Designing scaffolds for valvular interstitial cells: Cell adhesion and function on naturally derived materials

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Abstract: Valvular interstitial cells (VICs) possess many properties that make them attractive for use in the construction of a tissue-engineered valve; however, we have found that the surfaces to which VICs will adhere and spread are limited. For example, VICs adhere and spread on collagen and laminin-coated surfaces, but display altered morphology and do not proliferate. Interestingly, fibronectin (FN) was one adhesion protein that facilitated VIC adhesion and proliferation. Yet VICs did not spread on surfaces modified with RGD, a ubiquitous cell-adhesive peptide, nor with other FN-specific peptide sequences such as EILDV and PHSRN. Hyaluronic acid (HA) is a highly elastic polysaccharide that is involved in natural valve morphogenesis and possesses binding interactions with FN. Hyaluronic acid was modified to form photopolymerizable hydrogels, and VICs were found to spread and proliferate on HA-based gels, forming a confluent monolayer on the gels within 4 days. Modified HA retained its ability to specifically bind FN, allowing for the formation of gels containing both HA and FN. Valvular interstital cells cultured on HA surfaces displayed significantly increased production of extracellular matrix proteins, indicating that HA-based scaffolds may provide useful biological cues to stimulate heart valve tissue formation. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 71A: 172–180, 2004

Key words: tissue engineering; heart valve; hydrogel; valvular interstitial cells

INTRODUCTION

Diseased or dysfunctional heart valves are most often replaced with either mechanical valves or transplanted tissue valves, both of which are accompanied by serious limitations.[1,2] Problems with existing valve replacement options are especially pronounced in children, who may require multiple valve replacement surgeries to cope with rapid tissue valve degeneration or outgrowth of replacement valves.[1] A tissue-engineered heart valve has the potential to circumvent problems with biocompatibility and durability observed with existing valve replacements and would have the ability to grow and repair. While many research groups are investigating the development of a tissue-engineered heart valve,[3–8] few groups have attempted to use valvular interstitial cells (VICs) as the cell type in their valve scaffolds.

Selecting and obtaining an appropriate cell source may prove crucial to the successful creation of a tissue-engineered heart valve. Cell types used by other researchers for this application include arterial myofibroblasts, dermal fibroblasts, cardiac myocytes, and smooth muscle cells.[7,9,10] Although VICs are the most prevalent cell type in aortic heart valves, they are not well characterized in the literature.[11,12] This is due in part to the heterogeneous nature of the VIC population, which until recently was not identified.[13–15] However, we believe that VICs possess many properties that make them attractive for use in a tissue-engineered heart valve. These cells closely resemble myofibroblasts, which are found in most tissues and play important roles in tissue remodeling.[16,17] Additionally, VICs are highly migratory and contractile, and produce large amounts of extracellular matrix.[12,14,15] It has been documented that VICs actively participate in valvular response to injury to promote valve repair.[12] Vavular interstitial cells are also responsible for the synthesis of the extracellular matrix

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(ECM) components of the valve.[13,15] Heart valves consist of layers of histologically distinct tissue, with each layer containing a specific distribution of ECM components, which include glycosaminoglycans, elastin, and collagen.[13] Due to the harsh hemodynamic stresses withstood by heart valves, their complex ECM composition is constantly being remodeled and rebuilt, and VICs play a central role in this matrix remodeling.[12] A significant challenge in the synthesis of a tissue-engineered valve has been the recreation of the appropriate ECM distribution and composition.[7] For these reasons, as well as their ease of isolation, VICs display an advantage over other cell types used in tissue-engineered valve research.

While VICs are an appealing cell type for construction of a tissue-engineered heart valve, the challenge of creating a biomaterial on which they will function must first be met. The design of three-dimensional (3-D) scaffolds appropriate for VIC culture has not yet been explored in the literature. The majority of VIC characterization has been performed using tissue culture polystyrene as a culture surface.[14,18] In designing a scaffold for VICs in this study, emphasis was given to photopolymerizable hydrogels, as this class of materials possesses many properties that are attractive for tissue engineering applications.[19,20] Hydrogels provide an elastic framework that enables facile encapsulation of VICs and the ability to present a milieu of molecular cues. Both naturally derived and synthetic hydrogels have been explored as cell carriers for tissue regeneration. For creating a heart valve matrix, our first objective was to identify VIC interactions with ECM proteins, such that synthetic analogs could be created that capture the minimum necessary features to design a tissue-promoting cell niche. The preliminary goal in creating an appropriate hydrogel scaffold for VICs is the design of a 3-D material that supports VIC adhesion, growth, and ECM production. This study describes the progress made toward achieving this goal and identifies a potential suitable scaffold material for VICs, based on hyaluronic acid. Hyaluronic acid (HA) is the predominant glycosaminoglycan in native valves[21] and is a major component of the cardiac jelly from which the heart forms during embryogenesis.[22] Furthermore, HA is readily modified with methacrylate groups enabling photopolymerization into hydrogels.

