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The synthesis and degradation of collagenase-degradable poly(2-hydroxyethyl methacrylate)-based hydrogels and sponges for potential applications as scaffolds in tissue engineering

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ABSTRACT

A collagenase-cleavable peptide-based crosslinking agent was synthesized and was incorporated into PHEMA sponges, and P[HEMA-*co*-MeO-PEGMA] gels and sponges [HEMA 2-hydroxyethyl methacrylate, PHEMA = poly(2-hydroxyethyl methacrylate), MeO-PEGMA = poly(ethylene glycol) monomethyl ether methacrylate]. PHEMA and P[HEMA-*co*-MeO-PEGMA] sponges had polymer droplet morphologies where the dimensions of the morphological features were three to five times larger compared to sponges that were crosslinked with tetraethylene glycol dimethacrylate (TEGDMA), while the P[HEMA-*co*-MeO-PEGMA] gels had similar morphologies regardless of the crosslinking agent. The differences in the dimensions of the morphologies of the sponges were attributed to differences in hydrophilicities of the crosslinking agent. When incubated in a collagenase solution, PHEMA sponges did not degrade, but P[HEMA-*co*-MeO-PEGMA] gels took 28 days to degrade and the P[HEMA-*co*-MeO-PEGMA] sponges took 101 days to degrade to 8% dry weight remaining. A cytotoxicity assay showed that the hydrogels do not elicit any cytotoxic response in vitro.

1. Introduction

Hydrogels are a class of materials that have many uses in the biomedical field. One such hydrogel with a well established history is poly(2-hydroxyethyl methacrylate) (PHEMA) [1]. This history is partially attributed to the excellent biocompatibility of PHEMA, and, as such, PHEMA-based porous materials (sponges) are used as components of an artificial cornea [2], and of an orbital enucleation implant [3], and have been proposed as scaffolds for neural regeneration [4–8], and as drug delivery systems [9,10] or generally as tissue engineering scaffolds [11]. An added advantage of PHEMA is that it can be synthesized via a one-step photoinitiated polymerization-induced phase separation method to produce hydrogel sponges that are suited for cell growth [12–15] with interconnected pores with dimensions of the order of 10 μL [11,12,16,17].

Despite PHEMA sponges being highly biocompatible and macroporous, their use as materials for scaffolds for tissue engineering remains somewhat limited because PHEMA is not biodegradable, nor is it water-soluble at high molecular weights (>2000 Da) [18,19]. A key requirement of scaffolds for tissue engineering purposes is that the scaffold should be biodegradable, with the degradation products being water-soluble so they can be excreted via renal mechanisms [20]. Fortunately, PHEMA can be rendered water-soluble by incorporating low weight-percentage concentrations of poly(ethylene glycol) methyl ether methacrylate (MeO-PEGMA) to afford P[HEMA-*co*-MeO-PEGMA] copolymers. A consequence of the addition of MeO-PEGMA to the polymer network is the suppression of polymerization-induced phase separation, leading to the formation of gels instead of sponges. However, if NaCl is added to the polymerization solution, then P[HEMA-*co*-MeO-PEGMA] macroporous sponges can be obtained [11,17], giving rise to three different hydrogel formulations: PHEMA sponges, P[HEMA-*co*-MeO-PEGMA] gels, and P[HEMA-*co*-MeO-PEGMA] sponges. Although rendering PHEMA sponges water-soluble is relatively easy, rendering them biodegradable is not.

Hydrogels that are not biodegradable, such as PHEMA, can be rendered biodegradable by incorporating biodegradable elements into the polymer network. Such elements can be cleaved through specific

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processes, leading to breakdown of the polymer network. Short chain peptides [11,21–27] represent one class of biodegradable elements that have been successfully targeted to render a variety of polymer networks biodegradable. Typically, the short chain peptides are used as crosslinking agents, and the proteolytic cleavage of the peptide-based crosslinks leads to hydrogel degradation with the liberation of linear polymer fragments. An added advantage of peptide-based crosslinking agents is that the peptide sequences can be tailored for cleavage by specific enzymes, making the mode of degradation very specific, and thus helping to minimize nonspecific degradation processes.

Among the enzymes present in humans that could be used to degrade polymer networks, those remodeling the extracellular matrix are of particular interest. When a biomaterial/prosthesis is implanted, local trauma and inflammation are experienced at the implantation site, and are often followed by secretion of enzymes that are capable of remodeling the extracellular matrix from migrating cells present in the extracellular matrix [28,29]. The most abundant extracellular matrix protein is collagen, meaning that once a biomaterial/prosthesis is implanted, the collagen network is disrupted. This disruption triggers the secretion of collagenase – the enzyme that remodels collagen – to help reestablish the collagen matrix surrounding the implant. Due to the activity of collagenase during wound healing, collagenase was targeted for hydrogel degradation, which has led to the discovery that incorporating the Leu-Gly-Pro-Ala (LGPA) peptide sequence into a polymer network allows collagenase to degrade the network [26,27]; the LGPA is a substrate of collagenase and has a cleavage site between Leu (L) and Gly (G) [26,27,30].

