



ORIGINAL ARTICLE

# Self-healing injectable gelatin hydrogels for localized therapeutic cell delivery

Arbel M. Sisso<sup>1</sup> | Mary O. Boit<sup>2</sup> | Cole A. DeForest<sup>1,2,3,4</sup>

<sup>1</sup>Department of Bioengineering, University of Washington, Seattle, Washington

<sup>2</sup>Department of Chemical Engineering, University of Washington, Seattle, Washington

<sup>3</sup>Institute of Stem Cell & Regenerative Medicine, University of Washington, Seattle, Washington

<sup>4</sup>Molecular Engineering & Sciences Institute, University of Washington, Seattle, Washington

## Correspondence

Cole A. DeForest, Departments of Chemical Engineering and Bioengineering, University of Washington, Seattle, WA 98195.  
Email: profcole@uw.edu

## Funding information

Fulbright Association; National Institutes of Health, Grant/Award Number: T32EB001650

## Abstract

Self-healing injectable hydrogel biomaterials uniquely enable precise therapeutic deposition and deployment at specific bodily locations through versatile and minimally invasive processes that can preserve cargo integrity and cell viability. Despite the distinct advantages that injectable hydrogels offer in tissue engineering and therapeutic delivery, exceptionally few have been created using components naturally present in the cellular niche. In this work, we introduce a shear-thinning hydrogel based on guest-host complexation of gelatin. As a biocompatible, biodegradable, and nonimmunogenic biopolymer derived from the most abundant extracellular matrix protein (collagen), gelatin offers great utility as the structural component of biomaterials. Taking advantage of reversible guest-host interactions between  $\beta$ -cyclodextrin (CD) and adamantane (AD) on modified gelatins, we report the first strategy to afford a self-healing material based solely on a functionalized extracellular matrix protein. By varying the initial material formulation, hydrogels were synthesized with variable moduli and shear-thinability across a broad range. Gels were demonstrated to exhibit shear-thinning and self-healing properties, supporting protection of clinically relevant stem-cell-derived cardiomyocytes during injection. These materials are expected to expand clinical opportunities in cell delivery for in vivo tissue regeneration.

## KEYWORDS

cell delivery, guest-host complexation, hydrogel, injectable, shear-thinning

## 1 | INTRODUCTION

Hydrogels are comprised of hydrophilic macromolecular polymeric networks of natural, synthetic, or mixed origin that retain a high water content while preserving their 3D structural integrity (Hoffman, 2012). Over the past many decades, these materials have been extensively employed in a variety of biomedical applications including for the delivery of therapeutic agents (Badeau, Comerford, Arakawa, Shadish, & DeForest, 2018; Badeau & DeForest, 2019; Hoare & Kohane, 2008; Youngblood, Truong, Segura, & Shea, 2018); to support 3D cell culture and dynamically direct cell fate (Caliari & Burdick, 2016; DeForest & Anseth, 2012; Healy, Reznia, & Stile, 1999; Shadish, Benuska, & DeForest, 2019; Tibbitt & Anseth, 2012); as scaffolds for tissue engineering and regenerative medicine (Drury & Mooney, 2003; Ifkovits & Burdick, 2007; Khademhosseini &

Langer, 2007; Nicodemus & Bryant, 2008); and as components in biomedical devices, bioadhesives, and biosealants (Annabi, Yue, Tamayol, & Khademhosseini, 2015; Caló & Khutoryanskiy, 2015; Ghobril & Grinstaff, 2015). This growing list of applications stems directly from the materials' ability to be formulated in manners that are biocompatible, support nutrient diffusion, and offer tunable physiochemistry that mimics critical aspects of the native extracellular matrix (ECM; Seliktar, 2012). In addition to the base polymer composition, the crosslinking type (physical vs. chemical) and density, and overall network biodegradability can be chosen directly to dictate the mechanical, structural, and biological properties of the resultant hydrogels (Hoffman, 2012; Seliktar, 2012).

Though hydrogels have gained tremendous interest as uniquely powerful materials in biomedicine, on-going challenges hamper their clinical use. Though preformed hydrogels can be customized to

support cell transplantation and engraftment, invasive surgical procedures required for physical placement within patients increase risks of infection, complications, pain, patient discomfort, and medical care cost (Doenst, Diab, Sponholz, Bauer, & Färber, 2017). Injectable hydrogel biomaterials offer distinct advantages for many applications, readily taking the shape of irregular cavities while providing a minimally invasive module of delivery. Since these systems can be introduced to specific bodily locations via syringe/catheter injection, they can be precisely placed *in vivo* without the need for complicated surgeries. Furthermore, therapeutic agents (e.g., proteins, drugs, cells) can be readily mixed into the precursor solution, affording a route for simple and uniform incorporation prior to administration in a potentially cargo-protective manner. As such, injectable hydrogels are of increasing interest in the field of tissue engineering and therapeutic delivery (Guvendiren, Lu, & Burdick, 2012; Lee, 2018; Thambi, Li, & Lee, 2017).

One common approach for engineering an injectable hydrogel is to design liquid precursors that undergo chemical crosslinking and gel *in situ* during/after injection. Though this approach has proven beneficial in many systems, there are potential drawbacks in requiring an external stimulus to initiate gelation or a multibarrel syringe setup to mix reactants together during injection. Another important variable in this process is the sol–gel transition time; a rapid crosslinking of the hydrogel may result in catheter/syringe obstruction prior to the deployment of the hydrogel, whereas a lagging crosslinking process may lead to material dissipation upon injection prior to setting. Moreover, release of unreacted precursors outside of the target zone due to slow gelation raises the risk of emboli formation and off-site cytotoxicity (Guvendiren et al., 2012; Lee, 2018; Thambi et al., 2017).

To overcome the challenges of *in situ* crosslinking systems, hydrogels with the ability to flow under application of shear stress (i.e., shear-thinning) and reform upon stress removal (i.e., self-healing) have gained popularity. Optimal systems undergo a reversible sol-to-gel transition in response to shear forces introduced during injection and subsequently exhibit rapid self-healing. Such injectable biomaterials have been achieved through physical self-assembly promoted through numerous noncovalent chemical interactions (e.g., hydrogen bonds, electrostatic interactions, hydrophobic interactions). While each individual bond is weak, cumulatively they can result in formation of materials with high structural integrity (Guvendiren et al., 2012; Zhang, 2002).

Though self-healing injectable biomaterials offer promise in many areas, one particularly powerful application has been toward promoting cardiac regeneration following myocardial infarction (MI; Laflamme & Murry, 2011). In MI, occlusion of a coronary artery prevents oxygenated blood from reaching its intended destination in the heart. Tissue located downstream to the blockage becomes ischemic, with prolonged lack of oxygen resulting in irreversible necrosis of the heart muscle. As one of the least regenerative organs in the body, loss of heart tissue results in fibrotic scar formation that can cause significant deficit of contractile force and ultimately heart failure (Gerbin & Murry, n.d.). With cardiovascular diseases being a leading cause of death and the number one healthcare cost globally, significant effort has been put into developing techniques to promote cardiac regeneration (Bertero & Murry, 2018;

Vadakke-Madathil & Chaudhry, 2018). Researchers have sought after therapeutic strategies dedicated to improving myocardial regeneration and restoration of function including direct injection of cardiomyocytes (CM; Chong et al., 2014), stem cells (Amado et al., 2005), biomolecules (Korf-Klingebiel et al., 2015; Rebouças, Santos-Magalhães, & Formiga, 2016), and chemokines (Dobaczewski & Frangogiannis, 2008). Though demonstrating some positive effect, these techniques suffer from significant impediments such as nonspecific delivery, low cell survival, and insignificant engraftment rate in the desired targeted region (Roche et al., 2014). Efforts of tissue engineering researchers have focused on overcoming these drawbacks by applying injectable biomaterials designed to protect the therapeutic cargo from degradation, improve targeted engraftment, and increase cell survival during and post injection (Roche et al., 2014).

