

Electronic Supplementary Information

Device Fabrication

The design and fabrication of the microfluidic devices followed previously described soft lithography techniques.¹ The chamber that was utilized was a $1 \times 50 \times 0.07$ mm (W \times L \times H) straight channel. A negative master was fabricated and assembled at the George J. Kostas Nanoscale Technology and Manufacturing Research Center at Northeastern University using conventional photolithography techniques. To form the polymeric chambers, poly(dimethylsiloxane) (PDMS, Dow Corning) elastomer was mixed (1:10 ratio), poured onto the negative master wafer, degassed, and allowed to cure overnight. PDMS replicas were then pulled off the wafers, inlet and outlet holes were punched with a 19-gauge blunt-nose needle and exposed to oxygen plasma and then immediately placed in contact with glass cover slides in order to creating an irreversible bond between the PDMS and glass.

Alginate-RGDS Synthesis

The tetra-amino acid peptide sequence Arg-Glu-Asp-Ser (RGDS; American Peptide, Sunnyvale, CA) was conjugated to alginic acid stock using EDC-NHS chemistry² where 4.8mg of EDC (Pierce) and 13.2mg Sulfo-NHS (Pierce) were added to a stock solution of 45 mg mL⁻¹ alginic acid and allowed to react with 500 μ L of 1mg mL⁻¹ RGDS for 20hrs. The solution was then dialyzed for 2 days (3500 MWCO) to remove all unreacted reagents. The resulting solution was freeze dried and lyophilized for 2 days.

The resulting alginic acid-RGDS powder was dissolved in MES buffer to a concentration of 12mg mL⁻¹. To assist in dissolving, the solution was repeatedly vortexed and briefly incubated

at 37°C to hydrate. Additionally, a small portion of 10 N NaOH was added to the solution and was centrifuged very briefly to remove any residual bubbles.

The RGDS peptide content was quantified using Fourier Transform Infrared Spectroscopy (FT-IR, Perkin Elmer Spectrum 2000). Ten microliters of an aqueous sample of 100 $\mu\text{g mL}^{-1}$, 50 $\mu\text{g mL}^{-1}$ and 25 $\mu\text{g mL}^{-1}$ were analyzed on poly(tetrafluoroethylene) IR sample cards (Crystal Labs, Garfield, NJ) to develop a calibration. This was followed by dissolving 1 gram of alginate-RGDS in buffer, which was then analyzed. Curves were compared and the RGDS content was determined by the amide stretching peak at 638 cm^{-1} .

Coverslip Preparation

12 mm diameter circular glass coverslips were treated in a plasma chamber for 30 seconds and 2 mg mL^{-1} alginate or alginate-RGDS was pipetted onto each surface. The surfaces were immediately placed into a spin-coater machine and spun for 4 s at 5000 rpm to produce a uniform thin layer on the glass surface. Each coverslip was carefully removed from the device with tweezers and placed into a petri dish containing 100 mM CaCl_2 to gel the surface layer. Coverslips were kept overnight at 4 °C. Prior to use, coated coverslips were rinsed in sterile PBS, and then placed into a well plate. For control groups where cells were seeded onto untreated glass coverslips.

Coating of the microfluidic devices

Four different alginate monomer solutions were in 2-(N-morpholino)ethanesulfonic acid (MES) buffer (Pierce Biotechnology, Rockford, IL) (2, 6, 12, and 16 mg mL^{-1}) were investigated to

