

Array of Biodegradable Micrafts for Isolation and Implantation of Living, Adherent Cells

Supplementary Information

Yuli Wang,^a Colleen N. Phillips,^a Gabriela S. Herrera,^b Christopher E. Sims,^a Jen Jen Yeh,^{b,c}

Nancy L. Allbritton*^{a,b,d}

^aDepartment of Chemistry, University of North Carolina, Chapel Hill, NC 27599

^bLineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC
27599

^cDepartments of Surgery and Pharmacology, University of North Carolina, Chapel Hill, NC
27599

^dDepartment of Biomedical Engineering, University of North Carolina, Chapel Hill, NC 27599
and North Carolina State University, Raleigh, NC 27695

*Corresponding Author. E-mail: nlallbri@unc.edu; Fax: +1 (919) 962-2388; Tel: +1 (919) 966-
2291

1. Fabrication of the biodegradable micraft arrays

(1) Fabrication of the PDMS template imprinted with a microwell array

The PDMS template was fabricated by casting PDMS on an SU-8 master using standard soft lithography. The PDMS template was imprinted with microwell arrays with controlled depth and dimension. A typical dimension of the microwell was $100\ \mu\text{m} \times 100\ \mu\text{m} \times 40\ \mu\text{m}$ (length \times width \times depth), and inter-well gap (rim to rim) was $20\ \mu\text{m}$. The SU-8 master was fabricated by standard photolithography on a glass slide with $40\ \mu\text{m}$ thick SU-8. The surface of the master mold was treated to render it non-sticky to PDMS by spin coating 1 vol% octyltrichlorosilane in propylene glycol monomethyl ether acetate onto the master at 2000 rpm for 30 s, followed by baking at $120\ ^\circ\text{C}$ on a hotplate for 10 min. PDMS prepolymer (10:1 mixture of base:curing-agent of Sylgard 184 kit) was spread on the master mold, and degassed under vacuum to remove trapped air bubbles. To control the thickness of the PDMS, PDMS was spin-coated at 400 rpm for 30 s on the master to yield a $200\ \mu\text{m}$ -thick PDMS layer. The PDMS was cured by baking the master at $100\ ^\circ\text{C}$ on a hotplate for 30 min. The PDMS template forming the multiwell array was then obtained by peeling it from the master.

(2) Drain coating process

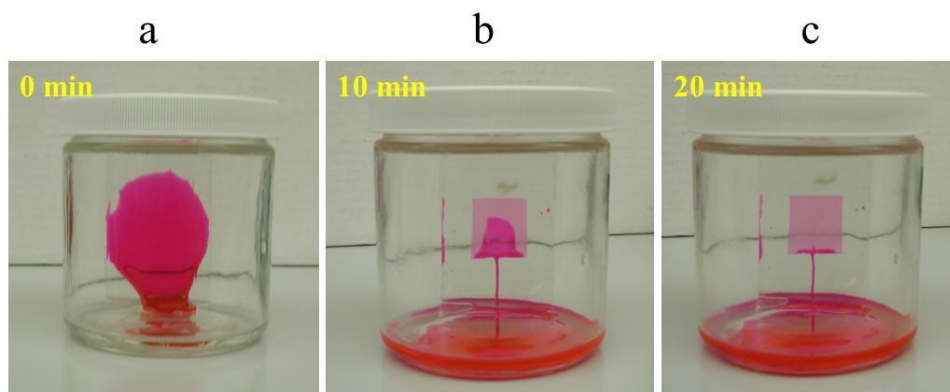


Fig. S1. Drain coating setup and process for fabricating biodegradable rafts. For illustration purposes, biodegradable polymer #10 (40 wt% in GBL) was mixed with rhodamine B (0.001 wt% of polymer).