A variety of self-healing hydrogel biomaterials have now been reported, including those to support enhanced cell delivery/engraftment in the treatment of MI. One such strategy utilized zwitterionic polycarboxybetaine-based microgels that self-assemble through charge–charge interactions around cells with tunable moduli (Sinclair et al., 2018). Other approaches have exploited the physical electrostatic crosslinking of alginate with divalent cations (e.g.,  $\text{Ca}^{2+}$ ) and have demonstrated enhanced cell viability during *in-gel* injection compared to that in phosphate-buffered saline (Aguado, Mulyasmita, Su, Lampe, & Heilshorn, 2012; Ruvinov & Cohen, 2016). A shear-thinning, engineered protein-based hydrogel with cell-adhesive domains has been reported to improve induced pluripotent stem cells derived endothelial cells viability during the injection process by protecting the cells from mechanical forces within the needle (Wong Po Foo, Lee, Mulyasmita, Parisi-Amon, & Heilshorn, 2009). In one particularly powerful example involving hydrogel derived from ECM-presented glycosaminoglycans, injectable hyaluronic acid-based materials formed through guest-host interactions were demonstrated to support endothelial progenitor delivery, enhance cell engraftment, and improve ischemic myocardium in rat and sheep MI models (Gaffey et al., 2015; Rodell et al., 2016).

Toward designing a shear-thinning biomaterial to further improve cell viability and engraftment following injection, as well as provide a suitable environment for the long-term culture of a variety of cells, we took inspiration from the native ECM, most abundantly comprised of collagen protein (Engler et al., 2008; Lockhart, Wirrig, Phelps, & Wessels, 2011). Though we expected a collagen-based material to provide appropriate physiochemical cues to support long-term cell culture, collagen in its natural form is highly viscous and difficult to handle. Moreover, intact collagen has been reported to induce immunogenic response in some patients (Lynn, Yannas, & Bonfield, 2004). Therefore, we hypothesized that utilizing gelatin, a biocompatible and non-immunogenic protein originating from denatured collagen with potentially tunable and easy-to-use properties, would promote cell survival when used as an injectable scaffold.

Gelatin is a protein cured from collagen that has been reported to have biocompatible, biodegradable, nonimmunogenic, and cell-integration properties (Nikkhah et al., 2016). Despite these advantages, gelatin is a liquid at body temperature, and therefore cannot be

used as a stable scaffold *in vivo*; chemical modifications are required to provide stability to its structure and enhance its mechanical properties while avoiding rapid degradation upon exposure to host-tissue (Nikkhah et al., 2016). Many chemical and enzymatic modifications have been utilized to alter gelatin's mechanical properties (e.g., glutaraldehyde, carbodiimide [EDAC], transglutaminase, methacrylation; Benton, DeForest, Vivekanandan, & Anseth, 2009; Kuijpers et al., 2000; Nichol et al., 2010; Nikkhah et al., 2012; Sung, Huang, Chang, Huang, & Hsu, 1999). Though these approaches can yield stable materials that have been beneficial for cell culture and drug delivery, there has yet to be developed a method that affords a stimuli-free, by-product free, single-barrel injection of an *in situ* self-healing gelatin.

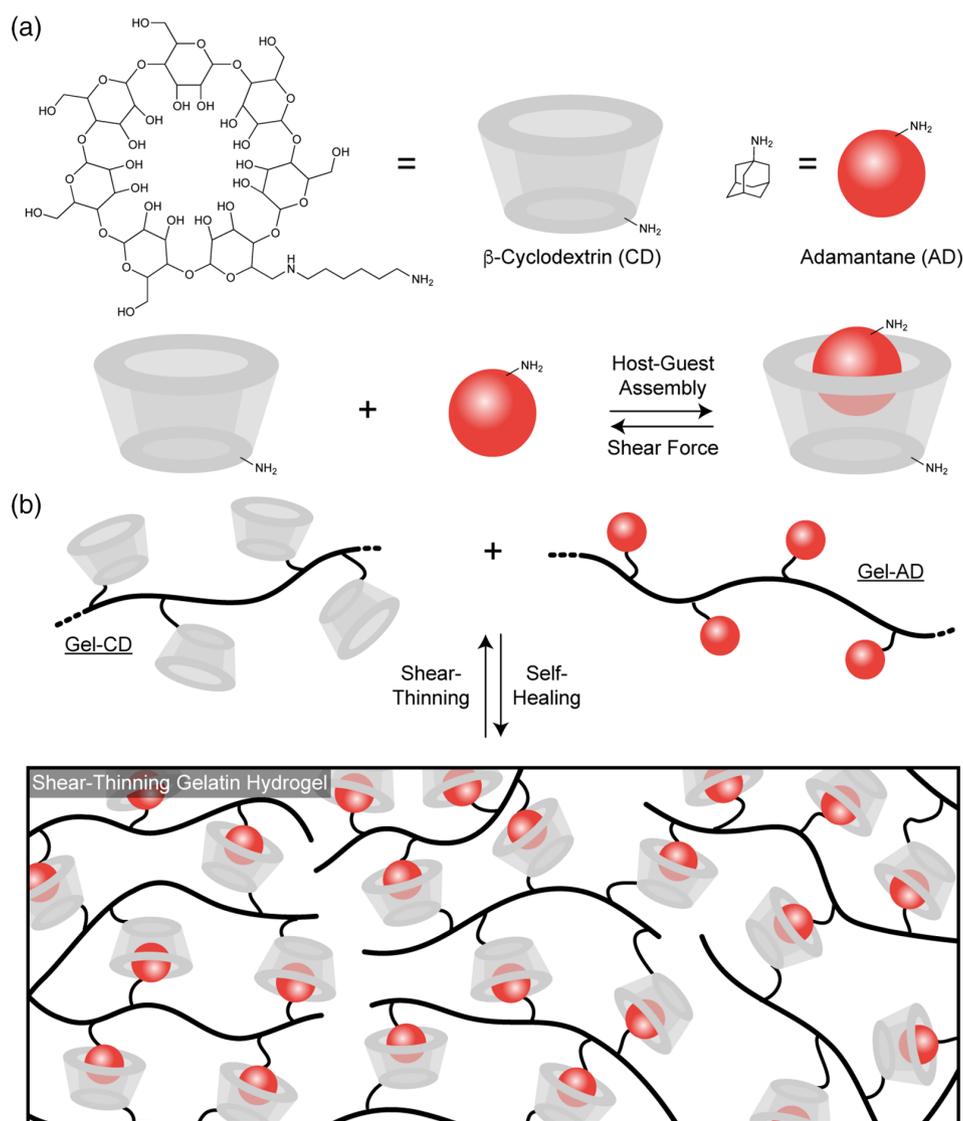
In this study, we sought to develop a self-healing and injectable gelatin-based platform for minimally invasive therapeutic delivery. Toward this, we functionalized gelatin with complementary association domains whose physical interaction could be disrupted with mild shear force and would rapidly reform upon force removal. We sought to employ guest-host chemistry based on physical hydrophobic bonding between a cyclic cup-shaped host (i.e.,  $\beta$ -cyclodextrin) and a

complimentary guest molecule (i.e., adamantane). These moieties were conjugated onto carboxylic acid moieties pendant to the gelatin biopolymer backbone using EDAC/NHS chemistry; rapid association and dissociation of the guest-host bonds upon application of low- and high-shear, respectively, resulted in the formation of a shear-thinning injectable gelatin-based platform that can potentially be used as a minimally invasive drug and cell delivery module (Figure 1).