Despite the appeal of targeting collagenase for enzymatic degradation of hydrogels, there has been little investigation into collagenase-degradable PHEMA-based hydrogels. There are, however, reports on PHEMA gels that are degradable by subtilisin [25], on peptide-modified PHEMA hydrogels that are biodegraded (to a little extent) by collagenase [20], and on P[HEMA-*co*-MeO-PEGMA] sponges and gels that are degradable by papain [11]. Although these reports show the potential for enzymatic degradation of PHEMA-based hydrogels, the enzymes used for degradation were either not native to humans (subtilisin, papain), or not effective enough (collagenase), limiting their wider use as scaffold for tissue engineering. To examine the feasibility of using PHEMA-based hydrogels for tissue engineering purposes, more appropriate modification of the hydrogels needs to be targeted for degradation by a relevant enzyme such as collagenase. The aim of this investigation was to determine the feasibility of using collagenase to degrade peptide-conjugate P[HEMA-*co*-MeO-PEGMA] hydrogels, in a non-porous (gels) or porous form (sponges). Initially, a peptide-based crosslinking agent that contained the collagenase-cleavable LGPA sequence was synthesized. This crosslinking agent was then used to form various PHEMA-based hydrogels, and the hydrogels were subsequently degraded by incubation in collagenase solutions. In addition, the cytotoxicity of the gels and sponges was examined qualitatively by growing corneal epithelial cells in the presence of the hydrogel samples.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) (min. 99.0%, from Bimax, USA) was distilled (b.p. 38–9 °C/0.1 mm Hg) and stored at −20 °C until use. $CaCl_2$ (AR) was purchased from Ajax, Australia, and used as received. Tetraethylene glycol dimethacrylate (TEGDMA) and sodium chloride (AR grade) were purchased from Fluka/Sigma-Aldrich, USA, and were used as received. 2,2-Dimethoxy-2-phenylacetophenone (DPAP) (Irgacure 651, 97%), rhodamine B isothiocyanate (RBITC), N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES) and collagenase (from *Clostridium histolyticum*) were all purchased from Sigma-Aldrich, USA, and used as received. Poly(ethylene glycol) methyl ether methacrylate (MeO-PEGMA, average MW ca. 1100, from Sigma-Aldrich) was recrystallized from hot ether to remove the inhibitor. Degassing of HPLC solvents was carried out by vacuum filtration through a 0.45 μm membrane. TESCA buffer was prepared using 50 mM TES, 0.36 mM $CaCl_2$ and 0.2 mg/mL NaN_3 at pH 7.4 in milliQ H_2O. Sterile ethanol (70%) was supplied by Orion Laboratories, Australia. Dulbecco's phosphate buffered saline solution (DPBS) without Ca and Mg was supplied by Invitrogen Life Technologies, Australia. Hank's buffered salt solution (HBSS) with Ca and Mg was supplied by Invitrogen Life Technologies, Australia. The defined cell culture medium for corneal epithelial cells, CnT-20, was supplied by CELLnTEC-Advanced Cell Systems, Switzerland, and supplemented with 1% antibiotic/antimycotic (Invitrogen Life Technologies, Australia). Triton X-100 and HEPES were supplied by Sigma, USA, and the nuclear stain Hoechst 33342 was supplied by Invitrogen Molecular Probes. A neutral buffered formalin solution (3.7%) was supplied by United Biosciences, Australia. The Iwaki™ tissue culture plastic (TCP) plates (24 wells) were supplied by Asahi Glass Co., Japan.

2.2. Synthesis of the peptide-based crosslinker **1**

The peptide-based crosslinker **1** containing the LGPA sequence was synthesized using solution phase Boc coupling procedures with HBTU as the coupling agent. Details for the synthesis of **1** are provided in the Supplementary data.

2.3. Preparation of PHEMA and P[HEMA-co-MeO-PEGMA] hydrogels

Polymer hydrogels crosslinked with the peptide **1** were made by photoinitiated polymerization using a modified procedure [11,17] according to the formulations in Table 1. Stock solutions of **1** (50 mg/mL), MeO-PEGMA (215 mg/mL), NaCl (138 mg/mL) and DPAP (16.6 mg/mL) were used to prepare the hydrogels. A general procedure for the hydrogel **H5** (see Table 1) is given here. A cylindrical quartz vial (I.D. of 8 mm) was charged with HEMA (40 μL), H_2O (10 μL) and stock solutions of **1** (50 μL), MeO-PEGMA (50 μL), and NaCl (50 μL). The contents of the vial were mixed until a homogeneous solution formed, then DPAP (5 μL) was added to the vial and mixed thoroughly then the vial was suspended in front a UV lamp (UVP, 365 nm, 120 W) for 30 min. Upon completion of the polymerization, the newly formed polymer disk (sponge for polymers **H1**, **H2**, **H5** and **H6**, gel for polymers **H3** and **H4**) was carefully removed from the vial and immersed in water, with the water being exchanged daily for 1 week to remove any unreacted monomers, **1** and NaCl. The polymer disks had a diameter of ca. 8 mm and thickness of ca. 5 mm. The samples were stored in water until needed. For samples polymerized in the absence of MeO-PEGMA and/or NaCl, water was used to make the final volume of up to 200 μL. To obtain the control samples, TEGDMA (0.9 μL) was added as a crosslinker instead of the stock solution of **1** to the pre-polymerization solution and additional H_2O was added to make the final pre-polymer solution volume of up to 200 μL.

Table 1
Hydrogel formulations.

Hydrogel sample	H_2O:HEMA:MeO-PEGMA[a]	Crosslinker	Hydrogel form
H1	80:20:00	**1**	Sponge
H2	80:20:00	TEGDMA	Sponge
H3	80:20:05	**1**	Gel
H4	80:20:05	TEGDMA	Gel
H5	80:20:05[b]	**1**	Sponge
H6	80:20:05[b]	TEGDMA	Sponge

[a] The hydrogels were prepared according to the ratio H_2O:HEMA:MeO-PEGMA (in parts by weight). The concentration of the initiator (DPAP) was 0.1 mol% relative to HEMA and the crosslinking agent concentration was 0.9 mol% relative to HEMA.
[b] Polymerization was carried out in 0.8 M NaCl solutions in place of water.