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A Controlled-Release Strategy for the Generation of Cross-Linked Hydrogel Microstructures

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In this communication we report a simple method of micromolding rapidly gelling, chemically cross-linkable hydrogels that is based on controlled release of the gelling agent from the mold. Hydrogels are a promising class of biomaterials since they can be easily tailored to produce desirable mechanical and chemical properties that resemble the native extracellular matrix and exhibit high permeability to oxygen, nutrients, and other water-soluble metabolites.[1,2] As such, hydrogels have been widely used in biomedical applications such as tissue engineering and drug delivery.[3,4] In particular, microscale hydrogels have been used for cell encapsulation, cell-based therapy, and bioprocess applications.[5,6] Microscale hydrogels can also be used to encapsulate and deliver drugs in a sustained manner.[7]

The ability to control the shape of microparticles can be used to tailor drug-release kinetics for drug delivery applications,[8] or assemble microconstructs for tissue engineering.[5,9] The most commonly used methods of forming microgels are emulsification,[10] microfluidics,[11] and shear-induced droplet formation.[12] While the latter two methods overcome the problem of polydispersity in particle size associated with emulsification, microfluidic generated particles are limited to photocross-linkable materials, and shear-induced droplets are limited to spherical particles. Therefore, a simple method for producing monodisperse hydrogel microparticles of controlled shape and size would be advantageous.

To date, this has been accomplished either via photolithographic techniques[9] or micromolding of photopolymerizable and temperature-dependent hydrogels.[13−15] However, these techniques cannot be applied to a wide variety of chemical or pH-dependent cross-linking hydrogels such as alginate, chitosan, fibrinogen, and some self-assembling peptides. Previously,[16] alginate microfluidic devices were fabricated by pouring the hydrogel precursor over a mold followed by the immersion of the setup in a bath containing the gelling agent. However, this procedure cannot be easily used to produce thin membranes of controlled height, nor can it be used to fabricate individual microparticles.

A significant challenge to micromolding of chemical or pH-dependent cross-linking hydrogels is their rapid gelling, which occurs immediately upon contact with the gelling agent. Controlling the shape and size of microparticles and microstructures made from these hydrogels has not been possible with the use of previously reported techniques. Here, we use controlled release of the gelling agent from a hydrogel mold to overcome this limitation. As shown in Figure 1A, the hydrogel precursor was molded with a hydrogel slab containing the gelling agent.

The slab provides a physical barrier while simultaneously inducing the gelation of the hydrogel precursor, resulting in the formation of both membranes and microparticles of controlled morphology.

We demonstrate the flexibility of this approach by generating microstructures of calcium alginate as a model ionically cross-linked hydrogel and of chitosan as a model pH-dependent hydrogel. Both alginate and chitosan are biocompatible hydrogels commonly used in tissue engineering, drug delivery, and cell culture applications[4] (see Supporting Information). In our process, alginate was molded between a plasma-cleaned PDMS mold and a calcium-containing agarose slab and subsequently gelled by the controlled release of calcium ions from the agarose. Similarly, chitosan hydrogel precursor (pH $\approx$ 6) was gelled upon contact with high-pH agarose (prepared by hydrating a dried agarose slab in a 5% w/v NaOH solution).

As shown in Figure 1, both alginate (Figure 1B,C) and chitosan (Figure 1D,E) could be micromolded to generate patterned membranes and particles. Figure 1 shows the basic schematic of this process, as it is applied to the fabrication of patterned membranes (Figure 1B,D) and microparticles (Figure 1C,E). Features with lateral dimensions between 5 and 2000 μm and vertical dimensions between 10 and 200 μm could be obtained. While patterned membranes were produced using a replica molding process by sandwiching the hydrogel precursor between a flat substrate and the agarose mold, the production of microparticles required microtransfer molding (μTM). In this procedure, a thin layer of hydrogel precursor was coated over the PDMS mold; after degassing in a vacuum chamber and scraping away excess material, we pressed the agarose slab containing the gelling agent against the mold. This procedure was required to overcome the weak seal formed between the PDMS mold and agarose, which otherwise led to the formation of a continuous film.

