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Array of Biodegradable Microraftsfor Isolation and Implantation of Living, Adherent Cells

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Abstract

A new strategy for efficient sorting and implantation of viable adherent cells into animals is described. An array of biodegradable micro-structures (microrafts) was fabricated using a polydimethylsiloxane substrate for micromolding poly(lactic-co-glycolic acid) (PLGA). Screening various forms of PLGA determined that the suitability of PLGA for microraft manufacture, biocompatibility and *in vitro* degradation was dependent on molecular weight and lactic/glycolic ratio. Cells plated on the array selectively attached to the microrafts and could be identified by their fluorescence, morphology or other criteria. The cells were efficiently dislodged and collected from the array using a microneedle device. The platform was used to isolate specific cells from a mixed population establishing the ability to sort target cells for direct implantation. As a proof of concept, fluorescently conjugated microrafts carrying tumor cells stably expressing luciferase were isolated from an array and implanted subcutaneously into mice. *In vivo* bio-luminescence imaging confirmed the growth of a tumor in the recipient animals. Imaging of tissue sections from the tumors demonstrated *in vivo* degradation of the implanted microrafts. The process is a new strategy for isolating and delivering a small number of adherent cells for animal implantation with potential applications in tissue repair, tumor induction, *in vivo* differentiation of stem cells and other biomedical research.

INTRODUCTION

Cell transplantation has wide-ranging applications in establishing xenograft tumor models,[1] studying *in vivo* differentiation of stem cells,[2] and repairing tissue for regenerative medicine.[3] Biodegradable microcarriers have been used to grow anchorage-dependent cells and as a means of delivering cells for tissue regeneration for liver,[4] cartilage,[5] skin,[6] and brain.[7] The use of microcarriers has shown benefits in prolonged survival and function of the transplanted cells.[8] These biodegradable microcarriers are typically small porous particles with diameters in the range of 100–400 μm made from either natural biomaterials including extracellular matrix (ECM) proteins (*e.g.* collagen and gelatin) and dextran, or synthetic polymers including poly(lactic acid), poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone.[3, 9] A variety of primary cells and stem cells (including adult and embryonic stem cells) have been cultured on biodegradable microcarriers for cell

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transplantation or *in vitro* differentiation, and are summarized recently in two excellent review papers.[3, 10]

When isolated from animal or human, cells are usually heterogeneous and the desired cell types must be selectively and viably isolated from the mixed population before implantation into another animal. Fluorescence-activated cell sorting (FACS) is a common method for isolation of non-adherent cells.[11] FACS requires large sample sizes of $10^6 – 10^7$ cells which may not be available from small animals such as mice or worms or from methods procuring small amounts of tissue such as needle biopsies; otherwise prolonged *in vitro* expansion is needed to generate sufficient number of cells for FACS. Additionally, FACS can impose significant stresses on cells as they move at very high speeds through the flow cytometer, which negatively impacts cellular physiology and viability.[12–14] Therefore it is desirable to develop alternative cell isolation strategies for small sample sizes or fragile, sensitive cells for transplantation which is stress-free and requires minimal *in vitro* expansion and handling. This is especially true when handling primary and stem cells as added manipulations and additional passages during culture contribute to irreversible alterations in gene expression, increases of phenotypic heterogeneity, and acquisition of pluripotency-limiting genetic abnormalities.[15–20]

To meet this goal, we have previously developed a microraft array platform that enables separation of live adherent cells with high viability.[21, 22] The array is composed of a large number of micron-scale elements made from a polymer such as polystyrene, each termed a microraft. Within the array, each microraft serves as a releasable culture site for individual cells or colonies. To isolate target cells, a microneedle is inserted through the compliant polymer substrate to dislodge a microraft and its attached cell(s). This strategy achieves cell isolation with excellent viability and purity; for example, single-cell cloning efficiencies of greater than 95% have been realized with 100% purity.[22] This method has numerous advantages over FACS - *i*) it is compatible with very small sample sizes, *ii*) target cells can be defined based on a wide range of selection criteria, *iii*) cells are isolated with high viability, *iv*) both single-cell or colony isolation can be accomplished, *v*) operation is simple and inexpensive, and *vi*) the array operations can be performed in an individual laboratory as opposed to a core facility.

