

Table of contents

Acknowledgements	7
Table of contents	9
List of figures	16
List of tables	20
List of appendices	21
Abstract	23
Resumen	24
Resum	25
CHAPTER 1	27
Introductory remarks	27
1.1 Introduction	29
1.2 Hypothesis and objectives	31
1.3 Thesis outline	33
CHAPTER 2	35
Hyaline cartilage: structure, pathologies and therapeutic alternatives	35
2.1 Summary	37
2.2 Hyaline cartilage	37
2.2.1 Embryological development of the articular cartilage and the growth plate	38
2.2.2 Anatomy of cartilaginous structures in long bones	39
2.2.2.1 Articular cartilage.....	39
2.2.2.2 Growth plate.....	42
2.2.3 Molecular components of the articular cartilage and the growth plate.....	43
2.2.3.1 Articular cartilage.....	43

Table of contents

2.2.3.2	Growth plate.....	46
2.2.4	Biomechanical properties of hyaline cartilage	48
2.3	Pathologies affecting hyaline cartilage.....	50
2.3.1	Articular cartilage.....	51
2.3.2	Growth plate.....	51
2.4	Treatments to restore hyaline cartilage	52
2.4.1	Noninvasive treatments.....	52
2.4.2	Invasive treatments.....	53
2.5	Hydrogels used for hyaline cartilage regeneration.....	54
2.5.1	Hyaluronic acid – gelatin hydrogels.....	56
2.5.1.1	Hyaluronic acid	56
2.5.1.2	Gelatin.....	57
2.5.1.3	Enzymatic cross-linking of hyaluronic acid – gelatin	58
2.5.1.4	Advantages of hyaluronic acid – gelatin hydrogels	60
2.5.1.5	Effect of hyaluronic acid – gelatin hydrogels in chondrogenesis.....	62
CHAPTER 3	64
Mechanobiological modeling of endochondral ossification	64
3.1	Summary	66
3.2	Introduction	67
3.3	Materials and methods	70
3.3.1	Experimental data.....	70
3.3.1.1	Tissue samples.....	70
3.3.1.2	Measurement of length and thickness	70
3.3.1.3	Alcian blue/Alizarin red staining	71
3.3.1.4	Histological and image analysis.....	71

3.3.1.5	Micro-CT.....	71
3.3.2	Mathematical model.....	72
3.3.2.1	Geometrical model and boundary conditions.....	72
3.3.2.2	Mechanical factors and material properties.....	73
3.3.2.3	Molecular regulatory system.....	74
3.3.2.4	VEGF diffusion.....	78
3.3.2.5	Growth rate.....	78
3.3.2.6	Bone collar formation.....	80
3.3.2.7	Bone elongation and ossification.....	80
3.3.2.8	Model Implementation.....	81
3.4	Results.....	83
3.4.1	Femur Morphogenesis.....	83
3.4.2	Biochemical Regulation.....	85
3.4.3	Hydrostatic Stress and Fluid Velocity Distribution	87
3.5	Discussion.....	89
3.6	Concluding Remarks.....	91
CHAPTER 4	93
	Biophysical stimuli: a review of electrical and mechanical stimulation in hyaline cartilage.....	93
4.1	Summary.....	95
4.2	Introduction.....	95
4.3	Electrical stimulation for tissue engineering hyaline cartilage.....	97
4.3.1	Electric fields.....	97
4.3.1.1	In vivo studies.....	97
4.3.1.2	In vitro studies.....	98
4.3.1.3	Studies performed in explants and 3-Dimensional cartilage constructs.....	101

