Micromolding of photocrosslinkable hyaluronic acid for cell encapsulation and entrapment

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Statement of Purpose: 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 additional 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.

Methods: Methacrylated HA was synthesized and stored as described elsewhere(1). To fabricate hydrogel microstructures, a 5 % w/v methacrylated HA solution containing 0.5% w/v Irgacure 2959 (Ciba Chemicals) was micromolded onto a methacrylated glass slide using a patterned PDMS stamp(2). Molded HA solution was gelled by exposure to 200 mW/cm^2 UV light (365 nm) for 180 s. To immobilize cells within the HA microstructures, trypsinized cells were plated on the HA microwells, allowed to settle, and subsequently rinsed to remove cells that had not docked within the microwells. To encapsulate cells within hydrogels, a solution of methacrylated HA and photoinitiator was added to an equal volume of media containing cells and molded on a methacrylated glass slide. Enzymatic degradation of HA structures was assessed with a solution containing 10,000 U/ml of hyaluronidase in DMEM. Cell viability was analyzed using calcein AM (2 μg/ml) and ethidium homodimer (4 μg/ml). Microtructures were analyzed using scanning electron microscopy, confocal microscopy and fluorescence microcopy.

Results / Discussion:
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 (Fig. 1), 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 (Fig.2).

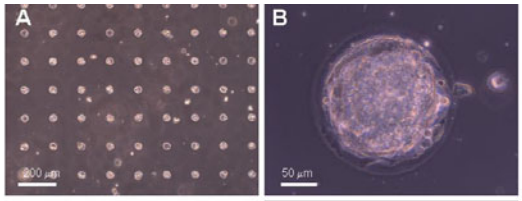

Fig. 1: ES cell docking within HA microwells after 24 hours. Cells were docked with good reproducibility within microwells ranging from 40 μm (A) to >200 μm (B) in diameter.

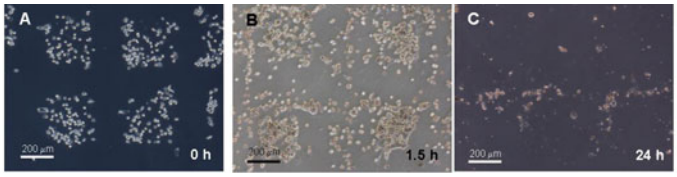

Fig. 2 Cell encapsulation within HA microstructures and *in vitro* degradation of molded HA structures using hyaluronidase. Images were taken at time 0 (A), after 1.5 (B) and 24 (C) hours, respectively.

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.

Conclusions: In conclusion, 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.

References:
1. Burdick, J. A., Chung, C., Jia, X., Randolph, M. A. & Langer, R. (2005) *Biomacromolecules* **6,** 386-91.

2. Khademhosseini, A., Yeh, J., Jon, S., Eng, G., Suh, K. Y., Burdick, J. A. & Langer, R. (2004) *Lab Chip* **4,** 425-30.