In the current study, we have used biodegradable materials to form microrafts, and utilized the array to identify, sort and implant adherent target cells into animals. Biodegradable materials were screened for suitability in manufacturing microrafts in a high density array format on a polydimethylsiloxane (PDMS) template by a simple drain coating process. PLGA materials were selected since they are the most commonly used biodegradable polymer for tissue engineering. The *in vitro* degradation rate of microrafts was measured. The biodegradable microraft array used as a substrate for culturing adherent cells and imaging of cells on the array by brightfield and fluorescence microscopy was demonstrated. The microrafts carrying cells of interest were identified and isolated from the array using a simple mechanical device. The isolated cells remained attached to the biodegradable microrafts during the separation process, after which time they could be directly implanted into mice. Microrafts carrying human pancreatic adenocarcinoma cells were subcutaneously injected in mice to establish a xenograft tumor model and to demonstrate the *in vivo* degradation of implanted microrafts.

MATERIALS AND METHODS

Materials

Thirteen biodegradable polyesters (Table 1) based on polylactic acid, polyglycolic acid and their copolymers PLGA were purchased from Sigma Aldrich (St. Louis, MO), Polysciences

(Warrington, PA), and Purac Biomaterials (Gorinchem, The Netherlands) and tested for their degradation rate and suitability for the drain coating process. Gamma-butyrolactone (GBL), rhodamine B, Hoechst 33342, octyltrichlorosilane, propylene glycol monomethyl ether acetate (PGMEA), dicyclohexylcarbodiimide, and dimethylaminopyridine were purchased from Sigma Aldrich. SU-8 photoresist was purchased from MicroChem Corp. (Newton, MA). PDMS was prepared from the Sylgard 184 silicone elastomer kit (Dow Corning, Midland, MI). Tide Fluor 3 amine (TF3 amine) was purchased from AAT Bioquest (Sunnyvale, CA). CellTracker Orange CMTMR, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from Invitrogen (Carlsbad, CA). Wild-type HeLa, H1299, and AsPC-1 cells were obtained from ATCC (Manassas, VA). H1299 cells stably expressing green fluorescent protein (GFP) and AsPC-1 cells stably expressing luciferase (AsPC-1-Luc) were prepared by standard transfection methods and maintained in presence of G418. All other reagents were from Fisher Scientific (Pittsburgh, PA).

Synthesis of PLGA-TF3 conjugate

TF3 amine fluorescent dye (excitation 554 nm, emission 578 nm) was covalently conjugated to the backbone of PLGA via the carboxyl-amine coupling reaction catalyzed by dicyclohexylcarbodiimide (DCC) following reported protocols.[23, 24] PLGA (2.5 g, sample #3 in Table 1, carboxyl acid terminated) and TF3 amine (1 mg) were dissolved in 6 mL anhydrous dimethylformamide in a glass bottle. DCC (58 mg) and dimethylaminopyridine (0.31 mg) were dissolved in 2 mL anhydrous dimethylformamide and then added to the polymer solution. The bottle was then purged with nitrogen for 10 s and sealed with a cap. The reagents were mixed by a magnetic stirrer at room temperature in the dark for 48 h. The reacted solution was diluted by 15 mL dichloromethane and precipitated in 80 mL methanol. The precipitated polymer was then re-dissolved in 20 mL dichloromethane and precipitated in 80 mL methanol. To completely remove un-conjugated dye, the dissolution and precipitation was repeated two additional times. The conjugated polymer was then dried under vacuum in the dark at room temperature for 24 h.

Fabrication of biodegradable microrafts by a drain coating process

A simple drain coating process requiring a few grams of material was used to fabricate the microraft array. Briefly, a solution was first prepared by dissolving biodegradable polyester in GBL solvent at a concentration of 40 wt% solid. A PDMS microwell array was formed via soft lithography on an SU-8 master (supplementary data). This microwell array was then used as template for drain coating. Polymer solution (3 mL) was added to the PDMS template, and trapped air bubbles within the microwells were removed by degassing for 1 min using a Leybold Trivac pump (Model # D2.5E). The PDMS template was then hung vertically inside a large wide-mouth glass bottle (supplementary data Fig. S1). Dewetting of polymer solution from the template produced isolated polymer pockets trapped inside each microwell. The PDMS template was placed in a 95 °C oven for 2 h to evaporate the GBL solvent. Complete evaporation of the GBL was achieved by a 1 h bake at 100 °C in a vacuum oven (−30 in. Hg). Following fabrication of the microraft arrays, the PDMS template was attached to a custom-made polycarbonate cassette, with the array facing toward the interior of the cassette (supplementary data Fig. S2). The detailed fabrication process and integration of the array with cassette can be found in the supplementary data.

