

Contents

1	Tissue Engineering & Articular Cartilage.....	1
1.1	The structure of articular cartilage	2
1.2	Cartilage disease, injury and management	4
1.2.1	Non-surgical approaches (focus on chondroitin sulfate)	5
1.2.2	Surgical approaches.....	6
1.3	Autologous chondrocyte implantation (ACI)	7
1.3.1	History and development of ACI	7
1.3.2	The limitations of ACI	8
1.4	3D culture systems.....	9
1.4.1	Preventing dedifferentiation in monolayer cultures.....	10
1.4.2	Biomimetic systems.....	11
1.4.3	Mechanical stimulation	12
2	Scope of the thesis	15
3	Materials and methods.....	19
3.1	Materials	19
3.2	Instruments.....	21
3.3	Protocols	24
4	Layer-by-Layer Films Made from Extracellular Matrix Macromolecules on Silicone Substrates	35
4.1	Current monolayer culturing techniques	35
4.2	Build-up of Col1/CS and Col1/HN films on PDMS.....	37
4.3	Effect of substrate on film build-up	41
4.4	Film topography.....	42

	4.5	Film thickness and stability.....	44
	4.6	Assessment of cell adhesion and integrin mediated spreading.....	46
	4.7	Chapter summary	51
5		Chondrocyte Culture in 3D Alginate Sulfate Hydrogels Promotes Proliferation While Maintaining Expression of Chondrogenic Markers ... 53	
	5.1	Improving chondrogenic performance of chondrocytes in 3D may be achieved using biomimetic materials.	53
	5.2	Preparation and characterization of sulfated alginate.....	55
	5.3	Morphology of chondrocytes encapsulated in sulfated alginate (DSs = 0.8)	57
	5.4	Assessment of cell proliferation within the hydrogels	59
	5.5	Decoupling stiffness from cell spreading.....	60
	5.6	RhoA and integrin signalling of chondrocytes in alginate sulfate	62
	5.7	Cyclin D1 expression is upregulated in alginate sulfate samples	64
	5.8	Expression of cartilage markers for dedifferentiated chondrocytes within alginate sulfate hydrogels.....	65
	5.9	Immunohistological staining and gross appearance of alginate sulfate hydrogels.....	66
	5.10	Chapter summary.....	69
6		GFOGER Modified MMP-Sensitive Polyethylene Glycol Hydrogels Induce Chondrogenic Differentiation of Human Mesenchymal Stem Cells	71
	6.1	Chondrogenic differentiation and the microenvironment.....	71
	6.2	Hydrogel modification did not affect mechanical properties	73
	6.3	Cell viability.....	74
	6.4	Cell morphology.....	75
	6.5	Cell proliferation was highest in RGD and GFOGER degradable hydrogels	77

6.6	Gene expression.....	78
6.7	GAG production in peptide-modified gels	79
6.8	Histology and immunostaining	80
6.9	Chapter summary	84
7	Probing the Microenvironmental Conditions for Induction of Superficial Zone Protein Expression	85
7.1	Chondrocyte dedifferentiation and superficial zone protein.....	85
7.2	Both SZP and Col2 undergo dedifferentiation during serial passaging	87
7.3	Redifferentiation of serially passaged primary chondrocytes.....	88
7.4	Design and evaluation of a 3D tension and compression chamber compatible with the strex strain machine	90
7.5	Application of cyclic mechanical strain to 2D and 3D constructs.....	92
7.6	Effect of mechanical strain on SZP expression	94
7.7	Effect of oxygen tension on expression of SZP.....	95
7.8	Cell morphology and the expression of SZP	98
7.9	Chapter summary	105
8	Conclusions and outlook.....	107
9	References	109
	Curriculum Vitae	125