MOSAIC HYDROGELS: ONE-STEP FORMATION OF MULTISCALE SOFT MATERIALS

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ABSTRACT

We introduce the one-step, continuous formation of mosaic hydrogel sheets. A microfluidic device allows controllable incorporation of secondary biopolymers within a flowing biopolymer sheet followed by a cross-linking step that retains the microscale composition. Information is encoded, mosaic stiffness and diffusivity patterns are created, and tessellations are populated with biomolecules, microparticles and viable primary cells. The extension to 3D soft material assemblies is demonstrated.

KEYWORDS

Hydrogels, hierarchical materials, biomaterials, encoding, 3D cell culture.

INTRODUCTION

Soft materials with a spatially non-uniform composition that is closely linked to their function are abundant in nature. Such materials often possess a hierarchical architecture that extends from cell to tissue scales in several directions. A number of strategies have been developed with the aim of creating soft materials with a microscale composition that mimics the hierarchical organization found in nature. Bottom-up approaches are often based on polymer microparticles with a homogeneous or heterogeneous composition that are continuously prepared in various shapes and compositions, and locally provide 3D microenvironments at the cellular scale. Such particles constitute building blocks for the tissue-scale organization in one or two directions [1], and their assembly was demonstrated along fluid interfaces or through confined packing within microfluidic channels [2]. In addition, fibers with homogeneous and heterogeneous composition were continuously defined in a microfluidic format and subsequently woven into planar or three-dimensional assemblies [3]. Alternative top-down approaches are replica molding [4] and subsequent stacking, and the layer-by-layer fabrication of lithographically defined 3D biomaterials [5]. However, extending these efforts to tissue scales is not straightforward and currently relies on sequential, discontinuous approaches with limited scalability. We present a microfluidic-based approach that enables the scalable and continuous formation of planar to 3D soft materials with mosaic material and cellular composition [6].

EXPERIMENT

A multilayered microfluidic device containing seven on-chip reservoirs enables the incorporation of up to seven distinct secondary biopolymers into a continuously extruded base biopolymer solution exiting the device into a liquid-filled reservoir (Fig. 1a,b). Flow from the on-chip reservoirs is defined by computer-controlled solenoid valves. Upon a cross-linking reaction, the composition of the base biopolymer and the incorporated secondary biopolymer is retained, with the obtained soft material sheet collected onto a rotating drum which enables control over the sheet thickness (Fig. 1c).

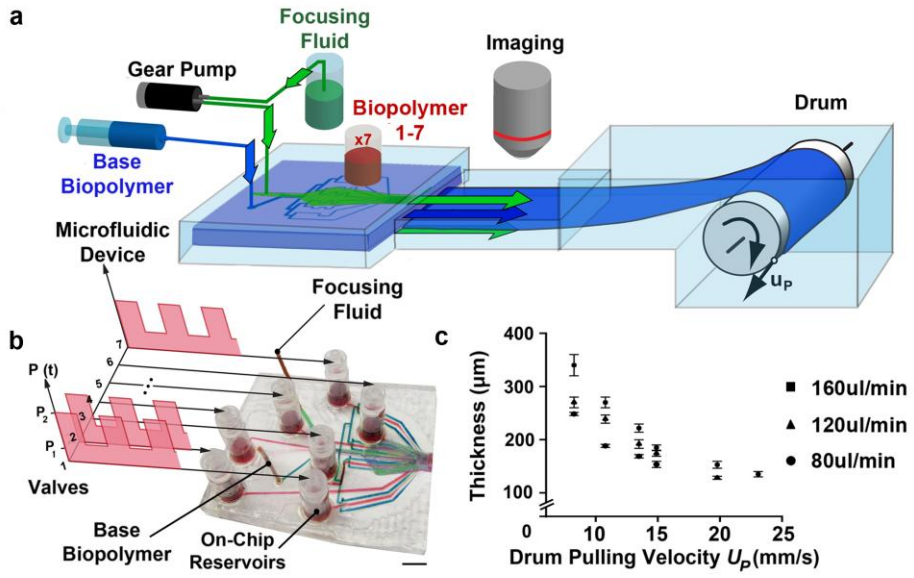

Figure 1. Continuous formation of mosaic hydrogels. (a) Experimental setup includes fluidic control, liquid filled reservoirs, and collecting drum. (b) Photograph of microfluidic device with seven on-chip reservoirs. (c) Control over sheet thickness. Scale bar 5mm (b).

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RESULTS AND DISCUSSION

As an illustration of the spatio-temporal control achieved, we encoded information in the form of letters and 7-bit ASCII code, and simultaneously read the written pattern using a line-camera (Fig. 2a-d). The formation of soft material sheets of heterogeneous material properties was demonstrated by the diffusion of various molecular weight dextran molecules as a payload, and bulk material stiffness dependence on heterogeneous material patterns (Fig. 3a-e, g,h). The secondary biopolymer may consist of the same solution as the focusing fluid, in which case a sheet with void spaces can be generated (Fig. 3f).