qualitatively determine the efficiency of adsorption. To each solution, 100 μL of fluorescein isothiocyanate (FITC; Vector Laboratories, Burlingame, CA) was added as a fluorescence indicator. Each solution was injected into a microfluidic device and allowed to adsorb for 1 hr. The solution was then rinsed with MES buffer at 10 $\mu\text{L min}^{-1}$ for 10min followed by a 100 mM CaCl_2 (Sigma Aldrich) solution at 10 $\mu\text{L min}^{-1}$ for 10 min to form a thin layer of alginate gel on the walls of the microchannels. Each device was imaged under a Nikon Eclipse TE2000 inverted microscope at 10 \times magnification using a fluorescein (480 \pm 30 nm/535 \pm 40 nm) excitation/emission filter. It was determined that there was no fluorescence at concentrations of 2 and 6 mg mL^{-1} . Additionally, 16 mg mL^{-1} concentration was found to be too viscous a working solution for injection; therefore for all subsequent alginate and alginate-RGDS experiments 12 mg mL^{-1} monomer solutions in MES buffer were utilized. Both alginate and alginate-RGDS adsorptions were allowed to adsorb for 1 hr. and rinsed with MES buffer at 10 $\mu\text{L min}^{-1}$ for 10min followed by a 100 mM CaCl_2 (Sigma Aldrich) solution at 10 $\mu\text{L min}^{-1}$ for 10 min. to form the hydrogel layer.

Cell Culture

Neonatal (1 to 2 days-old) Sprague–Dawley rats were euthanized according to the procedure approved by the University of Toronto Committee on Animal Care. The hearts were removed, quartered, and the cells were isolated by an overnight treatment with trypsin (4 $^\circ\text{C}$, 6,120 U mL^{-1} in Hank's Balanced Salt Solution, HBSS), followed by a serial collagenase digestion (220 U mL^{-1} in HBSS) as described in previous work.³ The supernatants from five collagenase digests of the tissues were collected and centrifuged at 750 rpm (94 \times g) for 4min, resuspended in culture medium, and then preplated into T75 flasks (Falcon) for 1hr followed by aspiration of the

supernatant. To obtain pure cardiac fibroblasts, the cells that remained attached after one preplating step were expanded for 7 days. The cardiomyocyte culture (CM) medium consisted of Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g L⁻¹ glucose, 4 mM L-glutamine, 10 % fetal bovine serum (FBS), 100 U mL⁻¹ penicillin, 100 µg mL⁻¹ streptomycin, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES). Cells were isolated (passages 2-8) and resuspended in serum free media at a concentration of $10 \pm 0.1 \times 10^4$ cells mL⁻¹ for all subsequent flow experiments. For static experiments cardiac fibroblasts were trypsinized and resuspended in serum-free medium such that the required number of cells was seeded carefully onto each surface in a 15 µL volume. Coverslips were placed into a 5% CO₂ humidified incubator at 37 °C for 30 minutes.

Static Experiments

A volume 500 µL of medium was carefully added to each coverslip/well (Wash 1) and the plate gently agitated. The coverslips were removed to a fresh plate with 1 mL CM medium per well. The medium in the wells was then collected (Wash 2) and the coverslips carefully rinsed with PBS to remove any. Coverslips were removed to a fresh plate where 500 µL of a specified concentration of ethylene diamine tetraacetic acid (EDTA; resuspended in buffer or CM medium) or trypsin-EDTA at 37 °C was added to each well to resolubilize the alginate and alginate-RGDS and remove cells from the glass. The plates were placed on an orbital shaker for 15 minutes at a speed of 70 rpm. Following this, 1 mL of CM medium was added to quench the trypsin. The liquid was collected, the coverslips were again rinsed in PBS and this additional liquid pooled. Coverslips were re-examined to ensure total cell removal. Samples with cells remaining were imaged again under an optical microscope.

Flow Experiments

Following gel formation the CaCl₂ solution was rinsed from the device at 5 $\mu\text{L min}^{-1}$ for 10min. with MES buffer. Cell suspensions were flowed through microfluidic devices at a flow rate of 5 $\mu\text{L min}^{-1}$ for a period of 20min, respectively, using a Harvard Apparatus PHD 2000 syringe pump (Holliston, MA). Cell adhesion within the devices was measured using a field finder (with 1 mm \times 1 mm grids) placed under the microfluidic chamber. Adhered cells were manually counted at selected points along the device axis under a Nikon Eclipse TE2000 inverted microscope. Cell counts were taken between 5 and 25 mm from the device inlet, along the device axis. All flow experiments were performed at room temperature. Next, a 50 mM solution of EDTA was injecting the device at 5 $\mu\text{L min}^{-1}$ for 10 min followed by another manual cell count.

