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Crosslinked hyaluronan scaffolds as a biologically active carrier for valvular interstitial cells

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Abstract

Hyaluronic acid (HA), a major component of the cardiac jelly during heart morphogenesis, is a polysaccharide that upon modification can be photopolymerized into hydrogels. Previous work in our lab has found that photopolymerizable HA hydrogels are suitable scaffolds for the culture and proliferation of valvular interstitial cells (VICs), the most prevalent cell type in native heart valves. The physical properties of HA gels are easily modified through alteration in material crosslink density or by copolymerizing with other reactive macromolecules. Degradation products of HA gels and the starting macromers significantly increased VIC proliferation when added to cell cultures. With low molecular weight HA (<6700 Da) exhibiting greatest stimulation of VIC proliferation. Low molecular weight HA degradation products added to VIC cultures also resulted in a four-fold increase in total matrix production and a two-fold increase in elastin production over untreated controls. VIC internalization of HA, as shown by cellular uptake of fluorescently labeled HA, likely activates signaling cascades resulting in the biological responses seen here. Lastly, VICs encapsulated within HA hydrogels remained viable, and significant elastin production was observed after 6 weeks of culture. This work shows promise for the creation of a tissue-engineered heart valve utilizing the synergistic relationship between hyaluronic acid and VICs.
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1. Introduction

Hyaluronic acid (HA) is a polysaccharide with numerous attractive qualities for tissue engineering applications and, specifically, formation of a tissue-engineered heart valve. HA is non-immunogenic, non-thrombogenic, and can be readily crosslinked via a variety of mechanisms to form hydrogels [1–4]. Composed of repeating units of *N*-acetyl glucosamine and glucuronide, HA is involved in numerous biological events in vivo. Angiogenesis is promoted by degraded HA [5], and HA is also involved in the differentiation, proliferation, and migration of cells during wound healing [6]. Hyaluronan-rich matrices are found during morphogenesis of embryonic organs and during developmental events such as mesenchymal cells invading the primary corneal stroma, mesenchymal cells proliferating in embryonic limb development, tendon regeneration, and fetal wound repair, and most important for the current application, during migration of cushion cells from endocardium to myocardium in the formation of heart valves [7].

Recently, HA was identified as an essential component in cardiac morphogenesis [8,9]. Embryonic heart

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valves develop from a hyaluronan-rich "cardiac jelly" [10]. Embryos genetically deficient in hyaluronic acid synthase 2 (Has2) are unable to produce HA, and in the absence of HA, heart valves fail to form resulting in a lethal cardiac defect [8]. Administration of exogenous HA to Has2-deficient mice rescues valve formation and allows proper development. This finding demonstrates the importance of HA in heart morphogenesis and provides support for using HA as a scaffold in the regeneration of a heart valve in vitro, where it may provide biological signals that mimic in vivo heart valve development. Current tissue engineering and biomaterials design strategies do not often consider the extracellular matrix environment that is present during in vivo organ morphogenesis. Yet in the creation of neotissues, it seems logical to investigate the environment and biological cues provided to developing tissues in vivo and then design tissue engineering scaffolds that recreate these conditions.

In the current study, photopolymerized HA hydrogels were investigated as a scaffold material for cardiac valvular interstitial cells (VICs). Photopolymerization was chosen as the HA crosslinking method as it allows both spatial and temporal control of crosslinking, and is mild enough to enable the encapsulation of cells. VICs were chosen as the cell type for the study as they are the most prevalent cell type in native valves [11] and possess many characteristics that may facilitate development of a tissue-engineered valve. VICs closely resemble myofibroblasts, which are found in many tissues and play important roles in tissue remodeling [12,13]. Additionally, VICs actively participate in the response to injury and promote valve repair [14], and they are also responsible for the synthesis and remodeling of the extracellular matrix (ECM) components of the valve [11,15]. A significant challenge in the synthesis of a tissue-engineered valve has been the re-creation of the appropriate ECM distribution and composition [16]. For these reasons, as well as their ease of isolation, VICs display an advantage over other cell types used in tissue-engineered valve research. This study examines the use of photopolymerizable HA-based hydrogels as scaffolds for VICs and investigates the ability of these matrices to affect or enhance VIC behavior.

