Highly Porous Bioresorbable Scaffolds with Protein Controlled Release for Tissue Regeneration Applications

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Introduction: The tendency in the last years in Tissue Engineering is to develop and study controlled release of bioactive agents from scaffolds for tissue regeneration and growth. The main obstacle to successful protein incorporation and delivery from biodegradable scaffolds is potential inactivation of bioactive molecules by exposure to high temperatures or harsh chemical environments during polymer processing. In order to address this issue, in the present study we developed and studied highly porous protein-eluting bioresorbable films for tissue regeneration applications. The high porosity was designed to enable tissue growth into the scaffold.

Methods: Poly(DL-lactic–co–glycolic acid) (PDLGA) films were prepared using the freeze drying of inverted emulsions technique, in which the continuous organic phase contains PDLGA dissolved in an organic solvent and the dispersed aqueous phase contains protein dissolved in double distilled water. This technique enables to preserve the emulsion's microstructure and to incorporate very sensitive bioactive molecules without exposing them to organic so as not to loose their activity. The enzyme horseradish peroxidase (HRP) was used as a protein source in order to examine the effect of the emulsion's formulation on the film's microstructure and on the resulting cumulative protein release. The microstructure was studied using scanning electron microscopy (SEM), and the HRP release profiles were determined using spectrophotometer for measuring the HRP concentration in the release medium at certain time points during four weeks. In addition, we investigated the effect of these films on cell adhesion and growth of human fibroblasts in culture.

Results: (a) Microstructure: A porous structure with high porosity (72%-93%) and partially interconnected pores was obtained. Some of the specimens exhibited a single pore population, but most specimens exhibited two pore populations, large and small. The specimens' microstructure was significantly affected by the emulsion formulation. An increase in the polymer content in the organic phase or in the polymer's initial molecular weight resulted in a decrease in the mean diameter of the small pore population whereas an increase in the lactic acid content in the copolymer composition resulted in a decrease in the emulsion stability and an increase in the mean diameter of the small pore population. An increase in the enzyme content resulted in thermodynamically stabilization of the emulsion and a homogenous structure. An increase in the phase ratio resulted in a decrease in the mean diameter of the small pore population and a decrease in porosity. Polyethylene glycol (PEG) addition barely affected the microstructure.

(b) Release profile and cell growth: The release profiles usually exhibited an initial burst effect, accompanied by a decrease in release rate with time, as is typical for diffusion-controlled systems. The release profile was mostly affected by the polymer's initial molecular weight and the enzyme content. An increase in the polymer's initial molecular weight resulted in a decrease in the initial burst affect and a decrease in the release rate due to changes in the microstructure decrease the enzyme's diffusion from the film. An increase in the enzyme content resulted in an increase in the initial burst effect and an increase in the release rate due to an increase in the driving force for diffusion. The other parameters had minor effects on the release profile in the examined range.
We observed cell adherence and growth with characteristics of fibroblasts on the film.

Conclusions: We have demonstrated that appropriate selection of the inverted emulsion's parameters result in desired release profile which can be adjusted to the specific application. The films developed in this study are biocompatible and can be used as a controlled release system for bioactive agents in tissue regeneration applications.

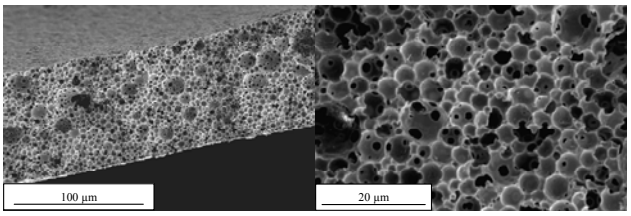

Figure 1: The microstructure of the highly porous protein-eluting scaffolds.

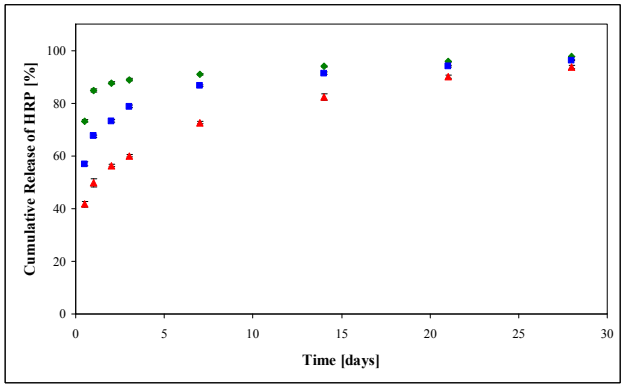

Figure 2: The effect of PDLGA's initial molecular weight on the release profile of HRP from the scaffolds:
◆ - 50,000 Da, ■ - 83,000 Da, ▲ - 185,000 Da.