To demonstrate the applicability of this process to tissue engineering, cells were encapsulated in rectangular hexahedron microparticles (Figure 2A) and in alginate micropatterned membranes (Figure 2B). A wide range of cell densities was used (10^3−10^8 cells/mL) to generate microstructures with high cell viability (>80%). In addition, the mechanical properties of the micropatterned hydrogels could be altered by varying the precursor concentration and gelling conditions. In all cases, features remained stable after long-term (>2 weeks) incubation in cell culture media at 37 °C (see Supporting Information). To further explore the potential use of cell-laden micromolded hydrogels to control cell−cell interactions in vitro (B and C of Figure 2) Cell-Tracker Blue-

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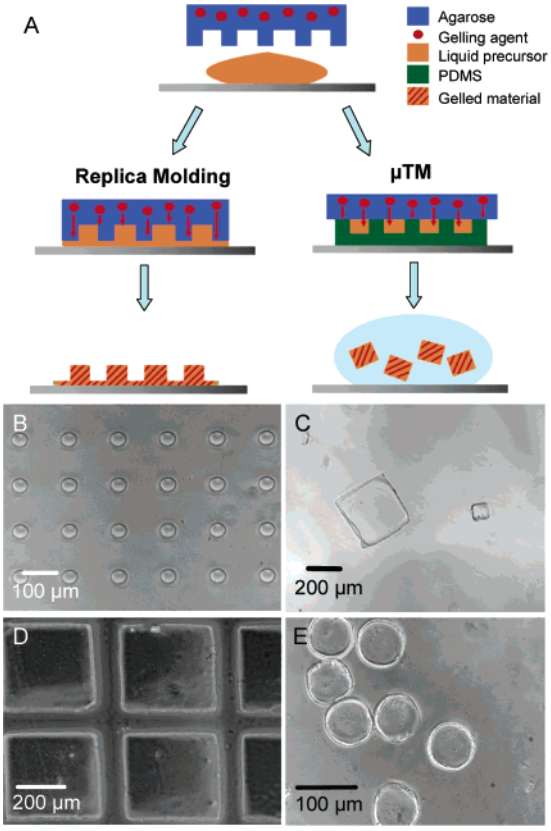

Figure 1. (A) Schematic of the two controlled-release molding processes used. Replica molding generated patterned (B) calcium alginate and (D) chitosan membranes with features in relief while μTM generated (C) calcium alginate and (E) chitosan microgels. (C) Particles obtained with molds of different sizes.

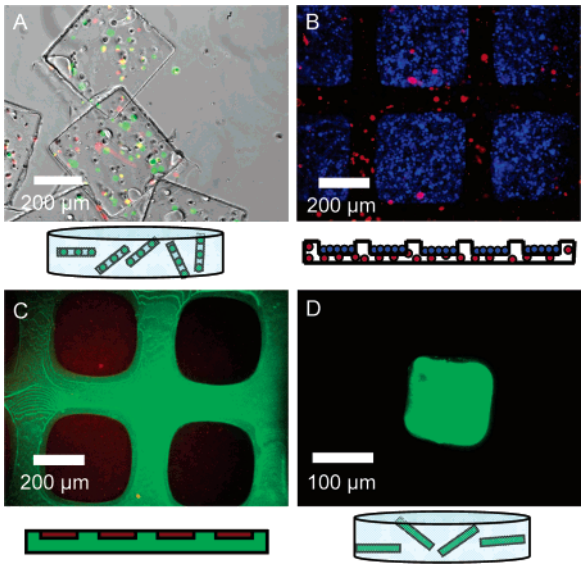

Figure 2. (A) Micrograph of NIH-3T3 cell-laden alginate microparticles overlaid with live/dead staining (green/red, respectively). (B) Co-culture of AML12 cells, stained with PKH26 (red) and mES cells, stained with CellTracker Blue. AML12 cells were encapsulated within the alginate membrane, while mES cells were seeded within wells. (C) Composite hydrogel of FITC-BSA (green)- and rhodamine (red)-loaded alginates. (D) Alginate microparticle loaded with FITC-BSA.

stained mouse embryonic stem cells (blue) were seeded within microwells formed from alginate hydrogels embedded with PKH26-stained AML12 hepatocytes (red). The ability to control heterotypic cell−cell interactions in a 3D environment within natural hydrogels can be used to mimic the native tissue complexity and architecture in culture.

This approach was also used to produce multilayered hydrogel constructs by sequential molding of hydrogels (Figure 2C). In this process alginate microgels containing FITC-BSA or rhodamine were molded on each other by first fabricating a thin patterned layer of gel containing one of the dyes and subsequently filling the void regions with a gel containing the second dye. Such 3D constructs could be important for the study of cell behavior and migration and for fabricating constructs with complex architectures for tissue engineering. In addition, we demonstrate the use of this process to synthesize microparticles for drug delivery by micromolding alginate or chitosan microgels containing a fluorescently labeled model protein (FITC-BSA) (Figure 2D). The ability to engineer the size, shape, and network density of the hydrogel particles can be used to control the release kinetics of the encapsulated molecules.

While in our work we focused on calcium alginate and chitosan, the technique we present, based on replica molding or μTM by controlled release of the gelling agent, should be applicable to any chemically cross-linkable and pH-dependent hydrogels. This soft lithographic approach is easy to implement and can be used to fabricate microparticles and micropatterned membranes for a variety of applications, such as scalable cell culture systems, diagnostics, drug delivery, and tissue engineering.

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Supporting Information Available: Detailed fabrication and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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