



Preparation and characterization of a three-dimensional printed scaffold based on a functionalized polyester for bone tissue engineering applications

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ABSTRACT

At present there is a strong need for suitable scaffolds that meet the requirements for bone tissue engineering applications. The objective of this study was to investigate the suitability of porous scaffolds based on a hydroxyl functionalized polymer, poly(hydroxymethylglycolide-co- ϵ -caprolactone) (pHMGCL), for tissue engineering. In a recent study this polymer was shown to be a promising material for bone regeneration. The scaffolds consisting of pHMGCL or poly(ϵ -caprolactone) (PCL) were produced by means of a rapid prototyping technique (three-dimensional plotting) and were shown to have a high porosity and an interconnected pore structure. The thermal and mechanical properties of both scaffolds were investigated and human mesenchymal stem cells were seeded onto the scaffolds to evaluate the cell attachment properties, as well as cell viability and differentiation. It was shown that the cells filled the pores of the pHMGCL scaffold within 7 days and displayed increased metabolic activity when compared with cells cultured in PCL scaffolds. Importantly, pHMGCL scaffolds supported osteogenic differentiation. Therefore, scaffolds based on pHMGCL are promising templates for bone tissue engineering applications.

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1. Introduction

Tissue engineering combines the principles of engineering and life sciences to provide substitutes that can restore, maintain or augment tissue function [1]. For tissue engineering the availability of biodegradable and biocompatible scaffolds is needed [2,3]. These scaffolds serve as a temporary construct and should possess certain surface characteristics, particularly hydrophilicity, to promote cell adhesion. Further, scaffolds should have adequate mechanical properties to maintain the three-dimensional (3-D) structure and gradually degrade when the new tissue and an extracellular matrix is formed. Scaffolds should be highly porous with large interconnected pores to facilitate cell growth and diffusion of nutrients and waste products into and out of the scaffold [4–6].

There are many conventional techniques to make 3-D scaffolds for tissue engineering applications, such as solvent casting, gas foaming, particulate leaching and electrospinning [7,8]. However, these technologies all have some limitations, such as the difficulty of producing an interconnected porous structure for cell growth. Among scaffold fabrication techniques those based on rapid prototyping (RP) methods, including stereolithography [9], 3-D printing

[10], selective laser sintering [11], fused deposition modeling [12] and direct wiring [13], are preferred, since these methods result in the formation of complex, well-defined and reproducible constructs via a computer-aided design (CAD) model and computer controlled (CAM) tool handling [4,14]. Another advantage is that these complex structures can be designed according to the needs of individual patients using their 3-D medical scan data [2]. Out of these RP techniques, 3-D printing (melt plotting) is an attractive method to fabricate scaffolds for tissue engineering applications, since this technology does not use possibly toxic solvents [15,16].

The preferred polymer used for RP techniques is poly(ϵ -caprolactone) [2,15,17,18]. This aliphatic polyester is a biocompatible and biodegradable polymer with a degradation time ranging from 2 to 4 years [19]. However, its surface characteristics are not favorable for cell adhesion and proliferation due to its intrinsic hydrophobicity and lack of bioactive functional groups [20,21]. In our group we have developed a new co-polyester based on PCL, i.e. poly(hydroxymethylglycolide-co- ϵ -caprolactone) (pHMGCL). This co-polymer is more hydrophilic than PCL and features a tunable degradation rate, as well as available hydroxyl functional groups for further functionalization with, for example, peptides [22,23]. We have also shown that human mesenchymal stem cells (MSC) exhibited good adherence to pHMGCL films compared with the more hydrophobic PCL surfaces. The cells survived and differentiated towards the osteogenic lineage on pHMGCL surfaces [23]. In

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the present study 3-D scaffolds of this novel hydroxyl-functionalized polyester and PCL were fabricated by means of 3-D plotting. Both polymers were synthesized and characterized for structural, thermal and molecular weight properties and the thermal and mechanical properties of scaffolds based on these polymers were studied. Furthermore, the compatibility of human MSCs with these 3-D structures was also evaluated by investigating the attachment, differentiation and metabolic activity of seeded cells.

2. Materials and methods

2.1. Materials

All chemicals used in this study were purchased from Aldrich and used as received, unless stated otherwise. All solvents were purchased from Biosolve (Valkenswaard, The Netherlands) except acetone (Merck, Darmstadt, Germany), hexane (Antonides-Interchem, Oosterzee, The Netherlands), toluene and sulfuric acid (Acros, Geel, Belgium). Toluene was distilled from P_2O_5 and stored over 3 Å molecular sieves under argon. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. 3S-benzyloxymethyl-1,4-dioxane-2,5-dione (benzyl-protected hydroxymethyl glycolide (BHMg)) was synthesized as described by Leemhuis et al. [24,25]. ϵ -Caprolactone (CL) and silica gel (0.035–0.070 mm, 60 Å) were obtained from Acros (Geel, Belgium). CL was distilled from CaH_2 . O-benzyl-L-serine was supplied by Senn Chemicals (Dielsdorf, Switzerland). Sodium nitrite ($NaNO_2$) and dimethylaminopyridine (DMAP) were purchased from Fluka (Zwijndrecht, The Netherlands). Sodium sulfate (Na_2SO_4), triethylamine, sodium carbonate (Na_2CO_3) and benzyl alcohol (BnOH) were provided by Merck (Darmstadt, Germany). Bromoacetyl bromide, tin(II) 2-ethylhexanoate ($SnOct_2$) and Pd/C (palladium, 10 wt.% (dry basis) on activated carbon, wet (50 wt.% water), Degussa type E101 NE/W) were obtained from Aldrich (Zwijndrecht, The Netherlands).