MATERIALS AND METHODS

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

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.[23] The resulting VIC suspension was poured through a 100-μm cell strainer, centrifuged, then plated into tissue culture dishes in VIC culture medium, consisting of 15% fetal bovine serum (FBS), 2% penicillin/streptomycin, and 0.2% gentamicin in Medium 199 (Invitrogen Corp., Carlsbad, CA). Vavular interstitial cells were cultured at 37°C in a 5% CO_2 environment and used between passages 3 and 6 in all experiments. NIH 3T3 fibroblasts were used as a control cell type in all adhesion experiments. 3T3s were cultured in Dulbecco's Modified Eagle Medium (Invitrogen) containing 10% calf serum in a 37°C, 5% CO_2 environment.

VIC adhesion to collagen, fibronectin, and laminin-coated surfaces

Untreated 24-well plates were coated with 5 μg/cm^2 of bovine plasma fibronectin or 2 μg/cm^2 laminin (ICN, Costa Mesa, CA) diluted in phosphate buffered saline (PBS), and then allowed to incubate at 4°C overnight. Wells were rinsed twice with PBS prior to cell seeding. Thin gels of collagen I were formed in untreated 24-well plates by combining Vitrogen 100 (Cohesion Technologies, Palo Alto, CA) with 10× PBS in a 10:1 ratio and adjusting to pH 7.4 using 0.1*N* NaOH. Gelation occurred after 45 min incubation at 37°C. Vavular interstitial cells were seeded into the wells at 25,000 cells/cm^2. Phase contrast photomicrographs were taken of the cells at 24, 48, and 72 h postseeding on a Nikon Eclipse TE300 microscope.

VIC adhesion to PEG-peptide gels

After screening VIC adhesion and spreading on natural protein surfaces, a synthetic polymer, poly(ethylene glycol) (PEG), was modified with fibronectin-derived peptide sequences. Poly(ethylene glycol) is a non–cell adhesive material that provides an ideal surface to selectively incorporate and test the effects of peptide modification on cell adhesion. Acryloyl-PEG-*N*-hydroxysuccinimide (Nektar Therapeutics, Huntington AL) was reacted in an equimolar ratio with either GRGDS (Bachem, King of Prussia, PA) or EILDV (Bachem) for 2 h at room temperature in 50 m*M* sodium bicarbonate buffer, pH 8.4. The products were dialyzed (MWCO 1000 Da) and lyophilized. Acryloyl-PEG-GRGDS and -EILDV were combined with a 15% (w/v) solution of PEG-diacrylate (MW 4600, synthesized as described in Ref. 24), sterile filtered, and exposed to UV light (365 nm, 5 mW/cm^2) for 5 min in the presence of 0.1% Irgacure 2959 (Ciba Specialty Chemicals, Tarrytown, NY) to form gels that contained PEG-peptide in concentrations of 0.5, 1.5, and 5 m*M*. Gels containing combinations of PEG-GRGDS and

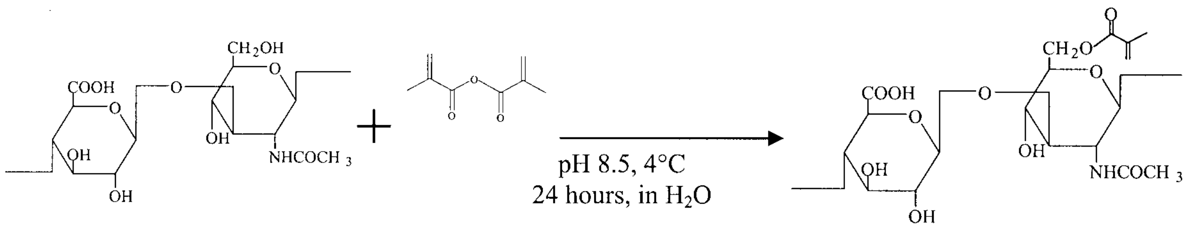

Figure 1. Hyaluronic acid (HA) is reacted with a 5-fold excess of methacrylic anhydride to form methacrylated HA (HA-MA), which can be photocrosslinked to form hydrogels.

PEG-EILDV were also fabricated to examine possible synergistic effects of the two peptides in VIC adhesion. Gels were placed in wells of a 24-well plate, VICs were seeded upon the gels at a concentration of 25,000 cells/cm^2, and cell spreading was examined at 24, 48, and 72 h postseeding.