2. Materials and methods

2.1. VIC isolation and culture

Fresh porcine hearts were generously donated by Quality Pork Processors, Inc. (Austin, MN) and used within 24 h of slaughter. Aortic valve leaflets were excised from the hearts and subjected to two collagenase digestions, the second of which yields VICs [17]. The resulting VIC suspension was poured through a 100 μm cell strainer, centrifuged, and the resulting cell pellet was then plated into tissue culture dishes in VIC culture medium, consisting of 15% FBS, 2% penicillin/streptomycin, and 0.2% gentamicin in Medium 199 (Invitrogen Corp., Carlsbad, CA). VICs were cultured at 37 °C in a 5% CO_2 environment and used between passages 3–6 in all experiments.

2.2. Synthesis and photopolymerization of methacrylated HA

The sodium salt of HA (from *Streptococcus equi*, MW ~5 MDa by gel permeation chromatography) was methacrylated using five-fold excess methacrylic anhydride relative to free HA hydroxyl groups following a procedure outlined in [1,18]. The methacrylated hyaluronic acid (HA-MA), was precipitated twice into 95% ethanol, dried, and dialyzed for 2 days against diH_2O. ^1H NMR analysis was performed on HA-MA dissolved in deuterated water (Cambridge Isotopes, Andover, MA) to quantify the extent of methacrylation. Gels of HA-MA were formed by making a 2% solution (w/v) of the polymer in phosphate buffered saline (PBS) and exposing to UV light at an intensity of 5 mW/cm^2 for 3 min with 0.05% Irgacure 2959 (Ciba Specialty Chemicals, Tarrytown, NJ) present as a photoinitiator.

2.3. Characterization of HA-MA hydrogels

Swelling, enzymatic degradation, and compressive moduli of HA-MA hydrogels were examined. HA-MA with various degrees of methacrylate modification or HA-MA copolymerized with 5% or 10% poly(ethylene glycol) diacrylate (PEG-DA, MW 4600, synthesized according to [19]) was used to make hydrogels with a wide range of properties. The swelling properties of HA-MA or HA-MA + PEG-DA hydrogels were examined by polymerizing gels, lyophilizing them to obtain their dry weight, then re-swelling in PBS and examining changes in gel weight over time. Degradation characteristics of HA-MA ± PEG-DA hydrogels were also investigated. Gels were polymerized, swollen in PBS overnight, and then degraded in PBS containing 5 U/ml bovine testis hyaluronidase (HAse). Gels were weighed at various time points to assess degradation. Lastly, the compressive modulus of elasticity of HA-MA gels was compared with that of HA-MA plus either 5% or 10% PEG-DA, in addition to 10% PEG-DA alone. Measurements were made using a dynamic mechanical analyzer (DMA-7, Perkin-Elmer, Shelton, CT) in unconfined compression at room temperature in a humidified environment to prevent de-swelling. Samples were initially unloaded, and then subjected to a static load that increased at a rate of 100 mN/min. The compressive modulus was determined by analyzing the linear region

of the stress vs. strain curve on samples at low deformation (<20% strain).

2.4. Effect of HA degradation products on VIC proliferation

Uncrosslinked HA-MA was dissolved in diH_2O at a concentration of 1 mg/ml and degraded in 50 U/ml HAse overnight. HA-MA was also polymerized as described earlier to form hydrogels which were immediately subjected to degradation in 50 U/ml HAse. After overnight incubation of gels in HAse at 37 °C, the HAse was deactivated by heating to 80 °C for 1 h. The molecular weight distribution of the degradation products was analyzed via gel permeation chromatography (GPC, Waters Corp., Milford, MA), with dextran standards and HA degradation samples run in 10 mM sodium bicarbonate buffer. All molecular weights reported in this work are number averages. VICs were seeded in 24-well tissue culture plates at a density of 10,000 cells/cm^2; at 6 h post-seeding, cells were re-fed with serum-free media, and degradation products from both uncrosslinked and crosslinked HA-MA were added to VIC cultures at a concentration of 3 μg/ml every 24 h. After 3 and 20 days of culture, cell proliferation was assessed by performing cell counts on a Coulter Counter (Beckman–Coulter Corporation, Miami, FL). To investigate the optimal HA molecular weight for increased VIC proliferation, unmodified HA was degraded overnight at 37 °C in 10, 50, 100, 500, or 1000 U/ml HAse to yield degradation products with a range of molecular weights. Degraded HA was analyzed via GPC and added to cell cultures as described above. To eliminate the possibility of cell reaction to HAse, a set of wells contained corresponding amounts of HAse, but no HA. Control wells containing no HA treatment were also seeded for all experiments.