Human bone marrow aspirates were obtained during total hip replacement procedures with informed consent and approval of the local medical ethical committee. The mononuclear cell fraction was isolated by centrifuging on Ficoll-paque. Subsequently the cells were plated in growth medium containing α -MEM (Gibco) supplemented with 0.2 mM L-ascorbic acid 2-phosphate (Sigma),

10% heat-inactivated fetal bovine serum (Invitrogen), $100 U ml^{-1}$ penicillin with $100 \mu g ml^{-1}$ streptomycin (Invitrogen) and $1 ng ml^{-1}$ rhB-FGF (R&D Systems). Cells were passaged at subconfluence and seeded onto the scaffolds before passage 5. The medium was refreshed every 3–4 days during cell expansion and scaffold culture.

The isolated cells were characterized for accepted characteristics of human MSCs, such as their multilineage potential and the CD surface marker profile. Multilineage potential was confirmed by differentiation into the adipogenic, osteogenic and chondrogenic lineages as previously described [26]. Further, the MSCs were FACS analyzed for the absence or presence of established MSC markers [27]. They were negative for CD31 and CD45 and showed >90% positivity for CD73, CD90, and CD105 and thus exhibited a marker profile that agrees with that for MSCs.

2.2. Synthesis of poly(ϵ -caprolactone)

PCL was synthesized via ring opening polymerization (ROP) using BnOH and $SnOct_2$ as initiator and catalyst, respectively. In a typical procedure ϵ -caprolactone (5.78 ml, 65.9 mmol), BnOH (5.6 mg, 0.052 mmol) and $SnOct_2$ (10.5 mg, 0.026 mmol) were loaded into a dry Schlenk tube under a dry nitrogen atmosphere. The tube was evacuated for 1 h, then closed and immersed in an oil bath which was heated at 130 °C. Polymerization was performed overnight and the polymer formed was dissolved in chloroform, precipitated with cold methanol and dried overnight under vacuum. The obtained PCL was characterized by 1H NMR, gel permeation chromatography (GPC) and differential scanning calorimetry (DSC).

2.3. Synthesis of random co-polymer of CL and BHMg

(poly(benzyloxymethyl glycolide-co- ϵ -caprolactone), pBHMgCL)

A random co-polymer of BHMg and ϵ -caprolactone was synthesized by a ROP method. Briefly, ϵ -caprolactone (6.54 ml, 59 mmol) and BHMg (1.2 g, 5.1 mmol) were introduced into a dry Schlenk tube, equipped with magnetic stirrer, under a dry nitrogen atmosphere. Next, BnOH (23.14 mg, 0.214 mmol) and $SnOct_2$ (43.3 mg, 0.107 mmol) were added as initiator and catalyst, respectively.

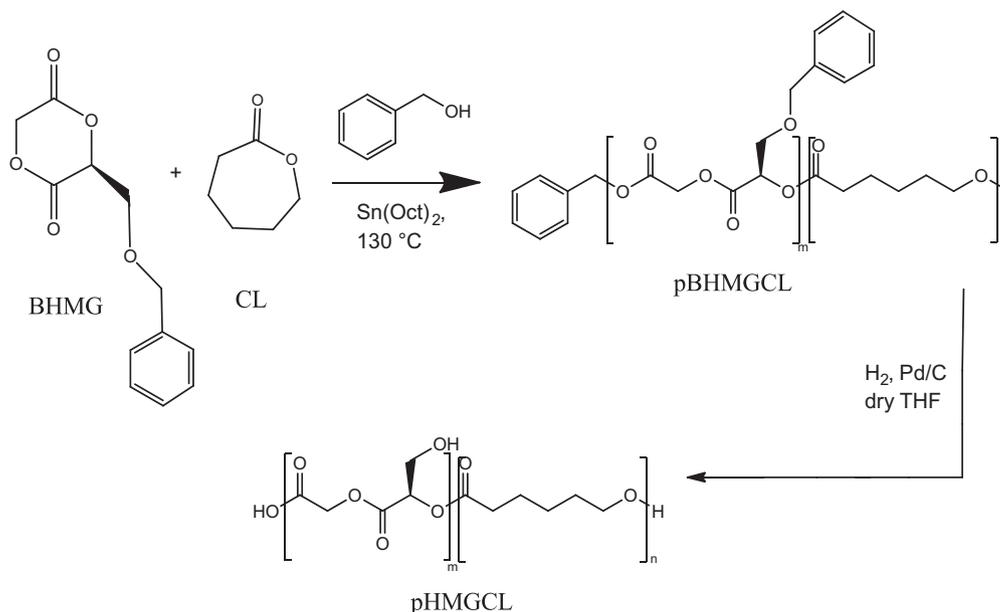


Fig. 1. Synthesis of poly(benzyloxymethyl glycolide-co- ϵ -caprolactone) (pBHMgCL) and poly(hydroxymethyl glycolide-co- ϵ -caprolactone) (pHMgCL) random co-polymers.

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