RGD has been shown to exhibit synergistic binding with another FN-derived peptide sequence, PHSRN.[25,26] GRGDS and PHSRN were coupled to acrylated PEG according to the procedure outlined above. In order to investigate the effect of this synergy on VIC adhesion, hydrogels containing PEG-PHSRN, PEG-GRGDS, and both PEG-PHSRN and PEG-GRGDS were made in the same manner as that described above. Hydrogels containing either GRGDS or PHSRN were synthesized with a final peptide concentration of 0.05 m*M* and 0.50 m*M*. Hydrogels containing both GRGDS and PHSRN were synthesized with either 0.025 m*M* or 0.25 m*M* of each peptide. Gels were placed in wells of a 24-well plate and VICs were seeded upon the gels at a concentration of 25,000 cells/cm^2. After 24 h, gels were fixed in 10% formalin and adherent cells were counted under the microscope. Four gels were counted per condition.

VIC adhesion to crosslinked polysaccharide gels

Hyaluronic acid (HA, from *Streptococcus equi,* MW ≈5 MDa by gel permeation chromatography) was methacrylated following a procedure outlined in Ref. 27 and depicted in Figure 1. Briefly, a 2% solution (w/v) of HA in diH_2O was prepared and reacted with a 5-fold excess of methacrylic anhydride. The pH of the reaction mixture was adjusted to 8.5 using 5*N* NaOH, and the reaction was allowed to proceed overnight at 4°C. The product, methacrylated hyaluronic acid (HA-MA), was precipitated at a 1:10 ratio two times into 95% ethanol, dried, and dialyzed for 2 days against diH_2O. ^{1}H NMR analysis was performed on HA-MA dissolved in D_2O (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 PBS and exposing to UV light at an intensity of 5 mW/cm^2 for 3 min with 0.05% Irgacure 2959. Vavular interstitial cells were seeded upon the gels at 25,000 cells/cm^2 and examined at 24, 48, and 72 h postseeding.

Interactions of HA-MA with fibronectin

Methacrylated hyaluronic acid with various degrees of methacrylate modification and nonmethacrylated HA (as a control) were dissolved in diH_2O and combined with 2 mg/mL bovine plasma fibronectin (FN). These solutions were diluted 1:2 with Native PAGE sample buffer (0.0625 *M* Tris-HCl pH 6.8, 30% (v/v) glycerol, and 0.1% (w/v) bromophenol blue in diH_2O) and electrophoresed on 4% Ready-Gel precast polyacrylamide Tris-HCl gels (Bio-Rad, Hercules, CA) using a vertical electrophoresis system (Mini-Protean II, Bio-Rad) at 200 V in Native PAGE running buffer (3 g Tris base and 14.4 g glycine per 1 L diH_2O). Fibronectin bands were detected via a Silver Stain Plus kit (Bio-Rad), and band intensity was quantified on Kodak 1D software. Fibronectin bands indicate free or unbound FN, while the FN complexed with HA appears as a higher molecular weight band. Results are reported as the difference in FN band intensity of the FN + HA-MA sample from pure FN divided by the difference in band intensity of the FN + HA sample from pure FN.

The ability of polymerized HA-MA hydrogels to retain encapsulated FN was also examined. Fibronectin was coupled with sodium periodate-activated horseradish peroxidase (HRP) via reductive amination using sodium cyanoborohydride (kit from Roche Applied Science, Indianapolis, IN). Unreacted HRP was removed via dialysis (MWCO 100,000). Horseradish peroxidase–labeled immunoglobulin G (IgG) was used as a control. Either FN-HRP or IgG-HRP was encapsulated within HA-MA hydrogels, which were polymerized as described earlier. The hydrogels were rinsed in PBS for 4 days, then placed in a solution containing an HRP chromogen (AEC, Dako Corporation, Carpinteria, CA) to visually detect the presence of either FN-HRP or IgG-HRP. Hydrogels not containing any protein were also formed, rinsed, and exposed to the AEC solution and used as a negative control.

VIC extracellular matrix (ECM) production on HA-modified surfaces

Hyaluronic acid was covalently attached to polystyrene 96-well plates functionalized with 1×10^{14} reactive hydrazide groups per square centimeter (Carbo-BIND plates, Corning Incorporated Life Sciences, Acton, MA) to achieve stable HA coatings. Hyaluronic acid was dissolved in MES buffer, pH 4.6, at concentrations ranging from 1.25 μg/mL to 1.25 mg/mL and combined with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) in a 1:3 molar ratio of HA disaccharide units to EDC. The reaction was allowed to proceed for 4 hs, at which point