2.5. Effect of HA degradation products on VIC ECM production

The effects of degraded HA on ECM production by VICs was investigated through incorporation of ^{3}H-glycine into glycoprotein, elastin, and collagen portions of the ECM as determined by sequential enzyme digestion (TEC assay [20]). Unmodified HA was degraded with various amounts of HAse, as described above, to produce degradation products with a range of molecular weights. VICs were seeded in 24-well tissue culture plates at a density of 10,000 cells/cm^2; at 6 h post-seeding, cells were re-fed with serum-free media containing 1 μCi/ml ^{3}H-glycine, and HA degradation products were added to VIC cultures at a concentration of 3 μg/ml every 24 h for 20 days. Cells were re-fed with fresh media every 3 days. The same procedure was followed for cells intended for counting, except that the media was not supplemented with ^{3}H glycine. At time points of 3 and 20 days, the cells in non-radioactive plates were trypsinized and counted on a Coulter Counter. The matrix on the remaining plates was dehydrated and matrix components were sequentially digested by incubation with trypsin, elastase, and collagenase as described in [20]. Radioactivity in samples from each digestion step was determined by scintigraphy (Beckman LS 6500, Beckman Instruments Inc., Fullerton, CA). Control wells received no HA treatment.

2.6. VIC internalization of HA

To investigate the interaction between HA and VICs, the ability of VICs to internalize fluorescein-labeled HA (FL-HA, Molecular Probes, Eugene, OR) was observed. VICs were seeded in 24-well plates at 10,000 cells/cm^2 and incubated with 150 μg FL-HA/ml media for 2 h. Wells were briefly rinsed for 5 min with a 10 U/ml HAse solution prior to observation of VICs in order to remove any pericellularly bound FL-HA. Internalization of FL-HA was observed under a fluorescent microscope (Nikon Eclipse TE300).

2.7. Encapsulation of VICs in HA-MA hydrogels

A cell suspension of VICs in cell culture media was combined with a 2% solution of HA-MA in PBS to achieve a final cell density of 40×10^6 cells/ml. The solution was photopolymerized as described earlier to encapsulate the VICs, and hydrogels were incubated at 37 °C, 5% CO_2 on an orbital shaker plate in 6-well plates containing media. At various time points, hydrogels were prepared for histological sectioning by rinsing in PBS and fixing in 10% formalin. Following standard procedures, paraffin sections (10 μm) were unmasked with pepsin and immunostained for elastin using monoclonal mouse anti-elastin (Sigma, Clone BA-4) primary antibody and a VectaStain Elite ABC kit (Vector Labs, Burlingame, CA). Vector NovaRed (Vector Labs) was used as the peroxidase chromogen, resulting in a reddish-brown color to indicate positive staining. Staining controls were treated similarly, except that they did not contain either the primary antibody or the secondary antibody. Photomicrographs were taken of immunostained sections on a phase contrast microscope (Nikon Eclipse TE300).

2.8. Statistical analysis

Data were compared using two-tailed, unpaired *t*-tests or one-way analysis of variance (ANOVA) where indicated. p Values less than or equal to 0.05 were considered statistically significant. Data are presented as mean ± standard deviation.

Table 1
The physical properties of HA-based hydrogels

Macromer formulation[a]	Equilibrium swelling ratio (Q)	Compressive modulus (kPa)	Degradation time (h)
2% HA(1.5)[b]	79.9 ± 16.23	12.61 ± 0.37	
2% HA(54)	66.02 ± 9.62	18.08 ± 1.66	48
2% HA(100)	23.76 ± 0.19	48.7 ± 2.01	
2% HA(54) + 5% PEG	15.98 ± 0.30	32.2 ± 2.8	285
2% HA(54) + 10% PEG	9.69 ± 0.66	54.7 ± 14	10% mass loss after 300 h
10% PEG	15.9 ± 1.24	6.5 ± 1.2	NA

[a]Solution composition in weight percent. Percent methacrylation is indicated in parentheses.
[b]Percent methacrylation refers to the number of methacrylate groups per HA disaccharide unit.

