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Covalent attachment of a three-dimensionally printed thermoplast to a gelatin hydrogel for mechanically enhanced cartilage constructs

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ABSTRACT

Hydrogels can provide a suitable environment for tissue formation by embedded cells, which makes them suitable for applications in regenerative medicine. However, hydrogels possess only limited mechanical strength, and must therefore be reinforced for applications in load-bearing conditions. In most approaches the reinforcing component and the hydrogel network have poor interactions and the synergetic effect of both materials on the mechanical properties is not effective. Therefore, in the present study, a thermoplastic polymer blend of poly(hydroxymethylglycolide-co-ε-caprolactone)/poly(ε-caprolactone) (pHMGCL/PCL) was functionalized with methacrylate groups (pMHMGCL/PCL) and covalently grafted to gelatin methacrylamide (gelMA) hydrogel through photopolymerization. The grafting resulted in an at least fivefold increase in interface-binding strength between the hydrogel and the thermoplastic polymer material. GelMA constructs were reinforced with three-dimensionally printed pHMGCL/PCL and pMHMGCL/PCL scaffolds and tested in a model for a focal articular cartilage defect. In this model, covalent bonds at the interface of the two materials resulted in constructs with an improved resistance to repeated axial and rotational forces. Moreover, chondrocytes embedded within the constructs were able to form cartilage-specific matrix both in vitro and in vivo. Thus, by grafting the interface of different materials, stronger hybrid cartilage constructs can be engineered.

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1. Introduction

Hydrogels consist of hydrophilic polymer networks that are able to hold large amounts of water in a three-dimensional (3-D) structure, while maintaining their shape [1,2]. Thereby they can provide a 3-D aqueous environment to embedded cells, analogous to the extracellular matrix of natural tissues [3,4]. For tissue engineering purposes, stem cells [5,6], tissue specific cells [7] and/or bioactive factors [5,8,9] can be incorporated in the temporary hydrogel scaffold to direct specific tissue regeneration [3,10]. By doing so, hydrogel systems have already been clinically applied for the regeneration of a range of tissue types including skin, blood vessels, neural tissues and cartilage [3,11]. Unfortunately, hydrogels have limited mechanical strength due to their high water content [12,13], which may result in construct failure of the hydrogel under mechanically challenging conditions, as found in the load-bearing bone and cartilage tissues. One approach to overcoming this limitation is by stimulation of extracellular matrix formation of the cells prior to implantation, resulting in a stiffer implant [14]. However, this requires long in vitro preconditioning periods, limiting clinical translation. The most straightforward approach to directly enhance the stiffness and strength of a hydrogel is by increasing its polymer concentration and crosslinking density [15,16]. Nevertheless, this also impacts the diffusion rates of bioactive factors, nutrients and cell metabolites through the hydrogel, thereby most likely also the regenerative capacities of embedded cells [1,16,17]. Therefore, alternative strategies have been explored to increase the strength of hydrogel constructs, e.g. through the creation of interpenetrating polymer networks (IPNs) [18–22] or the incorporation of solid particles [23–25] and nanofibers/nanotubes [26–28]. Mechanical properties of hydrogels can also be improved by integrating a secondary scaffold material, such as fiber mats [29–31], randomly organized porous polylactic acid scaffolds [32,33] non-woven meshes [34] or electrospun polymer networks [35–39]. Moreover, with advances

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in biofabrication techniques, a rigid thermoplastic polymer network can be fabricated layer-by-layer along with the hydrogel construct [40–45], allowing for a tailored reinforcement of hydrogels. In addition, when mechanical stability is provided by this secondary rigid network, hydrogels with a lower crosslink density can be processed [41], which is beneficial for the viability and proliferation capacity of embedded cells [1,16,17,46]. Reinforcing the cell-laden hydrogel with a secondary scaffold material may also reduce the often-experienced swelling or shrinking of the hydrogel-based implant, and this can lead to imperfect filling of the tissue defect [47].

In the present generation of constructs, however, the reinforcing network and the hydrogel show insufficient adhesion, mostly because of their different physicochemical properties [42,48], which leads to construct disintegration upon application of mechanical stresses. Hence, the introduction of chemical bonds between both components may improve the construct strength sufficiently to withstand the challenging mechanical conditions in the musculoskeletal system.

Therefore, this study aims to mechanically enhance cartilage implants by grafting a hydrogel to a 3-D-fabricated biodegradable thermoplastic polymer network. To achieve this, the polyester poly(hydroxymethylglycolide-co-ε-caprolactone) (pHMGCL [49]) was functionalized with methacryl groups coupled to the hydroxyl side groups of this polymer. In previous studies, we have shown that pHMGCL is more hydrophilic than e.g. PCL due to the presence of the pendant hydroxyl groups [49], facilitating cell attachment and matrix deposition [50], and resulting in an increased degradation rate [51]. pHMGCL is thus more likely to balance its degradation to the formation of neo-tissue [52] compared to PCL [41–43]. However, pHMGCL containing more than 20% HMG is an amorphous polymer with a low glass transition temperature (T_g) and therefore has low dimensional stability. PCL, on the other hand, is semi-crystalline, which is beneficial for dimensional stability, but has no reactive groups to allow, for example, surface grafting. To increase the dimensional stability of pHMGCL, it can be blended with PCL [53]. The blending also improves the physical properties of the material for scaffold fabrication techniques such as electrospinning [54] and 3-D fiber deposition (3-DF) [50].

