Development and Characterisation of Completely Degradable Composite Tissue Engineering Scaffolds

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Barcelona, July 2007

Chapter 8: Conclusions

- This thesis is an in-depth study of the development and characterisation of scaffolds for tissue engineering. Two processing techniques have been applied: solvent casting and phase separation. The materials used are polylactic acid (PLA) and a soluble calcium phosphate glass. The PLA is meant to give a highly porous structure, and the glass particles are meant to reinforce the mechanical and biological properties of the scaffolds. The properties of the scaffolds made via both methods, have been analysed and compared.

- The Solvent Casting method is easy to implement in the laboratory. The total porosity and pore size can be easily controlled by means of the amount and size of the NaCl particles respectively. The addition of glass in the scaffolds, does not affect the porosity. The mechanical properties of the scaffolds are rather low (100 - 300 kPa); the glass particles further weaken the structure.
- The Phase Separation procedure is more difficult to implement and to control than solvent casting. Once the processing parameters are mastered, however, they can tailor the porosity, pore size, and polymer crystallinity. The addition of H_2O in the solvent mix adds microporosity within the pore walls, and reduces the coating of the glass particles by the PLA. The mechanical properties of the phase-separated scaffolds are higher than the solvent cast ones (approx. 10MPa); the addition of glass particles reinforces the structure.

- The degradative behaviour of the scaffolds is clearly dependent on the scaffold processing parameters (despite having the same composition). Scaffolds made by solvent casting are more affected by 10 weeks of degradation than phase-separated ones. The mechanical properties and the porosity of the solvent cast scaffolds vary during degradation, whereas they do not for phase separated ones. In both cases, however, there is a 10% weight loss, probably due to the loss of glass particles that fall out of the structure.
- The surface characterisation of the scaffolds was performed on composite films that were meant to mimic the pore walls of the 3D structures. The addition of the

glass particles influences the surface properties of the films, especially the hydrophilicity that increases significantly. This is only true, however, if the glass particles are not completely coated with the PLA, if they are, they only contribute to the physical roughness and not to the chemistry of the surface. The surface energy of the films correlated well with the protein adsorption they sustained.

- Both types of scaffolds sustain cell attachment, growth, and differentiation in static culture conditions. Solvent cast scaffolds allowed for higher cell penetration; cells spread and dwelled within the interior of the scaffolds. Phase separated scaffolds tended to induce a thick outer layer of cells, without much cell penetration. Phase separated scaffolds sustained higher cell differentiation.

- The different processing techniques produce scaffolds with advantages and disadvantages in different aspects. Regarding fabrication, although the solvent casting procedure is simpler to implement, both techniques require approximately the same time to produce a scaffold.
- The phase-separated scaffolds have higher mechanical and degradative properties, but both parameters may not be decisive in certain tissue engineering applications. The 10MPa stiffness of the phase-separated scaffolds is weak for most load bearing applications, thus, they would probably be used accompanied by some other form of stabilisation. The degradative properties also depend on the requirements of the implantation site. Thus, both degradative behaviours could be adequate.
- Being able to sustain cell penetration and growth without the need of dynamic culture conditions and without creating at thick layer of cells on their surface is a fundamental advantage of the solvent cast scaffolds. This characteristic would enormously simplify cell cultures prior to implantation if the scaffolds were meant to be implanted with cells in vivo. It could also enhance tissue in-growth in vivo and maybe reduce the possibility of fibrous encapsulation.
- Given the solvent cast scaffolds excellent cell penetration properties, they would be the best candidates for tissue engineering.

Chapter 9: Future work

The conclusions of this PhD thesis are not meant to be final, rather they are an outline of the significant results and inferences stemming from the research performed during the duration of the thesis. There remain, evidently, many other studies to perform as well as expanding and developing those that have been undertaken. This section will depict some ideas on future work that have evolved from this thesis.

- The phase-separation processing technique can be further optimised and mastered, concentrating especially on controlling the roughness of the pore walls and the orientation and morphology of the pores. Scaffolds with equivalent compositions, and mechanical properties, but differing in aspects such as those mentioned above would be a unique cell culture substrata, and could potentially elucidate cell response to specific phenomena such as certain aspects of roughness or exact pore size.
- The macroscopic phase separation techniques must also be improved: development of mould designs, moulding and demoulding techniques and mould materials. The objective would be to produce defect-free scaffolds with controlled pore orientation.
- Research into other applicable processing techniques which could perhaps enhance the PLA-Glass synergy could be undertaken. Scaffolds have been produced via gas-foaming with these materials, but experiments were discontinued due to doubts on pore interconnectivity.

- The loss of glass particles during degradation is a point that requires further investigation (Chapter 4). A degradation protocol should be developed, ensuring fallen glass particles are completely collected in order to determine what proportion of the glass lost is due to dissolution and what proportion is due to simple falling out of the structure. Mechanisms that strengthen the PLA-glass bond, both physically and chemically should be looked into. Perhaps by treating the surface of the glass particles, altering the fabrication technique (applying a vacuum for example), or modulating the size and quantity of glass particles

integrated into the PLA matrix. The loss of glass particles during mechanical testing should also be studied, to evaluate whether glass particles tend to loosen during elastic strain.

- The surface characterisation study as has been described in Chapter 5 can potentially give far more in-depth information. Its interpretation is limited however due to the large scatter in the results. Despite this fact, complementary studies could shed light on other interesting aspects of the materials such as the magnitude of the effects of sterilisation on the PLA as opposed to the glass particles, the effects of glass particle size on the distribution of roughness parameter such as skewness and kurtosis and their effect on cell behaviour, or the correlation between the films and pore wall morphology. Furthermore, a complete characterisation of surface energy, including the acid and base components, could reveal unique information on the chemical properties of the materials and their interaction with cells.

- This thesis only contains a preliminary protein adsorption study. Further studies characterising the nature, conformation and magnitude of protein adsorption in time and in different media would give fundamental information on the surface characteristics of the composite material. It would be interesting to elucidate the effect of the calcium phosphate glass on protein adsorption, whether it preferentially adsorbs certain proteins, and if so how would that correlate with its osteoblast-differentiating potential.

- Further cell cultures must be pursued in order to validate the results described in this thesis. Cell cultures must be performed both with MG63 cells and mesenchymal stem cells, paying special attention to the differentiation of the cells and studying the histology. The ability of the solvent cast scaffolds to sustain cell penetration without external stimulation must be characterised: maximum scaffold thickness, cell penetration rate, histological staining to