Hyaluronic acid-based shape-memory cryogel scaffolds for focal cartilage defect repair

Tengfei He[a], Boting Li[a], Thibault Colombani[b], Kasturi Joshi-Navare[b], Shikhar Mehta[a], John Kisiday[c], Sidi A. Bencherif[a,b], Ambika G. Bajpayee[a,d]

[a]Department of Bioengineering, [b]Chemical Engineering and [d]Mechanical Engineering
Northeastern University, 805 Columbus Ave, Boston, MA, USA

[c]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

Author Emails:

Tengfei He | he.te@northeastern.edu | 617-459-0611
Boting Li | li.bot@northeastern.edu | 857-919-9540
Thibault Colombani | t.colombani@northeastern.edu | 617-818-7261
Kasturi Joshi-Navare | k.joshinavare@northeastern.edu | 617-373-2000
Shikhar Mehta | mehta.shi@northeastern.edu | 781-439-2095
John Kisiday | john.kisiday@colostate.edu | 970-297-5071
Sidi A. Bencherif | s.bencherif@northeastern.edu
Ambika G. Bajpayee | a.bajpayee@northeastern.edu

Corresponding Authors:

Sidi A. Bencherif
336 Mugar Life Sciences Building
330 Huntington Avenue, Boston, MA, USA
+1 617-373-7103 (Phone)
s.bencherif@northeastern.edu

Ambika G. Bajpayee
ISEC Room 216
805 Columbus Avenue, Boston, MA, USA
+1 617-373-7018 (Phone)
a.bajpayee@northeastern.edu

Running Title: Injectable Shape Memory Chondrocyte-Loaded Cryogels

Keywords: injectable scaffolds; hyaluronic acid; cryogel; shape-memory; cartilage repair; tissue engineering

Abstract

Traumatic joint injuries can result in significant cartilage defects, which can greatly increase the risk of osteoarthritis development. Due to the limited self-healing capacity of avascular cartilage, tissue engineering approaches are required for filling defects and promoting cartilage regeneration. Current approaches utilize invasive surgical procedures for extraction and implantation of autologous chondrocytes, therefore injectable biomaterials have gained interest in order to minimize the risk of infection as well as patient pain and discomfort. Here, we engineered biomimetic, hyaluronic acid (HA)-based cryogel scaffolds that possess shape-memory properties as they contract and regain their shape following syringe injection to non-invasively fill cartilage defects. The cryogels, fabricated with HA and glycidyl methacrylate at -20 °C, resulted in an elastic, macroporous and highly interconnected network that provided a conducive microenvironment for chondrocytes to remain viable and metabolically active following injection through a syringe needle. Chondrocytes seeded within cryogels and cultured for 15 days exhibited enhanced cell proliferation, metabolism and production of cartilage extracellular matrix glycosaminoglycans compared to in HA-based hydrogels. Furthermore, immunohistochemical staining revealed production of collagen type II from chondrocyte-seeded cryogels, indicating the maintenance of cell phenotype. These results demonstrate the potential of chondrocyte-seeded, HA-based, injectable cryogel scaffolds to promote regeneration of cartilage tissue for non-surgically invasive defect repair.

Impact Statement: Hyaluronic acid-based shape memory cryogels provide a conducive microenvironment for chondrocyte adhesion, proliferation and matrix biosynthesis for use in repair of cartilage defects. Due to their sponge-like elastic properties, cryogels can fully recover their original shape back following injection while not impacting metabolism or viability of encapsulated cells. Clinically, they provide an opportunity for filling focal cartilage defects by using a single, minimally invasive injection of a cell encapsulating biocompatible 3D scaffold that can return to its original structure to fit the defect geometry and enable matrix regeneration.

INTRODUCTION

Traumatic joint injuries can result in focal cartilage defects that regenerate poorly due to a lack of blood supply and low chondrocyte density in cartilage [1]. Cartilage defects, if left untreated, can cause the tissue to lose its mechanical integrity, while the joint loses lubrication between bones, ultimately leading to severe pain and disability [2]. Currently, the most promising FDA-approved treatment for regeneration of cartilage defects is MACI® - a matrix-applied implantation of autologous chondrocytes cultured on a porcine collagen membrane [3]. Despite the clinical improvement it has provided, this technique has a number of limitations. Primarily, MACI® requires two surgical procedures – an arthroscopy for chondrocyte collection and a mini-arthrotomy for implantation. Secondly, the membrane used for culturing cells is comprised of collagen types I and III derived from porcine tissue which increases the risk of an immune response following implantation [3]. Thus, an improved design that can limit the number of surgeries or bypass

surgical implantations, while improving biocompatibility is desirable for cartilage defect repair.

Hydrogels (e.g., PEG, alginate, self-assembling peptides, hyaluronic acid (HA), collagen) have been extensively studied as conventional 3D scaffolds for their biocompatible characteristics, mimicking the extracellular matrix [4-10]. However, hydrogels often exhibit a quasi-nonporous network which limits cell proliferation, mechanical flexibility and diffusion of nutrients, oxygen and cellular waste [11-13]. Implantation of preformed hydrogels typically requires invasive surgical procedures such as arthrotomic surgery [14, 15], thus injectable hydrogels have gained interest as a method for filling defects due to their minimally invasive nature [16-19]. However, due to the fact that a pre-gelation liquid has to be delivered to the defect, issues with gelation time can cause leakage to adjacent tissues or body fluids resulting in potential toxicity or changes in tissue mechanical properties [9, 20-23]. Thus, injectable scaffolds possessing shape-memory properties (the ability to contort within the needle and then recover back to the original shape of the defect immediately) are desired for maintaining mechanical properties and for preventing leakage from occurring [4, 12, 24-28]. Cryogels, a subclass of hydrogel, formed from crystallization at freezing temperatures, have been shown to offer shape-memory properties and have several advantageous features such as a macroporous architecture with highly interconnected pores (for unrestricted flow of nutrients and matrix proteins), and adequate mechanical strength and swelling capacity (for shape-memory and integrity) [12, 23, 24]. These properties allow efficient flow of nutrients and cellular trafficking which in turn facilitate tissue integration [29]. Cryogels can be fabricated using naturally derived polymers, thus reducing risk of toxicity. For example, HA-based cryogels are

biocompatible and biodegradable, thereby limiting an immune response and allowing for the scaffold to be replaced by repaired tissue [2]. Therefore, due to their injectable shape-memory properties and their biocompatibility, the use of cryogels as cell-seeding scaffolds for cartilage defect repair is promising [30, 31].

