Micromolding of photocrosslinkable hyaluronic acid for cell encapsulation and entrapment

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Abstract: Micropatterning of hydrogels is potentially useful for a variety of applications, including tissue engineering, fundamental biological studies, diagnostics, and high-throughput screening. Although synthetic polymers have been developed for these applications, natural polymers such as polysaccharides may have advantages for biological samples and cell-based devices because they are natural components of the *in vivo* microenvironment. In this study, we synthesized and used hyaluronic acid (HA) modified with photoreactive methacrylates to fabricate microstructures as functional components of microfabricated devices. To demonstrate the universality of this approach, two types of microstructures were formed. In the first approach, HA microstructures were fabricated and used as docking templates to enable the subsequent formation of cell microarrays within low shear stress regions of the patterns. Cells within these microwells remained viable, could generate spheroids, and could be retrieved using mechanical disruption. In the second approach, cells were encapsulated directly within the HA hydrogels. Arrays of viable embryonic stem (ES) cells or fibroblasts were encapsulated within HA hydrogels and could later be recovered using enzymatic digestion of the microstructures. These results demonstrate that it is possible to incorporate photocrosslinkable HA, a natural, versatile, degradable, and biocompatible biopolymer, into microelectromechanical systems. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res 79A: 522–532, 2006

Key words: biomaterials; micropatterning; hyaluronic acid; photocrosslinking; embryonic stem cells

INTRODUCTION

Research in biology, medicine, and drug discovery is becoming increasingly dependent on the use of *in vitro* systems for screening and analysis. However, most *in vitro* tissue culture techniques lack the complexity and the architecture associated with tissues in the body. *In vivo*, cells are exposed to tightly controlled conditions in three-dimensional (3D) architectures regulated by factors such as cell–cell, cell–extracellular matrix (ECM), and cell-soluble factors. Microscale approaches such as cell patterning are powerful tools for *in vitro* studies of 3D architectures. These techniques can be used to study cell behavior with precise control of cell-microenvironment interactions in a spatially and temporally regulated manner.[1] In addition, microscale approaches can be used to miniaturize assays and perform high-throughput experimentation,[1–3] thereby enabling the study of complex biological systems by testing the influence of many factors simultaneously.

Microscale approaches have been used to control topographical features and spatial presentation of surface molecules for the development of cell and protein arrays. These arrays have been used for drug discovery, diagnostic assays, and biosensors.[4] Cells and proteins have been patterned on various substrates using self-assembled monolayers (SAMs),[5–11] microstamped proteins,[12] biological[13–16] and comb polymers,[17] microfluidic channels,[18] and elastomeric membranes.[19,20] In most of these approaches, cells are patterned on 2D

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surfaces, which lack a 3D microenvironment and the appropriate cell-ECM interactions.

Hydrogels are becoming widely used as an attempt to provide a more *in vivo*-like environment for tissue engineering and biology.[21] Recently, the ability to use crosslinkable biomaterials has led to methods of fabricating patterned 3D microenvironments. Polyethylene glycol dimethacrylate (PEGDM) is a photocrosslinkable hydrogel that has been commonly used in fabricating 3D features. PEGDM is inert, hydrophilic, and non-toxic and has been used with photolithography to encapsulate cells in 3D microgels.[22,23] Furthermore, PEG-based microstructures have been used to serve as templates for immobilizing cells on 2D surfaces[24,25] or within microfluidic channels.[26] One potential disadvantage of using PEG is that it does not degrade (unless modified using chemical means), and that it is a relatively inert component of the cell environment, unable to initiate cell signaling. Thus, the fabrication of naturally based alternatives to synthetic microfabrication polymers such as PEG may be advantageous to cells.

Hyaluronic acid (HA), or hyaluronan, is a natural glycosaminoglycan that is nonimmunogenic and nonadhesive.[27] HA is a linear polysaccharide composed of repeating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid and is found in many tissues. HA is a biocompatible and enzymatically degradable polymer that is useful for many applications in drug delivery, tissue engineering, and viscosupplementation.[28–31] As a component of the ECM, HA plays an important role in lubrication, watersorption, water-retention, and a number of cellular functions such as attachment, migration, and proliferation.[32,33] We have previously shown that HA can be immobilized on hydrophilic surfaces using a molding process for the fabrication of cell arrays[34] and patterned co-cultures.[35] However, these arrays were limited to 2D substrates because the molded HA structures washed away during the rinsing step, leaving a film that was a few nanometers in depth. More recently, photocrosslinkable HA gels have been synthesized for various tissue engineering applications.[36–38] The mechanical properties and degradation rates of these gels can be controlled by altering the hydrogel crosslinking density.

In this paper, we present and characterize a method of combining natural photocrosslinkable polymers such as HA with micromolding approaches to fabricate hydrogels capable of immobilizing cells. To demonstrate the universality of the approach, cells were immobilized either by directly encapsulating the cells within the microstructures or by docking the cells within low shear stress regions generated by the microstructures. We have shown that the cells maintain their viability using either of the two approaches and that they can form aggregates of controlled sizes and shapes. Also, cells can be released from the HA microstructures upon enzymatic degradation of the polymer for subsequent analysis or for tissue engineering.