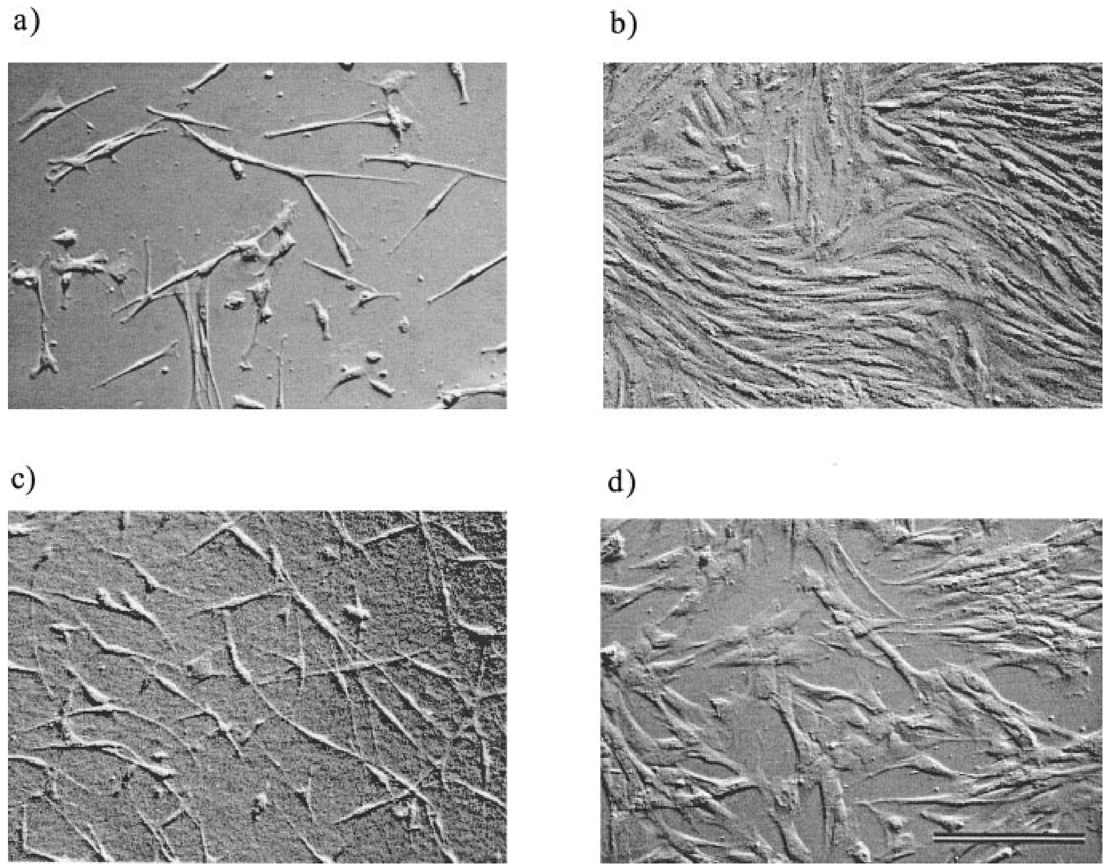

Figure 2. Valvular interstitial cells (VICs) were seeded upon various protein-coated substrates such as (a) laminin, (b) fibronectin, (c) collagen, and (d) control tissue culture polystyrene, in order to evaluate these proteins for use as VIC scaffold materials. After 2 days of culture, fibronectin-coated surfaces were the most successful in supporting VIC adhesion and proliferation. $n = 4$ samples per condition. All images are shown at the same magnification. Scale bar represents 100μm.

wells were rinsed four times with PBS and stored dry at 4°C until use. Valvular interstitial cells were seeded upon the HA-coated surfaces at a density of 25,000 cells/cm^2, and extracellular matrix (ECM) production 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[28]). After 5 days of culture, cells were counted and radioactivity in samples from each digestion step was determined by scintigraphy (Beckman LS 6500, Beckman Instruments Inc., Fullerton, CA). Valvular interstitial cells were also seeded upon unmodified Carbo-BIND wells as a control.

Statistical analysis

Data were compared using two-tailed, unpaired *t* tests. *p* values less than or equal to .05 were considered statistically significant. Data are presented as mean ± standard deviation.

RESULTS

VIC adhesion to collagen, fibronectin, and laminin-coated surfaces

Vavular interstitial cell adhesion and spreading upon protein-coated surfaces were examined to identify suitable biological signals that could be used as a basis for the synthesis of a tissue-engineered scaffold material. While VICs adhered and spread on both collagen gels and laminin-coated surfaces, their morphology was greatly altered compared to that on tissue culture polystyrene, as depicted in Figure 2, and they never reached confluency, even after weeks in culture. In contrast, VICs seeded on fibronectin-coated surfaces appeared healthy and displayed normal VIC morphology. However, covalent tethering of a large molecule such as fibronectin (MW 550 kDa) to scaffolding materials can be quite cumbersome. For this reason, shorter fibronectin-derived peptide sequences were examined as a means of enhancing VIC adhesion.