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Gelatin type A (porcine skin, ~275 bloom, 40–50 kDa),  $\beta$ -cyclodextrin (CD), 1-adamantylamine hydrochloride (AD), *p*-Toluenesulfonyl Chloride (TsCl), *o*-Phthalaldehyde (OPA), and 1,6-Hexanediamine (HDA) were purchased from Acros Organics (Fair Lawn, NJ). Sodium hydroxide (NaOH), acetone, acetonitrile, glycine, sodium tetraborate, ethanol, and anhydrous diethyl ether were purchased from Fischer Scientific (Fair



**FIGURE 1** Rapid association and dissociation of guest-host complementary bonds. (a) Transient hydrophobic bond formation occurs between the host moiety ( $\beta$ -cyclodextrin, CD) and its guest molecule (adamantane, AD). (b) Gelatins that have been covalently modified with CD and AD react to form a physically cross-linked hydrogel that exhibits both shear-thinning and rapid self-healing

Lawn, NJ). Ammonium chloride was purchased from JT Baker (Phillipsburg, NJ). Anhydrous dimethyl sulfoxide (DMSO), and di-tert-butyl dicarbonate (Boc) were purchased from Alfa Aesar (Tewksbury, MA). *N*-hydroxysuccinimide (NHS), 2-mercaptoethanol, and *N,N*-dimethyl formamide (DMF) were purchased from Sigma-Aldrich (St. Louis, MO). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) was purchased from AK Scientific (Union City, CA). *d*<sub>6</sub>-DMSO was purchased from Cambridge isotope laboratories (Andover, MA). Hydrochloric acid (HCl, 12M) was purchased from Avantoc (Center Valley, PA). All chemicals were used as received unless stated otherwise.

UV analysis was performed with SpectraMax M5 UV-VIS spectrophotometer (Molecular Devices, San Jose, CA). <sup>1</sup>H NMR spectra performed in DMSO-*d*<sub>6</sub> using Avance 300 MHz instrument (Bruker, Billerica, MA). Freeze drying of the polymer derivatives occurred by means of FreeZone 2.5plus lyophilizer (Labconco, Kansas City, MO). Rheology experiments performed on Physica MCR301 Rheometer (Anton Paar, Graz, Austria). Cell viability analysis was performed on a NC-200 NucleoCounter®.

## 2.2 | Synthesis of Boc-protected adamantane-modified gelatin (Gel-AD-Boc)

Adamantane-modification of gelatin was performed by adaptation of a previously reported synthesis describing conjugation of 2-aminoethyl methacrylate to carboxylic acid side chains of gelatin (Van Nieuwenhove, Stubbe, Graulus, Van Vlierberghe, & Dubrue, 2014). Briefly, the synthetic route involves Boc-protection of gelatin's primary amines, NHS-activation of the Boc-protected gelatin, amide formation through reaction with AD, and final Boc deprotection (Figures S1 and S2). Here, Gelatin A (5 g, 1.46 mmol amine of lysine and hydroxylysine (Kale & Bajaj, 2010), 4.23 mmol carboxylic acids (Mark & Kroschwitz, 1987, 1 equiv) was dissolved in 50 ml anhydrous DMSO at 55°C under nitrogen atmosphere and reflux conditions. After obtaining a homogeneous solution, Boc (635.1 mg, 2.91 mmol, 2 equiv) was added and the reaction mixture was stirred for 80 min. Molar excess of Boc was considered with respect to free amino groups of gelatin based on a content of 0.291 mmol free amine groups per gram of gelatin type A (Kale & Bajaj, 2010). EDAC (991 mg, 6.38 mmol, 1.5 equiv) was added and the solution was stirred for 5 min. NHS (979.5 mg, 8.51 mmol, 2 equiv) was included in the reaction mixture followed by stirring for 45 min. 1-Adamantylamine (AD; 835.7 mg, 5.53 mmol, 1.3 equiv) was added and the reaction occurred overnight (20 hr) while stirring at 55°C under nitrogen atmosphere with reflux conditions. Molar excesses of EDAC, NHS, and AD were considered in respect to carboxylic acid side chains based on a content of 0.851 mmol carboxylic acid groups per gram gelatin type A (Mark & Kroschwitz, 1987). Mixture was precipitated in a 10-fold excess (500 ml) of cold acetone and filtered onto a glass filter (M). The residue was dissolved in minimal double-distilled water (DDW) at 50°C and dialyzed (molecular weight cut-off: 3,500 Da) against DDW for 48 hr at 42°C. Dialyzed product was frozen and lyophilized for 48 hr and a light-yellow spongy material was obtained (1.75 g, denoted Gel-AD-Boc).

## 2.3 | Synthesis of Boc-protected β-cyclodextrin-modified gelatin (Gel-CD-Boc)

### 2.3.1 | Synthesis of 6-(6-aminohexyl)amino-6-deoxy-β-cyclodextrin (CD-HDA)

6-(6-Aminoethyl)amino-6-deoxy-β-cyclodextrin (CD-HDA) synthesis was performed by adaptation of previously reported similar syntheses (Fetter, Salek, Sikorski, Kumaravel, & Lin, 1990; Kaya & Mathias, 2010; Rodell, Kaminski, & Burdick, 2013) and shown in Figure S3. A round-bottom flask was charged with CD (1 equiv, 20 g, 17.6 mmol) and DDW (150 ml), and cooled on ice to 0°C for 1 hr. NaOH (3.1 equiv, 2.2 g, 55 mmol) was dissolved in 6.67 mL of DDW and added dropwise. By the end of the addition of NaOH, the solution turned completely homogeneous and clear. The suspension was stirred for 30 min at room temperature. TsCl (1.25 equiv, 4.2 g, 22 mmol) was dissolved in minimal acetonitrile (10 ml) and added dropwise to the CD suspension. Immediate precipitation was observed upon TsCl addition. The suspension was stirred at room temperature for 2 hr. pH was adjusted to 8–8.5 by addition of approximately 10 g of solid ammonium chloride (NH<sub>4</sub>Cl) and suspension was cooled at 4°C overnight. Reaction precipitate was collected by glass filter (M funnel) using vacuum pump and rinsed with DDW (400 ml), washed by acetone (300 ml), and dried under vacuum to afford the 6-*o*-monotosyl-6-deoxy-β-cyclodextrin intermediate (5.09 g, 3.948 mmol, 22.4% [molar]) as a white powder. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>), δ = 7.76 (d, 2H), 7.44 (d, 2H), 5.89–5.56 (m, 14H), 4.84 (s, 5H), 4.78 (s, 2H), 4.12–4.55 (m, 9H), 3.8–3.42 (m, 28H), 3.41–3.06 (m, overlaps with H<sub>2</sub>O), 2.42 (s, 3H; Figure S4).

A round-bottom flask was charged with 6-*o*-monotosyl-6-deoxy-β-cyclodextrin (5 g, 3.878 mmol, 1 equiv) and 1,6-hexanediamine (HDA; 20 g, 172.1 mmol, 45 equiv) and dissolved in DMF (25 ml) under nitrogen conditions with a reflux condensing setup and a temperature-controlled silicon oil bath (80°C). Reaction was carried out overnight (20 hr) prior to room temperature cooling for 1 hr. Product was precipitated using cold acetone (500 ml, 4°C) and collected under vacuum through a glass filter (M funnel). Precipitate was redissolved in DMF (25 ml) and then reprecipitated with acetone (500 ml), a process repeated three times. Precipitate was washed by cold diethyl ether (200 ml, 4°C) twice and filtered by vacuum on M glass funnel as a pale-yellow slurry. Product was dried under vacuum for 2 hr to yield species CD-HDA (3.06 g, 2.48 mmol, 63.9% [molar]) as a light-brown powder. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>), δ = 5.71 (br s, 14H), 4.83 (br s, 7H), 4.46 (br s, 6H), 3.79–3.5 (m, 28H), 3.5–3.17 (m, overlaps with H<sub>2</sub>O), 1.6–1.08 (m, 12H; Figure S5).

