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Enhancing the mechanical properties and physical stability of biomimetic polymer hydrogels for micro-patterning and tissue engineering applications

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ABSTRACT

Low mechanical strength and rapid degradation of photo-crosslinkable polymers are the major obstacles for their applications. The aim of this study was to address these issues by fabricating hybrid polymeric hydrogels from a biopolymer (gelatin) and a synthetic polymer. Methacrylated gelatin (GelMA) and poly(lactic-ethylene oxide fumarate) (PLEOF) were photo-crosslinked, using Irgacure and poly(ethylene glycol)-diacrylate. The optimum hybrid hydrogel was produced when using 200 mg/ml PLEOF and 100 mg/ml GelMA. These hydrogels possessed porosity in the range of 90%, also comprised of micro (~20 μm) and macro pores (540 μm), which are suitable for nutrients mass transfer and osteoblast cell proliferation, respectively. The compression modulus of GelMA-PLEOF hydrogels was more than 200 kPa, which is paramount compared to GelMA hydrogels. Moreover, fabrication of hybrid hydrogel substantially enhanced the structural stability of gelatin in simulated physiological environment from one week to more than 28 days. In vitro studies showed that primary human osteoblast cells adhered and proliferated into PLEOF/GelMA hydrogel. Additionally, micro-patterns with 10 × 100 μm dimensions were created on the surface of these hydrogels to promote the cellular alignment. These results demonstrated the potential of using this hybrid construct for in vitro regeneration of load-bearing tissues.

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1. Introduction

The current paradigm of tissue engineering is commonly focused on the application of cell seeded scaffolds for in vitro generation of tissues and subsequent in vivo implantation [1,2]. Surface topography has been used for stimulating the cellular alignment and controlling the biological activity of cells within the structure of constructs [3,4]. Different lithography approaches such as micro-moulding and photolithography have been used for micro-patterning of scaffolds [5–7]. Hydrogels are the materials of choice as scaffold since they exhibit superior water uptake properties and high permeability of nutrients, proteins and oxygen [8–12]. The physicochemical and biological properties of the hydrogel can be modulated by changing the degree of crosslinking or addition of different materials such as other polymers and graphene. The ultimate result is a series of hybrid structures with tuneable properties to meet the specific biomechanical properties and functional requirements for regeneration of different tissues [13–16].

We have recently fabricated a relatively robust hydrogel by interpenetrating polymer network (IPN) of natural and synthetic polymers for bone regeneration applications. This IPN hydrogel was fabricated by simultaneous chemical crosslinking of poly(lactide-co-ethylene oxide fumarate) (PLEOF) and physical crosslinking of gelatin [17].

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This IPN hydrogel, however, is not suitable for micropatterning and cell laden applications due to its slow gelation time and use of redox crosslinking reagents. In addition, more than 40% of gelatin was leached out from this construct within 72 h of incubation in phosphate buffered saline (PBS), which is not favourable for long term in vitro studies. Moreover, all the previously developed hybrid hydrogels of photo-crosslinkable gelatin with other biomaterials still exhibited low mechanical strength and very fast degradation rate. For instance, hybrid hydrogels of gelatin-methacrylate (GelMA) and silk fibroin possessed 70 kPa compression modulus and 70% mass loss within 7 days of incubation in PBS [18]. Fast degradation of these hydrogels lead to high wearing off rate of micropatterns and lack of mechanical strength overtime, which are not favourable for in vitro regeneration of load-bearing tissues [19,20].

The aim of this study was to develop a biomimetic hydrogel with high physical stability and mechanical strength, superior biological activity and rapid gelation time. We hypothesised that the fabrication of a hybrid hydrogel by photocrosslinking of methacrylated gelatin and PLEOF is an effective approach to achieve these criteria. To photo-crosslink gelatin, different ratios of primary amine groups in this biopolymer were conjugated with methacrylated groups. The effects of different factors such as the degree of methacrylation, the concentrations of PLEOF and crosslinking agents on the characteristics of these hydrogels were determined. The feasibility of using photolithography to generate micropatterns on the surface of hydrogels was also investigated. In addition, human osteoblast cells were cultured on the surface of hybrid hydrogels to assess their potential for bone regeneration applications.

