Conclusions

The physical properties and the degradability make PEEA copolymers good materials for use in medicine. Endothelial cells have been shown to attach and grow on PEEA copolymers. These copolymers are processable into highly porous scaffolds, which make them suitable materials for tissue engineering.

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References


PREPARATION OF INTERCONNECTED HIGHLY POROUS POLY(D,L-LACTIDE) STRUCTURES BY SUGAR TEMPLATING AND FREEZE-DRYING

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Introduction

Tissue engineering aims at developing functional substitutes for damaged or diseased tissues, and has attracted much attention since the last decade [1–3]. In tissue engineering, three-dimensional biodegradable polymeric scaffolds play an important role as active, temporary supports for the transplantation of specific tissue cells. Besides the chemical composition of the scaffolding material, careful design of the microstructure and morphology of the porous structures is of critical importance for their success.

In general, a high porosity of the scaffold is desired in order to obtain a large specific area for cell attachment and tissue in-growth. Furthermore, the pore morphology can affect the growth of cells and even alter cell function [4]. Interconnected pores larger than the dimensions of the cells are essential for allowing infiltration of the cells into the scaffold, whereas smaller pores may influence the exchange
of nutrients and cellular waste products. Therefore, an appropriate pore size range and distribution of pore sizes is beneficial to the viability and function of the cells within the tissue-engineering scaffold.

Although several porosifying techniques (particulate leaching, gas foaming, thermally induced phase separation methods, three-dimensional printing techniques and fused deposition modelling) have been used to prepare porous polymer scaffolds, it remains a challenge to prepare scaffolds of high porosity with excellent interconnectivity and independent control of pore size.

In this study we have developed a simple and effective method for the preparation of such porous polymer structures that makes use of a leachable sugar template and freeze-drying. The sugar template ensures interconnectivity of the porous network, while the freeze-drying allows a high porosity scaffold to be obtained.

Materials and methods

Poly(D,L-lactide) (PDLLA with an L- to D-lactide ratio of 50/50, M_w = 116,000, T_g = 52.3 °C) was dissolved in 1,4-dioxane (Merck, Germany) at room temperature to make 3–15% (w/v) solutions. The sugar cube templates (Van Gils Maxi-klontjes, the Netherlands, 27.5×18×12 mm in size, crystal sugar density: 1.587 g/cm³, bulk density: 1.007 g/cm³, porosity: 36.5%) were immersed into the polymer solutions and the interstices of the templates were filled by repeated application of a low vacuum (30 mbar). The sugar does not dissolve in 1,4-dioxane.

The sugar cube templates filled with the PDLLA solution were immediately frozen at temperatures of 6–8°C and −25°C for 20 h. The solvent was then removed by freeze-drying at 0.04 mbar for 2–3 days. Subsequently, the structures were placed in gently stirred demineralised water for a period of 4–5 days to leach out the sugar templates. The resulting polymer scaffolds were vacuum dried for a period of 5 days and stored under vacuum before use.

For comparison, PDLLA scaffolds were also prepared by freeze-drying the 1,4-dioxane solutions without the sugar cube templates. The PDLLA solutions were poured into polypropylene containers (height: 70 mm, inner diameter: 10 mm, wall thickness 1 mm) and frozen at 6°C and −25°C for 20 h followed by freeze-drying.

Results and discussion

Fig. 1 shows a SEM micrograph of the fracture surface of a sugar cube template. It can be seen that it consists of a sintered structure in which the sugar particles, ranging 100–600 μm in size, are well connected. As the sugar template will be leached out with water after the freeze-drying step, this structure will reflect the connected pore network of the polymer scaffold.

Typical SEM micrographs of PDLLA scaffolds prepared by freeze-drying a 10-wt% polymer solution in 1,4-dioxane in the presence of a

Fig. 1. SEM micrograph of the fracture surface of the sugar template.
sugar template are shown in Figs. 2A and 2C. The same polymer solution freeze-dried in the absence of a template is shown in Figs. 2B and 2D.