### 2.3.2 | Gelatin modification with CD-HDA

Synthesis of Boc-protected β-cyclodextrin-modified gelatin was performed by adaptation of the Gel-AD-Boc synthesis, with the single change of addition of 1.3 equiv CD-HDA instead of 1.3 equiv 1-adamantylamine. The synthetic routes are shown in Figures S1 and

S6. A light-brown powder was recovered (Gel-CD-Boc, 3.524 g product per 5 g of gelatin).

## 2.4 | Synthesis of adamantane- and $\beta$ -cyclodextrin-modified gelatins (Gel-AD and Gel-CD)

Boc deprotection was performed by adaptation of previously reported procedure (Van Nieuwenhove et al., 2014), and shown in Figures S2 and S6. Gel-AD-Boc and Gel-CD-Boc ( $100 \text{ mg ml}^{-1}$ ) were each dissolved individually in DDW to which 0.5 v/v% of an aqueous HCl solution (12M) was added. The quantity of protective groups removed from modified gelatin was determined indirectly by evaluating the difference between the amine content in gelatin prior to and after Boc deprotection. Amine concentration was determined spectrometrically using *o*-phthalaldehyde (OPA). The reaction between primary amines and OPA resulted in the formation of isoindol chromophores possessing a maximum absorbance  $\lambda_{\text{max}}$  at 340 nm. The deprotection reaction proceeded over several days at 4°C, and amine concentration was quantified relative to a calibration curve based on glycine standards (Figure S7). Solution A was prepared by dissolving OPA in ethanol (5 mg in 2.5 ml) and diluting the mixture to 15 ml using DDW. 30 ml of borate buffer pH = 10 were prepared separately by mixing 13 ml of NaOH 0.1 M solution and 17 ml of Sodium Tetraborate solution (0.05 M in DDW). Solution B was prepared by adding 15  $\mu\text{l}$  2-mercaptoethanol to previously prepared 30 ml borate buffer. The two solutions were then combined to prepare a 40 ml stock solution made of 10 ml solution A and 30 ml solution B. Samples at concentration of  $100 \text{ mg ml}^{-1}$  were diluted to  $1 \text{ mg ml}^{-1}$  with DDW prior to measurement. About 100  $\mu\text{l}$  of natural or modified gelatin samples ( $1 \text{ mg ml}^{-1}$ ) at different time points throughout the deprotection reaction were added to a 96-well microplate. About 200  $\mu\text{l}$  stock solution was added to each well (300  $\mu\text{l}$  total volume in each well) and absorbance at  $\lambda_{\text{max}} = 340 \text{ nm}$  was measured 2 min after addition of stock solution to the samples. Blank (i.e., mixture with water instead of gelatin) measurements were then subtracted from the sample measurements. Similar measurements were performed with glycine (0.03–0.5 mM) standards to obtain a calibration curve (Figure S7). Calculation of the amount of free amine groups remaining after the modification enabled determination of the deprotection degree of the Boc-protected gelatins. Once the absorbance measurement reached the measurement of the unmodified gelatin, the samples were dialyzed for 48 hr (molecular weight cut-off: 3,500 Da) at 42°C against DDW, frozen, and lyophilized prior to further use. Final products were denoted Gel-AD and Gel-CD.

## 2.5 | Rheological analysis of gelatin hydrogels

### 2.5.1 | Assessing shear-thinability

The hydrogels were formed on the rheometer plate by mixing varying volume ratios of Gel-CD in DDW ( $400 \text{ mg ml}^{-1}$ ) with Gel-AD in DDW

( $400 \text{ mg ml}^{-1}$ ) between parallel plates (8 mm diameter). An overall volume of 40  $\mu\text{l}$  was used to form hydrogels with 0.5 mm thickness. Silicone oil was used to seal the sample and prevent sample drying during analysis. The storage and loss moduli ( $G'$  and  $G''$ , respectively) were recorded at constant angular frequency ( $10 \text{ rad s}^{-1}$ ) with cyclic strain varying from low and high strain (10 and 400%, respectively) at room temperature. Angular frequency, low, and high strain were determined according to angular frequency sweep and strain amplitude sweep, respectively, below the limit of the linear viscoelastic region.

### 2.5.2 | Oscillatory time sweeps of gelatin hydrogels varying in Gel-AD:Gel-CD ratios

$G'$  and  $G''$  of hydrogels were measured in hydrogels formed with varying ratios of Gel-AD:Gel-CD (5:1–1:5) and a constant combined total mass concentration ( $400 \text{ mg ml}^{-1}$  in DDW). Measurements were conducted 30 min following sample mixing on the rheometer plate and spanned over 5 min at an angular frequency of  $10 \text{ rad s}^{-1}$  and oscillatory strain of 10%. Two hydrogels of each formation were measured, and an average and standard deviation of the results over time was determined.

## 2.6 | Stem-cell derived cardiomyocyte injection and viability assays

WTC11 cell line of induced pluripotent stem-cell-derived cardiomyocytes (iPSC-CMs) were gifted (Charles Murry Laboratory, University of Washington, Pathology) on Day 18 of directed differentiation and immediately used for injection. To evaluate cell protection during injection under needle flow, iPSC-CMs were suspended and encapsulated ( $4 \times 10^6$  cells  $\text{ml}^{-1}$ ) within pro-survival cocktail solutions through gentle mixing of Gel-AD and Gel-CD (2:1,  $400 \text{ mg ml}^{-1}$  total). Pro-survival cocktail was prepared in RPMI media as previously reported (Laflamme et al., 2007) and contained 50% (v/v) growth factor-reduced Matrigel™, benzyloxycarbonyl-Val-Ala-Asp(O-methyl)-fluoromethyl ketone (100  $\mu\text{M}$ ), Bcl-XL BH4 (50 nM, cell-permeant TAT peptide), cyclosporine A (200 nM), IGF-1 (100 ng  $\text{ml}^{-1}$ ), and pinacidil (50  $\mu\text{M}$ ). The gelatin-cell constructs and control suspensions, both in pro-survival cocktail, were carefully transferred to 1 ml syringes and injected at 100  $\mu\text{l s}^{-1}$  through a 28-G needle. Cells were tested for viability using a NC-200 NucleoCounter® for live/dead cell counts and imaged. Final cell populations were assayed twice with  $n = 5$  samples.