MATERIALS AND METHODS

Materials

All tissue culture media and serum were purchased from Gibco Invitrogen Corporation, and cell lines were purchased from American Type Culture Collection. All chemicals were purchased from Sigma, unless otherwise indicated.

HA synthesis

Methacrylated HA was synthesized using a process described previously.[36,39] Briefly, methacrylic anhydride was added to a 1% w/v solution of HA (Lifecore, 50 kD) and reacted for 24 h in deionized water adjusted to a pH of 8 with 5*N* NaOH. The solution was then dialyzed for purification and then lyophilized. The white powder macromer was stored at −20°C until use.

PDMS fabrication

Polydimethylsiloxane (PDMS) (Essex Chemical, Sylgard 184) molds were fabricated by mixing silicone elastomer and curing agent in a 10:1 ratio. The mixture was poured onto the silicon masters that were patterned with SU-8 and cured at 60°C for 4 h. The PDMS molds were then peeled from the silicon surfaces and cut prior to use. In some experiments, the surfaces of PDMS molds were made hydrophilic by plasma treatment for 3 min (60 W, PDC-32G, Harrick Scientific).

Cell culture

All cells were manipulated under sterile tissue culture hoods and maintained in a 95% air/5% CO_2 humidified incubator at 37°C. NIH-3T3 cells were maintained in 10% fetal bovine serum (FBS) in Dulbecco's modified eagle medium (DMEM). Confluent dishes of NIH-3T3 cells were passaged and fed every 3–4 days. Murine embryonic stem (ES) cells (R1 strain) were maintained on gelatin treated dishes with media comprised of 15% ES qualified FBS in DMEM knockout medium. ES cells were fed daily and passaged every 3 days at a subculture ratio of 1:4.

Methacrylation of glass surfaces

To ensure that the molded gels adhered onto the glass surfaces, the slides were methacrylated using poly(3-trimethoxysilyl)propyl methacrylate (TMSMA) treatment. Glass slides were plasma-treated for 5 min (60 W, PDC-32G, Harrick Scientific), coated with a 98% TMSMA solution, and incubated at 100°C for 30 min. Afterwards, the slides were separated and heated at 110°C for 10 min. After cur-

ing, the glass slides were rinsed with distilled water to remove excess TMSMA and dried.

Hydrogel microstructure formation

To fabricate hydrogel microstructures, a 5% w/v methacrylated HA solution was prepared by dissolving the lyophilized macromer in PBS. A 0.5% w/v Irgacure 2959 solution was added as photoinitiator (Ciba Chemicals). To fabricate the microstructures using micromolding, a few drops of a solution containing the HA macromer and photoinitiator were placed onto a methacrylated glass slide. The PDMS mold was then brought into contact with the solution and gently pressed. Finally, the mold was exposed to 300 mW/cm^2 UV light (EFOS Ultracure 100ss Plus, UV spot lamp) at a wavelength of 365 nm for 180 s to cure the gel. After exposure, the PDMS mold was peeled from the surface, and the hydrogel was immediately placed in PBS.

Cells encapsulated within HA hydrogels

To encapsulate cells within micropatterned hydrogels, a solution of 10 wt % methacrylated HA in PBS and 1% w/v Irgacure 2959 photoinitiator was added to an equal volume of media containing cells. The solution was pipetted onto the PDMS mold, and the mold was then inverted and placed on a methacrylated glass slide. The HA solution containing cells were allowed to mold into the features of the stamp. Subsequently, HA was gelled by exposure to 200 mW/cm^2 UV light at 365 nm for 180 s. The mold was then removed, and the cells/microstructures were placed in media. For experiments in which the cells settled to the bottom of the microgels, the gels were left undisturbed for 10 min prior to UV exposure.

Cell viability analysis

To analyze cellular viability, a live/dead assay was performed consisting of calcein AM and ethidium homodimer (Molecular Probes). The two components were added to PBS at a concentration of 2 μg/mL and 4 μg/mL, respectively. The micropatterns that contained cells were then placed in the solution for 10 min and visualized under a fluorescent microscope (Zeiss, Axiovert 200). With this assay, live cells stain green while dead cells uptake the red dye.

Docking cells within the microwells

To immobilize cells within the HA microstructures, cells were trypsinized and resuspended in medium at a concentration of ~4 × 10^6 cells/mL. The cells were then plated on the HA substrate and allowed to settle overnight. The cultures were subsequently washed with a gentle flow of medium to remove cells that had not docked within the microwells.

Scanning electron microscopy

To perform scanning electron microscopy (JEOL 6320FV), samples were mounted onto aluminum stages, sputter coated with gold to a thickness of 200 Å, and analyzed at a working distance of 20 mm.

Cell staining protocol

Cells were stained with the membrane dye, carboxyfluorescein diacetate, succinimidyl ester (CFSE). CFSE passively diffuses into cells where it becomes fluorescent by reacting with intracellular esterases. The succinimidyl ester group reacts with intracellular amines, forming fluorescent conjugates within the cell. To stain with CFSE, trypsinized cells were resuspended to a concentration of 1 × 10^6 cells/mL within the staining solution, which consisted of CFSE at 10 μg/mL in PBS. The cells were rinsed in PBS twice before being used for experiments.