Here, we engineered macroporous biomaterials with shape-memory properties in order to provide a conducive environment for chondrocyte adhesion, proliferation and matrix formation. We first synthesize HA-based cryogel scaffolds with and without RGD, a cell adhesion peptide, to evaluate cell-cryogel interactions, and then test their mechanical properties for comparison with conventional HA-based hydrogels. We then seed primary chondrocytes within the gels to provide the cells with a physiologically relevant microenvironment for in vitro culture. We hypothesize that (i) chondrocytes infused within HA-based cryogels will remain metabolically active following injection through a small-pore needle and (ii) that the macroporous and highly interconnected structure of cryogels will provide an unhindered environment for cell growth and diffusion of oxygen and nutrients, thereby enhancing chondrocyte metabolism and matrix production compared to that of mesoporous HA-based hydrogels.

MATERIALS AND METHODS

Materials

Ammonium persulfate (APS), N, N, N′, N′-Tetramethylethylenediamine (TEMED), Hyaluronic acid (HA), glycidyl methacrylate (GM), dimethylmethylene dye (DMMB), resazurin sodium salt, Griess reagent, Chloramine T and 4-(Dimethylamino) benzaldehyde (DMAB), Paraformaldehyde (PFA), Triton™ X-100, 4′,6-Diamidine-2′-

phenylindole dihydrochloride (DAPI) and other salts were purchased from Sigma-Aldrich (St. Louis, MO). Acrylate-PEG-N-hydroxysuccinimide (Acrylate-PEG-Succinimidyl Valerate, MW 3,400) was purchased from Laysan Bio Inc. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was purchased from Biobots. Trypan Blue and High-glucose Dulbecco's Modification of Eagle's Medium (DMEM) were purchased from Corning (Corning, NY). Fetal bovine serum (FBS) was purchased from GE Healthcare (Chicago, IL). HEPES, non-essential amino acids (NEAA), Penicillin-streptomycin Antibiotic-Antimycotic (PSA), L-proline, ascorbic acid and NHS-Rhodamine were purchased from Thermo Fisher Scientific (Waltham, MA). Quant-iT™ PicoGreen™ dsDNA Assay Kit was purchased from Invitrogen (Cambridge, MA). ViaQuant™ Far-Red Fixable Dead Cell Stain Kit and Alexa Fluor 488-phalloidin stain kit were purchased from GeneCopoeia (Rockville, MD). The integrin binding peptide (Gly) 4-Arg-Gly-Asp-Ser-Pro (RGD) was purchased from Commonwealth Biotech (Midlothian, VA). Staining antibodies were provided by ServiceBio (Wuhan, China).

Fabrication of Hyaluronic Acid-based Cryogels and Hydrogels

Chemical modification of hyaluronic acid

HA was conjugated to GM to incorporate polymerizable methacrylate residues along the polymer backbone [32]. The resulting product, hyaluronic acid glycidyl methacrylate (HAGM), was characterized by ^{1}H NMR as previously described [1]. Rhodamine-labeled HAGM (R-HAGM) was prepared by reacting NHS-rhodamine with amine-terminated HAGM (NH_2-HAGM) based on a method previously reported [1]. R-HAGM was incorporated into hydrogels and cryogels to stain the polymer network for

imaging via confocal microscopy. G4RGDSP (containing the RGD sequence) was conjugated to acrylate-PEG-N-hydroxysuccinimide to form acrylate-PEG-G4RGDSP (APR), as previously reported [24]. This approach allows functionalized G4RGDSP to be covalently incorporated into the gels (hydrogels and cryogels) during polymerization to promote cell adhesion.

Synthesis of HA cryogels and hydrogels

HA-based cryogels were prepared with 3.2% (wt/v) HAGM and either 0.8% (wt/v; ~2 mM) APR (**Cryo RGD+**) or 0.8% (wt/v) methoxy-PEG-acrylate (MPA; **Cryo RGD-**) based on methods previously described [24] (**Fig. 1A**). Briefly, the polymers were first dissolved in pre-cooled deionized (DI) water. Next, 14% (wt/v) APS (20 µL/mL) and 7% (v/v) TEMED (10 µL/mL) were then added and mixed well. Next, the prepolymer solution was transferred into cold and sterile Teflon cylindrical molds (6 mm diameter × 1.5 mm height) and incubated overnight at -20 °C to allow for cryogelation. The next day, Cryo RGD+ and Cryo RGD- were thawed at room temperature to remove ice crystals, then washed and stored at 4 °C in DI water for future use.

HA-based hydrogels (**Hydro RGD+**) were fabricated by first mixing 3.2% (wt/v) HAGM, 0.8% (wt/v) APR, 0.5% (wt/v) LAP photoinitiator in DI water. Next, the prepolymer solution was transferred into Teflon cylindrical molds (6 mm diameter × 1.5 mm height) and photopolymerized for 30 seconds under a UV light (405 nm) with a 10W UV lamp. To fabricate cell-laden hydrogels, Hydro RGD+ were also photopolymerized with primary bovine chondrocytes as described in section 2.4.

Scanning electron microscopy (SEM) imaging and characterization of mechanical properties

For SEM analysis, cryogels were lyophilized and sputter-coated with platinum/palladium up to 5 nm thickness [33]. Gels were imaged using a Hitachi S-4800 scanning electron microscope (Hitachi High-Technology Corporation, Japan) at 3 KV and 10 μA.

Swelling ratio (Q_M) and pore connectivity were determined for further characterization [33]. For swelling ratio, gels were swelled in phosphate-buffered saline (PBS) at 37 °C under gentle shaking for 1 day to reach equilibrium. Then, the wet weights of gels were measured after removal of excess surface liquid. The gels were lyophilized and weighed to get the dry wright. The swelling ratio, $Q_{M,}$ was calculated by using the following equation:

$$Swelling\ ratio\ (\mathrm{Q_M}) = \frac{Wet\ weight - Dry\ weight}{Dry\ weight}$$

The Pore connectivity was calculated as following:

$$Pore\ connectivity\ (\%) = \frac{Wet\ weight - Wicked\ weight}{Wet\ weight} \times 100$$

where wet weight is the weight of fully hydrated gels, and wicked weight is obtained by measuring the gel weight after the free water is wicked away using absorbent wipes from the gel surface.