## 3 | RESULTS

### 3.1 | Chemical modification of gelatin

#### 3.1.1 | Synthesis of modified gelatin derivatives

This study aims to develop novel shear-thinning gelatin derivatives that mix to form an injectable gel that can be used for therapeutic

cargo delivery. Gelatin contains many chemical functionalities in the side chains of the different amino acids which can be exploited for bioconjugation. Amine functionalities are present in the side chains of lysine and hydroxylysine; carboxylic acids in glutamic and aspartic acid; and hydroxyl functionalities in serine, threonine, and hydroxylysine (Mark, 2009). While most reported gelatin-modification strategies exploit gelatin's primary amines as their reactive anchor to introduce nonnatural chemical functionalities (Van Hoorick et al., 2019), we elected to use the carboxylic acid moieties as they are more abundant (7.7%  $-\text{COOH}$  relative to 3.3%  $-\text{NH}_2$ , based on amino acid compositional make-up; Mark, 2009). In order to introduce  $\beta$ -cyclodextrin (CD) as a pendant moiety on the gelatin backbone, CD was modified to contain a primary amine by addition of a single 1,6-hexanediamine (HDA) chain to the cyclic CD carbohydrate. This was achieved by initial mono-tosylation of CD, according to previously reported syntheses, under basic aqueous conditions (Fetter et al., 1990; Kaya & Mathias, 2010; Rodell et al., 2013). Chemical structure of product was verified using  $^1\text{H}$  NMR (Figure S4) prior to overnight reaction with excess HDA in anhydrous DMF under dry  $\text{N}_2$  conditions at  $80^\circ\text{C}$ .  $^1\text{H}$  NMR was used to confirm the structure of the product CD-HDA (Figure S5). The modification of carboxylic acid functionalities present in the glutamate and aspartate side chains of the gelatin was carried out using conventional carbodiimide coupling in anhydrous DMSO under dry  $\text{N}_2$  conditions at  $55^\circ\text{C}$  overnight. First, the primary amine side chains of gelatin were protected using di-*tert*-butyl dicarbonate (Boc) in order to prevent crosslinking between activated carboxylic acid side chains with primary amine side chains. Then, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) was introduced, followed by addition of *N*-hydroxysuccinimide (NHS) to stabilize the activated carboxylic acid groups (Figure S1). Using a primary amine as a nucleophile (either 1-adamantylamine or CD-HDA), a nucleophilic substitution was realized, and a stable amide bond was formed. As a result, Adamantane (AD) or CD were chemically grafted onto the gelatin backbone (Figures S2 and S6).

### 3.1.2 | Deprotection of primary amines of modified gelatin

The quantity of incorporated Boc amino-protective groups in gelatin derivatives was determined by evaluating the difference between the amine content in modified compared to unmodified gelatin. As a result of primary amine protection with Boc, less amines in gelatin are available for reaction with OPA resulting in lower absorbance values at 340 nm. Amine deprotection was achieved through extended mild acid treatment (0.5% v/v of 12 M HCl) and evaluated spectrometrically using OPA. As deprotection progressed over time, more primary amines become available to react with OPA, which results in higher absorbance values at 340 nm. Full Boc deprotection was achieved after  $\sim 2$  weeks of mild acid treatment for all samples (Figure S8). Importantly, unmodified gelatin that has been acid-treated does not show an increase in amine content compared to untreated gelatin over the course of the treatment, indicating that no acid-

catalyzed gelatin backbone degradation occurs. Moreover, untreated samples showed no significant increase in primary amine content, confirming stability of Boc-protection and justifying acid addition for deprotection. A calibration curve of amine concentration (mM) to absorbance was constructed using glycine standards of increasing concentrations (Figure S7). From this linear correlation, we determined that our unmodified gelatin contained 0.291 mmol primary amines per gram. This finding is in line with previous primary amine measurements of gelatin type A that reported 0.284–0.288 mmol primary amines per gram of gelatin (Kale & Bajaj, 2010).

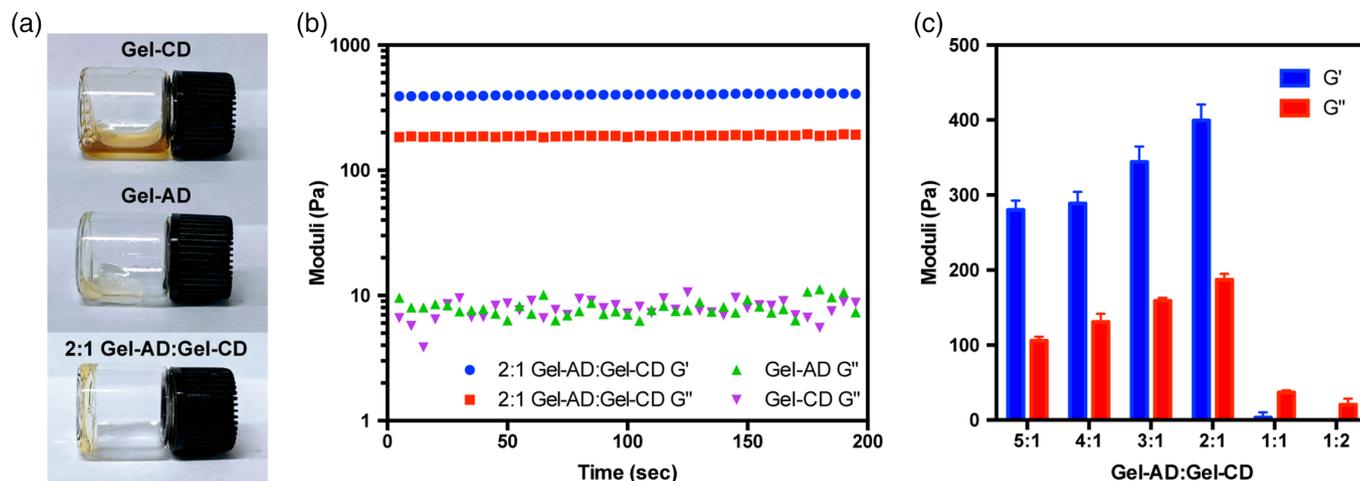
## 3.2 | Formation of gelatin guest-host hydrogels with tunable initial moduli

Though solutions of Gel-CD and Gel-AD were free-flowing liquids at room temperature, we anticipated that mixtures of Gel-CD with Gel-AD would result in soft hydrogel biomaterials formed through guest-host complexation based on physical hydrophobic bonding between CD and AD. Initial tests involved tube-inversion assays, with Gel-CD and Gel-AD mixed at varying ratios while keeping the total gelatin content within a sample constant. Reaction mixtures containing excess Gel-AD yielded stable hydrogels, remaining bound in its solid form to the bottom of scintillation vials when subjected to gravitational forces while inverted (Figure 2a).

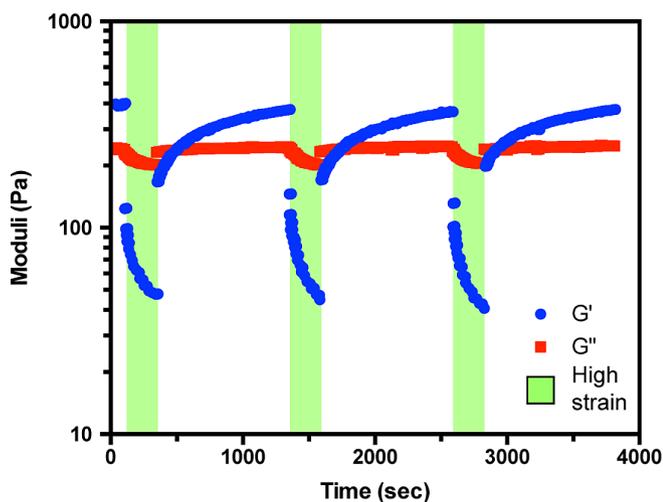
To further characterize the viscoelastic properties of the formed gels, we performed rheological oscillatory time sweep experiments of gels of varying composition. Storage ( $G'$ ) and loss ( $G''$ ) moduli were determined for mixtures of Gel-AD:Gel-CD (spanning ratios 5:1–1:5). The concentration of each individual solution was  $400\text{ mg ml}^{-1}$  regardless of the ratio of AD to CD, such that the any change in material properties could be directly attributed to changes in guest-host complexation. Analysis demonstrated that gels formed with 2:1 Gel-AD:Gel-CD yielded a solid-like material with  $G' \sim 400\text{ Pa}$  and  $G'' \sim 10\text{ Pa}$  (Figure 2b). As the Gel-AD:Gel-CD ratio increases, a reduction in storage modulus was observed, with both the 4:1 and 5:1 samples yielding solid materials with similar properties (Figure 2c). Interestingly, mixtures with excess Gel-CD relative to Gel-AD did not result in gel formation, as indicated by behavior of  $G'' > G'$  or an absence of storage modulus. These results indicate that stable hydrogel formation is dependent on the quantity of guest species compared to host moieties present in the gel. The ability to tune the mechanical properties of the gel, by controlling the ratio of host to guest and therefore controlling the physical crosslinking density, is an important feature that may allow a formation of a hydrogel with the desired mechanical properties suitable to the relevant application.