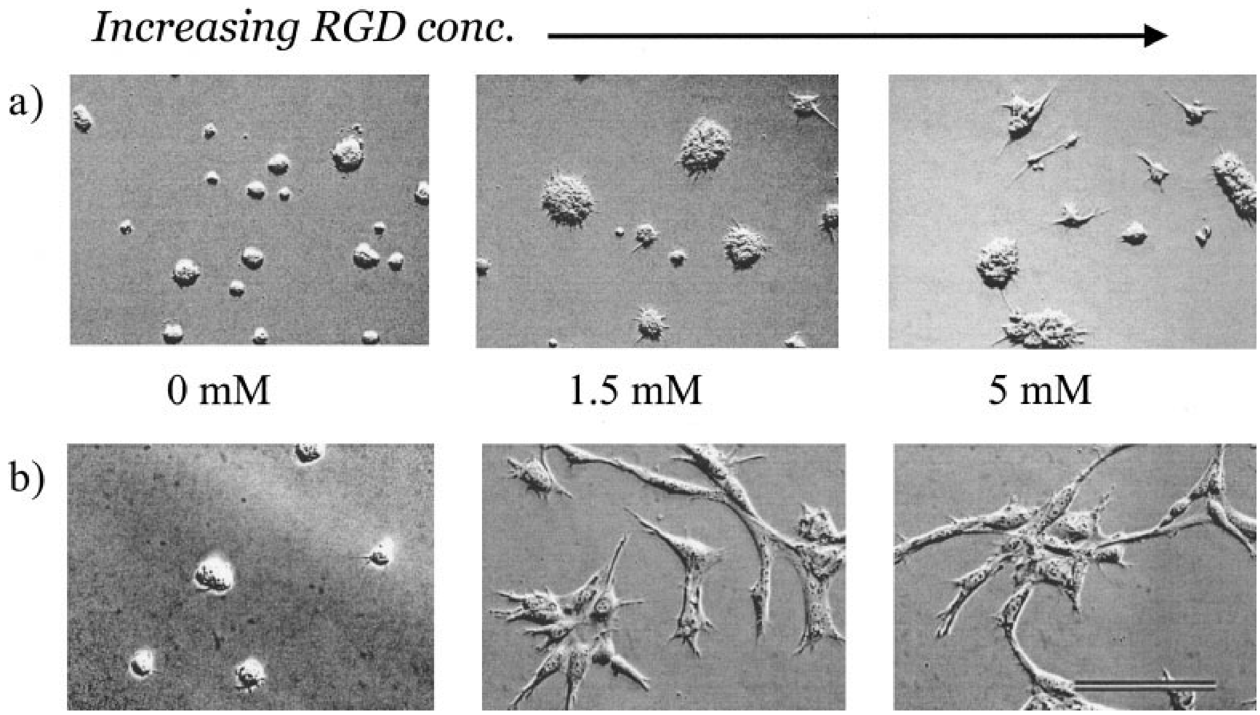

Figure 3. Non-cell-adhesive poly(ethylene glycol) (PEG) hydrogels were modified to contain RGD, a fibronectin-derived cell adhesive peptide sequence. RGD-modified surfaces did not support the adhesion and spreading of VICs at 2 days postseeding (a). Control 3T3 fibroblasts (b) did adhere and spread on the RGD-modified surfaces, confirming the material bioactivity and implying that lack of VIC adhesion was a cell type specific response. $n = 4$ samples per condition. All images are shown at the same magnification. Scale bar represents 100 μm.

VIC adhesion to PEG-peptide gels

Poly(ethylene glycol) hydrogels were modified to contain the ubiquitous cell adhesion peptide sequence RGD. However, adhesion and spreading of VICs on RGD-modified surfaces was only slightly improved over that of VICs on control PEG-diacrylate gels, as depicted in Figure 3(a) Surfaces modified to contain similar RGD concentrations have been shown to promote the spreading of several other cell types.[29–32] EILDV found in the alternatively spliced type III connecting segment of fibronectin, interacts with the $\alpha_4\beta_1$ integrin.[33,34] The combination of RGD with EILDV did not significantly improve VIC adhesion (data not shown). 3T3 fibroblasts were used as a positive control for cell adhesion experiments. Seeding 3T3s on the various modified surfaces demonstrated that the lack of VIC adhesion was cell-specific [Fig. 3(b)] and verified that the PEG materials did contain active peptide sequences.

While RGD and EILDV did not significantly aid VIC adhesion to PEG hydrogels, possible benefits of using PHSRN, another fibronectin-derived peptide sequence, in conjunction with RGD were explored. Use of PHSRN in conjunction with RGD has been shown to significantly enhance cell adhesion compared to RGD alone, as this synergism is necessary for full adhesive activity of the $\alpha_5\beta_1$ binding receptor.[26] Cell counts performed on hydrogels composed of PEG-PHSRN, PEG-RGD, and both PEG-PHSRN and PEG-RGD (Fig. 4) demonstrated that the number of adhered cells was not significantly increased in the presence of RGD and PHSRN compared to control hydrogels containing no peptide. In addition, there was no significant difference seen between the number of cells adhered to hydrogels containing just RGD and hydrogels containing RGD and PHSRN.