Bulk compressive elastic modulus of gels was examined by using stress-relaxation testing on an ElectroForce 5500 mechanical testing apparatus (TA instruments, DE). Cryogels were subjected to multiple successive stress-relaxation cycles of 10% strain per minute, followed by static holds of 800 s [33], whereas hydrogels were subjected to a single

cycle for the same strain rate. For each cycle, the compression displacement and load of the scaffolds were recorded with WinTest7 software. Equilibrium stress values were extracted from relaxation curves using a custom MATLAB script adapted from previous methods [34], and the modulus was calculated from the linear region of equilibrium stress-strain curves with 10% strain rate for 10 cycles per minute.

Primary chondrocyte isolation, seeding in gels and culture

Cartilage slices were obtained from immature bovine femoral condyles [35] using a scalpel and then placed in a petri dish containing PBS (**Fig. 1B**). Slices were then chopped using a sterile blade before digesting in 50 mL of 2 mg/mL pronase solution in culture medium for 1 h at 37 °C and in 50 mL of 0.25 mg/mL collagenase II solution in culture medium overnight at 37 °C in a spinner flask. Isolated chondrocytes were filtered from the rest of tissue using a 70 µm pore-size cell strainer and further purified using a 40 µm cell strainer, which was then rinsed twice using sterile PBS. Chondrocytes were then resuspended in culture media containing high-glucose DMEM, 10% FBS, 1% HEPES, 1% non-essential amino acids, 1% antibiotic-antimycotic, 0.4% proline and 0.4% ascorbic acid. Viable primary chondrocytes were counted using Trypan Blue. On the same day, cells were seeded into cryogels and hydrogels as described below.

Prior to cell seeding, cryogels were first sanitized with 70% ethanol for 30 min and subsequently washed several times with sterile PBS. Next, cells were seeded dropwise into two types of cryogels (Cryo RGD+ or Cryo RGD-): 10 µL of cell suspension containing 8×10^5 cells in the culture media were seeded into cryogels in a dropwise manner in a 48-well plate and kept for 30 min in a 37 °C in 5% CO_2 environment before adding culture

media. Hydro RGD+ containing cells were fabricated by first mixing cells (8×10^5 cells) with the polymer and LAP photo-initiator in complete cell culture medium. Next, the cell-containing polymer solution was transferred into a cylindrical mold (6 mm diameter $\times$ 1.5 mm height). Cell-laden hydrogels were then formed via photopolymerization under UV light (6.9 mW/cm^2, 320 – 390 nm) for 30 seconds.

Cryogels and hydrogels were cultured in 500 µL of culture media at 37 °C in 5% CO_2 environment for 15 or 21 days. Media was changed every 3 days and used for a variety of biochemical analysis to measure nitrite release, DNA content, cell metabolic activity, glycosaminoglycans (GAG) and collagen content in gels (**Fig. 1C**) after 15 days, which is described in section **2.7**. Gels were fixed for histology and immunostaining as described in section 2.8.

Injectability test

Cell-laden cryogels (Cryo RGD+) (2×10^5 cells/construct) were prepared and incubated overnight in culture media to allow for cell adhesion to the polymer walls. Cryogels were then loaded individually into syringes containing 50 µL of culture media [33], and then injected through a hypodermic 16-gauge needle. Next, syringe-injected cell-laden cryogels were cultured for another 24 h and the cell viability, cell metabolism and nitrite release were assessed and compared to a control group (non-injected). Cryo RGD- was not evaluated due to its similar mechanical structure and composition with Cryogel RGD+ while Hydrogel RGD- was not injectable. Cryo RGD+ was also injected through a 16-gauge needle and deposited directly into an ex vivo bovine articular cartilage defect (6 mm diameter x 1.5 mm thickness) for evaluation of shape-memory property

(**Supplemental Video**). The defect was created by punching a full thickness 6 mm diameter core into a 9 mm diameter x 1.5 mm thick condylar cartilage explant.

Biochemical analysis

Media was changed every three days throughout the culture duration. At Day 0 and Day 15, cell-laden gels were incubated in media containing 1x resazurin sodium salt (AlamarBlue assay) for 3 h in dark at 37 °C and 5% CO_2. Then, the cell metabolic activity was quantified following the manufacturer's recommendation. For injectability test, nitrite released into the media was measured using the Griess assay. Briefly, equal volumes of Griess reagent and culture media collected were mixed and incubated at room temperature for 15 min. Next, the absorbance at 540 nm was measured using a microplate reader. Sodium nitrite was used as a standard [36].

At the end of culture, gel samples were frozen at -80 °C and then lyophilized. Gels were then digested in 500 µL of 5% wt/v proteinase K for 2 h at 57 °C in 1M tris buffer (pH=8.0). DNA content in gels was analyzed using the PicoGreen assay following the manufacture's protocol. The total GAG content in gels was measured using the DMMB assay [37]. Total collagen content was determined using the Hydroxyproline assay [36].

Cell viability and cytoskeleton staining in gels using confocal microscopy

At Day 0 and Day 15, cell-laden cryogels and hydrogels were washed with 1 mL of PBS and subsequently incubated for 15 min in the presence of a ViaQuant™ Fixable Far-Red Dead Cell Staining Kit according to the manufacturer's instructions. Next, the gels were rinsed with 1 mL of PBS, fixed with 500 µL of 4% PFA for 20 min at RT in dark,

and washed with 1 mL of PBS. Next, the cells were permeabilized using 500 µL of PBS supplemented with 0.1% Triton X-100 for 5 min, then stained with 4′,6-diamidino-2-phenylindole (DAPI) (nucleus, blue) and Alexa Fluor 488-phalloidin (cytoskeleton, green). Confocal microscopy images were obtained using a Leica TCS SP5 X WILL confocal microscope (Buffalo Grove, IL, USA), and processed using Image J. Percentage of cell viability was defined as the fraction of number of viable cells to the total number of cells. For each condition, one representative stacked image was recorded with a 5 µm separation in between z-stacks. Cryogels were stained with rhodamine during fabrication as described previously [33].

Histology and immunohistochemistry

Cell-laden gels at Day 21 were fixed in 4% formalin, embedded in 0.75% agarose for ease of handling, dehydrated in a graded series of ethanol and xylenes, and embedded in paraffin. Transverse sections of gels were taken at 6 µm thickness. Sections were then immunostained with anti-collagen II mouse monoclonal antibody (1:700) (ServiceBio, GB12021) before an HRP-labeled goat anti-mouse secondary antibody (ServiceBio, G1214) was used (1:2000). Using 3,3'-diaminobenzidine, sections were stained in order to develop a strong signal. For nuclei staining, sections were further stained with hematoxylin. Stained sections were imaged using a 3D scanner (MIDI II HISTECH).