## 3.3 | Shear-thinability and self-healing of gelatin hydrogels

Having demonstrated that guest-host assembly of functionalized gelatins resulted in stable hydrogel formation, we sought to assess their responsiveness to high shear and their ability to undergo self healing



**FIGURE 2** Tunable gelatin hydrogel formation through guest-host complexation. (a) Inversion tests of Gel-AD, Gel-CD, and 2:1 Gel-AD:Gel-CD (each mixture totaling  $400 \text{ mg ml}^{-1}$ ). (b) Oscillatory rheological time sweeps of Gel-AD, Gel-CD, and 2:1 Gel-AD:Gel-CD with  $G'$  and  $G''$  measured at 10% strain, and an angular velocity of  $10 \text{ rad s}^{-1}$ . (c) Storage and loss moduli for gels formed with varying Gel-AD:Gel-CD ratios. Error bars indicate standard deviation of measurements about the mean for experimental duplicates



**FIGURE 3** Shear-thinning behavior of guest-host gelatin hydrogels. Rheology measurements of  $G'$  (storage modulus; blue circles) and  $G''$  (loss modulus; red squares) at cycles of high oscillatory strain (400%, green background) followed by low oscillatory strain (10%, white background) of hydrogel composed of 2:1 Gel-AD:Gel-CD ( $400 \text{ mg ml}^{-1}$ ). Data collected at  $10 \text{ rad s}^{-1}$

(Figure 3). Toward this, hydrogels of varying AD:CD ratios (5:1–2:1) were subjected to cycles of high oscillatory strain (400%) followed by low oscillatory strain (10%). At low shear, solid-like materials persisted as previously discussed (Figure 2). Under high strain, the loss moduli overtook the storage moduli in all samples, indicating a near-instantaneous disassembly for the preformed network and a gel-to-sol transition. Though not explicitly measured in these types of rheological studies, such experimental findings correlate well with gel injectability (Guvendiren et al., 2012).

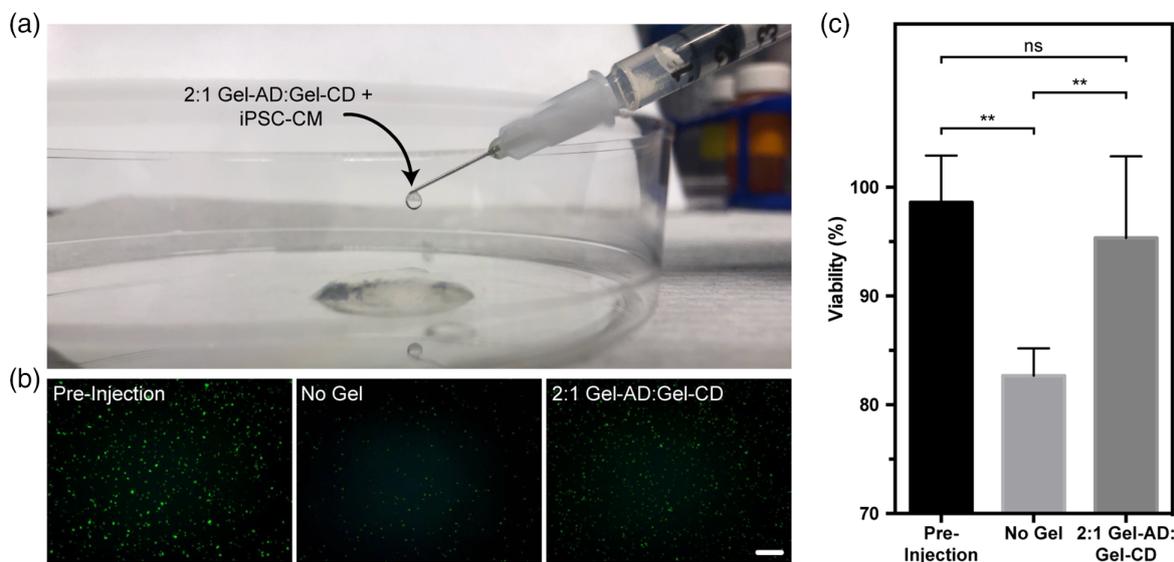
Upon cessation of high strain conditions, an elastic solid-like behavior occurred ( $G' > G''$ ), and a gradual recovery to the initial

storage modulus was observed. The  $t_{1/2}$  constant of recovery was  $\sim 5 \text{ min}$ , corresponding to the time required for gelatin chain diffusion and AD/CD complexation to occur. Similar self-healing mechanical characteristics were observed regardless of the number of cycles performed. These results indicate that the materials are capable of recovery following high-shear events such as injection, that will potentially allow for retention of the matrix with the intercalated therapeutic cargo at the desired target site.

### 3.4 | Gelatin materials support cell injection

Encouraged by the shear-responsiveness of the gelatin-based hydrogel materials, we sought to directly assess their injectability. Mixtures of Gel-AD and Gel-CD were prepared in 1 ml syringes outfitted with 28 G needles (0.184 mm inner diameter). We anticipated that the shear forces associated with manual extrusion would induce the same reversible gel-to-sol transition that was observed rheologically. Indeed, gel mixtures were readily injectable, transitioning back to a solid form shortly after injection ( $100 \mu\text{l s}^{-1}$ ; Figure 4a).

Finally, we tested the ability of the Gel-AD/Gel-CD materials to support cell delivery. Knowing that direct injection of stem-cell-derived cardiomyocytes into the heart muscle following MI has proven to be an effective therapy in nonhuman primate models (Chong et al., 2014), even in spite of poor engraftment rates and cell survival through the injection process (<20%), we tested the ability of Gel-AD/Gel-CD materials to deliver and protect iPSC-CMs during injection under needle flow. iPSC-CMs were suspended in pro-survival cocktail, a collection of growth factors previously optimized to maximize cell engraftment (Lafamme et al., 2007), and injected through a 28-G needle at  $100 \mu\text{l s}^{-1}$  as a free cell suspension or in the 2:1 Gel-AD:Gel-CD mixture. Following injection, iPSC-CMs in the 2:1 Gel-AD:Gel-CD mixture were determined to be  $95 \pm 7\%$  viable, a



**FIGURE 4** Guest-host gelatin hydrogel protect cells during injection. (a) 2:1 Gel-AD:Gel-CD mixtures containing encapsulated iPSC-CMs are injectable. (b and c) Cell injected in gelatin materials exhibit higher survival and viability than those injected in free suspension in pro-survival cocktail alone. (b) Live cells are stained in green for each experimental condition. (c) Quantification of iPSC-CM viability. Error bars correspond to  $\pm 1$  SD about the mean for  $n = 5$  experimental replicates (\*\* $p < .01$ , unpaired two-tailed  $t$  test; ns indicates nonsignificance). Scale bar = 100  $\mu\text{m}$ . iPSC-CM, induced pluripotent stem-cell-derived cardiomyocyte

value that was statistically indistinguishable with the iPSC-CM population suspended in pro-survival cocktail prior to injection ( $98 \pm 4\%$ ). Free cell suspension of iPSC-CMs exhibited a statistically significant loss in viability immediately following injection ( $83 \pm 3\%$  viable), attributed to cell-damaging forces during injection that shear-thinning materials are known to help dissipate. We anticipate that the protective nature of the shear-thinning guest-host gelatin gels could have significant clinical implications in cardiac regeneration and beyond.