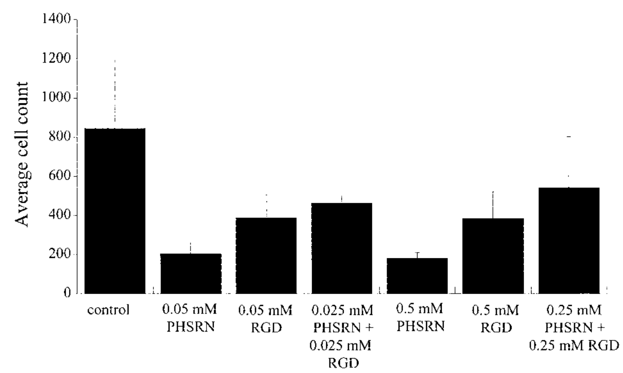

Figure 4. Poly(ethylene glycol) hydrogels were modified to contain the adhesive peptides sequences RGD or PHSRN, or a combination of the two, which has been shown to synergistically increase cell attachment in other systems. However, neither RGD nor PHSRN, nor both peptides presented in conjunction was able to stimulate VIC attachment.

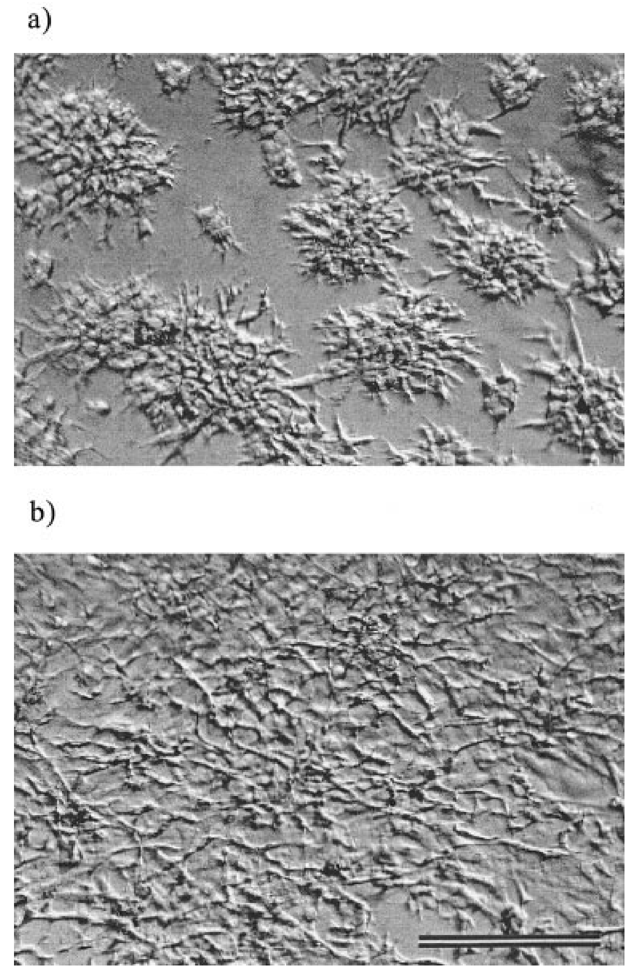

Figure 5. Valvular interstitial cells seeded upon HA-MA hydrogels were spread after (a) 2 days of culture, and confluent by (b) 4 days. $n = 4$. All images are shown at the same magnification. Scale bar represents 100 μm.

VIC adhesion to crosslinked polysaccharide gels

Hyaluronic acid was next investigated as a VIC scaffold material, as it possesses many attractive qualities, including specific binding interactions with FN, a protein that was observed to support VIC growth. Photopolymerization of methacrylated HA resulted in rapid formation of firm, yet elastic, transparent hydrogels. Valvular interstitial cells adhered to the HA-MA gels [Fig. 5(a)], although the cell morphology differed from that of VICs on control tissue culture surfaces [Fig. 2(d)]. However, unlike the VICs seeded upon collagen and laminin-coated surfaces, VICs on HA-MA readily proliferated, forming a confluent monolayer within 4 days [Fig. 5(b)].

Interactions of HA-MA with fibronectin

Hyaluronic acid possesses specific binding interactions with fibronectin (FN), and this property was retained after modification of HA to form HA-MA. When FN is combined with HA or HA-MA, decreased staining intensity for FN on native PAGE gels indicates FN binding to HA or HA-MA. The results in Figure 6(a) are reported as a percent of the control binding. The degree of FN association with HA-MA was dependent upon the extent of HA-MA methacrylation, with a higher degree of modification corresponding to decreased FN binding. The percentage methacrylation of HA-MA refers to the number of methacrylate groups per HA disaccharide unit.

