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Designing porosity and topography of poly(1,3-trimethylene carbonate) scaffolds

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Abstract

Using phase separation micromolding (PSμM) we developed porous micro-patterned sheets from amorphous poly(1,3-trimethylene carbonate) (PTMC). The use of these PTMC sheets can be advantageous in tissue engineering applications requiring highly flexible constructs. Addition of poly(ethylene oxide) (PEO) in various amounts to PTMC casting solutions provides PTMC sheets with tailored porosity and pore sizes in the range 2–20 μm. The pore-forming effect of PEO during the phase separation process is evaluated and glucose transport measurements show that the pores are highly interconnected. Additionally, tailoring the micro-pattern design yields PTMC sheets with various surface topographies. Cell culturing experiments with C2C12 pre-myoblasts revealed that cell attachment and proliferation on these sheets is relatively high and that the micro-pattern topography induces a clearly defined cell organization.

Keywords: Tissue engineering scaffold; Phase separation; Micro-patterning; Poly(trimethylene carbonate); Cell attachment and proliferation

1. Introduction

In tissue engineering the choice of scaffold material plays an important role in the functionality of the designed scaffold for various applications. Characteristics of the material should correspond to the required properties at the site of implantation, e.g. suitable flexibility, mechanical strength and degradation in pace with tissue growth [1,2].

Amongst synthetic biodegradable biomaterials poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) and their co-polymers have been intensively studied [3,4]. However, the stiffness and degradation profiles of these materials can be unfavorable, especially when considering certain soft tissue engineering applications. Poly(1,3-trimethylene carbonate) (PTMC) is a good alternative material in these cases, due to its elasticity combined with slow degradation in aqueous solutions and rapid degradation *in vivo* via enzymatic degradation without leading to the formation of acidic products, as in the case of, for example, PLA [5,6]. Also, selecting high molecular weight PTMCs yields relatively good mechanical properties [7]. Alternatively, one can synthesize co-polymers of PTMC with other polymers, e.g. PLA and poly(ε-caprolactone) (PCL), to obtain

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a co-polymer that has improved flexibility and degradation properties compared with, for example, PLA and an increased Young modulus compared with PTMC [8–11]. *In vivo* studies using PTMC-based scaffolds have revealed the suitability of these materials for application in, for example, protein delivery systems [12] and the prevention of post-operative adhesions [13].

Besides the choice of material, scaffold design is very important for the functionality of the engineered tissue [2,14]. One of the most important requirements is ensuring a good nutrient supply to the cells, which can be achieved via scaffold inner porosity. Another important issue is cell organization, which can be influenced by varying the scaffold topography. In this way, the organization of the cells can be tuned, thereby improving the functionality of the grown tissue [15,16].

In a previous work we showed that phase separation micromolding (PSμM) enables fabrication of porous micro-patterned sheets that can be stacked into three-dimensional scaffolds [17]. Various polymers, such as poly(L-lactic acid) (PLLA), poly(D,L-lactic acid) (PDLLA) and poly(ε-caprolactone) (PCL), were processed and the most suitable PLLA fabricated sheets were selected to analyze the functionality of the micro-patterned porous sheets as scaffold sheets.

In this work we used PSμM to fabricate porous micro-patterned PTMC scaffold sheets. Porosity was introduced and tuned by adding various amounts of poly(ethylene oxide) (PEO) to PTMC casting solutions. The pore forming effect of PEO during the phase separation process of the PTMC sheets was studied and various micro-patterned PTMC sheets were manufactured by casting on properly designed molds. Micro-pattern replication and pattern and morphology stability of these PTMC sheets were studied systematically. We successfully fabricated micro-patterned porous PTMC sheets that supported cell attachment, proliferation and morphology very well.

2. Materials and methods

2.1. PTMC synthesis and characterization

PTMC synthesis is slightly modified with respect to previously reported protocols [7,11]. Briefly, in an argon atmosphere a freshly silanized dried glass ampoule (silanized with SERVA silicone solution in isopropanol; SERVA Electrophoresis GmbH, Heidelheim, Germany) was charged with trimethylene carbonate (TMC) (1,3-dioxan-2-one) monomer (polymer grade; Boehringer Ingelheim) and 1.8×10^{-4} mol stannous octoate catalyst (stannous 2-ethylhexanoate, $SnOct_2$; Sigma) per mol monomer was added. The ampoule was purged three times with dry argon, heat-sealed under vacuum and conditioned in a pre-heated oil bath. To ensure homogeneous mixing of monomer and catalyst the ampoule was shaken vigorously for the first hour of reaction. Polymerization was carried out at 130 °C for 3 days. To purify the obtained polymer was dissolved in chloroform (2% w/v solution, analytical quality; Merck), filtered and precipitated into a fivefold volume of ethanol (analytical quality, Merck). Subsequently, the precipitated polymer was collected and dried prior to use.

Monomer conversion of the synthesized polymer was characterized by nuclear magnetic resonance (NMR) spectroscopy. ^{1}H NMR (Varian Unity 300 MHz) spectra were recorded using solutions of the polymer in deuterated chloroform ($CDCl_3$; Merck).

The molecular weight, molecular weight distribution and intrinsic viscosity of PTMC were determined by gel permeation chromatography (GPC) using chloroform (p.a., filtered through a 0.2 μm Whatman Schleicher & Schuell RC58 membrane filter prior to use) as eluent. The GPC set-up consisted of a GPCmax VE-2001 GPC solvent/sample module, a series of ViscoGEL I columns and a TDA 302 triple detector array comprising a light scattering detector (RALS and LALS), a differential refractive index detector and a four-capillary differential viscometer. A polystyrene standard ($M_n = 6.4 \times 10^4$ g mol^{-1}) with a narrow molecular weight distribution was used for calibration.

Thermal properties of the pure polymers and their blends were evaluated by differential scanning calorimetry (DSC) using a Perkin Elmer Pyris1 (USA). Samples of 5–10 mg were heated from −100 °C to 100 °C at a heating rate of 10 °C min^{-1}. The glass transition temperature (T_g) was obtained from the midpoint of the heat capacity change and the melting range and peak temperature (T^m) were determined from the melting endotherm. Cyclohexane, indium and lead were used as standards for temperature calibration.

2.2. Scaffold preparation

PSμM, which was used as the fabrication method, has been described in detail in previous works [17,18].

2.2.1. PTMC

In this study the synthesized high molecular weight PTMC was dissolved in chloroform (analytical grade; Merck) at a concentration of 3 wt.%. In order to prepare porous PTMC sheets PEO (M_w 6×10^6 g mol^{-1}; Aldrich) was added to the 3 wt.% PTMC–$CHCl_3$ solution, as PEO is water soluble and can, therefore, be leached out after the phase separation process. Concentrations varying from 0.1 to 1.0 wt.% with respect to the total final casting solution were used. Table 1 gives an overview of the various casting solution compositions and the notations that will be used from this point on. The polymer solutions were cast on flat or micro-patterned molds to obtain non-patterned or micro-patterned sheets, respectively. Two micro-pattern designs were applied, which are illustrated in Fig. 1. Table 2 lists their dimensions. A mixture of ethanol (analytical grade; Merck,) and Milli-Q water at a ratio of 8:1 at room temperature (ranging from 18 to 21 °C) was

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