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Injectable, porous, and cell-responsive gelatin cryogels

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ABSTRACT

The performance of biomaterials-based therapies can be hindered by complications associated with surgical implant, motivating the development of materials systems that allow minimally invasive introduction into the host. In this study, we created cell-adhesive and degradable gelatin scaffolds that could be injected through a conventional needle while maintaining a predefined geometry and architecture. These scaffolds supported attachment, proliferation, and survival of cells *in vitro* and could be degraded by recombinant matrix metalloproteinase-2 and -9. Prefabricated gelatin cryogels rapidly resumed their original shape when injected subcutaneously into mice and elicited only a minor host response following injection. Controlled release of granulocyte-macrophage colony-stimulating factor from gelatin cryogels resulted in complete infiltration of the scaffold by immune cells and promoted matrix metalloproteinase production leading to cell-mediated degradation of the cryogel matrix. These findings suggest that gelatin cryogels could serve as a cell-responsive platform for biomaterial-based therapy.

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1. Introduction

Implantable biomaterials have been proposed to locally deliver or recruit cells, or provide sustained release of therapeutic molecules for applications such as tissue engineering, drug delivery, gene therapy, and vaccines [1–4]. The clinical implantation of prefabricated biomaterials for these purposes typically requires trained physicians, causes patient distress, creates potential scarring, poses a risk of infection, and often causes inflammation at the surgical site that may inhibit the performance of the implant. Biomaterials that can be introduced in a minimally invasive manner are thus of interest in many therapeutic applications.

Injectable hydrogels have been used as biomaterial implants without the need for surgery [5,6]. Many of these materials systems involve the injection of a polymer solution and subsequent crosslinking of the polymer chains by chemical or physical means to form a solid [7,8]. The use of liquid precursors may result in leakage from the implant site to unwanted tissues and poses difficulties in generating the desired implant geometry. We have recently created preformed hydrogel scaffolds with a defined geometry and microstructure that can be introduced to the body in a minimally invasive manner through a conventional needle [9]. These hydrogels are formed by cryopolymerization of methacrylated alginate using radical polymerization at sub-zero temperatures. Although these scaffolds are capable of delivering cells and biomolecules in a non-invasive manner, their ability to be remodeled and degraded locally by cells is limited, which could reduce their ability to integrate with host tissue in certain applications. Additionally, these hydrogels require modification of the alginate polymer with cell-adhesive peptides to allow cell attachment, which requires additional synthesis steps.

We hypothesized that using a material with inherent cell-responsive (e.g. cell binding and enzymatically degradable) elements may improve the performance of cryogel implants by allowing direct cell attachment and local remodeling. Gelatin, derived from collagen, contains inherent peptide sequences that facilitate cell adhesion and enzymatic degradation. Additionally, its low cost, lack of immunogenicity, and safety record in medicine (hemostatic agent, blood volume expander etc.) makes gelatin an attractive implantable biomaterial. Modification of gelatin with pendant methacrylate groups (GelMA) allows crosslinked hydrogels to be formed using radical polymerization, which have been used extensively in cell culture and tissue engineering studies [10–15]. In this report, we fabricated and characterized scaffolds formed by cryopolymerization of GelMA (cryoGelMA). We studied the bulk mechanical behavior, structure, and degradation of the cryoGelMA

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gels, as well as the ability of these scaffolds to facilitate cell attachment, proliferation, and survival. Finally, the ability of gelatin cryogels to locally deliver a chemoattractant protein, recruit host cells, and undergo cell-mediated degradation *in vivo* was explored.

2. Materials and methods

2.1. Mice

All work with C57BL/6J and C57BL/6J-Tyr$^{c\text{-}2J}$ mice (female, aged 6–8 weeks; Jackson Laboratories) was performed in compliance with National Institutes of Health and institutional guidelines.

2.2. Methacrylated gelatin synthesis

Methacrylated gelatin (GelMA) was synthesized (Fig. 1-A) by allowing Type A porcine skin gelatin (Sigma) at 10% (w/v) to dissolve in stirred Dulbecco's phosphate buffered saline (DPBS; GIBCO) at 50 °C for 1 h [10,12]. Methacrylic anhydride (Sigma) was added dropwise to a final volume ratio of 1:4 methacrylic anhydride:gelatin solution. This resulted in GelMA with a degree of substitution of 79% (Fig. S1). The solution was stirred at 50 °C for 1 h, and then diluted 5× with DPBS. The resulting mixture was dialyzed in 12–14 kDa molecular weight cutoff tubing (Spectrum Labs) for 4 d against distilled water (dH_2O) with frequent water replacement. The dialyzed solution was lyophilized, and the resulting GelMA was stored at −20 °C until use. Rhodamine-labeled GelMA, created from the reaction of GelMA with NHS-rhodamine (Thermo Scientific), was purified using an identical dialysis and lyophilization process.

2.3. Gelatin cryogel preparation

Cryogels were formed by dissolving GelMA in dH_2O to the final desired concentration in the presence of 0.5% (w/v) ammonium persulfate (APS; Bio-Rad) and 0.1% (w/v) tetramethylethylenediamine (TEMED; Bio-Rad). This prepolymer solution was pipetted into cylindrical (5 mm diameter, 2 mm thickness) polystyrene molds and placed in a freezer set to −12 °C (Fig. 1-B). Cryopolymerization was allowed to proceed for 18 h, and the resulting cryogels were thawed and hydrated in dH_2O prior to use.