Confocal microscopy

For confocal microscopy, CFSE-stained cell samples were fixed with Fluoromount-G and covered with a No. 1 thickness coverslip. Confocal images were taken at 10× magnification through a FITC filter with a maximum focal depth of 248 μm. CFSE was excited with an Argon laser at 488 nm.

Enzymatic degradation of HA structures

To assess the enzymatic degradation of HA structures, samples were immersed in a solution of hyaluronidase at 10,000 U/mL in DMEM media (with 10% PBS and 1% penicillin streptomycin) and incubated at 37°C and 5% CO_2. Microscope images were taken at regular intervals to monitor the progression of degradation.

RESULTS AND DISCUSSION

Fabrication of the HA microstructures

Developing microfabrication methods for natural polymers using photochemistry may be beneficial for a variety of applications such as tissue engineering and high-throughput diagnostics. Here we fabricated HA microstructures using the process outlined in Figure 1. In this process, also known as capillary force lithography,[40] a balance of forces, including surface interactions, viscous forces, and the molding pressures, was used to mold a layer of the polymer into the void regions of a PDMS stamp. The molding technique has a number of advantages over other approaches such as photolithography in that it can be used to control surface chemistry as well as to-

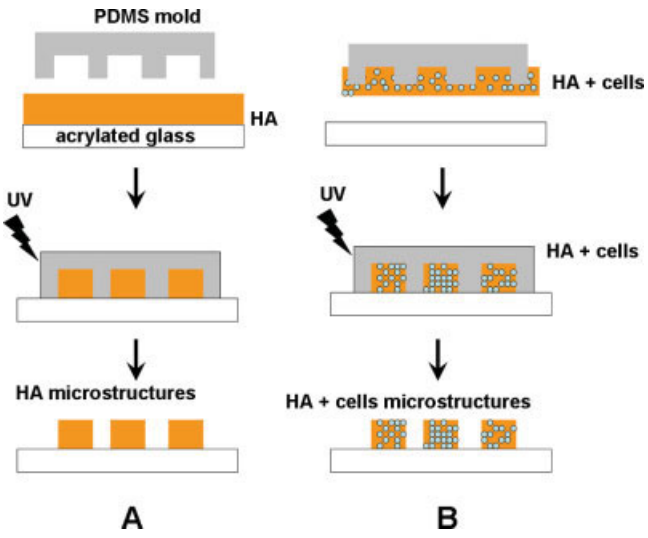

Figure 1. Schematic diagram of the HA micromolding process. PDMS molds were used to mold a layer of HA into the void regions of the stamp. The polymer was then cured with exposure to UV light to fabricate HA microstructures. To fabricate HA microstructures without cells, a thin polymer film on the substrate was molded (A). To fabricate HA microstructures that encapsulated cells (B) the HA solution was transferred from the PDMS mold onto the substrate and subsequently crosslinked. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pography.[41] In addition, capillary molding minimizes the need for expensive clean rooms and photolithographic equipment, thereby making this approach more scalable for widespread use in biological and medical laboratories. In this process, the fabrication of silicon masters still required the use of clean room facilities and photolithographic equipment; however, once a master was fabricated it was used repeatedly to generate PDMS molds. Subsequently, the prepolymer was molded into the void regions of the mold and UV light was used to crosslink the polymer. Once the hydrogel was formed, the PDMS mold was removed and the microstructures were immersed in an aqueous solution to remain hydrated.

The fabricated HA microstructures were visualized and characterized using scanning electron microscopy (SEM) and light microscopy (Fig. 2). HA microstructures could be made into various shapes by using PDMS molds of different topographies. Representative images of HA microstructures are shown in the shape of raised boxes or as HA layers containing circular microwells (Fig. 2). Figure 2 (A,B) shows light microscope images of the microstructure patterns that were fabricated using PDMS molds with protruding grids and posts, leading to HA boxes and microwells. Both light and SEM images indicate that the patterns could be formed with high fidelity. While the PDMS structures had protruding features of around 50 μm in height, the resulting SEM images show a much thinner structure due to the dehydration process. The water content of the HA hydrogels used was ~95%.