A dip coating solution was prepared by dissolving biodegradable polyesters in GBL at a concentration of 40 wt% solid. Three milliliters of the polymer solution was added to the PDMS template, and the trapped air bubbles within the microwells were removed through degassing for 1 min by a Leybold Trivac pump (Model # D2.5E) in a glass desiccator (Wheaton Dry-Seal Vacuum Desiccators, Model 365882). The PDMS template was then hung vertically in a large wide-mouth glass bottle with the help of a paper clip and a nylon string (Fig. S1-a). For ease of visualization, the biodegradable polymer solution (40 wt% in GBL) was mixed with rhodamine B (0.001 wt% of polymer). During drain coating, the bottle cap was sealed tightly to minimize the evaporation of GBL. Polymer solution flowed downward due to gravity. Dewetting of polymer solution from the template produced isolated polymer pockets trapped inside each microwell (Fig. S1-b). Complete dewetting of polymer solution from the array required about 20 min (Fig. S1-c). Only 18 mg polymer solution (equivalent to 7.2 mg solid polymer) was trapped on the array. Polymer solution collected in the bottle could be re-used to fabricate the next array. The PDMS mold was then removed and placed in a $95\ ^\circ\text{C}$ oven for 2 h to evaporate GBL.

Further evaporation of the GBL was achieved by a 1 h bake at 100 °C in a vacuum oven (-30 in. Hg).

(3) Plastic cassettes for micraft array and collection

Following fabrication of the micraft arrays, the PDMS template was attached to a custom-made polycarbonate cassette, with the array facing toward the inside of the cassette (Fig. S2). The array cassette had a chamber of 25.4 mm × 25.4 mm area for housing the culture medium, and a round outer wall with a diameter of 53 mm to maintain sterility in the form of a Petri dish. In-plane stretching of the PDMS mold was used to further reduce array sagging. The PDMS was stretched along the axes parallel to the array surface to offset the out-of-plane bowing (z-axis). PDMS prepolymer was applied to glue the array to the cassette and cured at room temperature for 3 days. A cover for the Petri dish (60 mm × 15 mm) was used for the array cassette.

Fisherbrand 50 mm dish (Fisher Scientific Catalog # 09-753-53A) was used as a collection dish for the released micraft. Its surface was oxidized with air plasma for 2 min to promote cell migration from the micraft and attachment to the dish.

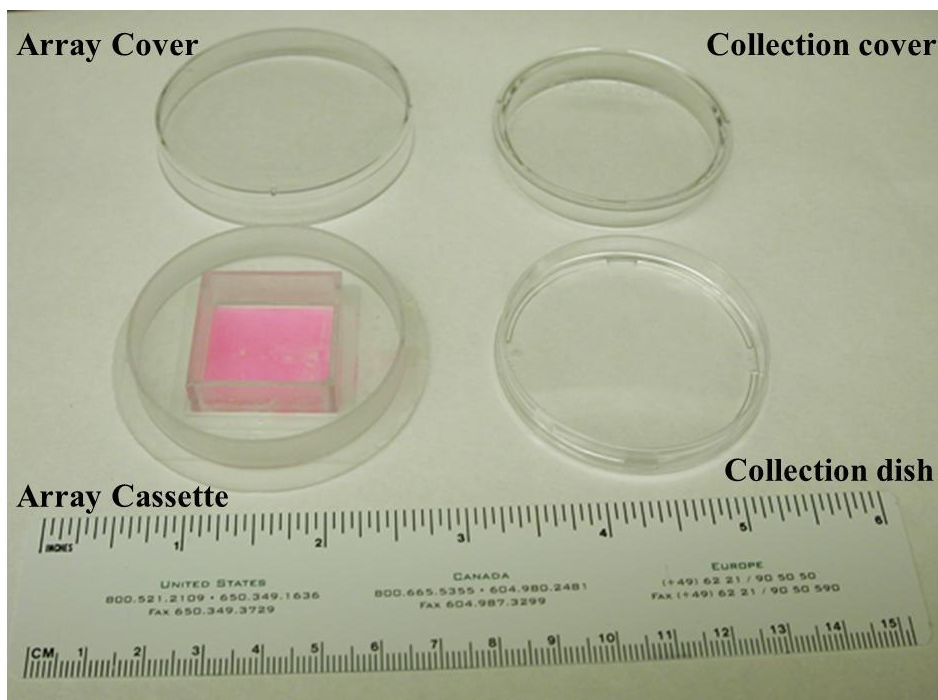


Fig. S2. Plastic cassettes for micraft array and collection. For visualization purposes, the array (pink area) was made using biodegradable polymer #10 doped with rhodamine B (0.001 wt% of polymer).

