Micromolding of Fast-gelling Hydrogels for 3D In Vitro Studies and Bottom-up Tissue Engineering

Giovanni Talei Franzesi[1], Yibo Ling[1,2], Bin Ni[3], Ali Khademhosseini[1,4]

[1]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, USA
[2] Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, USA
[3] Department of Biology, Massachusetts Institute of Technology, Cambridge, USA
[4]Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, USA

Statement of Purpose:
Among the most exciting possibilities opened up by a microscale use of hydrogels are the development of novel, biomimetic 3D in vitro setups for studying cell-cell interactions while integrating as free parameters substrate chemical composition (as many synthetic hydrogels are easily modified with prosthetic groups), mechanical properties and microtopography. A further possible application of micropatterned hydrogels is bottom-up tissue engineering, by which building blocks composed of sub-millimeter cell-laden hydrogels are put together to form clinically relevant, macroscopic constructs either using robotic microassemblers or the principles of self-assembly. However, some of the most useful and better studied fast gelling ionically and pH-crosslinked hydrogels are not amenable to standard micromolding and micropatterning techniques.

Methods:
We chose calcium alginate as a model ionically crosslinked hydrogel, a material commonly used in tissue engineering, drug delivery and cell culture applications and not amenable to previously described techniques. 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. 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. A similar approach was used to obtain chitosan (as model pH-controlled hydrogel) micropatterns, with high-pH agarose instead of the calcium-laden agarose.

Results/Discussion:

Features with lateral dimensions between 5 and 2000 µm, and vertical dimensions between 10 and 200 µm could be reliably obtained, and the cells embedded within (10^3- 10^8 cells/ml) showed >80% viability. As shown in figure 1C types could be co-cultivated, either in two separate hydrogel phases micromolded on top of each other or one cell type could be encapsulated in a micromolded hydrogel, while the other is seeded on top of the structure. The particular configuration demonstrated in Figure 2C is

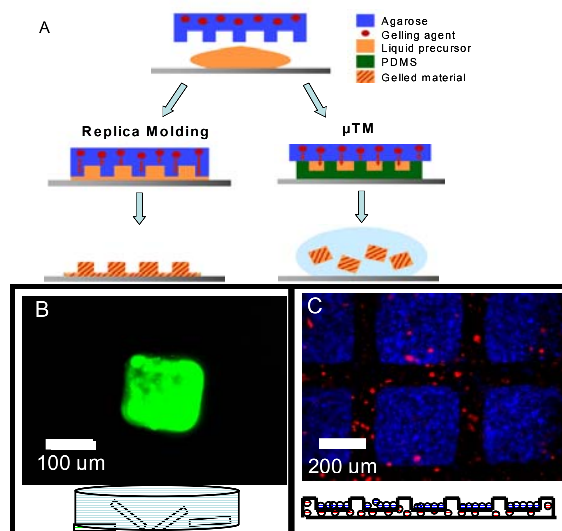

Figure 1: A schematic of the micromolding process. B individual FITC-labeled alginate microparticle. C model patterned cell coculture.

potentially useful for studying the effect of fibroblast (embedded in the alginate wells) co-culture with embryoid bodies of controlled size. Another application is studying the joint influences of heterotypic cell-cell interactions (e.g. fibroblasts and myocites), surface microtopography (such as microridges or micropillars) and substrate/marix stiffness. By varying the precursor concentration and gelling conditions we can precisely control the mechanical properties of the micropatterned hydrogels. As necessary for long-term studies in all cases features (often as small as 10µm) remained stable after >2 weeks incubation in cell culture media at 37°C. Preliminary work on self-assembly of hydrogels has shown that mesoscale alginate particles assemble naturally in ordered structures; work is underway to extend this approach to the microscale.

Conclusions:
We have presented a novel method for the creation of microscale biologically and clinically relevant hydrogels, not amenable to patterning with previously described techniques, of controlled size and morphology. We also demonstrated some of its potential applications as proof of principle.