

Index

<u>Abstract</u>	<u>1</u>
<u>Resumen</u>	<u>3</u>
<u>Resum</u>	<u>5</u>
<u>General objective and specific purposes</u>	<u>7</u>
<u>1. Introduction</u>	<u>9</u>
1.1. <u>Connective tissue: Bone and cartilage</u>	<u>11</u>
1.1.1. <u>Bone</u>	<u>11</u>
1.1.2. <u>Cartilage</u>	<u>15</u>
1.2. <u>Regenerative capacity of bone and cartilage</u>	<u>18</u>
1.2.1. <u>Bone healing</u>	<u>18</u>
1.2.2. <u>Cartilage healing</u>	<u>20</u>
1.3. <u>Cell free and cell based approaches in cartilage and bone tissue engineering</u>	<u>22</u>
1.3.1. <u>Bone tissue engineering cell-free approach: Biomaterials and mineral composite scaffolds</u>	<u>25</u>
1.3.2. <u>Cartilage tissue engineering construct approach: Polymer/hydrogel scaffolds and cells</u>	<u>31</u>

1.3.2.1	Cells and cell culture protocols for cartilage tissue engineering. Cell-scaffold constructs	34
1.3.2.1.1.	Oxygen level tension	38
1.3.2.1.2.	Mechanical loading stimulation	40
1.3.2.1.3.	Coculture	41
2.	Materials and methods	45
2.1.	Materials	47
2.1.1.	Polymers and chemicals	47
2.1.1.1.	Polycaprolactone	47
2.1.1.2.	Polylactic acid	47
2.1.1.3.	Elvacite 2043	47
2.1.1.4.	Hydroxyapatite	48
2.1.1.5.	Bioglass [®] 45S5	48
2.1.1.6.	Hyaluronic acid	48
2.1.1.7.	Divinyl sulfone	49
2.1.1.8.	Tyramine	49
2.1.1.9.	Alginate	49
2.1.1.10.	Fibronectin	50
2.1.2.	Cells and culture medium	50

<u>2.1.2.1. Cells</u>	<u>50</u>
<u>2.1.2.1.1. Cell lines:</u>	<u>50</u>
<u>2.1.2.1.1.1. MC3T3-E1.</u>	<u>50</u>
<u>2.1.2.1.2. Primary cell culture</u>	<u>51</u>
<u>2.1.2.1.2.1. Human chondrocytes</u>	<u>51</u>
<u>2.1.2.1.2.2. Bovine chondrocyte</u>	<u>51</u>
<u>2.1.2.1.2.3. Mesenchymal stem cells</u>	<u>52</u>
<u>2.1.2.2. Cell culture medium</u>	<u>52</u>
<u>2.2. Methods</u>	<u>54</u>
<u>2.2.1. Scaffold manufacture</u>	<u>54</u>
<u>2.2.1.1. Polymeric scaffolds obtained through freeze-extraction and particle leaching mixed technique.</u>	<u>54</u>
<u>2.2.1.2. Polymer-ceramic composite scaffolds fabrication</u>	<u>55</u>
<u>2.2.1.3. Polycaprolactone scaffold composites obtained by biomimetic apatite coating</u>	<u>56</u>
<u>2.2.1.4. Polycaprolactone scaffold coating with hyaluronic acid</u>	<u>58</u>
<u>2.2.1.5. Tyramine substituted hyaluronic acid tyramine substituted hyaluronic acid</u>	<u>61</u>
<u>2.2.1.5.1. Tyramine substituted hyaluronic acid crosslink</u>	<u>61</u>
<u>2.2.2. Physico-chemical characterization</u>	<u>62</u>

<u>2.2.2.1. Scanning electron microscopy and cryo scanning electron microscopy</u>	<u>62</u>
<u>2.2.2.2. Energy dispersion X-ray analysis</u>	<u>62</u>
<u>2.2.2.3. Differential scanning calorimetry</u>	<u>63</u>
<u>2.2.2.4. Ceramic content in composite samples</u>	<u>63</u>
<u>2.2.2.5. Polymer content in blends and hybrid samples</u>	<u>64</u>
<u>2.2.2.6. Porosity measurement by gravimetry</u>	<u>65</u>
<u>2.2.2.7. Water absorption behaviour</u>	<u>66</u>
<u>2.2.2.8. Mechanical analysis: Compression assays</u>	<u>66</u>
<u>2.2.2.9. Dynamic mechanical analysis: Equilibrium and dynamic modulus</u>	<u>67</u>
<u>2.2.2.10. Stability in physiological medium</u>	<u>68</u>
<u>2.2.2.11. Degradation study</u>	<u>69</u>
<u>2.2.3. Cell Culture</u>	<u>69</u>
<u>2.2.3.1. Disinfection protocol, sample preconditioning and cell seeding protocol</u>	<u>69</u>
<u>2.2.3.2. Normoxia and hypoxia</u>	<u>73</u>
<u>2.2.3.3. Hydrostatic pressure</u>	<u>74</u>
<u>2.2.3.4. Co-culture</u>	<u>75</u>
<u>2.2.4. Biological characterization</u>	<u>77</u>