## 4 | DISCUSSION

In this work, we have added gelatin-based materials to the growing arsenal of shear-thinning biomaterials. Derived from the most abundant ECM protein, these materials offer unique advantages with respect to biocompatibility, biodegradability, and nonimmunogenicity, as well the ability to support 3D cell culture and function. Hydrogel precursors (Gel-AD and Gel-CD) are synthesized through simple, straight-forward, and scalable chemistries, and can be lyophilized and stably stored until the point of use. Through altered molar ratios and total biopolymer content, materials with different mechanical properties can be accessed. Self-healing materials are assembled through noncovalent guest-host interactions that have been previously applied to stabilize networks based on synthetic polymers and proteoglycans, and have found utility both in vitro and in vivo. Though not explicitly assessed as part of this study, given prior results with other base materials (Gaffey et al., 2015; Rodell et al., 2016), the guest-host chemistry itself is not expected to elicit an immune response should our gelatin-based system be applied in vivo.

In addition to exhibiting shear-thinability and self-healing as engineered, the gelatin guest-host hydrogels were demonstrated as

injectable through needles of clinically relevant sizes and at a constant rate. Cells encapsulated in gels were protected from membrane-disrupting shear forces present during injection, exhibiting higher viability in gels compared to cells injected directly as a suspension. Given challenges with cell engraftment and poor viability associated with current delivery protocols, the introduced materials are likely to further cell transplantation as a viable therapy for variety of debilitating diseases. Given our promising findings with stem-cell-derived cardiomyocytes, coupled with recent cell therapy successes in nonhuman primate models (Chong et al., 2014), we are excited at the prospect of using these materials to aid in the treatment of MI.

## 5 | CONCLUSION

In this work, we have demonstrated that the incorporation of CD and AD moieties onto the gelatin backbone enables facile generation of physically cross-linked hydrogel biomaterials with tunable mechanical properties. Since CD and AD guest-host complexation is rapid and reversible, this chemistry enables unique materials to be generated that are injectable and exhibit repeatable self-healing. In studies involving induced pluripotent stem-cell-derived cardiomyocytes, we have demonstrated material utility in supporting cell protection during injection in a clinically relevant cell type. As the first self-healing hydrogel platform to be based solely on ECM proteins, as well as derived from the most abundant protein in the human body, these materials are well positioned for a variety of applications in cell/therapeutic delivery and tissue engineering.

## ACKNOWLEDGMENTS

The authors recognize and thank Dr. C. Murry (University of Washington) for providing iPSC-CMs and for helpful discussion involving cardiac cell transplantation. This work was supported by a University of Washington Faculty Startup Grant (C.A.D.), a Fulbright Scholarship (A.M.S.), and a University of Washington Bioengineering Cardiovascular Training Grant (National Institutes of Health Institutional National Research Service Award, T32EB001650, awarded to M.O.B.).

## ORCID

Mary O. Boit  <https://orcid.org/0000-0001-9990-6950>

Cole A. DeForest  <https://orcid.org/0000-0003-0337-3577>

## REFERENCES

- Aguado, B. A., Mulyasmita, W., Su, J., Lampe, K. J., & Heilshorn, S. C. (2012). Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers. *Tissue Engineering. Part A*, 18, 806–815.
- Amado, L. C., Saliaris, A. P., Schuleri, K. H., St. John, M., Xie, J. S., Cattaneo, S., ... Hare, J. M. (2005). Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 11474–11479.
- Annabi, N., Yue, K., Tamayol, A., & Khademhosseini, A. (2015). Elastic sealants for surgical applications. *European Journal of Pharmaceutics and Biopharmaceutics*, 95, 27–39.
- Badeau, B. A., Comerford, M. P., Arakawa, C. K., Shadish, J. A., & DeForest, C. A. (2018). Engineered modular biomaterial logic gates for environmentally triggered therapeutic delivery. *Nature Chemistry*, 10, 251–258.
- Badeau, B. A., & DeForest, C. A. (2019). Programming stimuli-responsive behavior into biomaterials. *Annual Review of Biomedical Engineering*, 21, 241–265.
- Benton, J. A., DeForest, C. A., Vivekanandan, V., & Anseth, K. S. (2009). Photocrosslinking of gelatin macromers to synthesize porous hydrogels that promote valvular interstitial cell function. *Tissue Engineering Part A*, 15, 3221–3230.
- Bertero, A., & Murry, C. E. (2018). Hallmarks of cardiac regeneration. *Nature Reviews. Cardiology*, 15, 579–580.
- Caliri, S. R., & Burdick, J. A. (2016). A practical guide to hydrogels for cell culture. *Nature Methods*, 13, 405–414.
- Caló, E., & Khutoryanskiy, V. V. (2015). Biomedical applications of hydrogels: A review of patents and commercial products. *European Polymer Journal*, 65, 252–267.
- Chong, J. J. H., Yang, X., Don, C. W., Minami, E., Liu, Y.-W., Weyers, J. J., ... Murry, C. E. (2014). Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature*, 510, 273–277.
- DeForest, C. A., & Anseth, K. S. (2012). Advances in bioactive hydrogels to probe and direct cell fate. *Annual Review of Chemical and Biomolecular Engineering*, 3, 421–444.
- Dobaczewski, M., & Frangogiannis, N. G. (2008). Chemokines in myocardial infarction: Translating basic research into clinical medicine. *Future Cardiology*, 4, 347–351.
- Doenst, T., Diab, M., Sponholz, C., Bauer, M., & Färber, G. (2017). The opportunities and limitations of minimally invasive cardiac surgery. *Deutsches Arzteblatt International*, 114, 777–784.
- Drury, J. L., & Mooney, D. J. (2003). Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials*, 24, 4337–4351.
- Engler, A. J., Carag-Krieger, C., Johnson, C. P., Raab, M., Tang, H.-Y., Speicher, D. W., ... Discher, D. E. (2008). Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: Scar-like rigidity inhibits beating. *Journal of Cell Science*, 121, 3794–3802.
- Fetter, R. C., Salek, J. S., Sikorski, C. T., Kumaravel, G., & Lin, F. T. (1990). Cooperative binding by aggregated mono-6-(Alkylamino)- $\beta$ -cyclodextrins. *Journal of the American Chemical Society*, 112, 3860–3868.
- Gaffey, A. C., Chen, M. H., Venkataraman, C. M., Trubelja, A., Rodell, C. B., Dinh, P. V., ... Atluri, P. (2015). Injectable shear-thinning hydrogels used to deliver endothelial progenitor cells, enhance cell engraftment, and improve ischemic myocardium. *The Journal of Thoracic and Cardiovascular Surgery*, 150, 1268–1276.
- Gerbin, K. A., & Murry, C. E. (2015). The winding road to regenerating the human heart. *Cardiovascular Pathology*, 24, 133–140.
- Ghobril, C., & Grinstaff, M. W. (2015). The chemistry and engineering of polymeric hydrogel adhesives for wound closure: A tutorial. *Chemical Society Reviews*, 44, 1820–1835.
- Guvendiren, M., Lu, H. D., & Burdick, J. A. (2012). Shear-thinning hydrogels for biomedical applications. *Soft Matter*, 8, 260–272.
- Healy, K. E., Rezaia, A., & Stile, R. A. (1999). Designing biomaterials to direct biological responses. *Annals of the New York Academy of Sciences*, 875, 24–35.
- Hoare, T. R., & Kohane, D. S. (2008). Hydrogels in drug delivery: Progress and challenges. *Polymer (Guildf)*, 49, 1993–2007.
- Hoffman, A. S. (2012). Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, 64, 18–23.
- Ifkovits, J. L., & Burdick, J. A. (2007). Review: Photopolymerizable and degradable biomaterials for tissue engineering applications. *Tissue Engineering*, 13, 2369–2385.
- Kale, R., & Bajaj, A. (2010). Ultraviolet spectrophotometric method for determination of gelatin crosslinking in the presence of amino groups. *Journal of Young Pharmacists*, 2, 90–94.
- Kaya, E., & Mathias, L. J. (2010). Synthesis and characterization of physical crosslinking systems based on cyclodextrin inclusion/host-guest complexation. *Journal of Polymer Science, Part A: Polymer Chemistry*, 48, 581–592.
- Khademhosseini, A., & Langer, R. (2007). Microengineered hydrogels for tissue engineering. *Biomaterials*, 28, 5087–5092.
- Korf-Klingebiel, M., Rebold, M. R., Klede, S., Brod, T., Pich, A., Polten, F., ... Wollert, K. C. (2015). Myeloid-derived growth factor (C19orf10) mediates cardiac repair following myocardial infarction. *Nature Medicine*, 21, 140–149.
- Kuijpers, A. J., Engbers, G. H. M., Krijgsveld, J., Zaat, S. A. J., Dankert, J., & Feijen, J. (2000). Cross-linking and characterisation of gelatin matrices for biomedical applications. *Journal of Biomaterials Science. Polymer Edition*, 46, 520–230.
- Laflamme, M. A., Chen, K. Y., Naumova, A. V., Muskheli, V., Fugate, J. A., Dupras, S. K., ... Murry, C. E. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nature Biotechnology*, 25, 1015–1024.
- Laflamme, M. A., & Murry, C. E. (2011). Heart regeneration. *Nature*, 473, 326–335.
- Lee, J. H. (2018). Injectable hydrogels delivering therapeutic agents for disease treatment and tissue engineering. *Biomaterials Research*, 22, 27.
- Lockhart, M., Wrigg, E., Phelps, A., & Wessels, A. (2011). Extracellular matrix and heart development. *Birth Defects Research Part A: Clinical and Molecular Teratology*, 91, 535–550.
- Lynn, A. K., Yannas, I. V., & Bonfield, W. (2004). Antigenicity and immunogenicity of collagen. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 71, 343–354.
- Mark, H. F., & Kroschwitz, J. I. (1987). *Encyclopedia of polymer science and engineering*. New York: John Wiley & Sons, Inc.
- Mark, J. E. (2009). *Polymer data handbook*. Oxford: Oxford University Press.
- Nichol, J. W., Koshy, S. T., Bae, H., Hwang, C. M., Yamanlar, S., & Khademhosseini, A. (2010). Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials*, 31, 5536–5544.