Statistical methods

Data is presented as Mean ± Standard Deviation. For all studies, n = 3-5 gels per condition were used and experiments were repeated at least three times. Confocal images shown are representative of 3 samples per condition. For comparisons between different treatment conditions, Tukey's Honestly Significant Difference test was used. $P < 0.05$ was considered statistically significant.

EXPERIMENT

Synthesis and characterization of gels

Gelation of HAGM (with or without RGD) at -20 °C resulted in ice crystal formation (**Fig. 1A**). After thawing at RT, 3D cryogel scaffolds with an interconnected macroporous architecture were created. Using SEM, an average pore size of 50 ± 0.5 μm was determined for 'dry' cryogels (pre-swelling) both with and without RGD (**Fig. 2A**), confirming their macroporous architecture. As shown in **Fig. 2B**, Hydro RGD+ had the highest swelling ratio (Q_M= 50.2 ± 2.6), while Cryo RGD+ and Cryo RGD- exhibited 2x lower swelling ratios compared to Hydro RGD+ (Q_M = 22 ± 3.7 and 31 ± 2.6, respectively). Additionally, the degree of pore connectivity for Cryo RGD+ and Cryo RGD- (83.5 ± 2.8 and 85.0 ± 0.8 respectively) was approximately 7 times higher than that of Hydro RGD+ (**Fig. 2C**). The mechanical stiffness for Cryo RGD+ and Cryo RGD- was 4.8 ± 0.3 kPa and 5.5 ± 0.4 kPa, respectively, both of which were significantly lower than that of hydrogels, which measured at 65 ± 10 kPa (**Fig. 2D).** Cryogels, thus, exhibited a macroporous network with high pore connectivity and low swelling.

Injectability of cryogels

The cell-laden cryogels restored their original shape rapidly, fitting into the same size (6 mm diameter × 1.5 mm thickness) cylindrical defect in a cartilage explant after being injected through a 16-gauge needle (**Figs. 3A-B**). This feature demonstrates their shape-memory properties (**Supplemental Video**). In addition, chondrocytes encapsulated within cryogels remained metabolically active and viable (**Fig. 3C**) (same as untreated control) and showed no inflammatory response following their injection through the 16-gauge needle, as evidenced by the similar levels of nitrite release (**Fig. 3D**).

Biological response

After 15 days of culture, cryogels did not show a significant reduction in percentage of chondrocyte viability compared to Day 0, indicating that the cryogel had a microenvironment that was conducive to maintaining chondrocyte health over a 2-week period (**Fig. 4A**). Hydro RGD+ showed a slight decrease in cell viability from 83.8% to 73.7% over 15 days, although this difference was not significant.

Additionally, chondrocytes in cryogels exhibited higher cell proliferation rates (up to 1.5x greater) than hydrogels over 15 days (**Fig. 4B**). By Day 15, at least 2x higher GAG content was measured in cryogels compared to hydrogels, whether the data was normalized by DNA content or not (**Figs. 4C-D**). Further, a greater percentage of GAGs was found to be retained within the cryogels compared to ~~from~~ Hydro RGD+, indicating the superior conducive microenvironment for matrix production provided by cryogels (**Supplemental Fig. S1**). Additionally, there was no significant difference in total collagen content in cryogels or hydrogels by Day 15 (**Fig. 4E).** Chondrocyte metabolism in all three types of gels increased significantly by Day 15 compared to that at Day 0 ($p < 0.003$); cell

metabolism in cryogels was 2x higher compared to that in hydrogels at Day 15 (**Fig. 4F**), indicating its superior micro-environment for chondrocytes. These data suggest that cryogels provide a superior micro-environment for maintaining chondrocyte phenotype, while supporting cell growth and promoting matrix regeneration. The presence of RGD in cryogels, however, did not significantly enhance the number of chondrocytes that remained adherent within the scaffold following 24 h of cell seeding (**Supplemental Fig. S2**). Additionally, the presence of RGD did not improve biosynthesis rates as shown by no difference in GAG or collagen content in the two types of cryogels by Day 15.

Cell attachment, survival, and spreading in the gels

Chondrocytes in Hydro RGD+ remained dispersed over 15 days, exhibiting less cell-cell interaction and no characteristics of forming an intricate tissue-like structure (**Fig. 5**). When cultured in RGD-containing and RGD-free cryogels, chondrocytes exhibited different behaviors. Chondrocytes seeded in both types of cryogels were homogenously distributed and simultaneously formed cellular aggregates at Day 0. For Cryo RGD+, cells interacted with the polymer network and displayed more of a spindle-shaped morphology by Day 15 rather than maintaining their initial spheroid-like shape. On the other hand, chondrocytes in Cryo RGD- self-organized and formed large 3D cylindrical organoids throughout the construct by Day 15. The organoids displayed a sophisticated architecture with spherical cells in the center surrounded by stretched spindle-shaped cells interacting with the polymer walls.

Histological and immunohistochemical analysis

The three gel types were assessed by immunohistochemistry for collagen type II content after 21 days of culture (**Fig. 6**). Collagen type II staining indicated that both hydrogels and cryogels stimulated chondrocytes for the synthesis of de novo collagen as evidenced by the brown color. Distinct differences in stain color between nuclei and collagen II were not visible in hydrogels due to their mesoporous structure, whereas the macroporous structure of cryogels allowed for vivid dark blue and brown staining of the nuclei and collagen II, respectively. Total collagen levels did not show any difference between Cryo RGD+ and Cryo RGD-, thereby supporting the data from the biological analysis (Fig. 4E).

DISCUSSION

Here, we have designed syringe-injectable, HA-based shape-memory cryogels that provide a conducive microenvironment for chondrocyte adhesion, proliferation and matrix biosynthesis for use in the repair of cartilage defects. These shape-memory cryogels provide an opportunity for improving the standard of care as they require only a single, minimally invasive injection of a biocompatible 3D scaffold that can return to its original structure (fitting to cartilage defect size and shape) following injection [38]. The large interconnected macropores of cryogels (~50 μm) (**Fig. 2A**), may provide the unobstructed transport of nutrients, solutes and waste as evidenced by the increased cell metabolism and GAG synthesis from chondrocytes over 15 days. In addition, their high pore interconnectivity is able to enhance the regulation of cell adhesion, viability, proliferation and metabolism while providing the mechanical strength required for injectability and shape-memory properties [37]. The interconnected macropores of the designed cryogels

375 also aid in recreating the tissue microenvironment for chondrocyte growth and function,
376 while providing compositional similarity with the native cartilage ECM due to its HA-based
377 nature. These characteristics have also been previously shown to improve ECM protein
378 production for tissue engineering [29, 39].