Culture and imaging of cells on biodegradable microrafts

The microraft array and cassettes were sterilized by exposure to ultraviolet (UV) light for 30 min in a tissue culture hood. Phosphate buffered saline (PBS, 2 mL) was added to the array, and the trapped air bubbles within the microwells were removed through degassing for 2 min by an oil-free diaphragm vacuum pump (Vacuubrand, Fisher Scientific). PBS buffer

was aspirated, and the array was rinsed 1× with DMEM supplemented with FBS (10%), L-glutamine (584 mg/L), penicillin (100 units/mL) and streptomycin (100 μg/mL). Cells used in these studies included wild-type HeLa cells (a human ovarian carcinoma cell line), wild-type H1299 cells (a human non-small cell lung carcinoma cell line), H1299 cells stably expressing GFP, and AsPC-1-Luc (human pancreatic adenocarcinoma cells). To plate cells on the microraft array, cells in suspension were added to the array and allowed to settle and adhere to the microrafts over 24 h in a 37 °C incubator with a 5% CO_2 atmosphere.

Cells plated on microrafts were imaged by both brightfield and fluorescence microscopy using a cooled charge-coupled device (CCD) camera (Photometrics CoolSNAP HQ2, Tucson, AZ) mounted on an inverted epifluorescence microscope (Nikon TE200-U, Melville, NY). HeLa cells were co-labeled with Hoechst 33342 DNA dye and the cytoplasmic stain CellTracker Orange CMTMR per supplier protocol.

To image cells by scanning electron microscopy (SEM), cells cultured on the arrays were rinsed gently with PBS and then fixed with 2.5 wt% glutaraldehyde in PBS for 30 min. The sample was washed with deionized water and dried in air. The fixed cells were observed by SEM (FEI Quanta 200 ESEM, FEI Company).

Release and collection of biodegradable microrafts

A manual system was built to release the targeted microrafts from the array with a needle (supplementary data Fig. S3-a). An XYZ micromanipulator was mounted on the stage of 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 cassettes (see below). To release a targeted microraft, the needle was aligned with the center of the microraft. The user lowered the needle a specified distance to pierce the PDMS and extrude the microraft from the array and into the collection dish.

Prior to cell selection, the arrays were washed ×2 with DMEM. Fresh culture medium (7 mL) was added to completely fill the cell culture chamber in the array cassette so that a convex fluid surface was formed. The collection dish was mated directly to the cell culture chamber. The cassette assembled in this manner formed an enclosed compartment housing the array that was filled with culture medium and lacking air bubbles. The assembly was then inverted and placed in a 100 mm Petri dish and the dish was placed on the microscope stage. Microrafts containing selected cells (*e.g.* those with fluorescent cytoplasm) were released, whereupon the microrafts settled on the collection plate by gravity. 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 microscopy.

In vitro degradation of microrafts

Arrays of biodegradable microrafts were immersed in PBS buffer and placed in a 37 °C incubator with a 5% CO_2 atmosphere. The appearance of the microrafts was monitored by brightfield microscopy over a period of 20 days. At the end of day 20, the microrafts on the array were rinsed with deionized water, dried in air, and observed by SEM.

Establishment of subcutaneous tumor models in mice by using PLGA-TF3 microrafts as cell microcarriers

AsPC-1-Luc cells were plated onto an array at a density of 10 cells/raft. PLGA-TF3 microrafts (dimension = 200 × 200 × 40 μm) were used. After 48 h in culture, 1000

microrafts carrying cells were released from the array. The suspension of released cells/microrafts in medium was transferred to a 15-mL conical centrifuge tube. The microrafts settled to the bottom of tube after 5 min, and the medium was aspirated. The microrafts were then mixed with 100 μL cold Matrigel on ice, and the mixture was subcutaneously injected into two mice (50 μL for each mouse) using a 16G syringe needle. The *in vivo* proliferation of AsPc-1-Luc cells was imaged using an IVIS® Kinetic Optical System (PerkinElmer, Waltham, MA). Mice were sacrificed at the end of 1 and 3 months, and the tumors were harvested and freshly frozen. The tumors were then cut by a microtome into 7-μm thick tissue sections which were imaged by fluorescence microscopy equipped with a Cy3 filter set to reveal *in vivo* degradation of the implanted PLGA-TF3 microrafts. Some sections were stained with hematoxylin and eosin (H&E) according to standard protocols.