2. Experimental

2.1. Materials

((3S)-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione) (L-LA, 98%), Tin (II) 2-ethylhexanoate ($Sn(Oct)_2$, 95%), anhydrous dichloromethane (DCM, $\geqslant$99.9%), polyethylene glycol (PEG, M_n 3500 g/mol), phosphate buffered saline (PBS) tablet, PEGDA (M_n 700 g/mol), K_2CO_3, gelatin type A (from bovine skin), methacrylic anhydride (MA, 99%) and N-vinyl-2-pyrrolidone (NVP) were all purchased from Sigma–Aldrich. Diethylene glycol (DEG), diethyl ether AR grade and fumaryl chloride (FuCl) were purchased from Merck. Food grade CO_2 (>99.9%) was purchased from BOC gases.

2-Hydroxy-1-(4-(hydroxyethoxy)phenyl)-2-methyl-1-propanone (Irgacure 2959®, 98%) was supplied by Ciba Geigy. L-LA was dried overnight at 40 °C under reduced pressure (<1 mmHg). PEG was purified by azeotropic distillation using toluene. K_2CO_3 was dried overnight at 100 °C under reduced pressure (<1 mmHg). All other chemicals were used as received without further purifications. Osteoblast Growth Media (SupplementMXC-39615) was purchased from PromoCell. MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) assay was purchased from Promega. PBS solution was prepared by dissolving one PBS tablet in 200 ml MilliQ water to acquire pH 7.4.

2.2. Synthesis of methacrylated gelatin (GelMA)

GelMA with different degree of methacrylation was synthesised as described, by Van Den Bulcke et al. [21]. Briefly, 10 g gelatin was dissolved in 100 ml PBS at 60 °C under gentle stirring at 300 rpm. After which, 2–20 ml MA was added to the gelatin solution at the rate of 0.5 ml/min. After 1 h stirring at 50 °C, the methacrylation reaction was ceased by addition of 500 ml of pre-warmed PBS to the solution. The diluted solution was then dialyzed against deionized water (changed on a daily basis) using a 12–14 kDa cut-off dialysis tubes (Sigma–Aldrich) for 7 days at 40 °C in a static condition. The purified GelMA solution was then lyophilized for 4 days to form white foam and stored at −80 °C until further application.

The degree of methacrylation in the synthesised GelMA polymers was quantified using proton nuclear magnetic resonance (^{1}H NMR) analysis [22]. The ^{1}H NMR spectra were collected at 35 °C in deuterium oxide (Adelaide) at a frequency of 500 MHz, using Varian INOVA NMR spectrometer. Phase and baseline correction were applied before obtaining the integral of peaks, and the analysis was repeated at least triple.

2.3. Synthesis of PLEOF

The synthesis of PLEOF involved two steps: (1) ring opening polymerization of L-LA monomers and (2) condensation polymerisation for chemical bonding between precursors PLA, PEG, and fumarate. Low molecular weight PLLA and also PLEOF were synthesised according to the methods developed in-house [23].

Briefly, for PLLA synthesis, a known amount of L-LA, DEG and $Sn(Oct)_2$ were added into a custom made high pressure vessel (70 mL) and the synthesis was conducted at 80 °C and 160 bar for a period of 18 h. To acquire PLLA with M_n of 1800 g/mol, M_w of 2160 g/mol and PDI of 1.2, the molar ratios of DEG to $Sn(Oct)_2$ and L-LA to DEG were 1:3 and 13:1, respectively. For the production of PLEOF with M_n of 7710 g/mol, M_w of 12,400 g/mol and PDI of 1.61, the synthesis was conducted using a CO_2 gas expanded system in which dichloromethane was used as a solvent. For this reaction PEG (3.5 g, 1.03 mmol), PLLA (1.5 g, 0.83 mmol), K_2CO_3 (0.35 g, 2.52 mmol), FuCl (0.18 ml, 1.68 mmol) and DCM (1.34 ml/g of reactants) were added into a custom made high pressure vessel (70 ml) at 25 °C and 50 bar for a period of 24 h under constant stirring at 150 rpm. To confirm the synthesis of PLLA and PLEOF polymers, different techniques such as ^{1}H NMR and gel permeation chromatography analyses were used as previously described [23]. The synthesis of PLLA and PLEOF polymers was confirmed, using ^{1}H NMR spectra with evidence of proton peaks from all main segments in the polymers.

2.4. Fabrication of PLEOF/GelMA hybrid hydrogel

GelMA (0–150 mg) and Irgacure 2959® (1 mg) were dissolved in 1 ml PBS at 70 °C, under gentle mixing in an amber container to avoid light exposure and consequently release of free ions. After 10 min, the known amount of PLEOF (100–

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