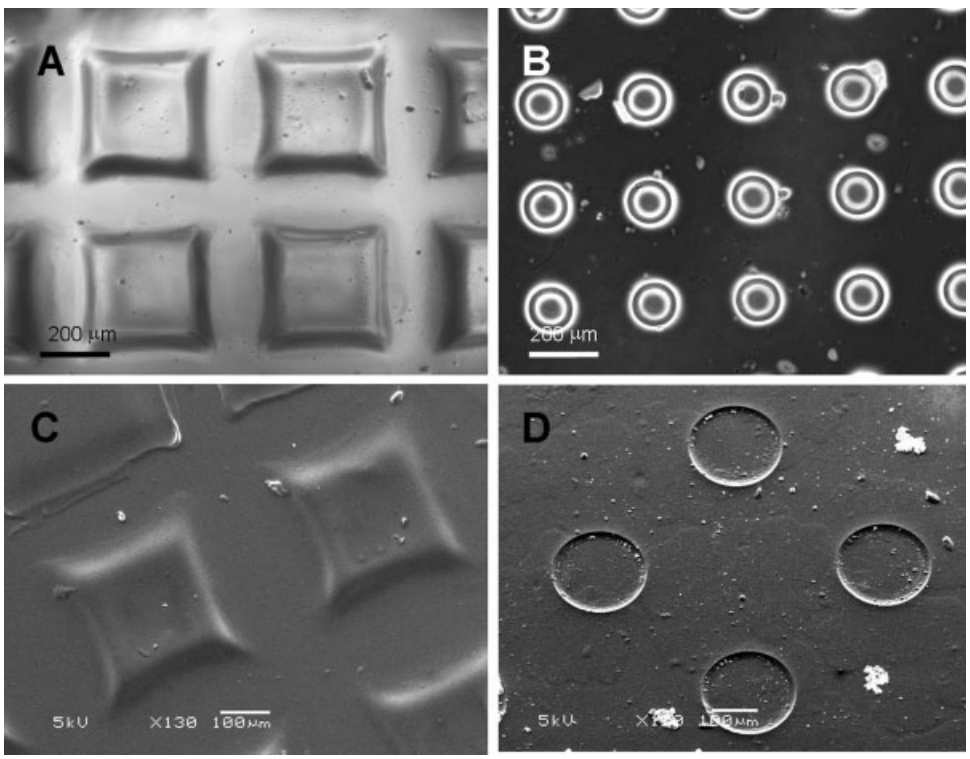

Figure 2. Characterization of HA microstructures. Light microscopy (A–B) and scanning electron microscopy (C–D) images of HA microstructures in the shape of raised square blocks (A, C) and circular microwells (B, D).

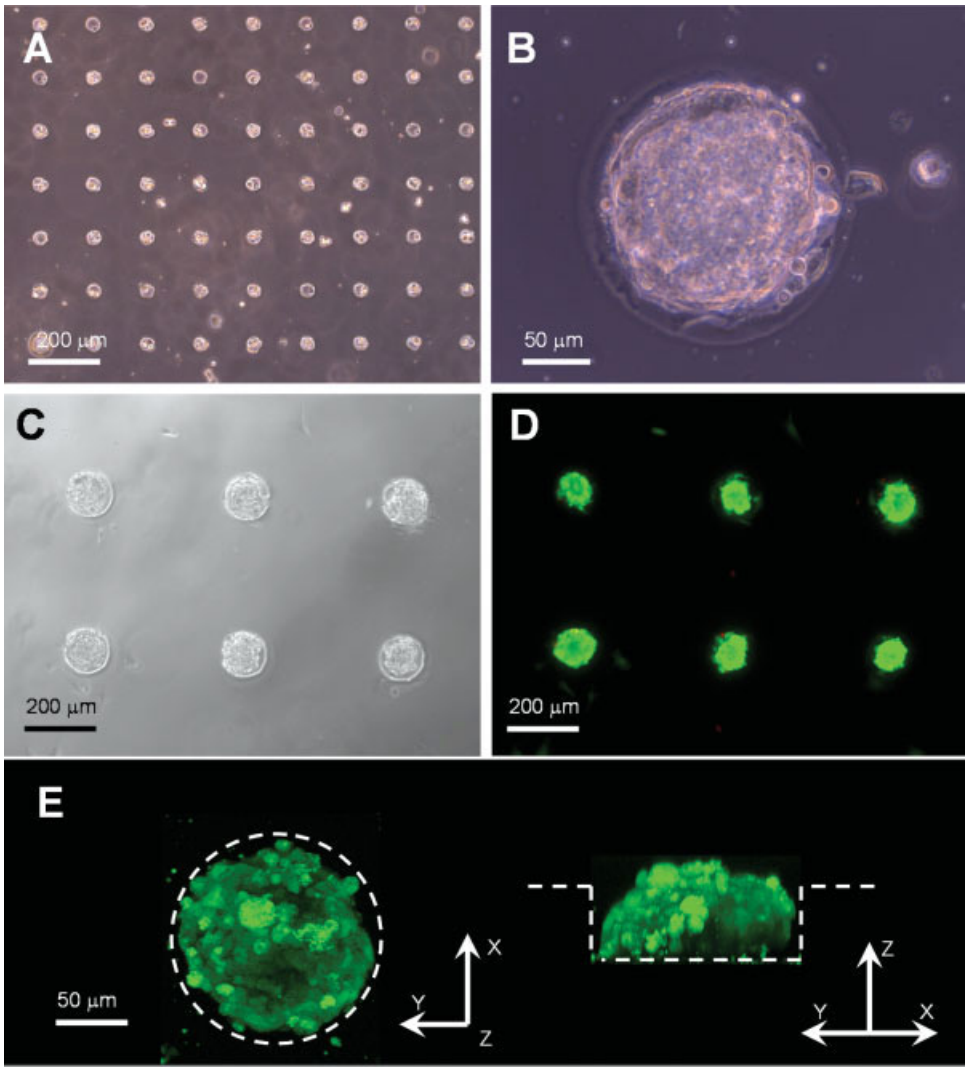

Figure 3. Cell docking and viability within HA microwells after 24 h. Light micrographs of HA microwells used as templates for ES cells aggregate formation (A–C). Cells were docked with good reproducibility within microwells ranging from 40 μm (A) to >200 μm (B) in diameter. Figures (C–D) are light and fluorescence images of the same region stained with live/dead assay, indicating that the cells remained alive (green) inside the HA microwells. Figure (E) represents confocal image of CFSE stained ES cells in HA microwells viewed from the top and side. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