Table of contents

4.3.1.4	Effect of EFs over the growth plate.....	101
4.3.2	Electromagnetic fields.....	101
4.3.2.1	In vivo use of EMFs to heal osteoarthritis.....	103
4.3.2.2	In vitro studies using cell cultures.....	103
4.3.2.3	Studies performed in explants 3-Dimensional cartilage constructs.....	103
4.3.2.4	Effect of the EMFs over the growth plate	104
4.4	Mechanical Stimulation for Tissue Engineering Hyaline Cartilage.....	105
4.4.1	Compressive loads.....	105
4.4.1.1	In vivo studies	105
4.4.1.2	In vitro studies: tissue cultures	108
4.4.1.3	In vitro studies: cell cultures	108
4.4.2	Tension.....	109
4.4.3	Hydrostatic pressure.....	109
4.4.3.1	In vitro studies: tissue cultures	109
4.4.3.2	Studies performed in cartilage explants and 3- Dimensional constructs	110
4.4.4	Shear stress.....	110
4.5	Cellular mechanisms involved in transduction of biophysical stimuli	112
4.6	Perspectives.....	114
CHAPTER 5	116
An <i>in vitro</i> chondrocyte electrical stimulation framework....		116
5.1	Summary	118
5.2	Introduction	118
5.3	Materials and methods	120
5.3.1	Chondrocyte isolation and cell culture.....	120

5.3.2	Chondrocyte culture medium characterization and EF estimation	122
5.3.2.1	Capacitance and permittivity of the cell culture medium (C_{cm} , ϵ_{cm})	122
5.3.2.2	Complex permittivity and conductivity of the cell culture medium (ϵ_{jcm} , σ_{cm})	123
5.3.2.3	Estimation of EFs	124
5.3.3	Electrical stimulation device	125
5.3.4	Electrical stimulation assay	126
5.3.5	Proliferation and cell death assay	126
5.3.6	Glycosaminoglycan quantification.....	126
5.3.7	Statistical analysis	127
5.4	Results	127
5.4.1	Cell culture medium characterization and EF estimation	127
5.4.2	Proliferation and cell death.....	129
5.4.3	Glycosaminoglycans synthesis.....	129
5.5	Discussion	133
5.6	Concluding Remarks	137
CHAPTER 6.....		138
Capacitively coupled electrical stimulation of rat chondroepiphysis explants.....		138
6.1	Summary	140
6.2	Introduction	140
6.3	Materials and methods	144
6.3.1	Chondroepiphysis isolation and in vitro culture... ..	144
6.3.2	Electric field estimation	144
6.3.3	Electrical stimulation assay	145

6.3.4	Growth plate histological analysis	148
6.3.4.1	Masson's trichrome staining	148
6.3.4.2	Quantitative analysis of growth plate zones	148
6.3.5	Statistical analysis	149
6.4	Results	150
6.4.1	Estimation of EFs	150
6.4.2	Histomorphometric of the growth plate	152
6.4.2.1	Thickness and morphology of growth plate zones 152	
6.4.2.2	Columnar analysis of growth plate zones.....	155
6.5	Discussion	159
6.6	Concluding Remarks	161
CHAPTER 7		163
Electrical stimulation enhances chondrogenic differentiation of mesenchymal stem cells cultured in hyaluronic acid – gelatin hydrogels		163
7.1	Summary	165
7.2	Introduction	166
7.3	Materials and methods	168
7.3.1	Materials.....	168
7.3.2	Synthesis of HA-GEL hydrogels.....	170
7.3.3	Tyramine substitution degree in HA and GEL.....	171
7.3.4	Hydrogels preparation	171
7.3.5	Rheological measurements of HA–GEL hydrogel	172
7.3.6	Dielectric constants of HA–GEL hydrogels and culture media	172
7.3.7	Estimation of EFs	173
7.3.8	Mesenchymal stem cell isolation and cell culture	174

7.3.9	Electrical stimulation assay	175
7.3.10	Morphology and cell proliferation assay	175
7.3.11	Assessment of chondrogenic markers	176
7.3.12	Gene expression assay	177
7.3.13	Statistical analysis	178
7.4	Results	178
7.4.1	HA molecular weight	178
7.4.2	Tyramine substitution degree in HA and GEL.....	179
7.4.3	Mechanical properties of the hydrogels	180
7.4.4	Electrical properties of the hydrogel	182
7.4.5	Distribution of EFs	184
7.4.6	Morphology and cell proliferation	185
7.4.7	Chondrogenic differentiation	187
7.5	Discussion	191
7.6	Concluding remarks	195
CHAPTER 8	196
Conclusions and perspectives	196
Bibliography	202
Appendices	234