2.2.4.1. Cytotoxicity determined by MTS	77
2.2.4.2. Sample enzymatic digestion for biochemical assays	78
2.2.4.3. DNA content	79
2.2.4.4. Sulfated glycosaminoglycans content	79
2.2.4.5. Hydroxyproline content	80
2.2.4.6. Collagen type II and X ELISA	81
2.2.4.7. Alkaline phosphatase analysis	82
2.2.4.8. Scanning electron microscopy	83
2.2.4.9. Sample inclusion	83
2.2.4.10. Immunostaining	84
2.2.4.11. Histochemistry	86
2.2.4.12. Dynamic mechanical analysis of cell-scaffolds constructs.	87
2.3. .Statistical analysis	87
3. Macroporous PCL composite scaffolds for bone tissue engineering:	
Results and discussion	91
3.1. Abstract	93
3.2. Characterization and validation of ceramic-polymer composite scaffolds	95
3.3. Development and evaluation of polymer based composite scaffolds:	
Development of composite scaffolds series	97
3.3.1. Morphology and microstructure	99

3.3.2. Crystallinity and ceramic content	104
3.3.3. Mechanical properties	105
3.3.4. Discussion	107
3.3.5. Conclusions	110
3.4. Development and evaluation of polymer based composite scaffolds: PCL/PLLA composite scaffolds degradation study	111
3.4.1. Morphology and physico-chemical properties	113
3.4.2. Morphology and microstructure evolution	115
3.4.3. Weight loss evolution	117
3.4.4. Mechanical properties	119
3.4.5. Discussion	121
3.4.6. Conclusions	123
3.5. Annex: <i>In vitro</i> and <i>in vivo</i> scaffold evaluation as potential spinal fusion strip	125
3.5.1. Discussion	126
3.6. Chapter discussion	127
4. Macroporous PCL constructs for cartilage tissue engineering: Results and discussion.	129
4.1. Abstract	131

<u>4.2. Development of PCL+HA hybrid scaffolds: characterization and validation.</u>	<u>133</u>
<u>4.2.1. Scaffold and coating structure</u>	<u>135</u>
<u>4.2.2. Compression properties</u>	<u>137</u>
<u>4.2.3. Cell morphology and behaviour</u>	<u>138</u>
<u>4.2.4. Quantitative biochemical assays</u>	<u>143</u>
<u>4.2.5. Discussion.</u>	<u>147</u>
<u>4.2.6. Conclusions</u>	<u>151</u>
<u>4.3. Characterization of hypoxia as culture condition to develop an <i>in vitro</i> construct</u>	<u>153</u>
<u>4.3.1. Mechanical properties</u>	<u>155</u>
<u>4.3.2. Cell behaviour and differentiation</u>	<u>157</u>
<u>4.3.3. Discussion</u>	<u>164</u>
<u>4.3.4. Conclusions</u>	<u>167</u>
<u>4.4. Characterization of mechanical stimulation to improve hypoxia effect</u>	<u>169</u>
<u>4.4.1. Mechanical properties</u>	<u>171</u>
<u>4.4.2. Cell behaviour and differentiation</u>	<u>173</u>
<u>4.4.3. Discussion</u>	<u>181</u>
<u>4.4.4. Conclusions</u>	<u>184</u>
<u>4.5. Characterization of coculture as culture condition to develop an <i>in vitro</i> construct</u>	<u>185</u>

4.5.1. Characterization of materials	190
4.5.2. Initial adhesion of MSC to HA or FN-modified PCL scaffolds	192
4.5.3. DNA and proliferation	195
4.5.4. Extracellular matrix synthesis	197
4.5.5. Histological stains	200
4.5.6. Expression of collagen type II	204
4.5.7. Expression of hypertrophic marker	206
4.5.8. Discussion	208
4.5.9. Conclusions	212
4.6. Chapter discussion	215
5. Conclusions	219
Glossary	225
Figure index	227
Table index	235
Literature	237