Methacrylated hyaluronic acid gels were also shown to specifically retain noncovalently bound, photoencapsulated FN-HRP as evidenced by the color change upon exposure to the HRP chromogen [Fig. 6(b)]. To demonstrate that the binding was specific to FN, gels containing IgG-HRP, a protein that should not bind to HA, did not stain positively for protein. Furthermore, negative control gels that did not contain any protein did not exhibit any color change when exposed to the HRP chromogen. These results demonstrate that FN is still able to associate and bind with HA-MA that has been crosslinked to form a hydrogel matrix.

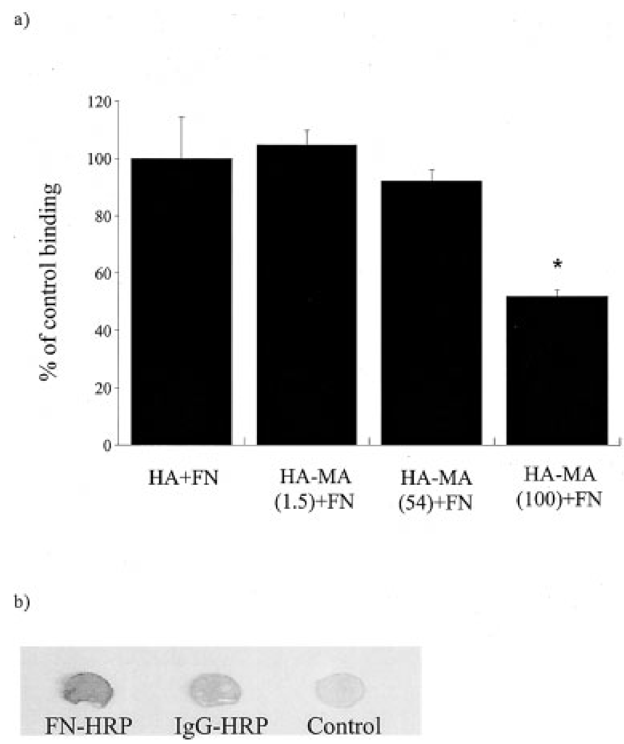

Figure 6. Methacrylated HA retained its ability to specifically bind to fibronectin. A native PAGE analysis of HA (a) demonstrated that FN binding to HA decreased with increasing extent of HA methacrylation (% methacrylation shown in parentheses). $^*p < .005$, $n = 3$ samples per condition. Photoencapsulated FN-HRP was specifically retained within HA-MA hydrogels and responded to an HRP chromogen while a non-HA-binding protein readily diffused out of the gels (b).

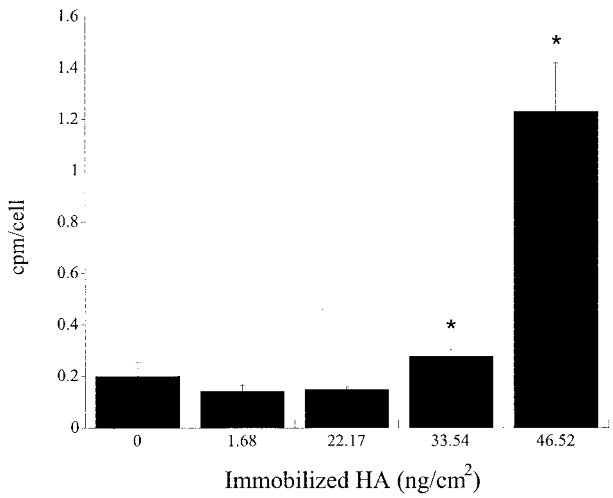

Figure 7. Extracellular matrix production by VICs seeded on HA modified surfaces was assessed after 5 days by measuring ^{3}H-glycine incorporation into glycoprotein, elastin, and collagen. The total radioactivity present in the three fractions was normalized by cell number. Increasing amounts of immobilized HA resulted in significantly increased overall matrix production by VICs when compared to the control. $*p < .005$, $n = 7$ samples per condition.

VIC ECM production on HA-modified surfaces

Valvular interstitial cells were cultured on HA-modified surfaces and their ECM production was examined after 5 days of growth by measuring the ^{3}H-glycine incorporated into glycoprotein, elastin, and collagen during ECM synthesis by the cells. Extracellular matrix production by VICs was found to be dependent upon the amount of immobilized HA, where greater amounts of immobilized HA resulted in significantly increased total matrix production by VICs (Fig. 7). This increase in overall matrix production occurred without a significant change in matrix composition (data not shown).