3. Results

3.1. Synthesis of HA-MA

^{1}H NMR analysis of HA-MA demonstrated degrees of methacrylation ranging from 1.5% to >100%. The percent methacrylation refers to the number of methacrylate groups per HA disaccharide unit. Because any hydroxyl group on each disaccharide has the ability to react with methacrylic anhydride to form a methacrylate group, there is the potential to achieve more than one methacrylate per disaccharide (>100% methacrylation). The functionality of the HA influences the final network structure and properties upon photopolymerization.

3.2. Characterization of HA-MA hydrogels

The physical properties of HA-MA hydrogels were significantly altered by changing the extent of HA methacrylation or by copolymerizing with PEG-DA, as shown in Table 1. Swelling decreased with increasing % methacrylation, while compressive modulus and degradation time both increased, as expected with a higher degree of crosslinking. Copolymerization of the ionically charged HA with the neutrally charged photopolymerizable PEG material also had the effect of increasing crosslink density. Gels containing HA-MA alone swelled four-fold more than HA-MA + 5% PEG, and seven-fold more than HA-MA + 10% PEG. Additionally, the degradation time of the gels was extended, as HA-MA alone degraded in a HAse solution within 2 days, while HA-MA + 5% or 10% PEG-DA took over 1 week to completely degrade. Finally, combination of HA-MA with PEG-DA resulted in a synergistic increase in mechanical stiffness, as evidenced by the compressive moduli.

3.3. Effect of HA degradation products on VIC proliferation

Addition of HA-MA degradation products to VIC cultures significantly increased VIC proliferation over untreated and HAse-treated controls (Fig. 1). Degradation products from both crosslinked and uncrosslinked HA-MA created a proliferative response. To investigate this result, the molecular weight of all degradation products was examined via GPC, and the magnitude of the cell proliferation increase was related to the HA molecular weight. To further explore the relationship between VIC proliferation and HA molecular weight, HA degradation products with ranging molecular weights were added to VICs, and VIC proliferation was found to increase with decreasing HA molecular weight ($p<0.01$; Fig. 2).

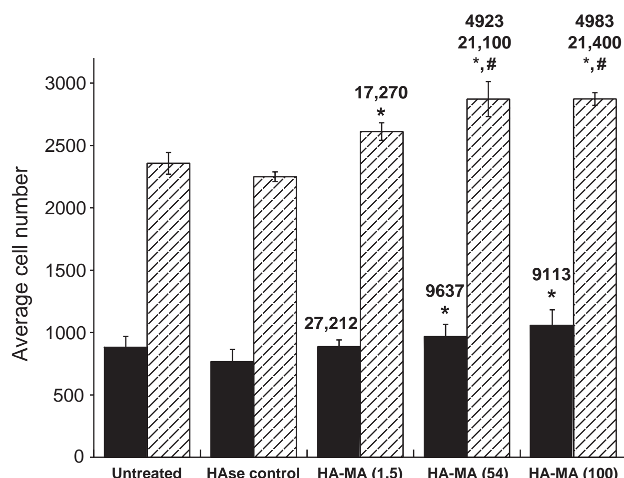


Fig. 1. HA-MA with varying degrees of methacrylation (% methacrylation given in parentheses) was degraded both prior to and following photopolymerization, then added to VIC cultures at a concentration of 3 μg/ml for 3 days. VIC proliferation was significantly increased by the addition of either ■ uncrosslinked, degraded HA-MA, or ▨ crosslinked, degraded HA-MA. This increase in proliferation was dependent upon the molecular weight of the degradation products, shown above the corresponding bars in the graph. * $p<0.05$ compared to HAse treatment alone; # $p<0.01$ compared to HA-MA (1.5). $n=4$ samples per condition.