Viability

Cell viability was examined for cells captured, and subsequently released, within the microfluidic devices or coverslip. Viability was also checked prior to experimentation for comparison. For viability analysis pre- and post-experimentation, cells were incubated in a 4 μM EthD-1 (dead cell indicator) and 2 μM calcein (live cell indicator) solution in media for 1 hr. Live and dead cells were visualized and counted at 10 \times magnification using fluorescein and rhodamine filters, respectively, on the Nikon microscope. To ensure that EDTA would not adversely affect cell viability in post-experimental assays, cells detached from the device were collected in centrifuged tubes containing 1 mL of CM medium (20 \times dilution) to neutralize EDTA.

Supplementary Material (ESI) for Lab on a Chip
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References:

1. Y. N. Xia and G. M. Whitesides, *Angew. Chem. Int. Ed.*, 1998, **37**, 551-575.
2. G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, Boston, MA, 1996.
3. M. Radisic, H. Park, H. Shing, T. Consi, F. J. Schoen, R. Langer, L. E. Freed and G. Vunjak-Novakovic, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 18129-18134.

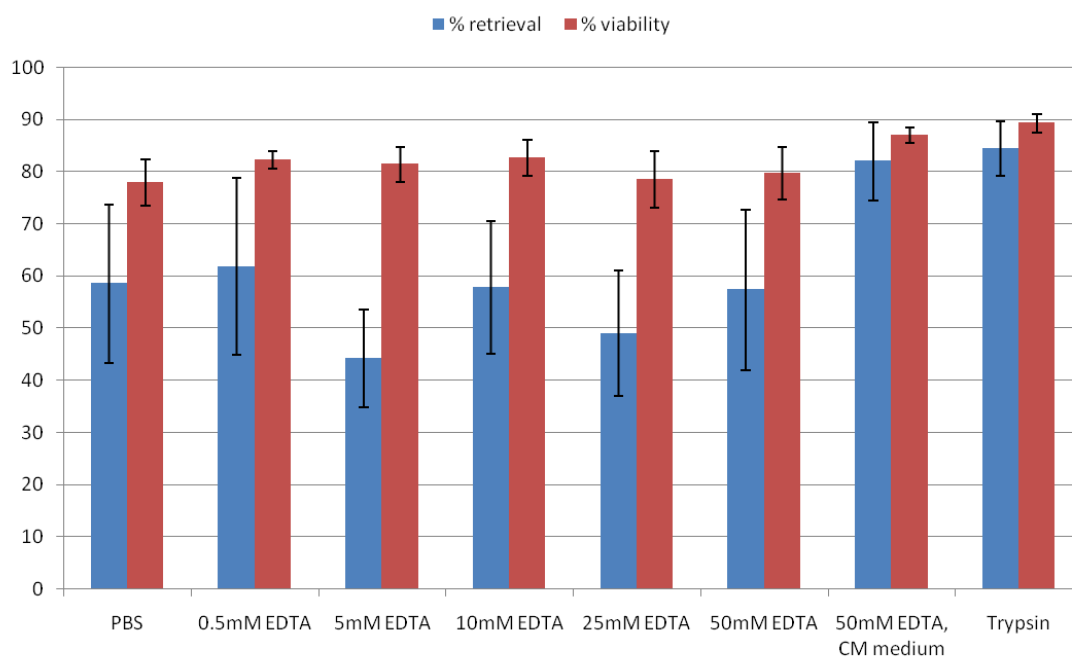


Fig S1: Cell retrieval and viability with 100,000 cells in suspension incubated for 15 minutes on an orbital shaker at 70 RPM. Results show that 50mM EDTA in cell media resulting in comparable release to that of the positive control, trypsin. In addition it should be noted that all experiments maintain a viability above 75 %. Error bars denote standard errors for four replicates of each experiment.