from the rest of the disc (Stokes & Iatridis, 2005). The CEP has a composition very similar to articular cartilage in the knee which is also hyaline cartilage (Stokes & Iatridis, 2005). The CEP is thought to be important in maintaining a uniform stress distribution across the boundary of the disc (Setton et al., 1993). It also plays a significant role in allowing the diffusion of nutrients into the disc and has a direction-dependent fluid flow resulting from the constriction of the CEP by vertebral pores (Ayotte et al., 2000). Calcification of the CEP is potentially one of the primary mechanisms of IVD degeneration (Urban et al., 2004). As the CEP acts as the interface between the bulk of the IVD and the adjacent vertebrae it is likely that any tissue engineering attempts at repairing a whole disc will need to take the CEP into account in order to bond the tissue-engineered structure to the vertebrae and ensure correct nutrient flow.

1.2.4 Future investigations

The IVD exhibits properties which stem from its unique hierarchical structure and are not usually found together in bulk engineering materials, nor even in typical engineering composites (Strange & Oyen, 2011b). A summary of the structure-property relationships needed to mimic the IVD is presented in figure 1.8. It is worth pointing out that these basic relationships are not fully understood. Only limited measurements of some of the key material properties such as the elastic modulus and Poisson's ratio of the NP have been made, due to the difficulties involved in carrying out well-controlled tests on biological tissues. Furthermore, this review has largely only commented on the macroscale behaviour of the tissues. There is still significant uncertainty over the nanoscale properties and how these properties relate to the macroscale; cells may be more influenced by nanoscale topography than macroscale. A limited amount of work has been performed applying nanoindentation (Lukes et al., 2009), atomic force microscopy (AFM) indentation (Lewis et al., 2008) and microindentation (Umehara et al., 1996) to the disc, but significant differences between the measurements were observed.

1.2 The intervertebral disc

IVD



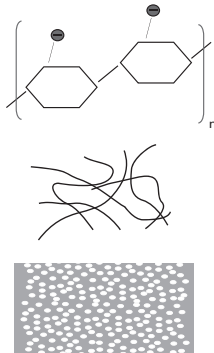
Functionally graded structure

Gradual changes in microstructure result in a more uniform stress state within the material, reducing stress concentrations

Tensile-compressive structure

By transferring compressive load in the NP to tension in the AF the structure becomes much stiffer in compression than its individual components

NP



Highly charged macromolecules

Hydrophilic macromolecules swell with large amounts of water creating a swelling pressure that can resist compressive forces

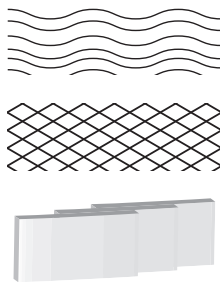
Random fibrous network

The fibrous network can resist osmotic swelling pressures in the absence of external forces

Porous permeable structure

Frictional drag of fluid through the tissue leads to a poroelastic material response which may protect the tissue from sudden shock loads

AF



“Crimped” Fibres

In tension, fibres are compliant under small strains but stiff at greater strains

Cross-ply Fibres

Laminates are anisotropic and are optimised for their loading conditions

Multi-laminate architecture

Ground substance between laminates resists reorientation of fibres, increasing circumferential stiffness under low loads

CEP



Direction dependent permeability

Direction-dependent fluid permeability ensures fluid and nutrients are rapidly recovered when load is removed

Figure 1.8: A summary of structure-property relationships that are present in the IVD.

The structure-property relationships presented above are a good starting point when creating biomimetic scaffolds of the IVD, but there is still significant work that must be done.

1.3 Biomimetic scaffolds

The aim of tissue engineering of the IVD is to replace degenerated tissue with a functional engineered disc, ultimately to reduce back pain and improve quality of life. There have been many different approaches to this problem, based on the severity and particulars of the disease. Several authors have investigated regenerating the IVD by injecting cells and growth factors directly into the degenerated disc (Walsh et al., 2004). In many cases though, the degeneration of the disc has proceeded too far and it is necessary to replace either a portion or the whole IVD with a cell-seeded scaffold.

In the following section, attempts at creating biomimetic scaffolds for the NP, the AF and the whole disc will be reviewed. The focus is on materials and processes used for IVD tissue engineering efforts and therefore materials such as ceramics have not been considered. A particular emphasis is placed on how the scaffolds mimic the structure and function of native tissue and the properties and performance that arise from these unique structures.

1.3.1 Materials

Polymers

Polymers are long-chained repeating molecules (figure 1.9). They are a staple of both the ECM (proteins and GAGs) and of tissue engineering efforts. Polymers that have previously been widely used as biomaterials, including UHMWPE (Shikinami et al. 2004), polylactic acid (PLA; Mizuno et al., 2004), polyglycolic acid (PGA; Mizuno et al., 2004), and more recently polycaprolactone (PCL; Nerurkar et al., 2010c) and poly(vinyl alcohol) (PVA; Wang & Campbell, 2009), are now being investigated as scaffolds for IVD tissue engineering. These synthetic polymers can be processed into a number of forms, including fibres and porous foams. Properties such as molecular weight, crystallinity, and crosslinking density

1.3 Biomimetic scaffolds

can be carefully controlled during processing, resulting in structures with desired mechanical and chemical properties (Drury & Mooney, 2003).

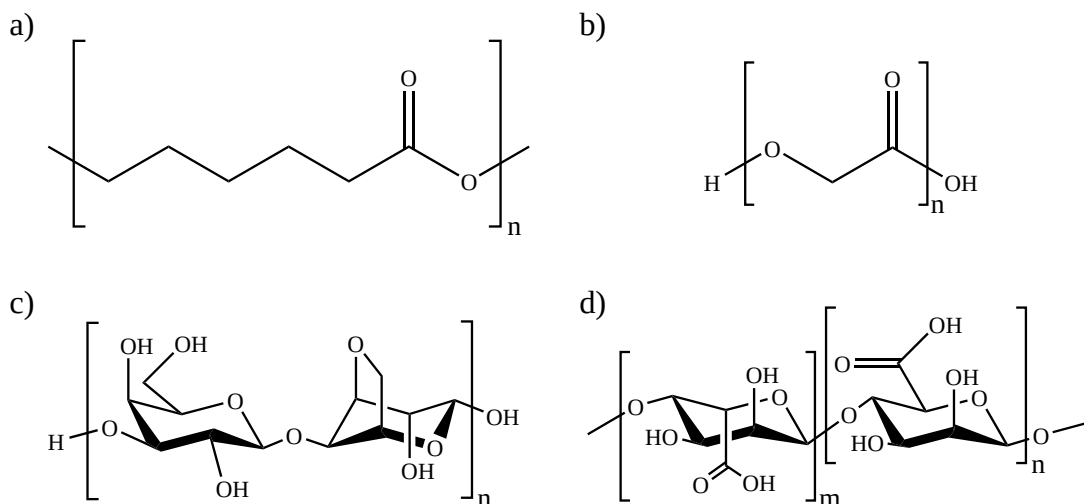


Figure 1.9: Chemical structures of four polymers: (a) polycaprolactone, (b) polyglycolic acid, (c) agarose and (d) alginate.

Although synthetic polymers can be manufactured with well-controlled material properties, they lack much of the chemical and biological complexity of natural polymers. Researchers are concurrently investigating the use of natural polymers, including collagen (Bowles et al., 2010), silk (Park et al., 2012), gelatin (Cloyd et al., 2007), agarose (Gruber et al., 2006) and alginate (Bron et al., 2011), as materials for tissue engineering. Collagen and silk are proteins with repeating peptide subunits, while alginate and agarose are hydrophilic linear polysaccharides. Gelatin is denatured collagen. Natural polymers are advantageous because they may possess properties similar to the natural ECM (Drury & Mooney, 2003). For example, collagen contains amino acid sequences that facilitate cell adhesion through the binding of proteins known as integrins (Jokinen et al., 2004). Collagen can also be induced to form fibrillar architectures similar to those found *in vivo* (Bowles et al., 2010). The protein can additionally be degraded by proteases, enzymes that are secreted by cells, facilitating cell migration while maintaining the structural integrity of the scaffold (Alberts et al., 2008). However, there are still issues with the possible immunogenicity of natural polymers that must be

resolved. Furthermore, there is substantially more variability in the properties of natural polymers than synthetic polymers, due to the wide range of sources that they are derived from. Researchers are also developing polymers that combine the advantages of synthetic and natural polymers, either by directly synthesising polypeptides (Moss et al., 2010), or by chemically modifying synthetic polymers with natural moieties (Ma, 2008).

Hydrogels

Hydrogels consist of hydrophilic polymer networks that swell to many times their own weight with water (figure 1.10). Many hydrogels contain similar concentrations of water to the ECM and possess similar mechanical, structural and transport properties as native ECM. They can be formed from both synthetic (e.g. PVA, Wang & Campbell, 2009; Polyethylene glycol, PEG, Moss et al., 2010) or natural polymers (e.g. alginate, Baer et al., 2001; agarose, Gruber et al., 2006; collagen, Bron et al., 2009).

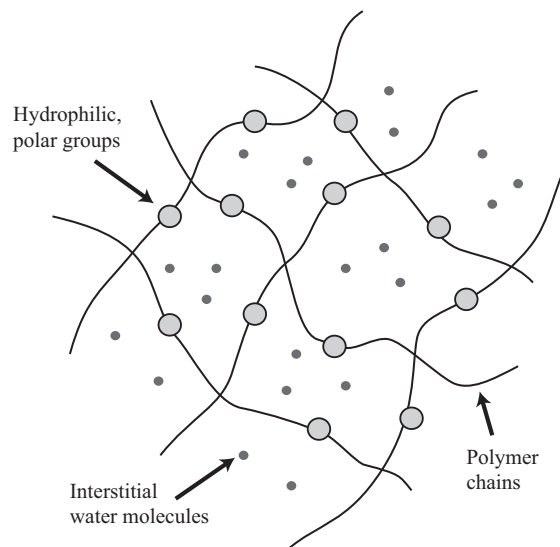


Figure 1.10: Schematic of a hydrogel.

When a hydrogel is placed in an aqueous environment it will swell until the osmotic pressure is balanced by the tensile forces of the polymer network. The polymer network is held together by crosslinks between the polymer chains. These

1.3 Biomimetic scaffolds

crosslinks can be permanent covalent chemical bonds between chains (such as those found in PEG hydrogels) or reversible physical bonds.

Physical bonds can arise from molecular entanglements between chains or from secondary bonds such as ionic bonds or hydrogen bonds (Van Vlierberghe et al., 2011). Agarose will form a hydrogel with molecular entanglements on a reduction of temperature in water, while PVA will form a physically crosslinked hydrogel with repetitive freeze-thaw cycles. Both alginate and gellan gum will form ionically bonded hydrogels when placed in a solution containing divalent cations such as Ca^{2+} (Kuo & Ma, 2001; Silva-Correia et al., 2011). The cation will bind to two polymer molecules, forming a bridge between them.

Some polymers that naturally form physically crosslinked hydrogels have been chemically modified to contain covalent crosslinker points in order to form covalently bonded hydrogels. Such gels include gellan gum, gelatin and alginate (Chou et al., 2009; Leone et al., 2007; Nichol et al., 2010; Silva-Correia et al., 2011). These modified gels can then be crosslinked using standard chemistry techniques, allowing for further control of mechanical and biodegradation properties, as well as enabling novel processing routes such as photo-polymerisation.

Decellularised materials

As much of the difficulty in creating biomimetic scaffolds extends from encouraging natural and synthetic components to assemble into structures similar to the IVD, Mercuri et al. (2011) approached this problem from another angle: the team created scaffolds by obtaining fresh porcine NPs and removing nearly all of the cellular components of the tissue using detergents and ultrasonification. While there was a trade off between complete decellularisation and damage to the ECM, Mercuri et al. were able to achieve a 98.44% reduction in DNA content, while the majority of ECM remained intact. Several challenges still remain, including proving the long-term biocompatibility of this scaffold and lack of any immune response. However, this may be a promising route of obtaining scaffolds that closely mimic the ECM of the NP. Similar methods of using decellularised tracheae as scaffolds have already proved clinically successful (Macchiarini et al., 2008).

1.3.2 Processing

Many of the properties of tissue engineering scaffolds are achieved via chemical modification. However, it is becoming increasingly apparent that the structure of a scaffold is equally important to its performance. Here, some of the processing routes used to produce scaffolds with controlled porosity and multi-scale architectures are discussed.

Foams

Porous foams can be formed from a variety of methods including particle-leaching techniques and freeze drying (lyophilisation). In a particle leaching process, a porogen (such as sodium chloride) is mixed into a polymer solution. The polymer solution is cast and solidified. The porogen is then rinsed away leaving pores in its place. The porosity and pore size of the foam can be controlled by varying the concentration and size of the porogen (Ma, 2004). Particle leaching has been used to fabricate porous silk scaffolds (Chang et al., 2007) and poly(L-lactide-co-glycolide) (PLGA) scaffolds (Kim et al., 2012) for IVD tissue engineering with porosities greater than 90% and pore sizes larger than 100 μm . Electrospun nanofibrous scaffolds (see section 1.3.2) with larger micro pores have also been created by co-spinning sacrificial fibres as porogens (Baker et al., 2008).

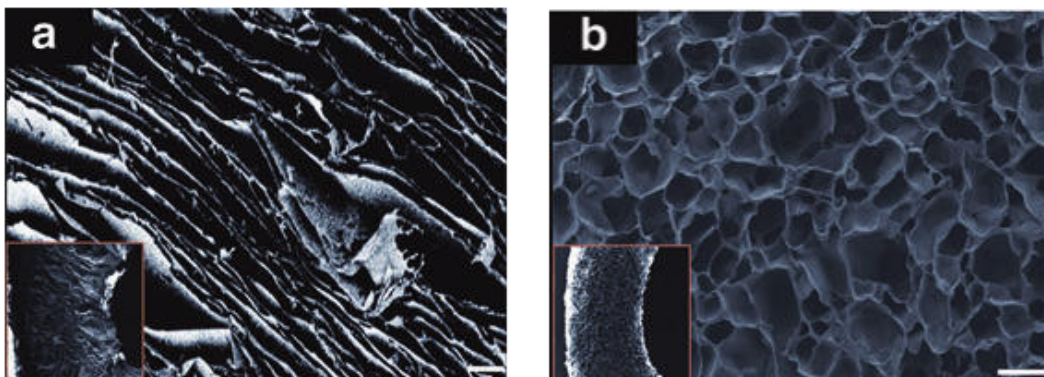


Figure 1.11: SEM images of (a) lamellar and (b) isotropic porous silk scaffolds prepared using a freeze-drying process. Scale: 200 μm . From Park et al. (2012) with permission from Mary Ann Liebert, Inc.

1.3 Biomimetic scaffolds

Porous foams can also be formed by freezing a polymer solution and sublimating out the solvent (figure 1.11). The process has been used to form foams of synthetic polymers and other materials (Mizuno et al., 2004; Sato et al., 2003), and even to form foams that explicitly mimic the composition of the AF. A type 1 collagen and GAG slurry was freeze dried and then crosslinked to form a porous scaffold with pore size on the order of 100 μm (Rong et al., 2002).

Rapid prototyping

One of the challenges of designing a functional scaffold is ensuring it can be implanted into a patient. Medical imaging and rapid prototyping techniques can be used to form materials with geometries that accurately match patient-specific geometries (Hutmacher, 2001). Bowles et al. (2012, 2011) utilised microcomputed tomography (μCT) and MRI images to 3D print an injectable mould with a geometry that matched the NP. A hydrogel was then injected into the mould to construct anatomically shaped implants that differed by less than 7% from native discs.

Rapid prototyping techniques can also be used to form scaffolds with multi-scale architectures and minimum feature sizes on the order of 1 μm (Hollister, 2005). These techniques have been used to form polymer or hydrogel scaffolds for articular cartilage tissue engineering (Billiet et al., 2012). However, as of yet these techniques have not been utilised for IVD tissue engineering.

Non-woven materials

Polymer scaffolds with fibrous architectures can be fabricated using technologies developed for the textile industry. Fibres with diameters on the order of 10–100 μm are formed using an extrusion process. The fibres can then be arranged into a mesh and mechanically entangled or thermally or chemically bonded together (Ratner et al., 2004). Non-woven fabrics have been made from a range of polymers including PGA (Freed et al., 1994) and alginate (Shao & Hunter, 2007). Non-woven PGA-PLA scaffolds are commercially available (Reza & Nicoll, 2008). These fibrous materials are extremely porous ($> 90\%$ porosity) with long interconnected pores.

Electrospinning

One of the most promising techniques for creating biomimetic structures is electrospinning. Nanofibres with diameters similar to those of collagen fibres found *in vivo* (~ 100 nm) can be formed by applying a large electric field to a polymer solution so that it forms a polymer jet that spins and elongates as it travels to an earthed plate (figure 1.12a). It is possible to produce mats of aligned nanofibres by collecting the polymer jet onto a rotating mandrel which aligns the fibres in the direction of spinning (figure 1.12b). Koepsell et al. (2011) produced electrospun PCL with varying degrees of alignment by varying the rotation speed of the mandrel. Nerurkar et al. (2009) formed cross-ply $\pm 30^\circ$ orientated fibre laminates with a fibre structure similar to the AF by fabricating aligned fibre mats, cutting them at an angle to the prevailing fibre direction and joining them together.

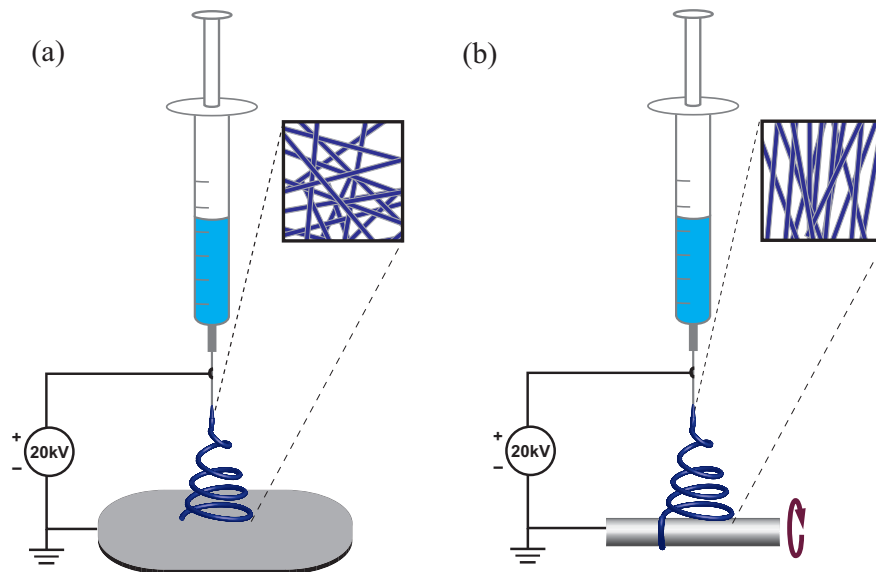


Figure 1.12: Diagram demonstrating the principle of electrospinning: (a) A random mesh of nanofibres is collected on a grounded plate. (b) Aligned nanofibres are collected on a rotating mandrel.

Miscellaneous

Other processing techniques of note include collagen gel contraction, whereby cells are seeded into a collagen gel that surrounds a cylinder. The cells apply

1.3 Biomimetic scaffolds

forces to the gel and the collagen contracts around the cylinder, with the collagen fibrils aligning in the circumferential direction (Bowles et al., 2010). There are a number of other processing techniques to form tissue-like scaffolds, which so far have not been utilised to form IVD scaffolds; the reader is referred to Hutmacher (2001) or Stella et al. (2010) for a review.

Although the processing techniques listed above have been discussed in isolation, many of the techniques have been combined to form complex architectures with multiple scales of hierarchy. A number of researchers have surrounded hydrogels with fibre-like architectures in order to replicate the tensile-compressive structure of the IVD (Bowles et al., 2010; Mizuno et al., 2004; Nerurkar et al., 2010c; Nesti et al., 2008; Park et al., 2012). Other investigators have created composites of hydrogels and fibre networks, leading to materials with altered swelling ratios and increased elastic moduli (Borges et al., 2010, 2011; Moss et al., 2010).

1.3.3 Properties

In this section, several important properties governing the mechanical performance of IVD scaffolds are presented. The list is not exhaustive; many properties (including biological functionality and biodegradability) that likely have a significant impact on the long-term success of an IVD scaffold are not considered and the reader is referred to Ma (2008) or Yang & Li (2009) for a more detailed treatment of these areas. The emphasis is on structure-property relationships that affect the mechanical behaviour of an IVD scaffold. For a detailed comparison of the mechanical properties of current IVD scaffolds and native IVD tissue, the reader is referred to Nerurkar et al. (2010a).

Elastic mechanical properties

The majority of mechanical characterisation of IVD scaffolds has involved measuring the elastic modulus of the material, whether in unconfined compression, tension or shear. The elastic modulus of foams (produced via particle leaching or freeze-drying) is a function of the porosity of the material and the modulus of the dense polymer (Gibson & Ashby, 1999; see section 2.5.2 in the following chapter

1.3 Biomimetic scaffolds

for further details); polymer foams with porosities of 95–99% porosity have moduli on the order of 10s of kPa to MPas, similar to that of the AF and NP (table 1.4a). For a given material the modulus can be varied by simply increasing or decreasing the porosity, although this may affect other material properties such as time-dependent behaviour, biodegradability and cellular response.

Material	$E_{tensile}$ (MPa)	E_{comp} (kPa)	References
PCL (Electrospun)	2-18		Koepsell et al., 2011; Nerurkar et al., 2007
PLA/PGA (Foam)		1-20	Mizuno et al., 2004; Moran et al., 2003
Silk (Lamellar)	0.26	164.8	Mandal et al., 2011; Park et al., 2012
Silk (Foam)	1.30-1.41	293.78-347.58	Mandal et al., 2011; Park et al., 2012

(a)

Material	E_{comp} (kPa)	$ G^* $ (kPa)	References
Alginate	2.3-13.02	0.75	Baer et al. 2001; Cloyd et al. 2007
Agarose	13.45		Cloyd et al. 2007
Gellan gum	56.2-151.2 [†]		Silva-Correia et al., 2011
PVA	1-1303		Wang & Campbell 2009
Silk	9		Park et al., 2011
Type 1 Collagen		0.5-8	Bron et al., 2009

(b)

[†]The modulus reported here is actually the compressive storage modulus.

Table 1.4: Elastic mechanical properties of (a) polymer scaffolds and (b) hydrogels for IVD tissue engineering. The elastic moduli in tension, $E_{tensile}$ and compression, E_{comp} and dynamic shear moduli $|G^*|$ are reported where applicable.

Like the NP, the elastic moduli of hydrogels are largely in the kPa range (table 1.4b). The modulus of a hydrogel is largely a function of its water content. Wang & Campbell (2009) fabricated PVA hydrogels with compressive elastic moduli ranging from 1 kPa to 1521 kPa as the concentration of PVA increased from 3% to 40%. To a lesser extent, the modulus of hydrogels depends on the density and nature of crosslinks in the polymer network. Silva-Correia et al. (2011) formed

1.3 Biomimetic scaffolds

ionically crosslinked methacrylated gellan gum hydrogels with compressive storage moduli (at 1 Hz) of 89.5 kPa and covalently crosslinked hydrogels with moduli of 122.8-151.2 kPa. Wang & Campbell (2009) demonstrated that the elastic modulus of 40% PVA hydrogels could be increased from 1521 MPa to 2117 MPa by increasing the number of freeze-thaw cycles, as each cycle increased the degree of physical crosslinking. The nature of the structure-property relationships governing the elastic modulus of hydrogels and isotropic porous polymers is reasonably well understood.

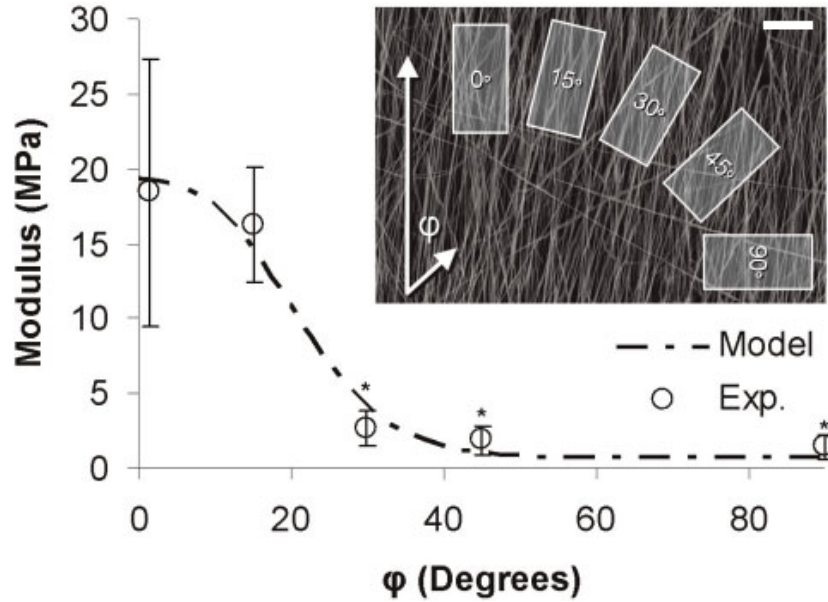


Figure 1.13: Anisotropic tensile modulus of electrospun PCL fibre mats. The fibres can be cut at $\pm 30^\circ$ to obtain lamella that mimic the structure of the AF. Scale: $25 \mu\text{m}$. From Nerurkar et al. (2007) with permission from John Wiley and Sons.

It is less well understood how to fabricate structures with similar anisotropic elastic moduli and tensile-compressive nonlinearities to the IVD. Nerurkar et al. (2007) fabricated aligned PCL electrospun fibres and investigated how the tensile moduli of the scaffolds varied with fibre orientation (figure 1.13). The same group also demonstrated that the matrix in between laminates of cross-ply electrospun fibres played a crucial role in resisting the reorientation of the fibres (Nerurkar et al., 2009). The tensile modulus of cross-ply laminates separated by a layer

of agarose increased by 3.5 MPa when the concentration of agarose increased from 2 to 6%, although the modulus of agarose only increased by approximately 0.1 MPa. Park et al. (2012) and Mandal et al. (2011) created isotropic porous silk foams and silk foams with an anisotropic lamellar structure using a freeze-drying process (figure 1.11). The isotropic porous silk foam demonstrated a substantial tensile-compressive nonlinearity while the anisotropic lamellar structure did not (table 1.4a). The reasons behind this were not clear, but likely stem from the different architectures of the materials.

Similarly, the confined compression behaviour of hydrogels has for the most part been overlooked, even though this mode of deformation is of particular importance for the mechanical function of the NP. Moss et al. (2010) measured the aggregate modulus of a composite gel consisting primarily of hyaluronan, an essential ECM component, and found that it was still an order of magnitude smaller than that of the NP. Cloyd et al. (2007) measured the Poisson's ratio, ν of a number of common gels and found it to lie within the range of 0.1–0.4. While hydrogels have a large concentration of water, they do not necessarily have the same large concentration of highly charged macromolecules and large osmotic swelling pressure that varies with dilation.

Frequency and time-dependent mechanical properties

An engineered IVD will undergo complex time-dependent and frequency dependent loading. However, information about the time-dependent and frequency-dependent stiffness of polymer foams and electrospun fibres is sparse and the majority of investigations have involved hydrogels. These investigations have largely been performed using dynamic mechanical analysis. A frequency dependent complex shear modulus, $|G^*|$ and a loss tangent, $\tan \delta$, which is a measure of viscous dissipation of the material, is reported.

The loss tangent of hydrogels varies widely between materials, e.g. collagen (0.12; Bron et al., 2009), alginate (0.05–0.24; Baer et al., 2001; Bron et al., 2011), and modified hyaluronic acid (0.05–0.3; Gloria et al., 2012). Some gels demonstrate comparable dissipation to the NP in shear (0.24; Iatridis et al., 1999), while others are nearly elastic. For a single hydrogel material, the preparation method

1.3 Biomimetic scaffolds

(which likely affects the degree of crosslinking) can make a substantial difference to the degree of viscous dissipation; ionically crosslinked alginate prepared using a diffusion method and an *in situ* diffusion method had loss tangents of 0.2 and 0.1 respectively (Bron et al., 2011). However, unlike the elastic modulus, experiments with collagen, alginate and gellan gum show little variation in loss tangent with water concentration (Bron et al., 2011, 2009; Silva-Correia et al., 2011).

The viscoelastic behaviour of hydrogels in the time domain has been investigated to a lesser extent than in the frequency domain, although similar trends have been noted. Cloyd et al., 2007 observed a wide range in the degree of stress-relaxation between gelatin, alginate and agarose hydrogels. The study also reported little variation in the degree of stress-relaxation as the water concentration of alginate gels was varied.

Like the NP, the time-dependent behaviour of hydrogels arises as a result of fluid flow—poroelasticity—and the rearrangement of the polymer network—flow-independent viscoelasticity. However, there has been little investigation into the poroelastic behaviour of materials for IVD tissue engineering. Mizuno et al. (2006) characterised composite disc-like structures in confined compression using poroelastic theory and determined an effective permeability for the whole composite that was larger than the individual permeabilities of the AF and NP. Moss et al. (2010) attempted to characterise the time-dependent behaviour of a thiol-modified hyaluronan and elastin-like polypeptide composite hydrogel during confined compression, but found that poroelastic theory could not adequately explain the time-dependent behaviour of these gels, potentially because of the presence of flow-independent viscoelasticity. Zhao et al. (2010) has demonstrated that covalently crosslinked alginate gels demonstrate predominately poroelastic behaviour, while ionically crosslinked alginate gels demonstrate a mixture of poroelastic and viscoelastic behaviour.

Both fluid pressurisation and fluid shear stress have been shown to affect the response and morphology of AF cells (Reza & Nicoll, 2008; Wang et al., 2011), while nutrient transport likely plays a significant role in the success of an IVD scaffold (Nerurkar et al., 2010c). Furthermore, poroelastic and viscoelastic time-dependent behaviour can result in very different responses to applied loading

conditions (Huang et al., 2003). Decoupling poroelastic and viscoelastic mechanisms is crucial to understanding how these materials will behave *in vivo* both mechanically and as scaffolds for IVD tissue engineering.

Shape and structure

While shape and structure would not normally be considered to be functional properties in their own right, they have considerable influence on the behaviour of cells in a tissue engineering scaffold. AF and NP cells have been shown to behave differently when cultured in two-dimensional monolayer conditions versus being encapsulated in alginate; pressure is applied over the full surface of the cell and spatial gradients of nutrients exist within an alginate scaffold (Kluba et al., 2005). AF cells have also been demonstrated to align preferentially along the direction of electrospun nanofibres and collagen fibrils. Sanders et al. (2002) demonstrated that fibrous capsules were much more likely to form *in vivo* on polymer fibres with diameters larger than 6 μm , than on polymer fibres with diameters less than 6 μm , regardless of the material. Hence, whether a cell is cultured on a monolayer, in a porous foam, a hydrogel or a fibrous scaffold can have a significant impact on the success of the tissue engineered implant (Tibbitt & Anseth, 2009).

1.3.4 Performance

The aim of IVD tissue engineering is to alleviate pain resulting from failure of the IVD over the course of a patient's lifetime. This is difficult to evaluate and instead the performance of a tissue engineering scaffold is more often measured using three interrelated metrics: the biological performance of the scaffold, the success of the implant at encouraging *de novo* tissue growth with similar structures and properties to native healthy tissue, and the functional mechanical performance of the engineered tissue. The biological performance can be measured by examining cell proliferation, morphology and DNA expression. Tissue growth is often determined by the presence and quantity of ECM macromolecules such as collagen and GAGs. The biomechanics of an engineered tissue can be evaluated by comparing the basic mechanical properties of the tissue (such as unconfined compression

1.3 Biomimetic scaffolds

modulus) with mechanical properties of the IVD (e.g. Nerurkar et al., 2010a) or by using more functional indicators such as the range of motion of a spine containing the engineered disc (e.g. Kranenburg et al., 2012). For the remainder of this review, these three different aspects of performance are considered with a focus on how they are influenced by biomimetic scaffold architectures.

Cross-ply collagen laminates

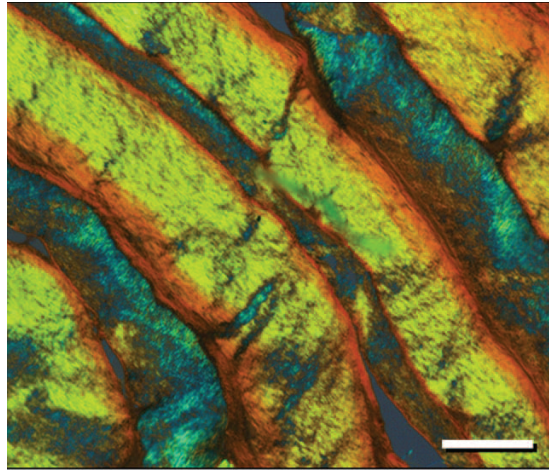


Figure 1.14: Polarised light micrograph of Picrosirius Red-stained section of tissue engineered annulus fibrosus cultured for 6 weeks with mesenchymal stem cells. The alternating hues show the opposing orientations of collagen fibres in adjacent lamellae that were deposited during the culture period. Scale: 250 microns. From Nerurkar et al. (2010a) with permission from Wolters Kluwer Health.

Nerurkar et al. (2009) demonstrated that cross-ply laminates of type I collagen can be grown *in vitro* with tensile circumferential moduli near native AF values. Electrospun PCL aligned fibre scaffolds were seeded with cells and cultured separately for two weeks. Laminates with opposing fibre orientations were then pressed together for one week after which enough ECM had built up at the interface between the laminates that the constraints could be removed. After ten weeks of culture, the engineered tissues were characterised. Polarising light microscopy revealed that a collagen-rich ECM had been deposited which mimicked the $\pm 30^\circ$ multilaminate structure of the AF (figure 1.14). The engineered tissue

had a tensile modulus that was within 15% of native values (14.2 ± 2.5 MPa versus 17.3 MPa; Nerurkar et al., 2009).

Koepsell et al. (2011) produced electrospun PCL scaffolds with varying degrees of anisotropy. The researchers found that the degree of anisotropy correlated with the degree of alignment of seeded AF cells and deposited collagen content. There is still much that can be improved upon in these studies, including increasing cell infiltration, creating a functionally graded scaffold and determining the time-dependent and compressive properties of the scaffold. Still, these studies exemplify the potential for a biomimetic method to produce engineered tissues that are functionally equivalent to native tissues.

Multi-phase architecture

Study	NP hydrogel	AF scaffold
Mizuno et al., 2004, 2006	alginate	non woven PLA-PGA mesh
Nesti et al., 2008	hyaluronic acid	random electrospun PLA
Nerurkar et al., 2010a	agarose	cross-ply electrospun PCL
Bowles et al., 2011, 2010	alginate	type 1 collagen gel
Park et al., 2012	fibrin/hyaluronic acid	lamellar silk structure

Table 1.5: Materials used for the generation of total IVD scaffolds, containing separate NP- and AF-like regions.

A number of investigators have fabricated scaffolds that contain separate AF- and NP-like regions (table 1.5). Mizuno et al. (2004, 2006), Nesti et al. (2008), and Nerurkar et al. (2010a) constructed composite discs with hydrogel centres surrounded by a fibrillar annulus. The composites were seeded with cells and cultured for several weeks. In all cases substantially more collagen was detected in the fibrillar region than in the hydrogel core, while the level of GAGs was comparable. Mizuno et al. (2004) and Nesti et al. (2008) also determined that the fibrillar region predominately contained type I collagen, similar to the AF, while the hydrogel region predominately contained type II collagen. Nesti et al., 2008 noted that cells seeded in the fibrillar region began to elongate and became layered in a concentric fashion similar to native AF cells, while cells in the gel region become rounded and encapsulated. Both Mizuno et al. (2006) and Nerurkar et al.

1.3 Biomimetic scaffolds

(2010a) investigated the compressive mechanical properties of the composites. Nerurkar et al. (2010a) noted that the compressive modulus of the composite disc-like structures was an order of magnitude greater than that of the agarose gel used to make up the core, although it was still substantially smaller than that of a native IVD. The compressive properties of the fibrillar region were not individually measured so it is not known whether the composites were truly behaving in a similar manner to the IVD, distributing compressive load in the NP to tensile hoop stress in the AF.

Bowles et al. (2011, 2010) also fabricated a disc-like structure by contracting a collagen gel around the outside of an alginate gel. The discs were cultured for six months *in vivo*. Unlike previous studies, at the end of the culture period the collagen and proteoglycan content of the engineered tissue was comparable to native AF and NP. Furthermore, the compressive mechanical properties were comparable to the native disc. Bowles et al. (2011) investigated the compressive properties of functional spine units (two vertebrae and one disc) containing the engineered IVDs. The group found that the stiffness and energy dissipation of these functional spine units was similar to native functional spine units.

However, it is unclear whether the comparative success of the tissue engineered IVD in the Bowles et al. (2011) study relative to similar studies (such as Nerurkar et al., 2010a) is a result of the different scaffold architectures and materials, or other factors. Bowles et al. (2011) cultured the scaffolds *in vivo*, while Nerurkar et al. (2010a) cultured the scaffold *in vitro*. Furthermore, Bowles et al. (2011)'s scaffolds had a much smaller diameter than Nerurkar et al. (2010a)'s scaffolds, which were still much smaller than a human IVD (4.1 mm versus 10 mm). Nerurkar et al. (2010a) found that ECM accumulation was much lower in the NP of the composite disc than in an equivalent engineered NP cultured without an artificial AF, indicating the challenges of delivering nutrients to larger constructs. It is also unknown how the engineered tissues in either study would perform in other deformation modes such as bending and torsion, as these are equally important to the function of the spine. Nevertheless these studies are promising as they demonstrate that composite discs with AF- and NP-like regions can be engineered.

Integration into the spine

The physiological attachment of IVD scaffolds to vertebrae has only been investigated to a limited extent. Bowles et al. (2012, 2011) designed scaffolds that were slightly larger than the IVD space and were a ‘press-fit’. After a six month culture *in vivo*, collagen bundle networks were observed to cross the boundary between the vertebrae interface and engineered tissue. Hamilton et al. (2006) specifically created an engineered CEP tissue. Cartilage cells were seeded onto a calcium polyphosphate scaffold and allowed to produce neo-cartilage for two weeks. NP cells were then seeded onto the neo-cartilage and cultured for eight weeks, by which time ECM resembling the NP had been formed. Hamilton et al. (2006) performed shear tests on the interfaces of the constructs and found that the peak load and energy-to-failure obtained in the three layer construct containing a CEP was significantly higher than that needed for a NP-calcium polyphosphate construct. Before any scaffold is applied clinically it is essential that there is further investigation into how to attach these constructs to the adjacent vertebrae.

1.4 Summary and thesis outline

There has been a surge of interest in biomimetic materials, which can facilitate the growth of new tissues. These efforts are promising and have demonstrated that cells can be enticed to form materials with a composition and structure similar to that of the IVD. Even so, little is known about the functional properties of these materials. The disc’s primary function is mechanical and so it is important to understand how the mechanical properties of engineered tissues compare with those of the native disc. Properties such as anisotropy, viscoelasticity, permeability, aggregate modulus and Poisson’s ratio are particularly important for these engineered tissues, but have only been investigated to a limited extent. A number of authors have assumed that engineered tissue with a similar composition to the native disc will have similar mechanical properties, and have neglected the complex structures that form the basis of the IVD. Future work needs to identify which features are sufficient to replicate native tissue behaviour and use this knowledge to create biomimetic scaffolds. Only then will it be possible to

1.4 Summary and thesis outline

develop clinically relevant tissue engineering options that can help the millions of people suffering from chronic back pain.

The overriding aim of this thesis was to create materials with structure-property relationships similar to those observed in the IVD, and to better understand those relationships. In the following chapter (chapter 2), an overview of the fundamental mechanical principles used throughout this work are presented. These include experimental characterisation methods (uniaxial tension, unconfined compression, confined compression and indentation testing), viscoelastic and poroelastic theories for time-dependent deformation and the mechanics of composite materials.

In the following two chapters (chapters 3 and 4), frameworks for the design of materials with tuneable time-dependent responses and separate tensile and compressive properties are reported. In chapter 3, the manufacture of composite hydrogels of agar, alginate, and gelatin is described. Indentation and unconfined compression experiments are performed on these hydrogels and the results are analysed using both viscoelastic and poroelastic frameworks. It is demonstrated that the degree of time-dependent behaviour of these composite hydrogels can be tuned independently of their elastic stiffness. In chapter 4, a method to create thick electrospun fibre-reinforced hydrogels with substantial tensile-compressive nonlinearities is discussed. The composites exhibit substantial strain-to-failures. It is shown that the compressive stiffness of the fibre-reinforced hydrogels can be altered almost independently of their tensile stiffness. These two experiments demonstrate that materials can be created with sophisticated mechanical behaviour that mimics some of the complex structure-property relationships present in the native IVD.

In order to better understand these structure-property relationships, improved methods for characterising the mechanical properties of biomimetic materials are needed. In the second half of this thesis (chapters 5 and 6), new methods for characterising the time-dependent behaviour of hydrogels are presented. In chapter 5, a finite element (FE) model of spherical indentation of a poroelastic hydrogel is developed. Using this model, it is shown that the load-relaxation of a poroelastic material can be described by a simple expression, which takes account of the finite-ramp time used to indent the material.

1.4 Summary and thesis outline

In chapter 6, the load-relaxation of poroviscoelastic (PVE) hydrogels is investigated. It is shown that the load-relaxation responses of PVE materials can be separated into poroelastic and viscoelastic relaxation responses. Using this insight, an approach for rapidly characterising PVE materials and predicting their load-relaxation response is presented. The approach is then used to examine the time-dependent behaviour of an agar hydrogel. The methods developed here will allow for a better understanding of how time-dependent behaviour can be tuned and optimised in materials for IVD tissue engineering. Furthermore, they provide physical insight into the mechanical behaviour of hydrogels and soft tissues.

The last chapter (chapter 7) provides a summary of the thesis. Original contributions of the work are reviewed with an emphasis on how the work fits into current research. Finally, an outline of future directions is discussed.

Chapter 2

Material characterisation methods and analytical techniques

This chapter introduces the key mechanics concepts that are used throughout the thesis. It begins with a discussion of the three material models considered in this work: elasticity, viscoelasticity, and poroelasticity. Tension, compression and indentation testing were all used to evaluate the properties of materials and identify their salient features; the advantages and disadvantages of each test method are discussed in section 2.3. Both viscoelastic and poroelastic methods for analysing the time-dependent behaviour of a material are reviewed in section 2.4. Finally, as many of the materials produced in this work are composites, a brief introduction to composite models is given in section 2.5.

2.1 Introduction

Soft tissues and tissue engineering scaffolds exhibit complex mechanical behaviour. In order to fully describe the behaviour of these materials, one would need to account for at least nonlinear elasticity, microstructural anisotropy, poroelasticity, flow-independent viscoelasticity and environmental effects including temperature and ionic concentration. However, there is an inherent trade-off between a model's generality and accuracy and its ease of application.

Furthermore, the ability to fully characterise these materials is often limited by the difficulty of obtaining samples and problems associated with sample preparation. It is more challenging to grip, machine, and test soft tissues and hydrogels than metals and stiff polymers (Anseth et al., 1996; Liu et al., 2009). Multiple tests must be performed because soft tissues are inherently variable and measurements are likely to have large standard deviations. The simplest model that captures relevant data is often preferable to a model which captures all phenomena but is impractical to characterise. In this work, there is a particular emphasis on characterising the time-dependent behaviour of the materials and three material models are considered: elasticity, viscoelasticity, and poroelasticity. It was decided not to investigate the nonlinear elastic characteristics of these materials and most of the characterisation has been done at small strains.

2.2 Material models

2.2.1 Elasticity

The deformation of a material is fundamentally related to the applied loading by the stress and strain at a point in the material. The stress, σ is defined as the load per unit area, i.e.

$$\sigma = \frac{P}{A}, \quad (2.1)$$

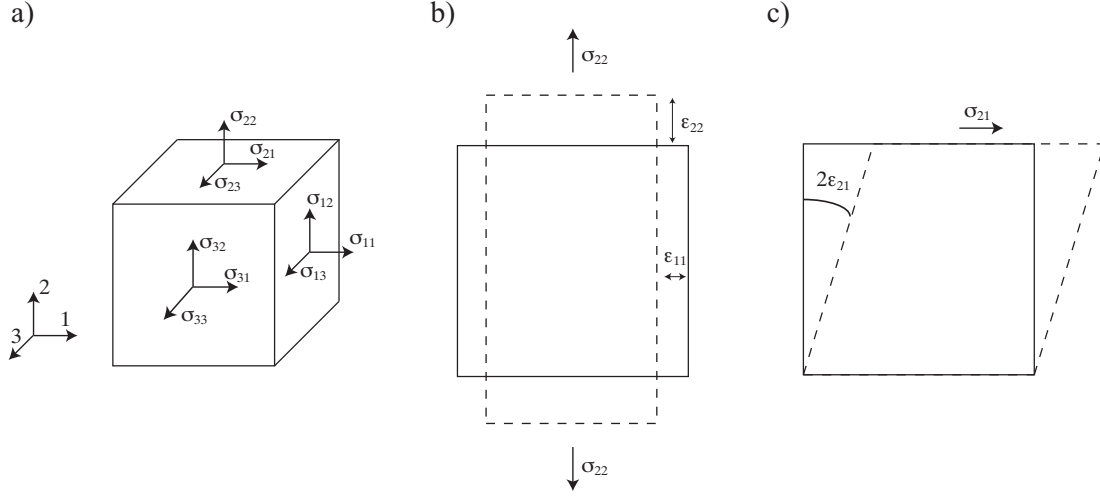


Figure 2.1: Schematic of elastic material illustrating: (a) the six components of stress at any point (as $\sigma_{12} = \sigma_{21}$, $\sigma_{13} = \sigma_{31}$ and $\sigma_{23} = \sigma_{32}$) and (b) the uniaxial deformation and (c) shear deformation of the elastic material.

where P is the load and A is the area. The strain, ϵ is defined as the change in length per original length, L at a point, i.e.

$$\epsilon = \frac{\Delta L}{L}. \quad (2.2)$$

There are six components of stress (three normal stresses and three shear stresses) and six components of strain at any point (figure 2.1). In a linearly isotropic elastic material, uniaxial stress is related to strain through the elastic modulus, E :

$$\sigma = E\epsilon. \quad (2.3)$$

When a uniaxial tensile strain is applied to a material, it contracts laterally. This deformation can be described by the Poisson's ratio, ν :

$$\nu = -\frac{\epsilon_{22}}{\epsilon_{11}}, \quad (2.4)$$

where ϵ_{22} is the applied strain, and ϵ_{11} is the strain in the perpendicular direction. For isotropic materials, the Poisson's ratio is bounded from -1 to 0.5, although most materials have Poisson's ratios between 0 and 0.5.

2.2 Material models

	E, ν	G, ν	H_A, ν	K, ν
$E =$	E	$2G(1 + \nu)$	$\frac{H_A(1+\nu)(1-2\nu)}{1-\nu}$	$3K(1 - 2\nu)$
$G =$	$\frac{E}{2(1+\nu)}$	G	$\frac{H_A(1-2\nu)}{2(1-\nu)}$	$\frac{3K(1-2\nu)}{2(1+\nu)}$
$H_A =$	$\frac{E(1-\nu)}{(1+\nu)(1-2\nu)}$	$\frac{2G(1-\nu)}{(1-2\nu)}$	H_A	$\frac{3K(1-\nu)}{1+\nu}$
$K =$	$\frac{E}{3(1-2\nu)}$	$\frac{2G(1+\nu)}{3(1-2\nu)}$	$\frac{H_A(1+\nu)}{3(1-\nu)}$	K
$E_R =$	$\frac{E}{1-\nu^2}$	$\frac{2G}{1-\nu}$	$\frac{H_A(1-2\nu)}{(1-\nu)^2}$	$\frac{3K(1-2\nu)}{1-\nu^2}$

Table 2.1: Common relationships between elastic constants. Adapted from Mow et al. (2005).

These two constants (E, ν) are sufficient to describe the mechanical behaviour of any linearly isotropic elastic materials, though it is often convenient to express stress-strain relationships in terms of other elastic contents depending on the applied loading. Other elastic constants of interest are the shear modulus G , which relates shear stresses to shear strains; the aggregate modulus H_A , which relates the applied axial stress to the strain in confined conditions ($\epsilon_{22} = \epsilon_{33} = 0$); the bulk modulus K , which relates an applied uniform pressure to the dilation (volumetric strain) of a material and finally the reduced modulus, E_R , which relates an applied stress to an applied strain in plane strain conditions ($\epsilon_{33} = 0$). Relationships between the constants are given in Table 2.1. It should be noted that a material with $\nu = 0.5$ is nearly incompressible and both the bulk modulus and aggregate modulus are infinite. For further information on the mechanics of elastic solids the reader is referred to Timoshenko & Goodier (1970).

2.2.2 Viscoelasticity

An elastic framework considers the response of the material to be independent of time. The material properties of soft tissues and tissue engineering scaffolds are generally far from time-independent. The effective stiffness that is measured during tension or compression will depend on the strain rate used to deform the material. At fixed strain, stress-relaxation will be observed, while at fixed

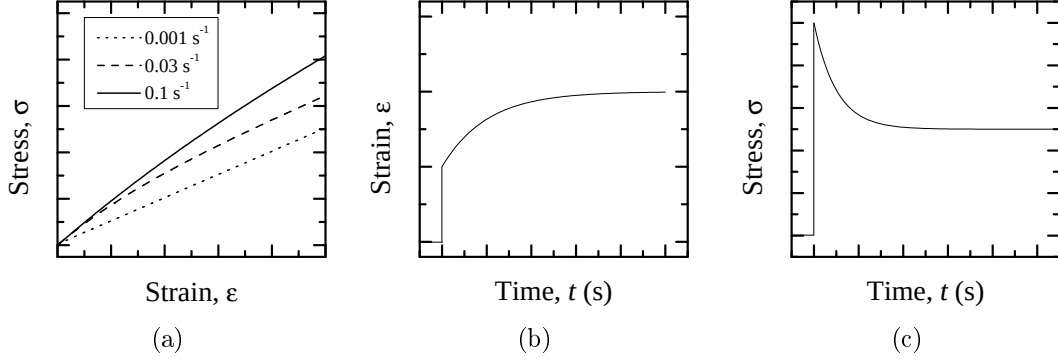


Figure 2.2: (a) Stress-strain responses of a viscoelastic material subjected to different strain-rates. (b) Idealised creep response of a viscoelastic material subjected to a sudden applied stress. (c) Relaxation response of a viscoelastic material subjected to a sudden applied strain.

stress, strain-creep will occur (figure 2.2). Physically, these effects may occur as a result of the physical rearrangement of molecules or fluid flow, among other reasons (Lakes, 2009). Together they can be modelled using a linear viscoelastic framework, which provides a phenomenological description of the material based upon the determination of a creep or relaxation function. Viscoelastic behaviour can be considered in both the time domain and the frequency domain. This work will be limited solely to a discussion of viscoelastic effects in the time domain.