RESULTS AND DISCUSSION

A new strategy is proposed for the isolation and delivery of adherent cells for implantation based on a recently developed microarray technology for culture and selection of adherent cells.[21] The conceptual scheme for this strategy is shown in Fig. 1. Biodegradable microelements (termed microrafts due to the similarity in their shape and function to rafts) are fabricated in a high-density array format on a flat PDMS microwell template (Fig. 1-a). These elements have dual functions as a culture surface and a microcarrier for living, adherent cells. Cells plated on the array selectively attach to the surface of the microrafts (Fig. 1-b) where they are visualized using standard imaging techniques, including brightfield and epifluorescence microscopy. Microrafts possessing cells of interest are identified by fluorescence signature, morphology or other criteria, and then are easily dislodged from the array by a microneedle inserted through the PDMS substrate and then are collected (Fig. 1-c). The cells residing on the released microrafts are available for direct transfer, for example by syringe (Fig. 1-d), for subcutaneous injection (Fig. 1-e). Cells isolated in this manner continue to survive *in vivo* while the microrafts degrade (Fig. 1-f) into metabolizable small organic molecules.

Fabrication of biodegradable microraft arrays by a drain coating process

We have recently reported the fabrication of microraft arrays via discontinuous dewetting on a PDMS microwell template, and their use for isolating living, adherent cells.[21, 22] The microrafts were made from conventional polymers such as epoxy, polystyrene, poly(styrene-co-acrylic acid), and their composites with magnetic nanoparticles. These materials have been shown to be biocompatible as cells can be cultured directly on their surface or after coating with ECM proteins in a manner equivalent to standard tissue culture surfaces. However, the microrafts made from these materials are not suitable for cell transplantation applications because they are not biodegradable. For cell isolation and implantation as outlined in Fig. 1, there are several requirements for the materials used in fabricating microrafts. First, the material must be biocompatible *in vitro* for cell attachment and proliferation prior to implantation, and biodegradable *in vivo* post transplantation. Second, the material must have adequate mechanical strength to withstand the needle release and subsequent manipulation without breaking. Third, the material must be transparent and of low-autofluorescence for high quality imaging of cells on the microraft surface. Lastly, the material must be suitable for the micromolding process via discontinuous dewetting on the PDMS microwell template.

A variety of biodegradable materials were screened, including ECM proteins (*e.g.* collagen and gelatin), natural polymers (*e.g.* dextran, chitosan), and synthetic polymers including polylactic acid, PLGA and polycaprolactone. ECM proteins and natural polymers were excluded since they were generally soluble or swelled in medium during cell culture and as a result did not have the mechanical properties needed to withstand mechanical manipulation

during release and transfer. Some synthetic biodegradable polymers were excluded because they were either non-transparent (such as polycaprolactone) or were not commercially available. For these reasons, a commonly used biodegradable polymer matrix, PLGA and its family, polylactic acids and polyglycolic acids, were chosen for further testing. Of the biodegradable polymers of PLGA, 13 types with a variety of molecular weights and lactic/glycolic ratios (Table 1) were screened for the suitability in the micromolding process via discontinuous dewetting on a PDMS microwell template. All were commercially available and cost effective.