- Nicodemus, G. D., & Bryant, S. J. (2008). Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Engineering. Part B, Reviews*, 14, 149–165.
- Nikkhah, M., Akbari, M., Paul, A., Memic, A., Dolatshahi-Pirouz, A., & Khademhosseini, A. (2016). *Biomaterials from nature for advanced devices and therapies* (pp. 37–62). New Jersey: Wiley Blackwell.
- Nikkhah, M., Eshak, N., Zorlutuna, P., Annabi, N., Castello, M., Kim, K., ... Khademhosseini, A. (2012). Directed endothelial cell morphogenesis in micropatterned gelatin methacrylate hydrogels. *Biomaterials*, 33, 9009–9018.
- Rebouças, J. d. S., Santos-Magalhães, N. S., & Formiga, F. R. (2016). Cardiac regeneration using growth factors: Advances and challenges. *Arquivos Brasileiros de Cardiologia*, 107, 271–275.
- Roche, E. T., Hastings, C. L., Lewin, S. A., Shvartsman, D. E., Brudno, Y., Vasilyev, N. V., ... Mooney, D. J. (2014). Comparison of biomaterial delivery vehicles for improving acute retention of stem cells in the infarcted heart. *Biomaterials*, 35, 6850–6858.
- Rodell, C. B., Kaminski, A. L., & Burdick, J. A. (2013). Rational design of network properties in guest-host assembled and shear-thinning hyaluronic acid hydrogels. *Biomacromolecules*, 14, 4125–4134.
- Rodell, C. B., Lee, M. E., Wang, H., Takebayashi, S., Takayama, T., Kawamura, T., ... Gorman, R. C. (2016). Injectable shear-thinning hydrogels for minimally invasive delivery to infarcted myocardium to limit left ventricular remodeling. *Circulation. Cardiovascular Interventions*, 9, e004058.
- Ruvinov, E., & Cohen, S. (2016). Alginate biomaterial for the treatment of myocardial infarction: Progress, translational strategies, and clinical outlook: From ocean algae to patient bedside. *Advanced Drug Delivery Reviews*, 96, 54–76.
- Seliktar, D. (2012). Designing cell-compatible hydrogels for biomedical applications. *Science*, 336, 1124–1128.
- Shadish, J. A., Benuska, G. M., & DeForest, C. A. (2019). Bioactive site-specifically modified proteins for 4D patterning of gel biomaterials. *Nature Materials*, 18, 1005–1014.
- Sinclair, A., O'Kelly, M. B., Bai, T., Hung, H. C., Jain, P., & Jiang, S. (2018). Self-healing zwitterionic microgels as a versatile platform for malleable cell constructs and injectable therapies. *Advanced Materials*, 30, 1870291.
- Sung, H. W., Huang, D. M., Chang, W. H., Huang, R. N., & Hsu, J. C. (1999). Evaluation of gelatin hydrogel crosslinked with various crosslinking agents as bioadhesives: in vitro study. *Journal of Biomedical Materials Research*, 46, 520–530.
- Thambi, T., Li, Y., & Lee, D. S. (2017). Injectable hydrogels for sustained release of therapeutic agents. *Journal of Controlled Release*, 267, 57–66.
- Tibbitt, M. W., & Anseth, K. S. (2012). Dynamic microenvironments: The fourth dimension. *Science Translational Medicine*, 4, 160ps24.
- Vadakke-Madathil, S., & Chaudhry, H. W. (2018). Cardiac regeneration time to revisit nature. *Circulation Research*, 123, 24–26.
- Van Hoorick, J., Tytgat, L., Dobos, A., Ottevaere, H., Van Erps, J., Thienpont, H., ... Van Vlierberghe, S. (2019). (Photo-)crosslinkable gelatin derivatives for biofabrication applications. *Acta Biomaterialia*, 97, 46–73. <https://doi.org/10.1016/j.actbio.2019.07.035>
- Van Nieuwenhove, I., Stubbe, B., Graulus, G.-J., Van Vlierberghe, S., & Dubruel, P. (2014). Protein functionalization revised: N-tert-butoxycarbonylation as an elegant tool to circumvent protein crosslinking. *Macromolecular Rapid Communications*, 35, 1351–1355.
- Wong Po Foo, C. T. S., Lee, J. S., Mulyasmita, W., Parisi-Amon, A., & Heilshorn, S. C. (2009). Two-component protein-engineered physical hydrogels for cell encapsulation. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 22067–22072.
- Youngblood, R. L., Truong, N. F., Segura, T., & Shea, L. D. (2018). It's all in the delivery: Designing hydrogels for cell and non-viral gene therapies. *Molecular Therapy*, 26, 2087–2106.
- Zhang, S. (2002). Emerging biological materials through molecular self-assembly. *Biotechnology Advances*, 20, 321–339.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Sisso AM, Boit MO, DeForest CA. Self-healing injectable gelatin hydrogels for localized therapeutic cell delivery. *J Biomed Mater Res*. 2020;108A: 1112–1121. <https://doi.org/10.1002/jbm.a.36886>