Relaxation modulus and creep compliance

When a step strain, $\epsilon(t) = \epsilon_0 \mathcal{H}(t)$, is applied to a viscoelastic material, the stress in the material will not remain constant but will relax with time ($\mathcal{H}(t)$ is the Heavyside step function). The uniaxial relaxation function, $E(t)$, describes how the stress in the material will decrease in time in response to the step strain:

$$E(t) = \frac{\sigma(t)}{\epsilon_0}. \quad (2.5)$$

Similarly, the uniaxial creep function of a viscoelastic material, $J_E(t)$, describes how the strain in a material will creep with time when a step stress, $\sigma(t) = \sigma_0 \mathcal{H}(t)$, is applied:

2.2 Material models

$$J_E(t) = \frac{\epsilon(t)}{\sigma_0}. \quad (2.6)$$

Response to arbitrary loading profile

The creep and relaxation functions can be used to predict the response of the material to an arbitrary loading profile. The relaxation response of a material can be expressed as the response to a superposition of an infinite number of step strains, which leads to the relationship (Lakes, 2009):

$$\sigma(t) = \int_0^t E(t-u) \frac{d\epsilon(u)}{du} du, \quad (2.7)$$

known as the Boltzmann hereditary integral. The corresponding relationship describing creep is:

$$\epsilon(t) = \int_0^t J_E(t-u) \frac{d\sigma(u)}{du} du. \quad (2.8)$$

Exponential creep and relaxation functions

Although the form of the creep and relaxation functions is not completely arbitrary and must obey a number of restrictions including conservation of energy (Lakes, 2009), the functions can take a variety of forms depending on the material, including power laws, stretched exponentials and logarithms. The relaxation function of biological materials is thought to be well described by a series of exponentials such that:

$$E(t) = C_0 + \sum_{i=1}^n C_i \exp(-t/\tau_i), \quad (2.9)$$

where C_i and τ_i are constants (Oyen, 2005a). Similarly the creep function of a biological tissue can be represented by :

$$J_E(t) = D_0 - \sum_{i=1}^n D_i \exp(-t/\tau_i). \quad (2.10)$$

The creep and relaxation functions appear qualitatively similar, but the time constants, τ_i , are not equivalent. Instead, the creep function and relaxation function are related by the formula:

$$E(s)J_E(s) = \frac{1}{s^2}, \quad (2.11)$$

where $E(s)$ and $J_E(s)$ are the Laplace transforms of the creep and relaxation functions (Lakes, 2009).

The coefficients of exponential functions can be related to the stiffness and damping coefficients of a spring dashpot model. If $n = 1$, both the creep and relaxation functions correspond to the standard linear solid shown in figure 2.3. Having said that, these spring-dashpot models are merely a convenient description of viscoelastic behaviour and do not necessarily convey real physical insight that can be related to the underlying microstructure.

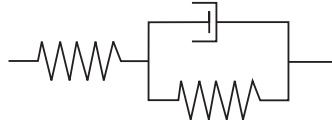


Figure 2.3: The standard linear solid model.

Instantaneous and equilibrium shear moduli

While the constants in the creep and relaxation functions give information about the shape of the response, frequently only the instantaneous and equilibrium moduli are reported for the sake of brevity. These can be determined from $E(t)$ or $J_E(t)$ at $t = 0$ and $t = \infty$:

$$E_0 = \left(C_0 + \sum_{i=1}^n C_i \right) = \frac{1}{(D_0 - \sum_{i=1}^n D_i)}, \quad (2.12)$$

and

$$E_\infty = C_0 = \frac{1}{D_0}. \quad (2.13)$$

2.2 Material models

The ratio between the equilibrium and instantaneous moduli (i.e. the viscoelastic ratio: E_∞/E_0) is a measure of viscoelastic extent, where $E_\infty/E_0 = 1$ corresponds to an elastic solid and $E_\infty/E_0 = 0$ corresponds to a viscous fluid.

Bulk, shear and uniaxial viscoelasticity

So far the relationships presented here relate uniaxial stress to uniaxial strain, in terms of the relaxation function, $E(t)$, and creep function, $J_E(t)$. Viscoelastic deformation also affects the bulk and shear behaviour of the material and can be described by a shear relaxation function, $G(t)$, and bulk relaxation function, $K(t)$, as well as equivalent creep compliances. This has the consequence that Poisson's ratio, ν , can be a function of time, $\nu(t)$. For an isotropic viscoelastic material, knowing any two material parameters will fully define the others: $G(t) = E(t)/(1 + \nu(t))$ and $K(t) = E(t)/[3(1 - 2\nu(t))]$.

Although it is possible to separately identify two time-dependent relaxation functions, in practice this is difficult. Frequently one of two assumptions is made so that only one time-dependent function needs to be identified. Either, it is assumed that only deviatoric deformation is time-dependent and that the bulk modulus is time-independent (Lakes, 2009) with the added consequence that the Poisson's ratio will increase with time (as $\nu(t) = \frac{3K-2G(t)}{2(3K+G(t))}$). Or, it can be assumed that Poisson's ratio is time-independent such that $E(t) \propto G(t) \propto K(t)$. This latter assumption will be adopted here as it allows for simple conversions between viscoelastic functions, particularly in the case of indentation (Cheng & Cheng, 2005; Oyen, 2006).

2.2.3 Poroelasticity

While viscoelastic creep and relaxation functions describe the behaviour of a time-dependent material, these models reveal little about the actual structural and physiological reasons for viscous creep and relaxation. In contrast, poroelastic models assume that the material consists of a coherent porous solid skeleton filled with a freely moving fluid. Time-dependent deformation results from the frictional drag of interstitial fluid flow through the solid skeleton. When a compressive load is applied to a poroelastic material, the modulus is initially much

larger than the eventual equilibrium modulus as the bulk of the load is supported by viscous drag (figure 2.4). Hydrostatic pressure is generated in the fluid. As time progress, the fluid flows out of the tissue until the pressure in the fluid reaches equilibrium and the load is redistributed among the solid phase.

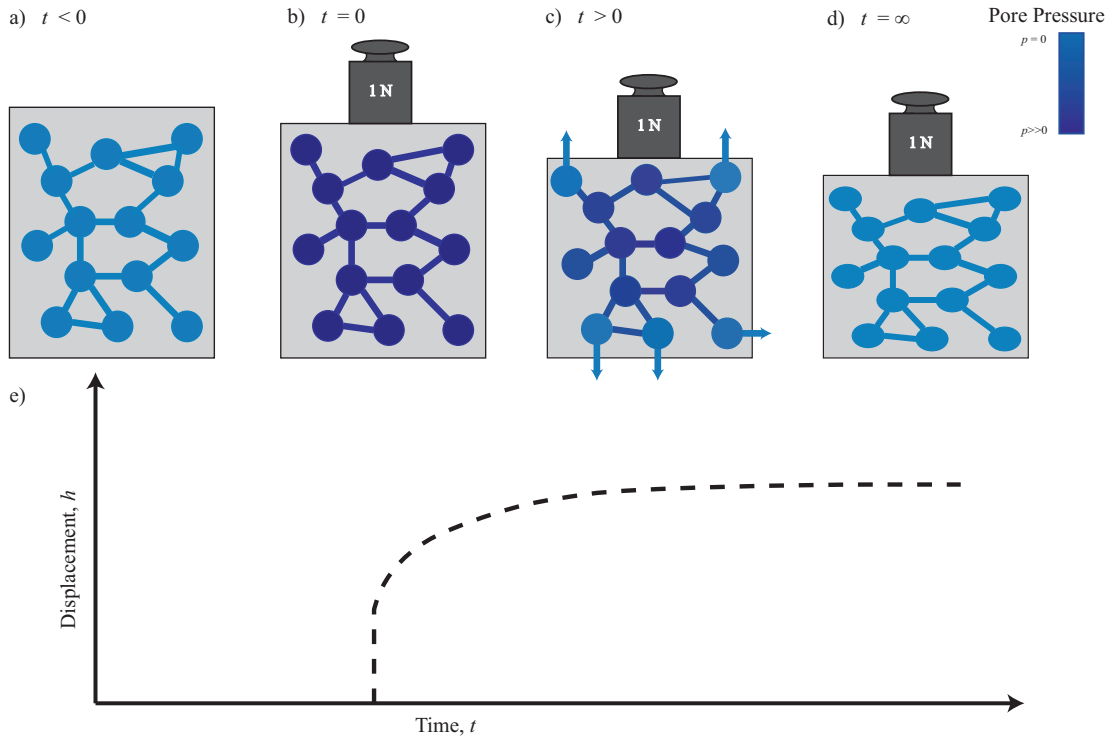


Figure 2.4: Schematic depicting the response of a poroelastic material to a step-load. Initially the material deforms little (b) but the pore pressure in the material increases. Fluid then begins to flow out of the material (c) until the pore pressure in the material has equilibrated with the applied load (d). Adapted from figure provided by Matteo Galli.

A full set of constitutive equations for poroelastic behaviour was first fully described by Biot (1941). However, poroelasticity was first described in the context of cartilage and other soft-tissues under the name of biphasic theory by Mow et al. (1980). It has since been shown that poroelasticity and biphasic theory are equivalent when the fluid is assumed to be inviscid (which is a reasonable assumption for soft-tissues and hydrogels), and as a result solutions from both theories will be presented interchangeably (Simon, 1992; Wu et al., 1997).

2.2 Material models

Constitutive equations

Poroelastic materials can be modelled as a continuum, where the length scale of interest is much greater than the length scale of the fluid-filled pores. The constitutive equations of a poroelastic material couple the elastic stresses and strains in the material with the pore pressure and fluid volume in those pores. The state of the material is described in terms of the stress, σ_{ij} , strain, ϵ_{ij} , pore pressure, p and increment of fluid content ζ . Stress and strain are analogous to their elastic counterparts, while pore pressure is equivalent to the pressure of the fluid occupying that point in space. The increment of fluid content, ζ , is equal to the flow of fluid into an element divided by the volume of that element.

Two constitutive equations are needed to relate these variables:

$$\epsilon_{ij} = \frac{1}{2G} \left[\sigma_{ij} - \frac{\nu}{1+\nu} \sigma_{kk} \delta_{ij} + \frac{1-2\nu}{1+\nu} \alpha p \delta_{ij} \right], \quad (2.14)$$

$$\zeta = \frac{\alpha}{K} \frac{\sigma_{kk}}{3} + \frac{\alpha^2 (1-2\nu)(1+\nu_u)}{3K(\nu_u - \nu)} p, \quad (2.15)$$

where G is the shear modulus, K is the drained bulk modulus ($K = \frac{2G(1+\nu)}{3(1-2\nu)}$), ν_u is the undrained Poisson's ratio, ν is the drained Poisson's ratio, and α is the effective stress coefficient that relates volumetric strain to the increment of fluid content under constant pressure.

For a full discussion of these constitutive equations, the reader is referred to Detournay & Cheng (1993) or Wang (2000), but it is worth mentioning two things. First, linear poroelasticity only affects the volumetric behaviour of the material; equation 2.14 shows that shear strains solely depend on the shear modulus and applied shear stress and not pore pressure. This is in direct contrast to the assumptions for viscoelasticity adopted in section 2.2.2. Second, a poroelastic problem can often be thought of as transitioning between two elastic states: the instant immediately after the application of a step load before fluid has begun to flow ($\zeta = 0$) and the equilibrium drained case where the pressure in the material has dissipated ($p = 0$). When the increment of fluid content, ζ is 0, it can be shown that the material behaves elastically with a Poisson's ratio equivalent to the undrained Poisson's ratio. When the pore pressure, p is 0 the material behaves

elastically with a Poisson's ratio equal to the drained Poisson's ratio. (This result can be readily seen from equation 2.14; the former can be shown by substituting 2.15 into 2.14, assuming $\zeta = 0$.)

Darcy's law

The constitutive equations describe the state of the material at any one point in time, but do not describe how fluid will flow through the material with time. Fluid flows away from regions of high pressure to regions of low pressure and this behaviour can be described using Darcy's law. Neglecting gravity:

$$q_i = -\kappa \frac{dp}{dx_i}, \quad (2.16)$$

where q_i is the fluid flux (discharge per unit area) and κ is the hydraulic permeability. The hydraulic permeability is a measure of the material's resistance to fluid flow and is analogous to the diffusion coefficient, but under a pressure gradient rather than a concentration gradient.

Material constants

The deformation of a poroelastic material can be characterised by 5 material constants: shear modulus, G , undrained Poisson's ratios, ν_u , drained Poisson's ratio ν , hydraulic permeability, κ , and the effective stress coefficient, α . Here, the undrained condition refers to the instantaneous material properties of the tissue after the application of a load but prior to the onset of fluid flow. The drained condition describes the state of the material after the internal pore pressure has reached equilibrium with the applied load.

Poroelastic soft tissues and hydrogels are generally modelled as incompressible fluids mixed with incompressible solids: ν_u is assumed to equal 0.5 and α (which is related to the compressibility of the solid) is assumed to equal 1. As this work only considers hydrogels and soft tissues, the equations presented here will be simplified using these assumptions rather than presenting their more general forms (where they exist). Therefore, when characterising the linear isotropic

2.2 Material models

poroelastic behaviour of a soft tissue or hydrogel we generally seek to identify three materials parameters: G , ν , and κ .

Permeability

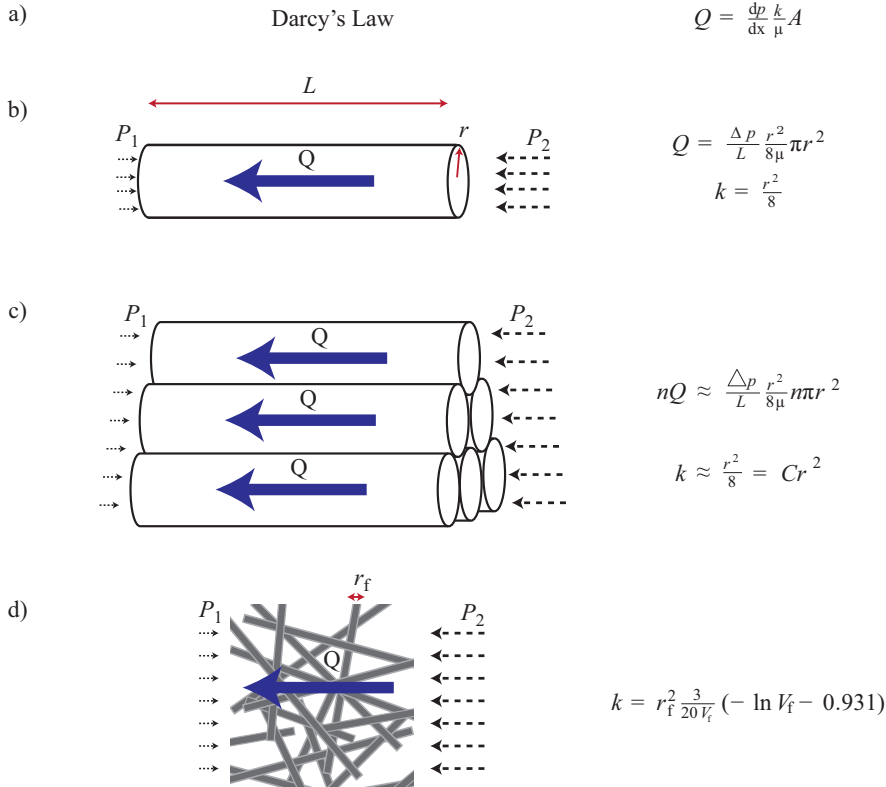


Figure 2.5: Schematic showing various microstructures and their theoretical permeabilities, including: (b) a thin-walled pipe, (c) a cylindrical porous network of thin-walled pipes and (d) Jackson & James (1986)'s model for random fibrous networks.

The hydraulic permeability of a material, κ , characterises the viscous drag that results as a fluid flows through a solid matrix; κ is a property of the structure of the matrix and the viscosity of fluid. The hydraulic permeability can be related to the intrinsic permeability—which is solely a function of the structure of the material—according to:

$$k = \mu\kappa, \quad (2.17)$$

where μ is the dynamic viscosity of the fluid.

The intrinsic permeability is indicative of the effective pore size of the material. This can be illustrated by considering the permeability of an idealised material consisting of a thin-walled pipe (figure 2.5). The flow through a poroelastic material is assumed to be laminar, due to the small length scales involved. The pressure drop in a laminar fluid flowing through a pipe is governed by the Poiseuille equation:

$$\Delta p = -\frac{8\mu L Q}{\pi r^4}, \quad (2.18)$$

where Δp is the pressure drop across the pipe, Q is the volumetric flow rate ($Q = qA$), L is the length of the pipe and r is the radius of the pipe. By comparing the Poiseuille equation with Darcy's law ($\frac{dp}{dx} \propto \mu q$), it can be seen that for this structure, $k = \frac{r^2}{8}$. A material consisting of several thin-walled pipes would have approximately the same permeability as a single pipe: the volumetric flow rate through the material would be greater but the total cross-sectional area would also increase proportionally (assuming the pipe wall area is negligible). More generally, a material will be composed of pores with varying fluid paths and the solid skeleton will comprise a significant proportion of the cross-sectional area. Therefore:

$$k = Cr^2, \quad (2.19)$$

where C is a constant depending on the geometry and r is the effective pore radius of the material (Mow et al., 1984).

The above illustration relates the permeability of a material to an effective pore size, but for fibrous networks it can be more convenient to relate the permeability to the fibre radius. Jackson & James (1986) investigated the permeability of a wide range of fibrous networks (including hydrogels and polymers) and showed that the permeability of a fibrous network scales with:

$$k = r_f^2 f(V_f), \quad (2.20)$$

where r_f is the fibre radius and V_f is the solid volume fraction of the material. $f(V_f)$ depends on the arrangement of the fibrous network. For isotropic fibrous networks, they used theoretical calculations based upon the Navier-Stokes equa-

2.3 Mechanical testing

tion to show that:

$$f(V_f) = \frac{3}{20V_f} (-\ln V_f - 0.931). \quad (2.21)$$

This result agrees well with more recent calculations of permeability through fibrous lattices using three-dimensional computations (Nabovati et al., 2009).

2.3 Mechanical testing

In this section the advantages and disadvantages of four mechanical test methods for characterising the elastic behaviour of solids will be discussed (figure 2.6). The test methods used in this work are uniaxial tension, uniaxial (unconfined) compression, confined compression, and indentation. In section 2.4, analysis methods to determine viscoelastic and poroelastic material parameters will be reviewed.

2.3.1 Uniaxial tension and unconfined compression

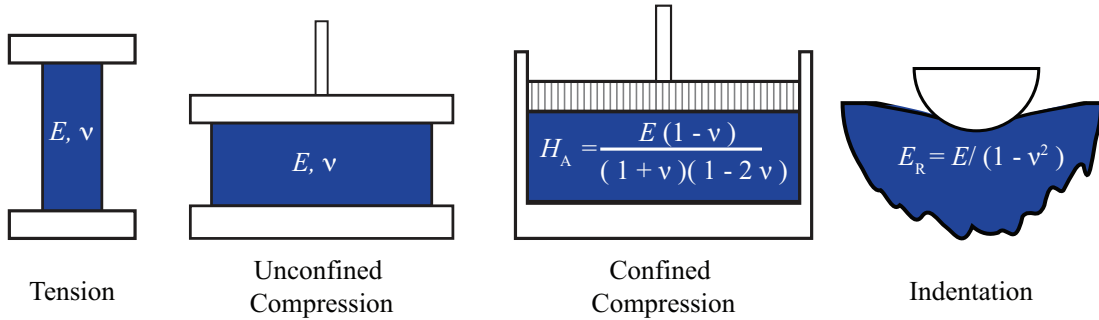


Figure 2.6: Diagram depicting the four mechanical test methods that will be used to characterise the materials in this work. The confined compression setup is shown here with a permeable platen, impermeable bottom and impermeable side walls as would be used to characterise the poroelastic properties of a material. See section 2.4.2 for details.

Tensile testing is a direct way of determining the uniaxial stress-strain behaviour and Young's modulus of a material, E . The applied stress is directly related to the applied strain by E ; hence the load, P is related to the sample extension,

h according to:

$$P = E \frac{hA}{L}, \quad (2.22)$$

where A is the cross-sectional area of the sample and L is the sample length (or height). Poisson's ratio can also be directly measured using optical methods to determine the lateral strain. Failure strength can be quantified, as long as the failure occurs near the middle of the sample, which is facilitated by machining the sample into a dog-bone shape that reduces the stress concentration and likelihood of failure at the grips. Material anisotropy can also be characterised.

Particular challenges exist for testing soft tissues and hydrogels in tension. It is often difficult to machine samples into their characteristic dog-bone shape necessitating the use of rectangular samples instead. It can be particularly difficult to grip hydrated samples without applying significant pressure and slippage at the grips is not uncommon. It is also not entirely clear where the initial loading point of the sample is; slack must first be removed from the sample, but the sample may exhibit nonlinear stress-strain behaviour which qualitatively looks very similar (Drury et al., 2004). Although tensile testing is the gold standard for testing stiff materials, in this work it is only used when testing electrospun fibrous materials and related materials in chapter 4.

In unconfined compression testing a compressive uniaxial stress or strain is applied to a material; a compressive elastic modulus can be determined from equation 2.22. In isotropic materials with no tensile-compressive nonlinearity this is equivalent to the tensile elastic modulus. Unconfined compression tests are performed on cylindrical samples that have free boundaries. Compression testing is often preferred to tensile testing of hydrogels as there is no need to grip the sample. It is also relatively easy to perform the tests in a fully hydrated environment, which more closely mimics *in vivo* conditions. Still, the sample surfaces must be parallel and flat—determining the contact point of the platen with the sample can be difficult otherwise. Friction between the platen and the sample must also be minimised so that the lateral expansion of the material is not confined (Miller, 2005). Because the lateral expansion of the tissue is unconfined, it is possible to directly measure the Poisson's ratio of the tissue during unconfined compression using optical methods (Jurvelin et al., 1997).

2.3 Mechanical testing

2.3.2 Confined compression

Confined compression tests are often performed to measure the aggregate modulus, H_A of the material as the compressive load is related to the applied displacement according to:

$$P = H_A \frac{hA}{L}. \quad (2.23)$$

The cylindrical sample is placed in a container where lateral deformation is prevented. The container must be relatively frictionless in order to not prevent vertical deformation. The sample surface must also be relatively flat and the gap between the platen and container walls must be small, otherwise the sample will not be initially confined when contact is made (Gervaso et al., 2007). Even though it is a difficult test to perform, confined compression testing has become a popular testing technique because it provides a direct measurement of the aggregate modulus and because a well defined solution for the time-dependent behaviour of a poroelastic material undergoing confined compression exists (section 2.4.2).

2.3.3 Indentation

Indentation involves measuring the load, P required to press a tip into a sample to a displacement, h . It requires minimal sample preparation. The sample only needs to have a reasonably flat surface. Because the deformation involved in indentation is confined to a small area, multiple indents can be performed on the same sample and property maps can be developed. Furthermore, using indentation, a variety of scales of microstructure can be tested. Nanoindentation instruments have displacement ranges on the order of nanometres to micrometres and universal testing machines have displacement ranges of micrometres to millimetres.

Unlike compression or tension tests, the load-displacement relationships measured during indentation tests cannot be directly related to stress-strain relationships (Herbert et al., 2001); the deformation experienced within the material is not uniform. However, there are a variety of analytical and numerical models that can be fitted to the load-displacement data in order to extract relevant material properties (Johnson, 1985; Oliver & Pharr, 1992; Sneddon, 1965).

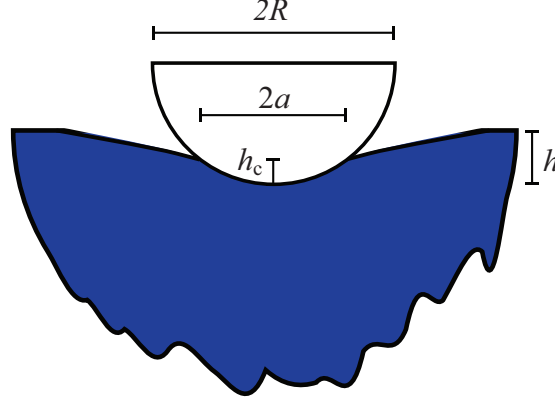


Figure 2.7: Schematic diagram of spherical indentation of a bulk half-space showing the tip radius, R , contact radius, a , indenter displacement, h and contact depth, h_c .

The most well known analytical model is the elastic Hertz solution, between a sphere and a bulk half-space (Johnson, 1985). According to the Hertz solution, if the modulus of the indenter tip is much greater than the modulus of the material in question, the indenter load and displacement are related such that:

$$P = \frac{4\sqrt{R}}{3} E_R h^{3/2}, \quad (2.24)$$

where E_R is the reduced modulus,

$$E_R = \frac{E}{1 - \nu^2}, \quad (2.25)$$

and R is the radius of the spherical tip (Johnson, 1985). The reduced modulus is directly identified by the indentation test and the elastic modulus cannot be determined on its own without assuming *a priori* a value of the Poisson's ratio, ν .

Huber (1904) derived expressions for the state of stress under a Hertzian indenter. These stress and strain fields are shown in figure 2.8. The size of the stress and strain fields is a function of the contact radius between the sphere and half-space, a . For Hertzian contact, the contact radius is related to the indentation depth according to:

2.3 Mechanical testing

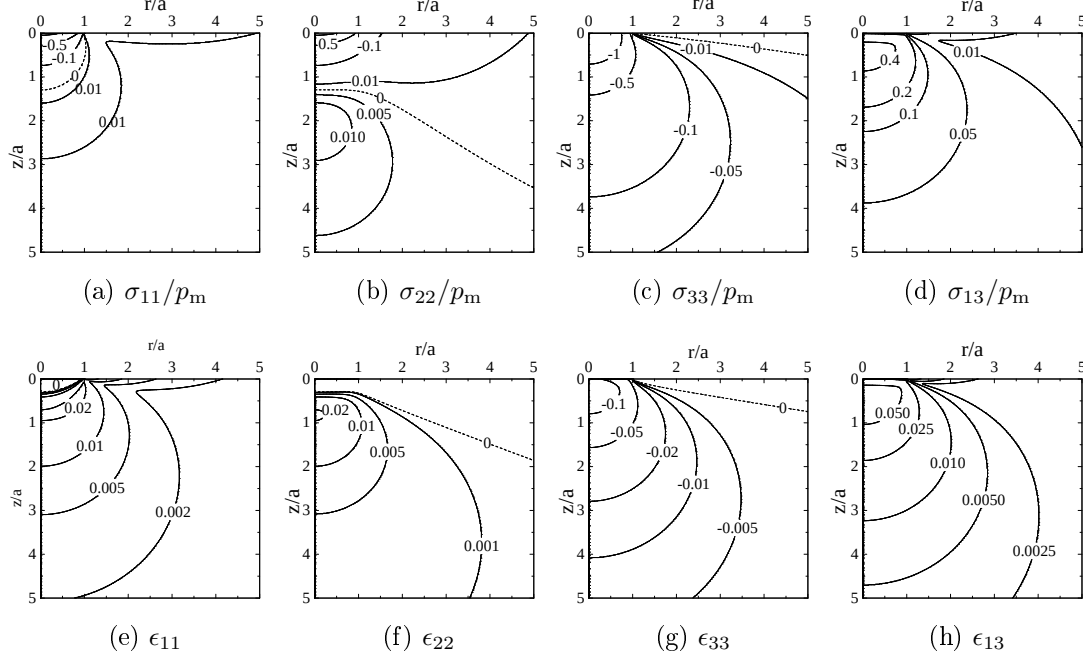


Figure 2.8: Principle stress and strain fields under a Hertzian indenter plotted as a function of the contact radius, a . The fields were calculated using the equations in Fischer-Cripps (2000); Huber (1904) for $\nu = 0.26$ and $a/R = 0.25$. Stresses are normalised by the mean contact pressure, $p_m = \frac{4}{3\pi} E_R (a/R)$.

$$a = \sqrt{Rh}. \quad (2.26)$$

The magnitude of the stress and strain is a function of the ratio of contact radius to the indenter radius (a/R) and the sample reduced modulus, E_R . The magnitude of stress and strain gets progressively smaller as the distance from the contact region increases.

Although, the strain field is not uniform, Tabor (1951) proposed that a representative indentation strain, ϵ be used as a benchmark of the magnitude of the indentation strain field, where

$$\epsilon \approx 0.2 \frac{a}{R}. \quad (2.27)$$

The indentation strain has been used to correlate spherical indentation measurements with the tensile stress-strain curves of elastic-plastic metals beyond the linear elastic regime (Field & Swain, 1995; Swain & Lawn, 1969; Tabor, 1951).

FE studies have also demonstrated that indentation strain can be used to characterize the strain field of a hyperelastic material (Lin et al., 2009).

The Hertz model has a simple algebraic form, though it makes a number of assumptions which may differ from the reality of an experiment. Many of these assumptions are only valid for small indentation strains. The model assumes that the shape of the indenter tip can be described by a paraboloid (i.e. not a true sphere) though this effect is small when the indentation depth is small relative to the contact radius (see chapter 5 for further details). The Hertz model also assumes that finite-deformation effects can be neglected. Experiments using compliant photo-elastic materials as well as FE simulations of elastic-plastic materials have shown that finite-deformation effects can become significant when $a/R > 0.25$ (i.e. at indentation strains greater than 0.05) although below this the Hertz load-displacement relationship provides a reasonable description of observed behaviour (Fessler & Ollerton, 1957; Johnson, 1982; Mesarovic & Fleck, 1999). Similarly, the Hertz model assumes the material is governed by linear elasticity with uniform material properties. The effect of non-linear elastic behaviour can be significant, though FE simulations of hyper-elastic materials with properties typical of synthetic gels have shown that the Hertz model provides a reasonable description of the behaviour of materials for indentation strains less than 0.05 (Lin et al., 2009).

The Hertz model assumes that contact between the indenter and half-space is frictionless with no adhesion. Solutions of spherical indentation with finite friction reveal that the total load needed to produce a contact radius of a given size can only be a maximum of 5% larger than that predicted by the Hertz solution (Johnson, 1985). However, the presence of friction can substantially alter the strain distribution, increasing the magnitude of radial tensile strain at the edge of contact (Johnson, 1982; Mesarovic & Fleck, 1999). Adhesion between the sample and tip can have a substantial effect on the measured load-displacement relationship. The presence of adhesion is typically noted by the presence of a pull-off force when the indenter is removed from the sample surface. This pull-off force can be used to characterise the strength of the adhesive interaction, although that is beyond the scope of this work (Johnson, 1985).

2.3 Mechanical testing

Finally, the Hertz model assumes that the material is a bulk half-space. Given that the stress field spreads out from the initial indentation site and its magnitude far from the contact region is small (figure 2.8), several investigators have suggested that a bulk half-space condition can be assumed if the contact radius or indentation depth, relative to the specimen thickness, is small (i.e. $a < 6L$ or $h < 0.1L$) and the indent is performed far from the edge of the sample (a distance of at least $6-8a$; Oyen et al., 2004; Tabor, 2002). For materials where this is not the case, models of indentation of materials with finite thicknesses have been developed (Dimitriadis et al., 2002; Hu et al., 2011; Yu et al., 1990). These models also allow the error in assuming a bulk half-space condition to be quantified. If the contact radius, a is less than 0.2 times the sample thickness, L , the difference in load predicted between the Hu et al. (2011) and Hertz solutions is less than 10%. If a is less than 0.1 times the sample thickness, then this difference is less than 1%. The Hu et al. (2011) model assumes that the material being indented has been placed on a rigid frictionless substrate. While the precise boundary conditions between the sample and its surroundings can affect the load-displacement relationships for finite-thickness samples, FE simulations have demonstrated that the boundary conditions of the material are inconsequential when the boundary is far from the indentation site (Mesarovic & Fleck, 1999).

Although the indentation method is more complicated to analyse than unconfined compression or tension results, it is used frequently throughout this work because of the limited sample preparation required. Effort is made to remain within a Hertzian regime and indentation strains are limited to 0.05 while the sample thickness is at least five times greater than the indentation contact radius, if not larger. The effects of friction and adhesion are limited by performing the indentation measurements in a fully-hydrated environment. Still, given that the materials investigated in this work are complex, caution must be taken when interpreting these results as unexpected deviations from the Hertz model may occur. Hence, these measurements have largely been performed in conjunction with another testing technique such as unconfined compression or tension.

2.4 Analysis

2.4.1 Viscoelasticity

For a viscoelastic material, we seek to identify the constants in the viscoelastic creep or relaxation function (equation 2.9) and determine the instantaneous and equilibrium uniaxial, shear or bulk moduli. For many viscoelastic problems, analytical expressions governing the deformation of a material can be determined by solving the Boltzmann hereditary integral (equation 2.7) for the applied loading conditions. The analytical expressions can then be fit to experimental data to identify the relevant material parameters.

Unconfined compression and tension

In this work, either a constant strain rate profile is used to deform the material or a ramp-hold profile is used. If a constant strain rate, $\dot{\epsilon}$, is used to deform the material:

$$\frac{d\epsilon_{11}(u)}{du} = \dot{\epsilon}, \quad (2.28)$$

and the relaxation function takes the form of a series of exponentials (equation 2.9) the uniaxial stress will vary with time according to:

$$\sigma_{11}(t) = \dot{\epsilon} \left[C_0 t + \sum_{i=1}^n C_i \tau_i (1 - \exp(-t/\tau_i)) \right]. \quad (2.29)$$

This equation can then be fit to an experimentally observed stress response, to determine the coefficients C_i and τ_i , and hence $E(t)$.

A ramp-hold profile (figure 2.9) is advantageous for characterising time-dependent behaviour as the relaxation observed in the hold period is less sensitive to any discrepancies that occur in the ramp such as contact. If a ramp-hold profile is used to characterise a viscoelastic material, the stress during the hold period will be:

$$\sigma_{11}(t) = \epsilon_{11} \left[C_0 + \sum_{i=1}^n RCF_i C_i \exp(-t/\tau_i) \right], \quad t \geq t_R, \quad (2.30)$$

2.4 Analysis

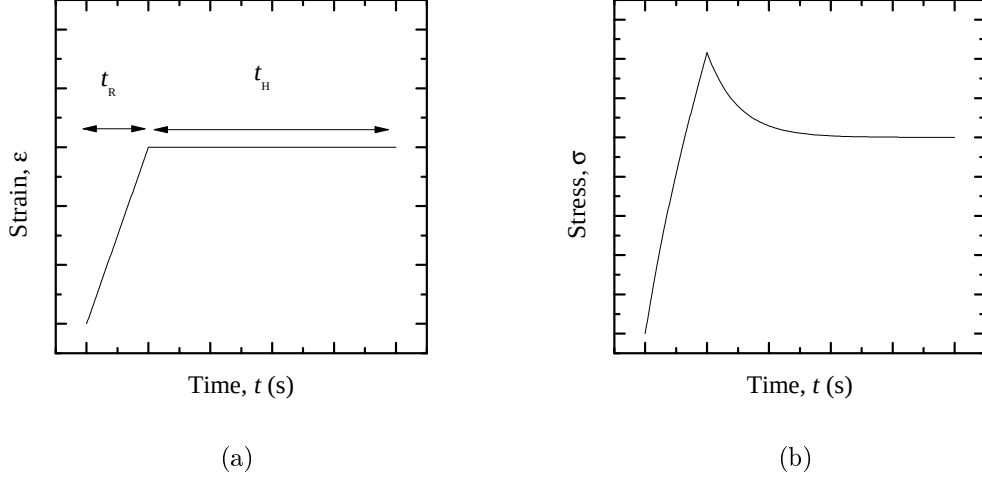


Figure 2.9: Schematic of a ramp-hold profile showing both the strain-time loading (a) and the resultant material stress-time (b) curve. The strain was increased at a constant rate for t_R seconds and then held for t_H seconds.

where:

$$RCF_i = \left[\frac{\tau_i}{t_R} (\exp(t_R/\tau_i) - 1) \right], \quad (2.31)$$

and t_R is the ramp time. The solution differs from that of a step displacement by the ramp correction factor RCF_i , which accounts for any relaxation that occurs during the ramp phase (Oyen, 2006).

Indentation

For unconfined compression and tension experiments, it is simple to determine the constants of the creep or relaxation function as the stress and strain of the sample are known. For indentation experiments, the stress and strain in the material are not uniform. Nevertheless, by using the viscoelastic correspondence principle, whereby viscoelastic operators are replaced for their equivalent elastic counterparts in Laplace space, the creep behaviour of material indented with a spherical indenter under load control can be determined. It is necessary to assume a constant Poisson's ratio and it is convenient to work with the shear relaxation, $G(t)$, and creep functions, $J_G(t)$. Assuming $\nu = 0.5$ and replacing P/G in the

Hertz solution (equation 2.24) with the Boltzmann hereditary integral operator for creep, the viscoelastic response of the material is (Oyen, 2005a, 2006)¹:

$$h^{3/2}(t) = \frac{3}{16\sqrt{R}} \int_0^t J_G(t-u) \frac{dP(u)}{du} du. \quad (2.32)$$

An analytical solution can then be derived that describes how the material creeps with time in response to the applied loading.

A similar equation can be derived for the relaxation response of a solid indented using displacement control (Mattice et al., 2006):

$$P(t) = \frac{16\sqrt{R}}{3} \int_0^t G(t-u) \left[\frac{d}{du} h^{3/2}(u) \right] du. \quad (2.33)$$

Unlike the equation for load control, this equation has no analytical solution. Instead, Mattice et al. (2006) proposed a semi-analytical solution for viscoelastic solids modelled with an exponential relaxation function (equation 2.9):

$$P(t) = \frac{16\sqrt{R}}{3} h_{\max}^{3/2} \left(C_0 + \sum_{i=1}^n RCF_i C_i \exp(-t/\tau_i) \right), \quad t \geq t_R, \quad (2.34)$$

where $RCF_i = \left[\frac{\tau_i}{t_R} (\exp(t_R/\tau_i) - 1) \right]$, $h_{\max}$ is the peak displacement and t_R is the ramp time. As indicated by the inequality, the function can only be fitted to the hold portion of a displacement controlled indent. The solution has recently been validated with FE simulations (Qiang et al., 2011).

While the above analysis of indentation assumes that $\nu = 0.5$ implying that the material is incompressible, this need not be the case; ν only must be constant. The incompressible modulus, G^I , which assumes $\nu = 0.5$, can be related to a shear

¹In the original work of Oyen (2005a, 2006), $P/2G$ is replaced with the hereditary integral for creep, instead of P/G . Similarly, in Mattice et al. (2006), $2Gh^{3/2}$ and not $Gh^{3/2}$ is substituted for the hereditary integral for relaxation. In all of these studies $J_G(t)$ and $G(t)$ are defined such that $G_0 = 2G(0) = \frac{1}{2J_G(0)}$. In this work, for simplicity, it was decided to define $J_G(t)$ and $G(t)$ to be directly equivalent to their elastic analogues (section 2.2.2).

2.4 Analysis

modulus computed with Poisson's ratio, ν , according to:

$$G = 2G^I(1 - \nu). \quad (2.35)$$

2.4.2 Poroelasticity

For a poroelastic material, we seek to determine the shear modulus G , drained Poisson's ratio ν and hydraulic permeability κ . These parameters can be determined by fitting expressions for load-relaxation or creep to experimental data. However, although poroelasticity provides a micromechanical basis for much of the time-dependent behaviour of a hydrated material, the equations governing this behaviour are often difficult to solve analytically. Generally, only cases involving simple geometries where the governing equations can be uncoupled lead to closed form solutions; FE or numerical methods must be used instead (Galli & Oyen, 2009). Practically, there is often a trade-off between the ease of analysis and the difficulty in sample preparation and setup.

Given that many poroelastic problems consider a material transitioning from the undrained elastic case (immediately after a step load or displacement) to the drained elastic case (after a significant length of time), solutions describing poroelastic behaviour are often given in terms of a non-dimensional load, P^* (relaxation) or displacement, h^* (creep), which relates the transition between these two states

$$\text{Relaxation : } P^* = \frac{P(t) - P_\infty}{P_0 - P_\infty} \quad (2.36)$$

$$\text{Creep : } h^* = \frac{h(t) - h_0}{h_\infty - h_0} \quad (2.37)$$

where P_0 , P_∞ , h_0 and h_∞ are the respective instantaneous (undrained) and equilibrium (drained) load and displacements.

Similarly, the time scale of the test is also normalised by a constant carrying the material parameters, the consolidation coefficient c , as well as a characteristic drainage length, L_D where:

$$t^* \propto \sqrt{\frac{ct}{L_D^2}}, \quad (2.38)$$

$$c = \frac{2\kappa G(1-\nu)}{(1-2\nu)} = H_A \kappa. \quad (2.39)$$

When normalised, the time-dependent behaviour of a poroelastic material collapses onto a single curve, regardless of the elastic and poroelastic properties of the material. This normalisation suggests that the time to equilibrium has a fundamental length scale dependency. This can be seen from Darcy's law (equation 2.16); for the same pressure difference across a sample, the larger the sample is the smaller the flow-rate will be, resulting in a greater time to equilibrium. The length scale of interest is the characteristic distance over which the pressure difference occurs, whether it be the sample height (confined compression with a permeable platen), sample radius (unconfined compression with an impermeable platen) or contact radius (indentation). The larger this length scale is, the longer it will take for the sample to equilibrate as the pressure gradient will be smaller.

Confined compression

Confined compression is an advantageous method for testing poroelastic materials. A permeable platen is used to compress the sample into the confinement chamber. Flow solely occurs through the permeable platen and the characteristic drainage length is equal to $2L$, where L is the sample height. The total stiffness of the material in a confined compression setup depends on the aggregate modulus, H_A which tends to infinity when $\nu = 0.5$ and to E when $\nu = 0$ (table 2.1). As the material transitions from an undrained to a drained state, a large change in stiffness occurs and substantial creep or relaxation can be observed.

Furthermore, in load control, a uniform uniaxial stress state exists (although strains are not uniform) and full solutions for the displacement and pore pressure throughout the sample are available (Detournay & Cheng, 1993). The non-dimensional displacement of the sample, subjected to a step load, is equal to:

$$h^*(t^*) = 1 - \sum_{n=1,3,\dots}^{\infty} \frac{8}{n^2 \pi^2} (1 - \exp(-n^2 \pi^2 t^{*2})). \quad (2.40)$$

This solution was further extended by Galli et al. (2009) who modified the solution

2.4 Analysis

to take into account the finite ramp time:

$$h^*(t^*) = 1 - \sum_{n=1,3,\dots}^{\infty} \frac{8}{n^4 \pi^4 t^{*2}} (1 - \exp(-n^2 \pi^2 t^{*2})), \quad 0 \leq t^* \leq t_R^*, \quad (2.41)$$

$$h^*(t^*) = 1 - \sum_{n=1,3,\dots}^{\infty} \frac{8}{n^4 \pi^4 t_R^{*2}} [\exp(-n^2 \pi^2 (t^{*2} - t_R^{*2})) - \exp(-n^2 \pi^2 t^{*2})], \quad t^* \geq t_R^*, \quad (2.42)$$

where t_R^* is the non-dimensional ramp time.

A load-relaxation solution is also available for displacement control (Soltz & Ateshian, 1998). However, for a material where $\nu_U = 0.5$, a step displacement cannot be applied to the material; in the undrained state the material is incompressible and is infinitely stiff when confined. Therefore only a solution including a ramp exists.

One of the principle advantages of performing a displacement-controlled experiment on an elastic material is that the strains are uniform and are specified by the test. However, the strains in a poroelastic material undergoing displacement-controlled confined compression are not uniform; the material is initially incompressible and only the surface (where flow occurs) can compress. Very large strains can be observed at the surface, even when the strain in the bulk of the material is negligible (Holmes et al., 1985). The distribution of strains will depend on the interplay between the ramp rate and material parameters which are not known *a priori*. Hence, load-controlled confined compression is often preferable to displacement-controlled confined compression when strains are to be kept small.

Unconfined compression

Unconfined compression experiments are ubiquitous when testing elastic, hyperelastic or viscoelastic materials because of their well-defined strain states and simple analytical behaviour. This is not necessarily the case for poroelastic materials. In an unconfined compression experiment, the characteristic drainage length is equal to the radius of the material, and as fluid flows out of the material the Poisson's ratio changes from the outside in and the radial strains are not

uniform. Furthermore, the observable time-dependent behaviour of a poroelastic material is limited during unconfined compression, as the stiffness of the material is governed by the elastic modulus which can only change by a maximum of 33% for a material with a drained Poisson's ratio, $\nu = 0$ (Miller, 1998). The pressure gradient is also smaller than that of confined compression for the same applied stress, and as a result poroelastic behaviour proceeds more slowly.

Still, unconfined compression experiments allow the Poisson's ratio of the material to be measured directly (if the radial displacement is recorded during the experiment using a camera). Analytical solutions for displacement-controlled unconfined compression have been developed by Armstrong et al. (1984), Cohen et al. (1998) and Cai et al. (2010) and can be fit to stress-relaxation experiments to determine poroelastic properties.

Indentation

Indentation has great potential as a method to rapidly determine the poroelastic properties of a material. The contact size of the indenter is small and hence the drainage length of the experiment is also relatively small. Relaxation proceeds quickly relative to compression tests, and the time-dependent behaviour proceeds more quickly the smaller the contact size, opening up possibilities for AFM testing and nanoindentation to rapidly identify the permeability of materials in a matter of minutes (rather than hours or days).

However, the equations describing the behaviour of an indenter indenting a poroelastic half-space cannot be solved analytically. Agbezuge & Deresiewicz (1974) (who first proposed that indentation could be used to determine permeability in 1974) numerically solved the equations describing impermeable and permeable spherical indenters indenting a half-space with an applied step load for the particular case of $\nu = 0$. Numerical solutions for flat punch indentation in both load and displacement control were presented by Mak et al. (1987) and Mow et al. (1989). Hu et al. (2010) curve fit non-dimensional solutions (section 2.4.2) generated from FE methods for displacement-controlled indentation using a variety of indenter shapes (conical, cylindrical, spherical) and Hu et al. (2011) presented more accurate curve fits for spherical indenters. All of these

2.4 Analysis

solutions can then be fit to experimental data to determine poroelastic material parameters.

Inverse FE methods have also been used to directly extract the material properties of the material from load-time and displacement-time curves; the material properties of an FE model of the indentation process are updated until the resultant load-time curve matches the observed behaviour (Kauer et al., 2002). Inverse FE analysis has the advantage that it can be accurately used to apply the precise boundary conditions of the experiment (including taking into account finite depths and ramp times; Galli et al., 2009; Miller & Morgan, 2010) although it is computationally expensive and time consuming.

Inverse FE methods are time consuming while numerical solutions generally only exist for specific boundary conditions. Galli & Oyen (2009) generated a database of master-curves with varying ramp times from FE models to rapidly analyse indentation results performed under load control. Similarly, Hu et al. (2011) developed a database of master-curves for displacement controlled indentation of materials with finite depth. These master-curve approaches can be used to quickly identify parameters for a variety of conditions.

Hence, although analytical solutions do not exist for indentation, there are a variety of methods which can be used to accurately determine material parameters of varying complexity. However, there are still cases which are lacking in-depth solutions, including ramp corrected displaced-controlled indentation.

Direct permeability measurement

The permeability of a material can be directly measured using a permeometer (rather than inferred from time-dependent behaviour). Fluid is pumped at a constant flow rate through a cylinder of material contained within a tube, and the pressure difference across the thickness of the cylinder is measured (Heneghan & Riches, 2008). Darcy's law can then be used to calculate the hydraulic permeability (equation 2.16). The experiment can also be performed by keeping the fluid on one side of the sample at a constant pressure, and measuring the flow rate of fluid that passes through the material (Mansour & Mow, 1976). Although the experiment gives a direct measurement of the permeability, in order to measure

appreciable flow rates, large pressures may need to be applied to the material. These pressures can compress the pores of compliant materials and lower the measured permeability. Some author's have attempted to quantify this effect and measure a strain-dependent permeability (Heneghan & Riches, 2008; Mansour & Mow, 1976).

2.5 Composite models

Many natural materials and tissue engineering scaffolds are composite materials. Composite models allow us to predict the behaviour of the composite from the properties of the constituent materials that form the composite and their respective volume fractions. In their simplest form, composite models do not require an understanding of the interactions between the phases, but solely a brief understanding of their geometrical arrangement. Several of these models will be considered here.

2.5.1 Elastic behaviour

The Voigt-Reuss bounds (also known as the rule of mixtures) provide upper and lower bounds on the elastic modulus of a two-phase composite material (Chawla, 1998):

$$E_U = V_2 E_2 + (1 - V_2) E_1, \quad (2.43)$$

$$E_L = [V_2/E_2 + (1 - V_2)/E_1]^{-1}, \quad (2.44)$$

where E_1 and E_2 are the elastic moduli of the two phases, and V_2 is the volume fraction of the second phase. The upper bound, E_U corresponds to geometrical arrangements where both phases undergo equal strains, i.e. the phases are arranged parallel to the applied loading (figure 2.10). The lower bound, E_L corresponds to geometrical arrangements where both phases undergo equal stress (the phases are arranged perpendicular to the applied loading). The model neglects strains that could occur as a result of differing Poisson's ratios between the materials, although in general these effects are small (Chawla, 1998).

2.5 Composite models

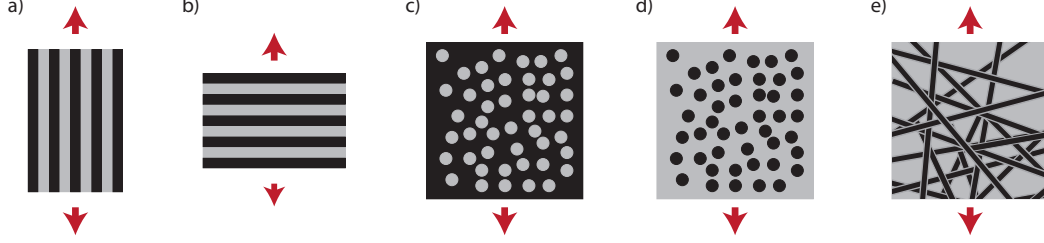


Figure 2.10: Theoretical microstructures corresponding to various composite bounds: (a) Voigt, (b) Reuss, (c) upper Hashin-Shtrikman, (d) lower Hashin-Shtrikman, (e) 2-D Christensen and Waals. The black phase corresponds to E_2 and the grey phase corresponds to E_1 where $E_2 > E_1$.

Very few isotropic composites are likely to be arranged in a similar manner to the cases predicted by the Voigt-Reuss bounds. The Hashin-Shtrikman (Hashin & Shtrikman, 1963) bounds predict the behaviour of a composite consisting of particles surrounded by a continuous matrix. The upper Hashin-Shtrikman bound occurs when the matrix is the stiff phase ($G_1 > G_2$) while the lower bound occurs when the matrix is the complaint phase ($G_2 > G_1$). Most isotropic composites will lie in between the Hashin-Shtrikman bounds. The bounds are calculated according to:

$$K_{\text{HS}} = K_1 + \frac{V_1}{(K_2 - K_1)^{-1} + (1 - V_1)(K_1 + \frac{4}{3}G_1)^{-1}}, \quad (2.45)$$

$$G_{\text{HS}} = G_1 + \frac{V_1}{(G_2 - G_1)^{-1} + \frac{2(1-V_1)(K_1+2G_1)}{5G_1(K_1+\frac{4}{3}G_1)}}, \quad (2.46)$$

$$E_{\text{HS}} = \frac{9K_{\text{HS}}G_{\text{HS}}}{3K_{\text{HS}} + G_{\text{HS}}}, \quad (2.47)$$

where G_1 , G_2 , K_1 and K_2 are the shear and bulk moduli of each phase. For composites where the Poisson's ratio of both phases is equal to 0.5 the bounds can be simplified to:

$$G_{\text{HS}} = G_1 + \frac{V_1}{(G_2 - G_1)^{-1} + \frac{2(1-V_1)}{5G_1}}, \quad (2.48)$$

2.5 Composite models

$$E_{HS} = 3G_{HS}. \quad (2.49)$$

Christensen & Waals (1972) considered the modulus of composites consisting of randomly oriented fibres distributed within a matrix and presented relationships for the composite modulus of both two-dimensional (all the fibres lie within a plane) and three-dimensional geometries. For small fibre volume fractions ($V_f < 0.2$), the full expressions can be approximated as:

$$E_{2-D} \approx \frac{1}{3}E_2V_2 + E_1(1 + V_2), \quad (2.50)$$

$$E_{3-D} \approx \frac{1}{6}E_2V_2 + E_1(1 + (1 + \nu_1)V_2), \quad (2.51)$$

where E_2 and V_2 are the elastic modulus and volume fraction of the fibre phase, and E_1 and ν_1 are the elastic modulus and Poisson's ratio of the matrix.