One parameter that was optimized was the concentration of photocrosslinkable HA for micromolding. The concentration of HA investigated was between 2 and 15%. Although higher HA concentrations were desirable because they enhanced the mechanical integrity of the microstructures, high concentrations negatively affected the molding process as the viscous forces became stronger than the surface interaction forces. In addition, higher concentrations were cytotoxic for cell encapsulation,[36] which prevented the use of these concentrations for that purpose. In these studies we found that a 5% w/v solution of HA in water or PBS was the optimal concentration for the various applications outlined.

One advantage of the molding process is that it allows control of many properties of a pattern by simple modifications to surface properties such as hydrophobicity. Therefore, complex structures that require multistep photolithographic processes can be simply fabricated by micromolding. For example, to fabricate isolated HA posts, the surfaces of the substrate and the PDMS mold were made hydrophobic. The two hydrophobic surfaces repelled the polymer solution as the mold was pressed on the substrate, and the excess solution was dewetted into the void regions of the PDMS mold. Therefore, the polymer solution was crosslinked only within the mold patterns. Alternatively, to fabricate microstructure patterns while maintaining a layer of HA on the substrate, the PDMS was plasma cleaned to increase its hydrophilicity. Thus, a thin layer of the gel was retained between the surface of the mold and the substrate. Additionally, the thickness of the initial polymer film and the molding pressure are other

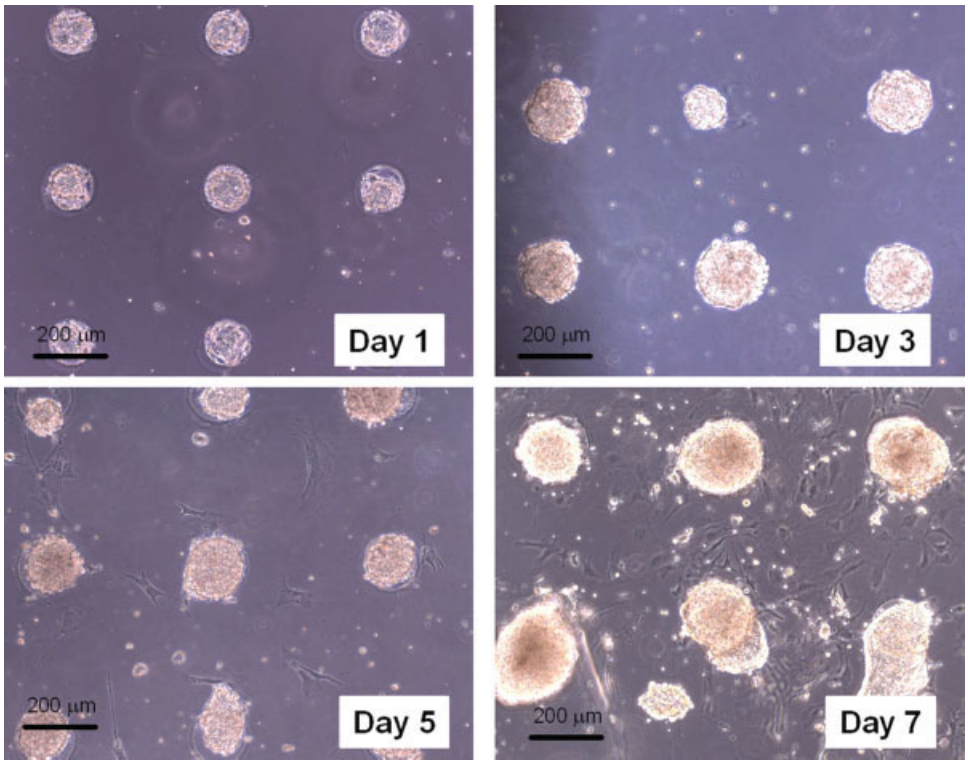

Figure 4. Stability of ES cell spheroid arrays within HA microwells after 1, 3, 5, and 7 days in culture. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

factors that can be used to further control the shape of the structures.

Cell analysis within HA microwells

Polymeric microstructures provide a potentially useful platform for controlling cell–cell interactions. To test the feasibility of using HA microstructures for immobilization on surfaces, we fabricated HA microwells that ranged in size from ~40 μm to > 200 μm in diameter and ~50 μm in depth. These circular wells provided low shear stress regions where cells could dock.

ES cells or NIH-3T3 fibroblasts were plated onto the microwells after the gels were fabricated and washed with PBS. HA is hydrophilic and has been previously shown to resist cell adhesion for a few hours.[34,35,42] Once the cells were seeded, they did not immediately attach to the surface of the HA gels. As the cells settled on the surfaces, the cells that deposited within the microstructures (i.e., microwells) were not washed away with the subsequent rinsing steps. Therefore, HA microwells were suitable templates for the fabrication of cell arrays. To form these cell arrays, cells were added in suspension, incubated for 12 h, and then washed to remove excess cells. Shear protected cells remained within the microwells and formed uniform arrays (Fig. 3). Cell aggregates found within HA microwells ranged in size from ~40 μm (Fig. 3A) to ~200 μm (Fig. 3B) depending on the size of the well. To analyze whether the cells in these microwells remained viable, a Live/Dead cell viability assay was used. As shown in Figure 3C and 3D, >99% of the cells were viable 24 h after the initial seeding, as determined by the percentage of cells that fluoresced green. These aggregates conformed to the features of the microwells and were imaged using confocal microscopy (Fig. 3E).