379 While cryogels have been designed with different types of polymers (i.e. hyaluronic
380 acid, chitosan, gelatin, chondroitin sulfate (CS), PEG, collagen, alginate, agarose and
381 their blends) for various tissue engineering applications, cartilage defect repair remains a
382 largely unexplored area [2, 4, 29, 30, 39-43]. Gupta et al. designed a cell-free chitosan-agarose-
383 gelatin cryogel scaffold which was able to promote the regeneration of cartilage in a rabbit
384 osteochondral defect [4]. However, their non-injectable cryogels required surgical
385 implantation, most likely due do their high modulus (~44 kPa) and poor compressibility. A
386 chondrocyte-seeded gelatin-chondroitin-6-sulfate-hyaluronan cryogel scaffold designed
387 by Kuo et al. also regenerated cartilage in rabbit osteochondral defects [41]. However, the
388 scaffold's ability to retain its structure following injection was not evaluated. Recently, a
389 gelatin-HA-PEG acrylate cryogel was shown to be repeatedly injectable and stretchable
390 for use in adipose tissue engineering applications [44]. Thus, this is the first time that
391 injectable shape-memory cryogels have been considered for their ability to promote
392 cartilage regeneration for defect repair.

393 Hyaluronic acid is a natural occurring polysaccharide that has been widely studied
394 and used for tissue engineering [24]. It is the polymer backbone of aggrecan – the primary
395 proteoglycan that comprises the dense protein meshwork existing in cartilage tissue, and
396 it is also the main solid component of synovial fluid [45]. Further, chondrocytes have been
397 shown to efficiently adhere to HA due to the presence of CD44 receptors on the cell

surface, which can enhance chondrocyte proliferation and matrix synthesis [46]. Therefore, HA is an ideal candidate biomaterial for cartilage tissue regeneration [47]. However, the function of HA is limited due to the in vivo enzymatic degradation caused by multiple metabolic pathways [48]; therefore requiring strategies to crosslink chemically modified HA to decrease degradation [49]. Previous studies have added methacrylate derivatives to HA so as to create photopolymerizable HAGM macromers for further crosslinking. The crosslinked networks of such conjugates have been reported to have high biocompatibility, low inflammatory response and considerable vascularization at an optimized polymer concentration [50, 51]. The cryogels used in this study were previously optimized for the degree of methacrylation (~30%) and HAGM macromonomer concentration (3.2% wt/v) in order to enable shape-memory following injection [24]. In this study, HAGM is utilized as the main component of the polymer for both hydrogels and cryogels. In hydrogels, chondrocytes were encapsulated during the photopolymerization process while they were seeded dropwise on the preformed cryogels. This way, the relatively low Young's modulus (5.5 ± 0.4 kPa), combined with high crosslink density and interconnected macropores, make cryogels injectable with shape-recovering ability. While the cryogels possess shape-memory, it is important to note that the gels are poroelastic in nature, as a result of fluid flow through the matrix.

Our biological analysis shows that the HAGM cryogel can provide a superior microenvironment for chondrocytes compared to the hydrogel. Following syringe injection of cell-seeded cryogels, cells exhibited similar metabolic activity and viability as prior to injection (**Figs. 3C-D**), which is consistent with previously reported findings [24, 33]. Shape-memory cryogels can therefore be used as a tissue-engineered construct to be injected

421 into chondral defects without surgical implantation. Its highly interconnected macroporous
422 structure enables efficient transport of nutrients, waste and macromolecules (i.e. matrix
423 proteins), thereby providing ideal physical support for chondrocytes to adhere, remain
424 functional and synthesize their own extracellular matrix (GAGs and collagen). Thus, these
425 cryogels allow for the complete integration of implanted 3D substrates compared to
426 conventional hydrogels (**Figs. 4B, D, E**). Conversely, our previous work has shown that
427 conventional hydrogels possess a mesoporous structure (pore size: ~2-50 nm) with low
428 pore interconnectivity (Fig. 2C) which limits cellular infiltration and ease of injectability [24],
429 further emphasizing the superior physical support provided by cryogels to chondrocytes.
430 To provide a bioactive surface for chondrocytes to proliferate, adhesion peptides
431 (RGD) were grafted to the cryogels [24, 33]. However, the cell-substrate interaction
432 performed similarly in HAGM cryogels with or without RGD likely due to the interactions
433 between CD44 receptors present on the surface of chondrocytes and the HA component
434 of the gel structure. This strong bioadhesion may overcome the adhesion effects
435 expected from the presence of RGD peptide [27, 33], resulting in similar DNA content
436 between Cryo RGD+ and Cryo RGD-. It is important to note that under compression
437 (during injection), presence of RGD may influence long-term cellular adhesion and
438 production of GAG and collagen, therefore warranting a future study investigating the
439 long-term effects post-injection. Interestingly, chondrocytes were able to form large
440 spheroids with fibroid matrix connecting to the polymer walls in cryogels without RGD,
441 while chondrocytes formed smaller spheroids in the presence of RGD (**Fig. 5**). The
442 presence of integrin-binding ligands (RGD) can enhance cell interaction with the
443 synthesized extracellular matrix and polymer walls, leading to a reduced number of cells

444 in clusters. These chondrocyte clusters are involved in the reparative process due to the
445 overexpression of RUNX1, a hematopoietic lineage determining transcription factor,
446 which likely upregulates the expression of aggrecan and lubricin, a chondroprotective
447 molecule [52, 53]. Additionally, it has been shown that chondrocyte clusters form to initiate
448 the repair response in a partial thickness cartilage defect rabbit model [54], confirming the
449 cells' chondroprogenitor phenotype [53]. Therefore, in our study, the clusters formed in
450 cryogels may contribute to the enhanced cell proliferation, metabolism and production of
451 cartilage extracellular matrix.

452 A limitation of this study was the exclusion of hydrogels without RGD, as the
453 primary objective was to evaluate chondrocyte behavior in cryogels compared to
454 hydrogels following injection. Further, there were no significant differences in chondrocyte
455 proliferation, metabolism or matrix production between Cryo RGD+ and Cryo RGD-,
456 despite their improvement over hydrogels. Previous studies have also indicated that
457 presence of RGD in PEG-hydrogels positively influences cartilage matrix synthesis and
458 gene expression, however only in response to dynamic mechanical stimulation [55]. Thus,
459 since our gels were not subjected to mechanical loading, we would expect to see similar
460 trends between hydrogels with or without RGD.