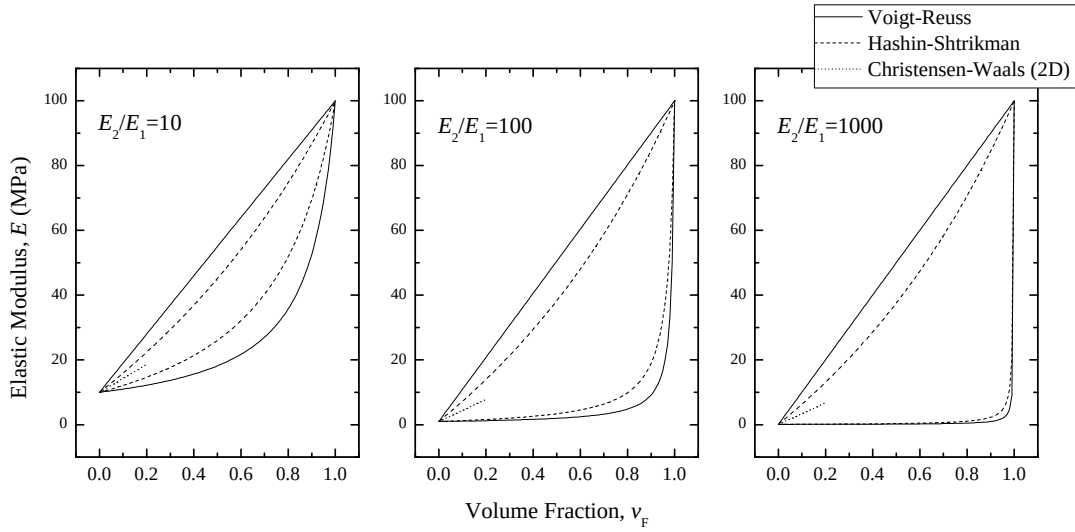


Figure 2.11: Composite bounds on the elastic modulus plotted for a material with $E_2 = 100$ MPa and $E_1 = 10, 1$ or 0.1 MPa; $\nu_1 = \nu_2 = 0.5$. Adapted from Oyen (2005b).

All of these composite models are compared in figure 2.11, assuming both constituent phases are incompressible ($\nu_1 = \nu_2 = 0.5$). It can be seen that a large range of properties is possible, for constituents with a large modulus mismatch.

2.6 Summary

2.5.2 Models of porosity

Composite models can also provide us with an indication of the stiffness of a porous material. The composite bounds can be used as normal, except that one of the phases is assumed to have negligible stiffness. For the Voigt bound this would predict that the stiffness of a porous material will scale linearly with the volume fraction of solid ($E_\phi = V_s E_s$). However, Gibson & Ashby (1999) developed several models for cellular solids (such as honeycomb foams), and showed that many three-dimensional porous cellular solids had moduli that were related to the square of the volume fraction:

$$E_\phi \approx V_s^2 E_s. \quad (2.52)$$

These relationships also allow us to make predictions of the porosity and relative density of a material if its elastic modulus is known; $\phi = 1 - \frac{\rho}{\rho_0} = 1 - V_s$ where ϕ is the porosity, ρ is the density of the porous material and ρ_s is the density of the fully-dense solid material.

2.5.3 Viscoelastic composites

The performance of viscoelastic composites can also be modelled using the viscoelastic correspondence principle, whereby elastic variables in the composite bounds (section 2.5.1) can be replaced with viscoelastic operators. The composite bounds for the limiting instantaneous or equilibrium elastic behaviour of a viscoelastic composite can be calculated using the instantaneous or equilibrium moduli of each phase. For further information on the properties of viscoelastic composites, the reader is referred to Lakes (2009).

2.6 Summary

In this chapter, several methods of mechanical characterisation have been described. A summary of the advantages and disadvantages of these techniques is presented in table 2.2. The techniques will be used in this work to characterise various aspects of the materials that are fabricated.

Two different analytical frameworks for characterising time-dependent behaviour have been reported: viscoelasticity and poroelasticity. Viscoelasticity provides a convenient description of time-dependent behaviour, while poroelasticity attempts to relate the observed behaviour to the flow of fluid through the material microstructure. Methods for characterising poroelastic materials using indentation will be further developed in chapter 5. Here viscoelasticity and poroelasticity have been considered as separate frameworks, although in reality many soft tissues and hydrogels are likely to exhibit poroelastic and flow-independent viscoelastic behaviour (i.e. PVE behaviour). More complex material models are needed to describe this behaviour, one of which will be presented in chapter 6.

Finally, simple methods of predicting the elastic behaviour of composites have been presented. In chapter 4, the fabrication of fibre-gel composites is described and in the following chapter (chapter 3) the mechanical behaviour of mixed hydrogel systems is examined. The composite bounds are used to predict and analyse their performance.

	Elastic	Viscoelastic	Poroelastic
Tension	+ Direct measurement of E – Difficult to grip soft sample – Sample preparation difficult	+ Analytical solution available	
Confined Compression	+ Direct measurement of H_A – Contact issues – Large frictional area		+ Small changes in ν lead to large changes in force or displacement. – Relaxation relatively slow
Unconfined Compression	+ Direct measurement of E – Contact issues	+ Analytical solution available	+ Direct measurement of ν – Little observable relaxation
Indentation	+ Little sample preparation needed + Can perform local measurements – Non-uniform strain state – Must assume ν to determine E	+ Approximate relationship for displacement-controlled indentation	+ Drainage length is small + Fast relaxation – No analytical solutions
Permeometer			+ Direct measurement of k – Large pressures may be needed

Table 2.2: Summary of the advantages (+) and disadvantages (–) of various mechanical characterisation techniques.

2.6 Summary

Chapter 3

Composite hydrogels

There has been limited work that has characterised the time-dependent mechanical behaviour of hydrogel scaffolds for NP tissue engineering. Poroelastic behaviour, which plays a key role in nutrient transport, is of particular importance. In this chapter, the time-dependent mechanical properties of three hydrogels (agar, gelatin and alginate), and gelatin-agar and gelatin-alginate composites, are investigated. The time-dependent properties of these hydrogels are explored using viscoelastic and poroelastic frameworks. Several gel formulations demonstrate comparable equilibrium elastic behaviour to the NP under unconfined compression, but have permeability values that are much greater than those of the native tissue. A range of time-dependent responses are observed in the composite gels examined, presenting the opportunity for targeted design of custom hydrogels with combinations of mechanical properties optimised for tissue engineering applications.

3.1 Introduction

Due to similarities in their compositions, hydrogels have frequently been utilised for tissue engineering of the NP. These water-swollen polymer networks have been used to study the response of NP cells to applied loading conditions (Wang et al., 2011), as mechanical analogues to determine the response of NP cells to their substrates (Wang et al., 2001), and as biomaterials/scaffolds which can be implanted into the disc to support mechanical load (Bao & Yuan, 2002; Borges et al., 2010; Yang & Li, 2009). A number of polymers have been used to form NP hydrogel scaffolds, including agarose (Gruber et al., 2006), alginate (Baer et al., 2001), and gelatin (Cloyd et al., 2007).

Hydrogels transmit biochemical and micromechanical cues to encapsulated cells that are similar to those *in vivo* (Tibbitt & Anseth, 2009) and for this reason both the chemical and mechanical qualities of tissue engineering scaffolds have been the subject of recent interest. In terms of chemistry, gelatin is a protein derived from the denaturation of collagen; agar and alginate are polysaccharides. Both gelatin and agar set from a molten solution to a solid on a reduction in temperature, while alginate sets when crosslinked with Ca^{2+} and other divalent cations. In terms of mechanical behaviour, a scaffold must be sufficiently robust to withstand physiological loading, although it is unclear whether the ideal scaffold matches the properties of the NP or if the regeneration of tissue would be better facilitated by mechanical properties different to those of the NP. Substrate stiffness (Engler et al., 2006), viscoelasticity (Murrell et al., 2011) and poroelasticity (Trappmann et al., 2012) have all recently been shown to affect cell response.

Although mechanical behaviour is crucial to the function of scaffolds, for the most part mechanical characterisation of hydrogel scaffolds has been limited to characterising their equilibrium compressive behaviour using unconfined compression (Nerurkar et al., 2010b). It is not difficult to form a hydrogel that has a NP-like elastic modulus in the kPa range—most can be formed this way with a small enough polymer concentration—but the degree and mechanisms for time-dependent behaviour and the Poisson’s ratios of gels vary considerably, and this has not been widely investigated (Cloyd et al., 2007). In particular, the intrinsic permeability, which is related to the physical pore size of the scaffold, is a critical

parameter in avascular tissues and corresponding scaffolds due to its importance in both hydraulic nutrient transport and in regulating local fluid pressurisation.

As the NP is a composite material consisting of both proteins and polysaccharides, it is possible that the required scaffold properties for successful tissue regeneration will not be found in a single phase hydrogel and that composites must be created. Composites of gelatin and agar have been examined to a limited extent for use in the food industry, although their time-dependent behaviour has not been described in great detail (Clark et al., 1983; Gotlieb et al., 1988; Watase & Nishinari, 1980). Similarly, gelatin-alginate composites have been investigated for controlled drug release although their mechanical properties have not been extensively characterised (Dong et al., 2006).

In this chapter, it is reported that hydrogels with different time-dependent mechanical properties were combined to form composite hydrogels with a range of behaviours not observed in any single phase system. It is demonstrated that a rapid testing and analysis approach can be used to screen many compositions for desired time-dependent mechanical responses, with an objective of optimising hydrogel properties for use as NP scaffolds. Base materials were the protein gelatin and the polysaccharides agar and alginate. Mechanical testing was primarily conducted via spherical indentation load-relaxation testing. A select group of single-component and composite gels were also tested using unconfined compression. Data analysis was conducted within a linearly viscoelastic framework for all indentation and compression testing datasets. Effective poroelastic properties, including shear modulus, Poisson's ratio and permeability, were also computed from the same indentation and unconfined compression data. By appropriate design of protein and polysaccharide components and proportions, it is demonstrated that a hydrogel can be created with a range of stiffness–time-dependence combinations, illustrating the utility of the composite hydrogel approach for materials design in tissue engineering.

3.2 Materials and methods

3.2.1 Materials

Single phase hydrogels

Gelatin (180g bloom), agar and alginate were obtained from Sigma-Aldrich Company Ltd. (UK). Gelatin (Gel; 7.5%, 15%, and 30% w/w) and agar (1%, 2%, and 3% w/w) gels were prepared by soaking granules in distilled water at room temperature. The mixtures were stirred in a water bath at 65 °C (gelatin) or 90 °C (agar) until dissolved. Solutions were left in the water bath without stirring until the majority of trapped gas had been released. They were then poured into moulds to cool for one hour before being placed in a refrigerator for 48 hours. Set hydrogels were weighed prior to being soaked in phosphate buffered saline (PBS) at room temperature (Sigma-Aldrich Company Ltd., UK) for a further 24 hours.

Alginate gels (Alg; 1% w/w) were prepared by mixing sodium alginate into distilled water at room temperature and pouring the solutions into porous foam moulds covered with filter paper and wire mesh. These moulds were then soaked in 200 mM CaCl₂ solution (Sigma-Aldrich Company Ltd., UK) for 24 hours while refrigerated. Crosslinked gels were removed from moulds, weighed and placed in PBS for 24 hours.

Composite hydrogels

Gelatin, agar and alginate solutions were prepared as for the single phase gels (section 3.2.1). Mixed gelatin-agar gels were prepared as follows: gelatin and agar solutions were mixed together by volume fraction (V_f) of melt solution. Thus, a composite gel of 30%Gel-1%Agar with gelatin $V_f = 0.8$ consisted of 80% by volume the 30% gelatin solution and 20% by volume the 1% agar solution. Mixtures were stirred moderately at 75 °C for 5 minutes. Melts were poured into moulds and allowed to cool, set and soak following the same time-schedule as the single phase gels. For some analyses, the composite gels were re-examined in terms of the solids fraction of each component, where the gelatin solids fraction S_f is the gelatin weight in the mixture divided by the total weight of solids in the mixture.

Mixed gelatin (30% w/w) - alginate (1% w/w) gels, i.e. 30%Gel-1%Alg, were prepared by heating the alginate solution to 65 °C. The gelatin and alginate solutions were mixed by volume fraction and stirred moderately at 65 °C for 5 minutes. Solutions were then poured into moulds and allowed to cool for one hour, and then placed in the refrigerator for 24 hours. Set gels were then divided in two; one half was soaked in 200 mM CaCl₂ solution for 24 hours (crosslinked), while the other half was soaked in distilled water for 24 hours (uncrosslinked). Both types of gels were then removed from the refrigerator and soaked in PBS at room temperature for a further 24 hours.

Test specimens

Hydrogels of varying geometries were produced for different test types. Rectangular sheets, approximately 8 mm thick, were prepared for indentation testing by pouring molten gels into trays. A scalpel was used to cut out approximately 30 mm x 30 mm rectangular slabs. Cylindrical gels for unconfined compression testing were cast in silicone moulds with a radius (r) of 7.8 mm and height (L) of 10 mm. The formation of a meniscus was minimised by pouring the gels into the moulds until they were flush with the mould top.

3.2.2 Swelling

The weight after 24 hours soaking, W_1 , was measured immediately prior to testing. This was used along with the initial weight of the gel, W_0 , to calculate the percent increase in weight, % W according to:

$$\%W = \left(\frac{W_1 - W_0}{W_0} \right) \times 100\%. \quad (3.1)$$

3.2.3 Mechanical testing

All mechanical tests were performed on an Instron 5544 universal testing frame (Canton, MA, USA) with a 5 N load cell. Tests were performed in displacement control using a ramp-hold profile: the displacement (h) is ramped at a constant rate to a fixed level ($\dot{h} = h_{\max}/t_R$) and then held at the peak displacement ($h_{\max}$)

3.3 Theory

for a fixed hold time (t_H). The load (P) is recorded for the duration of the test. Samples were fully immersed in PBS during each test.

Indentation

Indentation tests were carried out using a stainless steel spherical tip with a radius (R) of 4.7 mm. Prior to testing, samples were fixed to the bottom of the test chamber using cyanoacrylate. The test chamber was then filled with PBS. A small preload (0.5 mN) was applied to the test sample, followed by a 10 s ramp to a peak displacement of 0.3 mm. This corresponds to an indentation strain of 0.05 (equation 2.27). The indenter was held at $h_{\max}$ for 300 s. Two to four samples were used for each test and three to five indents were performed on each sample, spaced at least 15 mm apart.

Unconfined compression

Unconfined compression tests were then performed using an impermeable steel platen. Prior to testing, gels were photographed on graph paper using a camera equipped with a Canon close-up lens (Canon 250D, Tokyo, Japan). The radius, r , of the swollen gels was determined from the photographs using FIJI (available at <http://fiji.sc>). Samples were then placed into the test chamber filled with PBS. A small preload (0.01 N) was applied to the test sample, from which the height of the sample was determined, followed by a 10 s ramp to a peak displacement of 0.5 mm, corresponding to an approximate strain of 0.05. The peak displacement was held for 900 s. Three samples were tested for each gel formulation.

3.3 Theory

3.3.1 Viscoelastic analysis

Unconfined compression

Viscoelastic analysis was undertaken using the Boltzmann hereditary integral approach for a ramp-hold profile detailed in section 2.4.1. The relaxation function was assumed to take the form of a two-term Prony-series exponential decay:

$$E(t) = C_0 + \sum_{k=1}^2 C_k \exp(-t/\tau_k), \quad (3.2)$$

In extensive coordinates (P, h, t) , the solution for the holding phase ($t > t_{\text{ramp}}$) is (section 2.4.1):

$$P(t) = \frac{\pi r^2}{L} h_{\text{max}} \left[C_0 + \sum_{k=1}^2 RCF_k C_k \exp(-t/\tau_k) \right], \quad (3.3)$$

where RCF_k is a ramp correction factor defined by:

$$RCF_k = \frac{\tau_k}{t_r} [\exp(t_r/\tau_k) - 1]. \quad (3.4)$$

Equation 3.3 was fit (MATLAB, The MathWorks, Natick, MA, USA) directly to the experimental data to obtain the relaxation function parameters (C_0, C_k, τ_k) using the nonlinear least-squares optimisation function (`lsqnonlin`) in the optimisation toolbox. The function seeks to minimise least-squares error between the experimental data and the model. The optimisation function was provided with initial guesses of C_0, C_k, τ_k . An initial guess of C_0 was provided by solving equation 3.3 for $t = \infty$, assuming that $P(\infty)$ was equal to the load at the end of hold (which is equivalent to assuming that the material is fully relaxed by the end of the hold). Initial guesses of C_1 and C_2 were fixed to 20% and 10% of C_0 , respectively. The time constants, τ_1 and τ_2 were estimated as the rise and hold times. Bounds for C_0, C_k and τ_k were provided such that all identified values were greater than 0. The limiting values of the relaxation function, $E(0) = E_0$ and $E(\infty) = E_\infty$, are reported as the instantaneous and equilibrium modulus values.

Equation 3.3 was fit only to the hold portion of the experiment. Fits to only the first third of the hold were carried out, although no difference was found when only the first 300 s of the hold was fit compared with the full 900 s hold time.

Indentation

The relaxation function $G(t)$ is assumed to take the same form as the Prony series used for compression above, taken in shear (where $\nu = 0.5$ has been assumed):

3.3 Theory

$$G(t) = E(t)/3 = \left[C_0 + \sum_{k=1}^2 C_k \exp(-t/\tau_k) \right] / 3. \quad (3.5)$$

As described in section 2.4.1, there is no closed-form analytical solution describing the relaxation of a viscoelastic material indented in displacement control with a ramp-hold profile. Instead, an approximate solution, based on the analogy between step- and ramp-loading via the RCF_k parameter defined in equation 3.4, was fit to the hold data (Mattice et al., 2006):

$$P(t) = \frac{16\sqrt{R}}{3} h_{\max}^{3/2} \left[C_0 + \sum_{k=1}^2 RCF_k C_k \exp(-t/\tau_k) \right]. \quad (3.6)$$

Initial guesses of C_0, C_k, τ_k were obtained using the same assumptions as for unconfined compression. Reported parameters again include the limiting values of the instantaneous and equilibrium shear modulus, G_0 and G_∞ and the ratio G_∞/G_0 ; for comparison with the compression data, the values are converted from G_i to E_i via the factor of 3 assumed based on $\nu = 0.5$.

3.3.2 Poroelastic analysis

Indentation

Experimental indentation data were analysed in order to identify the shear modulus G , the drained Poisson's ratio ν , and the hydraulic (Darcy) permeability κ , following a modified method of Hu et al. (2010). The published method (Hu et al., 2010) determines poroelastic parameters from the transition in load between the undrained and drained elastic responses (see section 2.4.2). The method assumes step displacement conditions such that P_0 (the elastic undrained response of the material) can be directly identified. It also assumes that the test time is on the order of hours and that the sample fully relaxes by the end of the test such that the equilibrium indentation force P_∞ can also be directly identified. Shear modulus, G can then be calculated from the value of P_0 using the Hertz solution for $\nu_u = 0.5$. The drained Poisson's ratio can be determined from the ratio between P_∞ and P_0 :

$$\nu = 1 - \frac{P_0}{2P_\infty}. \quad (3.7)$$

An expression generated using FE methods (Hu et al., 2010) describing the non-dimensional load-time response of a poroelastic material indented using a step displacement can then be fit to non-dimensional experimental data to identify κ :

$$P^* = 0.791 \exp(-0.213t^*) + 0.209 \exp(-0.95t^{*2}), \quad (3.8)$$

where:

$$P^* = \frac{P(t) - P_\infty}{P_0 - P_\infty}, \quad (3.9)$$

and:

$$t^* = \sqrt{\frac{ct}{Rh_{\max}}}. \quad (3.10)$$

where c is the consolidation coefficient (equation 2.39).¹

In this work, indents with finite ramp times and short hold times were performed; P_0 and P_∞ (and therefore G and ν) could not be directly identified. Instead, P_0 and P_∞ were initially estimated from the loads at the beginning and end of the hold, respectively. Experimental data were converted to non-dimensional load-time data using these parameters and an initial permeability guess of $10^{-14} \text{ m}^4 (\text{Ns})^{-1}$. Equation 3.8 was then fit to an experimental non-dimensional curve (from the ramp time, t_R to the end of the hold), using MATLAB. P_0 , P_∞ and κ were allowed to float in the fits but the ratio between P_0 and P_∞ was bounded between one and four in order to constrain the Poisson's ratio to a value between -1 and 0.5. κ was bounded to be positive.

Unconfined compression

Poroelastic material properties (G , ν , κ) were determined from unconfined compression data. The hold portions of experimental data were fit with an analytical

¹Hu et al. (2010) defined $t^* = (ct) / (Rh_{\max})$ rather than $\sqrt{(ct) / (Rh_{\max})}$. An alternate definition of t^* has been used here in order to be consistent with the rest of this thesis. Equation 3.8 has been modified accordingly. Further details on the reasons for this definition are given at the end of section 5.2.1.

3.3 Theory

expression for stress-relaxation during unconfined compression with a ramp-hold profile (Cohen et al., 1998)¹. In terms of non-dimensional time ($t^* = \sqrt{\frac{ct}{r^2}}$), the non-dimensional load is:

$$P^* = \left(\frac{2(1-\nu)}{t_R^{*2}} \sum_{n=1}^{\infty} \frac{\exp(\alpha_n^2(t_R^{*2} - t^{*2})) - \exp(-\alpha_n^2 t^{*2})}{\alpha_n^2 [(1-\nu)^2 \alpha_n^2 - (1-2\nu)]} \right), \quad (3.11)$$

where α_n are the roots of the equation:

$$J_1(x) - \frac{(1-\nu)xJ_0(x)}{1-2\nu} = 0, \quad (3.12)$$

and J_n are Bessel functions of the first kind.

P_0 and P_∞ were calculated using linear elastic theory for the undrained and drained material (e.g.. $P_\infty = 2G(1+\nu)\pi r^2/L$). The fits were performed using the nonlinear optimisation function in MATLAB and the chebfun toolbox (available from <http://www2.maths.ox.ac.uk/chebfun/>) was used to determine roots of equation 3.12.

Permeability

The Darcy hydraulic permeability can be converted to the intrinsic permeability k , a geometrical and microstructural property of the solid skeleton, via the fluid dynamic viscosity, μ :

$$k = \kappa\mu, \quad (3.13)$$

assuming a value for the viscosity of water of 0.001 N s m^{-2} . The solid skeleton effective pore size was estimated as $k^{1/2}$.

3.3.3 Composite models

The viscoelastic (G_0 and G_∞) values obtained for indentation data were plotted within the Voigt-Reuss composite bounds corresponding to the modulus values

¹The original equation was presented for a transversely isotropic material and has been simplified here. Furthermore, there was an error in Cohen et al. (1998)'s original manuscript, in the definition of the constant Δ_3 , which should read $\Delta_3 = (1-2\nu_{31})^2 \Delta_2 / \Delta_1$. The equations presented have been corrected accordingly.

for the single phase gel components, where V_f was taken as the volume fraction of the gelatin phase:

$$G_U = V_f G^{x\%Gel} + (1 - V_f) G^{x\%Agar}, \quad (3.14)$$

$$\frac{1}{G_L} = \frac{V_f}{G^{x\%Gel}} + \frac{(1 - V_f)}{G^{x\%Agar}}. \quad (3.15)$$

The data were also plotted within the Hashin-Shtrikman bounds (see section 2.5.1 for equations). For these calculations, the volume fraction values utilised were those defined by mixing the composite gel and they therefore do not reflect differential swelling after gel manufacture.

3.4 Results

3.4.1 Swelling

Hydrogel swelling is shown in figure 3.1, as quantified by percent weight gain, for composite gels after 24 hours swelling in PBS. Pure agar gels did not swell at all, gelatin gels demonstrated substantial swelling (up to $48.8 \pm 1.9\%$) and crosslinked alginate gels decreased in weight by $23.8 \pm 5.5\%$. The gelatin-agar composite gel systems demonstrated significantly different swelling responses depending on the base gel composition. When agar was the stiff phase (7.5%Gel-3%Agar system), its non-swelling response dominated the composite gels and there was substantial swelling only in gels with gelatin volume fractions of 0.8 and above. This overall trend in swelling was nonlinear with composition. For gels based on the stiff gelatin system (30%Gel-1%Agar system), the swelling was directly proportional to gelatin content and thus approximately linear with gelatin volume fraction. For gelatin-alginate gels, there were both composition and crosslinking effects on the swelling response with an inversion in response between 0.2 and 0.4 volume fraction gelatin.

3.4 Results

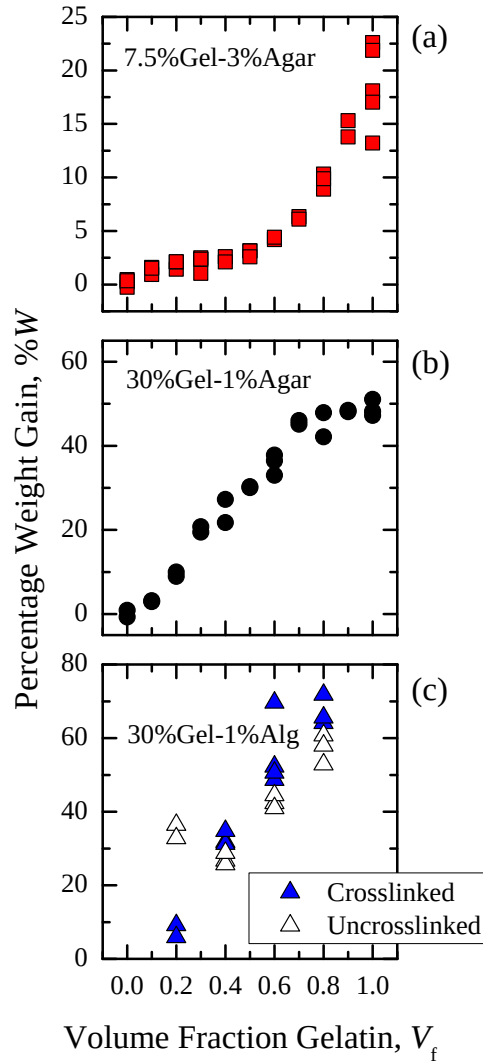


Figure 3.1: Hydrogel swelling, as quantified as percent weight gain, for (a,b) gelatin-agar composite gels after 24 hours swelling and for (c) gelatin-alginate gels after 48 hours crosslinking and swelling. There was substantial swelling only in gels with gelatin volume fraction (V_f) 0.8 or more for the 7.5%Gel-3%Agar system, while for gels in the 30%Gel-1%Agar system the swelling was proportional to gelatin content. Apart from composites with gelatin volume fraction (V_f) 0.2, crosslinked gelatin-alginate gels swelled more than uncrosslinked gels.

3.4.2 Viscoelastic results

The baseline responses of homogeneous agar and gelatin gels were examined as a function of gel concentration, and the results are shown in figure 3.2. Shear modulus values, both instantaneous (G_0) and equilibrium (G_∞) shear moduli, increased as a function of gel concentration (w/w) for both gel systems (figure 3.2a,b). The viscoelastic ratio (G_∞/G_0) values for each gel are constant with concentration (figure 3.2c,d). Equilibrium modulus values for gelatin and agar gels were in the same range, but significant differences in viscoelastic responses were apparent in both the instantaneous modulus values, which were much larger for agar, and the viscoelastic ratios, which were much smaller for agar. Alginate gels (1%) had equilibrium shear modulus values (1.01 ± 0.20 kPa) in the same range as the 7.5% gelatin and 1% agar gels, but had viscoelastic ratios (0.51 ± 0.06) in between the two. Gelatin gels, while not elastic *per se*, thus demonstrated a significantly more "elastic" response than agar and alginate gels.

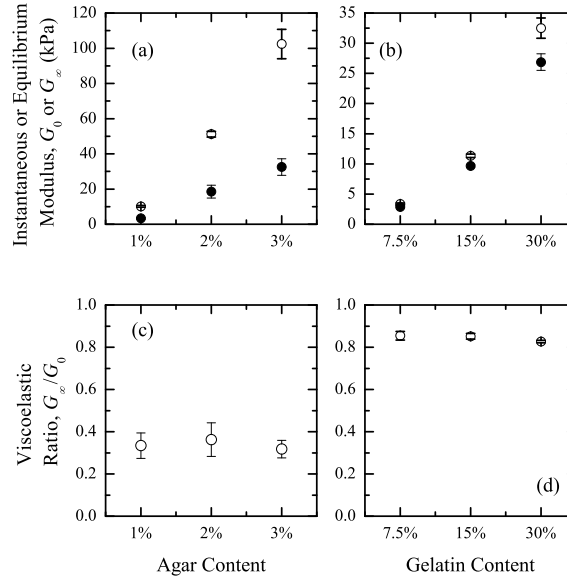


Figure 3.2: Instantaneous (G_0 , hollow markers) and equilibrium (G_∞ , solid markers) shear moduli as a function of gel concentration (w/w) for (a) agar and (b) gelatin gels as measured by microindentation testing. The equilibrium moduli increase with concentration, while the viscoelastic ratio (G_∞/G_0) values for each gel are constant (c, d).

3.4 Results

Mechanical properties for composite gelatin-agar gel systems are shown in figure 3.3 for both elastic and viscoelastic responses. In both composite gel systems, the equilibrium shear modulus (G_∞) was near the upper Voigt-Reuss and Hashin-Shtrikman composite bounds, indicating that the stiffer gel was always contributing proportionally. The variations in instantaneous shear modulus (G_0) values with composite gel composition were more complicated, and in fact the picture is clearer when considering the viscoelastic ratio (G_∞/G_0) results. In the 30%Gel-1%Agar system (figure 3.3a), the viscoelastic response is dominated by gelatin, giving ratio values the same as pure gelatin for gels from volume fractions 0.2 to 0.9 gelatin. In the 7.5%Gel-3%Agar system, the viscoelastic response is gelatin-like only for gels with gelatin volume fractions 0.8 and above, smoothly transitioning to an agar-like response at around 0.2 gelatin fraction.

Although the vast majority of gels considered here were in the gelatin-agar family, a set of gelatin-alginate gels were examined for comparison (figure 3.4). The composite alginate gels are interesting because they must be crosslinked with a calcium (Ca^{2+}) solution in order to set, and because of this their contribution to the overall composite gel can be queried by comparing crosslinked and uncrosslinked composite gels (figure 3.4). Overall, the mechanical results indicated that the alginate gel was not contributing significantly to the mechanical properties of the overall gel, which are dominated by the 30% gelatin component and closely resemble those for 30%Gel-1%Agar (figure 3.3a). Only at 0.2 volume fraction gelatin was there any meaningful difference between crosslinked and uncrosslinked composite gels suggesting a real alginate contribution, and this is also where the swelling behaviour was most intriguing (figure 3.1c).

The results presented thus far were obtained via indentation testing. Comparisons were made between indentation testing and unconfined compression tests for gels in the 7.5%Gel-3%Agar system, and the results are shown in table 3.1. Typical load-displacement curves for both indentation and unconfined compression are shown in figure 3.5. There was reasonable agreement between testing modes for the equilibrium modulus (E_∞), but the instantaneous modulus (E_0) was nearly always smaller when measured by compression testing than by indentation.

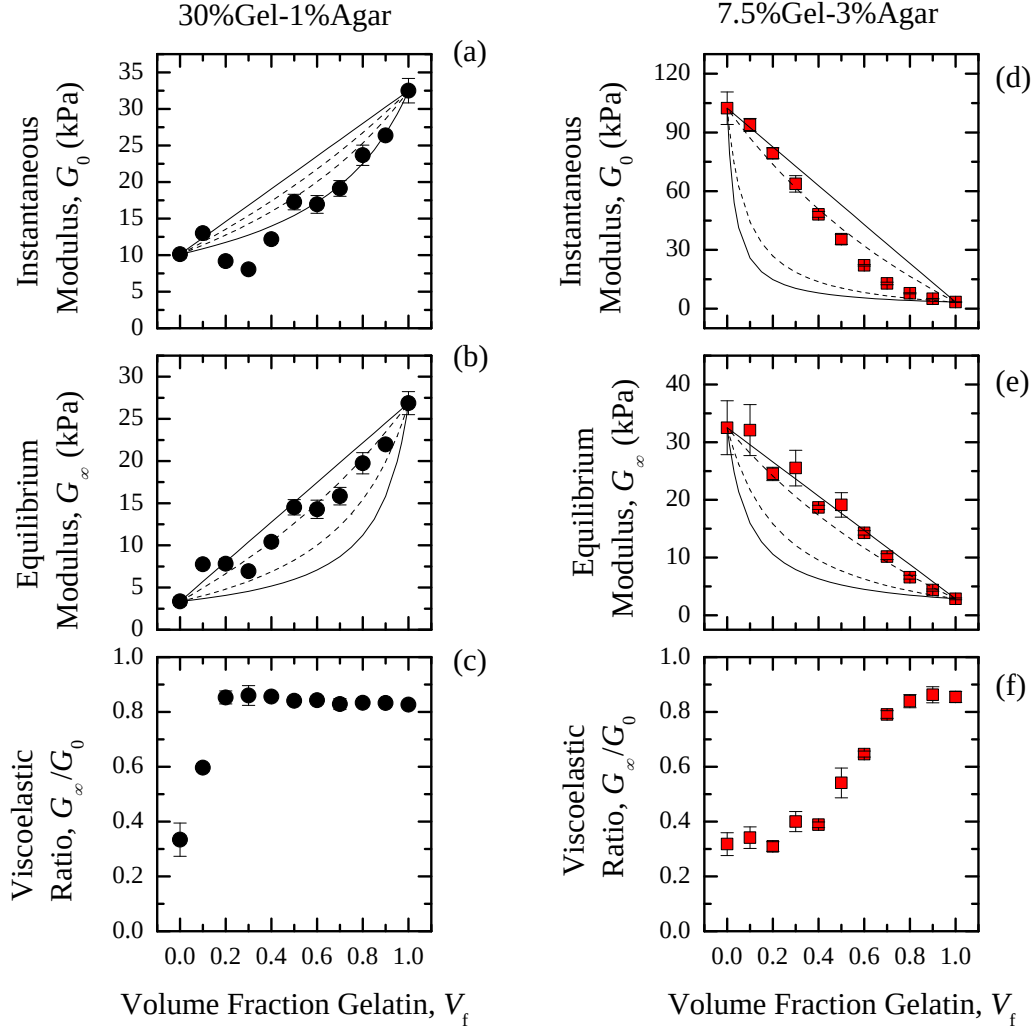


Figure 3.3: Instantaneous (G_0) shear modulus, equilibrium (G_∞) shear modulus and viscoelastic ratio (G_∞/G_0) values plotted as a function of gelatin concentration for composite gels in a (a-c) 30%Gel-1%Agar system and (d-f) 7.5%Gel-3%Agar system. Solid lines are the Voigt-Reuss composite bounds on modulus, while dashed lines are the Hashin-Shtrikman composite bounds. The viscoelastic response of the 30%Gel-1%Agar system (c) is dominated by gelatin for gels with gelatin volume fractions (V_f) of 0.2 and greater. In contrast the viscoelastic response of the 7.5%Gel-3%Agar (f) is dominated by the gelatin response for gelatin volume fractions of 0.2 and smaller, dominated by the agar response for gelatin volume fractions of 0.8 and greater, and varies smoothly between the extremes in the V_f range 0.3-0.7.

3.4 Results

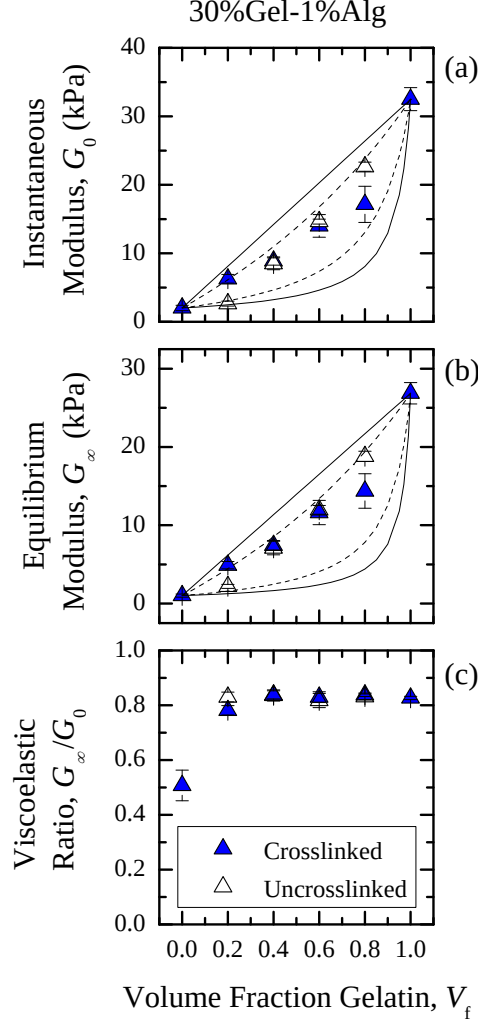


Figure 3.4: (a) Instantaneous (G_0) shear modulus, (b) equilibrium (G_∞) shear modulus and (c) viscoelastic ratio (G_∞/G_0) values plotted as a function of gelatin concentration for composite gels in a gelatin-alginate system in which the alginate component is or is not crosslinked. Solid lines are the Voigt-Reuss composite bounds on modulus, as computed from the homogeneous 30% gelatin and crosslinked 1% alginate gels. The gelatin response dominates the composite response for gelatin volume fractions (V_f) of 0.4 and greater, with a small contribution from the crosslinked alginate only visible at $V_f = 0.2$.

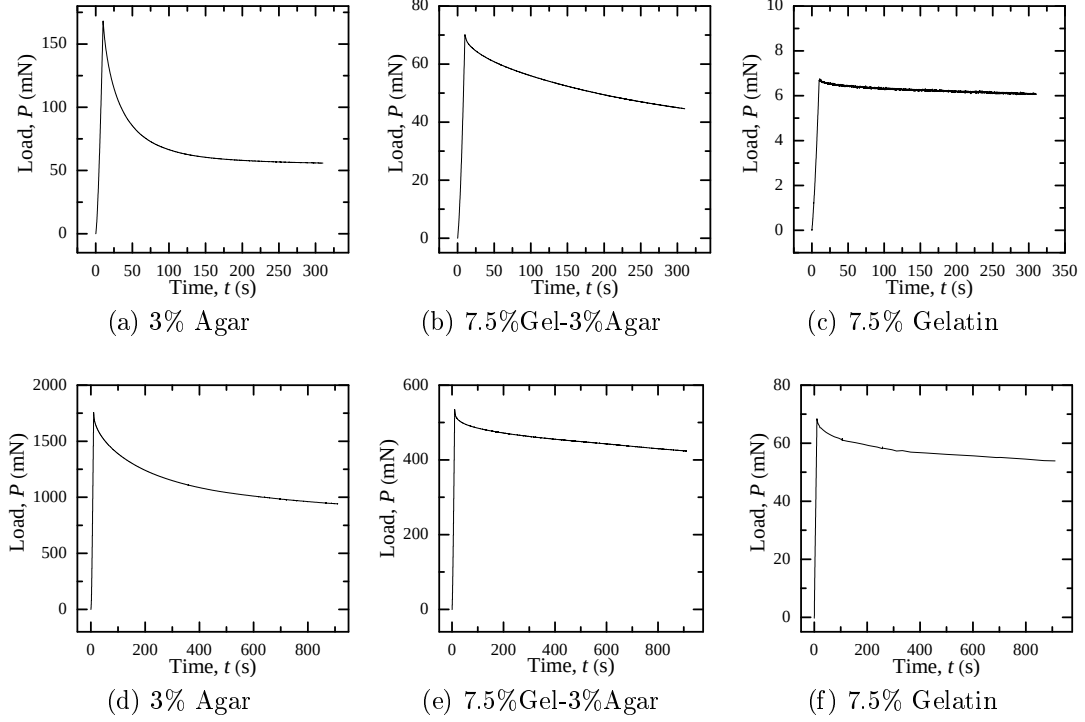


Figure 3.5: Typical load-displacement curves measured through indentation (a-c) and unconfined compression (d-f) for two homogeneous gels and one composite gel ($V_f = 0.5$).

	Unconfined Compression	Indentation
7.5% Gelatin		
E_0 (kPa)	6.86 ± 0.38	10.05 ± 0.49
E_∞ (kPa)	5.60 ± 0.30	8.59 ± 0.46
E_∞/E_0	0.82 ± 0.01	0.85 ± 0.02
7.5%Gel-3%Agar, $V_f = 0.5$		
E_0 (kPa)	54.64 ± 10.88	106.26 ± 7.80
E_∞ (kPa)	36.53 ± 13.64	57.42 ± 6.38
E_∞/E_0	0.65 ± 0.13	0.54 ± 0.05
3% Agar		
E_0 (kPa)	170.84 ± 19.14	307.16 ± 24.93
E_∞ (kPa)	89.97 ± 9.53	97.51 ± 13.99
E_∞/E_0	0.53 ± 0.00	0.32 ± 0.04

Table 3.1: Comparison between unconfined compression and indentation tests for two homogeneous gels and one composite gel.

3.4 Results

3.4.3 Poroelastic results

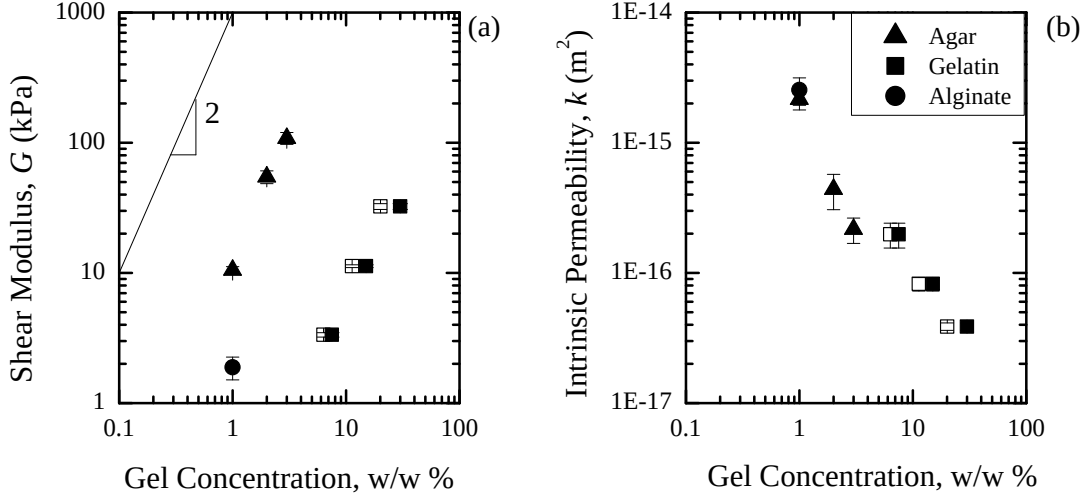


Figure 3.6: (a) Shear modulus, G , and (b) intrinsic permeability, k , determined using poroelastic analysis of agar, alginate and gelatin gels plotted as a function of their concentration pre-swelling (dark markers). Gelatin properties are also plotted against the gelatin concentration post-swelling (hollow markers), which was determined by dividing the original gelatin solids content by the weight of the gel post-swelling. The shear modulus values for both agar and gelatin were approximately proportional to the square of the concentration.

To this point, the gels have been considered within an empirical viscoelastic framework, when in reality they are two-phase poroelastic materials in which fluid moves through pores in the solid skeleton (Galli & Oyen, 2009). The (poroelastic) shear modulus values of pure gelatin and agar gels were found to vary approximately with the square of their concentration, especially when the concentration was modified to account for swelling in gelatin (figure 3.6a). The Poisson's ratio of the pure gels did not vary substantially with concentration: gelatin gels had a mean (drained) Poisson's ratio of 0.40 while agar gels had a mean Poisson's ratio of -0.81. Alginate gels had a mean Poisson's ratio of -0.0167. The gels had intrinsic permeabilities ranging from 3.87×10^{-17} to $2.16 \times 10^{-15} \text{ m}^2$ indicating an effective pore size on the order of nanometres to tens of nanometres. Agar and alginate gels were found to be more permeable than their approximately

equivalent-stiffness gelatin counterparts (figure 3.6b).

A wide range of poroelastic modulus, Poisson's ratio, and permeability values were observed for the 30%Gel-1%Agar and 7.5%Gel-3%Agar composite gel systems (figure 3.7). Shear modulus values from poroelastic analysis (figure 3.7a, d) and equilibrium values from viscoelastic analysis (figure 3.3b, e) were in good agreement for gels predicted to have a large drained Poisson's ratio determined from poroelastic analysis. However, when the drained Poisson's ratio was small or negative, differences were observed in the shear modulus values as the viscoelastic analysis assumed a Poisson's ratio of 0.5. The trend in drained Poisson's ratio values for composites (figure 3.7b, e) was identical to that seen in the viscoelastic ratio (figure 3.3c, f). The intrinsic permeability of the 30%Gel-1%Agar composites was found to decrease with increasing gelatin content. In contrast, the permeability of the 7.5%Gel-3%Agar composites was approximately equal for the limiting pure gelatin and pure agar cases, but reached a minimum at a volume fraction of 0.5 gelatin.

Similar trends were noted in poroelastic properties of gelatin-alginate gels as were observed using viscoelastic analysis. The mean Poisson's ratio for both the crosslinked and uncrosslinked composites with gelatin volume fractions (V_f) between 0.2 and 0.8 was 0.39, which is characteristic of a pure gelatin gel. The permeability of uncrosslinked and crosslinked gelatin-alginate composites did not differ substantially and ranged from 5.71×10^{-17} to 2.32×10^{-16} m², increasing with decreasing gelatin content. This range is comparable to the range observed for pure gelatin gels with concentrations ranging from 7.5% to 30% (figure 3.6). Poroelastic analysis of composite gelatin-alginate gels indicate that the mechanical properties of the overall gel were dominated by the 30% gelatin component.

Poroelastic parameters determined from unconfined compression experiments were substantially different to those from indentation. The poroelastic shear moduli measured in unconfined compression were always smaller than those measured by indentation while the intrinsic permeabilities were always larger (table 3.2). Poisson's ratios were comparable for the composite gel but otherwise differed, although it should be noted that a negative Poisson's ratio was identified for the 3% Agar gel in both unconfined compression and indentation.

3.4 Results

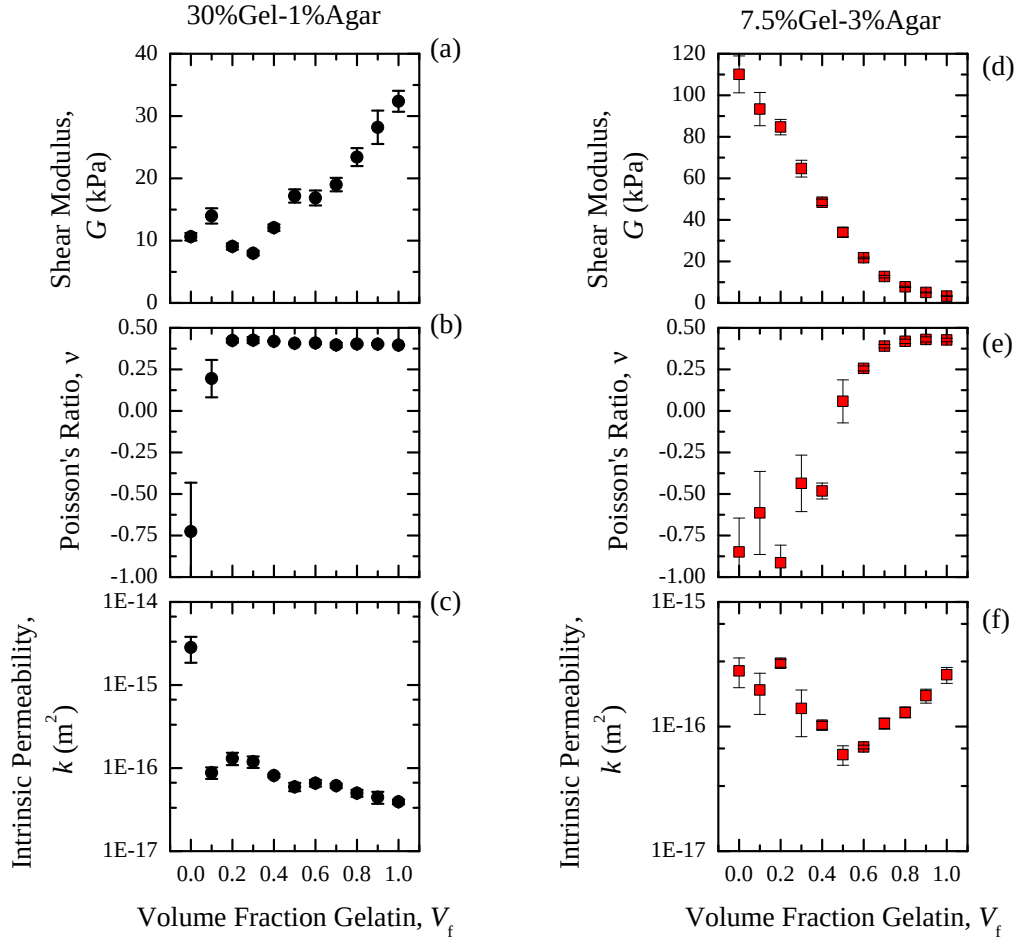


Figure 3.7: Poroelastic parameters identified from spherical microindentation tests on composite gels in a (a-c) 30%Gel-1%Agar system and (d-f) 7.5%Gel-3%Agar system showing the shear modulus, G , drained Poisson's ratio, ν and intrinsic permeability, k .

	Unconfined Compression	Indentation
7.5% Gelatin		
G (kPa)	2.23 ± 0.15	3.35 ± 0.13
ν	0.24 ± 0.03	0.40 ± 0.01
k (10^{-15} m ²)	9.28 ± 2.79	0.198 ± 0.0426
7.5%Gel-3%Agar, $V_f = 0.5$		
G (kPa)	17.62 ± 3.69	34.00 ± 2.23
ν	0.08 ± 0.18	0.00 ± 0.14
k (10^{-15} m ²)	1.51 ± 1.20	0.0542 ± 0.0115
3% Agar		
G (kPa)	56.55 ± 6.09	108.33 ± 11.33
ν	-0.17 ± 0.01	-0.92 ± 0.17
k (10^{-15} m ²)	0.93 ± 0.04	0.22 ± 0.5

Table 3.2: Comparison between poroelastic properties determined for unconfined compression and indentation tests for two homogeneous gels and one composite gel.