3.4. Effect of HA degradation products on VIC ECM production

After 3 days of culture, overall matrix production by VICs in response to addition of degraded HA did not

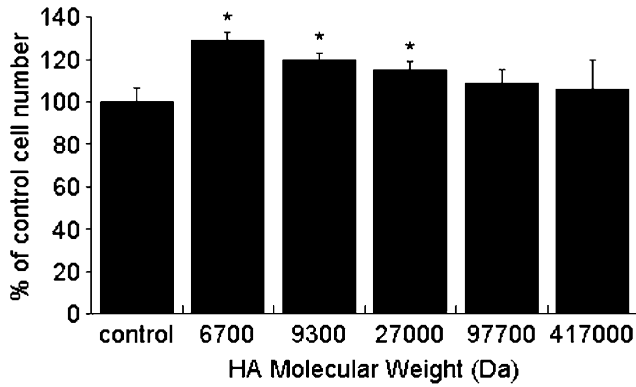


Fig. 2. To investigate the relationship between VIC proliferation and HA molecular weight, unmodified, degraded HA with a range of molecular weights was added to VICs at 3 μg/ml for 3 days. VIC proliferation increased significantly with decreasing HA molecular weight. * $p<0.01$ compared to untreated control. $n=4$ samples per condition.

differ from the control (Fig. 3a). However, degraded HA did significantly alter the composition of the ECM produced by VICs, with lower molecular weight HA stimulating significantly more elastin production ($p<0.03$) than in wells treated with high molecular weight HA or no HA (Fig. 3b). Interestingly, after 20 days of culture and treatment with degraded HA, there were significant differences in both total matrix production (Fig. 3a) and matrix composition (Fig. 3c) in response to HA degradation products. Total matrix production significantly increased ($p<1\times10^{-12}$ by ANOVA) with decreasing HA molecular weight (Fig. 3a). Furthermore, a drastic increase in elastin production was observed across all conditions at this late time point, with increased elastin production linked to lower molecular weight HA ($p<1\times10^{-9}$ by ANOVA). Samples receiving the lowest molecular weight HA (MW 6700 Da) continued to secrete significantly more elastin ($p<0.03$) than all other conditions (Fig. 3c).

3.5. VIC internalization of HA

There are two receptors through which cells may internalize HA; these receptors are CD44 and receptor for hyaluronic acid-mediated motility (RHAMM), and they are not actively expressed in all cell types. Uptake of FL-HA by VICs was observed by fluorescence microscopy (Fig. 4), indicating the ability of VICs to internalize HA.

3.6. Encapsulation of VICs in HA-MA hydrogels

VICs encapsulated in HA-MA hydrogels remained viable following photopolymerization (Fig. 5a). Furthermore, significant elastin production throughout the scaffolds was observed after 6 weeks of culture through the use of immunoperoxidase staining for elastin (Fig. 5b). Here, reddish-brown staining indicates areas positive for elastin.