Highly porous structures, with porosities over 97% have been prepared. Figs. 2A and 2C show that highly interconnected porous structures are obtained with large pore sizes of 100–600 \( \mu m \). This is due to the fact that the relatively large sugar particles of the template are well bound; therefore the pores obtained after leaching of the template are open and well interconnected. At the same time much smaller pores are obtained by the freeze-drying process. Figs. 2B and 2D show the effect of temperature on pore morphology as a result of freeze-drying only. It can be seen that these pores are not well interconnected. The average pore size from freeze-drying increases with increasing freezing temperature [5,6]. The final pore structure of the scaffolds is determined by the characteristics of the bound sugar particles of the template as well as by the solvent-crystal morphology after freezing of the 1,4-dioxane solution.

Conclusions

Three-dimensional porous PDLLA structures with high porosities and a well-interconnected pore network have been prepared by freeze-drying polymer solutions in the presence of a sugar template followed by leaching of the template. The final scaffold architecture consists of large interconnected pores reflecting the sugar template, and small pores resulting from the freeze-drying.
SEARCHING FOR WAYS TO IMPROVE THE INTEGRATION OF A CARTILAGE TRANSPLANT AND THE ADJACENT CARTILAGE

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Introduction

Surgical intervention in cartilage is associated with chondrocyte death in wound edges leading to the formation of an acellular band [1,2]. Integrative cartilage repair, necessary for good outcome of repair strategies (i.e. transplantation or in situ cartilage tissue generation) is probably hindered by the lack of matrix producing cells in the area where integration is required. To increase the initial adhesion of cells or cartilage tissue to wound edges, several enzymatic digestions [3,4] of wound edge surfaces have been used in an experimental setting. In addition, enzymatic extracellular matrix digestion of intact cartilage has indicated induced cell proliferation [5].

It is our aim to find a method to increase the amount of vital and matrix producing cells in cartilage wound edges. In the present study we evaluated effects of enzymatic treatment on articular cartilage wound edges. In an in-vitro explant culture model, tissue viability and integration was studied after hyaluronidase or collagenase treatment.

Methods

Full thickness cartilage discs were harvested aseptically from the metacarpophalangeal joints of 6 months old calves. Explants were pretreated with medium with 2% FCS (control), medium with 2% FCS supplemented with 0.1–3% hyaluronidase and/or medium with 2% FCS supplemented with 10–600 U/ml highly purified collagenase VII for periods varying from 1 to 48 hours. Explants were subsequently washed thoroughly and cultured for 14 days in medium with 10% FCS and 25 μg/ml ascorbic acid. Samples were harvested before treatment (t=0), after the enzymatic treatments and after 14 days of culture.

From each condition discs were fixed in formalin and embedded in paraffin. Sections (5 μm) were cut and stained with thionin for proteoglycans and HE for determination of cell death and viability. The cartilage (total thickness about 600 μm) was divided in a superficial zone (100 μm from the surface down), a deep zone (150 μm from the base up) and a middle zone (250 μm between superficial and deep zones). Thionin staining and cell numbers were measured at 200× magnification using a grid containing 50×50 μm boxes. Nuclear changes indicating cell death were scored as described by Kim et al. [6]. Wound area was defined as a band of 150 μm from the cutting edge. Thionin staining and cell numbers in this area were compared with the unwounded cartilage in the middle part of the explant at time point t = 0.

To test the integrative capacity, a central punch was taken out of a larger explant, both pieces of cartilage were treated with enzymes and the central punch was re-implanted and cultured for another 4 weeks.

Results

A gradual depletion of thionin staining was observed at lesion edges starting immediately after explantation. In addition, a rapid loss of vital cells was observed at the lesion edges, most prominent at the superficial layer. Early nuclear changes were observed in explants that were fixed within one hour after harvesting. The loss of chondrocytes lead to the formation of a low chondrocyte density band over approximately 150 μm.

In the unwounded part of the cartilage explant no cell death was observed during the experiment and the average number of vital cells remained constant.