3.5 Discussion

3.5.1 Flexible design space

In this investigation, the time-dependent mechanical behaviour of a range of composite protein-polysaccharide hydrogels was characterised. Single phase hydrogels with a significant mismatch in moduli and viscoelastic response were combined to form composite gels with widely varying mechanical properties. It was found that over a very narrow composition range, gelatin-agar and gelatin-alginate composites exhibited a steep change in time-dependent response. Since this sharp transition coincided with a small change in equilibrium elastic modulus, this produces an exciting opportunity for the design of gels with specific sets of mechanical properties across a two (or three) component parameter space but within a narrow composition range.

The behaviour exhibited in the two gelatin-agar composite systems demonstrates that it is possible to separately tune the elastic and viscous behaviour of the composite. When both gelatin-agar systems are plotted with respect to the solid fraction of gelatin (defined in section 3.2.1), both systems fall on the

3.5 Discussion

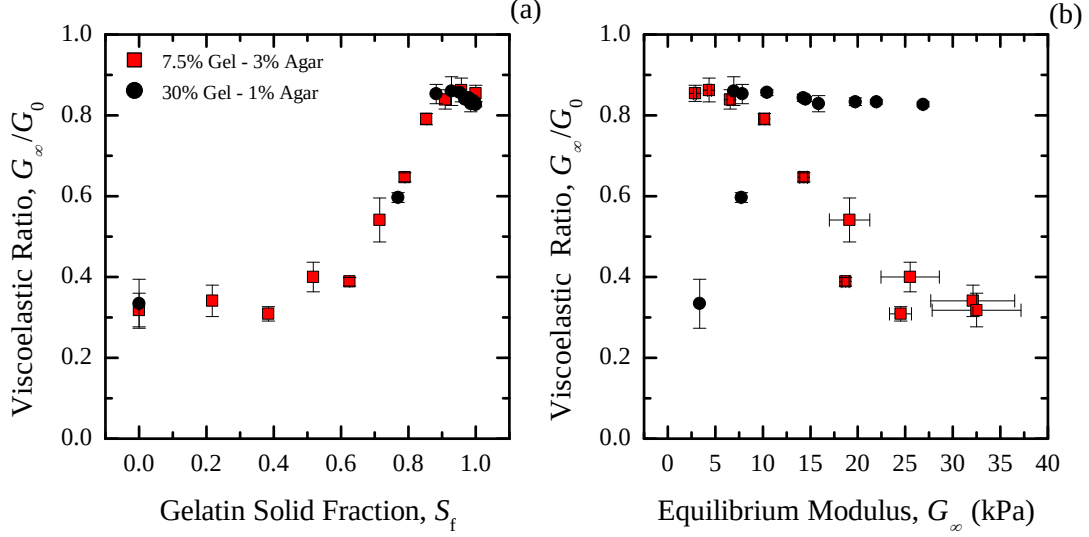


Figure 3.8: (a) Composite gel viscoelastic ratio (G_{∞}/G_0) results (figure 3.3) re-plotted as a function of the gelatin solids fraction (S_f). The data fall on a universal curve demonstrating a transition from agar-like response to gelatin-like response at gelatin solids fractions greater than 0.6. (b) Composite gel viscoelastic ratio (G_{∞}/G_0) plotted against equilibrium shear modulus (G_{∞}). A wide range of mechanical responses are observed with just two composite gel systems.

same curve for viscoelastic ratio (figure 3.8a). However, the two systems still have significantly different equilibrium moduli as the overall concentrations of agar and gelatin in each composite are different. The design of a composite gel scaffold thus has significant flexibility: the elastic behaviour can be altered by increasing the overall concentration of gelatin and agar in the gel, while the viscous behaviour can be tuned by varying their relative proportions (figure 3.8b). This opens up a flexible design space for hydrogel composite materials. A wide range of hydrogel mechanical properties could be varied systematically, either to determine the effect of mechanical properties on cellular responses (e.g. Engler et al., 2006; Trappmann et al., 2012) or to find a material which will provide optimal biomechanical support.

Here, the mechanical results were considered in a composite materials context in order to better understand the composite gel microstructures. The proximity of modulus values to the upper or lower composite bound is indicative of phase continuity (Oyen et al., 2008). Using optical microscopy, Clark et al. (1983) ob-

served that 1% agar (w/w) gels with 2% or less gelatin (w/w) consisted of an agar matrix surrounding gelatin spherical inclusions. Gels of 1% agar and 3% gelatin (w/w) or greater consisted of a gelatin matrix surrounding agar spherical inclusions. At approximately 1% agar and 2.5% gelatin a phase inversion occurred, where an interpenetrating gel composite could be observed. This corresponds to a gelatin solids fraction, S_f of 0.71. It is possible that the gelatin-agar composites produced here had similar microstructures with a similar phase inversion; the viscoelastic ratio transitioned from agar-like values at $S_f = 0.6$ to gelatin-like values at $S_f = 0.9$. Regardless, the equilibrium moduli of the gels here remain near the upper composite bound for both gelatin-agar systems at all volume fractions (with the possible exception of large volume fractions of gelatin in the 7.5%Gel-3%Agar system). This suggests that the stiff gel phase was continuous at these volume fractions, whether as a matrix containing more compliant particles or as an interpenetrating phase. This observation is supported by the fact that the 7.5%Gel-3%Agar system did not substantially swell until the gelatin volume fraction was at least 0.8. For this to happen, the stiff agar phase had to restrict the gelatin phase; it could only do this if it was continuous. Further study of these gel microstructures in the transition region is needed.

It is interesting that a significant alginate contribution was not observed in the gelatin-alginate composite system. It is possible that this system mirrored that of the 30%Gel-1%Agar system, where a viscoelastic transition occurred at a gelatin volume fraction of 0.1, and hence was not observed. It is also possible that the manufacturing process may have affected the mechanical properties of alginate. The degree of polymerisation of alginate solutions can decrease after sterilisation at temperatures above 80 °C and it is possible that heating the alginate solutions to 65 °C may have caused similar effects (Leo et al., 1990; Oates & Ledward, 1990). In addition, alginate gels were prepared by crosslinking them in CaCl_2 solution, in order to be able to compare crosslinked and uncrosslinked gels. This method of crosslinking often results in inhomogeneous gels, with a polymer concentration gradient developing from the edge of the gel to the centre. Preparing alginate gels by mixing the sodium alginate solution with a slurry of CaCO_3 and GDL (Glucono- δ -lactone) produces more homogeneous gels with improved mechanical properties, due to the substantially longer crosslinking time (Kuo &

3.5 Discussion

Ma, 2001). Preparing gelatin-alginate composites by incorporating an alginate-calcium carbonate suspension into the gelatin melt could potentially result in gels with a more significant alginate contribution to mechanical properties.

3.5.2 Characterisation techniques

The use of indentation testing allowed for rapid and repeatable characterisation of a large number of composite gels. Hundreds of individual indentation tests were carried out in the course of this study, for 33 distinct gel compositions. While the analysis of indentation is more complicated than that of unconfined compression, there are a number of published analytical and numerical solutions available (Galli et al., 2009; Hu et al., 2010; Lee & Radok, 1960; Mattice et al., 2006; Oyen, 2005a, 2006) to easily fit the time-dependent behaviour observed, allowing for a full range of mechanical properties to be characterised. The manufacture of specimens for indentation testing was also more straightforward than for compression testing.

Unconfined compression is advantageous in that it permits direct measurement of the elastic modulus without the need to assume a value of the Poisson's ratio *a priori* ($\nu = 0.5$ was assumed for indentation viscoelastic analysis). However, it is necessary to produce samples with a controlled cylindrical geometry even after swelling. In this investigation, the equilibrium elastic modulus values (obtained using either viscoelastic or poroelastic analysis) measured in unconfined compression were smaller than those measured via indentation (table 3.1, table 3.2). It is likely that the increased compliance is the result of the formation of a small meniscus on the hydrogel cylinders, even though efforts were taken to prevent surface anomalies.

Alternatively, adhesion may have increased the effective modulus measured by indentation though no pull-off forces were noted. Non-linearities (both material and geometric) may also account for some of the discrepancy between measurements, due to the different strain regimes that the materials were under. Despite these slight discrepancies between measurements, the trends in elastic modulus and viscoelastic ratio with gel concentration were the same for both measurement techniques, indicating that indentation—with its ease of use—is a promising method for gel characterisation.

Both indentation and unconfined compression testing were analysed using separate poroelastic and viscoelastic frameworks in order to describe the time-dependent behaviour of the hydrogels. Viscoelastic analysis is straight forward to apply, and allows for simple comparisons of the extent of time-dependent behaviour, though it is an empirical approach which tells us little about the mechanisms that drive the relaxation function. In contrast, poroelastic analysis relates the time-dependent deformation of the material to structural properties, including the intrinsic permeability and the drained Poisson's ratio, which can be directly characterised. However, a poroelastic analysis approach is less universally applicable to gel mechanical behaviour when gels act as PVE systems, in which time-dependent deformation can occur without fluid flow (Galli et al., 2011). Agar, gelatin and alginate have all been considered as PVE systems (Galli et al., 2011; Kalyanam et al., 2009; Olberding & Francis Suh, 2006; Zhao et al., 2010), and hence the poroelastic properties identified here should be considered to be effective properties.

Poroelastic analysis of both indentation and unconfined compression experiments predicted that agar has a negative Poisson's ratio. It is not impossible that agar gels could have negative Poisson's ratios: their solid skeletons are porous and may have re-entrant structures similar to those observed in foams that have negative Poisson's ratios (Lakes, 1987). Furthermore, the Poisson's ratio is the ratio of the bulk modulus to the shear modulus; in the limit of very long times the bulk modulus could be smaller than the shear modulus (figure 3.9). Unlike rubbers, (which have large bulk moduli) hydrogels undergo very large volume changes due to hydrostatic swelling pressures, which is indicative of very low drained bulk moduli.

Still, the Poisson's ratio determined here is likely an artefact of assuming a purely linearly poroelastic framework. Purely poroelastic analysis assumes that the solid skeleton is linearly elastic and does not exhibit any viscoelastic behaviour on its own. As a result the total extent of relaxation is assumed to relate to the difference between the drained (ν) and undrained (ν_u) Poisson's ratio values. Any flow-independent viscoelastic relaxation would contribute to a smaller perceived Poisson's ratio. Material nonlinearities could also affect the degree of relaxation. The presence of friction or adhesion could increase the difference

3.5 Discussion

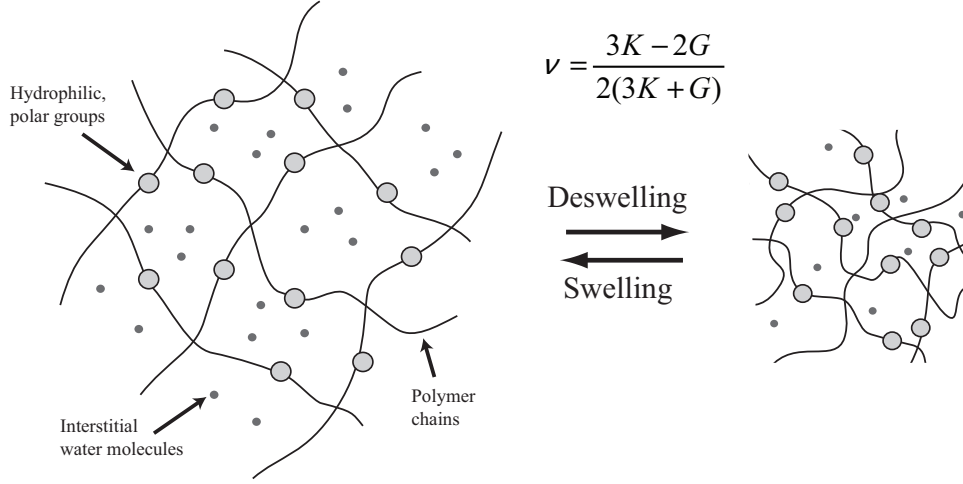


Figure 3.9: Illustration of a mechanism by which hydrogels could have negative Poisson's ratios.

between the undrained and drained states. Here, pure agar and 7.5%Gel-3%Agar composites with large volume fractions of agar were predicted to have negative Poisson's ratios because they demonstrated significant relaxation; the Poisson's ratio estimated here should be treated as a lower bound on the actual Poisson's ratio of the gel.

Regardless, the permeability of agar measured here by indentation (e.g. 2%: $4.4 \times 10^{-16} \text{ m}^2$) was in good agreement with experimental values reported for agarose previously measured via direct permeation ($3.5 \pm 0.95 \times 10^{-16}$; Johnson & Deen, 1996). It also was comparable to theoretical permeability values calculated using relationships for isotropic fibrous networks using fibre radii of 3-6 nm for the agarose polymer ($2 - 8 \times 10^{-16} \text{ m}^2$; equation 2.21; Waki et al., 1982), lending some credibility to the poroelastic approach taken here.

A reasonable identification of permeability can still be made from indentation measurements even if the Poisson's ratio is identified incorrectly. Hydraulic permeability, κ is determined from the consolidation coefficient, c , which is the sole material parameter that determines the rate of poroelastic relaxation. In terms of the equilibrium reduced modulus, $E_{R\infty}$, the hydraulic permeability, κ and the drained Poisson's ratio, ν , the consolidation coefficient is,

$$c = \frac{\kappa E_{R\infty}(1 - \nu)^2}{(1 - 2\nu)}. \quad (3.16)$$

The equilibrium reduced modulus is uniquely determined from the equilibrium load measured during indentation, P_∞ . Therefore, if reasonable identifications are obtained for the consolidation coefficient and equilibrium load, incorrect identification of Poisson's ratio can only affect the identification of permeability by little more than an order of magnitude (figure 3.10).

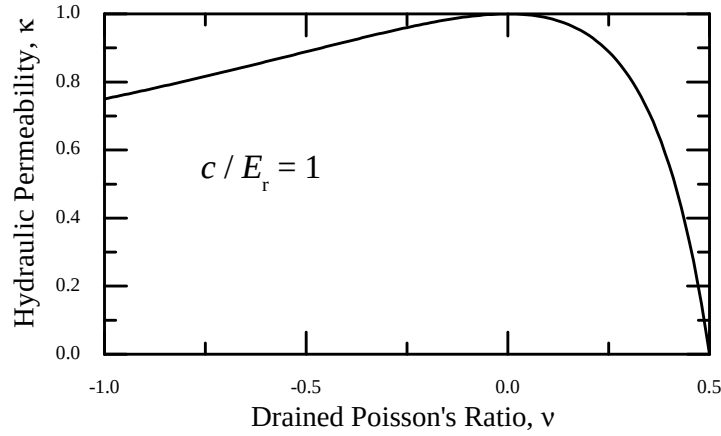


Figure 3.10: A plot of identified κ vs ν for constant ratio of consolidation coefficient to equilibrium reduced modulus determined from equation 3.16. The permeability varies by one order of magnitude over the full range of drained Poisson's ratios.

Unconfined compression testing was also used to determine the poroelastic properties of a small subset of gels, although with markedly different results. As noted in section 2.4.2, unconfined compression is not an ideal method for characterising the poroelastic behaviour of materials, particularly if the materials exhibit some degree of viscoelastic behaviour. Viscoelastic relaxation affects the total relaxation observed during indentation and unconfined compression equally (assuming the shear modulus relaxes and ν remains constant), i.e., if $G_\infty/G_0 = 0.75$, the observed load-relaxation, P_∞/P_0 will be the same whether indentation or unconfined compression has been performed. Poroelastic relaxation, on the other hand, results in greater observable relaxation in indentation than unconfined compression for the same drained Poisson's ratio. If $\nu = 0$, then $P_\infty/P_0 = 0.50$

3.5 Discussion

for indentation while $P_\infty/P_0 = 0.67$ for unconfined compression. If both poroelastic and viscoelastic mechanisms were to be present, then the poroelastic mechanism would dominate during indentation but would be comparable to the viscoelastic mechanism during unconfined compression.

The convolution of viscoelastic and poroelastic responses during unconfined compression is further exacerbated by the fact that the rate of poroelastic relaxation is much slower for unconfined compression than for indentation, due to the larger drainage lengths. Using equations 3.8 and 3.11 in the limit of a step displacement, it can be shown that it will take 225 s for 90% percent of relaxation to occur during an indentation test but 1900 s during an unconfined compression test assuming the same geometrical parameters as those used here and that $G = 100$ kPa, $\nu = 0.0$ and $k = 10^{-15}$ m².

Hence, the poroelastic parameters obtained from unconfined compression should be treated with extreme caution. The discrepancy between the results of indentation and unconfined compression provides evidence that there is viscoelastic behaviour that has not been accounted for. However, it is not clear to what extent this affects the identification of parameters during indentation. This will be discussed further in chapters 5 and 6.

3.5.3 Comparison to native tissue properties

Information linking the microstructure of a scaffold to that of native tissue can be gained through the use of poroelastic analysis. A poroelastic framework relates the time-dependent deformation of the hydrogel to its intrinsic permeability, a structural property that is a function of pore size. This has advantages when designing a scaffold for tissue engineering in which the effective pore size can be matched to that found in the native tissue (table 1.3) or otherwise optimised to promote the desired cellular response. The effective intrinsic permeability of gels determined here through indentation was on the order of 10^{-17} to 10^{-15} m², one to three orders of magnitude greater than NP values measured through direct permeation (Heneghan & Riches, 2008) and confined compression experiments (Périeré et al., 2005). This suggests that the gels have a different structure to that of the NP. Furthermore, these larger permeabilities may result in a different

mechanical response *in vivo*, where much of the load carried by the NP is initially sustained with fluid pressurisation.

The composite gels considered here had equilibrium elastic moduli ranging from 3 to 97.5 kPa depending on gel composition. This is a wider range than observed values for the NP (table 1.3). Furthermore, homogeneous gelatin and agar gels could both be made with moduli on the order of 10 kPa or less, with a small enough solid fraction. However, the elastic or shear modulus is not necessarily the property of functional importance *in vivo*, where the NP is confined by the vertebral bodies and AF. A number of models have provided evidence to suggest that this confinement is crucial to overall mechanical performance of the disc (Joshi et al., 2009; Strange et al., 2010). The NP is known to have an aggregate (confined compression) modulus, H_A that is significantly greater than its elastic modulus, E . In an isotropic material as the Poisson's ratio approaches 0.5, H_A tends to infinity (table 2.1). The gelatin gels measured here had a mean Poisson's ratio of 0.40 and would therefore have an aggregate modulus approximately two times greater than their elastic modulus. However, as increasing volume fractions of agar were mixed with the 7.5% gelatin the Poisson's ratio value of the composite decreased. A 7.5%Gel-3%Agar composite with a gelatin volume fraction of 0.6 had an effective Poisson's ratio of 0.20 resulting in an aggregate modulus which was not substantially greater than the elastic modulus. Still, in the composite gel systems described here at a fixed permeability of $\sim 5 \times 10^{-17} \text{ m}^2$, H_A ranged from 60 to 190 kPa. For a fixed value of $H_A \sim 50 \text{ kPa}$, hydraulic permeabilities were found ranging from 6×10^{-17} to $1.6 \times 10^{-16} \text{ m}^2$, although as noted before these permeabilities were far too large when compared with the native NP. It is not known which exact parameters would be best suited to engineer a NP tissue, but this approach provides an interesting opportunity for designing gels for specific applications and with optimised property combinations for tissue engineering applications in which confinement and flow behaviour are important.

The effective aggregate modulus of the NP is thought to result from the osmotic swelling force of the tissue, which increases as the tissue is compressed (Johannessen & Elliott, 2005). When the NP is excised and placed in 0.15 M NaCl solution *in vitro*, it can increase in volume by nearly 50%, and even more

3.6 Conclusions

in smaller concentrations of NaCl (Urban & Maroudas, 1981). Thus, it is particularly interesting that the agar did not swell when placed into the solution. Agar can contain sulphated polysaccharides, although agarose, which comprises the bulk of the agar, is electrically neutral and as a result unlikely to swell like the NP. This may also be a factor associated with the substantially greater permeability of these gels.

So far, the discussion has focused on the flexibility that a composite approach gives a scaffold designer in tuning the scaffold's mechanical response. Other factors, such as the biocompatibility and biodegradation of the gels, also have an important role in determining the success of these materials for use as tissue engineering scaffolds (Drury & Mooney, 2003). Some of these factors, including biodegradation, are dependent on many of the same properties as the mechanical response and have been shown to be tuneable using a composite approach (Dong et al., 2006). These factors become increasingly important as the materials begin trials *in vivo*.

3.6 Conclusions

Gelatin-agar and gelatin-alginate systems have been used here to demonstrate the wide range of mechanical properties and, more importantly, mechanical property combinations that can be engineered into hydrogels for tissue engineering applications. These systems are illustrative, but not themselves ideally suited for use *in vivo*. The techniques developed here, however, represent a framework for the mechanical testing and analysis of new hydrogels and hydrogel composites better suited for *in vivo* utilisation. Indentation testing, with its ease of implementation and established analytical framework, facilitated the identification of abrupt property transitions in narrow composition ranges in gel composites, opening up an exciting parameter space for design of unique gels ideally suited for specific tissue engineering applications. In the following chapter, composites of hydrogel and electrospun polymer fibres will be developed, providing further opportunities to create materials optimised for tissue engineering applications.

Chapter 4

Electrospun fibre-reinforced hydrogels

The formation of thick, randomly aligned polycaprolactone electrospun fibre structures infiltrated with alginate is reported in this chapter. The composites are characterised using both indentation and tensile testing and demonstrate substantially different tensile and compressive moduli, i.e. tensile-compressive nonlinearities. The composites are mechanically robust and exhibit large strain-to-failures. The method presented here provides a way to create large biomimetic scaffolds that more closely mimic the composite structure of natural tissue, with tuneable tensile and compressive properties.

4.1 Introduction

The IVD, like many soft tissues, is a fibre-reinforced composite. Both the AF and NP consist of collagen fibrils (AF: type 1, NP: type 2) embedded in a gel-like ground substance, consisting largely of proteoglycans and water. In the AF, these fibres are aligned at $\pm 30^\circ$ to the circumferential direction and serve to constrain the NP. The role of the collagen fibrils in the NP is less clear, although they likely resist the outward deformation of the tissue generated by the internal osmotic swelling pressure. Fibre-reinforced composites demonstrate a unique set of structure-property relationships. By mimicking the structures present in the IVD, it may be possible to create materials that support tissue regeneration while maintaining comparable mechanical function (Nerurkar et al., 2011).

There are a number of techniques for forming fibrous networks similar to those found *in vivo* (Stella et al., 2010). Electrospinning is a widely used technique that can be employed to form fibre networks from a range of polymers. The fibre diameter, pore-size, and anisotropy of electrospun fibre networks can be controlled and matched to native tissue, influencing tensile mechanical properties and cell behaviour (McCullen et al., 2012; Nerurkar et al., 2009). However, with conventional electrospinning techniques it is difficult to achieve material thicknesses greater than 1 mm, preventing this technique from being applied to larger tissues like the NP. Hong & Kim (2011) recently demonstrated that it was possible to create porous electrospun PCL fibre structures over 20 mm thick by immersing the ground electrode of the electrospinning apparatus into an ethanol bath with a rising fluid level.

The porosity of electrospun fibrous networks is crucial for cell infiltration, although porous fibrous networks are likely to buckle when a substantial compressive load is applied, demonstrating significantly reduced mechanical properties in compression rather than in tension. Furthermore, there are a number of characteristic mechanical properties that arise from the interactions between fibre and ground matrix, which are not present for purely electrospun fibre structures (Nerurkar et al., 2011). Moutos et al. (2007) fabricated woven fibre networks with 104 μm diameter PGA yarn and infiltrated the network with various hydrogels. The composites demonstrated comparable tensile-compressive nonlinearity,

anisotropy and viscoelastic behaviour to cartilage. Agrawal et al. (2012) manufactured fibre-reinforced gels (with fibre-diameters $\approx 100\text{ }\mu\text{m}$) and demonstrated that the fibre-reinforced gels had significantly greater tensile strain-to-failures than unreinforced gels. Infiltrating fibre networks with cell-seeded hydrogels also likely improves the penetration of cells into the fibrous network (Ameer et al., 2002; Ekaputra et al., 2008; Marijnissen et al., 2002).

Here, thick electrospun fibre structures are formed and infiltrated with alginate hydrogels of varying compositions. The composites are characterised using tensile and indentation testing. It is shown that the tensile modulus and failure properties of these fibre-reinforced composites is strongly influenced by the fibre network, while the indentation properties (which are representative of compressive properties) are largely influenced by the material properties of the hydrogel. The results presented in the chapter illustrate a methodology to create fibre-reinforced composites with varying degrees of tensile-compressive nonlinearity.

4.2 Methods

4.2.1 Electrospinning apparatus

An electrospinner was designed and built for the production of thin fibre membranes. The electrospinner was initially set up in a horizontal configuration (figure 4.1a). A Chemyx Fusion 100 Syringe pump (Stafford, TX, USA) capable of flow rates from $0.001\text{ }\mu\text{l hr}^{-1}$ – 90 ml min^{-1} was acquired. A disposable BD plastic 50 ml plastic syringe (Cole-Parmer Instrument Co. Ltd., London, UK) containing the polymer and solvent to be spun, was placed into the syringe pump. An 18 G needle (BD Blunt Fill Needle, Cole-Parmer Instrument Co. Ltd., London, UK) was attached to the end of the syringe. The end of the needle was cut using a grinding wheel so that it was blunt and the needle face was perpendicular to the needle direction. A high voltage supply (EH20P5; Glassman Europe Limited, Hampshire, UK) capable of positive voltage outputs between 0–20 kV was obtained. The positive terminal of the voltage supply was connected to the syringe needle via a RG-8/U coaxial cable. A plastic stand wrapped in aluminium foil was placed approximately 15 cm from the tip of the needle. The aluminium foil

4.2 Methods

was connected to ground. All of the above apparatus (excluding the high voltage supply) was enclosed within a plastic container.

In order to electrospin fibres into a fluid bath, the above apparatus was modified to work in a vertical configuration (figure 4.1b). The needle was mounted 14 cm vertically above a glass Petri dish. The needle was connected to the syringe and syringe pump via a PTFE tube. The glass Petri dish contained an earthed copper plate, which was used instead of the plastic stand wrapped in aluminium foil. Fluid could be added to the glass Petri dish via a separate PTFE tube connected to a syringe. In preliminary experiments the flow rate of the ethanol syringe was controlled by hand, though this was found not to have sufficient repeatability and in the experiments reported here, the syringe was controlled using a Graseby 3150 syringe pump (Smiths Medical, Smiths Group PLC, UK).

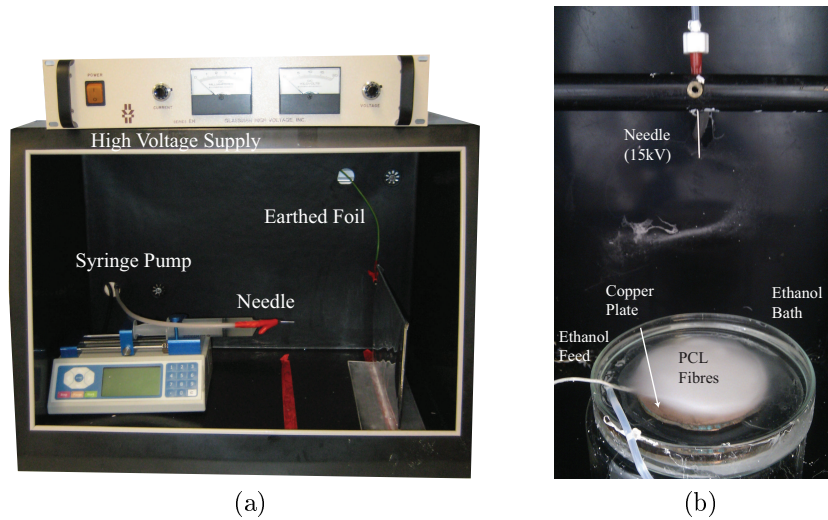


Figure 4.1: Electrospinner (a) set up in a horizontal configuration to produce thin fibre membranes and (b) set up in a vertical configuration in order to spin fibres into a fluid bath.

4.2.2 Sample preparation

Single phase materials

Porous PCL fibre structures over 1 mm thick were created following a similar method to that of Hong & Kim (2011). Seven grams of PCL ($M_n = 80,000$ g/mol) was dissolved into 50 ml of a 1:1 mixture of dimethylformamide and tetrahydrofuran (Sigma Aldrich, UK) and stirred for 48 hours. The mixture was placed in a 50 ml plastic syringe and loaded into the electrospinning apparatus. Prior to commencing the electrospinning process, ethanol (Sigma Aldrich, UK) was added to the Petri dish containing the earthed copper plate until the fluid was just covering the copper plate.

A voltage of 15 kV was applied between the needle and copper plate. The polymer solution was pumped through the needle at 0.02 ml min^{-1} . In preliminary experiments¹, ethanol was added to the Petri dish at approximately 5 minute intervals such that the fluid level gradually rose as more material was deposited. In the majority of experiments, ethanol was pumped into the Petri dish at 1.5 ml min^{-1} using a syringe pump. The diameter of the Petri dish was 11 cm; therefore, neglecting fluid evaporation and the deposited fibre volume, the fluid level rose at 0.16 mm min^{-1} . PCL fibre structures were collected after approximately one hour of electrospinning into ethanol.

Fabricated PCL fibre structures then underwent a process to infiltrate the structures with water. After fully submerging the structures in ethanol, the structures were centrifuged at 500 g to release any trapped air. An orbital shaker was then used to agitate the structures in distilled water for at least 24 hours until they were no longer buoyant; the distilled water was changed regularly over the 24 hour period. The resultant PCL fibre structures were stored in water until testing or until use for composite manufacture.

Alginate hydrogels (1%, 3%, and 5%) and homogeneous fully-dense PCL samples were manufactured as controls. Alginate gels were fabricated using a modified method to that presented in section 3.2.1: 1%, 3%, and 5% alginate solutions were prepared by stirring alginate granules into distilled water. The solutions were poured into moulds, kept in a -20°C freezer overnight to control

¹Preliminary experiments were carried out with assistance from Khaow Tonsomboon

4.2 Methods

their shape and then placed into a beaker containing 200 mM CaCl_2 solution and crosslinked for at least 12 hours.

In order to make fully-dense PCL samples, PCL pellets were placed into a Petri dish and heated in an oven at 80 °C until molten. A smaller Petri dish was pressed into the molten PCL to form a flat sheet approximately 1.5 mm thick. The molten PCL was then cooled at room temperature until it had solidified.

Composite fibre-hydrogels

Composite PCL-alginate structures (PCL-Alg) were produced by placing PCL fibre structures into solutions of 1%, 3% or 5% alginate for 6 hours. The solutions were gently agitated using a magnetic stirrer. The alginate-containing structures were then placed into 200 mM CaCl_2 solution and crosslinked for at least 12 hours while agitated using an orbital shaker. These composites were then stored in 200 mM CaCl_2 solution until testing.

4.2.3 Characterisation

Thickness and density measurements

The thickness of electrospun PCL fibres and PCL-Alg composites was determined using digital callipers as the mean thickness of three points. Measurements were made on five samples of each composition. The density of alginate hydrogels, PCL fibres, and composites was determined using the Archimedes principle. Multiple samples were weighed together on a balance to determine their mass. Their volume was determined by placing the samples into a graduated cylinder and recording the volume of the displaced water.

Fourier transform infrared spectroscopy (FTIR)

A Spectrum 100 FTIR spectrometer (Perkin Elmer, Waltham, MA, USA) was used to obtain attenuated total reflection infrared spectra of the samples. Samples were compressed against the spectrometer crystal while a scan was performed, using the inbuilt plunger. Spectra were recorded over the wave number range 4000-575 cm^{-1} at a resolution of 4 cm^{-1} from 16 scans. The absorbance spectra

were normalised to the largest peak (e.g. $A_{\text{norm}} = A/A_{\text{peak}}$, where A is the absorbance), in order to facilitate comparisons between samples. Spectra were obtained from multiple positions on a sample and averaged together; hence a mean spectrum is reported for each sample type.

Scanning electron microscopy (SEM)

Two electrospun PCL fibre structures and one sample of each type of composite were examined using electron microscopy. Sections were obtained by cutting the samples with a scalpel while wet. Samples were then dried in a desiccator for one week. Finally, samples were coated in gold for imaging at 15 kV using a Carl Zeiss (UK) EVO LS scanning electron microscope.

Mechanical testing

Indentation and tensile testing were performed to characterise the elastic modulus and tensile strain-to-failure of the composites and single phase materials using an Instron 5544 universal testing frame with both 5 N and 500 N load cells. Five samples of each composition were used for each test.

Indentation experiments were performed using a method similar to that described in section 3.2.3. Samples for indentation were sliced into rectangular slabs. PCL fibre and PCL-Alg samples were indented in the through-thickness direction, which was perpendicular to the predominant fibre plane. Indentation tests were carried out using a stainless steel spherical tip with a radius (R) of 4.7 mm on all sample types except fully-dense PCL, for which a 1.5 mm radius tip was used. A small preload (0.5 mN) was applied to the test samples, followed by a 10 s ramp to a peak displacement, h_{max} of 0.3 mm (for the 4.7 mm indenter) or 0.1 mm (for the 1.5 mm indenter). These displacements correspond to indentation strains of 0.05.

Viscoelastic indentation responses were examined using the elastic-viscoelastic correspondence approach described in section 3.3.1 and further detailed in section 2.4.1. Using this approach, the viscoelastic relaxation function, $G(t)$ of all materials was determined assuming $\nu = 0.5$, except for the fully-dense PCL samples, for which a value of 0.3 was assumed as is typical of a glassy polymer (Greaves

4.2 Methods

et al., 2011). From $G(t)$, the limiting instantaneous ($E_0 = 2G(0)(1 + \nu)$) and equilibrium ($E_\infty = 2G(\infty)(1 + \nu)$) elastic modulus values were calculated, as well as the ratio E_∞/E_0 , which is a measure of the extent of viscoelastic deformation. The identified Prony series for viscoelastic indentation were also used to predict an equivalent effective elastic modulus that would be measured during tensile testing at a constant strain rate, $\dot{\epsilon}$. This was determined using equation 2.29 such that:

$$E_{\text{eff}} = \sigma(t_T)/\epsilon(t_T) = \left(C_0 + \sum_{i=1}^n C_i \tau_i (1 - \exp(-t/\tau_i)) / t_T \right), \quad (4.1)$$

where t_T is the time over which the tensile strain was applied and $\sigma(t_T)$ and $\epsilon(t_T)$ were the measured stress and strain at this time.

For tensile testing, all samples, except for fully-dense PCL specimens, were cut into rectangular bars approximately 20 mm $\times$ 7 mm $\times$ 4 mm in size with a scalpel. Samples were clamped in grips lined with sandpaper such that the sample gauge length was approximately 15 mm. Prior to loading, the geometry of each test specimen was determined. The thickness of the samples was determined as the mean thickness of three separate points, using digital callipers. The gauge length and width of the samples was determined from images of the sample clamped in the grips taken with a PixeLINK PL-B776F 3.1 megapixel camera (Ottawa, Ontario, Canada). A preload of 0.05 N was applied to the sample followed by an increasing tensile extension (0.1 mm s⁻¹) until failure. Strain was determined as the cross-head displacement divided by the gauge length. The elastic modulus of the samples was calculated by fitting the stress-strain curve in the region of 0 to 0.1 strain with a straight line using Microsoft Excel (Microsoft, Richmond, USA).

Fully-dense PCL specimens were cut into 5 mm wide dog-bone shapes with a 20 mm gauge length using a steel die. Two small marks were made on the gauge length using a permanent marker. Samples were tested at the same displacement rate as the other materials, but with an initial preload of 0.5 N. A PixeLINK PL-B776F 3.1 megapixel camera was used to record images at 1 s intervals throughout the test. The sample strain was determined as the change in length between these marks divided by their original length. The elastic modulus of the samples was

only calculated from 0 to 0.05 strain as the stress-strain curve was no longer linear for larger strains.

4.3 Results

4.3.1 Formation of polycaprolactone structures and composites

Thick PCL fibre structures with a thickness of 1.91 ± 0.76 mm were formed using a similar method to that of Hong & Kim (2011) (figure 4.2). Although samples were not as large as those produced during preliminary experiments (full details of preliminary experiments are reported in Strange et al., 2012), their manufacture was much more repeatable.

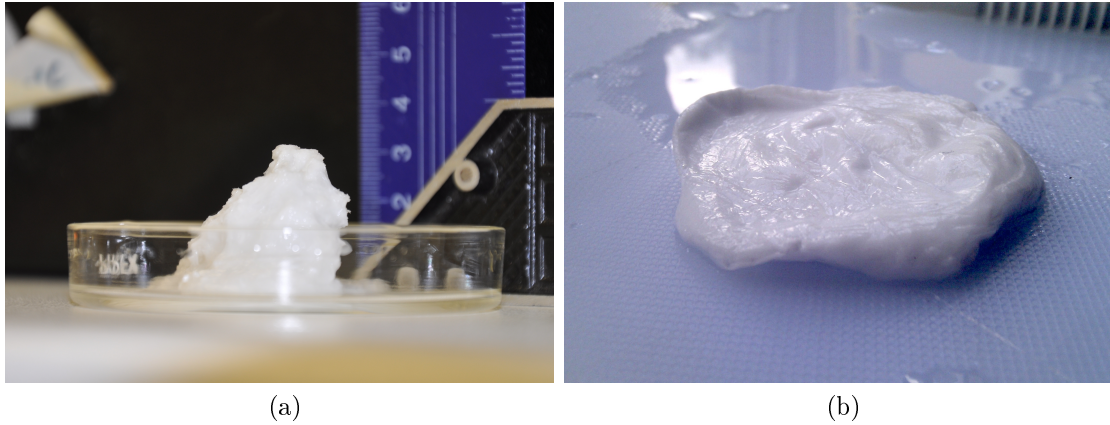


Figure 4.2: Images showing: (a) a PCL fibre structure fabricated in preliminary experiments, where the ethanol flow rate was controlled by hand and (b) a PCL fibre structure fabricated using the current method, where the ethanol flow rate was controlled with a syringe pump.

When electrospun PCL fibre structures were backfilled with alginate, the thickness of the samples increased from 1.91 ± 0.76 mm to 2.96 ± 0.45 mm for PCL-1%Alg composites and to 5.43 ± 1.06 mm for PCL-3%Alg composites. It was not possible to measure the thickness of the PCL-5%Alg composites as they swelled into a non-uniform shape and large volumes of alginate hydrogel remained attached to the composite which had to be removed with a scalpel (figure 4.3).

4.3 Results



Figure 4.3: Photograph of PCL-5%Alg composite. The composite swelled during the manufacturing process leading to an uneven distribution of fibre layers with large pockets of alginate.

A measurable change in density was not observed between composite systems and electrospun PCL fibre structures (the densities of PCL fibre structures, alginate hydrogels and composites were all within the range of $0.97\text{--}1.06\text{ g cm}^{-1}$). This is perhaps not surprising as the reported densities of PCL and alginic acid are 1.15 g cm^{-1} and approximately 1.60 g cm^{-1} respectively (Atkins et al., 2004a,b; Cipitria et al., 2011). With small volume fractions of both constituents, it would not be expected that the density of the composites would deviate substantially from the density of water. Still, samples were not buoyant in water, suggesting that the samples were completely infiltrated with water and/or alginate hydrogel and large air-pockets did not remain.

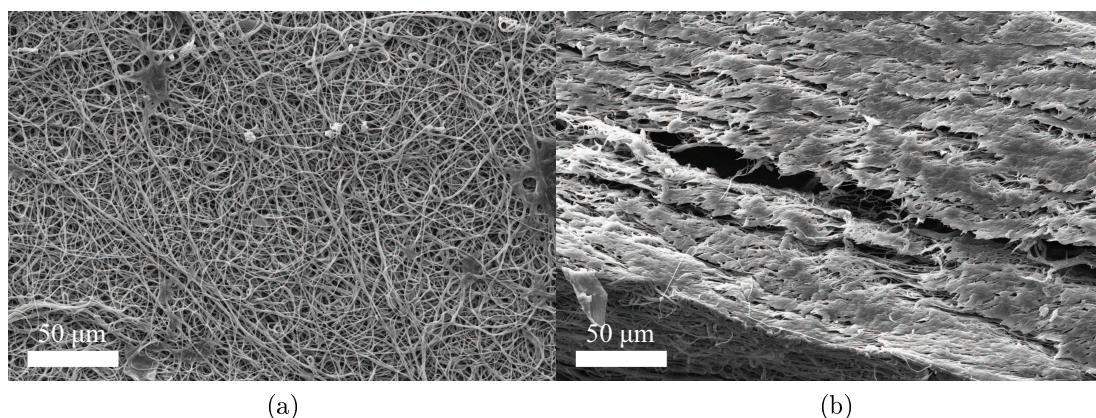


Figure 4.4: SEM micrographs of a PCL electrospun fibre structure, showing: (a) a top-down view and (b) a cross-section of the structure. In the cross-section, substantial plastic deformation is visible from where the scalpel cut through the sample.

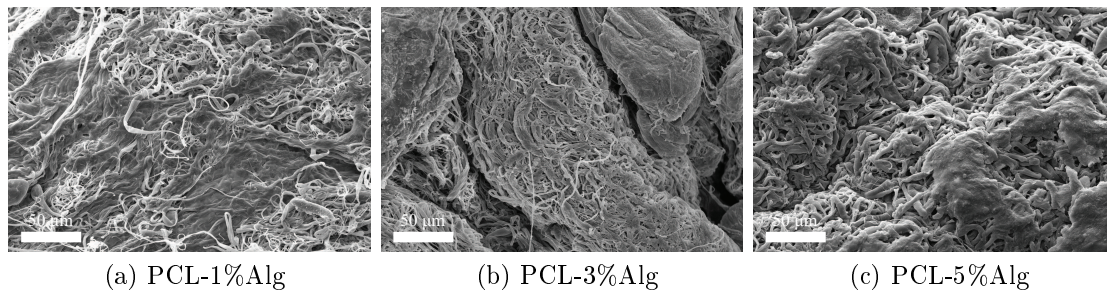


Figure 4.5: SEM micrographs of dehydrated PCL-Alg composites. The morphology of the composites appears to change with increasing alginate concentration although it is not clear whether this variation is a result of sample preparation or sample to sample variation.

4.3.2 SEM and FTIR spectroscopy

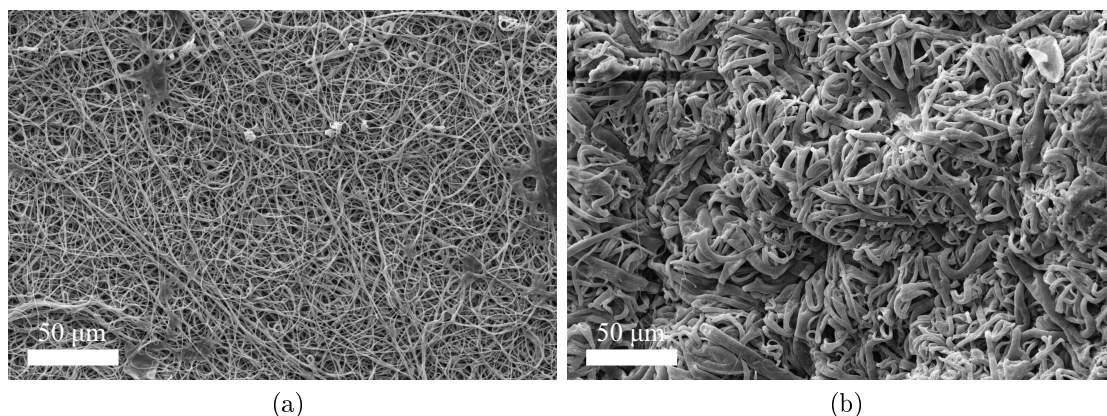


Figure 4.6: Side by side comparison of SEM micrographs of (a) a PCL electrospun fibre structure and (b) a PCL-5%Alg composite ($1000\times$ magnification). Although the dehydrated PCL-5%Alg composite still has a fibrillar structure when dried, the fibres are much larger than the electrospun PCL fibres and likely consist of a PCL fibre surrounded by a layer of dehydrated alginate.

Samples were examined using an SEM. PCL electrospun fibres consisted of a planar layers of randomly-orientated fibres with a diameter of $0.786 \pm 0.528 \mu\text{m}$ (figure 4.4a). A micrograph of a cross-sectioned sample shows apparent gaps between different layers of fibres (figure 4.4b). Substantial plastic deformation where the scalpel had cut through the sample was also visible. Both top-down and

4.3 Results

cross-sectioned micrographs revealed that the PCL structures were substantially porous, providing an opportunity to fill these structures with another material and create large hydrogel-electrospun composites.

Examination of dehydrated PCL-Alg composites using SEM demonstrated that alginate had permeated throughout the original PCL fibre structure (figure 4.5). In the PCL-1%Alg system, fibres were clearly visible in a solid matrix. In the PCL-5%Alg system, objects resembling fibres were predominately visible, though the diameter of these objects was substantially larger ($3.7 \pm 1.7 \mu\text{m}$) than the PCL fibres, suggesting that the fibres actually consisted of PCL fibres encased in a layer of alginate (figure 4.6). While there are possible differences in morphology in the composites for different alginate concentrations, only one sample was examined for each concentration and these differences could simply be the result of sample-to-sample variation.

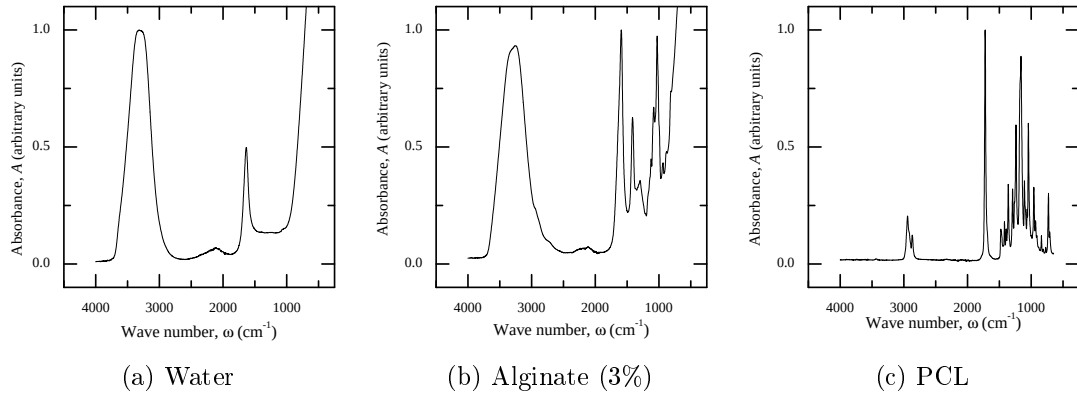


Figure 4.7: FTIR spectra of constituent phases found in the PCL-3%Alg composite system: (a) water, (b) 3% alginate and (c) PCL.

FTIR spectroscopy also revealed that alginate and water had permeated throughout the PCL fibre structure. The spectra of water, 3% alginate and PCL are shown in figure 4.7. Figure 4.8a shows the spectra obtained of electrospun fibre structures soaked in water, compared with the spectra of fully-dense PCL. The spectra of the electrospun fibre structures resembles that of fully-dense PCL with peaks characteristic of the O-H bonds of water (3368 and 1631 cm^{-1} ; figure 4.7a) superimposed on top, confirming that both phases were present. Similarly,

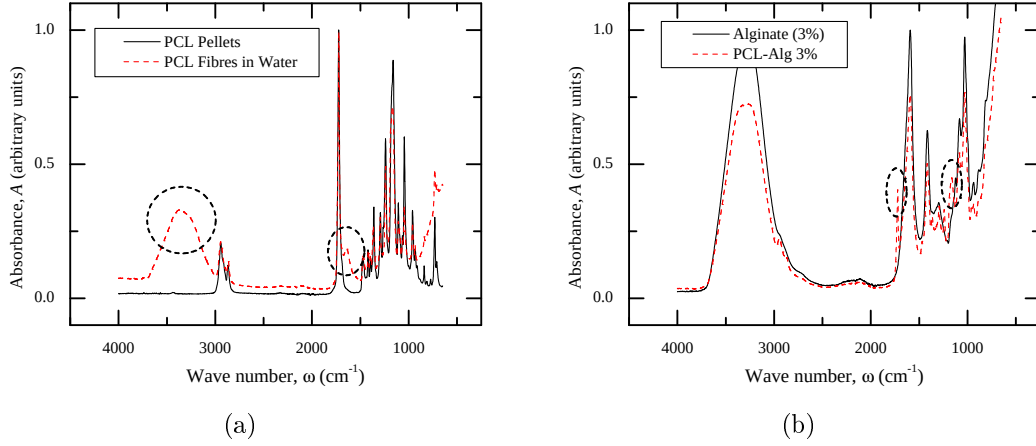


Figure 4.8: FTIR spectra of PCL fibres (a) infiltrated with water and (b) infiltrated with 3% alginate, compared with the spectra of their primary constituent. Bonds characteristic of (a) water and (b) PCL are encircled with a black dashed line.

the spectra of PCL-3%Alg composites mirrored that of a 3% alginate hydrogel, with additional peaks at 1720 and 1166 cm^{-1} , which are characteristic of the carbonyl and COC bonds found in PCL (Elzein et al., 2004). Substantial differences in spectra were not found between varying alginate concentrations for both single phase and composite materials. Although the Beer-Lambert law allows predictions of composition to be made (such as that made in Strange & Oyen, 2011a), the results obtained here are unlikely to be quantitative as the samples were compressed onto the FTIR crystal, likely changing their structure.

4.3.3 Mechanical characterisation

Fully-dense PCL had a tensile elastic modulus (E) of $219 \pm 11\text{ MPa}$, an instantaneous indentation modulus (E_0) of $245 \pm 18\text{ MPa}$ and an equilibrium indentation modulus (E_∞) of $174 \pm 11\text{ MPa}$. The agreement between the two measurement techniques is excellent: the equivalent effective tensile elastic modulus (equation 4.1) determined from indentation was $210 \pm 18\text{ MPa}$. Similarly, the elastic moduli of alginate hydrogels measured with tension and indentation were equivalent

4.3 Results

(table 4.1). However, an order of magnitude difference between tensile and indentation elastic moduli was observed for electrospun fibre structures, indicating that the structures have a substantial tensile-compressive nonlinearity. Representative tensile stress-strain and indentation force-displacement plots are shown in figures 4.9 and 4.10.

Material	Tension	Indentation		
	E	E_0	E_∞	E_{eff}
Dense PCL	219 ± 11 MPa	245 ± 18 MPa	174 ± 11 MPa	210 ± 18 MPa
1% Alginate	32 ± 10 kPa	41 ± 3.1 kPa	7 ± 0.8 kPa	29 ± 3 kPa
3% Alginate	179 ± 29 kPa	279 ± 73 kPa	51 ± 14 kPa	178 ± 48 kPa
5% Alginate	203 ± 38 kPa	346 ± 59 kPa	61 ± 10 kPa	219 ± 35 kPa
PCL Fibres	344 ± 133 kPa	15 ± 9 kPa	10 ± 7 kPa	14 ± 8 kPa

Table 4.1: Elastic and viscoelastic moduli of single phase materials measured using tension and indentation testing.