461 In our study, we evaluated the microenvironment for chondrocytes, however
462 clinically, success for chondrocyte-based tissue regeneration has been limited. A phase
463 III trial was recently launched with NeoCart, a product similar to MACI®, however with a
464 bovine type I collagen matrix as opposed to porcine collagen mix of type I and III. The
465 trial however did not meet its primary endpoint of change in physical functioning and pain
466 [56]. Hyalograft C, a hyaluronan-based scaffold seeded with autologous chondrocytes, was

investigated in prospective clinical trials with 5-12 years follow-ups, showing clinical improvement in patients at 32 ± 12 years old, with single and multiple defects (smaller than 4 cm^2), however, its use in salvage cases was not recommended. Concerns surrounding the manufacturing process and uncertainty of a positive benefit-risk balance eventually lead to the withdrawal of the product from the European market [57]. Thus, MACI® remains as the only scaffold-based product for repairing cartilage defects. The limited success of these trials and the risks and high costs associated with extraction and culture of autologous chondrocytes has resulted in the increased use of alternative cell types such as mesenchymal stem cells (MSCs) for cartilage regeneration. Injectable, shape-memory cryogels designed similar to ours have been used for delivering bioluminescent murine MSCs to mice via subcutaneous injection, resulting in enhanced survival and higher local retention compared to free cells [24]. MSCs are advantageous as they can be sourced from several areas including bone marrow, adipose tissue and umbilical cord using a syringe, and therefore, do not require surgery for collection [58]. Thus, combined with the injectable nature of cryogels, there is the potential to treat cartilage defects with MSCs entirely free of surgery, which would not only reduce patient discomfort but would also lower the risk of infection and incurred costs [59]. Further, induced pluripotent stem cells (IPSCs) which can be derived from somatic cells, have also shown the ability to differentiate into chondrocytes, and therefore have the potential to be seeded into our cryogels for cartilage defect repair [60]. Future studies will investigate the use of cryogels seeded with either chondrocytes, MSCs or IPSCs for in vivo cartilage defect implantation.

It is important to note that for cartilage regeneration, chondrogenic differentiation of MSCs and IPSCs must be induced in order to produce hyaline-like cartilage. Therefore,

these cells require treatment with factors such as TGFβ or Kartogenin for sustained periods of time to promote chondrogenic differentiation [61, 62]. This can be achieved using drug delivery systems which can enable charge-based interactions between the negatively charged hyaluronan matrix and positively charged drug carriers [63-67] that can provide controlled drug release such as multi-arm Avidin [68-70]. Combination of different cell sources, biomaterials and/or growth stimulators are directions to be considered going forward in cartilage tissue engineering. Additionally, bioadhesives may be required to keep the gels tightly secured within the cartilage defect site following their placement such that they can sustain cyclic mechanical loading pressures through the regeneration period, which will be incorporated in future design and tested using animal models. Optimization of cell harvesting and growth, improvements in ECM production, and reduction of cost from medical operation should be the focus of future research [59].

CONCLUSION

Hyaluronic acid based cryogels loaded with primary chondrocytes remain viable and metabolically active, while recovering their shape following injection. Compared to HA-based hydrogels, cryogels provide a more conducive microenvironment for cell adhesion, proliferation and matrix biosynthesis. The shape-memory feature of these cryogels allows for them to be injected into the joint space via syringe for the non-surgically invasive treatment and repair of cartilage defects. Use of these cryogels with various cell types and phenotype promoters can enhance cartilage tissue regeneration and allows for a broad range of applications in regeneration of other tissues of the body.

ACKNOWLEDGEMENTS

We would like to thank Professor Samuel Chung for providing access to their microscopes for histological imaging.

AUTHOR CONTRIBUTION STATEMENT

TH, BL, TC, KJN, SM, JDK, SB, AGB all contributed to the design of experiments and preparation and review of this manuscript.

AUTHOR DISCLOSURE STATEMENTS

The authors have no competing financial interests to disclose.

FUNDING STATEMENT

This work was supported by the United States Department of Defense through the Congressionally Directed Medical Research Programs (CDMRP) [W81XWH-17-1-0085]; and National Science Foundation [DMR 1847843].

REFERENCES

1. Bajpayee AG, Grodzinsky AJ. Cartilage-targeting drug delivery: can electrostatic interactions help? Nat Rev Rheumatol 2017;13:183.
2. Hwang Y, Sangaj N, Varghese S. Interconnected macroporous poly (ethylene glycol) cryogels as a cell scaffold for cartilage tissue engineering. Tissue Engineering Part A 2010;16:3033-3041.
3. MACI Safety: https://www.maci.com/patients/how-maci-works/the-maci-procedure.html. Accessed June 20, 2020.
4. Gupta A, Bhat S, Jagdale PR, et al. Evaluation of three-dimensional chitosan-agarose-gelatin cryogel scaffold for the repair of subchondral cartilage defects: an in vivo study in a rabbit model. Tissue Engineering Part A 2014;20:3101-3111.