To analyze the stability of spheroid arrays within HA microwells, ES aggregates were maintained in HA microwells and analyzed over time. Figure 4 shows timecourse light microscopy images of the cells within HA wells. The cells remained viable in the wells throughout the 7 days of culture analyzed. ES cell aggregates grew in size throughout the 1 week study to approximately double their initial size. An interesting property of the cultures was the emergence of cells from the microwells around day 5 of culture, which was further evident by day 7. The adhesion of these cells on the HA surfaces indicates that the non-biofouling properties of HA may be transient, similar to previously published results,[43] potentially as a result of the deposition of cell-adhesive positively charged proteins on the negatively charged HA molecules. Although the aggregates expanded to the size of the wells during the week-long experiment, they did not migrate through or compromise the structural integrity of the gels.

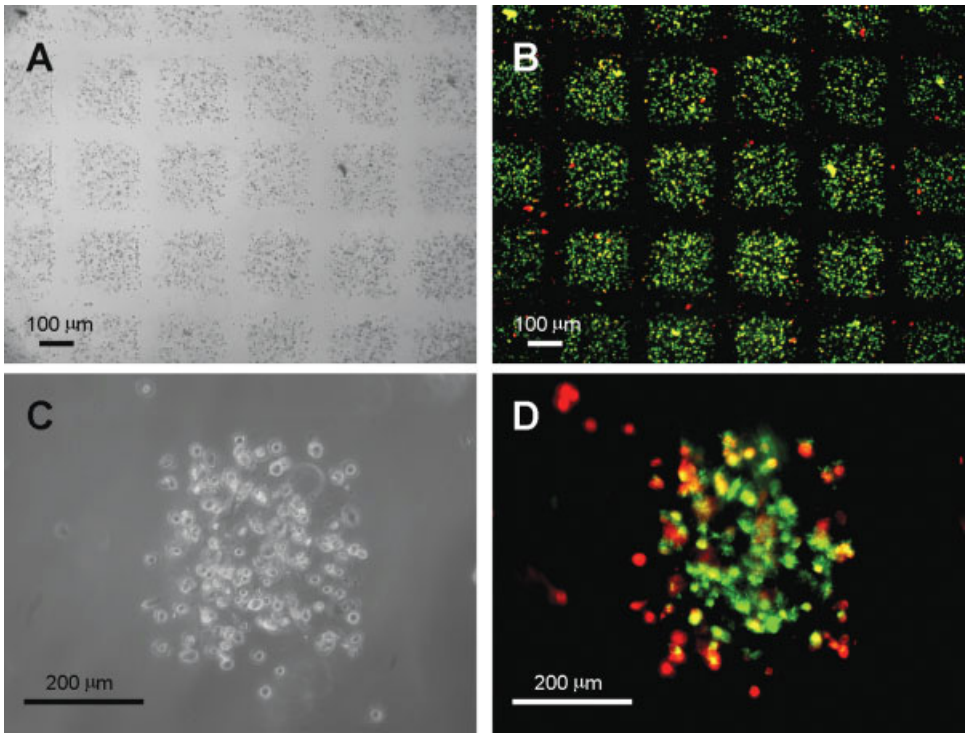

Figure 5. Light and fluorescent micrographs of encapsulated cells within micromolded photocrosslinked HA 6 h after photocrosslinking. Figures (A) and (C) are light micrographs of molded HA gels at low and high resolutions, and figures (B) and (D) are the corresponding fluorescent micrographs stained with the Live (green)/dead (red) viability assay. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

An interesting feature of the microwells introduced here is that the cells within them could be retrieved for tissue engineering applications, such as for the formation of embryoid bodies or hepatocyte spheroids. Here, cells were lodged within the microwells through a gentle flow of medium parallel to the surface and were maintained within the wells for at least one week. Alternatively, a vigorous flow of medium perpendicular to the surfaces of the gel could be used to remove the aggregates from the substrates to yield homogeneous cell spheroids (data not shown). Based on the micropatterns used, it may be possible to fabricate wells of various geometries. For example, microfabricated lanes could be used as templates for forming tissue fibers. In other words, the microwell technique is shown to be a versatile method of forming and controlling the aggregate sizes for prolonged culture.

Cellular encapsulation within HA microstructures

To test the feasibility of photoencapsulating cells in the HA microstructures, ES cells and fibroblasts were suspended directly in the HA hydrogel precursor solution. To initiate the molding process, the solution containing cells was either placed on the substrate or the PDMS stamp. Although both approaches were successful, we found that by placing the solution on the PDMS mold, it was easier to eliminate the cells from the spaces in between the void regions of the stamp. Solutions were molded and UV-crosslinked to fabricate the hydrogel microstructures containing cells. Cells were suspended at various concentrations in a solution of 5% w/v HA and 0.5% w/v photoinitiator in PBS. As shown in Figure 5 (A,C), cells could be directly encapsulated within HA microstructures in a reproducible manner over large areas.