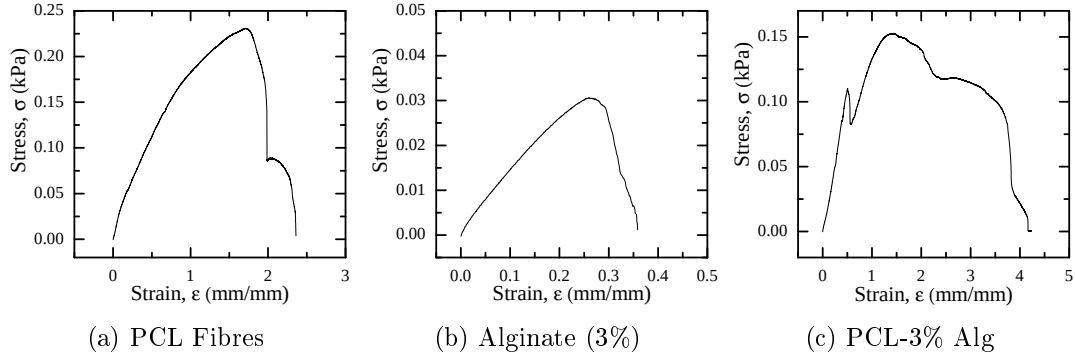


Figure 4.9: Representative tensile stress-strain curves of (a) PCL fibres, (b) 3% alginate and (c) PCL-3%Alg composite.

The modulus of alginate hydrogels increased with concentration (table 4.1). Alginate was also significantly more viscous than PCL. Fully-dense PCL and electrospun PCL fibres demonstrated similar viscoelastic ratios (0.71 ± 0.01 vs. 0.61 ± 0.09), whereas alginate gels had a mean viscoelastic ratio of 0.18 ± 0.024 . The viscoelastic ratio did not vary with concentration.

When electrospun fibre structures were backfilled with alginate hydrogels to form PCL-Alg composites, the indentation modulus increased. It can be seen from

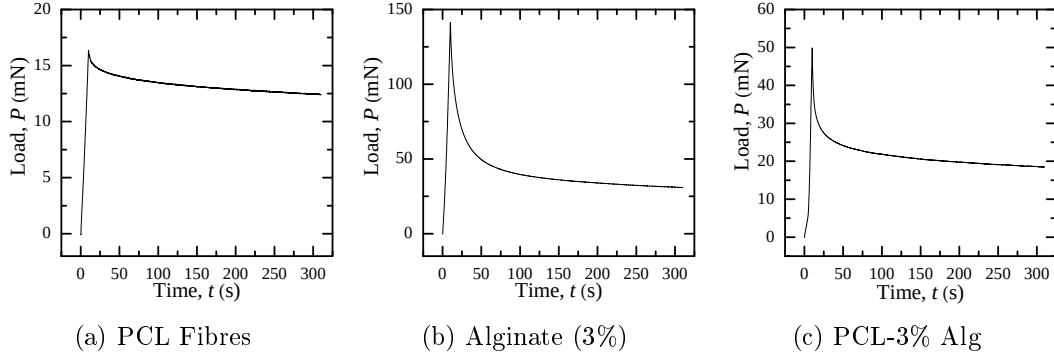


Figure 4.10: Representative indentation load-displacement curves of (a) PCL fibres, (b) 3% alginate and (c) PCL-3%Alg composite.

figure 4.11 that the indentation modulus of the PCL-Alg composites is dominated by the alginate phase. Little difference can be observed in both E_0 and E_∞ of the PCL-3%Alg composite and a pure 3% alginate hydrogel. Similar behaviour was noted for the other composite systems. The viscoelastic ratio of the composites (0.22 ± 0.07) was much more similar to that of pure alginate hydrogels than that of PCL fibres.

In contrast, with the possible exception of the PCL-3%Alg composite system, the tensile moduli of PCL-Alg composites did not vary substantially from that of the electrospun fibre structures (figure 4.12a). As the tensile modulus of the composites was similar to that of the PCL fibres and the indentation modulus was similar to that of the alginate phase, increasing the concentration (and hence modulus) of the alginate phase in the composites reduced the discrepancy between the measured tensile and indentation modulus.

PCL-Alg composites also demonstrated different failure behaviour to PCL and alginate (figure 4.12b; figure 4.13). During tensile testing, alginate gels exhibited brittle failure with moderate strain-to-failures, regardless of concentration. In contrast, PCL fibre structures exhibited extremely large strain-to-failures (> 3.0). During tensile testing, stable necking would occur until the structure appeared to be connected by a sole fibre bundle which would eventually break. PCL-Alg composites exhibited behaviour in between these two extremes: PCL-1%Alg composites failed similarly to PCL fibre structures, PCL-3%Alg composites failed

4.4 Discussion

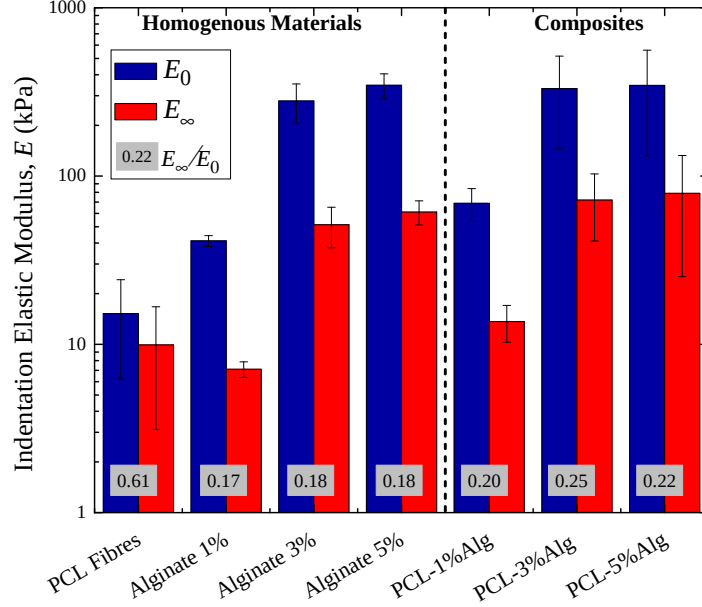


Figure 4.11: Viscoelastic moduli of single phase materials and composites tested in indentation.

after the formation of a small number of fibre bundles (4.13d) and PCL-5%Alg composites failed after the formation of multiple fibre bundles throughout the matrix, although little visible macroscopic necking was observed. As the concentration of alginate increased in the composites, the strain-to-failure decreased although it was still markedly greater than pure alginate even in the PCL-5%Alg system.

4.4 Discussion

In this work, thick electrospun fibre structures were rapidly fabricated using a method similar to Hong & Kim (2011). These structures were infiltrated with water and then with alginate in order to form fibre-reinforced hydrogels with varying tensile-compressive properties. These composites exhibit unique mechanical properties that are strongly influenced by their structure.

The porosity of the electrospun fibre structure can be estimated from the

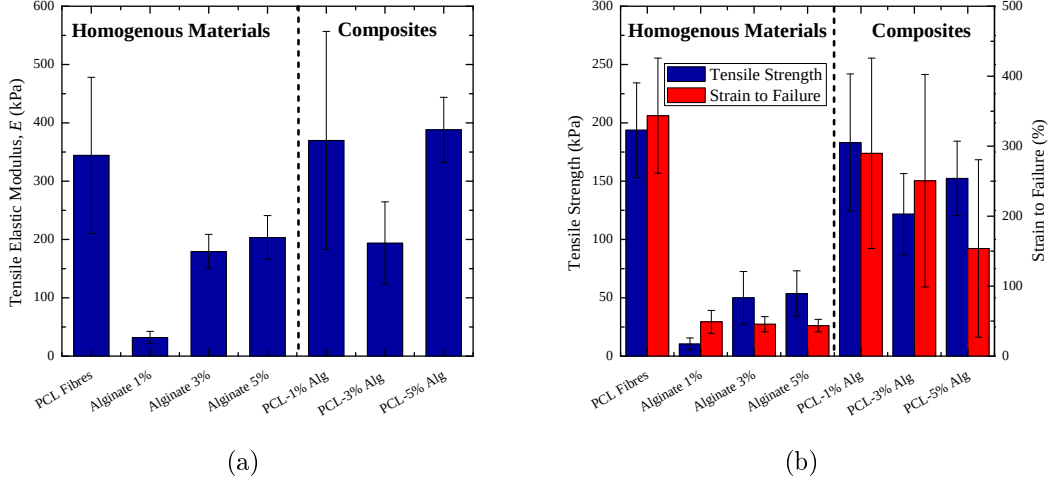


Figure 4.12: (a) Elastic modulus, (b) strength and strain-to-failure of single phase materials and composites tested in tension.

tensile modulus of the PCL fibre structure (344 kPa) and the elastic modulus of fully-dense PCL (219 MPa) using the relationships discussed in section 2.5.2. If the fibres are assumed to be randomly orientated in a two-dimensional structure, the porosity estimated from the Christensen & Waals (1972) composite bounds is 99.5% (equation 2.50; $E_1 = 0$). This is likely an upper bound on the porosity. The estimated porosity of the PCL fibre structure, assuming a three-dimensional cellular solid structure, is 96%, which provides a reasonable lower bound on the porosity (equation 2.52; Gibson & Ashby, 1999). These bounds should only be treated as estimates, given that the material microstructures were not homogeneous and likely differ from the theoretical situations. Regardless, the fibre structures are extremely porous, which makes them excellent candidates to infiltrate with hydrogel matrix.

When the composite structures were impregnated with alginate, they swelled in volume. This would have reduced the effective volume fraction of PCL. In the case of the PCL-3%Alg system, the total material volume increased by 280%, assuming the length and width remained constant and only the thickness changed. During swelling the volume of PCL would remain constant. Hence, the volume fraction of PCL in these composites, post swelling, can be estimated from the

4.4 Discussion

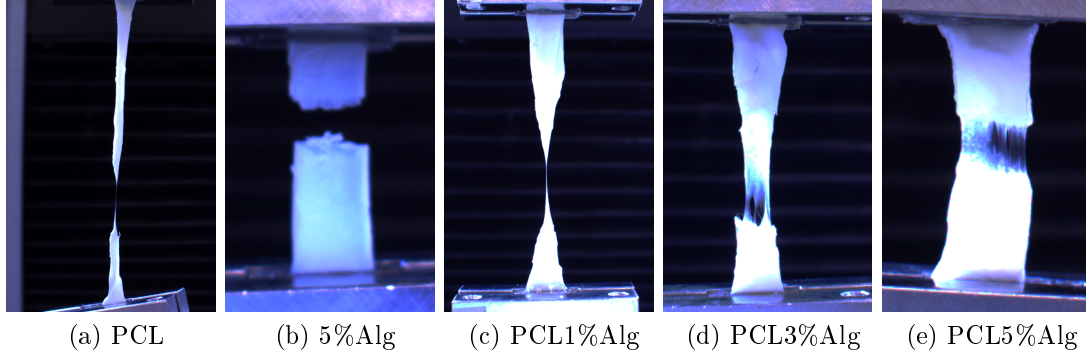


Figure 4.13: Images of single phase materials and composites undergoing tensile testing, at the point of failure.

porosity and swelling ratio as being between 0.2–1.4%. The fibres comprise an extremely small fraction of the composite. Nevertheless, because of the substantial modulus mismatch between dense PCL and alginate, the PCL dominates the tensile mechanical properties of the materials.

4.4.1 Elastic properties

Here, the tensile properties of composites were compared with their compressive properties (as determined with indentation). The tensile and compressive moduli of the composites may differ because of differences between the two measurement techniques. The magnitude of strains measured in tension and indentation differed. Finite-deformation effects were neglected even though the strains were relatively large. In addition, the modulus measured through indentation is potentially greater than what would be measured in an unconfined compression experiment as spherical indentation applies tensile strain to some of the material. The indentation measurement is also likely to be affected by any variation in microstructure near the surface, and may differ from the macroscopic behaviour measured in an unconfined compression test.

Still, the tensile stress-strain curve of all of the composites was observed to be linear between 0 and 0.1 strain and excellent agreement between tensile and indentation measurements of the homogeneous materials was obtained. Hence,

it is likely that the measurement performed here provides a reasonable estimate of the mechanical behaviour of the composites in tension and compression. Through these tests, it was found that the tensile properties of the composites were largely dominated by the presence of the electrospun PCL fibres. In contrast, the compressive properties were dominated by the modulus of the hydrogel phase.

This behaviour can be predicted from the composite bounds for elastic modulus. Figure 4.14 shows the Voigt-Reuss composite bounds on tensile elastic modulus for a PCL-1%Alg system and PCL-5%Alg system for PCL volume fractions of less than 5%. The figure also shows the Christensen-Waals bounds for two-dimensional and three-dimensional randomly orientated fibre networks (see section 2.5.1 for details of these composite bounds). For the small volume fractions of PCL considered here, the lower Voigt-Reuss bound, which represents a material where strains are applied perpendicular to the predominant fibre direction, is very sensitive to the modulus of the alginate phase but is not very sensitive to the volume fraction of PCL. In contrast, the upper Voigt-Reuss bound and both Christensen-Waals bounds are not very sensitive to the modulus of the alginate phase, but are linearly proportional to the volume fraction of PCL in the composite. Any increase in tensile modulus due to an increase in the modulus of alginate would likely have been offset by changes in the volume fraction of PCL due to swelling.

In the previous chapter, it was shown that the viscoelastic ratio of hydrogels could be tuned independently of elastic modulus. Here, the tensile modulus of the fibre-reinforced hydrogels could potentially be increased or decreased by varying the volume fraction of the PCL, either by changing the initial electrospinning parameters, or by controlling the swelling of the composites. Meanwhile, the compressive modulus, which is of primary importance for materials that mimic the NP, could be controlled by altering the concentration of the hydrogel matrix. In preliminary experiments, it was demonstrated that PCL fibre structures (and composites) could be formed with tensile moduli of between 500–1100 kPa (Strange et al., 2012).

4.4 Discussion

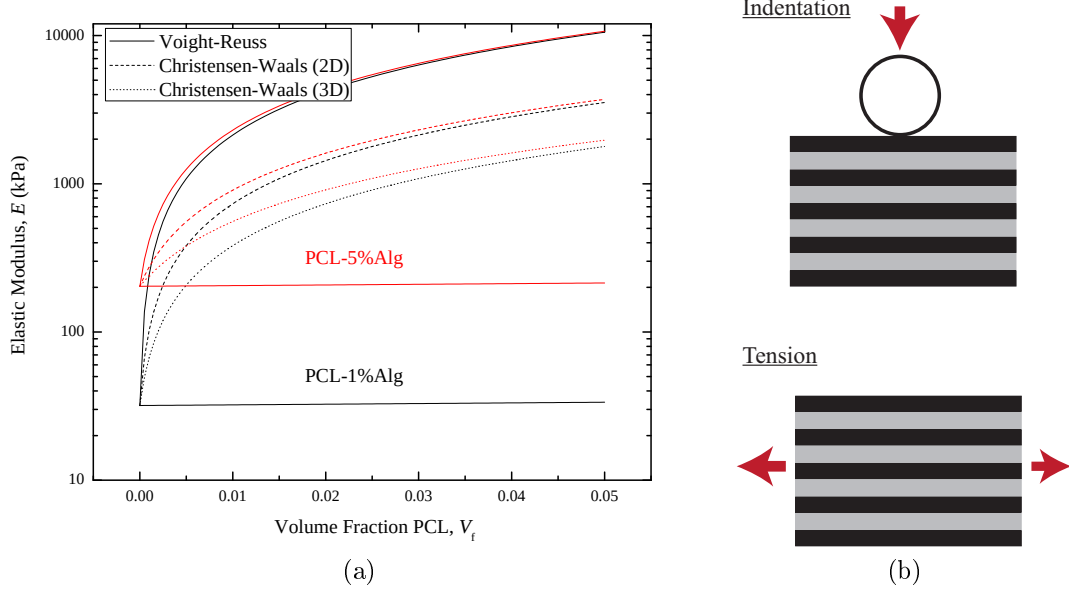


Figure 4.14: (a) Composite bounds calculated for two PCL-Alg composites from tensile elastic moduli. The upper and lower Voigt-Reuss bounds correspond to laminar composites undergoing equal stress and equal strain loading, respectively, while the Christensen-Waals bounds represent random fibrous networks orientated in a two-dimensional plane or three-dimensional volume. See section 2.5.1 for details. (b) The upper Voigt-Reuss and two-dimensional Christensen-Waals bounds are similar to the loading conditions found in tension, while the lower Voigt-Reuss bound is similar to what is observed during indentation.

4.4.2 Failure

Fibre-reinforced hydrogels were much more robust to failure than pure alginate hydrogels. Even the PCL-3%Alg system, for which the tensile modulus was comparable to 3% alginate, had a strain-to-failure which was five times larger than that of pure alginate. It has been observed that electrospun PCL fibre networks will reorientate along the principle loading direction at large strains (Koh et al., 2012). It is likely that the PCL fibre networks behaved similarly in tension tests. In the PCL-Alg composites, the alginate matrix would have resisted this fibre-reorientation, particularly for larger concentrations. This resulted in a more brittle failure, at a lower failure strain. Still, for the PCL-5%Alg system, fibre pull-out was observed throughout the matrix at failure.

Fibre pull-out contributes to the toughness of a material, by absorbing fracture energy during crack advance. The energy required for fibre pull-out is related to the interfacial shear strength, which could potentially be improved. Holloway et al. (2010, 2011) formed UHMWPE fibre-PVA hydrogel composites. They demonstrated that interfacial shear strength between UHMWPE fibres and PVA hydrogels could be improved by treating the UHMWPE fibres with oxygen plasma cleaning to increase their hydrophilicity, prior to infiltration with the hydrogel. Untreated PCL is also hydrophobic. The surface hydrophilicity of PCL can also be improved using oxygen plasma treatment (Martins et al., 2009). Treating the electrospun PCL fibre mesh with oxygen plasma may result in similar improvements in interfacial strength between the PCL and alginate and lead to a stronger composite.

The tensile failure strain of alginate was much lower than that of the PCL fibre network. Compared to many other gels, alginate is a tough gel with a reasonable strain-to-failure (Drury et al., 2004). Even still, Sun et al. (2012) recently demonstrated that extremely tough double network gels of ionically crosslinked alginate and covalently crosslinked polyacrylamide could be formed. These double network gels exhibited tensile strain-to-failures in excess of 2000%. It is possible that extremely tough and strong electrospun-fibre hydrogels could be formed by infiltrating the PCL with a similar double network gel.

In this study, the tensile strength of the composite has been considered and not the compressive or shear strength. For many applications, including NP tissue engineering, the compressive failure properties of the composite are likely to be more important. Fibre-reinforced composites can exhibit a variety of failure mechanisms in compression that are different to those observed during tension (Schultheisz & Waas, 1996). The failure behaviour of these composites should be further investigated. Nonetheless, the presence of these strengthening mechanisms in tension is promising as it suggests an opportunity to create hydrogel-like materials with sufficient toughness to withstand the loading encountered during implantation and *in vivo*.

4.4 Discussion

4.4.3 Time-dependent properties

PCL-Alg composites were much more time-dependent than PCL fibre structures, but were slightly less time-dependent than pure alginate. Indentation measurements performed on PCL fibres demonstrated that PCL was viscoelastic. Experiments by Zhao et al. (2010) have demonstrated that ionically crosslinked alginate exhibits both poroelastic behaviour arising from fluid flow and viscoelastic behaviour arising from the time-deformation of the polymer network. Here, the time-dependent behaviour of the composites arises from the viscoelasticity of the electrospun fibre network, viscoelasticity of the alginate polymer network, and poroelasticity of fluid passing through the composite.

Cottenden & Oyen (2011) used composite theory to show that the viscoelastic ratio of a viscoelastic matrix reinforced with elastic fibres could be markedly different from the viscoelastic ratio of the matrix; the elastic fibres suppressed time-dependent behaviour in the matrix for moderate fibre volume fractions. Here, the PCL fibres were much more ‘elastic’ than the alginate matrix, though there was little suppression of viscoelastic response, despite the significant modulus mismatch between the phases. This could potentially stem from the extremely small volume fraction of PCL fibres. As the volume fraction increases the viscoelastic ratio may also increase.

In contrast to viscoelastic theory, theories of poroelasticity that incorporate a tensile-compressive nonlinearity (e.g. Cohen et al., 1998; Soltz & Ateshian, 2000) predict that the extent of relaxation under compressive loading will be greater than that of materials without a tensile-compressive nonlinearity. This occurs because the fibres constrain the radial expansion of the material, increasing fluid pressurisation. This behaviour has been observed in articular cartilage.

Although the interacting effects are numerous, they could potentially be decoupled using an approach similar to Galli et al. (2011), who investigated the PVE properties of particle-reinforced gelatin through homogenisation for viscoelastic parameters and intrinsic permeability. Alternatively, a fibril-reinforced PVE FE model incorporating direct measurements of single phase properties may allow for accurate prediction of the time-dependent behaviour.

4.4.4 Comparison to native tissue properties

Many soft tissues consist of fibres embedded in a hydrogel-like matrix. The fibres provide stiffness and strength in tension, while also moderating the time-dependent response of the material in compression. The gel-phase swells and provides a mechanism to support and distribute compressive load, through fluid pressurisation.

In the IVD, the AF consists of alternating laminates of type 1 collagen fibrils aligned at $\pm 30^\circ$ to the circumferential direction of the disc. The tensile circumferential modulus of multilaminate AF samples is approximately 17.4 ± 14.3 MPa (Elliott & Setton, 2001), while the radial compressive aggregate modulus ranges from 0.27–0.44 MPa (Best et al., 1994). Here, electrospun fibres were randomly distributed in a plane, though they did still exhibit a tensile-compressive nonlinearity, with varying mechanical properties in the planar and through thickness directions. This nonlinearity could potentially be matched to that of the AF by increasing the concentration of fibres. Furthermore a laminated structure could potentially be created by following a method similar to Nerurkar et al. (2009, 2010c).

Unlike previous studies of AF-like fibre composites where electrospun fibre laminates were wrapped around a hydrogel (Nerurkar et al., 2009, 2010c), here the electrospun fibres were infiltrated with a hydrogel. This is likely to be important for the time-dependent behaviour of the material. One of the functions of the AF is to inhibit fluid flow away from the NP in order to maintain fluid pressurisation (Chiu et al., 2001; Ohshima et al., 1989). Although the permeability of the composites was not measured here, the time-dependent behaviour of the composites was substantially different from that of the electrospun fibre structures. This could have been due to restricted fluid flow through the alginate matrix. Infiltrating porous fibre structures with a semi-permeable hydrogel may be crucial to achieving a functional engineered IVD, with minimal culture time.

The NP is thought to be isotropic and it is not known whether it exhibits a tensile-compressive nonlinearity; the NP has never been tested in tension. Nevertheless, the NP does contain a network of randomly orientated collagen fibrils

4.4 Discussion

(2% wet weight; Ohshima et al., 1989) and the compressive mechanical properties of the NP have been correlated with the mean diameter of the collagen fibrils (Aladin et al., 2010). While the NP is semi-confined by the AF *in vivo*, when the NP is compressed there will still be some lateral expansion of the NP and the fibres could contribute to the fluid pressurisation. Nevertheless, fibrous networks and fibre-reinforced hydrogels have only been investigated to a limited extent as NP scaffolds (Nesti et al., 2008). The size of the NP is much larger (7–12 mm; Gilad & Nissan, 1986) than what can be achieved using a conventional electrospinning process and these scaffolds lack compressive mechanical integrity. The work carried out here provides a method to produce composites of comparable thickness to the NP with a similar proportion of hydrogel and fibres.

4.4.5 Future investigations

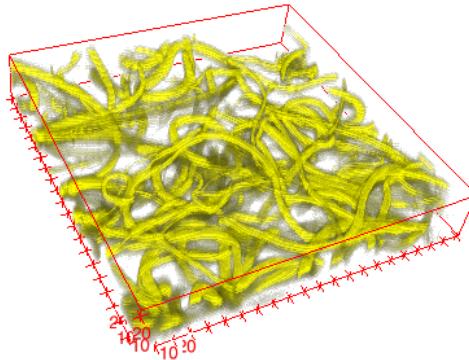


Figure 4.15: Three-dimensional section of fluorescent PCL fibre structure imaged with confocal microscopy. The sample was manufactured by the author and the confocal fluorescent microscopy was performed by Khaow Tonsomboon with the assistance of Peerapat Thongnuek.

The distinctive mechanical properties of the composites manufactured in this study stem from their particular structure. A deeper understanding of this structure could be obtained through the use of confocal fluorescent microscopy. Preliminary experiments performed in collaboration with Khaow Tonsomboon demonstrated that similar thick electrospun fibre structures could be produced and

imaged with the fluorescent dye Rhodamine 6G incorporated into the electrospinning solution (figure 4.15). However, the electrospinning process properties had to be altered and the resultant fibres had a much larger diameter ($\approx 6 \mu\text{m}$) than samples produced previously and the structure was less porous ($\approx 85\%$; the porosity was estimated using boneJ, available from <http://bonej.org/>). Still, alginate can also be covalently labelled with fluorescent markers (Strand et al., 2003) and incorporated into the electrospun fibre structure. This technique would allow direct quantification of the volume fraction of the fibres and alginate, and any porosity in a hydrated state. Mechanical tests could be performed on these fluorescent samples, in order to provide quantitative information of the structure-property relationships of fibre-hydrogel composites.

4.5 Conclusions

In this chapter, the creation of large three-dimensional electrospun fibre-hydrogel composites has been demonstrated. The composites exhibit a tensile-compressive nonlinearity with properties that can be tuned by varying the concentration and volume fractions of the constituent materials. The method presented here can be used to fabricate materials that mimic the fibrous nature of natural tissue with combinations of mechanical properties optimised for tissue engineering applications. In the following two chapters, methods to more accurately characterise the time-dependent behaviour of materials with a hydrogel phase will be developed.

Chapter 5

Indentation of poroelastic hydrogels

Spherical indentation has been shown to be a simple method for rapidly determining the poroelastic properties of hydrogels. Relaxation proceeds quickly compared to confined compression and unconfined compression tests due to the small drainage lengths involved. In this chapter, a finite element model of spherical indentation is developed. The model is used to investigate the effect of a finite ramp time on the relaxation of a poroelastic material. A concise expression describing the relaxation of a half-space is developed with analogy to the ramp correction factor approach for viscoelastic indentation.

5.1 Introduction

Indentation is a promising method of measuring the mechanical properties of poroelastic materials. Poroelastic relaxation proceeds more quickly compared to other methods due to the small distances over which fluid must flow. It also requires minimal sample preparation, which makes it ideal for testing soft-tissues and other materials that are difficult to handle. Indentation has been widely used to determine the poroelastic properties of cartilage (Miller & Morgan, 2010; Mow et al., 1989), bone (Oyen, 2008a), and a number of linear poroelastic hydrogels (Galli et al., 2009; Hu et al., 2010; Kalciglu et al., 2012), with comparable results to other test methods such as confined compression and direct permeation.

As indentation is a local measurement, it is also possible to map out heterogeneous properties of materials such as bone (Oyen & Ko, 2007). The size of the indenter tip can be varied such that different length-scales can be probed in hierarchical materials (Oyen et al., 2012; Stolz et al., 2004). Furthermore, in poroviscoelastic materials, it is possible to vary the size of the indenter in order to vary the drainage length of the material and determine the relative importance of poroelastic mechanisms over flow-independent viscoelastic mechanisms (Chiravambath et al., 2009; Hu et al., 2010; chapter 6).

Performing indentation tests in displacement control rather than load control is desirable as the indentation geometry (and therefore strain level and drainage length) can be controlled. Although the strain within the material is not uniform and not solely a function of the applied loading conditions, the peak strain can be approximately limited by choosing the indentation depth, h such that the indentation strain is constant (equation 2.27). Specifying the indentation strain during the test minimises variation resulting from nonlinear elasticity and strain-dependent permeability and allows for relevant comparisons between samples and tip sizes without prior knowledge of the material properties.

In displacement control, the contact radius (and drainage length) is fixed by the test parameters (indenter radius, R and displacement, h) and does not vary independently with time. Hu et al. (2010) demonstrated that non-dimensional force-relaxation curves for an applied step displacement took a simple form, which did not vary with Poisson's ratio. In contrast, in load control the contact radius is

dictated by the changing stiffness of the tissue; as a result the rate of poroelastic creep depends on the Poisson's ratio of the material.

As discussed in section 2.4.2, no analytical solutions exist for the indentation of a poroelastic material. Hu et al. (2010) and Hu et al. (2011) presented expressions for non-dimensional load, P^* as a function of non-dimensional time, t^* (section 2.4.2) generated from FE models for step displacement-controlled poroelastic spherical indentation. While in load control a step load is sometimes experimentally achievable, in displacement control the ramp is always finite. Inverse FE methods, which can account for a finite ramp time, have been used to determine material properties from experimental data, but these are time-consuming when hundreds of indents are performed.

The error that results from assuming that the displacement ramp-rate is infinite depends not only on the actual rate but also on the indenter size and the material properties. The degree of poroelastic relaxation that will occur during the ramp phase depends on the non-dimensional time, t^* :

$$t^* = \sqrt{\frac{ct}{Rh(t)}}, \quad (5.1)$$

where c is the consolidation coefficient, which depends on G , ν and κ (equation 2.39). The non-dimensional time, t^* is a function of the displacement, $h(t)$. As the displacement is itself a linear function of time during the ramp phase,

$$h(t) = \dot{h}t, \quad t \leq t_R, \quad (5.2)$$

paradoxically, the non-dimensional time does not depend on time, t . Instead the non-dimensional time depends on the ramp rate, $\dot{h}$ and is constant throughout the ramp phrase, i.e.,

$$t_R^* = \sqrt{\frac{c}{R\dot{h}}}. \quad (5.3)$$

The non-dimensional ramp time for a variety of indentation experiments is

5.1 Introduction

shown in table 5.1. The non-dimensional ramp time varies from 0.02–2.30 and shows little apparent correlation with the real ramp time in seconds, but instead depends heavily on the indenter tip size and material properties—the table contains data from micro, nano, and AFM-indentation experiments performed on biological tissues and hydrogels. These non-dimensional ramp times are compared with the degree of relaxation that is predicted had a step displacement of the same magnitude been applied and then held on the material for the non-dimensional ramp time (figure 5.1). The degree of relaxation that occurred during the ramp phase will have been less than what was predicted for the step hold phase and the discrepancies between these two assumptions could be large.

	G (kPa)	κ (mm ⁴ (Ns) ^{−1})	R (mm)	h (mm)
Agar (3%)	108.0	2.16×10^{-1}	4.7	0.3
Bone	4.30×10^5	4.65×10^{-9}	0.021	0.002
Cartilage	236	6.6×10^{-5}	0.239	0.003
Polyacrylamide	6.60	5.82×10^{-3}	10	0.1
Polyacrylamide	6.73	8.0×10^{-10}	0.0225	0.004

(a)

	t_R (s)	t_R^*	P_{Step}^*	Reference
Agar (3%)	10	0.47	0.62	Chapter 3
Bone	10	2.30	0.08	Oyen, 2008a
Cartilage	15	0.84	0.34	Oyen et al., 2012
Polyacrylamide	2	0.02	0.99	Kalcioglu et al., 2012
Polyacrylamide	0.08	0.35	0.73	Kalcioglu et al., 2012

(b)

Table 5.1: The non-dimensional ramp time calculated for a variety of indentation experiments. Material and experimental parameters are listed in (a), while the test ramp time, non-dimensional ramp time and calculated non-dimensional load-relaxation assuming step-loading are reported in (b). Poisson’s ratio is not reported, as it has a much smaller effect on the non-dimensional ramp time.

Hence, even with what appears to be an extremely fast ramp rate, substantial errors can be obtained by assuming that a step displacement has been applied, particularly at small length-scales where material relaxation occurs very quickly. This error is likely to result in incorrect identification of material parameters. Fur-

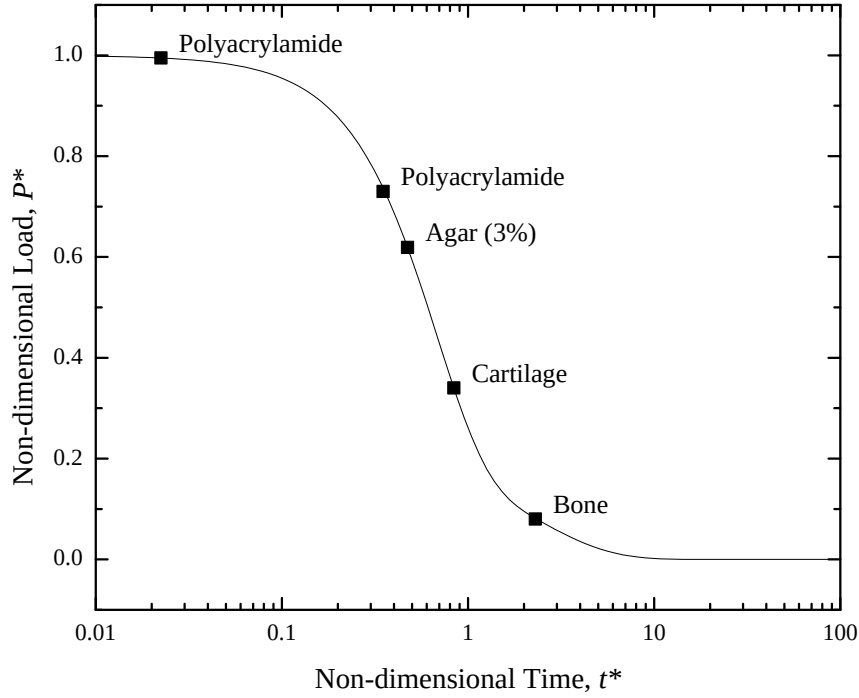


Figure 5.1: Non-dimensional load plotted against non-dimensional time for a spherical indenter under an applied step displacement (calculated using the expression of Hu et al., 2010). Specific points are highlighted that correspond to non-dimensional ramp times for indentation experiments that have been performed on hydrogels and biological tissues. See table 5.1 and text for further details.

thermore, the degree of error depends on the material parameters and cannot be predicted prior to the test. The error predicted here is a significant over-estimate as it is based upon values obtained from a step solution, but still highlights the need for a ramp displacement-controlled solution for spherical indentation. This will allow for improved characterisation and will allow errors in previous characterisations to be quantified.

A method to analyse creep for load-controlled indentation with a finite ramp time was previously proposed by Galli & Oyen (2009) who generated a series of master-curves for the creep behaviour of a poroelastic material. Each master-

5.2 Theory

curve corresponded to a different ramp time or Poisson's ratio. A similar approach could be used to develop a database for displacement-controlled indentation. It is also likely that a displacement-controlled database would take a simpler form than a load-controlled database and only depend on non-dimensional ramp time. As a result the master-curves could be described with a simple expression.

In this chapter, the development of an FE model of displacement-controlled indentation with a finite ramp is reported. Mesh-refinement studies were carried out to verify the elastic and poroelastic performance of the model. The model was validated against the solutions of Hu et al. (2010) and Hu et al. (2011) for a very short non-dimensional ramp time. The model was then used to generate a master-curve database of solutions for varying non-dimensional ramp rates. This database was curve fit with non-dimensional functions such that a concise description of the displacement-controlled indentation of a poroelastic material with a finite ramp rate can be presented. Finally, this expression was benchmarked against forward FE simulations and experimental data from the indentation of polyacrylamide and cartilage in order to quantify the error that arises from assuming a step-loading condition.

5.2 Theory

5.2.1 Poroelastic

In displacement-controlled indentation the non-dimensional load, P^* is a function of t^* and t_R^* , i.e.,

$$\frac{P(t, t_R) - P_\infty}{P_0 - P_\infty} = f\left(\sqrt{\frac{ct}{Rh_{\max}}}, \sqrt{\frac{c}{Rh}}\right). \quad (5.4)$$

In order to generate this function using FE analysis a family of non-dimensional curves spanning a large-range of non-dimensional hold times, t_H^* , must be produced for varying non-dimensional ramp times. However, it is not necessary to generate a separate non-dimensional curve to describe the ramp portion of the test (as was done by Galli & Oyen, 2009 for load-controlled indentation). In

displacement control, the ramp portion of the test is captured by a single non-dimensional ramp time (equation 5.3). Instead $P_R^* = f(t_R^*)$ can be generated from the beginning of each master-curve (equation 5.4). This is advantageous as the FE simulations will be most accurate when the most elements are in contact with the indenter, which will occur at the end of the ramp time/beginning of the hold.

As the FE model cannot directly generate non-dimensional curves, load-relaxation curves with varying ramp rates and hold times must be generated in the time-domain and then normalised. To perform this normalisation it is necessary to generate FE solutions for P_0 and P_∞ . These elastic limits could potentially be calculated from the Hertz formula for spherical indentation (equation 2.24) or similar, although this may result in small errors arising from the differing assumptions between the FE model and the analytical solutions. These are further discussed in section 5.2.2.

It must also be noted that the definition of non-dimensional time adopted here (equation 5.1), is different to that adopted by Hu et al. (2010) and Hu et al. (2011) for their expressions of displacement-controlled indentation with a step displacement. The non-dimensional time adopted in those studies is the square of the non-dimensional time adopted here ($t_{hu}^* = ct/[Rh_{max}]$). The definition presented in this study was adopted in order to be consistent with previous work on poroelasticity in both load-controlled indentation (Galli & Oyen, 2009) and confined compression (Detournay & Cheng, 1993). Furthermore, the definition adopted is advantageous—it can be seen from figure 5.1 that most relaxation occurs between 10^{-1} to 10^1 . If Hu et al. (2010) and Hu et al. (2011)’s definition was adopted, very small non-dimensional times would be needed to capture the early relaxation of the test and very large non-dimensional times would be needed to capture the later relaxation of the test.

5.2.2 Elastic

It is important to understand the limitations of the analytical solutions describing the spherical indentation of an elastic material, both to benchmark the FE model produced here and to understand any error in the elastic limits. The Hertz, Hay & Wolff (2001) and Sneddon (1965) analytical solutions are considered (figure

5.2 Theory

5.2b). The Hertz solution is widely used because of its algebraic simplicity, but it assumes simplified boundary conditions and a parabolic shape (instead of a true sphere). Hay & Wolff (2001) presented a small correction to the Hertz solution to account for the fact that the boundary conditions allow for an overlap between the rigid sphere and conforming surface outside the contact region:

$$P = \gamma \frac{4\sqrt{R}}{3} \frac{E}{1-\nu^2} h^{3/2}, \quad (5.5)$$

where

$$\gamma = 1 + \frac{2(1-2\nu)}{3\pi(1-\nu)} \sqrt{\frac{h}{R}}. \quad (5.6)$$

This correction, γ is equal to one when $\nu = 0.5$ and is only large for compressible materials when $\sqrt{\frac{h}{R}}$ is large, *i.e.* when the indentation strain is large (equation 2.27). Hay and Wolff's correction still assumes that the spherical indenter is a paraboloid.

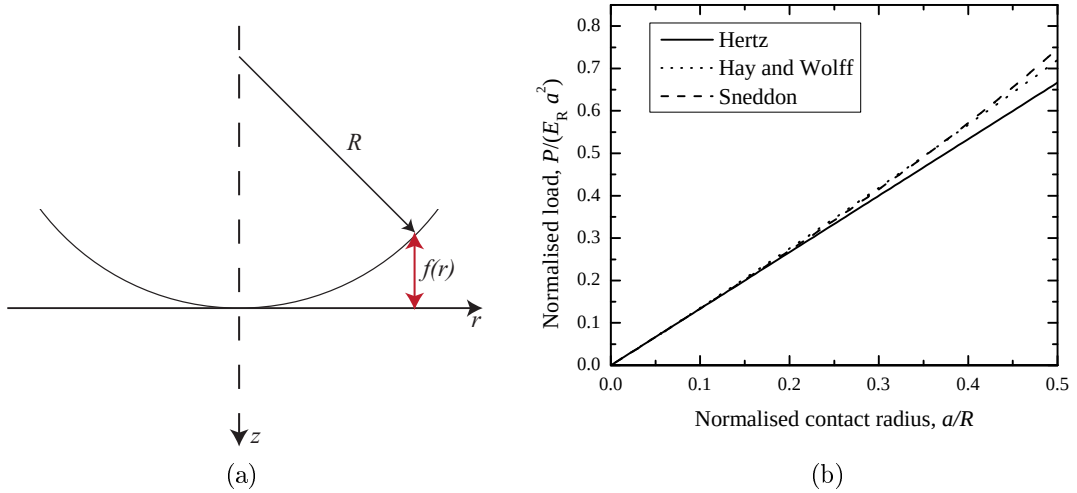


Figure 5.2: (a) Geometry of spherical contact. The separation between the sphere and half-space, $f(r)$ is calculated from equation 5.7 for the Sneddon expression and equation 5.8 for the Hertz expression (adapted from Hay & Wolff, 2001). (b) Normalised indentation load as a function of a/R calculated using the Hertz, Hay and Wolff, and Sneddon expressions, for a material with $\nu = 0.2$.

The vertical distance, z , between a spherical indenter and a flat surface is a

function of the distance from the centre of the indenter, r , such that (figure 5.2a),

$$z = f(r) = R \left(1 - \left(1 - \frac{r^2}{R^2} \right)^{0.5} \right). \quad (5.7)$$

A second-order Taylor expansion of this expression yields:

$$z = f(r) = \frac{r^2}{2R}, \quad (5.8)$$

which is equivalent to assuming that the indenter is a paraboloid rather than a sphere. This was the assumption adopted by Hertz (Johnson, 1985).

Sneddon (1965) derived an expression¹ for the indentation of a true sphere into a flat surface in terms of the contact radius, a :

$$P = \frac{E_R a^2}{2} \left[\left(1 + \frac{R^2}{a^2} \right) \ln \frac{(R+a)}{(R-a)} - \frac{2R}{a} \right]. \quad (5.9)$$

Sneddon also showed that the true contact radius of a sphere is related to displacement according to:

$$h = 0.5a \ln \left(\frac{R+a}{R-a} \right), \quad (5.10)$$

(where a Taylor expansion yields the relationship between a , R and h presented in equation 2.26). Sneddon's expressions are algebraically more complicated than the original Hertz equation which is why the former is preferred. The values of load only differ from that of Hertz for large values of $\frac{a}{R}$ and Sneddon's expressions still do not correct for inwardly radial displacements.

For large values of $\frac{a}{R}$, neither the Hertz, Hay & Wolff, nor Sneddon solutions provide a completely accurate description of the indentation load. Although the errors are small and are generally acceptable for the purposes of characterisation, FE methods will be used to generate the elastic limits for the master-curve database to avoid systematic errors in the database (similar to Galli & Oyen, 2009). Furthermore, all of the simulations will be performed with small values of $\frac{a}{R}$ so that there is little difference between the solutions.

¹There is a typographical error in the original equation (6.15) found in Sneddon (1965). A corrected equation is presented here.

5.3 Finite element model setup

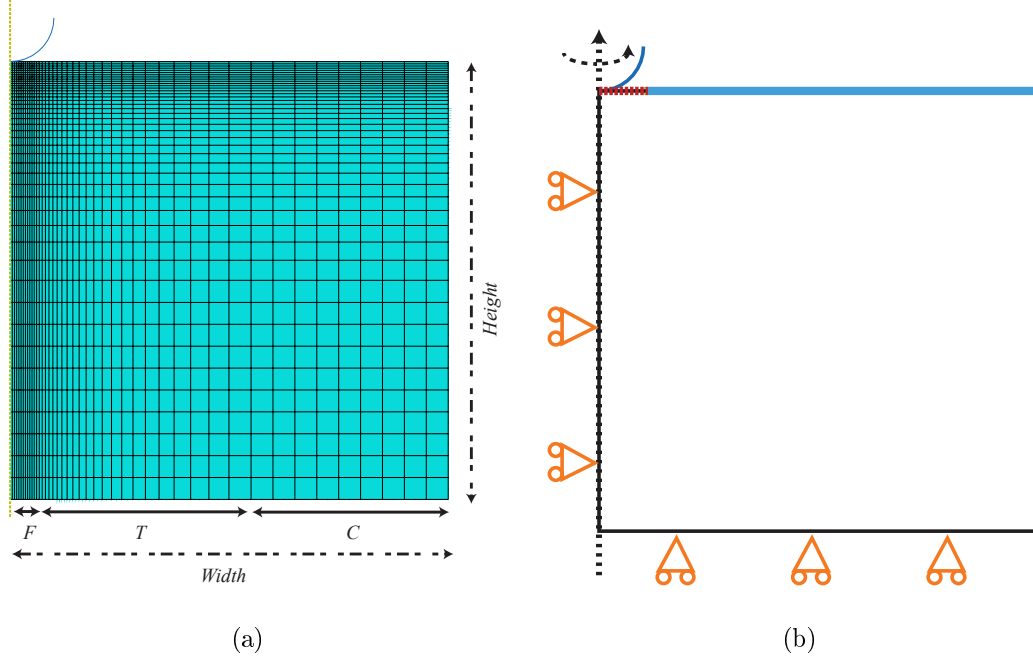


Figure 5.3: (a) Diagram illustrating the biased mesh used for the half-space model (not to scale). The half-space height and width were both 500 mm. The model was divided into a 5 mm long finely meshed region near the contact region (F) with constant element size of 0.1 mm, a 445 mm wide coarsely meshed region at the far edge of the model (C) with a constant element size of 5 mm, and a transition region (T) between them. (b) Boundary conditions applied to half-space model, including the zero pore pressure boundary condition (blue line) and impermeable contact user-defined boundary condition (red dashed-line) on the top surface. See section 5.3.4 for details.

5.3 Finite element model setup

The problem was modelled as a sphere in contact with a cylinder large enough to be considered a half-space. Both elastic and poroelastic models of displacement-controlled indentation were developed. In the poroelastic model, fluid was allowed to flow through the top surface of the half-space, apart from where the impermeable indenter contacts the half-space.

5.3.1 Model geometry and mesh

An axisymmetric model of the problem (figure 5.3a) was generated in Abaqus 6.11 (Dassault Systèmes, Vélizy-Villacoublay Cedex, France). The indenter radius was 50 mm. The indentation depth was 0.2, corresponding to an indentation strain of 0.013 (equation 2.27). The height, H and width, W of the half-space was much larger than the contact radius, a and depth of indentation, h ($H = W = 500$ mm).

The indenter was modelled as an analytical rigid surface, while the half-space was meshed with quadratic 8-node elements with reduced integration (CAX8RP). Quadratic elements generally yield more accurate results than linear elements for stress/displacement analyses (Abaqus Analysis User's Manual, 2011). Similarly, reduced integration typically provides greater accuracy than full integration (when quadratic elements are used). Pore pressure elements were used for poroelastic simulations while elastic hybrid elements were used for elastic simulations. Hybrid elements independently determine hydrostatic pressure and are necessary when modelling incompressible materials. It should be noted that unlike stress, pore pressure is not actually quadratic in quadratic pore pressure elements but is linearly interpolated from the edge nodes.

In order to capture the stress distribution and contact nonlinearities underneath the indenter, the half-space mesh was biased towards the contact region (figure 5.3a). The region under the contact area was finely meshed and the elements had a side length of 0.1 mm. The region farthest from the contact region was much more coarsely meshed and the elements were much larger (5 mm). Elements in the transition region increased in size the further they were from the contact region between the two extremes (fine and coarse). The aspect ratio of the element was limited to 50:1 which is less than the maximum recommended (Abaqus Analysis User's Manual, 2011). In total the mesh consisted of 31 684 elements.

5.3.2 Material properties

Material properties for a poroelastic base model and two elastic base models (P_0 and P_∞) are given below. Variations on these base models were used to generate the master-curve database, and these variations are discussed in section 5.4.1.

5.3 Finite element model setup

For elastic and poroelastic material models, G was specified to be 100 kPa and ν to be 0.3 (or alternatively 0.5, in elastic simulations for P_0). Abaqus requires that the drained elastic modulus, E (and Poisson's ratio) is specified instead of the shear modulus. E was determined from G and ν and set accordingly for each simulation using a Python script (Python Software Foundation, NH, USA; section 5.3.6). In the poroelastic model, the porous bulk moduli for the solid skeleton, K_s and fluid, K_f were not specified. This is equivalent to assuming both phases are incompressible.

For the poroelastic model, intrinsic permeability, k , was chosen to be $1 \times 10^{-17} \text{ m}^2$ and was specified to be independent of void ratio (Abaqus allows for k to be specified as a function of void ratio, the ratio of the void volume to the volume of solid material, such that k can vary with the applied strain). In Abaqus, an alternative definition of permeability, $\bar{k}$ (units of m/s), must be specified:

$$\bar{k} = \gamma \kappa = \frac{\gamma}{\mu} k, \quad (5.11)$$

where μ is the dynamic viscosity and γ is the specific weight of the liquid filling the pores (Abaqus Analysis User's Manual, 2011). $\bar{k}$ was calculated from k using approximate properties for water at room temperature: $\mu = 0.001 \text{ Pas}$ and $\gamma = 10\,000 \text{ N m}^{-3}$. The material parameters are summarised in table 5.2.

	G (kPa)	ν	k (m^2)	μ (Pas)	γ (N m^{-3})	K_s, K_f (kPa)
Elastic	100	0.5, 0.3				
Poroelastic	100	0.3	1×10^{-17}	0.001	10 000	∞

Table 5.2: Material parameters of the base poroelastic and elastic models.

5.3.3 Deformation and contact

The deformations of the poroelastic and elastic model were assumed to be linear. Geometrical nonlinearities were ignored and a small displacement formulation was used for the simulation; material strain was calculated relative to the reference configuration.

Although the deformation of the half-space was assumed to be linear, contact is inherently nonlinear. Contact between the indenter and the half-space

was modelled using a finite-sliding surface-to-surface contact formulation. The contact was assumed to be frictionless, which is a reasonable assumption for a submerged poroelastic material. The surface-to-surface contact formulation was used as it tends to provide more accurate stress and pressure results than the alternative node-to-surface formulation (Abaqus Analysis User's Manual, 2011). Finite-sliding was used instead of small-sliding as the latter may result in inaccuracies if the slave surface moves relative to the master surface, even though it is computationally less expensive.

5.3.4 Boundary conditions

Radial displacements and rotations of elements along the axial line of symmetry were fixed in order to enforce an axisymmetric boundary condition. The vertical displacement of elements at the bottom of the half-space were fixed, although radial displacements were permitted. Radial displacements were also permitted on the far edge of the half-space. (figure 5.3b)

In poroelastic simulations, the pore pressure of the top surface far from the contact region was set to 0 (allowing for free flow) while a pore pressure boundary condition was not applied to the bottom edge and far edge of the half-space, which is equivalent to assuming that the edges are impermeable ($\xi = 0$). A user-defined pore pressure condition was defined in the area directly beneath the indenter. The impermeable boundary condition prevented fluid flow in half-space regions in contact with the indenter but enforced a pore pressure of 0 in regions that were not in contact with the indenter.

The impermeable boundary condition between indenter and half-space was implemented using a user-defined subroutine (programmed in Fortran; Intel Fortran Compiler XE 12.1, Santa Clara, CA, USA).¹ The subroutine modified the seepage coefficient (analogous to surface hydraulic permeability) of the surface elements based upon whether they were in contact. At the end of each increment, the area of contact between the half-space and indenter was obtained and used to calculate the contact radius. Surface elements within the contact radius were assumed to be in contact with the indenter and had a seepage coefficient set to $0 \text{ mm}^4 (\text{Ns})^{-1}$

¹The subroutine was adapted from code provided by Matteo Galli and Tim Fletcher.