In the present study, covalent binding between methacrylated pHMGCL (pMHMGCL) blended with PCL and a methacrylated gelatin hydrogel (gelMA) through photopolymerization was investigated. The interface-binding strength between gelMA and pMHMGCL/PCL was studied and reinforced constructs as fabricated with 3-DF were tested with a repetitive loading regime. Finally, the in vitro and in vivo regenerative capacities of human chondrocytes within these fiber-reinforced constructs were analyzed.

2. Materials and methods

2.1. Materials

All reagents and solvents were purchased from Sigma–Aldrich (Zwijndrecht, the Netherlands) and used without purification, unless stated otherwise. Benzyl alcohol, acetone, triethylamine (TEA) and dimethyl sulfoxide (DMSO) were obtained from Merck (Darmstadt, Germany). Peptide-grade dichloromethane (DCM) and tetrahydrofuran (THF) were obtained from Biosolve (Valkenswaard, the Netherlands). *N,N'*-Dimethyl amino pyridine (DMAP) was purchased from Fluka (Zwijndrecht, the Netherlands). Irgacure 2959 was obtained from Ciba Specialty Chemicals Inc. (Basel, Switzerland). Type II collagenase was purchased from Worthington Biochemical Corp, (Lakewood, NJ). Phosphate-buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), ITS-X, penicillin, streptomycin and Picogreen DNA assay were provided by Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Biowhittaker (Walkersville, MD). FGF-2 and TGF-β2 were obtained from R&D Systems (Minneapolis, MN). Human serum albumin was provided by SeraCare Life Sciences (Milford, MA). Histoclear II was obtained from BiozymTC, the Netherlands. Buprenorphine (Temgesic) was purchased from Schering-Plough/Merck (USA). Antibody against collagen type II (1:100; II-6B3II) was obtained from Developmental Studies Hybridoma Bank, USA. Polylactic acid (PLA) filament for 3-D printing was obtained from Ultimaker Ltd. (Geldermalsen, The Netherlands).

2.2. Synthesis of PCL

PCL was synthesized by ring opening polymerization of ε-caprolactone (CL) using benzyl alcohol (BnOH) and stannous octoate ($SnOct_2$) as initiator and catalyst, respectively, according to a previously described method [49]. The molar ratio of CL/BnOH was 300/1 in the feed. The obtained PCL was characterized by proton nuclear magnetic resonance (^{1}H NMR), gel permeation chromatography (GPC) and differential scanning calorimetry (DSC).

2.3. Synthesis of pMHMGCL

pHMGCL was synthesized by ring opening polymerization of 3S-benzyloxymethyl-1,4-dioxane-2,5-dione (benzyl protected hydroxymethyl glycolide (BMG)) and ε-caprolactone in the melt using BnOH and $SnOct_2$ as initiator and catalyst, respectively, followed by removal of the protecting benzyl groups by catalytic hydrogenation, as described previously [49]. pHMGCL was synthesized by melt polymerization in a 40/60 M ratio of HMG/CL and the monomer to initiator molar ratio was 300. These random copolymers were further functionalized, aiming for derivatization of 50% of the available hydroxyl groups of pHMGCL with methacrylate groups (Fig. 2). In a typical reaction, pHMGCL (4.2 g, 13.2 mmol OH groups) was dissolved in freshly distilled THF (40 ml), in an aluminum foil covered, dry round-bottom flask. After dissolution, DMAP (39.3 mg, 0.32 mmol) and TEA (897 μl, 6.4 mmol) were added as catalyst and base, a combination that has shown to efficiently functionalize hydroxyl groups with methacryloyl groups [55]. Next, methacrylic anhydride (960 μl, 6.4 mmol, feed ratio methacrylic anhydride/$OH_{polymer}$ = 0.5) was added. To prevent

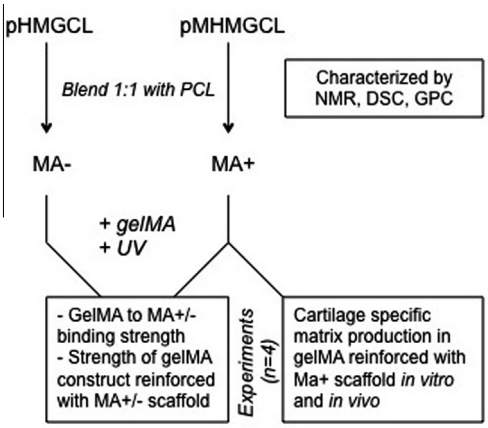

Fig. 1. Overview of the performed experiments in the present study. pHMGCL, pMHMGCL and their corresponding blends with poly-ε caprolactone (MA− and MA+, respectively) were characterized with NMR, DSC and GPC. Reinforced constructs of MA− and MA+ with gelMA were UV-irradiated and the interface-binding strength and strength of the reinforced construct was tested. Constructs of MA+/gelMA were further tested in vitro and in vivo for cartilage-specific matrix production. All mechanical, in vitro and in vivo experiments were performed in quadruplicate.

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