DISCUSSION

The present study investigates the synthesis of materials appropriate for the growth of VICs, with the ultimate goal of creating a tissue-engineered heart valve. Although VICs are the most prevalent cell type in native heart valves and possess remarkable properties in terms of matrix remodeling and wound repair, this is the first communication in which potential VIC scaffold materials have been explored. In this study, we first examined VIC attachment to collagen, laminin, and fibronectin surfaces, as well as cell adhesion peptide sequences RGD, EILDV, and PHSRN. The use of peptide-modified PEG hydrogels has proven successful in several other tissue engineering applications,[29,30] and it was the initial goal of this project to employ similar materials for use with VICs. However, because fibronectin was the most compatible protein with respect to VIC adhesion and growth, and FN-derived peptides were not adequate in encouraging VIC adhesion, a different approach toward creating a scaffold for VICs was taken.

Hyaluronic acid is a polysaccharide with numerous attractive qualities with respect to tissue engineering, and specifically, VIC culture. Recently, HA was identified as an essential component in cardiac morphogenesis.[35,36] In the absence of HA, heart valves failed to form resulting in embryonic lethality. 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. Hyaluronic acid is also nonimmunogenic, biocompatible, nonthrombogenic, and can be methacrylated to form photocrosslinked hydrogels. These resulting hydrogels possess many desirable physical and mechanical properties from a heart valve scaffold perspective. Furthermore, using HA as a scaffold material allows for facile incorporation of fibronectin into the matrices, as HA possesses specific binding interactions with FN. This combination of natural materials may be advantageous, as FN-coated surfaces were found to perform well with respect to supporting VIC adhesion and proliferation.

Initial studies discussed in this article imply that photopolymerized HA-based materials may be suitable substrates for the culture of VICs. While VIC morphology on HA hydrogels differed from that of VICs on tissue culture polystyrene, the cells proliferated to confluency and produced significantly more extracellular matrix proteins than VICs on control surfaces. A significant challenge in the creation of tissue-engineered heart valves is the production of adequate and appropriate amounts of ECM proteins. A scaffold material that actively encourages VICs to produce matrix proteins may be desirable for tissue formation. Furthermore, methacrylated HA retained its ability to bind to FN, and HA hydrogels were also shown to specifically retain FN following photopolymerization of HA-MA in the presence of FN. These results indicate that a scaffold system for VICs containing multiple biological components can be formed, with the goal of optimizing scaffold contents to facilitate VIC growth, and hence, heart valve tissue formation.

The finding that VICs adhere to and spread upon HA-MA hydrogels was somewhat unexpected, as HA-based materials do not often support cell adhesion without modification to contain adhesive proteins or peptides.[37] Although the biological basis for adhesive interactions between VICs and HA was not a topic of investigation in the current study, we can postulate possible explanations for this response. There are two receptors through which cells may internalize HA; these receptors are CD44 and RHAMM (receptor for hyaluronic acid-mediated motility). CD44 is a cell surface adhesion receptor that is not actively expressed in all cell types[38] and whose primary binding ligand is HA.[39] We have observed that VICs are capable of internalizing HA (unpublished data), indicating the presence of HA receptors on VIC cell surfaces. This result implies the presence of active CD44 on VIC surfaces, and CD44-HA interactions can lead to cell-matrix attachment.[39] In addition to direct VIC-HA binding via CD44, it has recently been reported that cell adhesion to crosslinked hyaluronan hydrogels can be increased by hydrogel treatment with UV light.[40] It was found that exposure to UV light increased the texture of the HA gel surfaces, thereby significantly increasing cell adhesion. While the UV irradiation conditions in Ref. 40 differ from the UV exposure used in the present study, it is still possible that the HA-MA gels discussed here have been slightly texturized during UV irradiation, thus making their surfaces more amenable to VIC adhesion. An analysis of the surface characteristics of these HA-MA gels has not been performed.

The physical properties of hydrogels are similar to those of many biological tissues, making hydrogels appealing materials for use in tissue engineering applications.[19] In the specific case of heart valves, there is additional rationale for the use of hydrogels, as normal embryonic heart development stems from a cardiac jelly[41] that bears resemblance to a hydrogel. Moreover, hydrogels made from natural polymers, such as HA, are biodegradable. In the case of HA-MA, degradation occurs enzymatically via hyaluronidase. An advantage of enzymatic degradation is that it may allow scaffold degradation to be paired with tissue ingrowth. Additionally, the properties of these materials may be altered and optimized through copolymerization with other biocompatible, photopolymerizable materials, such as acrylated poly(ethylene glycol). Thus, both the physical and biological properties of photopolymerized HA-based scaffolds are attractive for heart valve tissue engineering, and the interactions of HA with VICs deserve further exploration.

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