To analyze the viability of the cells encapsulated within the HA microstructures, NIH-3T3 fibroblasts were stained with the Live/Dead assay and analyzed under a fluorescent microscope. As it can be seen in Figure 5, both live (green) and dead (red) cells were visible 6 h after encapsulation. Quantitative counting of the cells in the gels indicated that 82 ± 5% survived the photocrosslinking and the subsequent molding process. It was found previously that the choice of light intensity, photoinitiator, and photoinitiator concentration are important in maintaining the viability of cells in photopolymerized hydrogels.[44] In this fabrication process, the decrease in cell viability could be due to high photoinitiator concentration or light intensity chosen for a rapid formation of the hydrogels. Other potential cytotoxic factors for encapsulated cells include mass transfer limitations and drying during processing. Interestingly, as shown in Figure 5D, the cells at the periphery of the

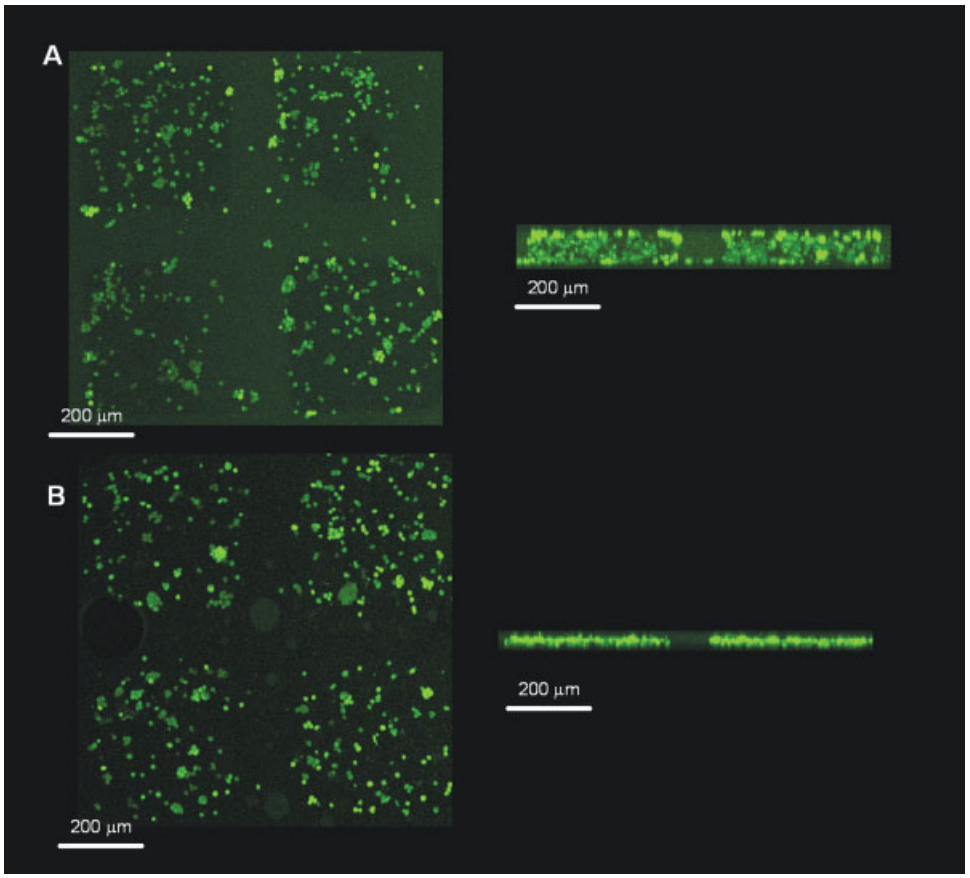

Figure 6. Confocal microscope images of cells encapsulated inside HA microstructures. (A) represents HA gels that were crosslinked immediately after molding while (B) represents HA gels that were crosslinked 10 min after molding. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

microgel were more likely to die in comparison to the cells at the interior of the gels. This observation suggests that the microstructures do not provide a significant barrier to oxygen and nutrient diffusion and that cell death is potentially associated with edge effects such as the drying of gels during processing or due to refraction within the PDMS, leading to increased local concentrations of UV and radicals along the edges of the gel. It is anticipated that by modifying the processing conditions, further enhancements in the cell viability could be achieved. These processing conditions include, limiting the exposure time of the cells to UV, minimizing the photoinitiator concentration as well as exposure to dry air.

To analyze the distribution of cells within the HA microgels, fibroblasts were stained with CFSE and imaged using confocal microscopy. Figure 6 demonstrates two different fabrication processes. In one condition the HA solution was crosslinked immediately after molding (Fig. 6A), while in the other condition the crosslinking process was delayed for 10 min (Fig. 6B). The cross-sectional image of the gels shows that the fixed patterns were ~50 μm in height in both cases, while significant differences were observed with respect to cell distribution in the gels. As shown in Figure 6A, photoencapsulated cells in which HA was immediately gelled after molding resulted in uniform distribution of cells throughout the gel, while in delayed crosslinking conditions the cells settled to the bottom (Fig. 6B). Therefore, the processing condition is shown to be particularly important for fabricating cells within hydrogels. Since encapsulation in hydrogels has been tested for other systems, this result demonstrates the importance of performing 3D imaging in order to confirm whether cells have distributed evenly.