Prior work demonstrated that the key for successful fabrication of microraft arrays depended on the selection of a solvent to dissolve the polymer, but which did not swell the PDMS template.[25] The solvent GBL successfully used in these prior studies dissolved PLGA and so it was selected as the solvent for the current work to fabricate arrays of biodegradable microrafts (Fig. 2a). Previously, a dip-coating process was used to generate high-density microraft arrays made of conventional polymers.[21, 22] Dip-coating requires a bath of coating solution with a minimum volume of 100 mL, in which the PDMS template could be fully immersed before being slowly withdrawn from the coating solution. This coating process was not suitable for testing various biodegradable polymers since they were far more expensive (*e.g.,* ~$30USD/gram) than conventional polymers (e.g., ~$0.05USD/gram). For low volumes (5 mL) of biopolymer solutions, a drain coating process was used to form the biodegradable microrafts (Fig. 2b). After pipetting the PLGA solution onto the microwell array surface, the PDMS template was hung vertically in a sealed glass bottle. The drainage of PLGA solution caused by its dewetting from PDMS left an array of isolated, convex pockets of PLGA solution on the microwell array (Fig. 2a-iii). The drainage required 10–30 min depending on the viscosity of the solution. The PLGA solution could be collected in the glass bottle and reused for fabrication of the next array (supplementary data Fig S1). Evaporation of the solvent at 95 °C solidified the PLGA in individual microwells to create the rafts (Fig. 2a-iv). The height of the rafts depended on the concentration of PLGA in the solvent, which for the present study was 20 – 40%, as well as the height of the microwells, which was 40–100 μm. Fig. 2c shows a representative PLGA microraft array (size: 100 μm) embedded in a template of a PDMS microwell array (size: 100 μm, height: 40 μm). The raft has a slightly concave shape, with a height at the edge of 19 μm and a height in the center of 15 μm. There was minimal adhesion between the PLGA rafts and PDMS, as suggested by the inserted SEM image in Fig. 2c showing a raft dislodged from a ruptured PDMS microwell. Among the 13 biopolymers listed in Table 1, only 10 types (sample #1–6: PLGA and sample #7–10: poly(D,L-lactic acid)) were suitable for the drain coating process since the others were either insoluble in GBL (sample #11: polyglycolic acid and sample #12: poly(L-lactic acid)), or the solution was too viscous to effectively dewet the PDMS template (sample #13: PLGA with intrinsic viscosity of 4.5 dl/g). To obtain the optimal result for drain coating, it was empirically determined that the intrinsic viscosity of PLGA and poly(D,L- lactic acid) needed to be below 0.5 dl/g.

The drain coating permitted placement of a small amount of biodegradable polymer into a high density array format via discontinuous dewetting. A typical dimension for the microwell in this study was 100 μm × 100 μm × 40 μm (length × width × depth), and the inter-well gap (rim to rim) was 20 μm. The array size was 25.4 mm × 25.4 mm (length × width) and consisted of 44,800 microwells. Theoretically 18 mg of PLGA solution (equivalent to 7.2 mg solid polymer if a 40% solution is used) was trapped on the array, so 1 gram of PLGA could be used to fabricate up to 130 arrays. Although discontinuous dewetting on a 3-D template (*e.g.,* the PDMS microwell array) has been utilized for generating isolated liquids, particles, and hydrogels for a variety of applications such as microlenses and nanoparticles for drug delivery,[26–28] the current study is the first to use this

phenomenon to generate an array of isolated biodegradable microelements for the purpose of sorting and implanting live, adherent cells.

In vitro degradation of biodegradable microrafts

To test *in vitro* degradation, microrafts were made from biodegradable polyesters (sample #1–10) and soaked in PBS buffer in an incubator at 37 °C for up to 20 days. The degradation of microrafts was directly observed by brightfield microscopy (Fig. 3a for sample #1–5). The degradation rate was found to be dependent on the molecular weight and lactic/glycolic ratio. The degradation rate of PLGA was inversely proportional to the molecular weight (MW) and PLGA with a 50:50 ratio of monomers yielded the fastest degrading material. The degradation rate decreased when either the lactic or glycolic content of the copolymer was increased. This observation was consistent with previous reports.[29–31] PLGA sample #1 (lactic:glycolic=70:30, MW of 5,000) and #2 (lactic:glycolic=50:50, MW of 7,000–17,000) show the fastest degradation on the array. The degradation started at day 1 (sample #1) or day 2 (sample #2), accompanied by the presence of a rough surface. PLGA sample #3 (lactic:glycolic=50:50, MW of 24,000–38,000) and #4 (lactic:glycolic=65:35, MW of 24,000–38,000) had intermediate degradation rates, with the apparent degradation starting at day 9 (sample #3) and day 12 (sample #4). As degradation proceeded, the microraft appeared darker suggesting bulk erosion instead of surface erosion, since as the microraft became porous the infiltration of water caused the microraft to appear dark due to the difference in refractive index of water (1.33) and PLGA (1.46).[32] The confirmation of a bulk erosion mechanism is also demonstrated by the SEM images (Fig. 3b) of microrafts incubated in PBS for 20 days: not only was the surface of microrafts rough, but the bulk material of the microraft was porous. Previous studies have shown that biodegradable polymers can undergo surface erosion or bulk erosion depending on the critical device dimension ($L_{critical}$) which is 7.4 cm for PLGA based on a theoretical model: if a matrix is larger than $L_{critical}$ it will undergo surface erosion, if not it will undergo bulk erosion.[33] The size of the microrafts (100 μm) is far less than $L_{critical}$, so that they can be expected to undergo bulk erosion. In PBS, PLGA degrades through hydrolysis of its ester linkages in the presence of water.[34] PLGA samples #5–10 (>75% lactic acid) showed slow degradation rates, without apparent degradation imaged by brightfield and SEM even at day 20 (data not shown).

