Diffusion-based microfluidics for fabrication of hydrogels with well-defined physical features

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Introduction: Tissue engineering is hampered by the lack of methods that provide control over micro- and macroscale physical features.[1] Emulsion-driven microfluidics enables production of hydrogels with well-defined physical architectures but lacks control over microscale features.[2] A novel diffusion-based microfluidics platform is presented for the fabrication of hydrogels with tailorable micro- and macroscale physical features.

Materials & Methods: Dispersed phase containing allylated gelatin; Gel-AGE (5-10wt%), crosslinker dithiothreitol (DTT; 18-36 mM), and co-initiators ruthenium (Ru; 0.1 mM) and sodium persulfate (SPS; 0-10 mM), was injected (150-450 µL/min, New Era pump) into a continuous oil phase (30-1000 µL/min) to form hydrogel precursor droplets. Droplets were exposed to light to induce photopolymerization (30mW/cm^2, 180s, 400-450nm) prior-to and during introducing an aqueous outer phase containing SPS ($[SPS]_{outer}$ = 5-20 mM, 500 µL/min) around the formed hydrogels, allowing diffusion of SPS across the liquid - gel interface (Figure 1A). Thereafter, the hydrogels were collected in PBS and cross-sectional hydrogel stiffness was determined (atomic force microscopy). Mesenchymal stromal cells (MSCs; 5*10^6 cells/mL) were encapsulated in hydrogels and cultured for 7 days in expansion media, assessing viability (Live/Dead®), cell morphology (F-actin) and cumulated cell length (AngioTool).

Results & Discussion: Spherical, "plug"-like modules and continuous filament hydrogels were fabricated through flow rate adjustments (length: 0.75±0.17 mm to ≥ 1.7 m, Ø: 0.51±0.03 mm to 1.51±0.05 mm, Figure 1B). SPS diffusion across the liquid-gel interface enabled the formation of a radial stiffness gradient across the resultant hydrogel cross-sectional surface (9.1 to 18 kPa for $[SPS]_{outer}$=10mM and 11.4 to 188.2 kPa for $[SPS]_{outer}$=20mM, Figure 1C) and enabled the fabrication of hollow hydrogels (wall thickness 132±1 and 219±4 mm for $[SPS]_{outer}$=5 and 10 mM, p=0.0318). The fabrication process did not affect MSC viability (84.2±7.9% in filaments versus 83.4±7.7% in cast control discs, day 1, p=0.8987). The presence of a radial stiffness gradient induced heterogeneous spreading of encapsulated MSCs (soft region: 1.61±0.5 mm, stiff region: 0.58±0.1 mm cumulated cell length, Figure 1D).

Conclusions: Diffusion-based microfluidics uniquely enabled the high-throughput fabrication of hydrogels with well-defined macroscale shape and size, and tailorable microscale physical features (*i.e.* radial stiffness gradients and controllable internal microarchitecture).

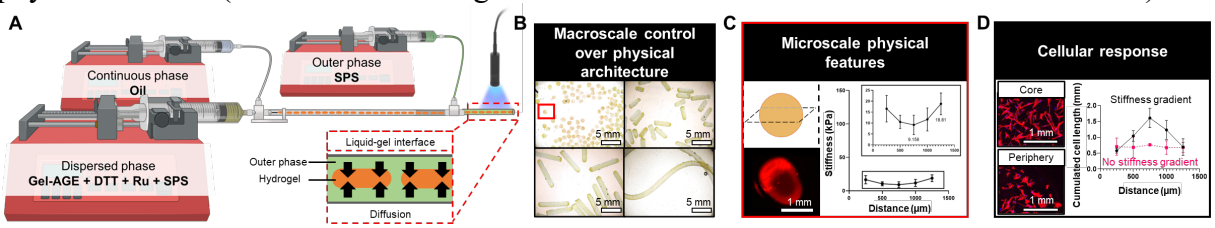

Figure 1: A. Diffusion-based microfluidics platform, enabling control over macro- (B) and microscale (C) physical features. D) Effect stiffness gradients on behaviour encapsulated MSCs.

References:
[1] Ingber, DE; et al. *Tissue Eng*. **2006,** 12(12):3265.
[2] Amirifar L; et al. *Biofabrication.* **2022,** 14(2):022001.