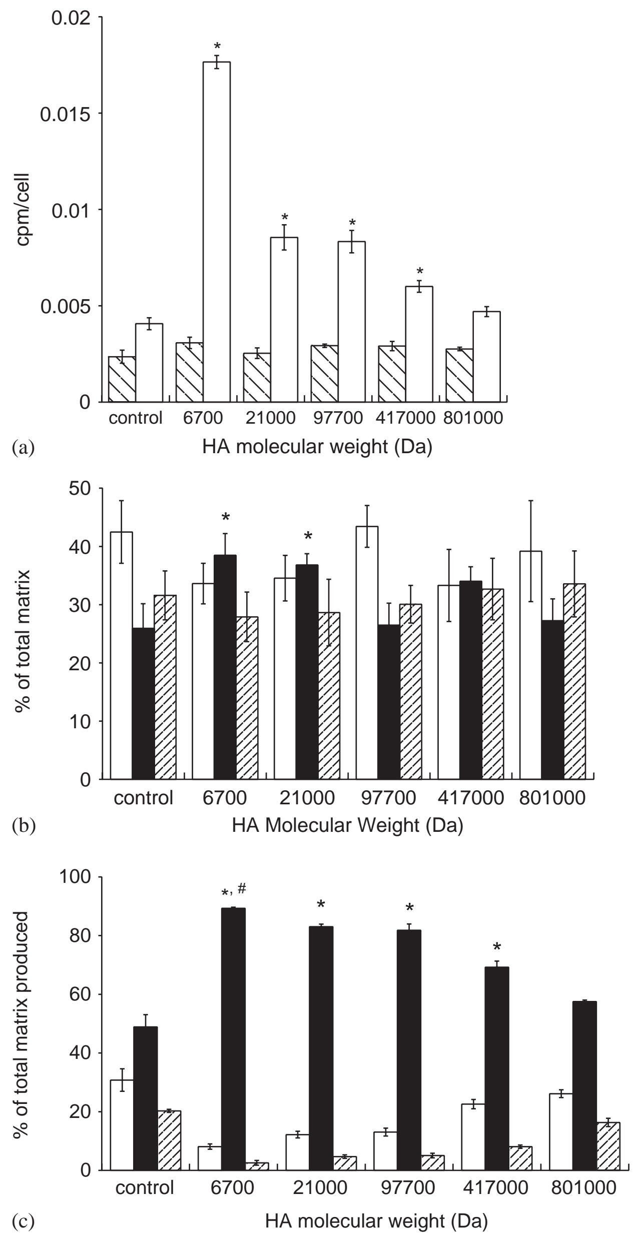


Fig. 3. VIC media was supplemented with 3 μg/ml of unmodified, degraded HA. (a) After ▧ 3 and □ 20 days of culture total matrix production was assessed. After 3 days degraded HA did not affect overall matrix production, however, after 20 days total matrix production was significantly increased compared to untreated controls. * $p<1\times10^{-12}$. (b) After 3 days, elastin production was significantly increased over untreated controls by the addition of 6700 Da and 21 kDa HA. * $p<0.03$. □, glycoproteins; ■, elastin; ▨, collagen. (c) After 20 days, the increase in elastin production seen by the addition of low molecular weight HA to VIC cultures was more apparent. * $p<0.01$ compared to untreated control; # $p<0.03$ compared to all other conditions. $n=3$ samples per condition. □, glycoproteins; ■, elastin; ▨, collagen.

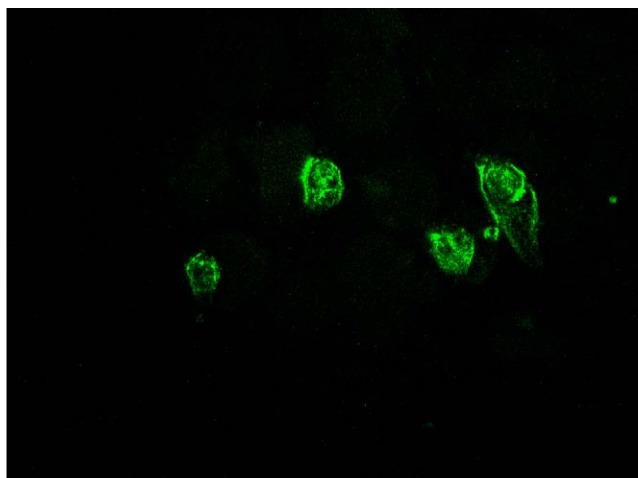
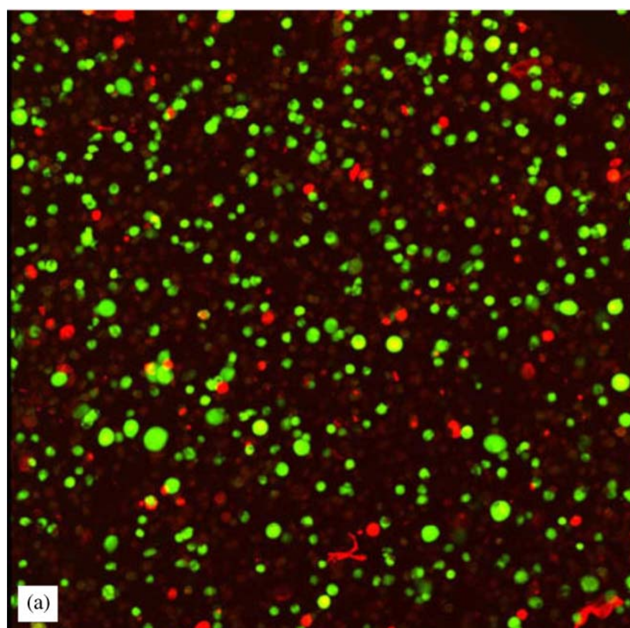


Fig. 4. FL-HA was internalized by VICs, indicating the presence of HA receptors on the VIC surface. A fluorescence photomicrograph (400 × magnification) shows a VIC that has internalized FL-HA.