An intermediate degradation rate for the microraft was desirable for the cell isolation and transplantation application: the material should have no apparent degradation during cell culture on the array in order to maintain its mechanical strength and optical clarity (usually in the range of a few hours to several days depending on the size of colonies to be sorted), yet the material must degrade relatively quickly after implantation *in vivo*. From the *in vitro* degradation data shown in Fig. 3, PLGA #2, #3 and #4 meet the initial requirement. PLGA #2 can be used for sorting single cells or small colonies that generally need only 1 day in cell culture on the array. PLGA #3 and #4 can be used for sorting larger colonies after 3–6 days in cell culture for adequate expansion. In the following experiments, only PLGA #2, #3 and #4 were used to fabricate biodegradable microrafts.

Cell culture and imaging on the biodegradable raft array

PLGA materials have previously been shown to be fully biocompatible,[35] and as a result they are excellent substrates for culturing adherent cells. H1299, HeLa and AsPc-1-Luc cells (15,000 cells) were plated on the microraft array (PLGA #4) (44,800 microrafts per array, microraft size = 200 μm). The distribution of cells on the microarray followed a Poisson distribution. At this cell density (cell:microraft = 1:3), theoretical calculation shows that 72% of microrafts will be empty, 24% microrafts will have single cells, and 4% microrafts will have ≥ 2 cells. Therefore, the majority of cells (>72%) are present as single cells on

individual microrafts. This distribution is important as it maximizes the number of sites with single cells, which can then form clonal colonies. H1299, HeLa and AsPc-1-Luc cells plated on the arrays attached to native PLGA microrafts relatively slowly, without apparent cellular spread within 6 h of plating; however, after 24 h, both cell types appeared fully attached to the surface based on their morphology. Cells cultured on arrays remained isolated on the microraft surface, and migration across the PDMS wall to adjacent microrafts rarely occurred during 48 h in culture, as observed by brightfield microscopy and SEM (Figs. 4a and 4b) consistent with prior findings.[21, 22] It is known that the native surface of PDMS does not support cell attachment unless it is treated with plasma or coated with ECM;[36] therefore, the PDMS wall effectively confined the cell proliferation and migration to individual microrafts.

If rapid cell adherence within a few hours is desired, it may be necessary to modify the microraft surface since the native PLGA surface possesses a neutral charge and has no recognition sites for cell attachment (*e.g.*, integrins). A variety of surface modification methods are available for PLGA to generate surface charges for cell affinity, such as the addition of a poly-L-lysine-g-PLGA graft copolymer,[37] chemical hydrolysis, or aminolysis.[38] These methods can be used to selectively modify the surface of PLGA without changing the surface properties of PDMS. Alternatively, plasma oxidation and ECM adsorption are commonly used to modify the surface of PLGA to promote cell attachment.[39] However, plasma treatment or the addition of an ECM (*e.g.*, collagen, fibronectin, gelatin, laminin, *etc.*) will also modify the surface of the PDMS walls which will reduce its barrier function. Plasma oxidization and ECM adsorption are recommended only when necessary for attachment and immediate cell sorting.

Once cells adhere to the microrafts, the cells of interest can be identified by their morphology, fluorescence markers, or other criteria. The PLGA microraft was optically clear which permitted imaging the cell morphology by brightfield, as demonstrated in Fig. 4c showing four HeLa cells growing on a microraft (PLGA #4) after 48-h in culture. PLGA demonstrated minimal autofluorescence.[40] As a demonstration, HeLa cells plated on microrafts were co-stained with a nuclear dye (Hoechst 33342, excitation/emission 350/461 nm) and a cytoplasmic dye (CellTracker Orange, excitation/emission 548/576 nm). Imaging by fluorescence microscopy demonstrated the visualization of cellular detail on PLGA microrafts (Fig. 4d and 4e).