5.3 Finite element model setup

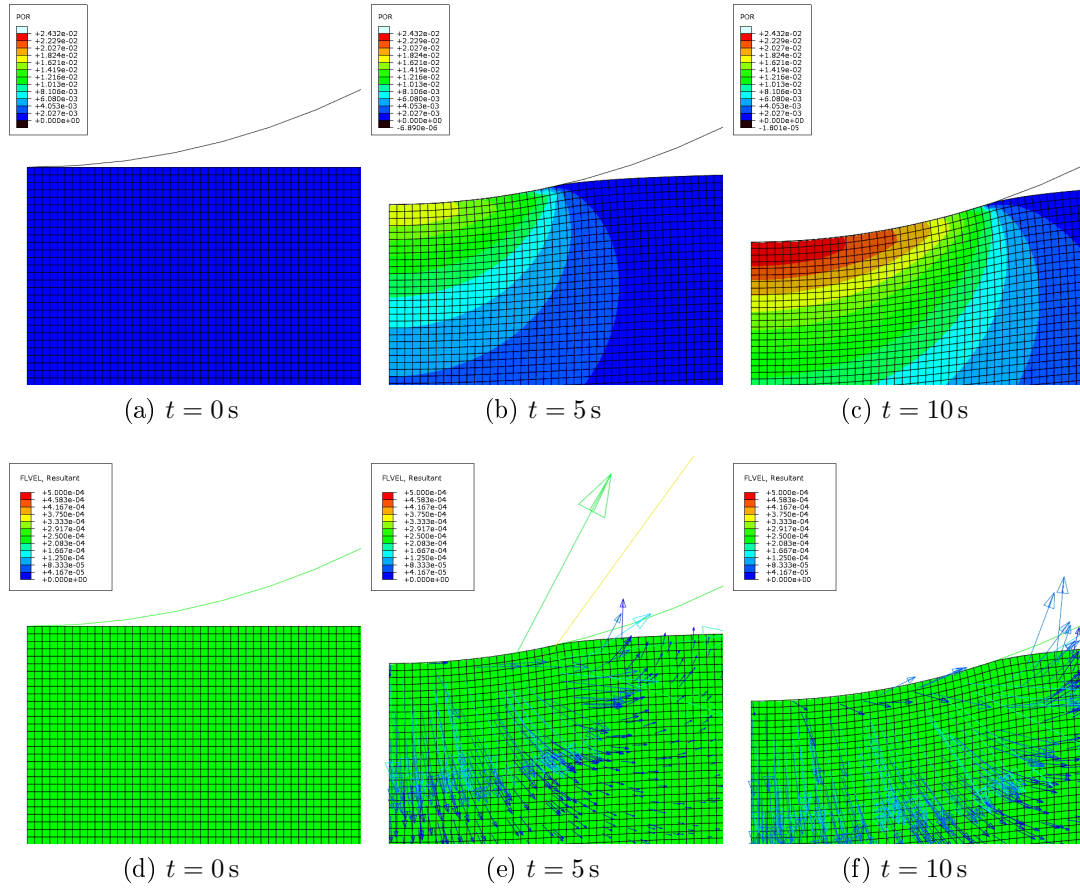


Figure 5.4: Pore pressure (a-c) and fluid velocity (d-f) in model during a ramp of 10 s with the user-defined impermeable indenter boundary condition. At the surface, pore pressure is only developed in elements directly in contact with the indenter; other surface elements have a pore pressure of 0. Fluid flows away from the indenter when elements are directly in contact.

(effectively impermeable). Surface elements with x-coordinates greater than the contact radius had a seepage coefficient set to $10^6 \text{ mm}^4 (\text{Ns})^{-1}$, enforcing a pore pressure of 0.

The effectiveness of the user-defined boundary condition was examined by simulating an indenter pressed into the material at a constant displacement rate, and comparing the results to that of a model with a permeable ($p = 0$) boundary condition. Non-zero pore pressure was maintained in the contact region (figure 5.4), and fluid flowed away from the indenter. The largest fluid velocities were observed at the edge of the contact radius where the half-space was just out of contact (figure 5.4).

It is worth noting that the accuracy of the impermeable boundary condition depends on the simulation time increment size as the subroutine uses the contact radius from the previous increment to calculate the seepage coefficients, and not the current contact radius. Decreasing the time increment size should result in increased accuracy up to a point but if the increment size is less than that required for a new node to come into contact, then the permeability of the surface will not be updated. Whereas in the majority of poroelastic simulations, the element size only needs to be small enough that the stress and pressure fields are calculated accurately at each time point, here, the element size can also affect the accuracy of time integration.

5.3.5 Time-stepping and integration

The accuracy of the time-dependent behaviour of a poroelastic material in Abaqus depends on the maximum pore pressure change allowed in each integration increment (Abaqus Analysis User's Manual, 2011). The finer the time-integration, the more accurate the solution will be. Because the rate of relaxation can change by orders of magnitude over the course of a poroelastic simulation, it is possible to set a limit on the maximum pore pressure change allowed per increment (UTOL); Abaqus will automatically adjust the time-stepping integration such that this limit is not exceeded. This is the recommended method of controlling time-stepping in a poroelastic simulation (Abaqus Analysis User's Manual, 2011).

5.3 Finite element model setup

However, the user-defined impermeable boundary condition was found to interfere with automatic time incrementation as the maximum pore pressure change in the simulation generally occurred at the edge of contact, where the pressure instantaneously changed from zero to a finite value when it came into contact with the indenter. If this pore pressure change was greater than the maximum permitted, the simulation would fail to converge even if the time increment was reduced.

Instead, time increments were specified for the simulations. During the ramp portion, only the final value of the response was saved (as all points during the ramp phase correspond to the same non-dimensional time and the final value will be most accurate). Nevertheless, the maximum increment during the ramp phase was limited to 1/50th of the total ramp time so that the pore pressure was accurately calculated during the ramp phase. Preliminary simulations demonstrated that the peak load at the end of the ramp phase with 50 increments was within 0.5% of the peak load at the end of the ramp phase with 190 increments. In contrast, simulations with only one increment differed by over 7% from simulations with 190 increments.

During the hold phase of the indenter, the indenter displacement is constant and the rate of relaxation depends on the material parameters as well as time throughout the test. Instead of limiting the maximum increment size, the time increments were specified at set non-dimensional times. This was done using a Python script detailed in section 5.3.6. One-hundred non-dimensional time-points were logarithmically specified between 10^{-2} and 10^2 in order to fully capture the relaxation behaviour. No discrepancies between the load-relaxation behaviour observed during the hold with 100 non-dimensional time-points and preliminary simulations with 200 non-dimensional time-points were found, suggesting that the accuracy of the simulation was not limited by the number of time-points.

5.3.6 Scripting

Simulations were scripted using Python in order to rapidly modify the Abaqus base model, run simulations and record the results. The Python script was capable of modifying the material parameters, geometry and mesh of the half-space,

as well as the radius and displacement of the indenter. In poroelastic simulations, non-dimensional time-points could be specified in order to control the non-dimensional ramp time and time-stepping during the hold portion. These would then be converted to real time-points by the script and set in the Abaqus model, before running a simulation.

At the end of a simulation, the Python script would record the indenter load, displacement and time in a (.csv) log file. In the case of a poroelastic simulation, the script would also record the non-dimensional times and loads in a log file, based upon the material parameters used to begin the simulation and a pre-created database of elastic simulations (for P_0 and P_∞). Additionally, the Python script recorded the parameters used for each simulation and the time taken to a log file. It saved a copy of the Abaqus input file used for each simulation so that the simulations could be rerun in the future if need be. (Abaqus output files were not kept for each simulation, due to the large amounts of memory this would require.) The Python script would also send an email to the user when it had completed a set of simulations. In this way, large sets of simulations with varying material and geometrical parameters could be run rapidly, in order to optimise the Abaqus model and create a master-curve database.

5.3.7 Model validation and mesh optimisation

A mesh refinement study was carried out to verify the accuracy of the simulation results. Elastic simulations with varying geometries and mesh refinements were run and plotted to see whether the results converged.

The accuracy of the simulations was most affected by the mesh refinement directly underneath the contact region but was insensitive to the mesh refinement far from the contact region. The indentation load converged as the size of the elements in the finely meshed region decreased and the number of elements in contact increased, but the load was unaffected by the size of this finely meshed region, as long as it was larger than the contact radius, a (figure 5.5a). Similarly, the load results were unaffected by the size and length of elements in the coarsely meshed region.

5.3 Finite element model setup

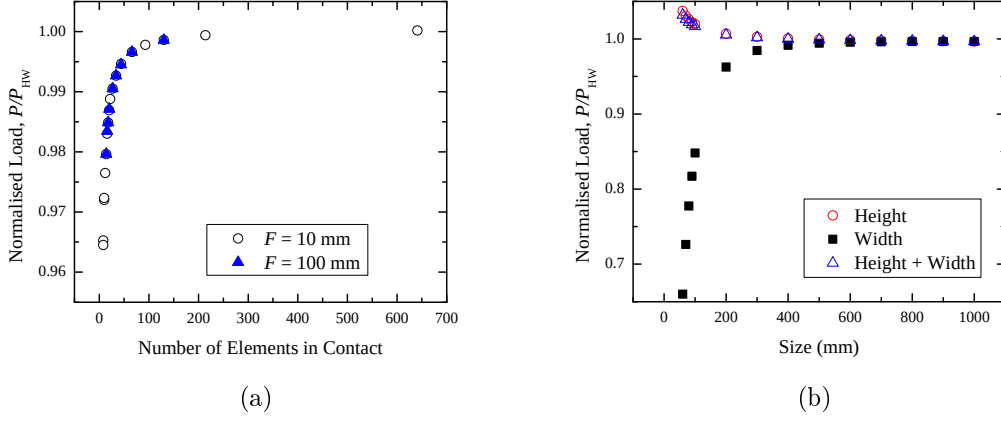


Figure 5.5: (a) The simulation load, P normalised by the load predicted by the Hay and Wolf expression (P_{HW} , equation 5.5) plotted against the number of nodes in contact with the indenter, for two sizes of the fine-mesh region, F (figure 5.3a). (b) P/P_{HW} plotted against the half-space height and width size. The base height and width were both 1000 mm in this case.

It is often assumed that a finite layer can be treated as a half-space when the indentation depth is less than 10% of the half-space thickness. Here it was found that when the height, or both the height and width of the half-space was ten times the indentation depth the error in indentation load was approximately 4% (relative to a model with a length and width equal to 1000 mm, which is 2000 times the indentation depth). However, in order to decrease this error to less than 1 %, it is necessary to have a height of at least 500 mm (figure 5.5b). In contrast it was found that the indentation load was very sensitive to the model width when the height remained constant at 1000 mm, although this may just be an artefact of having a small number of elements with extremely large aspect ratios stacked together.

Compared to analytical solutions, the indenter loads predicted by the base elastic models were within 1.5% of the Hertz and Sneddon solutions and within 0.4% of the Hay and Wolff solution. When Poisson's ratio was varied between 0 and 0.5 the loads were all within 2% of the Hertz and Sneddon solutions and 0.7% of the Hay and Wolff solution; the maximum error occurred when $\nu = 0$, which is when the Hay and Wolff correction is largest, and the error was smallest

when $\nu = 0.5$.

Poroelastic mesh refinement studies were not run to the same extent as elastic mesh refinement studies, due to the large computational expense involved in running each simulation. Simulations with a fine mesh element size of 0.05 were run for values of t_R^* of 0.01, 0.1 and 1. The maximum error between these simulations and the base model at any time point was a non-dimensional load of 0.013, indicating further mesh refinement was not necessary from the base fine-element size of 0.1. Similarly, simulations with a half-space height and width of 1000 mm were run for values of t_R^* of 0.01, 0.1 and 1 and compared with the base model with its height and width of 500 mm; the maximum error in P^* was 0.006. Galli & Oyen (2008) noted poroelastic simulations were more sensitive to finite-depth effects than elastic simulations, but the half-space modelled here was sufficiently large to be insensitive to these effects.

5.4 Results

5.4.1 Simulations

One hundred and one poroelastic simulations were run with non-dimensional ramp times logarithmically spaced between 10^{-2} to 10^2 in order to generate the master-curve database. Each simulation took approximately 40 minutes when run on eight 2.4 GHz Intel Xeon CPUs with up to 48 Gb of RAM available, leading to a total simulation time of approximately 63 hours.

In order to verify that separate master-curves were not needed for each value of Poisson's ratio (as is the case for load control), nine sets of simulations were run with varying non-dimensional ramp times logarithmically spaced between 10^{-2} to 10^2 . (Preliminary experiments confirmed that the indenter displacement, indenter radius, half-space shear modulus and permeability did not affect the the non-dimensional load-time curves.) For each non-dimensional ramp time, each simulation was run with a Poisson's ratio's of 0.15, 0.3 and 0.45. Elastic simulations corresponding to P_0 and P_∞ were run for each value of Poisson's ratio. Each of these elastic simulations took approximately 55 s.

5.4 Results

5.4.2 Non-dimensional master-curves

The full master-curve database is shown in figure 5.6a. The ramp-hold master-curves begin at a point that corresponds to the end of the ramp phase and then converge to the step displacement master-curve within approximately half-an-order of magnitude of t^* ; substantial relaxation is observed at the end of the ramp phase, which, perhaps counter-intuitively, proceeds more quickly than if a step displacement had been applied. Curves with very fast ramp times approximate the step-loading solution of Hu et al. (2011) and have a sigmoidal shape (figure 5.7). The rate of relaxation (on a log scale) changes little for $t^* < 0.1$ and then the rate of relaxation rapidly increases until approximately $t^* = 1$. The rate of relaxation then decreases once more until the material reaches the equilibrium drained state.

Curves with a finite non-dimensional ramp time exhibit substantial relaxation ($\geq 10\%$) during the ramp phase for t_R^* greater than 0.2; the non-dimensional ramp response of the material is shown as a dashed red line in figure 5.6a (bearing in mind that the material does not move along this curve as the ramp time progresses, but instead remains at a fixed point throughout, which is defined by the ramp-rate). The two curves (ramp and step) differ by less than $P^* = 0.03$ for $t^* < 0.1$ and $t^* > 10$. In the region of $0.1 \leq t^* \leq 10$ the ramp curve relaxes more slowly than that of the step curve, as would be expected, and the two curves differ by a maximum of approximately $P^* = 0.2$ near $t^* = 1$.

As predicted, the drained Poisson's ratio had little effect on the shape of the non-dimensional master-curves (figure 5.6b). Small discrepancies are visible between the curves, but these are most likely model artefacts, rather than real physical phenomena: the discrepancies are still visible at $t^* = 0.01 \approx 0$ where the material mostly acts as an undrained elastic material ($\nu = 0.5$) and the non-dimensional load should be unaffected by the drained Poisson's ratio.

The master-curves generated here in the limit of a very short ramp time show good agreement with Hu et al. (2011), especially in the region where the bulk of relaxation occurs ($0.1 \leq t^* \leq 1$; figure 5.7). For very small non-dimensional times, the master-curves produced here appear to underestimate the non-dimensional

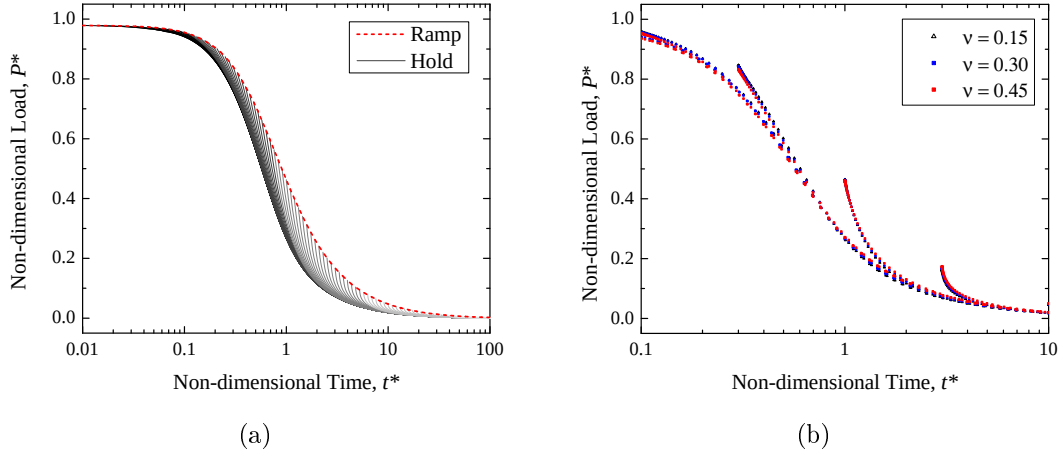


Figure 5.6: (a) The full master-curve database for non-dimensional ramp times ranging from 10^{-2} to 10^2 . The dashed red-line depicts the amount of relaxation that occurs during the ramp for each non-dimensional ramp time, t_R^* . (b) A series of master-curves with varying drained Poisson's ratios.

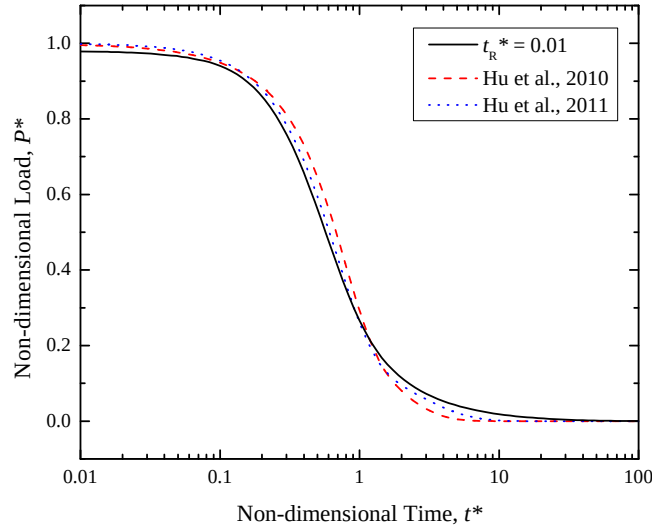


Figure 5.7: Comparison of the master-curve generated in this work for a very short ramp time ($t_R^* = 0.01$), with the step displacement solutions of Hu et al. (2010) and Hu et al. (2011).

5.5 Analysis and curve fitting

load, when compared to Hu et al. (2011). Furthermore, it appears that non-dimensional load of the master-curves tends to a value less than 1 for $t^* = 0$. This could stem from the difficulties in modelling very short ramp times due to the large changes in pressure with time. Furthermore, the elements used in the poroelastic FE model were not hybrid elements, which are necessary to model incompressible materials, although preliminary simulations found little difference between master-curves where hybrid elements were used and those where they weren't. Alternatively, Hu et al. (2011) does not report the range where they curve fit their results and it may be that their curve fit may not capture the very early time behaviour of the ramp well. Similar explanations are plausible for the differences observed between the later time behaviour of both models. Regardless, the agreement between the separate modelling approaches is good for the majority of time where relaxation occurs. The expression of Hu et al. (2010) demonstrates less agreement, although this is not surprising as the authors themselves admitted that Hu et al. (2011) provided a better fit to the data.

5.5 Analysis and curve fitting

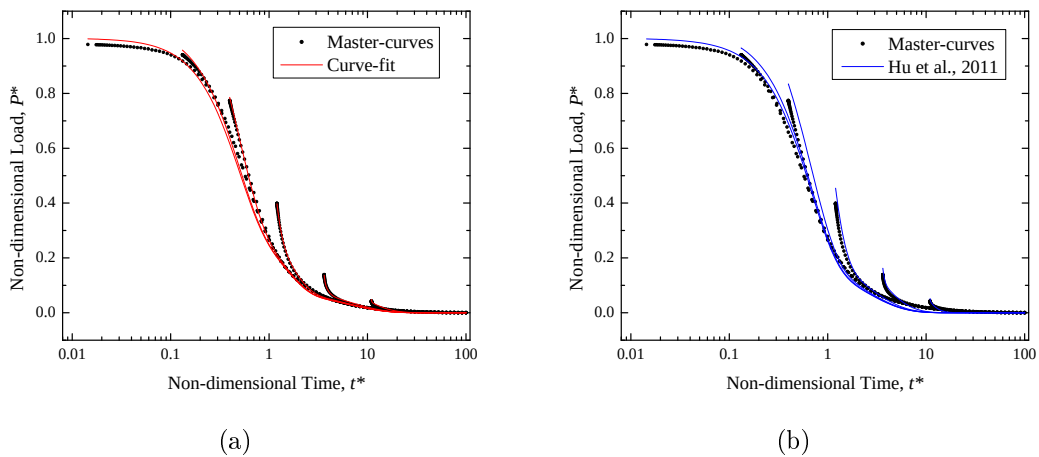


Figure 5.8: A series of master-curves generated from the FE model plotted with (a) a curve fit of a series of stretched exponential with a ramp correction factor, (b) the Hu et al. (2011) solution modified with a ramp correction factor.

While the methods of Galli & Oyen (2009) (for load-controlled indentation with a finite ramp) and Hu et al. (2011) (for displacement-controlled indentation of a finite thickness layer) are relatively simple and fast to implement once initial master-curve databases have been generated, the methods still require the user to generate their own set of master-curves. This is computationally expensive and requires significant effort to set up the initial model. It is desirable to present the results from the master-curves as a simple mathematical expression, which can then be fitted to experimental data.

The solutions for confined compression (both load and displacement-controlled) consist of infinite series of exponential decay terms, and it is possible that the processes here could be similarly described. Alternatively, a stretched exponential might be a useful function. Hu et al. (2010) fitted the results of FE simulations for varying indenter shapes with curves of the form $g(t^*) = A_1 \exp(-t^{*\beta_1}/\tau_1) + A_2 \exp(-t^{*\beta_2}/\tau_2)$, where $\beta_1 = 1$, $\beta_2 = 2$ and A_i and τ_i are constants determined during the curve fit. Hu et al. (2011) similarly fitted the numerical results of finite-depth simulations with a series of stretched exponentials, although also allowed β_1 and β_2 to float and as a result achieved better quality fits. While these expressions were chosen for convenience rather than for a physical reason, it is not surprising that they could describe the behaviour; stretched exponentials are able to capture relaxation that occurs over a larger spread of times than single exponentials (Lakes, 2009). Processes that appear as stretched exponentials can result from processes where there is a superposition of exponential decay functions.

When considering the indentation creep of a viscoelastic material, Oyen (2005a) proposed that a ramp correction factor could be used to modify the step-loading solution to take account of a finite ramp rate. As discussed in section 2.4.1, the ramp correction factor can be analytically derived for viscoelastic load-controlled indentation where the creep function is a series of exponentials, and takes the form $RCF_i = [(\tau_i/t_R)(\exp(t_R/\tau_i) - 1)]$. In displacement control, the integrals cannot be solved analytically, but it has been shown that the same ramp correction factor provides a good approximation (Mattice et al., 2006; Qiang et al., 2011).

As the solutions for poroelasticity often include series of exponentials, it is possible that a ramp correction factor would also be applicable in these instances.

5.5 Analysis and curve fitting

Galli et al. (2009) extended the step load solution for confined compression to take account of the finite ramp time. This solution can be rearranged and defined in terms of a step load solution and a ramp correction factor, such that:

$$h^*(t^*) = 1 - \sum_{n=1,3,\dots}^{\infty} \frac{8}{n^2\pi^2} [RCF_n \exp(-n^2\pi^2 t^{*2})], \quad t^* \geq t_R^*. \quad (5.12)$$

Here, the ramp correction factor takes the more general form,

$$RCF_i = \left[\frac{\tau_i}{t_R^{*\beta_i}} \left(\exp\left(t_R^{*\beta_i}/\tau_i\right) - 1 \right) \right], \quad (5.13)$$

where $\tau_i = \frac{1}{i^2\pi^2}$ and $\beta_i = 2$, which accounts for the exponent on the non-dimensional time. Similarly, the ramp load solution for unconfined compression (Cohen et al. (1998), 3.11) can also be rewritten in terms of a ramp correction factor.

It is plausible that a series of stretched exponentials with a ramp correction factor of the form shown in equation 5.13 may fit the data well and offer a concise description of the master-curve database, i.e,

$$P^* = \sum_{i=0} RCF_i A_i \exp\left(\frac{-t^{*\beta_i}}{\tau_i}\right). \quad (5.14)$$

5.5.1 Curve fitting method

Equation 5.14 was fit to a set of 52 master-curves with $0.01 \leq t_R^* \leq 5$. Master-curves with non-dimensional ramp times greater than 5 were not included in the set, as over 90% of relaxation had occurred during the ramp phase. In addition, equation 5.14 was fit solely to a near-step displacement master-curve ($t_R^* = 0.014$). The curve fits were performed in MATLAB using the nonlinear least-squares optimisation function (lsqnonlin) in the optimisation toolbox. Where 52 master-curves were fit simultaneously, the error was summed over the full set and passed to the optimisation function.

Fits with two, three and four stretched exponentials were performed to determine A_i , β_i and τ_i . In all cases it was enforced that A_i must sum to 1, so that the non-dimensional load at $t^* = 0$ was equal to 1. Hence, there was one less parameter to fit than three times the number of stretched exponentials. Initial guesses for A_i were set to $1/n$ where n was the number of stretched exponentials. Initial guesses for β_i were set to 1, while τ_i was logarithmically spaced between 0.1 and n . If the optimisation function did not converge using the initial guesses, the optimisation function was run using a reduced set of master-curves with $0.1 \leq t_R^* \leq 1$; the results from this optimisation were then used as initial parameters for the optimisation loop using the full set of 52 master-curves. Parameters were also bounded; A_i was bounded between 0 and 1, while β_i and τ_i were bounded between 0 and infinity.

5.5.2 Ramp correction factor expressions

A series of three stretched exponentials with ramp corrections (equation 5.14; hereafter referred to as the Ramp RCF expression) fit the master-curves generated from the FE model well (figure 5.8a). The parameters for the Ramp RCF expression are displayed in table 5.3a. When only two exponentials were used, a reasonable fit that matched the general shape of the master-curves was still achieved, but the sum of squared errors was much greater than when three exponentials were used (15.1 vs 4.3). On the other hand, increasing the number of stretched exponentials provided a negligible improvement over three; the sum of squared errors only decreased from 4.3 to 3.6. Furthermore, spurious oscillations were observed when four parameters were used for large non-dimensional times.

Two stretched exponentials fit to a single master-curve with a very short non-dimensional ramp time ($t_R^*=0.014$, table 5.3b; hereafter referred to as the Step RCF expression) also provided a reasonable description of master-curves from $0.01 \leq t_R^* \leq 5$. The sum of squared errors between the Step RCF expression and the set of master-curves was larger than for curves fit to the whole master-curve database (505 vs 15.1). However, this was primarily dominated by errors occurring in the six curves where $t_R^* > 3$. Similarly, the ramp correction factor was also applied to the stretched exponential solution determined by Hu et al.

5.6 Validation

i	A_i	β_i	τ_i	i	A_i	β_i	τ_i
1	0.6710	1.6378	0.2897	1	0.5623	1.8348	0.4091
2	0.2454	1.8087	2.0215	2	0.4377	0.7738	1.3234
3	0.0836	1.1615	8.6670				

(a) Ramp RCF
(b) Step RCF

Table 5.3: (a) Parameters determined from fitting three stretched exponentials with ramp correction factors (equation 5.14) to master-curves generated from the FE model with $0.01 \leq t_R^* \leq 5$. (b) Parameters determined from fitting two stretched exponentials with ramp correction factors to a master-curve generated from the FE model with $t_R^* = 0.014$.

(2011) and plotted against the same set of master-curves (figure 5.8b). The fit was in fact better than that achieved with the Step RCF expression (the sum of squared errors was 122.2 as opposed to 505.0). Hence, even without performing a new fit, it appears that the ramp correction factor can be used to account for a finite ramp time, when the non-dimensional relaxation is modelled with stretched exponentials.

5.6 Validation

In the previous sections, an expression describing the displacement-controlled ramp-hold indentation of a poroelastic material was developed. This expression can be fitted to experimentally observed load-relaxation curves to obtain the poroelastic properties of a material. The method offers an improvement in accuracy over the step displacement expression and is substantially faster than inverse FE methods. In order to determine how accurate this method is at identifying poroelastic properties, the equation was fit to load-relaxation data from forward FE models and experimental load-relaxation data of polyacrylamide hydrogels and cartilage.

5.6.1 Validation with forward finite element models

FE simulations

Two different cases of artificial data were used to validate the approach. Initially, ramp-corrected expressions for poroelastic indentation were fit to load-time curves from nine sets of simulations, run previously for different non-dimensional times with three different Poisson's ratios (section 5.4.1), as well as the full master-curve database. Prior to being fit, Gaussian noise with a maximum amplitude of 0.5% of the current load value was added to the dataset using MATLAB. Furthermore, in order to more closely mimic real experiments, the ramp-corrected equations were only fitted to a fraction of the generated hold data. The simulated hold-time was determined as a multiple of the non-dimensional ramp time (e.g. $t_H^* = 100t_R^*$).

	Contact Radius, a	Sphere Radius, R	Depth, h
AFM Indenter	9.49 μm	151 μm	598 nm
Nanoindenter	26.8 μm	425 μm	1.7 μm
Microindenter	1.19 mm	18.8 mm	75 μm

(a)

	Ramp Time, t_R	Hold Time, t_H
AFM Indenter	0.08 s	30 s
Nanoindenter	15 s	60 s
Microindenter	10 s	300 s

(b)

	Shear Modulus, G	Poisson's Ratio, ν	Permeability, κ
Bone	430 MPa	0.35	$8.00 \times 10^{-22} \text{ m}^4 (\text{Ns})^{-1}$
Cartilage	236 kPa	0.15	$6.60 \times 10^{-17} \text{ m}^4 (\text{Ns})^{-1}$
Agar	110 kPa	-0.92	$2.16 \times 10^{-13} \text{ m}^4 (\text{Ns})^{-1}$
Gelatin	30 kPa	0.39	$3.87 \times 10^{-14} \text{ m}^4 (\text{Ns})^{-1}$

(c)

Table 5.4: (a) Geometry (b) time points and (c) material parameters used for forward FE models of poroelastic relaxation by three different materials using three different indenter modes. The contact radii, experimental times and material parameters are representative of what has been reported in chapter 3 and in the literature.

In addition, data were generated using the Abaqus FE model described in

5.6 Validation

section 5.3 for hypothetical experiments on four different materials: 3% agar, 30% gelatin, cartilage and bone. For each material, contact radii and ramp times corresponding to microindentation, nanoindentation and AFM indentation, were simulated. In each case, the element sizes of the FE were scaled by the contact radius, so that the number of elements in contact with the indenter was constant. Furthermore the indenter displacement and radius were scaled by the contact radius, such that the indenter strain was a constant value (0.013) and the small strain assumption was not exceeded.

Parameter identification methods

Four different expressions were fitted to the data to identify poroelastic parameters to determine the robustness of each approach: the Hu et al. 2011 expression for step displacement indentation; the same expression multiplied by a ramp correction factor; an expression obtained from only fitting a near-step solution with $t_R^* = 0.014$ (table 5.3b; Step RCF) and an expression generated by fitting the 52 master-curves (table 5.3a; Ramp RCF).

The fitting process was carried using the nonlinear least squares optimisation function in MATLAB, following a similar method to that in chapter 3. Only the hold portion of the simulation was used for parameter identification. Initial guesses for G and ν were calculated using the Hertz equation, assuming that the load at the beginning and end of the hold represented the undrained and drained elastic states of the material, respectively. An initial κ guess of $10^{-14} \text{ m}^4 (\text{Ns})^{-1}$ was used for the optimisation, which is representative of the range of values found in hydrogels and soft-tissues. This value is also the value used in the FE simulations of the master-curve (section 5.3.2), although the effect of varying this guess from 10^{-20} to $10^{-10} \text{ m}^4 (\text{Ns})^{-1}$ was also investigated for $t_R^* = 1$. The values were also bounded such that $-1 \leq \nu \leq 0.5$, and $G > 0$. The permeability was bounded such that $\kappa \leq |10^{10} \kappa_{\text{guess}}|$. This very loose bound prevented occasional spurious identifications of permeability several tens of orders of magnitude away from the initial guess value.

In the optimisation loop, non-dimensional load was determined using equation 5.14 at non-dimensional times-points calculated at the forward FE time-points

using the current estimated values of G , ν and κ . The load was then calculated from the non-dimensional load using the Hay & Wolff (2001) equation for elastic indentation (equation 5.5), rather than the Hertz equation, in order to account for any small changes in load arising from non-zero strains. The equation is simple to use in displacement control and does not require any numerical iterations (as is the case for load control). The mean-squared error between the non-dimensional forward FE data and calculated load was then used to update the material parameters in the optimisation loop.

It is important to note that the non-dimensional relaxation expression was not calculated in the form shown in equation 5.14; instead the exponential terms in the ramp correction factor and step solution were multiplied together and their combined exponent was calculated first:

$$P^* = \sum_{i=0} \frac{\tau_i A_i}{t_R^{*\beta_i}} \left[\exp \left(\frac{t_R^{*\beta_i} - t^{*\beta_i}}{\tau_i} \right) - \exp \left(\frac{-t^{*\beta_i}}{\tau_i} \right) \right]. \quad (5.15)$$

Using this equivalent expression avoided floating-point errors (and infinite values) occurring during the calculation of the ramp correction factor for large values of $t_R^{*\beta} / \tau$.

Master-curve parameter identification

For long non-dimensional ramp times ($t_R^* \geq 3$), the error in identified poroelastic properties can be substantial, regardless of the non-dimensional hold time or the expression used to fit the forward FE simulations (figure 5.9). The identified permeabilities for all expressions differed by more than an order of magnitude from the simulation parameters when $t_R^* = 10$ and $t_H^* = 100$, while the shear moduli and Poisson's ratios differed by 20% and 50% from their respective simulation parameters. This is perhaps not surprising as over 85% of relaxation had occurred by $t_R^* = 3$ and the observable relaxation during the hold-period of the indentation simulation was limited.

Similarly, when the non-dimensional ramp time is particularly short ($t_R^* \leq 0.03$) and the hold time is not exceptionally long, the error in identified poroelastic properties can also be substantial (figure 5.9). Reliable identifications of Poisson's

5.6 Validation

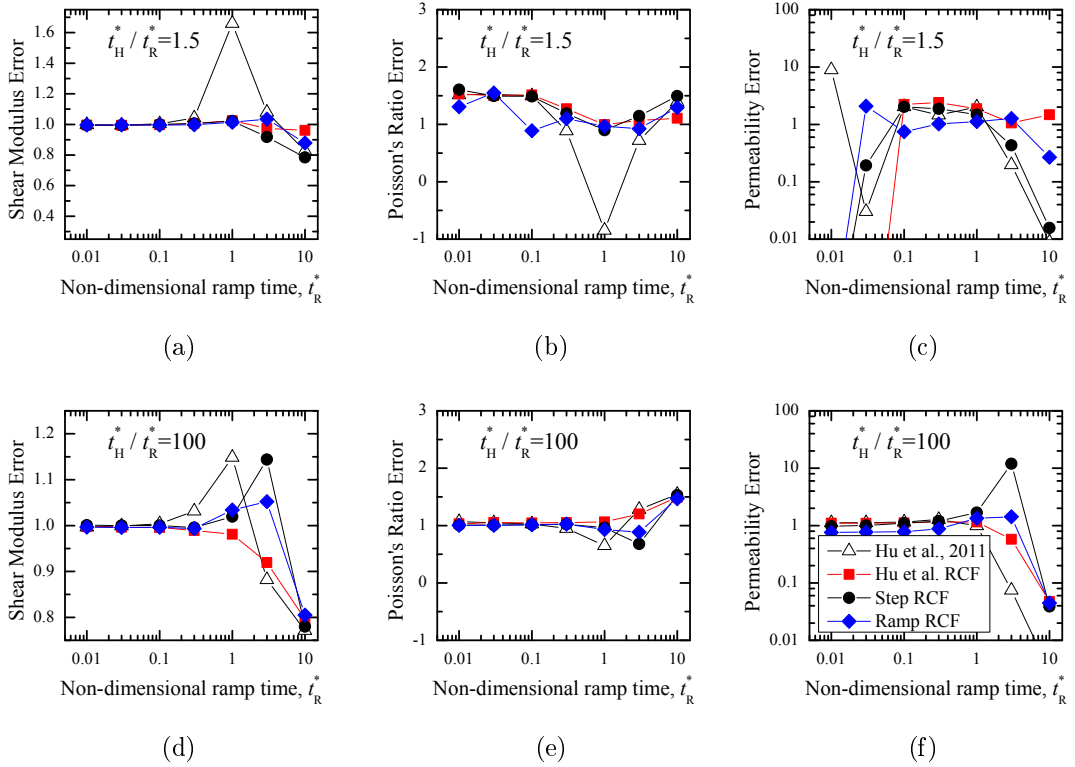


Figure 5.9: Error in identified poroelastic properties (e.g. G_{id}/G) obtained by fitting load-relaxation data from forward FE simulations with four separate expressions for non-dimensional load-time: the Hu et al., 2011 expression for step displacement indentation, the same expression modified with the ramp correction factor presented here, the Ramp RCF expression and the Step RCF expression. The error is plotted against the non-dimensional ramp time of each solution for two different hold time multiples (a-c: 1.5, d-f: 100).

ratio within 50% of the simulation parameter were not achieved when $t_R^* = 0.01$ and $t_H^* \leq 0.3$, regardless of the fitting expression. While this is a short non-dimensional hold time, it is a long experimental hold time, equivalent to 900 times the real ramp time as $t \propto t^{*2}$. The shear modulus and permeability identifications were accurate for moderate hold times ($t_H^* \geq 0.03$), but when $t_R^* = 0.01$, $t_H^* = 0.015$, the permeability differed by at least an order of magnitude for each solution. These errors arise because prior to $t^* \leq 0.1$ only a maximum of 5% of the total relaxation occurs; the behaviour observed during the hold time of such a test largely reflects the undrained elastic response of the material.

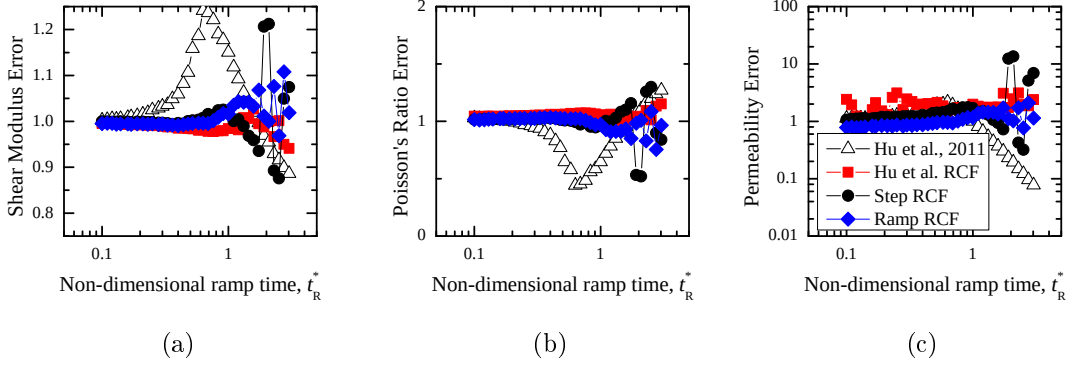


Figure 5.10: Error in identified poroelastic properties over the range of $0.1 \leq t_R^* \leq 3$ for simulations with a long non-dimensional hold time ($t_H^* = 100t_R^*$), determined for four expressions describing non-dimensional load-relaxation.

For moderate ramp times ($0.1 \leq t_R^* \leq 3$), expressions with a ramp correction factor offer an improvement in accuracy over the Hu et al. 2011 expression for step displacement indentation (figure 5.10). Even with an exceptionally long hold time ($t_H^* = 100t_R^*$, equivalent to $t_H = 10000t_R$), the shear modulus and Poisson's ratio identified with the Hu et al. 2011 expression differed by a maximum of 24% and 56% from their respective simulation parameters at $t_R^* = 0.63$. When a short non-dimensional hold time was used ($t_H^* = 1.5t_R^*$), the identified shear modulus differed by a maximum of 75%, while the Poisson's ratio differed by 193% (the identified value was negative). Conversely, shear modulus and Poisson's ratio values obtained using either of the three expressions with the ramp correction factor were within 15% and 35% of their respective parameters for simulations

5.6 Validation

with $0.2 \leq t_R^* \leq 3$ and $t_H^* = 1.5t_R^*$, although sporadic errors identifying Poisson's ratios occurred in the region of $0.1 \leq t_R^* \leq 0.2$ (data not shown).

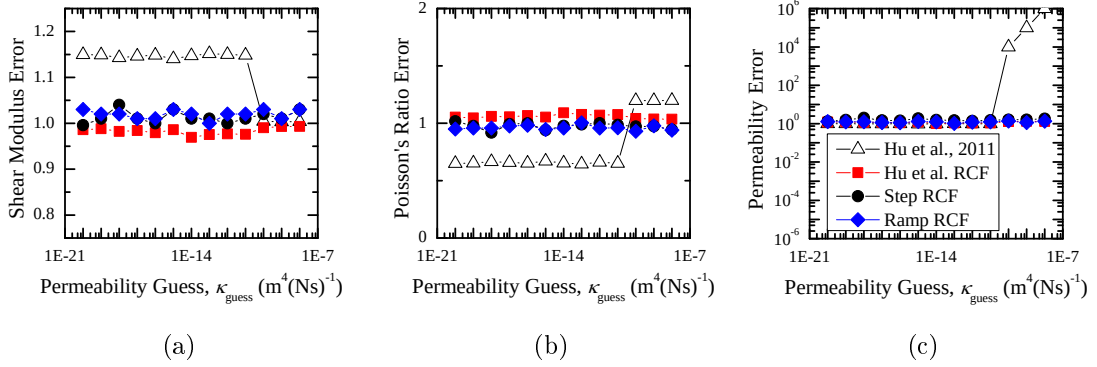


Figure 5.11: The effect of the initial permeability guess on the error in identified poroelastic properties for forward FE simulations with $t_R^* = 1$, $t_H^* = 100$.

The Hu et al., 2011 expression was also found to be more sensitive to the initial guess for permeability (figure 5.11). When $t_R^* = 1$ and $t_H^* = 100$, fits using the Hu et al., 2011 expression did not converge to a realistic solution for initial permeability guesses greater or equal to $10^{-10} \text{ m}^4 (\text{Ns})^{-1}$. In contrast, expressions with a ramp correction factor were more robust to the initial permeability guesses and the identified permeabilities were within 0.1 order of magnitude regardless of the initial guess (which spanned 12 orders of magnitude).

All three expressions with the ramp correction factor provided improved identifications of material properties, for moderate ramp times. Even the Hu et al., 2011 expression, which was obtained with an independent FE model with different underlying assumptions, provided comparable results when modified with the ramp correction factor, indicating that the ramp correction factor provides a good estimate of the effects of a finite ramp. Still, small differences were noted between the results obtained with each expression. The Ramp RCF expression (table 5.3a) consistently underestimated the permeability of the material by between 10% to 25% for $t_R^* \leq 0.40$. However, this expression provided more reliable identifications for $t_R^* > 1$ than the Step RCF expression (table 5.3b). The Step RCF (table 5.3b) provided the most accurate identifications for shear modulus and Poisson's ratio for $t_R^* < 1$ and $t_H^* = 100t_R^*$, with errors of less than 2% (shear modulus)

and 4% (Poisson's ratio) respectively, although it was more susceptible to small errors in permeability than the Hu et al. 2011 expression modified with the ramp correction factor. This expression also provided especially good identifications with maximum errors of 2% (shear modulus) and 6% (Poisson's ratio). Given the small differences between each solution, a single solution cannot be recommended as being superior to the others. However, for simplicity, in the remainder of this work the expression, Ramp RCF (table 5.3a), will be used for identifications and comparisons.

FE experiments parameter identification

The Ramp RCF expression and Hu et al. (2011) expression for a step displacement were fit to forward FE simulations of experiments corresponding to micro, nano and AFM indentation. Parameters identified from forward FE experiments are shown in figure 5.12. While in the majority of cases the Hu et al. (2011) expression offers comparable identification accuracy to the Ramp RCF expression, large differences were noted for certain material-testing mode combinations. The identified permeability differed by over an order of magnitude for gelatin indented with AFM and the identified shear modulus and Poisson's ratio differed by over 50% and 200% respectively for cartilage indented with nanoindentation. These material-testing mode combinations generally corresponded to non-dimensional ramp times, $t_R^* > 0.1$, although the Ramp RCF expression also provided more reliable identifications for extremely short ramp times, where the hold time was also reasonably short (cartilage microindentation).

Substantial errors in identified permeability for both equations occurred for simulations of agar indented with AFM and nanoindentation; the non-dimensional ramp time was too long and little poroelastic relaxation was observed. Similarly, the permeability of bone was not correctly determined for microindentation as the non-dimensional hold time was too short and very little poroelastic relaxation occurred.

Interestingly, it was not possible to predict whether material parameters were likely to be erroneous from the identified material parameters; the non-dimensional ramp time calculated from fitting the Ramp RCF equation to simu-

5.6 Validation

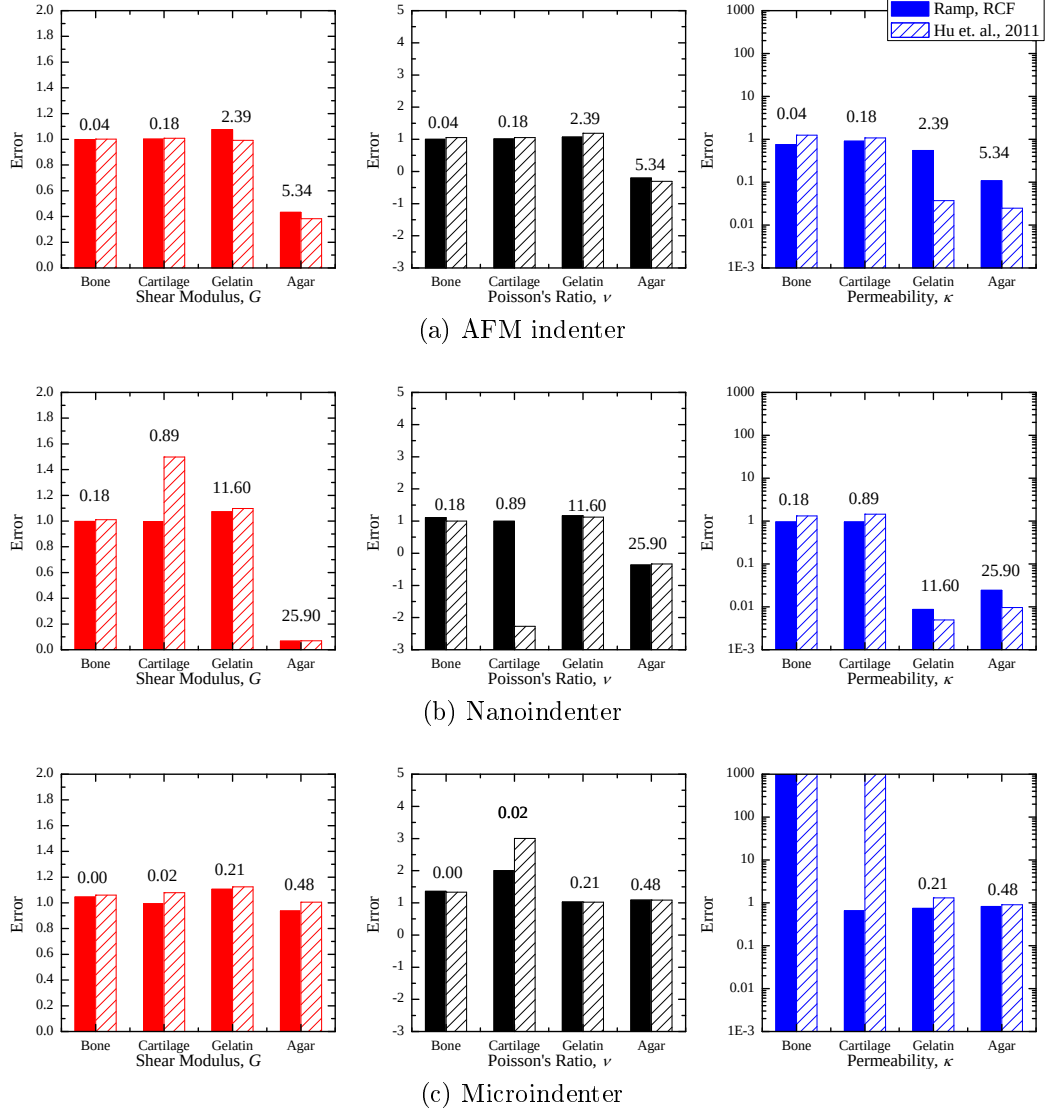


Figure 5.12: Error (e.g. G_{id}/G) in identified poroelastic parameters from forward FE models with properties described in table 5.4. The parameters were obtained by fitting load-relaxation data with a ramp-corrected expression (Ramp RCF, table 5.3a) and the Hu et al., 2011 expression for a step displacement. The non-dimensional ramp time of the FE model used to generate the data is written above each bar.

lated gelatin nanoindentation was equal to 1.4, which would likely result in reasonable identification of material parameters. However, the actual non-dimensional ramp time was equal to 11.60, which would not. Care must be taken when identifying poroelastic parameters using a single indent.

It should be stated that the FE validation performed here has only investigated how the ramp-corrected master-curve expression performs when the full load-displacement curve is not available and when there is scatter in the load-displacement curves. The effect of likely experimental deviations from the theoretical situation has not been investigated, including finite deformation, friction and non-ideal boundary conditions. Nor has the effect of non-ideal material behaviour (such as strain-dependent permeability, viscoelasticity and nonlinear elasticity) been investigated. Hence caution should be applied when interpreting experimental results, as is demonstrated in the following section.

5.6.2 Experimental validation

The ramp correction factor approach was used to identify the poroelastic properties of a hydrogel (polyacrylamide) and soft-tissue (cartilage). The poroelastic identification presented here demonstrates the power of the approach as a rapid tool for accurate poroelastic identification. It also indicates that researchers must be cautious when identifying poroelastic properties obtained using indentation at single length-scales.

Polyacrylamide

Experimental load-relaxation data obtained from microindentation and nanoindentation of polyacrylamide were provided by Michelle Oyen and Tamaryn Shean and is fully described in Galli et al. (2009). Briefly, 5 mm thick, 20% polyacrylamide gels were fabricated following established protocols. Microindentation was carried out on an Instron 5544 universal testing frame with a 5 N load cell. A stainless steel spherical tip with a radius (R) of 1.5 mm was brought into contact with the test sample, followed by a 10 s ramp phase (t_R) to a peak displacement, $h_{\max}$ of between 0.2 - 1 mm. The indenter was held on the sample for 300 s.

5.6 Validation

Nanoindentation was carried out using a Hysitron (Minneapolis, MN, USA) TI-700 Nanoindenter with a $400.83 \mu\text{m}$ radius spherical tip. Tests were performed with a ramp time, t_R of 5 s, hold time, t_H of 20 s and peak displacement, h_{max} of between $2.5\text{-}3.5 \mu\text{m}$.

The experimental data were fit with both the Ramp RCF expression (table 5.3a) and the Hu et al., 2011 expression for a step displacement, in order to identify poroelastic parameters. Poroelastic parameters identified using the Ramp RCF expression were comparable at both the micro and nanoscale (table 5.5). This demonstrates the promise of the ramp correction factor approach, given that the calculated non-dimensional ramp times differed by an order of magnitude (microindentation: $t_R^* = 0.08$; nanoindentation $t_R^* = 1.23$). The small differences between shear modulus and Poisson's ratio potentially stem from finite-depth effects as the largest microindentation displacements were 20% of the sample depth.