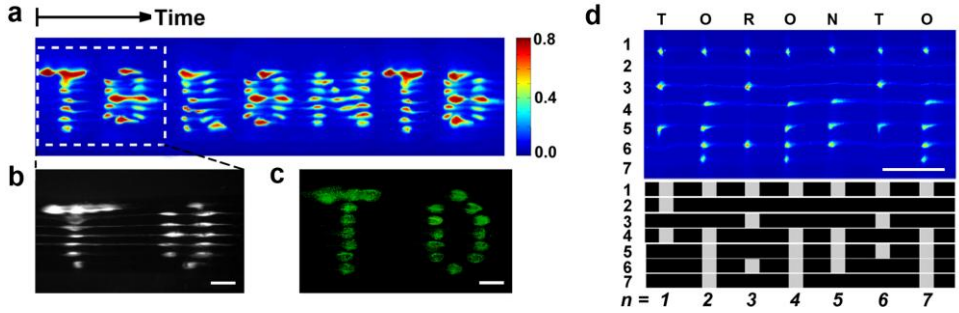

Figure 2. Demonstration of soft material patterning with precise spatiotemporal control. Fluorescence line-camera measurements demonstrating information encoding in the form of (a) letters, (b) imaged by fluorescence, (c) written with neonatal rat cardiomyocytes, or (d) 7-bit ASCII. Scale bars 1mm.

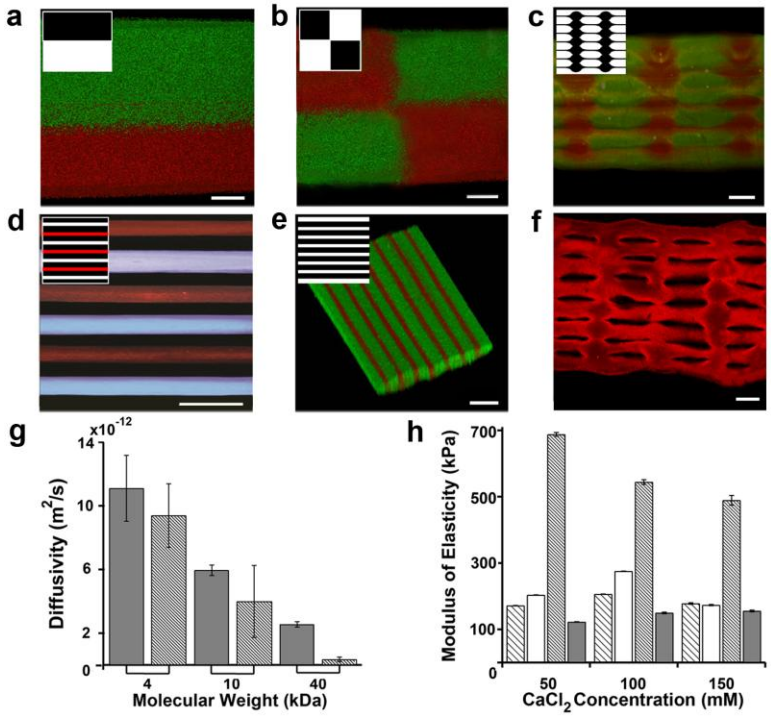

Figure 3. (a-f) Mosaic hydrogel patterns imaged with confocal and wide-field fluorescence microscopy. (g) Diffusivity of dextran molecules with three distinc molecular weight into 2%w.t. alginate (■) and 1%w.t. pectin-alginate (■). (j) Bulk elastic modulus of sheets consisting of 2%w.t. alginate (■), 1%w.t. pectin-alginate (■), and a combination of both, with patterns illustrated in c (▨) and e (□).

In a second case, the secondary solution may be a biopolymer complemented by a variety of payloads such as, biomolecules, microparticles, and cells. The incorporation of viable fibroblasts and cardiomyocytes within the biopolymer sheet and the subsequent attachment of fibroblasts within Day 5 of culture were demonstrated (Fig. 4a, b). The formation of cell-patterns for co-culture may be combined with a 6-bit barcode to track the various conditions patterned (Fig. 4a).

The platform presented may subsequently be scaled-up to 3D millimeter-size soft materials with defined heterogeneous composition. As an illustration, five layers of biopolymer sheets with parallel stripe patterns were stacked in an alternating orientation to create a 3mm[*w*]×3mm[*L*]×1.5mm[δ] biopolymer structure (Fig.4c). Multilayered sheets were obtained by collecting onto t he rotating drum a continuous sheet composed of two layers of biopolymers (green and red). Final bulk dimensions of 5mm[w] × 3.5mm[δ] (thickness δ obtained by ~18 double layers 200μm thick) with a total volume of 2 mL (Fig. 4d). Centimeter length tubular structures of homogeneous and heterogeneous composition were obtained by rolling onto a translating capillary tube (Fig. 4e-h).