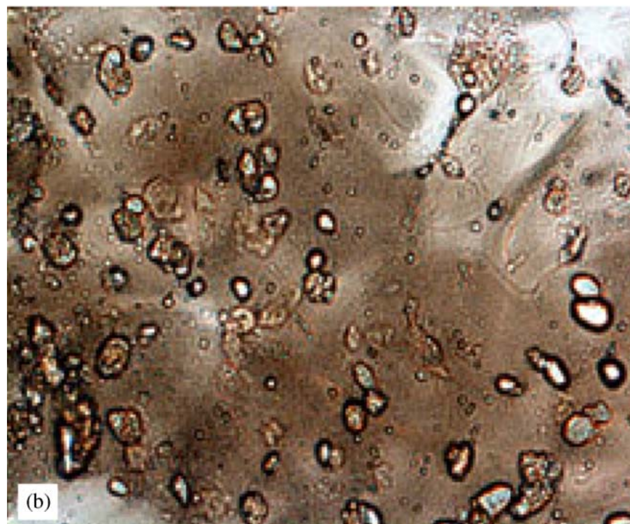


Fig. 5. VICs encapsulated in HA-MA gels (a) remained viable after 1 week of culture, as evidenced by live/dead staining, and (b) produced significant amounts of elastin after 6 weeks in culture, as displayed by reddish-brown staining throughout hydrogel sections.

4. Discussion

HA-based materials are emerging as a cell scaffold platform for tissue engineering applications, including the creation of tissue-engineered bone or cartilage [21–23]. Many unique properties of HA, including its biocompatibility, viscoelasticity, and lack of immunogenicity, have made it an appealing material for a variety of applications. The crucial involvement of HA in heart morphogenesis has only recently been described [8,9]. A HA-based scaffold may simulate important aspects of natural valve development, providing a biomimetic environment that presents signals to encourage formation of a tissue engineered valve. Other materials currently used as tissue-engineered valve scaffolds are not biologically active [16,24,25], and are therefore not able to provide biological cues for cell growth and differentiation without extensive modification.

In this study, HA was modified to form photopolymerizable hydrogels. Hydrogels are desirable materials for design of a tissue-engineered heart valve for several reasons. In the specific case of heart valves, normal embryonic heart development stems from a ‘‘cardiac jelly’’ which bears resemblance to a hydrogel and has a high HA content [7,10]. Photopolymerization occurs under relatively mild conditions and enables the encapsulation of cells within the scaffold material. This process eliminates the need for tedious or inefficient seeding methods that are necessary with other materials presently used for creation of a tissue-engineered heart valve [16,26,27]. Photopolymerization also allows the facile fabrication of complex shapes, such as a tri-leaflet structure. Other scaffold materials currently being investigated for use in heart valve applications must be thermally molded [16,27]. Moreover, hydrogels made from natural polymers, such as HA, are biodegradable. In the case of HA-MA, degradation occurs enzymatically via HAse. An advantage of enzymatic degradation is that it may allow scaffold degradation to be paired with tissue growth and remodeling.

The physical properties of HA-MA hydrogels are easily altered by changing either the degree of HA modification or through copolymerization with other photopolymerizable macromolecules. This ability to tailor the properties of hydrogel scaffolds is important, as it allows great flexibility in tuning the material properties to meet those needed by the application. Heart valves are exposed to a harsh hemodynamic

environment, necessitating a material that is both strong and flexible. We have demonstrated that HA-MA hydrogels can be synthesized to exhibit a wide range of physical properties.

HA degradation products are known to induce angiogenesis [5] and stimulate the proliferation of endothelial cells. However, prior to the present study, it was unknown whether HA degradation products would have any proliferative effect on VICs, and whether modification of HA to synthesize HA-MA would affect the potential biological activity of degraded HA. In this study, we demonstrated that both degraded HA and degraded HA-MA do indeed affect VIC proliferation. The amount of VIC growth stimulation is dependent upon the molecular weight of the HA degradation products, with lower molecular weight HA (<27,000 Da) inducing the most prominent increase in VIC proliferation. Furthermore, low molecular weight HA significantly stimulated the production of elastin by VICs after 3 days of culture, while both overall matrix production and elastin content were dramatically increased after 20 days of culture; addition of MW 6700 HA resulted in a four-fold increase in total matrix production and a two-fold increase in elastin at the 20 day time point. Also, while it may appear from Fig. 3c that the synthesis of glycoproteins and collagen has been slowed by the addition of degraded HA, this is not the case. While these two components comprise a lesser percentage of total matrix than in the control condition, the overall matrix produced (Fig. 3a) correspondingly increases dramatically, resulting in a net increase in the absolute amounts of collagen and glycoprotein produced. Recreation of the ECM of a native heart valve is a significant challenge in heart valve tissue engineering, with elastin being the most difficult component to produce in an in vitro environment. Current tissue-engineered heart valves created by other research groups have contained no detectable elastin when cultured in vitro [16,24], and only trace amounts when valves were implanted in vivo [16,25]. Recent studies have suggested that cell culture on HA substrates stimulates elastin production by neonatal aortic fibroblasts [28,29], and the present study has supported this finding by revealing that VICs can be stimulated to produce elastin through interaction with low molecular weight HA. These results demonstrate the ability of our scaffold material itself to provide important biological signals to the cells that influence their function.