In contrast, the differences in the parameters identified by the Hu et al. 2011 expression using micro and nanoindentation, while still small, were greater than what could likely be accounted for by experimental variation. The Poisson's ratio identified using nanoindentation with the Hu et al. 2011 expression differed by 0.25 from the corresponding microindentation value and by 0.13 from the corresponding nanoindentation value identified with the Ramp RCF expression. The ramp correction approach is a simple and fast method of obtaining consistent parameter identifications across different ramp times and scales.

Fitting Expression	Scale	G (kpa)	ν	κ ($\text{m}^4 (\text{Ns})^{-1}$)
Ramp RCF	Micro	142 ± 19.3	$0.43 \pm .04$	$5.51 \pm 2.16 \times 10^{-16}$
Ramp RCF	Nano	161 ± 17.8	$0.32 \pm .06$	$6.10 \pm 7.40 \times 10^{-16}$
Hu et al., 2011	Micro	142 ± 19.8	$0.44 \pm .04$	$8.94 \pm 3.56 \times 10^{-16}$
Hu et al., 2011	Nano	186 ± 13.3	$0.19 \pm .05$	$5.00 \pm 1.10 \times 10^{-16}$

Table 5.5: Polyacrylamide poroelastic parameters identified by fitting experimental polyacrylamide data with the Ramp RCF expression (table 5.3a) and the Hu et al., 2011 expression for a step displacement. The indentation data were obtained at two scales on the same gels, using a microindenter and nanoindenter.

Articular cartilage

Experimental indentation of articular cartilage was performed by the author. Articular cartilage has many similar structural features to the intervertebral disc and it exhibits many comparable mechanical behaviours including poroelastic and viscoelastic time-dependent behaviour and nonlinear stress strain behaviour. It has been more thoroughly characterised at a range of scales than the intervertebral disc and hence is more suitable as a validation material.

Three calf patellae were obtained and stored at -80°C . The patellae were defrosted and dissected using a scalpel. Strips of cartilage and bone approximately 3 mm thick were cut from the surface of the patellae and wrapped in saline-soaked gauze. Strips were indented within 36 hours of preparation. Prior to testing, the cartilage samples were soaked in PBS solution at room temperature for a minimum of half an hour.

Nanoindentation tests were performed on the samples using a Hysitron TI-700 Nanoindenter with a $238.86\text{ }\mu\text{m}$ diameter spherical fluid cell tip. Cartilage samples were mounted on petri dishes using cyanoacrylate and submerged in PBS solution such that there was a distance of approximately 2 mm between the sample and liquid surfaces. Indents were spaced a minimum of $10\text{ }\mu\text{m}$ apart.

Displacement-controlled tests were performed by first bringing the indenter tip to approximately $1\text{ }\mu\text{m}$ above the surface as determined by preliminary indents at a nearby location. From this initial height, a $4.5\text{ }\mu\text{m}$ indent into the surface at a rate of $0.225\text{ }\mu\text{m s}^{-1}$ was performed, resulting in an actual rise time of $15.04 \pm 4.55\text{ s}$ and peak depth of $3.40 \pm 1.03\text{ }\mu\text{m}$. The indent was then held for 60 seconds. Surface contact was determined using a custom written MATLAB script that analysed the change in the derivative of the load-time curve.

The cartilage load-relaxation curves were fitted with both the Ramp RCF expression and the Hu et al., 2011 expression to identify poroelastic material properties (figure 5.13a). Poroelastic parameters identified with the Ramp RCF expression (5.6) agreed well with poroelastic parameters identified using a master-curve database in load-controlled nanoindentation on the same samples (Oyen et al., 2012), as well as poroelastic parameters identified using inverse FE analysis on independent nanoindentation experiments performed on deep layers of cartilage

5.6 Validation

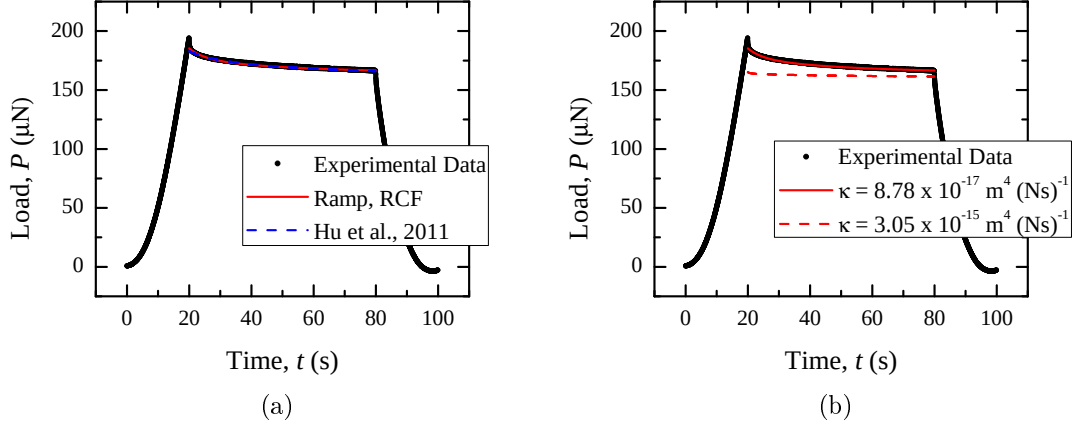


Figure 5.13: Experimental load-relaxation curves from nanoindentation of cartilage, compared with (a) fits obtained with Ramp RCF equation and the Hu et al. (2011) equation for step displacement indentation, (b) a fit obtained with the Ramp RCF equations ($\kappa = 8.78 \times 10^{-17} \text{ m}^4 (\text{Ns})^{-1}$), and a prediction of the poroelastic response of a material with the same shear modulus and Poisson's ratio, but a permeability representative of 'macroscale' cartilage ($3.05 \times 10^{-15} \text{ m}^4 (\text{Ns})^{-1}$).

(Miller & Morgan, 2010). In contrast, identification with the Hu et al., 2011 expression lead to a large overestimate of shear modulus (145%) and permeability (186%) and a large underestimate of Poisson's ratio. Direct measurements of drained Poisson's ratio of cartilage have been performed by Jurvelin et al. (1997) who reported a value of 0.185, similar to that identified here with the Ramp RCF expression. The calculated non-dimensional ramp time (from parameters identified using the Ramp RCF expression) is 0.92, so it not surprising that discrepancies arise between the Ramp RCF expression and the Hu et al., 2011 expression.

While the methods developed here identify poroelastic parameters that are consistent with recent nanoindentation work on cartilage (Miller & Morgan, 2010; Oyen et al., 2012), it must be pointed out that the permeabilities reported by these studies are at least an order of magnitude lower than permeability measurements using typical macroscale experiments such as confined compression and direct permeability measurements (Mow et al., 2005). Miller & Morgan (2010)

determined the permeability of cartilage using both confined compression and nanoindentation (with inverse FE methods) and determined a confined compression permeability of $3.05 \times 10^{-15} \text{ m}^4 (\text{Ns})^{-1}$ and a nanoindentation permeability of $6.6 \times 10^{-17} \text{ m}^4 (\text{Ns})^{-1}$. It is possible that the permeability of cartilage exhibits a structural length-scale dependence as these authors suggest, and as is the case for bone (Oyen et al., 2012). It is also plausible that there is a systematic error in the poroelastic characterisation of cartilage using nanoindentation.

The Ramp RCF equation can be used to rapidly predict and visualise how a cartilage-like material with macroscale properties will behave during nanoindentation. In figure 5.13b, the predictions from the identified nanoindentation poroelastic properties are plotted alongside experimental data, along with predictions for the same properties modified with a ‘macroscale’ permeability of $3.05 \times 10^{-15} \text{ m}^4 (\text{Ns})^{-1}$. It can be seen that predicted poroelastic relaxation for nanoindentation of a cartilage-like material with typical macroscale properties is negligible. The calculated non-dimensional ramp time for such a material is equal to 6.09, indicating that the majority of relaxation took place during the ramp phase. Cartilage is known to exhibit viscoelastic behaviour—which does not scale in time with contract radius—as well as poroelastic behaviour, and it is likely that this viscoelastic relaxation is what is observed and fit to during nanoindentation, resulting in a systematic discrepancy in identified poroelastic properties.

	G (kpa)	ν	κ ($\text{m}^4 (\text{Ns})^{-1}$)
Ramp RCF	204 ± 70	0.23 ± 0.10	$7.04 \pm 6.95 \times 10^{-17}$
Hu et al., 2011	296 ± 116	-0.15 ± 0.25	$1.30 \pm 0.97 \times 10^{-16}$
Miller & Morgan (2010)	319	0.16	6.6×10^{-17}
Oyen et al. (2012)	236 ± 116	0.15 ± 0.25	$6.6 \pm 4.7 \times 10^{-17}$

Table 5.6: Cartilage poroelastic parameters identified by fitting experimental cartilage nanoindentation load-relaxation data with the Ramp RCF expression (table 5.3a) and the Hu et al., 2011 expression for a step displacement. The parameters are compared with literature values obtained from nanoindentation of cartilage (Miller & Morgan, 2010; Oyen et al., 2012).

5.7 Discussion

In this work, an FE model of spherical indentation was created and used to generate a database of displacement-controlled indentation relaxation curves with varying non-dimensional ramp times. It was found that a ramp correction factor approach, analogous to viscoelastic indentation (Mattice et al., 2006), provided an excellent description of the resulting relaxation even for large non-dimensional ramp times. The ramp correction factor approach provides comparable predictions of poroelastic relaxation to forward FE models, whether it is applied with parameters generated by fitting the master-curve database produced here (i.e. Ramp RCF), or if it is applied to the description of step displacement poroelastic relaxation generated by Hu et al. (2011). The approach was used as a rapid alternative to inverse FE analysis, and its predictions were validated using forward FE models. Finally it was used to identify poroelastic parameters of polyacrylamide and cartilage, providing insight into the mechanical behaviour of these materials.

The ramp correction factor approach is appealing because of its simplicity. Unlike the master-curve approach proposed by Galli & Oyen (2009) for load-controlled poroelastic indentation, which required initially generating a large set of master-curves, the approach presented here only requires minor modification of existing analysis methods for step displacement poroelastic indentation. Even if the discrepancy between assuming a step displacement condition and finite-ramp is negligible (i.e. $t_R^* < 0.1$), the ramp correction factor approach provides comparable results to step displacement poroelastic indentation, both in terms of accuracy, speed, and ease of setup.

Furthermore, there are a number of experimental situations (including polyacrylamide and cartilage nanoindentation) where the non-dimensional ramp time is not negligible. The non-dimensional ramp time depends on the material consolidation coefficient, the experimental scale and the actual ramp time. Mitigating this effect of the ramp in the experimental design, by altering the ramp time or scale, would require prior knowledge of the material properties of the material. In the cases where the non-dimensional ramp time is non-negligible, errors from

assuming a step displacement are systematic: the identification error can be reduced by using a long hold time, but even with an exceptionally long hold time, an appreciable error can still remain.

There are also likely situations where neither the ramp correction factor approach nor the step displacement approach will provide reliable estimates of poroelastic properties. If the non-dimensional ramp time is extremely short (i.e. $t_R^* \leq 0.01$), the non-dimensional hold time is also likely to be short (even it corresponds to hours of experimental time), and the observable poroelastic relaxation will be negligible. Similarly, if the non-dimensional ramp time is particularly long ($t_R^* \geq 3$), the majority of poroelastic relaxation will have occurred during the ramp phase. In these instances, the ramp correction factor approach provides a means to rapidly predict what poroelastic relaxation would be expected at each scale, based on assumed properties, allowing for experimental data at different scales to be quickly validated and understood.

It is intriguing that complex poroelastic behaviour can be simply described with a ramp correction factor approach. A possible reason may be as follows: the Boltzmann hereditary integral principle for viscoelasticity is simply a linear relationship for the superposition of arbitrary relaxation functions. It applies regardless of the mechanism for relaxation, and therefore should apply to poroelasticity. When the hereditary integral is solved for ramp-hold loading with an exponential relaxation function, the ramp correction factor is apparent. Both poroelastic solutions for confined compression and unconfined compression consist of sums of exponentials that are functions of t^{*2} and hence t . It makes sense that the ramp correction factor provides a precise analytical description of ramp-hold behaviour in these instances.

There is no analytical solution to the Boltzmann hereditary integral for ramp-hold displacement to a material with a stretched exponential relaxation function, but the ramp correction factor provides a useful approximation of the underlying behaviour. The Boltzmann hereditary integral was numerically solved for the relaxation of a material with a stretched exponential relaxation function subjected to a peak strain of $\epsilon_{\max} = 1$ over a range of ramp times, t_R , i.e.,

5.7 Discussion

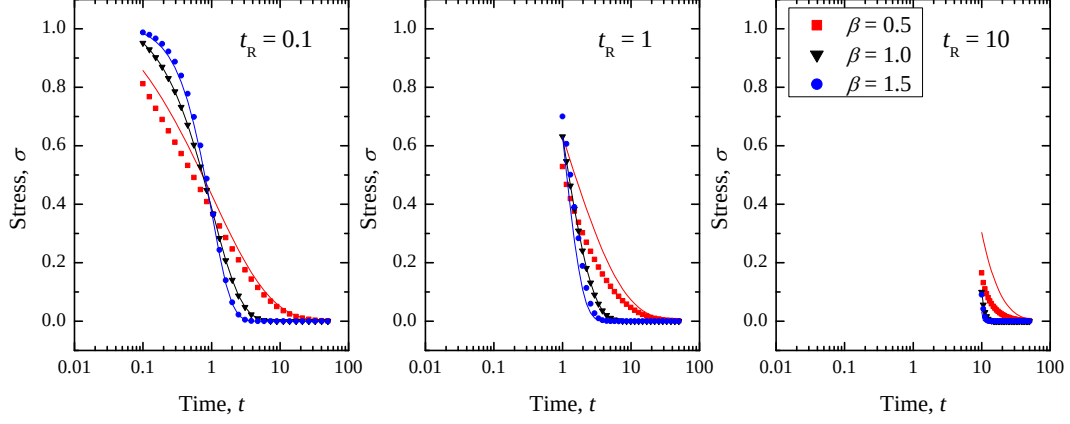


Figure 5.14: Stress-relaxation of a material with a stretched exponential relaxation function, with varying β , generated by numerically solving the Boltzmann hereditary integral (dots) and by calculating the response using a ramp correction factor approach (lines). The responses are calculated for three different ramp times.

$$\sigma(t) = \frac{\epsilon_{\max}}{t_R} \int_0^{t_R} E(t-u) du, \quad (5.16)$$

where $E(t) = \exp(-t^\beta)$. The results were compared with predictions calculated from the relaxation function multiplied by the ramp correction factor (equation 5.13) in figure 5.14. It can be seen that for $\beta < 1$, the predicted response lags behind the numerical response by a small degree, while for $\beta > 1$ it precedes the response. Regardless, the differences are small as long as β is near to 1.

Given that the ramp correction factor is a function of linear superposition and a stretched exponential function with $\beta \approx 1$, it is likely that it will generalise to more complex descriptions of poroelastic behaviour, including indentation of finite-thickness samples. Hu et al. (2011) presented a series of master-curve equations that approximate the relaxation observed for poroelastic samples of varying thickness. The equations take the form of stretched exponentials with $0.52 \leq \beta \leq 2.4$. A ramp correction factor could potentially be multiplied with these equations, to take into account finite-ramp time. This would provide a rapid method of characterising the vast majority of poroelastic materials regardless of size or scale.

5.8 Conclusions

A ramp correction factor approach that predicts the poroelastic response of a material tested with indentation, regardless of ramp time, was developed. The method can be used to both predict poroelastic relaxation and to identify poroelastic parameters. It could potentially be combined with existing master-curves for indentation of thin poroelastic materials. The approach provides a rapid alternative to inverse FE methods, allowing for hundreds of indents to be reliably characterised at multiple scales. This will lead to more robust identification of poroelastic parameters, whether for material characterisation of tissue engineered scaffolds, or for understanding the response of cells to complex mechanical behaviour. In the next chapter, it will be shown how this approach can be extended to materials that exhibit both poroelastic and viscoelastic relaxation mechanisms.

Chapter 6

Poroviscoelastic characterisation of hydrogels

The time-dependent behaviour of materials has so far been considered using either viscoelastic or poroelastic frameworks. However, when hydrogels are deformed, load-relaxation arising from the flow of fluid—poroelasticity—and from rearrangement of the polymer network—viscoelasticity—is observed concurrently. The physical mechanisms are different in that poroelastic relaxation varies with experimental length-scale while viscoelastic relaxation does not. In section 6.2, it is shown that poroviscoelastic load-relaxation is the product of the two individual responses. In section 6.3, it is demonstrated that the difference in length-scale dependence of the two mechanisms can be exploited to uniquely determine poroviscoelastic material properties from simultaneous analysis of multi-scale indentation experiments. The approach developed here is then applied to experimental indentation and unconfined compression data obtained for a 3% agar hydrogel (section 6.4). The chapter concludes with a discussion of the limitations of this approach and the opportunities it presents to better understand hydrogel physical behaviour.

6.1 Introduction

Hydrogels are increasingly being used as biomaterials and scaffolds (Strange & Oyen, 2012), substrates for cell culture (Trappmann et al., 2012) and soft flexible actuators (Zhao et al., 2011). Hydrogels consist of polymer networks swollen with fluid and they can exhibit substantial time-dependent relaxation. The relaxation results from the frictional drag of interstitial fluid through the polymer network—poroelasticity—and the intrinsic viscoelasticity of that polymer network. These effects can be understood using PVE theory, originally proposed by Biot (1956).

Mechanical characterisation of PVE materials is challenging for a number of reasons. At a minimum, characterisation requires describing the elastic (shear modulus, G_∞ and drained Poisson’s ratio, ν), poroelastic (hydraulic permeability, κ) and viscoelastic material parameters (normalised shear and bulk relaxation functions, $G(t)/G_\infty$ and $K(t)/K_\infty$). Solutions for creep and relaxation in Laplace space can be derived using the correspondence principle for problems where equivalent analytical poroelastic solutions already exist. These expressions in Laplace space can then be converted back to the time-domain and fit to experimental data using numerical methods (Hoang & Abousleiman, 2012; Mak, 1986). Inverse FE methods can also be used to identify PVE parameters, although these methods are time-consuming and difficult to implement for a large number of experiments (Kalyanam et al., 2009; Liu & Ovaert, 2011; Noailly et al., 2008; Olberding & Francis Suh, 2006). In both cases it is difficult to intuitively understand the effects of added viscoelastic behaviour to a poroelastic problem. Furthermore, there is no guarantee that unique PVE parameters can be identified from a single test, without *a priori* assuming parameter values (Kalyanam et al., 2009; Liu & Ovaert, 2011; Noailly et al., 2008; Olberding & Francis Suh, 2006).

Several authors have suggested that scaling arguments can be used to differentiate between poroelastic and viscoelastic behaviour and identify poroelastic parameters independently of viscoelastic material parameters and *vice versa* (Hu & Suo, 2012; Wang & Hong, 2012; Zhao et al., 2010). Viscoelastic relaxation results from rearrangements of the polymer network; if the experimental length-scale is much larger than the polymer mesh size, the time constant of viscoelastic relaxation will be independent of the geometry of the test (Hu et al., 2010). In

contrast, length-scale effects are inherent in the response of a poroelastic material. The rate of fluid flow through the network is related to the size of applied pressure gradient (Wang, 2000). However, the conditions under which separability occurs depend on both geometric and material parameters and are difficult to know prior to the test. A method is needed whereby the PVE parameters of a single material can be rapidly and robustly identified, using a minimum of experimental and computational time.

Here, an approach that separates the relative contributions of poroelastic and viscoelastic mechanisms of the relaxation behaviour was developed. Predictions of PVE load-relaxation, which compare well with forward FE simulations, were made from the product of the individual poroelastic and viscoelastic responses. The approach was then used to rapidly generate PVE load-relaxation curves which are fit to forward FE simulations and real experimental data on agar. The identification is simultaneously performed using indentation data across multiple scales in order to robustly identify PVE material properties.

6.2 Separable poroviscoelastic load-relaxation

6.2.1 Theory

It was postulated that viscoelastic and poroelastic contributions to time-dependent behaviour could be separated based upon their effects on the Poisson's ratio of the material. Poroelastic relaxation results in a change in compressibility of the material, which can be represented by a change in Poisson's ratio from the undrained, ν_u to the drained Poisson's ratio, ν (Wang, 2000). It was assumed that viscoelastic relaxation does not affect Poisson's ratio, which is equivalent to assuming that the normalised shear ($G(t)/G_\infty$) and bulk ($K(t)/K_\infty$) relaxation functions are equivalent (Lakes, 2009). Furthermore, it was assumed that the viscoelastic relaxation functions took the form $G(t) = G_\infty [1 + \sum g_i \exp(-t/\tau_i)]$, where g_i and τ_i are constants.

Using these assumptions, a relationship for PVE relaxation in terms of separate poroelastic and viscoelastic relaxation mechanisms was developed with analogy to an unconfined compression experiment (figure 6.1). It is shown that the

6.2 Separable poroviscoelastic load-relaxation

load-relaxation of a purely viscoelastic material can be considered as a function of an effective shear stiffness $G_{\text{eff}}(t)$, and that the load-relaxation of a purely poroelastic material can be determined as a function of a changing Poisson's ratio, $\nu_{\text{eff}}(t)$. Finally, by analogy, it is proposed that the load-relaxation of a PVE material separately depends on both the effective Poisson's ratio and shear modulus, and hence separately depends on poroelastic and viscoelastic load-relaxation.

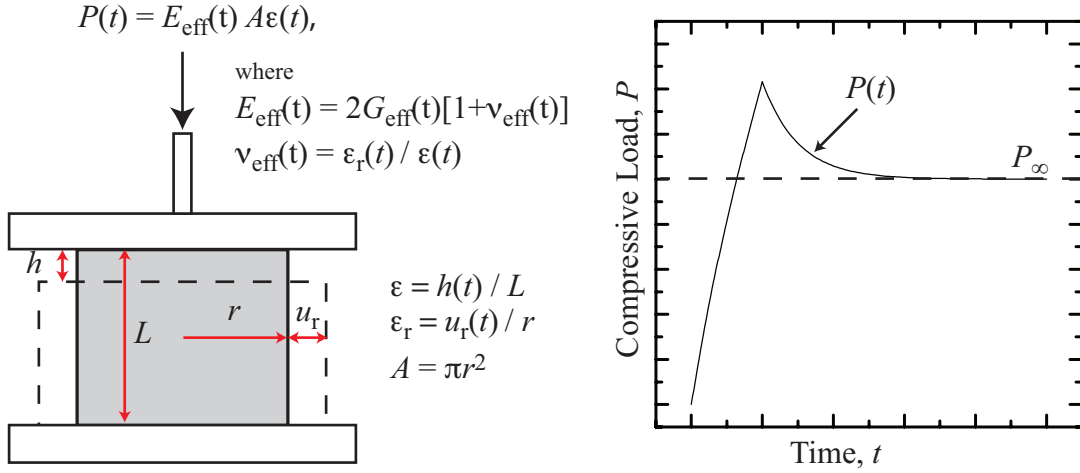


Figure 6.1: Schematic of ramp-hold displacement-controlled unconfined compression experiment, where the load-relaxation of a PVE material is expressed in terms of an effective shear stiffness, $G_{\text{eff}}(t)$, and effective Poisson's ratio, $\nu_{\text{eff}}(t)$.

Viscoelastic load-relaxation

A purely viscoelastic material with constant Poisson's ratio subjected to ramp-hold displacement-controlled unconfined compression demonstrates load-relaxation, $P_{\text{VE}}(t)$, where:

$$P_{\text{VE}}(t) = E_{\infty} A \epsilon_{\text{max}} \left[1 + \sum RCF_i g_i \exp(-t/\tau_i) \right], \quad t \geq t_R, \quad (6.1)$$

and $E_{\infty} = 2G_{\infty}(1 + \nu)$, A is the cross-sectional area of the cylinder, ϵ_{max} is the maximum applied strain and RCF is a ramp correction factor (section 2.4.1). The load at equilibrium is:

$$P_{\infty} = E_{\infty} A \epsilon_{\text{max}} = [2G_{\infty}(1 + \nu)] A \epsilon_{\text{max}}. \quad (6.2)$$

6.2 Separable poroviscoelastic load-relaxation

By analogy to an elastic material, the load at any time-point, $P_{VE}(t)$ can be expressed as a function of an effective shear stiffness, $G_{eff}(t)$. The effective shear stiffness is a function of material relaxation, $G(t)$ and the applied loading conditions (i.e. RCF), such that:

$$G_{eff}(t) = G_{\infty} \left[1 + \sum RCF_i g_i \exp(-t/\tau_i) \right]. \quad (6.3)$$

A simple relationship expressing the effective shear stiffness in terms of the current load, $P_{VE}(t)$ and equilibrium load, P_{∞} is found by dividing equation 6.1 by equation 6.2:

$$\frac{P_{VE}(t)}{P_{\infty}} = \frac{G_{eff}(t)}{G_{\infty}}. \quad (6.4)$$

Poroelastic load-relaxation

Similarly the time-dependence of a purely poroelastic material in response to a step displacement¹ can be expressed in terms of the effective Poisson's ratio, $\nu_{eff}(t)$, i.e. the current radial strain divided by the applied axial strain. The load-relaxation of a poroelastic material, $P_{PE}(t)$ is:

$$P_{PE}(t) = E_{\infty} A \epsilon_{max} \left[1 + \frac{(1-\nu)(1-2\nu)}{1+\nu} \sum_{n=1}^{\infty} \frac{\exp(-\alpha_n^2 t^{*2})}{(1-\nu)^2 \alpha_n^2 - (1-2\nu)} \right], \quad (6.5)$$

where t^* is the non-dimensional time and α_n are the roots of the equation 3.12 (Armstrong et al., 1984). The effective Poisson's ratio, $\nu_{eff}(t)$ is:

$$\nu_{eff}(t) = \left[\nu + (1-\nu)(1-2\nu) \sum_{n=1}^{\infty} \frac{\exp(-\alpha_n^2 t^{*2})}{(1-\nu)^2 \alpha_n^2 - (1-2\nu)} \right]. \quad (6.6)$$

Algebraic manipulation reveals that,

¹The step displacement poroelastic expression (Armstrong et al., 1984) is considered here instead of the ramp-hold solution (Cohen et al., 1998) as an expression for the radial displacement (and hence $\nu_{eff}(t)$) has only been derived for the step displacement case. It will be demonstrated in section 6.2.2 that the conclusions from this approach (e.g. equations 6.7 and 6.9) are equally valid for the ramp-hold displacements as for step displacements.

6.2 Separable poroviscoelastic load-relaxation

$$\frac{P_{\text{PE}}(t)}{P_{\infty}} = \frac{1 + \nu_{\text{eff}}(t)}{1 + \nu}. \quad (6.7)$$

Poroviscoelastic load-relaxation

By analogy, at any instant the response of PVE material undergoing unconfined compression, $P(t)$ can be represented in terms of an effective elastic stiffness, $E_{\text{eff}}(t)$, such that $P(t) = E_{\text{eff}}(t)A\epsilon_{\text{max}}$. Invoking isotropic elasticity, $E_{\text{eff}}(t) = 2G_{\text{eff}}(t)[1 + \nu_{\text{eff}}(t)]$, where $G_{\text{eff}}(t)$ is the effective shear stiffness determined from viscoelasticity and $\nu_{\text{eff}}(t)$ is the effective Poisson's ratio determined from poroelasticity. It follows that a PVE material will behave according to:

$$\frac{P(t)}{P_{\infty}} = \frac{G_{\text{eff}}(t)[1 + \nu_{\text{eff}}(t)]}{G[1 + \nu]}, \quad (6.8)$$

and therefore,

$$P(t) = \frac{P_{\text{VE}}(t)P_{\text{PE}}(t)}{P_{\infty}}, \quad (6.9)$$

where $P_{\text{VE}}(t)$, $P_{\text{PE}}(t)$ and P_{∞} are the viscoelastic, poroelastic and equilibrium load-responses calculated from the equilibrium material properties. A similar argument using the reduced modulus, $E_R = 2G/(1 - \nu)$ and aggregate modulus, $H_A = 2G(1 - \nu)/(1 - 2\nu)$ can be used to show that equation 6.9 holds for indentation and confined compression.

There is one caveat to the above expression: it neglects the influence of a changing shear modulus on the time taken for poroelastic relaxation. Purely poroelastic behaviour depends on non-dimensional time, $t^* = \sqrt{ct/L_D^2}$, where $c = 2\kappa G(1 - \nu)/(1 - 2\nu)$ and L_D is the drainage length (for unconfined compression, L_D is the radius, r). The dependence of poroelastic relaxation on viscoelastic load-relaxation can be taken into account by calculating t^* and $P_{\text{PE}}(t)$ using an effective consolidation coefficient,

$$c_{\text{eff}}(t) = \frac{2\kappa G_{\text{eff}}(t)[1 - \nu]}{1 - 2\nu}, \quad (6.10)$$

where $G_{\text{eff}}(t)$ is defined by equation 6.3. $P_{\text{VE}}(t)$ can still be calculated independently of $P_{\text{PE}}(t)$, as the linear viscoelastic relaxation rate does not depend on Poisson's ratio.

6.2.2 Comparison to forward finite element methods

The previous argument suggests that PVE relaxation can be determined as the product of expressions for poroelastic relaxation and viscoelastic relaxation, providing a rapid alternative to FE analysis. The accuracy of this approach was investigated by comparing its predictions with load-relaxation curves simulated using forward FE analysis. Load-relaxation was predicted and simulated for unconfined compression, spherical indentation and confined compression experiments with ramp-hold displacement-controlled loading.

FE models

Models of unconfined compression, spherical indentation and confined compression of a linear PVE material model were generated in Abaqus. All models were axisymmetric with quadratic elements (CAX8RP). The unconfined compression and confined compression model consisted of a cylinder with 30 000 quadratic elements with a height of 10 mm and radius of 7.5 mm. For unconfined compression, the bottom and top surfaces of the cylinder were impermeable but fluid could flow radially. An axial strain of 0.10 was applied over 10 s. For confined compression, the bottom and side surfaces were impermeable and fluid could only flow out the top surface. Furthermore, radial expansion was prevented. An axial strain of 0.10 was applied over 100 s.

The spherical indentation model was similar to that described in section 5.3. It consisted of an analytically rigid surface (radius = 9.4 mm) in contact with a cylinder large enough to be considered a bulk half-space (the width and height were fixed to be $150\times$ the indenter contact radius, a). The indents were simulated to a constant indentation strain of 0.05, applied over a 10 s ramp period. Fluid could flow out of the top of the half-space, but flow was prevented in the contact region, using a user-defined boundary condition (section 5.3.4). The spherical indentation model consisted of 31 684 elements biased towards the contact region such that 63 elements were in contact at peak displacement. Further mesh refinement of both models made an insignificant difference to the load-relaxation curves.

6.2 Separable poroviscoelastic load-relaxation

Semi-analytical expression for PVE

PVE load-relaxation was determined from equation 6.9 using analytical and semi-analytical solutions for P_∞ , $P_{VE}(t)$ and $P_{PE}(t)$, summarised in table 6.1. For unconfined compression, P_∞ was calculated using linear elastic theory (equation 6.2). $P_{VE}(t)$ was determined from equation 6.1, which was derived using a Boltzmann hereditary integral approach (section 2.4.1). $P_{PE}(t)$ was evaluated using equation 3.11, which is Cohen et al.'s solution to ramp-hold unconfined compression (simplified to the isotropic form), both for the case where $t^* = \sqrt{ct/r^2}$ and $t^* = \sqrt{c_{\text{eff}}(t)t/r^2}$ (equation 6.10). Similarly, for confined compression, P_∞ was calculated using linear elastic theory, while $P_{VE}(t)$ was derived using a Boltzmann hereditary integral approach. Poroelastic load-relaxation $P_{PE}(t)$ was determined using an analytical expression found in Soltz & Ateshian (1998), expressed here in terms of a ramp correction factor (table 6.1).

Elastic responses for indentation were calculated using the Hay and Wolff expression (equation 5.5; Galli & Oyen, 2009; Hay & Wolff, 2001). $P_{VE}(t)$ was calculated using a semi-analytical equation (2.34) based on the analogy between step and ramp loading (Mattice et al., 2006; Strange & Oyen, 2012). $P_{PE}(t)$ was calculated for indentation using the Ramp RCF expression developed in the previous chapter (chapter 5, table 5.3a). Like unconfined compression and confined compression, $P_{PE}(t)$ was determined both for $t^* = \sqrt{ct/a^2}$ and $t^* = \sqrt{c_{\text{eff}}(t)t/a^2}$.

Semi-analytical PVE versus forward FE methods

Load-relaxation responses predicted by equation 6.9 and FE were compared for a range of viscoelastic time constants ($\tau = 2 - 100\,000$ s), permeabilities ($\kappa = 10^{-16}, 7.5 \times 10^{-14}, 1 \times 10^{-12}$ m⁴(Ns)⁻¹) and Poisson's ratios ($\nu = 0, 0.15, 0.4$) for spherical indentation; a subset of these parameters was investigated for unconfined compression and confined compression. In each case, $P(t)$, $P_{VE}(t)$ and $P_{PE}(t)$ were determined and plotted, allowing for simple visualisation of when poroelastic and viscoelastic mechanisms are dominant. $P(t)$ was then compared with an FE generated response.

6.2 Separable poroviscoelastic load-relaxation

Experiment	Value	Expression
Unconfined Compression	P_∞	$= E_\infty A \epsilon_{\max}$
	$P_{\text{VE}}(t)$	$= P_\infty [1 + \sum_i RCF_i g_i \exp(-t/\tau_i)]$
	$P_{\text{PE}}(t)$	$= P_\infty + (P_0 - P_\infty) \left[\sum_{n=1}^{\infty} RCF_n \frac{2(1-\nu) \exp(-\alpha_n^2 t^{*2})}{(1-\nu)^2 \alpha_n^2 - (1-2\nu)} \right]$
Spherical Indentation	P_∞	$= \gamma E_{\text{R}\infty} \frac{4\sqrt{R}}{3} h_{\max}^{3/2}$ where $\gamma = 1 + \frac{2(1-2\nu)}{3\pi(1-\nu)} \sqrt{\frac{h_{\max}}{R}}$
	$P_{\text{VE}}(t)$	$= P_\infty [1 + \sum_i RCF_i g_i \exp(-t/\tau_i)]$
	$P_{\text{PE}}(t)$	$= P_\infty + (P_0 - P_\infty) \sum_{n=1}^3 RCF_n A_n \exp\left(\frac{-t^{*\beta_n}}{\tau_n^{\text{PE}}}\right)$
Confined Compression	P_∞	$= H_{\text{A}\infty} A \epsilon_{\max}$
	$P_{\text{VE}}(t)$	$= P_\infty [1 + \sum_i RCF_i g_i \exp(-t/\tau_i)]$
	$P_{\text{PE}}(t)$	$= P_\infty [1 + 8 \sum_{n=1}^{\infty} RCF_n \exp(-4n^2 \pi^2 t^{*2})]$

where $RCF_i = \left[(\tau_i/t_{\text{R}}^{*\beta_i}) \left(\exp(t_{\text{R}}^{*\beta}/\tau_i) - 1 \right) \right]$, τ_i is defined as the factor that divides time (or non-dimensional time) in the exponential function (i.e. $\tau_n = 1/(4n^2 \pi^2)$ for poroelastic confined compression), P_0 is the undrained load for a poroelastic material (i.e. $P_0 = 3GA\epsilon_{\max}$ for unconfined compression) and α_n are the roots of the equation: $J_1(x) - (1-\nu)xJ_0(x)/(1-2\nu) = 0$ where J_n are Bessel functions of the first kind.

Table 6.1: Equilibrium, viscoelastic and load-relaxation expressions used in equation 6.9 to determine PVE load-relaxation.

6.2 Separable poroviscoelastic load-relaxation

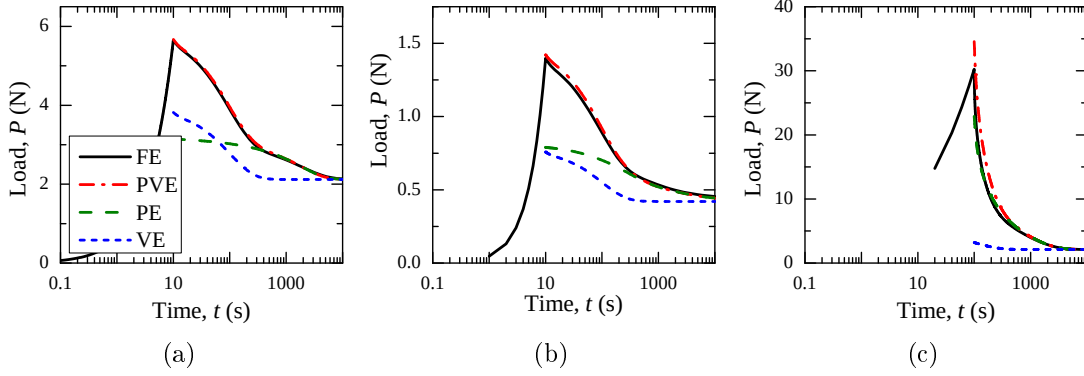


Figure 6.2: The load-relaxation response of a material with $G = 60$ kPa, $\nu = 0$, $\kappa = 7.5 \times 10^{-14} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 0.2$, $g_2 = 0.8$, $\tau_2 = 2$ s, $\tau_2 = 100$ s) calculated using equation 6.9 for (a) unconfined compression, (b) indentation and (c) confined compression. The consolidation coefficient c , was assumed to be constant and independent of viscoelastic relaxation. The calculated responses are compared with load-relaxation curves produced using FE simulations, as well as the response of a purely poroelastic or viscoelastic material.

The agreement between PVE predictions and forward FE simulations was excellent for unconfined compression and indentation even when poroelastic load-relaxation was calculated using a constant consolidation coefficient (figure 6.2). As long as the viscoelastic time constant was less than 1000 s the R^2 coefficient between the predictions and forward FE relaxation curves was always greater than 0.97. In cases where poroelastic relaxation finished well before viscoelastic relaxation occurred (e.g. $\tau = 100\,000$ s), or cases where the degree of viscoelastic relaxation was very large (e.g. $\sum g_i = 19$), the predicted PVE relaxation initially proceeded too slowly, leading to a small but observable error between the calculated and FE responses. When $P_{\text{PE}}(t)$ was calculated using a consolidation coefficient that depends on the effective shear modulus instead of the elastic shear modulus G_∞ (i.e. equation 6.10), the agreement between equation 6.9 and the FE simulations was near perfect (e.g. $R^2 > 0.9995$ for unconfined compression).

The accuracy of the predictions of PVE relaxation for confined compression was not as good as that of indentation or unconfined compression. This likely stems from the unrealistic strains in the material applied during displacement-controlled confined compression. A strain of 0.1 was applied to the material over

6.2 Separable poroviscoelastic load-relaxation

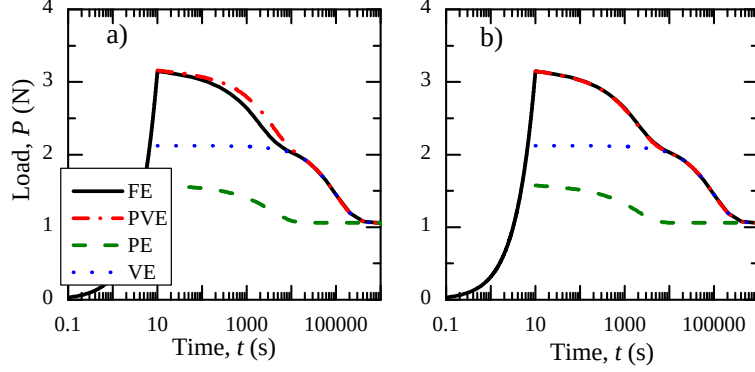


Figure 6.3: The load-relaxation response of a material with $G = 30$ kPa, $\nu = 0$, $\kappa = 7.5 \times 10^{-14} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 1$, $\tau_1 = 100\,000$ s) calculated using equation 6.9 for unconfined compression using (a) a constant consolidation coefficient, c and (b) an effective consolidation coefficient, $c_{\text{eff}}(t)$, compared with load-relaxation curves produced using FE simulations.

a 100 s ramp phase. At the end of this ramp phase, the calculated strain in the surface of the sample was 9.97, while the majority of the sample remained at less than 0.01 strain as this region was still nearly incompressible (section 2.4.2). The axial strain throughout the sample changed with time during the hold phase until equilibrium was reached. The viscoelastic relaxation of the material is calculated assuming the strains in the material are constant during the hold-phase and this would not have been the case. When a more realistic ramp time (1000 s) was used that did not overly strain the material (the peak strain at the end of the ramp phase was 0.27), the agreement between the predicted PVE relaxation and forward FE simulation was excellent (figure 6.4).

The example of confined compression reveals one of the limitations of this approach: viscoelastic relaxation is not completely independent of poroelastic relaxation, unless the applied strain directly determines the compressive strains within the sample (e.g. in unconfined compression). However, in the majority of realistic test situations (including those of confined compression, with slower strain rates), the axial strain in the material does not vary substantially during the hold phase and the assumption of separability is reasonable.

Calculating $P(t)$ from the separable responses (equation 6.9) was orders of magnitude faster than calculating it using FE methods, while achieving compar-

6.3 Identification of poroviscoelastic parameters

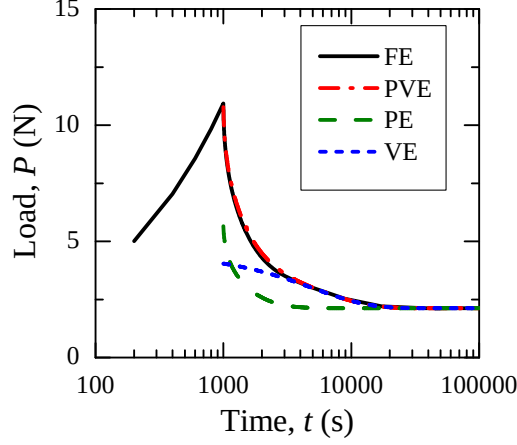


Figure 6.4: The load-relaxation response of a material in confined compression with 0.1 strain applied over a 1000 s ramp time. The load-relaxation response was calculated using equation 6.9 for an effective consolidation coefficient, $c_{\text{eff}}(t)$, and compared with load-relaxation curves produced using FE simulations. The material parameters were $G = 60$ kPa, $\nu = 0$, $\kappa = 7.5 \times 10^{-14} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 1$, $\tau_1 = 5000$ s.

able accuracy. It took approximately 40 minutes to run one FE simulation of PVE indentation on eight 2.4 GHz Intel Xeon CPU's with up to 48 Gb of RAM available. Solving the semi-analytical equation on a dual-core personal computer took 5 ms, including the time take to determine $P_{\text{VE}}(t)$, $P_{\text{PE}}(t)$ and P_{∞} . The approach developed here provides a rapid alternative to FE methods for a variety of experimental conditions, including those which lack analytical solutions. It can be used to simulate the response of the material and identify when viscoelastic or poroelastic contributions dominate, and to fit experimental data in order to identify PVE material properties.

6.3 Identification of poroviscoelastic parameters

The approach developed here provides a method to rapidly calculate PVE load-relaxation, though a robust method is still needed to identify unique PVE material properties. Previously attempted methods include simultaneously fitting creep and relaxation experiments performed on the same material (Olberding &

6.3 Identification of poroviscoelastic parameters

Francis Suh, 2006) and performing a combination of experiments such as shear and confined compression (Kalyanam et al., 2009). It is not clear in either case that the combination of experiments was sufficient to uniquely identify the PVE properties when the precise form of viscoelastic relaxation is unknown.

Scaling arguments can be used to differentiate between poroelastic and viscoelastic load-relaxation curves. Zhao et al. (2010) performed load-relaxation unconfined compression tests on both ionic and covalently crosslinked alginate cylinders of three different radii (5, 6 and 8 mm). The relaxation times of ionic gels did not vary substantially with indenter radius, while the relaxation times of covalent gels increased as the radius (and poroelastic drainage length) increased. When the stress-relaxation curves of the covalent gels were plotted as a function of $\sqrt{t}/R$, instead of time, the curves for the different sized cylinders collapsed onto a single curve indicating that the covalent gels largely exhibited poroelastic time-dependent behaviour.

Both Wang & Hong (2012) and Hu & Suo (2012) have argued that the scaling properties of poroelastic relaxation could be used to uniquely determine PVE material properties. In cases where the time for poroelastic relaxation is extremely fast (permeable specimens with small drainage lengths) or slow (impermeable specimens with large drainage lengths) the observed stress-relaxation behaviour will primarily be viscoelastic. By characterising the viscoelastic material properties it should be possible to uniquely determine the PVE properties of the material. Chiravambath et al. (2009) performed indentation at two scales on cartilage: one in which it was likely that poroelastic relaxation was negligible in order to determine viscoelastic properties, and one at a larger scale where PVE relaxation was observed, in order to determine the full PVE parameters using inverse FE analysis. The problem with these approaches is that the conditions where poroelasticity is negligible depend both on the material and the test methods. The conditions are difficult to know *a priori* and could potentially be experimentally difficult to achieve, requiring extremely small or large test scales. In the majority of experiments on PVE materials, poroelastic and viscoelastic relaxation occurs concurrently.

In this section, the previous results are used to show that scaling arguments can be used to deconvolve poroelastic and viscoelastic behaviour that occurs

6.3 Identification of poroviscoelastic parameters

concurrently. We apply the method to the case of multi-scale indentation; load-relaxation curves obtained using several different indenter sizes on the same material are simultaneously fit to uniquely identify PVE material parameters. The same approach can also be applied to gels with different radii (unconfined compression) and different heights (confined compression).

6.3.1 Theory

The elastic load measured during indentation scales with indenter size and depth according to the Hertz equation: $P_\infty \propto (R^{1/2}h^{3/2})$, where R is indenter radius and h is depth. The time constant of relaxation of a purely viscoelastic material is governed by the material relaxation function, which is independent of the geometry of the test set-up. The load-relaxation, $P_{VE}(t)$ of a viscoelastic material indented with different sized indenters, will be a function of time and $R^{1/2}h^{3/2}$:

$$\frac{P_{VE}(t)}{R^{1/2}h^{3/2}} = f(t). \quad (6.11)$$

In contrast, length-scale effects are inherent in the response of a poroelastic material. The load-response of a poroelastic material, $P_{PE}(t)$ indented with different sized indenters will vary quadratically in time with the contact radius, $a = \sqrt{Rh}$ such that:

$$\frac{P_{PE}(t)}{R^{1/2}h^{3/2}} = g\left(\frac{t}{a^2}\right). \quad (6.12)$$

The relaxation response of a PVE material can be determined from the product of the viscoelastic and poroelastic responses (equation 6.9), and thus the load-relaxation response will also depend on the contact radius:

$$\frac{P(t)}{R^{1/2}h^{3/2}} \propto \frac{P_{VE}(t)P_{PE}(t)}{(R^{1/2}h^{3/2})^2} = f(t)g\left(\frac{t}{a^2}\right). \quad (6.13)$$

Given that the poroelastic component of the response is solely responsible for the scaling behaviour, we can isolate the poroelastic response ($g(t/a^2)$) of the material by simultaneously fitting load-relaxation curves obtained using several different indenter sizes on the same material and hence determine the full PVE response.

6.3.2 Method

This approach was tested by simultaneously fitting load-relaxation curves generated with forward FE models for three different indenter radii (3.1 mm, 7.95 mm and 15.9 mm). Ramp-hold indentation experiments were simulated with a ramp time of 10 s to an indentation strain of 0.05 with a hold time of 3600 s. Gaussian noise (5% of the current load) was added to the generated load-relaxation curves in order to simulate adverse experimental conditions (Galli & Oyen, 2009). The material parameters of the base model were chosen to be representative of a hydrogel such as agar ($G = 60$ kPa, $\nu = 0.0$, $\kappa = 7.5 \times 10^{-14} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 1$, $\tau_1 = 100$). The permeability, κ was also varied from 1×10^{-16} to $1 \times 10^{-12} \text{ m}^4(\text{Ns})^{-1}$, ν from 0.00 to 0.40, and τ_1 from 10 to 1000 s, in order to test the robustness of the method. Simulations of materials with multiple viscoelastic Prony series terms were also examined.

For each set of material parameters, the three load-relaxation curves were simultaneously fit in MATLAB using the `lsqnonlin` optimisation function with a PVE load-relaxation curve generated using equation 6.9. $P_{VE}(t)$, $P_{PE}(t)$ and P_∞ were calculated as per section 6.2.2, using the effective consolidation coefficient, $c_{\text{eff}}(t)$. Initial guesses of ν , κ , g_1 and τ_1 were set to 0.2, $1 \times 10^{-15} \text{ m}^4(\text{Ns})^{-1}$, 0.1 and 50 s respectively. An initial guess of G_∞ was calculated from the load at 3600 s for the smallest indenter.

6.3.3 Identification results

PVE parameter identification of multi-scale forward FE models was excellent (table 6.2; figure 6.5). For materials with a permeability of $1 \times 10^{-15} \text{ m}^4(\text{Ns})^{-1}$ or greater, the shear modulus could be identified to within 6%, Poisson's ratio to within 0.08, permeability to within 28%, and viscoelastic parameters (G_1, τ) to within 14% (table 6.2a). Poroelastic parameters were not successfully identified for materials with a permeability of $1 \times 10^{-16} \text{ m}^4(\text{Ns})^{-1}$ although this is not surprising; the materials were so impermeable that only negligible poroelastic relaxation (and scaling) was observed during the hold time. All relaxation observed was largely due to viscoelastic mechanisms and the viscoelastic properties were correctly identified.

6.3 Identification of poroviscoelastic parameters

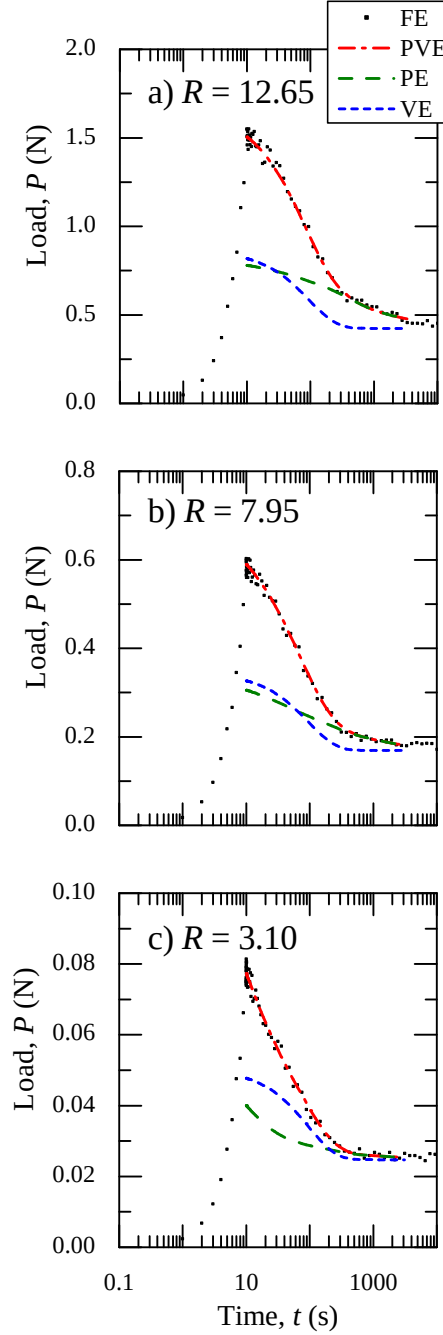


Figure 6.5: PVE parameter identification of multi-scale forward FE indentation. The model parameters are the base parameters discussed in section 6.3.2. The identified parameters are: $G = 60$ kPa, $\nu = 0.01$, $\kappa = 6.6 \times 10^{-14} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 0.96$, $\tau_1 = 94.9$. The PVE fit (red line) is excellent for all three indenter radii. The shape of the poroelastic contribution (green line) shifts in time as the indenter size and contact radius increases. In contrast, the shape of the viscoelastic contribution (blue line) remains constant.