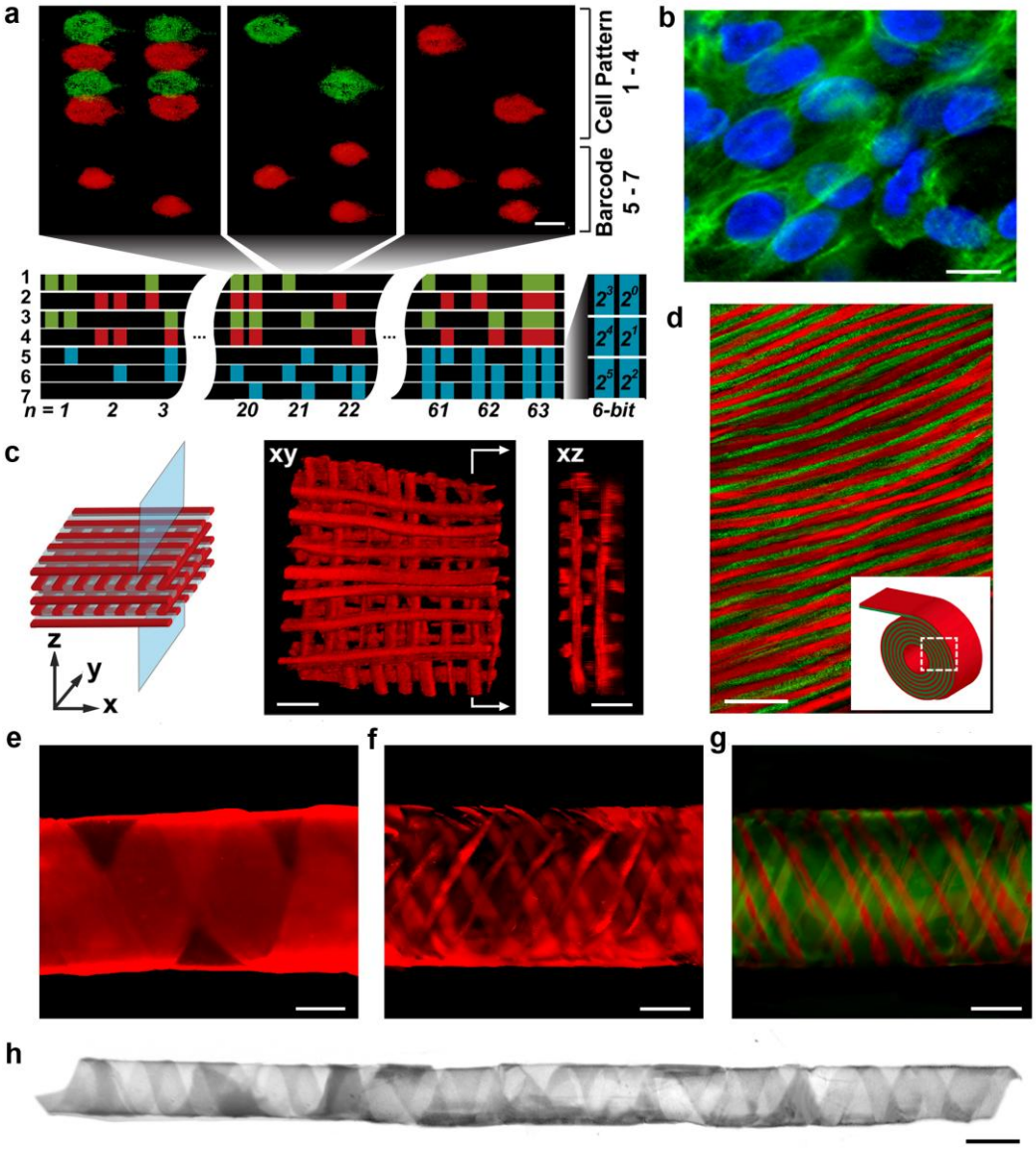

Figure 4. (a) Fluorescence image of patterned multiple cell types.Fibroblasts (red) and neonatal rat cardiomyocytes (green), combined with a 6-bit barcode. (b) Confocal fluorescence image demonstrating fibroblast attachment within a spot (Day 5). Example of millimeter-scale 3D structures obtained by stacking (c), rolling-up (d), and rolling-up with drum translation (e-h). Scale bars 500µm (a, d, e-g), 10µm (b), 1mm (c), 2mm (h).

CONCLUSION

The presented strategy may enable a fully-automated and continuous format for culturing cells in physiologically relevant microenvironments, the systematic investigation of cell-cell and cell-matrix interactions and, ultimately, define 3D functional tissues.

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