2.4. Interconnected porosity

To test for cryogels for interconnected porosity, scaffolds were first thawed and hydrated for 1 d. Hydrated scaffolds were weighed on a scale, and a Kimwipe was lightly applied to the scaffold surface for 30 s to wick away loosely held water, and the mass was again recorded. The interconnected volume was calculated as the mass of water wicked away divided by the total hydrated mass.

2.5. Scanning electron microscopy

For scanning electron microscopy, cryogels were serially transitioned from dH_2O into absolute ethanol with 20 min incubations in 30, 50, 70, 90, and 100% ethanol solutions. Samples were incubated in hexamethyldisilazane (Electron Microscopy Sciences) for 10 min and dried in a desiccator for 1 h. Dried cryogels were adhered onto sample stubs using carbon tape and coated with platinum/palladium in a sputter coater. Samples were imaged using secondary electron detection on a Carl Zeiss Supra 55 VP field emission scanning electron microscope (SEM). Cell-laden cryogels were fixed in 4% paraformaldehyde (PFA) and prepared for SEM as described above. Images were false-colored in Adobe Photoshop CS6 to highlight cells.

2.6. 2-Photon microscopy

To characterize the hydrated cryogel structure, rhodamine-GelMA cryogels were placed in dH_2O in a 35 mm glass-bottom culture plate (MatTek), and imaged on a Leica SP5 inverted laser scanning confocal microscope. 2-photon excitation was achieved using a Chameleon Vision 2 pulsed infrared (IR) laser (Coherent) at 820 nm, and fluorescence emission was collected through a 565–605 nm bandpass filter by a

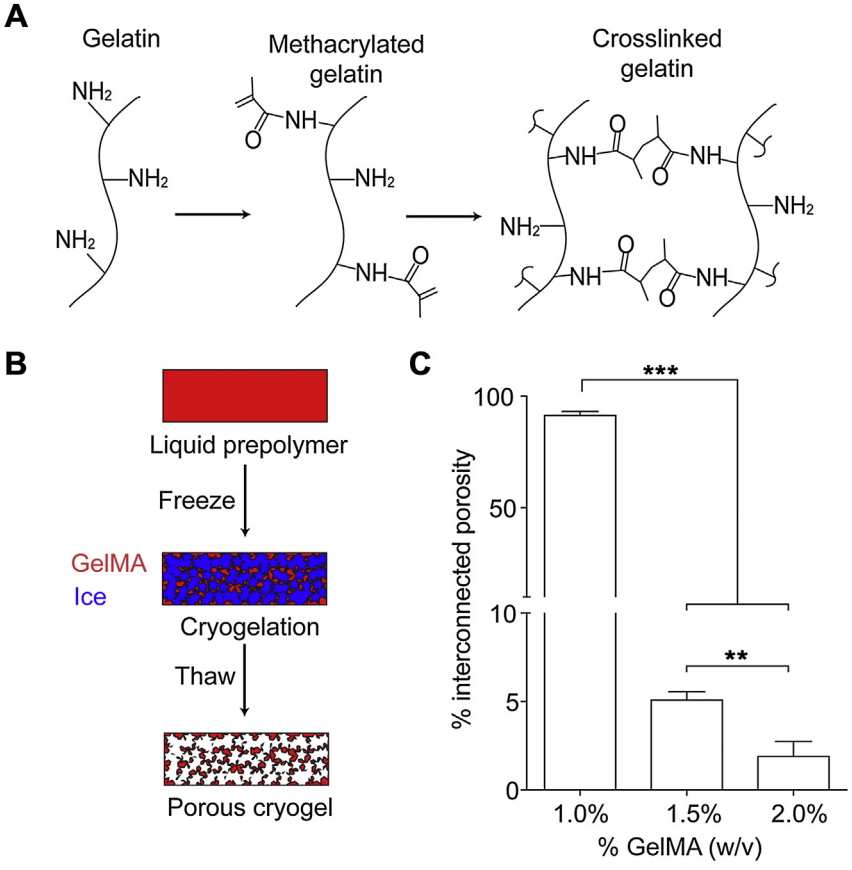

Fig. 1. Fabrication of gelatin cryogels with highly interconnected pores. (A) Schematic of GelMA synthesis and crosslinking. Pendant methacrylate groups are added primarily to the free amines of gelatin by reaction with methacrylic anhydride. Free radical polymerization results in crosslink formation between methacrylate groups. (B) Cryopolymerization of methacrylated gelatin. Freezing of methacrylated gelatin in the presence of radical initiators (APS and TEMED) allows polymerization to occur in the partially frozen state (cryopolymerization). Ice crystals formed during the freezing process and thawing after cryopolymerization results in the formation of a hydrogel with micron-scale pores. (C) Volume of interconnected pores in gelatin cryogels (normalized to total gel volume). Values represent mean and standard deviation ($n = 10$). Data were compared using ANOVA with Bonferroni's post-hoc test ($^{**}p < 0.01$, $^{***}p < 0.001$).

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