Degradable scaffolds have been employed in many tissue engineering applications [30,31]. Yet, whether they are hydrolytically or enzymatically degradable materials, most of these materials are used solely for their physical properties and ability to degrade over a reasonable time period to allow for the formation of new tissue. HA-based materials are unique in that the products of scaffold degradation are actually biologically active and can signal the encapsulated cells to perform specific functions. The ability of degraded HA-MA hydrogels to stimulate VIC proliferation and potentially alter VIC behavior in other manners, such as increasing ECM production, makes these materials more than just a structural support for VIC growth and heart valve tissue formation. In the present study, HA-MA hydrogels were degraded via administration of exogenous HAse; a topic of current experiments is the production of HAse by VICs, as well as the size of degradation products created by VIC-induced HA-MA hydrogel degradation. Additionally, while the hydrogels discussed here were all formed from high molecular weight HA-MA, it must be noted that any size HA can be methacrylated. It is possible that using a mid-range molecular weight HA-MA may facilitate hydrogel degradation by endogenous VIC HAse, and the production of low molecular weight degradation products that stimulate VIC functions would be more likely. Altering macromer molecular weight is another way that this system can be tuned to meet the needs of the forming tissue.

VICs encapsulated within HA-MA hydrogels were observed to produce significant amounts of elastin after 6 weeks in culture (Fig. 5b), but little elastin was observed at 4 weeks. Given the 2D ECM analysis finding that degraded HA dramatically increases elastin production by VICs, it is possible that encapsulated VICs were stimulated to produce elastin by low molecular weight HA produced by VIC-initiated HA gel degradation.

One way in which HA affects cellular events is through interaction with cell surface receptors that transduce intracellular signals. One such receptor is CD44, a widely distributed cell surface glycoprotein that is expressed as numerous isoforms that are not active on all cell types [32]. The intracellular domain of CD44 interacts with cytoskeletal proteins and regulates signaling, thereby providing a direct link between extracellular HA and the cell cytoskeleton [33]. Binding of HA to CD44 has been shown to activate intracellular signaling pathways that significantly alter cell proliferation and motility [33]. We have demonstrated that VICs possess HA receptors, likely including CD44, that enable the uptake of HA. It is possible that the responsiveness of VICs to HA and its degradation products is initiated due to the interaction of HA with CD44 on VIC surfaces. Not all cell types initiate signaling cascades in response to HA [34]. Activation of cell signaling pathways in VICs responding to HA treatment is evidenced in the present study by the dramatic effects of HA on the proliferation and ECM production on 2D surfaces, as well as the significant increase in elaboration of elastin by VICs encapsulated in HA-MA gels and cultured in this 3D matrix for 6 weeks.

5. Conclusion

Many unique properties of HA can be exploited in order to synthesize an appropriate scaffold material for the creation of a tissue-engineered heart valve. In the present communication, we demonstrate the compatibility of photopolymerizable HA-based hydrogels for this application. Reactive macromolecules based on HA, a major component of the cardiac jelly during heart morphogenesis, can be photopolymerized into hydrogels to serve as a bioactive carrier for VICs, the most prevalent cell type in the valve. Scaffold degradation products increase VIC proliferation and ECM production rate and may positively influence neotissue evolution when cell-gel constructs are cultured. These HA-based hydrogels are highly versatile as well as biologically active, and their potential for use in 3D valve tissue generation is promising.

Acknowledgments

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