5. Thornton AJ, Alsberg E, Hill EE, Mooney DJ. Shape retaining injectable hydrogels for minimally invasive bulking. The Journal of urology 2004;172:763-768.
6. Prata JE, Barth TA, Bencherif SA, Washburn NR. Complex fluids based on methacrylated hyaluronic acid. Biomacromolecules 2010;11:769-775.
7. Bencherif SA, Sheehan JA, Hollinger JO, Walker LM, Matyjaszewski K, Washburn NR. Influence of cross-linker chemistry on release kinetics of PEG-co-PGA hydrogels. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials 2009;90:142-153.
8. Kennedy S, Bencherif S, Norton D, Weinstock L, Mehta M, Mooney D. Rapid and extensive collapse from electrically responsive macroporous hydrogels. Advanced healthcare materials 2014;3:500-507.
9. Memic A, Abudula T, Mohammed HS, Joshi Navare K, Colombani T, Bencherif SA. Latest progress in electrospun nanofibers for wound healing applications. ACS Applied Bio Materials 2019;2:952-969.
10. Gsib O, Duval J-L, Goczkowski M, et al. Evaluation of fibrin-based interpenetrating polymer networks as potential biomaterials for tissue engineering. Nanomaterials 2017;7:436.
11. Vedadghavami A, Minooei F, Mohammadi MH, et al. Manufacturing of hydrogel biomaterials with controlled mechanical properties for tissue engineering applications. Acta biomaterialia 2017;62:42-63.
12. Bencherif SA, Braschler TM, Renaud P. Advances in the design of macroporous polymer scaffolds for potential applications in dentistry. Journal of periodontal & implant science 2013;43:251-261.
13. Fallahi A, Khadivi N, Roohpour N, et al. Characterization, mechanistic analysis and improving the properties of denture adhesives. Dental Materials 2018;34:120-131.
14. Kawamura S, Wakitani S, Kimura T, et al. Articular cartilage repair: rabbit experiments with a collagen gel-biomatrix and chondrocytes cultured in it. Acta Orthopaedica Scandinavica 1998;69:56-62.
15. Choi N-Y, Kim B-W, Yeo W-J, et al. Gel-type autologous chondrocyte (Chondron™) implantation for treatment of articular cartilage defects of the knee. BMC musculoskeletal disorders 2010;11:103.
16. Funayama A, Niki Y, Matsumoto H, et al. Repair of full-thickness articular cartilage defects using injectable type II collagen gel embedded with cultured chondrocytes in a rabbit model. Journal of orthopaedic science 2008;13:225-232.
17. Vinatier C, Gauthier O, Fatimi A, et al. An injectable cellulose-based hydrogel for the transfer of autologous nasal chondrocytes in articular cartilage defects. Biotechnology and bioengineering 2009;102:1259-1267.
18. Sepantafar M, Maheronnaghsh R, Mohammadi H, et al. Stem cells and injectable hydrogels: synergistic therapeutics in myocardial repair. Biotechnology advances 2016;34:362-379.
19. Béduer A, Braschler T, Peric O, et al. A compressible scaffold for minimally invasive delivery of large intact neuronal networks. Advanced healthcare materials 2015;4:301-312.
20. Yu L, Ding J. Injectable hydrogels as unique biomedical materials. Chemical Society Reviews 2008;37:1473-1481.

21. Guvendiren M, Lu HD, Burdick JA. Shear-thinning hydrogels for biomedical applications. Soft matter 2012;8:260-272.
22. Silva E, Mooney DJ. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. Journal of Thrombosis and Haemostasis 2007;5:590-598.
23. Eggermont LJ, Rogers ZJ, Colombani T, Memic A, Bencherif SA. Injectable cryogels for biomedical applications. Trends in biotechnology 2020;38:418-431.
24. Bencherif SA, Sands RW, Bhatta D, et al. Injectable preformed scaffolds with shape-memory properties. Proceedings of the National Academy of Sciences 2012;109:19590-19595.
25. Rogers ZJ, Bencherif SA, Cryogelation and Cryogels. Multidisciplinary Digital Publishing Institute: 2019.
26. Shih TY, Blacklow SO, Li AW, et al. Injectable, tough alginate cryogels as cancer vaccines. Advanced healthcare materials 2018;7:1701469.
27. Villard P, Rezaeeyazdi M, Colombani T, et al. Autoclavable and injectable cryogels for biomedical applications. Advanced Healthcare Materials 2019;8:1900679.
28. Memic A, Rezaeeyazdi M, Villard P, et al. Effect of Polymer Concentration on Autoclaved Cryogel Properties. Macromolecular Materials and Engineering 2020:1900824.
29. Han M-E, Kim S-H, Kim HD, et al. Extracellular matrix-based cryogels for cartilage tissue engineering. International journal of biological macromolecules 2016;93:1410-1419.
30. Kathuria N, Tripathi A, Kar KK, Kumar A. Synthesis and characterization of elastic and macroporous chitosan–gelatin cryogels for tissue engineering. Acta Biomaterialia 2009;5:406-418.
31. Bencherif SA, Sands RW, Ali OA, et al. Injectable cryogel-based whole-cell cancer vaccines. Nature communications 2015;6:1-13.
32. Rezaeeyazdi M, Colombani T, Memic A, Bencherif SA. Injectable Hyaluronic Acid-co-Gelatin Cryogels for Tissue-Engineering Applications. Materials (Basel) 2018;11.
33. Rezaeeyazdi M, Colombani T, Memic A, Bencherif S. Injectable hyaluronic Acid-co-Gelatin cryogels for tissue-engineering applications. Materials 2018;11:1374.
34. Eisenberg SR, Grodzinsky AJ. Swelling of articular cartilage and other connective tissues: electromechanochemical forces. Journal of Orthopaedic Research 1985;3:148-159.
35. Florine EM, Miller RE, Liebesny PH, et al. Delivering heparin-binding insulin-like growth factor 1 with self-assembling peptide hydrogels. Tissue Engineering Part A 2014;21:637-646.
36. Mehta S, Akhtar S, Porter RM, Önnerfjord P, Bajpayee AG. Interleukin-1 receptor antagonist (IL-1Ra) is more effective in suppressing cytokine-induced catabolism in cartilage-synovium co-culture than in cartilage monoculture. Arthritis research & therapy 2019;21:1-15.
37. Farndale RW, Sayers CA, Barrett AJ. A direct spectrophotometric microassay for sulfated glycosaminoglycans in cartilage cultures. Connective tissue research 1982;9:247-248.
38. Wang L, Shansky J, Borselli C, Mooney D, Vandenburgh H. Design and fabrication of a biodegradable, covalently crosslinked shape-memory alginate scaffold for cell and growth factor delivery. Tissue Engineering Part A 2012;18:2000-2007.
39. Han M-E, Kang BJ, Kim S-H, Kim HD, Hwang NS. Gelatin-based extracellular matrix cryogels for cartilage tissue engineering. Journal of Industrial and Engineering Chemistry 2017;45:421-429.