2. Selective release of biodegradable micrafts from the array by a mechanical needle release system

A manual system was built to release the targeted micrafts from the array with a needle (Fig. S3-a). An XYZ micromanipulator was mounted on an inverted microscope. An anodized

steel microneedle (150 μm base diameter, 17.5 μm tip diameter, Fine Science Tools, Foster City, CA) was mounted on a needle holder. The array was placed on the microscope stage in an inverted position with the needle above the base of the array. The cells remained immersed in media within the cassette created by mating the array and collection chambers (see below). To release the targeted structure, the needle was aligned with the micraft. The user simply lowered the needle a specified distance to pierce the PDMS and extrude the micraft.

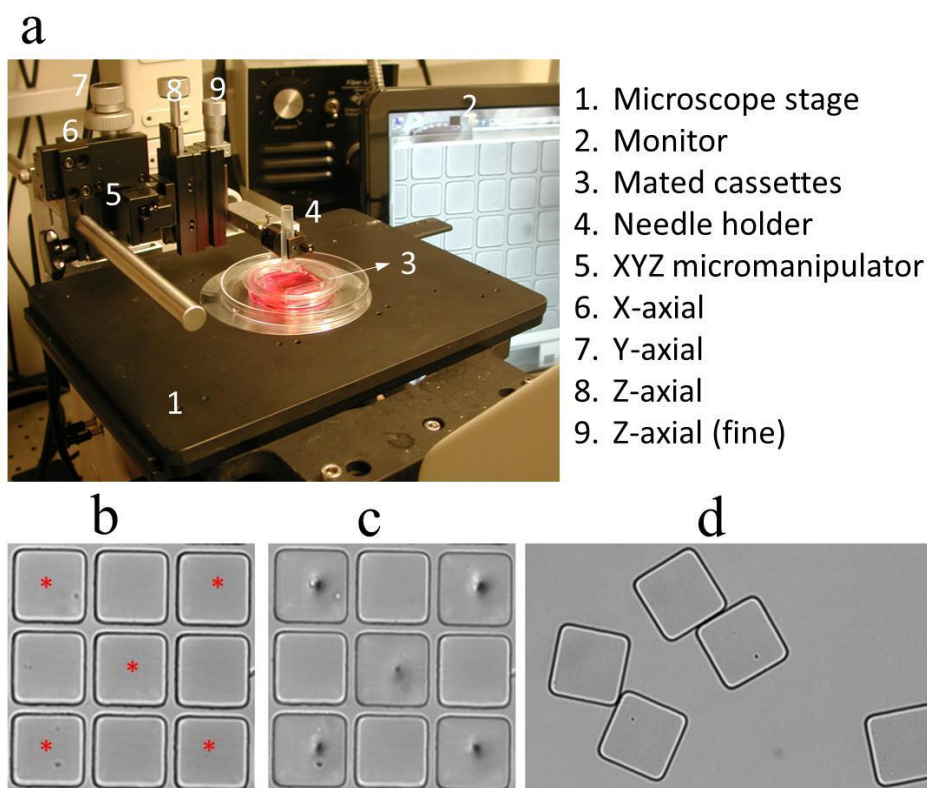


Fig. S3. Selective release of biodegradable micrafts (biopolymer #10) from the array by a mechanical needle release system. (a) Setup of the mechanical needle release system. (b)-(d) Five targeted micrafts were selectively released from the array and collected in a separate dish. (b) A 3×3 array before release. The targeted micrafts are denoted with red asterisks. (c) The same 3×3 array after release. Five micrafts were released leaving empty sites with puncture defects in the PDMS seen from the needle at the release sites. (d) The five micrafts were collected in a separate dish.

Prior to needle release, 7 mL of fresh culture medium was added to completely fill the cell culture chamber in the array cassette and form a convex fluid surface so that a bubble was not formed when mating the collection dish. The collection dish was mated directly to the cell culture chamber. Excess liquid overflowing the chamber as a result of mating the dish and chamber was aspirated at the conclusion of the experiment. The cassette assembled in this manner formed an enclosed compartment housing the array that was filled with culture medium and lacked air bubbles. The assembly was then inverted and placed in a 100 mm petri dish. The dish was placed on the microscope stage (Fig. S3-a). The targeted micrafts were released, whereupon the micrafts settled on the collection plate by gravity (Fig. S3 b-d). The collection

plate and array were then separated in a sterile environment and the collection plate containing the released cells/microrafts was transferred to a standard tissue culture incubator. The growth of the collected cells was monitored daily by brightfield imaging.