6.3 Identification of poroviscoelastic parameters

G	ν	κ	g_1	τ_1	G_{err}	ν_{err}	κ_{err}	g_{err}	τ_{err}
60	0.0	0.75	0.5	100	0.01	0.01	0.12	0.01	0.05
60	0.0	0.75	0.5	10	0.02	0.04	0.06	0.07	0.12
60	0.0	0.75	0.5	1000	0.04	0.01	0.22	0.02	0.13
60	0.4	0.75	0.5	100	0.01	0.00	0.04	0.00	0.01
60	0.0	10	0.5	100	0.01	0.02	0.15	0.00	0.03
60	0.0	0.01	0.5	100	0.02	0.04	0.28	0.00	0.01
60	0.0	0.001	0.5	100	0.02	0.46	3.24	0.015	0.08

(a)

G	ν	κ	g_1	τ_1	G_{err}	ν_{err}	κ_{err}	$g_{1,\text{id}}$	$\tau_{1,\text{id}}$	$g_{2,\text{id}}$	$\tau_{2,\text{id}}$
60	0.0	0.75	0.5	100	0.03	0.06	0.08	0.05	3.84	0.48	96.9
60	0.0	0.75	0.5	1000	0.03	0.01	0.27	0.25	989	0.25	1048
60	0.4	0.75	0.5	100	0.02	0.00	0.34	0.26	96.3	0.24	96.6

(b)

G	ν	κ	g_1	τ_1	g_2	τ_2	G_{err}	ν_{err}	κ_{err}	g_{id}	τ_{id}
60	0.0	0.75	0.2	2	0.4	100	0.02	0.06	0.18	0.46	85
60	0.0	0.75	0.5	2	0.5	100	0.03	0.07	0.13	0.34	58
60	0.0	0.75	0.5	10	0.5	100	0.08	0.06	0.19	0.43	28

(c)

Table 6.2: PVE parameter identification of multi-scale forward FE indentation for cases where (a) the fitted model had the same number of Prony series terms as the FE model, (b) the fitted model had more Prony series terms than the FE model and (c) the fitted model had fewer Prony series terms than the FE model. G_{err} , κ_{err} , g_{err} , τ_{err} are defined as $|A_{\text{id}}/A - 1|$ where A_{id} is the identified material parameter and A was the FE model parameter. As $\nu = 0$ in many cases, ν_{err} was defined as $|\nu_{\text{id}} - \nu|$. The units of G , κ and τ are kPa, $10^{-15} \text{ m}^4 (\text{Ns})^{-1}$ and s, respectively.

6.3 Identification of poroviscoelastic parameters

Identification was also largely insensitive to initial guess values. Occasionally, if the guesses were substantially different from the model parameters, the optimisation routine would fail to converge to the correct material parameters. This was apparent because the calculated fit did not match all three indenter responses. For these cases the correct identification could be obtained by varying the initial guess values.

The multi-scale approach was particularly successful, compared to fitting a single indent, when the equation was fit to a forward FE model with fewer or more Prony series terms than it. When the forward FE model used to fit the experimental data had more Prony series terms than the identification model, poroelastic material properties were reliably identified, while viscoelastic properties approximately represented the viscoelastic response given that they had one fewer term than was necessary (table 6.2c). When the identification model had one more Prony series term than the forward FE models, generally two of the time constants would converge to a single time constant (e.g. $\tau_1 = \tau_2$), or one would be set to a very low value ($\tau_2 \ll t_R$) such that its effect was minimal (table 6.2b).

Single-scale indents on a material with base parameters were also fit individually for comparison with the multi-scale approach. The identified shear modulus differed from the actual by a maximum of 32%, Poisson's ratio by 0.23, permeability by 527% and viscoelastic parameters (G_1, τ) by 34%. These identifications varied within the above range depending on the initial guess. Although the identified parameters differed from the actual, the predicted load-relaxation curves still accurately matched the single indent ($R^2 > 0.99$), although did not necessarily match the other load-relaxation curves generated with different indenter sizes. The identification errors resulting from non-unique solutions were further exacerbated when the identification model had more or fewer Prony series terms than the FE model, with identified permeability sometimes differing from the actual by multiple orders of magnitude.

It is encouraging that the multi-scale indentation approach is a much more reliable method at uniquely identifying PVE parameters than fitting single indents. Identifying PVE parameters from experiments on real materials would perhaps be more challenging, as a result of the variations in material properties between

samples and the variation encountered due to the presence of any geometric or material nonlinearities. Nevertheless, the amplitude of Gaussian noise simulated here is likely greater than that which would be encountered in an experiment.

The ability of the multi-scale indentation approach to reliably identify PVE parameters even when the number of Prony series parameters in the viscoelastic relaxation function differs from that of the FE model has great practical significance. In an experiment it is unlikely that the precise form of the viscoelastic material relaxation function will be known. With this approach, if the material relaxation function can be approximated with a Prony series, a correct identification will likely still be achieved.

The multi-scale approach has been demonstrated with indentation to uniquely identify PVE material parameters (of which there are a minimum of five). The method was demonstrated here with indentation, although it would likely work with compression tests on samples of different sizes as the scaling phenomena is equally applicable, but the test time would be appreciably longer due to the larger drainage lengths.

6.4 Experimental characterisation of agar

Agar (and agarose) gels have been used in a wide variety of tissue engineering experiments: as tissue engineering scaffolds for the IVD and articular cartilage (Moutos et al., 2007; Nerurkar et al., 2010c), as substrates to determine cellular responses to diffusion of nutrients (Horner & Urban, 2001) and as micro-environments to transmit forces to cells (Lee & Bader, 1997). These gels have even been used as controls to verify mechanical characterisation techniques at various scales (Stolz et al., 2004). Despite the wide use of agar, its material properties are still not well understood and there is little consensus on a full description of its mechanical behaviour.

Agar and agarose material properties have been determined using unconfined compression (Cloyd et al., 2007; Normand et al., 2000), tension (Normand et al., 2000; Subhash et al., 2011), confined compression (Gu, 2003; Mauck et al., 2000), rheology (Normand et al., 2000), indentation, (Olberding & Francis Suh, 2006; Stolz et al., 2004) and direct permeability measurements (Johnson & Deen, 1996)

6.4 Experimental characterisation of agar

using both elastic (Cloyd et al., 2007; Normand et al., 2000), viscoelastic (Roberts et al., 2011) and poroelastic (Gu, 2003; Mauck et al., 2000) frameworks. Several investigators have noted that many measurements performed using one experimental framework (e.g. confined compression, direct permeability) do not translate to other experimental frameworks (Gu, 2003; Mauck et al., 2000). Similarly, in chapter 3 it was noted that permeability measurements determined from indentation agreed well with literature values, but estimates of Poisson’s ratio were much less than 0 and were unlikely to correspond to unconfined compression experiments.

It was suggested that poroelasticity did not provide a full description of agar’s material behaviour and that flow-independent viscoelastic effects also needed to be taken account of. This has also been proposed by other authors (Mauck et al., 2000). Olberding & Francis Suh (2006) attempted to characterise the PVE properties of agar by simultaneously fitting creep and relaxation responses, although the identified parameters (particularly permeability, $\kappa \approx 5 \times 10^{-10} \text{ m}^4(\text{Ns})^{-1}$) still substantially differed from direct measurements and theoretical estimates of permeability. At the scales in which the experiments were performed, it seems likely that the identified poroelastic response would have comprised a negligible proportion of the overall PVE response, and hence the results should be treated with caution.

In this work, the approach developed in section 6.3 to identify PVE material parameters is used to characterise the linear PVE response of 3% agar. The results from this characterisation are compared to direct measurements of mechanical properties (Young’s modulus, E , drained Poisson’s ratio, ν and permeability, κ) obtained through unconfined compression and direct permeation experiments, in order to determine whether consistent mechanical predictions can be obtained for multiple experimental frameworks. Agar gel manufacture was performed by Helen Brawn and Khaow Tonsomboon. Helen Brawn performed the multi-scale indentation experiments and direct permeability measurements while Khaow Tonsomboon performed unconfined compression experiments. Analysis (including determining the variation in Poisson’s ratio from captured images) and PVE parameter identification was performed by the author.

6.4.1 Experimental methods

Agar gels (3% concentration) were fabricated using a similar method to chapter 3. Briefly, agar granules were soaked in distilled water at room temperature, and then heated and stirred in a water bath at 90 °C until dissolved. The molten mixture was poured into moulds, left to solidify for 24 hours and then soaked in distilled water for approximately 24 hours prior to testing. Samples were tested in distilled water.

Rectangular sheets approximately 10 mm thick were prepared for indentation testing. Unconfined compression cylinders (height 10 mm; radius 7.5 mm) were prepared by pouring the mixture into a PDMS mould and placing acetate over the top surface to prevent the formation of a meniscus. A small volume of red food dye was added to the mixture to enhance visibility. Permeability specimens were obtained by pouring the mixture directly into a 26.7 mm diameter syringe with a removable base, forming 20 mm thick cylinders.

Mechanical tests were performed on an Instron 5544 universal testing frame with a 5 N (indentation) and 500 N (unconfined compression) load cell. Spherical indentation testing was carried out with three different indenters on each sample with radii (R) of 3.1 mm, 7.95 mm, and 12.65 mm. Displacement-controlled tests with a ramp-hold profile were performed. The displacement was increased over a ramp time, t_R of 10 s to peak displacement $h_{\max}$ corresponding to an indentation strain, $\varepsilon = \sqrt{0.2h/R}$, of 0.05. The indenter was held on the gel until the gel had fully relaxed ($t_H = 1000\text{--}3600$ s). Mean load-relaxation curves were obtained by calculating the mean load at that time for multiple samples. Unconfined compression tests were carried out to a strain of 0.1. A preload of 0.25 N was initially applied to the sample to determine contact followed by a ramp time of 10 s and hold time of at least 2 hrs. The preload was substantially larger than the preload used previously when testing agar (section 3.2.3), in order to further minimise the effect of any surface imperfections. The predicted PVE response was calculated with indentation parameters and the effect of the preload was determined to be minimal.

Images of the bottom face of the samples was captured using a PixeLINK PL-B776F 3.1 megapixel camera at regular time intervals throughout the test,

6.4 Experimental characterisation of agar

following a method similar to Cloyd et al. (2007), in order to experimentally determine the radial strain and $\nu_{\text{eff}}(t)$. Images were analysed using FIJI. The radius of the sample was calculated from the area of a circle drawn through the edge of the cylinder. Only the green channel of each image was used for analysis and local contrast enhancement was used to enhance the clarity of the edge. The initial radius of the sample was determined from the mean radius from four images taken immediately prior to the onset of the ramp phase. The drained Poisson's ratio, ν was calculated from the measured strains after 7200 s hold.

Direct measurements of permeability were carried out by pumping fluid through a syringe containing the gel and calculating the permeability using Darcy's law ($\kappa = QL/A\Delta p$, where Q is the volumetric flow rate, Δp is the pressure difference across the material, L is the gel thickness and A is the area). Six tests were carried out at infusion rates of 0.05, 0.07, 0.09 and 0.11 ml hr⁻¹ using a Graseby 3150 syringe pump and a MPX5100AP/GP Freescale Semiconductor Pressure Transducer (Freescale Semiconductor Inc, Austin, TX).

6.4.2 Results

Multi-scale indentation experiments indicate that agar gels exhibit substantial scale-dependent relaxation: when the mean load-relaxation curves for three indenter sizes are normalised by the indenter geometry and plotted against time (equation 6.11; figure 6.6) the trends of each curve are similar, although offset in time. When the normalised load-relaxation curves are plotted against t/a^2 (equation 6.12; figure 6.6) the curves collapse onto a single curve in the long-term, indicating substantial poroelastic behaviour. At short times, the results do not normalise ideally, either due to the effects of viscoelastic behaviour or from differing degrees of poroelastic behaviour occurring during the ramp phase as the non-dimensional ramp time was not constant (i.e. figure 5.6). Without further analysis, it is not possible to differentiate between poroelastic and viscoelastic contributions to the relaxation responses.

A PVE model with one viscoelastic Prony series term was simultaneously fit to the average load-relaxation curves shown in figure 6.6, using equation 6.9. A fit to the experimental data could only be obtained if Poisson's ratio was allowed

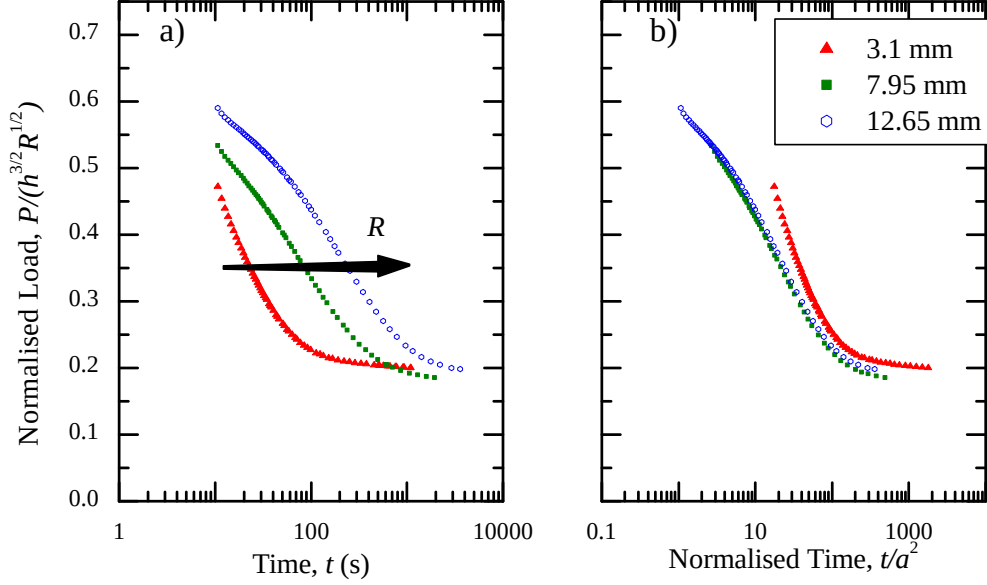


Figure 6.6: Average load-relaxation curves obtained from spherical indentation of agar hydrogels with three different indenter radii. Normalised load is plotted against real-time (a) and time normalised by contact radius (b).

to tend towards -1 . The identified parameters were $G = 98$ kPa, $\nu = -0.70$, $\kappa = 1.1 \times 10^{-13} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 0.12$ and $\tau_1 = 309$ s. Increasing the number of Prony series parameters from one to three provided a negligible improvement in the accuracy of the fit (the lowest R^2 value increased from 0.976 to 0.983) and the values of shear modulus, Poisson's ratio and permeability changed by 5% or less. Regardless of the number of Prony series parameters, the identification was insensitive to the initial guesses of parameters.

The PVE fits matched the load-relaxation of agar for all three indenter radii (figure 6.7). The fits predicted the full range of multi-scale behaviour with loads spanning from 0.03 N to 1.6 N, and relaxation times ranging from approximately 100 s to over 3600 s. It can be seen from figure 6.7 that the PVE relaxation at all scales was dominated by the poroelastic contribution to the response and that the viscoelastic contribution modified the response by a maximum of 10% (as $g_1 \approx 0.1$).

The identified PVE parameters were compared with direct measurements

6.4 Experimental characterisation of agar

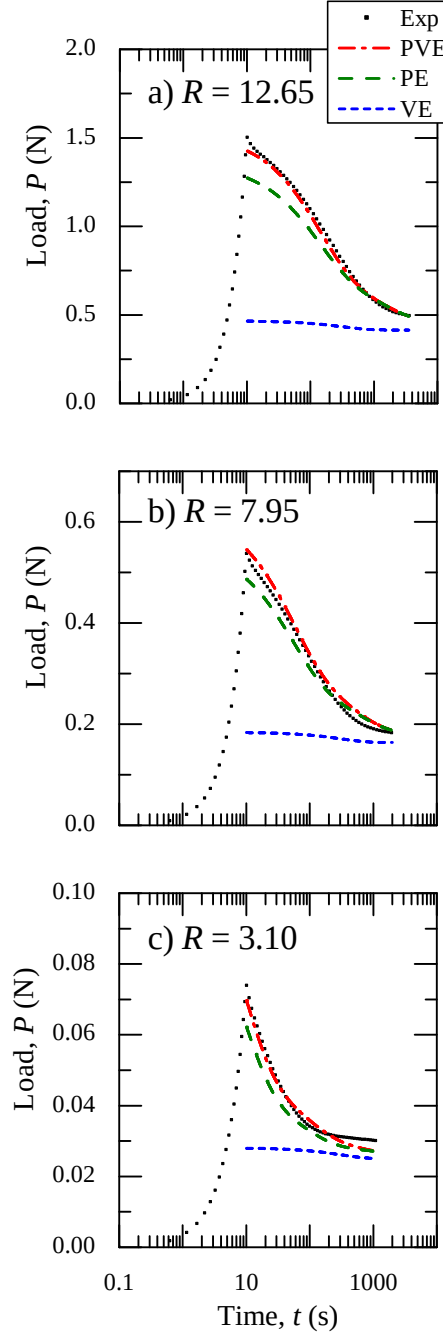


Figure 6.7: Experimental data from multi-scale indentation experiments, compared with fits of the data obtained by simultaneously fitting the experimental data with a semi-analytical expression for PVE load-relaxation (equation 6.9). The data is also compared with load-relaxation responses determined for a purely poroelastic and viscoelastic material response.

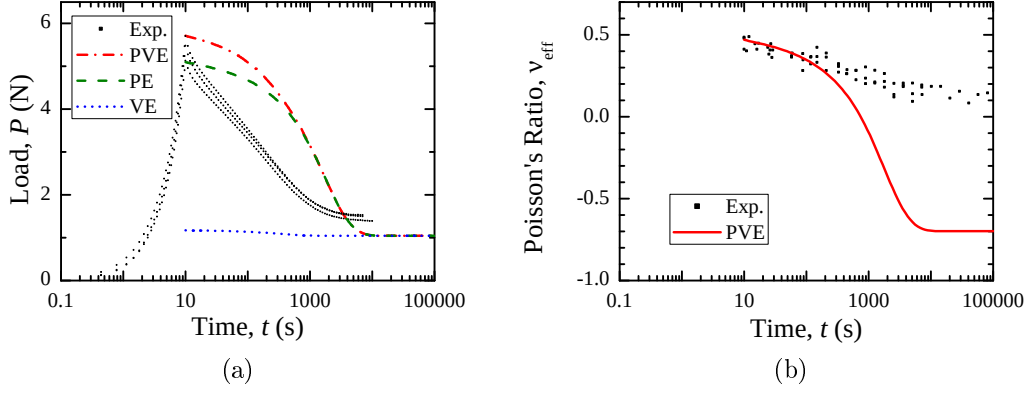


Figure 6.8: Experimental data from unconfined compression experiments on agar hydrogels showing both the change in load (a) and Poisson's ratio (b) with time. The data is compared with PVE predictions using parameters identified from indentation testing. The response for a purely poroelastic and purely viscoelastic material response are also shown in (a).

of equilibrium elastic modulus, E_{∞} , drained Poisson's ratio ν , and permeability κ measured with unconfined compression testing and direct permeability measurements. The permeability of the gels directly measured using a permeometer, $\kappa = 5.1 \pm 0.9 \times 10^{-14} \text{ m}^4 (\text{Ns})^{-1}$, compares well to the value identified from indentation tests. However, the equilibrium elastic modulus, E_{∞} (unconfined compression: 82.2 kPa, indentation: 55.8 kPa) and drained Poisson's ratio ($\nu = 0.17 \pm 0.03$) differed from the parameters identified from indentation measurements (figure 6.8).

The identified PVE parameters were also used to predict the response of the material under unconfined compression and compared to experimental measurements of $P(t)$ and $\nu_{\text{eff}}(t)$ (figure 6.8). The total amount of relaxation predicted, P_{∞}/P_0 , agrees well with the measured ratio during unconfined compression (figure 6.8a). The time to equilibrium is also similar, although the initial rate of relaxation differs substantially. Like indentation, the relaxation predicted by the PVE model is dominated by the identified poroelastic relaxation, with a large change of Poisson's ratio from the undrained Poisson's ratio of 0.5 to the drained Poisson's ratio of -0.7. In contrast viscoelastic relaxation contributes to a much smaller fraction of the total deformation.

6.4 Experimental characterisation of agar

6.4.3 Discussion of agar experiments

Multi-scale indentation provided a unique identification of PVE material parameters for agar. However, the identified material parameters differ from direct measurements of shear modulus, Poisson's ratio and permeability determined from unconfined compression and permeation experiments. The direct measurements of Poisson's ratio and permeability agree well with direct measurements on 2% agarose performed by other investigators (Cloyd et al., 2007; Kelly et al., 2006: $\nu = 0.12$; Johnson & Deen, 1996: $\kappa = 3.5 \pm 0.95 \times 10^{-13} \text{ m}^4 (\text{Ns})^{-1}$). Given that the approach correctly identified PVE parameters from multi-scale forward FE simulations (section 6.3), it is unlikely that the agar hydrogel demonstrates linearly isotropic PVE material behaviour.

Still, the approach provides significant insight into the material behaviour of agar. Predictions of indentation and unconfined compression load-relaxation using the identified PVE properties indicate that poroelastic relaxation dominates the material response (figure 6.7, figure 6.8). Both the rate of relaxation and time to relaxation for indentation and unconfined compression were experimentally observed to vary by orders of magnitude as the characteristic test size increased (figure 6.6). Indentation load-relaxation largely collapsed onto a single curve when time was normalised by contact radius. In the current framework, scaling relaxation can only be accounted for with poroelasticity, as there are no other time-dependent mechanisms that scale with contact radius. However, substantial scaling relaxation could result from a number of non-poroelastic mechanisms that are not taken into account in the current PVE framework and this will be discussed further on.

The identified negative Poisson's ratio is a manifestation of this substantial scaling relaxation, rather than a true material property. The ratio of the equilibrium to peak load (P_∞/P_0) of an isotropic poroelastic material with a positive Poisson's ratio is limited to a factor of 0.5 for indentation and 0.66 for unconfined compression. The mean measured load ratio for agar during unconfined compression was 0.27. In order to describe a scaling relaxation ratio this small using the current linearly isotropic PVE framework, the identified negative Poisson's ratio had to be negative.

6.4 Experimental characterisation of agar

In contrast, the identified permeability of the agar gels did agree well with direct measurements of permeability. The identified permeabilities are also very similar to those identified using purely linear poroelastic analysis in chapter 3 ($\kappa = 2.16 \times 10^{-13} \text{ m}^4 (\text{Ns})^{-1}$). The non-dimensional poroelastic time, t^* is proportional to the consolidation coefficient, c , which is very sensitive to changes in κ but is only slightly altered by an incorrect identification of ν . Given the accurate identification of κ , it is likely that the relaxation is scaling in time with a similar scale-dependence as poroelasticity. A substantial poroelastic contribution in agar is supported by observations by Mauck et al. (2000): when displacement-controlled confined compression experiments were performed on agar, substantial fluid pressure was generated at the end of the ramp phase and stress-relaxation equilibrium occurred only when the pressure subsided.

There are several plausible reasons why substantial relaxation could be occurring that is not consistent with a linearly isotropic PVE framework but that scales in an analogous manner. The bulk viscoelastic relaxation form may not be equivalent to the shear relaxation function, although, as is discussed in the following section, preliminary FE simulations demonstrated that this effect was minimal. Varying effects of sample thickness relative to depth and indenter radius could scale the relaxation rate, although would be unlikely to affect the relaxation ratio (Galli & Oyen, 2008; Hu et al., 2011). Similarly, the permeability of the hydrogel could be strain-dependent though this would only be expected to affect the relaxation rate and not the relaxation ratio. Finite-deformation effects and nonlinear elasticity could affect the degree of relaxation although the indentation strain was constant regardless of indenter size. Furthermore, agar stress-strain curves do not display substantial nonlinearities below strains of 0.1 (Normand et al., 2000). Adhesive effects would scale with indenter size, though it is unclear how these would affect the relaxation ratio.

The most likely cause of the observed behaviour is a tensile-compressive non-linearity in modulus. Poroelastic and PVE materials with differing compressive and tensile moduli can exhibit much greater relaxation ratios than isotropic poroelastic materials, when the compressibility of the material changes from the undrained ($\nu_{\text{eff}} = 0.5$) to drained state (in this case, $\nu = 0.17$; Cohen et al., 1998; Huang et al., 2003). Tensile-compressive nonlinearities have not previously been

6.5 Discussion

definitively reported for agar hydrogels though both Normand et al. (2000) and Subhash et al. (2011) noted a small discrepancy in the compressive and tensile moduli of agarose gels (as large as a factor of 1.7), but speculated that the discrepancy was likely the result of differing experimental protocols. Still, agar hydrogels are physical hydrogels and entanglements between chains could potentially stiffen in tension, and ‘give’ in compression (figure 6.9).

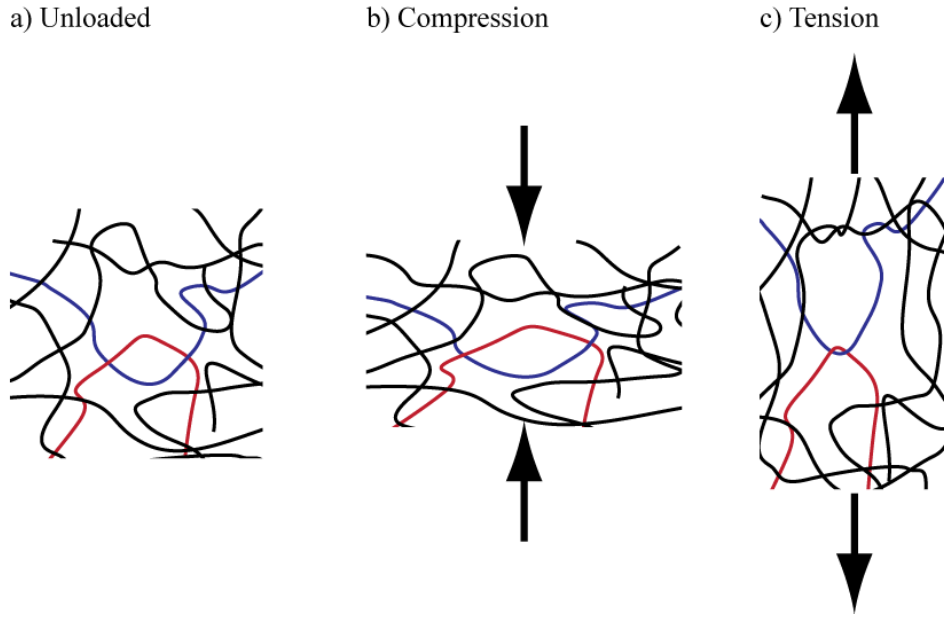


Figure 6.9: Schematic of a mechanism for a tensile-compressive nonlinearity in a physically crosslinked hydrogel. Compressive load (b) is primarily transferred through frictional interactions between polymer chains. In tension (c), strong entanglements could form in addition to the frictional interactions, resulting in a stiffer response. This is highlighted for the blue and red polymer chains.

6.5 Discussion

The key finding from this work is that the linearly poroelastic and viscoelastic responses are separable. An analogous case is found for some nonlinearly viscoelastic materials, for which the relaxation function can be separated into a time-dependent and strain-dependent component (Findley et al., 1989; Oyen et al., 2005). Just as is the case here, the material response is the product of the two

individual responses. This finding suggests an approach to identify PVE material properties, which has been demonstrated here with forward FE simulations and experiments on an agar hydrogel, using multi-scale indentation. In retrospect, it is perhaps not surprising that the two physical mechanisms (poroelasticity and viscoelasticity) can be decoupled, since one is scale-dependent and the other is not.

While there is a physical basis for why the two mechanisms should be separable, two significant assumptions were made in order to develop the approach used in this work. The first was that viscoelastic mechanisms did not affect the time-dependent Poisson's ratio while poroelastic mechanisms did. In practice, viscoelastic behaviour can result in increasing or decreasing Poisson's ratios with time. Constant Poisson's ratios have been observed for viscoelastic low-density foams, but Poisson's ratios that increase from 0.3 to nearly 0.5 have been observed for solid polymers (Lakes & Wineman, 2006). A time-varying Poisson's ratio will occur when the bulk relaxation function, $K(t)$ varies independently of the shear relaxation function, $G(t)$.

In practice, discrepancies arising from this assumption may not have a large effect on whether PVE load-relaxation is the product of poroelastic and viscoelastic contributions. The Poisson's ratio of an elastic material varies with shear and bulk moduli as follows:

$$v = 0.5 - \frac{3G}{2(3K + G)}. \quad (6.14)$$

If the PVE material is still in the undrained state while the viscoelastic relaxation occurs (i.e. the poroelastic contribution to the load-relaxation completes well after viscoelastic relaxation), then $K \gg G$; the second term in equation 6.14 is negligible and Poisson's ratio will be independent of the relation between the shear and bulk moduli. Even when poroelastic and viscoelastic relaxation occur concurrently it appears that there is little discrepancy between assuming that bulk relaxation is equivalent to shear relaxation or that there is no bulk relaxation (figure 6.10). It is only when poroelastic relaxation finishes well before viscoelastic relaxation that load-relaxation will be sensitive to the precise relationship between the shear and bulk relaxation functions. This sensitivity should

6.5 Discussion

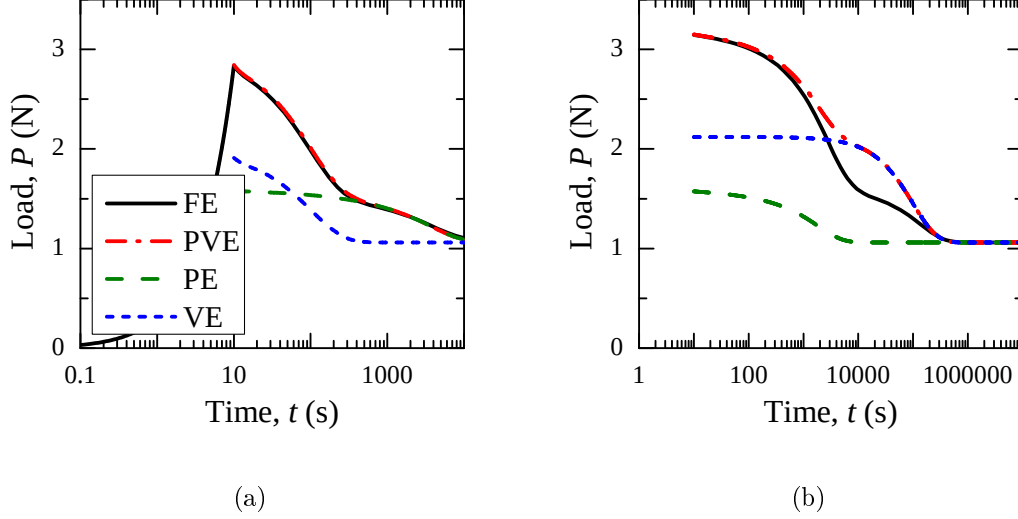


Figure 6.10: PVE relaxation calculated using equation 6.9 compared with FE simulations of a PVE material with a constant bulk relaxation function (i.e. $K(t) = K_\infty$) for situations where (a) viscoelastic and poroelastic relaxation occur currently ($G = 30$ kPa, $\nu = 0$, $\kappa = 7.5 \times 10^{-14} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 0.2$, $g_2 = 0.8$, $\tau_2 = 2$ s, $\tau_2 = 100$ s) and (b) where poroelastic relaxation completes well before viscoelastic relaxation ($G = 30$ kPa, $\nu = 0$, $\kappa = 7.5 \times 10^{-14} \text{ m}^4(\text{Ns})^{-1}$, $g_1 = 0.2$, $\tau_1 = 100\,000$ s).

be further investigated.

The second assumption made in this work was that the viscoelastic shear relaxation function took the form of a Prony series. This assumption was made for convenience as viscoelastic relaxation could be straightforwardly determined with analytical and semi-analytical expressions. Prony series are also frequently assumed for separable nonlinear viscoelasticity, although the separable relationship still holds even if that is not the case (Lakes, 2009). It is likely that the same is true here.

Even with the assumptions made, the work presents a general approach to rapidly and uniquely determine PVE material parameters. In contrast to previous methods, the approach isolates poroelastic behaviour through scaling, instead of isolating the viscoelastic response. This is advantageous over methods such as those proposed by Olberding & Francis Suh (2006) or Kalyanam et al. (2009), as the identified PVE results do not depend significantly on the assumed form of

the viscoelastic response.

6.6 Conclusions

Here, a relationship predicting linear PVE load-relaxation from separate poroelastic and viscoelastic parameters has been derived. It can be used as a rapid alternative to inverse FE methods, even for cases where no analytical solutions exist (e.g. indentation). Furthermore, the relationship provides a means for simple visualisation of when poroelastic or viscoelastic mechanisms are dominant, and could be used when validating a PVE model to check that both poroelastic and viscoelastic relaxation are present.

The relationship was simultaneously fit to indentation data at different scales, robustly identifying PVE parameters of forward FE simulations. Although the response of agar was not able to be fully determined using the current PVE framework, the permeability and time-scales for poroelastic relaxation were still correctly identified and new insight into the time-dependent behaviour of agar was obtained. Future work should verify whether agar does exhibit a tensile-compressive nonlinearity, which was a previously unknown response. The method here has been used to rapidly and robustly identify the PVE properties, providing substantial physical insight into the time-dependent behaviour of hydrogels.

Chapter 7

Conclusions and future directions

This chapter concludes the thesis. It begins by reviewing the state-of-the-art, before presenting a summary of the major findings in this work, both experimental and computational. Future directions for the work are discussed, with a focus on informing the design of intervertebral disc scaffolds and improving our understanding of the mechanics of hydrogels.

7.1 Conclusions

Back pain is a severely debilitating condition that has a profound socio-economic impact. Between 70–85% of people will suffer from back pain at some point in their lives (Andersson, 1999). In one study, over a quarter of American adults reported having suffered from back pain within the last three months (Deyo et al., 2006). While it is not life threatening and the majority of individuals recover without needing treatment, the substantial prevalence of back pain in society means that it has a significant economic consequences; over £1.6 billion was spent on treating back pain in the UK in 1998. A further £10.6 billion was estimated to have been lost as a result of indirect costs such as loss of production (Maniadakis & Gray, 2000).

Tissue engineering promises a better long-term outcome for patients than current treatments, which substantially alter the biomechanics of the spine. Much IVD tissue engineering research has focused on creating porous biocompatible materials that encourage cell growth (O’Halloran & Pandit, 2007). Although these materials may be excellent cell culture environments, they do not provide the same environmental cues as native tissue and cells do not respond in the same way as they would *in vivo*. To date, no study has been able to fully reproduce the mechanical functionality of the IVD using a tissue engineering approach.

More recent tissue engineering efforts have moved away from using traditional porous foam scaffolds for regeneration, which bear little resemblance to the natural ECM. Instead, researchers are designing biomimetic scaffolds with structural cues that significantly impact both the mechanical functionality of the scaffold and the progress of *de novo* tissue growth. Engineering a mechanically functional AF-like tissue with cross-ply collagen fibrils using an electrospun nanofibre scaffold is a key recent advance (Nerurkar et al., 2009, 2010a). The creation of an engineered IVD with compressive properties that are comparable to a healthy IVD, using an alginate hydrogel surrounded by a fibrillar collagen gel, is also promising (Bowles et al., 2011).

Yet there is still much that remains unresolved. Several modelling approaches have emphasised the role of poroelastic fluid flow and mechanical coupling in nutrient transfer in the IVD (Ferguson et al., 2004; Huang & Gu, 2008; Malandrino

et al., 2011). However, the time-dependent behaviour of scaffolds for both the NP and the AF has largely been overlooked. A tensile-compressive nonlinearity in the IVD may enhance shock absorption and nutrient transfer due to increased fluid pressurisation (Huang & Gu, 2007), but the use of fibre-reinforced composites for IVD tissue engineering has only been investigated to a limited extent. There is a need to develop materials with more sophisticated controllable mechanical properties that can be used to regenerate the IVD.

Nevertheless, the ideal mechanical properties of an IVD scaffold are not known. Mimicking the properties of the native IVD is a sensible strategy, but these properties may not be sufficient to encourage the development of mechanically functional tissue. Hence, this thesis has focused on creating materials with properties that can be systematically altered over a wide range. In chapter 3, it was demonstrated that time-dependent behaviour could be tuned by combining two hydrogels with different degrees of time-dependent behaviour. The work is certainly not the first to propose the use of composite gels, but it is the first to show that the time-dependent response of composite hydrogels can be systemically altered independently of the elastic modulus.

In chapter 4, the manufacture of thick electrospun fibre-reinforced hydrogels with varying compressive and tensile moduli was reported. Electrospun fibres have become increasingly popular materials for tissue engineering of soft tissues due to their similarity to the fibrous networks found *in vivo* (Cipitria et al., 2011; Koepsell et al., 2011; Nerurkar et al., 2007). However, the compressive properties of electrospun fibres have not been investigated previously. Here, the compressive properties of the fibre-reinforced hydrogels (including time-dependent behaviour) were dominated by the properties of the hydrogel phase, while the tensile properties were largely a function of the fibre volume fraction.

The large strain-to-failures of these composites compared to unreinforced alginate is also intriguing. Failure and strength have only been explored to a limited extent in this thesis, but these properties will be important to ensure the success of any implant. A recent study investigating the *in vivo* biomechanical performance of a hydrogel nucleus replacement noted that nearly 50% of the implants were damaged on retrieval (Smolders et al., 2012). The results presented here are promising as they suggest an opportunity to create hydrogel-like materials with

7.1 Conclusions

sufficient strength and toughness to withstand the loading encountered during surgical implantation and *in vivo* use.

The materials developed in chapters 3 and 4 are intended to be examples of how structure-property relationships present in the disc can be mimicked and systematically altered. Further characterisation and development would be required if any of the materials were to be used *in vivo*. In terms of biological factors, properties such as biocompatibility and biodegradability need to be determined. In terms of mechanical factors, the response of the materials at large-strains under physiological conditions requires investigation. The strain fields applied by the characterisation techniques used here (indentation, tension and unconfined compression) are likely to be different to those applied *in vivo*, where the NP exists in a semi-confined swollen state, and the AF is simultaneously placed under tensile and compressive forces. Effort has been made to relate these measurements to more physiologically relevant variables, such as the aggregate modulus measured in confined compression, though given the complex nature of these materials these efforts should only be treated as estimates. The characterisation has been performed assuming small strains and isotropic linear material properties though the IVD is anisotropic and highly nonlinear. *In vivo* strains in the AF could potentially be as large as 0.2 (Schroeder et al., 2006) and these properties are likely to have a significant impact on the performance of a successful IVD scaffold. Once desirable materials have been identified, more comprehensive and direct measurements should be performed on the materials to ensure that an accurate representation of the mechanical behaviour is obtained.

Nevertheless, in this study, in contrast to many previous studies, substantial characterisation of the time-dependent behaviour of the materials was performed. Time-dependent behaviour is crucial to the biomechanical function of the IVD and both the AF and NP are poroviscoelastic (Nerurkar et al., 2010a). Viscoelastic behaviour affects the frequency-dependent response of the IVD and provides a mechanism for energy dissipation (Costi et al., 2008; Race et al., 2000). Long term exposure to vibration is one of the strongest risk factors for IVD degeneration (Virtanen et al., 2007). Over longer time-scales, poroelastic behaviour plays a strong role in the nutrition of the vascular intervertebral disc; fluid transfer is associated with the transfer of nutrients and waste (Ferguson et al., 2004;

Huang & Gu, 2008; Malandrino et al., 2011). Poroelastic behaviour also plays a significant role in preventing mechanical damage. Clinically, IVD prolapse (where the NP bulges out through a weakness in the AF) is strongly associated with the repetition of lifting and bending motions (Kelsey et al., 2005). Measurements of hydrostatic fluid pressure in human subjects have shown that fluid pressure can increase by over four times when heavy objects are lifted (Wilke et al., 1999). During sustained loading or repetitive bending motions, fluid pressurisation is reduced as fluid flows out of the NP (over the course of hours) and more stress is transferred to the solid skeleton, increasing the likelihood of damage. (Adams & Dolan, 1996; Adams et al., 1996a). To further develop biomimetic materials for IVD tissue engineering, it will be necessary to better understand the structure-property relationships that lead to this time-dependent behaviour and improve current characterisation methods.

Part of the reason why knowledge of the time-dependent behaviour of materials for IVD tissue engineering is limited is due to the difficulty of characterising poroelastic and PVE mechanical properties. Methods to determine these properties have existed for decades (Biot, 1956; Mak, 1986). These methods have been used to characterise the mechanical behaviour of articular cartilage and, to a more limited extent, the IVD (Huang et al., 2003; Mow et al., 2005; Périé et al., 2005). Yet they have seldom been used to characterise the performance of tissue engineering scaffolds. These methods generally require complicated experimental setups (e.g. confined compression) and/or multiple test rigs (unconfined compression, and shear or tension), with correspondingly difficult sample preparation. Analysis of the experimental data of PVE materials frequently requires the use of FE methods. Even still, there are questions about the reliability of these methods (Chiravambath et al., 2009). While it is not too onerous to perform multiple types of characterisation on a single material, as was done for 3% agar in chapter 6, this becomes impractical when screening a wide range of materials and structures for desirable properties.

It was a primary aim of this work to develop methods to characterise the time-dependent behaviour of hydrogels that were rapid, robust and simple to implement. Agbezuge & Deresiewicz (1974), Galli & Oyen (2009), and Hu et al. (2010) have all demonstrated that spherical indentation is a promising method

7.1 Conclusions

for determining the poroelastic properties of a material. Poroelastic relaxation proceeds quickly during indentation due to the small length-scales over which fluid must travel. The existing methods for analysing displacement-controlled poroelastic relaxation are advantageous due to their simple form, but they neglect the effect of ramp time on relaxation, which as was demonstrated in chapter 5, can be significant. Here, a method to characterise the poroelastic response of a material tested with indentation, regardless of ramp time, was developed, based upon a semi-analytical ramp correction factor approach. The method ensures that consistent effective poroelastic properties will be obtained when indents are carried out at multiple scales, as the effect of ramp time varies with the size of the contact region.

Effective poroelastic properties can provide useful information about a material regardless of whether the material exhibits solely poroelastic behaviour. The permeability is related to the rate of relaxation and the Poisson's ratio is a function of the degree of relaxation. Mizuno et al. (2006), Moss et al. (2010) and Bowles et al. (2011) characterised effective poroelastic parameters of an IVD scaffold and compared these properties with those of the IVD to varying degrees of success. In chapter 3, the effective poroelastic properties of composites of alginate, agar and gelatin were determined. However, most scaffolds and tissues exhibit both poroelastic behaviour and flow-independent viscoelastic behaviour. Determining PVE properties will allow more accurate comparisons between materials to be made and will increase confidence in predictions of mechanical response in deformation modes other than those in which the measurement was made.

In chapter 6, it was shown that the load-relaxation responses of PVE materials can be separated into poroelastic and viscoelastic relaxation responses, providing intuitive insight into the time-dependent behaviour of these complex materials. With this understanding, a framework for rapidly characterising PVE materials and predicting their load-relaxation response was developed. The framework also provides a means for simple visualisation of the separate poroelastic or viscoelastic mechanisms. The approach was then used to examine the time-dependent behaviour of an agar hydrogel. It is hoped that this method will be adopted by researchers characterising the mechanical properties of IVD scaffolds—it takes only a slightly longer period of time than performing a single unconfined compression

test but provides substantially more information. It also does not require any extra equipment. The approach will facilitate the rapid screening of a wide range of materials and structures for desirable PVE properties. Once suitable materials have been identified, further characterisation can then be performed investigate their behaviour in more physiological conditions.

7.2 Future directions

7.2.1 Performance of an intervertebral disc scaffold

In this work, structure-property relationships for the design of an IVD scaffold have been explored. A particular emphasis has been placed on time-dependent behaviour. However, it is unclear how these properties will impact the performance of a full IVD scaffold. Future investigations should focus on determining how these properties influence biomechanical function, cell function and *de novo* tissue growth.

Mechanical functionality

The IVD must remain flexible in bending (where the AF is both in compression and tension), while being stiff in compression. It must also reduce the effect of shock loading on surrounding tissues through the viscous dissipation of energy. Achieving this functionality remains a significant challenge in IVD tissue engineering.

The material design frameworks presented in chapters 3 and 4 provide a means to experimentally determine the effect of the time-dependent mechanical properties and tensile-compressive nonlinearities of a total IVD scaffold on the biomechanics of a spinal motion segment. A scaffold could be created with a central hydrogel region surrounded by electrospun fibre laminates. The mechanical performance of the scaffold could be investigated both with and without hydrogel infiltrated electrospun scaffolds, and with both viscous and elastic hydrogels.

Further insight into the effect of systematically varying these properties could be obtained using FE modelling. Previous FE work by Joshi et al. (2009) investigated how the compressive mechanical properties of the IVD were affected by

7.2 Future directions

the stiffness and Poisson's ratio of a nucleus replacement. Similar studies that consider more complex material properties and structures have not yet been performed. Key computational results could then be validated using experimental models. These findings would inform the design of mechanically functional IVD scaffolds. They would provide insight into the degree by which infiltrating electrospun fibre laminates affects the bending flexibility of a total IVD scaffold, and the roles of poroelasticity and viscoelasticity in dissipating energy from shock loading.

Biological functionality

Mesenchymal stem cells are an advantageous cell source for IVD tissue engineering as there is a near unlimited supply that can be obtained from the body. The cells can be directed down AF and NP lineages. The differentiation of mesenchymal stem cells is affected by the mechanical properties of the substrate on which they are cultured. Engler et al. (2006) demonstrated that mesenchymal stem cells will commit to different phenotypes when cultured on polyacrylamide hydrogels of different stiffnesses. A recent study by Trappmann et al. (2012) (for which this author contributed mechanical characterisation results) demonstrated that mesenchymal stem cells cultured on polydimethylsiloxane (PDMS) were not affected by changes in stiffness. Stem cells seeded on polyacrylamide were, suggesting that the permeability or pore size of the polyacrylamide was potentially the deciding factor of cell fate. In contrast, work performed by Murrell et al. (2011) using PDMS elastomers has suggested that flow-independent viscoelastic effects may play a substantial role in guiding cellular behaviour.

Similarly, AF and NP cells are sensitive to the mechanical properties of their substrate (Baer et al., 2001). Furthermore, they exhibit different responses depending on their loading conditions, including dynamic versus static compression (Wang et al., 2007), and unconfined compression versus confined compression (Wang et al., 2011). The cells are sensitive to hydrostatic pressure and fluid shear stress (Reza & Nicoll, 2008; Wang et al., 2011). Future work must be performed to elucidate how stem cells and IVD cells respond to their substrate.

The composite gels created in chapter 3 and the PVE analysis framework developed in chapter 6 may provide a platform to further investigate how cells respond to PVE materials. The response of cells cultured on or within hydrogels with differing degrees of poroelastic and viscoelastic behaviour could be investigated. This would lead to a better understanding of how cell fate and behaviour can be influenced using a mechanical environment, providing guidelines for the design of scaffolds for IVD tissue engineering.

***De novo* tissue growth**

The primary motivation for this work is to create a functional engineered IVD that can be used to replace degenerated tissue and treat back pain. While there have been several recent advances in this field (Bowles et al., 2011; Nerurkar et al., 2010c), it is still questionable how well any scaffold will perform at regenerating tissue in a human; human IVDs are much larger than those of sheep or mice and the diffusion distances are therefore much larger. Increasing the diffusion distance by as little as 10% may have a substantial effect on oxygen levels (up to 57%) in the central NP (Malandrino et al., 2011). Nerurkar et al. (2010b) cultured electrospun scaffolds seeded with mesenchymal stem cells, while subjecting the scaffolds to dynamic loading using an orbital shaker. The group found that dynamic culture produced a large improvement in cellular survival throughout the scaffold and hypothesised that it may have enhanced nutrient waste exchange. Nutrient transport is affected by the applied loading conditions, the size of the material and the poroelastic and diffusion properties of that material.

The effect and importance of poroelastic behaviour in increasing nutrient transport and encouraging tissue growth could be evaluated by seeding scaffolds with stem cells and then culturing the constructs while they are subjected to mechanical loading, either in a bioreactor *in vitro*, or *in vivo*. The size and permeability of the scaffolds could be varied to determine the impact of increased diffusion distances (and the relative importance of poroelastic mechanisms) on the success of the IVD scaffold. Elucidating the role of poroelastic behaviour in nutrient transfer will help overcome the challenge of regenerating the largest avascular tissue in the human body.

7.2 Future directions

7.2.2 Mechanical behaviour of hydrogels and soft tissues

Understanding how the properties of IVD scaffolds influence biomechanical performance is part of a broader challenge in understanding the mechanical behaviour of hydrogels and soft tissues. These materials are difficult to handle and grip, and exhibit complex time-dependent behaviour. In chapters 5 and 6, significant advances were made in characterising the poroelastic and PVE properties of these materials.

Initially, the methods developed in these chapters could be used to gain further information about the hydrogel material systems developed in this work. Multi-scale indentation could be performed on several composite hydrogels to determine how their PVE properties vary with volume fraction. The Poisson's ratio of a smaller selection of composite gels could be directly characterised using unconfined compression testing. It was hypothesised that agar has a tensile-compressive nonlinearity in chapter 6. This hypothesis could be investigated by fixing the hydrogels to a platen using cyanoacrylate and then cycling between small compressive and tensile strains.

These methods are applicable to any soft tissue and hydrogel, and could be used to further investigate the mechanical properties of the NP. The NP demonstrates substantial viscoelastic behaviour in shear (Iatridis, 1997) and substantial poroelastic behaviour in confined compression (Johannessen & Elliott, 2005; Périé et al., 2006). Yet a full PVE description of the tissue is lacking. Multi-scale indentation experiments could be performed on the NP in order to determine the combined effect of poroelastic and viscoelastic relaxation, and verified against existing confined compression and shear rheometry experiments.

Similarly, Zhao et al. (2010) demonstrated that ionically crosslinked alginate exhibits PVE behaviour while covalently crosslinked alginate solely exhibits poroelastic behaviour, although the investigators did not characterise the PVE properties of these materials. The approach developed here would allow the poroelastic and viscoelastic mechanisms of the ionically crosslinked gels to be separated, elucidating whether the mechanisms of poroelasticity differ depending on the crosslinking method. This approach would provide fundamental insight into the structure-property relationships of hydrogels.

Hydrogels have applications in drug delivery, mechanical actuation and tissue engineering. Yet, their material properties have not been investigated to the same extent as metals or traditional polymers. Obtaining accurate information about the time-dependent properties of hydrogels and tissues will allow for better predictions of their mechanical response under a range of loading conditions to be made, increasing their utility as engineering materials. This knowledge will lead to the better design of implants and scaffolds and will result in improved clinical outcomes for conditions such as back pain.

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