628 40. Bhat S, Tripathi A, Kumar A. Supermacroprous chitosan–agarose–gelatin cryogels: in
629 vitro characterization and in vivo assessment for cartilage tissue engineering. Journal of the
630 Royal Society Interface 2011;8:540-554.
631 41. Kuo C-Y, Chen C-H, Hsiao C-Y, Chen J-P. Incorporation of chitosan in biomimetic
632 gelatin/chondroitin-6-sulfate/hyaluronan cryogel for cartilage tissue engineering. Carbohydrate
633 polymers 2015;117:722-730.
634 42. Little CJ, Bawolin NK, Chen X. Mechanical properties of natural cartilage and tissue-
635 engineered constructs. Tissue Engineering Part B: Reviews 2011;17:213-227.
636 43. Bölgen N, Yang Y, Korkusuz P, Güzel E, El Haj A, Pişkin E. 3D ingrowth of bovine articular
637 chondrocytes in biodegradable cryogel scaffolds for cartilage tissue engineering. Journal of
638 tissue engineering and regenerative medicine 2011;5:770-779.
639 44. Qi D, Wu S, Kuss MA, et al. Mechanically robust cryogels with injectability and
640 bioprinting supportability for adipose tissue engineering. Acta biomaterialia 2018;74:131-142.
641 45. Bajpayee AG, Wong CR, Bawendi MG, Frank EH, Grodzinsky AJ. Avidin as a model for
642 charge driven transport into cartilage and drug delivery for treating early stage post-traumatic
643 osteoarthritis. Biomaterials 2014;35:538-549.
644 46. Ishida O, Tanaka Y, Morimoto I, Takigawa M, Eto S. Chondrocytes are regulated by
645 cellular adhesion through CD44 and hyaluronic acid pathway. Journal of Bone and Mineral
646 Research 1997;12:1657-1663.
647 47. Tavsanli B, Okay O. Mechanically strong hyaluronic acid hydrogels with an
648 interpenetrating network structure. European Polymer Journal 2017;94:185-195.
649 48. Band P. Hyaluronan derivatives: chemistry and clinical applications. The chemistry,
650 biology andmedical applications of hyaluronan and its derivatives 1998:33-42.
651 49. Jeon O, Song SJ, Lee K-J, et al. Mechanical properties and degradation behaviors of
652 hyaluronic acid hydrogels cross-linked at various cross-linking densities. Carbohydrate polymers
653 2007;70:251-257.
654 50. Baier Leach J, Bivens KA, Patrick Jr CW, Schmidt CE. Photocrosslinked hyaluronic acid
655 hydrogels: natural, biodegradable tissue engineering scaffolds. Biotechnology and
656 bioengineering 2003;82:578-589.
657 51. Burdick JA, Chung C, Jia X, Randolph MA, Langer R. Controlled degradation and
658 mechanical behavior of photopolymerized hyaluronic acid networks. Biomacromolecules
659 2005;6:386-391.
660 52. Jay GD, Fleming BC, Watkins BA, et al. Prevention of cartilage degeneration and
661 restoration of chondroprotection by lubricin tribosupplementation in the rat following anterior
662 cruciate ligament transection. Arthritis & Rheumatism 2010;62:2382-2391.
663 53. LeBlanc KT, Walcott ME, Gaur T, et al. Runx1 activities in superficial zone chondrocytes,
664 osteoarthritic chondrocyte clones and response to mechanical loading. Journal of cellular
665 physiology 2015;230:440-448.
666 54. Bos P, Verhaar J, van Osch G, Age-related differences in articular cartilage wound
667 healing: A potential role for transforming growth factor β1 in adult cartilage repair. In *Tissue*
668 *Engineering*, Springer: 2006; pp 297-309.
669 55. Villanueva I, Weigel CA, Bryant SJ. Cell–matrix interactions and dynamic mechanical
670 loading influence chondrocyte gene expression and bioactivity in PEG-RGD hydrogels. Acta
671 biomaterialia 2009;5:2832-2846.

56. Crawford DC, DeBerardino TM, Williams III RJ. NeoCart, an autologous cartilage tissue implant, compared with microfracture for treatment of distal femoral cartilage lesions: an FDA phase-II prospective, randomized clinical trial after two years. JBJS 2012;94:979-989.
57. Brix MO, Stelzeneder D, Chiari C, et al. Treatment of full-thickness chondral defects with hyalograft C in the knee: long-term results. The American journal of sports medicine 2014;42:1426-1432.
58. Makris EA, Gomoll AH, Malizos KN, Hu JC, Athanasiou KA. Repair and tissue engineering techniques for articular cartilage. Nature Reviews Rheumatology 2015;11:21.
59. Mollon B, Kandel R, Chahal J, Theodoropoulos J. The clinical status of cartilage tissue regeneration in humans. Osteoarthritis and cartilage 2013;21:1824-1833.
60. Ko J-Y, Kim K-I, Park S, Im G-I. In vitro chondrogenesis and in vivo repair of osteochondral defect with human induced pluripotent stem cells. Biomaterials 2014;35:3571-3581.
61. Johnson K, Zhu S, Tremblay MS, et al. A stem cell–based approach to cartilage repair. Science 2012;336:717-721.
62. Liebesny PH, Byun S, Hung H-H, et al. Growth factor-mediated migration of bone marrow progenitor cells for accelerated scaffold recruitment. Tissue Engineering Part A 2016;22:917-927.
63. Vedadghavami A, Wagner EK, Mehta S, He T, Zhang C, Bajpayee AG. Cartilage penetrating cationic peptide carriers for applications in drug delivery to avascular negatively charged tissues. Acta biomaterialia 2019;93:258-269.
64. Vedadghavami A, Zhang C, Bajpayee AG. Overcoming negatively charged tissue barriers: Drug delivery using cationic peptides and proteins. Nano Today 2020;34:100898.
65. Young CC, Vedadghavami A, Bajpayee AG. Bioelectricity for Drug Delivery: The Promise of Cationic Therapeutics. Bioelectricity 2020.
66. Vedadghavami A, Mehta S, Bajpayee AG. Characterization of Intra-Cartilage Transport Properties of Cationic Peptide Carriers. JoVE (Journal of Visualized Experiments) 2020:e61340.
67. Wagner EK, Vedadghavami A, Jacobsen TD, Goel SA, Chahine NO, Bajpayee AG. Avidin grafted dextran nanostructure enables a month-long intra-discal retention. Scientific reports 2020;10:1-14.
68. He T, Zhang C, Vedadghavami A, et al. Multi-arm Avidin nano-construct for intra-cartilage delivery of small molecule drugs. Journal of Controlled Release 2020;318:109-123.
69. Bajpayee AG, Quadir MA, Hammond PT, Grodzinsky AJ. Charge based intra-cartilage delivery of single dose dexamethasone using Avidin nano-carriers suppresses cytokine-induced catabolism long term. Osteoarthritis and Cartilage 2016;24:71-81.
70. Zhang C, He T, Vedadghavami A, Bajpayee AG. Avidin-biotin technology to synthesize multi-arm nano-construct for drug delivery. MethodsX 2020:100882.

CORRESPONDENCE ADDRESS

Ambika G. Bajpayee
ISEC Room 216
805 Columbus Avenue, Boston, MA, USA

+1 617-373-7018 (Phone)
a.bajpayee@northeastern.edu