One of the attractive features of the HA hydrogels is that the microstructures can be enzymatically degraded in a controlled fashion with hyaluronidase exposure. It was previously found that the concentration of hyaluronidase and the length of exposure influence hydrogel degradation.[36] To monitor the progression of HA degradation, cells were encapsulated and then recovered using 10,000 U/mL hyaluronidase. As shown in Figure 7, HA hydrogels degraded as a function of time in the presence of hyaluronidase. Degradation was visible as the patterns became less distinct by 24 h and cells could be

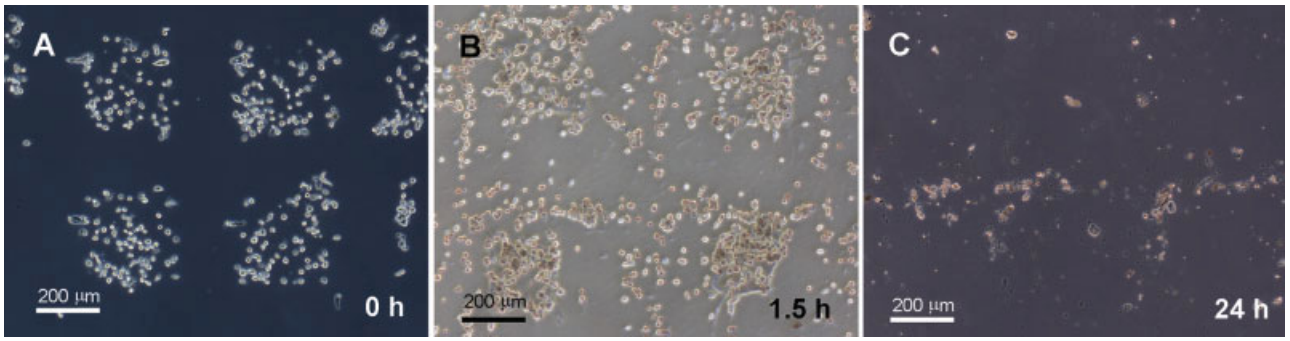

Figure 7. *In vitro* degradation of molded HA structures with encapsulated cells using hyaluronidase. Images were taken at times (A) 0 h, (B) after 1.5, and (C) 24 h, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

washed from the surface. In contrast, HA hydrogels that were incubated without hyaluronidase did not degrade during the same time periods (results not shown). Furthermore, since certain cell types produce hyaluronidase, it is expected that long-term culture of these cells within HA may lead to significant cell-mediated degradation of hydrogels. Although cell viability in these studies was not directly tested, we have released cells from the HA hydrogels and have demonstrated that cells remained viable after 24 h of treatment with the enzyme.

The system developed here has a number of potential advantages over previous approaches. For example, since micromolding of cells inside gels can be used to place cells inside individual microenvironments, the system could be used to investigate three-dimensional growth and characteristics of individual cells or aggregates within a matrix. Also, captured or encapsulated cells can be easily recovered from the gels through enzymatic degradation, which is not possible using traditional PEG hydrogels. This ability may be useful for high-throughput screening and diagnostic applications, in which cell retrieval is desired for subsequent analysis. In addition, natural, degradable gels may be incorporated within lab-on-a-chip devices for improved maintenance of cells such as hepatocytes. Another advantage of the molding approach we presented is that it avoids the need for complicated photolithographic systems and expensive clean room facilities. Therefore, capillary molding can be readily used as a soft lithographic tool within industrial and academic settings.

The ability to fabricate HA hydrogels is particularly appealing for tissue engineering since it is possible to use microscale techniques to fabricate these hydrogels with controlled shapes and spatial properties. For example, the ability to pattern fluids within microchannels can be merged with photopolymerization to form spatially oriented hydrogels.[23,45] Therefore, gradients of the photocrosslinkable macromers were formed within microfluidic channels and were subsequently gelled by exposure to UV light. Hydrogels were synthesized with gradients of signaling or adhesive molecules or with varying cross-linking density across the material. Such gels can be used to direct cell migration, adhesion, differentiation, or other cell behavior.[45,46] In addition, other polysaccharides can be functionalized with photoreactive groups and other functional groups,[36,39,47–49] thus this approach provides a general platform to fabricate microscale structures from natural materials. The merger of novel materials and microscale technologies provides valuable tools and approaches to understand biology, engineer tissues, and advance drug discovery by controlling the *in vitro* cellular microenvironment and by enabling high-throughput testing of desired conditions.

Summary

In summary, we have shown that photocrosslinkable HA, a natural and biocompatible polymer can be easily integrated with existing microfabrication techniques to fabricate microstructures. The technique presented here has been shown to be useful for capturing cells inside HA microwells or for encapsulating cells directly within the HA gels. In both approaches, the cells remained viable and could be retrieved for future analysis, either through mechanical forces or enzymatic digestion of HA.

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