Cell isolation using biodegradable rafts

The use of microrafts for cell sorting relies upon the ability to selectively release and collect targeted microrafts containing cells of interest. A manual system was built to release the targeted microrafts from the array with a needle (supplementary data Fig. S3-a). The system was composed of an XYZ micromanipulator for controlling the movement of an anodized steel microneedle that served to dislodge individual microrafts from the array (supplementary data Fig. S3-b). There were no defects in the released microrafts.

To demonstrate the cell sorting function of microrafts (PLGA #3 conjugated with TF3 dye), a cloning experiment was performed using a heterogeneous population of single cells plated on the array (Fig. 5). A minority population of H1299 cells stably expressing GFP was admixed with wild-type H1299 cells in suspension at a ratio of 1:100. The cells were plated at a density to maximize the number of microrafts containing single cells. After the cells were cultured for 72 h, microrafts (N=10) containing clonal colonies composed of cells with fluorescent cytoplasm were identified and released from the array (Fig. 5a). After the collection procedure, the released microrafts retained their attached cells (Fig. 5b). After an additional 72 h, cells on the collected microrafts were seen to migrate onto the collection dish, and expand into colonies in which all cells possessed fluorescent cytoplasm with no

non-fluorescent cells present (Fig. 5c). After cell sorting, the purity of GFP cells was increased from 1% (on the array) to 100% (in the collection dish). These data show the feasibility of using biodegradable microrafts to create highly purified clonal populations of cells from a heterogeneous population.

Implantation of cells using biodegradable microrafts

To demonstrate the feasibility of cell isolation and transplantation using the biodegradable microrafts, AsPC-1-Luc (to enable *in vivo* imaging of the cells) were plated on an array at a density of 10 cells/raft. An array of fluorescent microrafts (12,200 microrafts per array, microraft size = 200 μm) was fabricated using PLGA #3 conjugated with TF3 dye (to enable visualization of *in vivo* degradation of the microraft material). After culturing the cells for 48 h, proliferation of AsPc-1-Luc cells was confined to the microrafts and no cells grew on the PDMS walls (Fig. 6a, left). 1,000 microrafts were released from the array and collected in a Petri dish (Fig. 6a, right). Each microraft possessed on average 38±16 cells (N=20 counted). The collected microrafts were directly implanted into two nude mice as described in the Materials and Methods, and as a result ~19,000 cells were implanted in each mouse assuming no loss of cells/microrafts during manipulation. Bio-luminescence imaging of the luciferase showed *in vivo* growth of a mass of luciferase expressing cells in each mouse over time (Fig. 6b) consistent with the successful establishment of a xenograft tumor. The tumors were excised at 1 month from one mouse and 3 months from the second mouse, and subjected to histologic examination. Hematoxylin and eosin (H&E) staining of tissue sections obtained from the center of each mass revealed pathologic findings consistent with tumor formation resulting from the implanted AsPC-1-Luc cells (Fig. 6c). The tissue sections also revealed progressive *in vivo* degradation of the PLGA#3 microrafts (Fig. 6d). At 1 month, the microrafts were not fully degraded, and they were present in all tissue sections examined (n = 8). The average area of microrafts in a 7-μm-thick tissue section was 7304 ± 4167 μm^2 (number of microrafts = 12). At 3 months after implantation, only a few remnants of the implanted microrafts could be discerned in 3 of 8 tissue sections examined. The average area was 255 ± 232 μm^2(number of microrafts = 4). These data demonstrated that it was feasible to use the microraft arrays for cell implantation and confirmed the *in vivo* degradation of PLGA microrafts.

CONCLUSIONS

We have demonstrated a new strategy for isolation and delivery of living, adherent cells for transplantation into living animals using a microraft array composed of biodegradable PLGA. A simple drain coating process micromolded PLGA into a high density array format for culturing adherent cells. Three types of PLGA materials were identified as suitable for this application, with bulk erosion starting 2–12 days after immersion in buffer. Microrafts carrying cells were isolated from the array by a simple needle release device, and effectively implanted in a nude mouse model where *in vivo* degradation was confirmed over a 3-month period. The microrafts served a dual function as a culture surface for selective cell or colony isolation and as a microcarrier for cell implantation. Cells of a desired phenotype can be directly imaged and identified on the array based on broad selection criteria such as fluorescence signature, morphology, growth rate, or other attributes. The technique has potential applications when a small number of cells need to be isolated and then directly implanted into animal surrogates, such as for tumor induction, *in vivo* differentiation of stem cells, or other cell biomedical research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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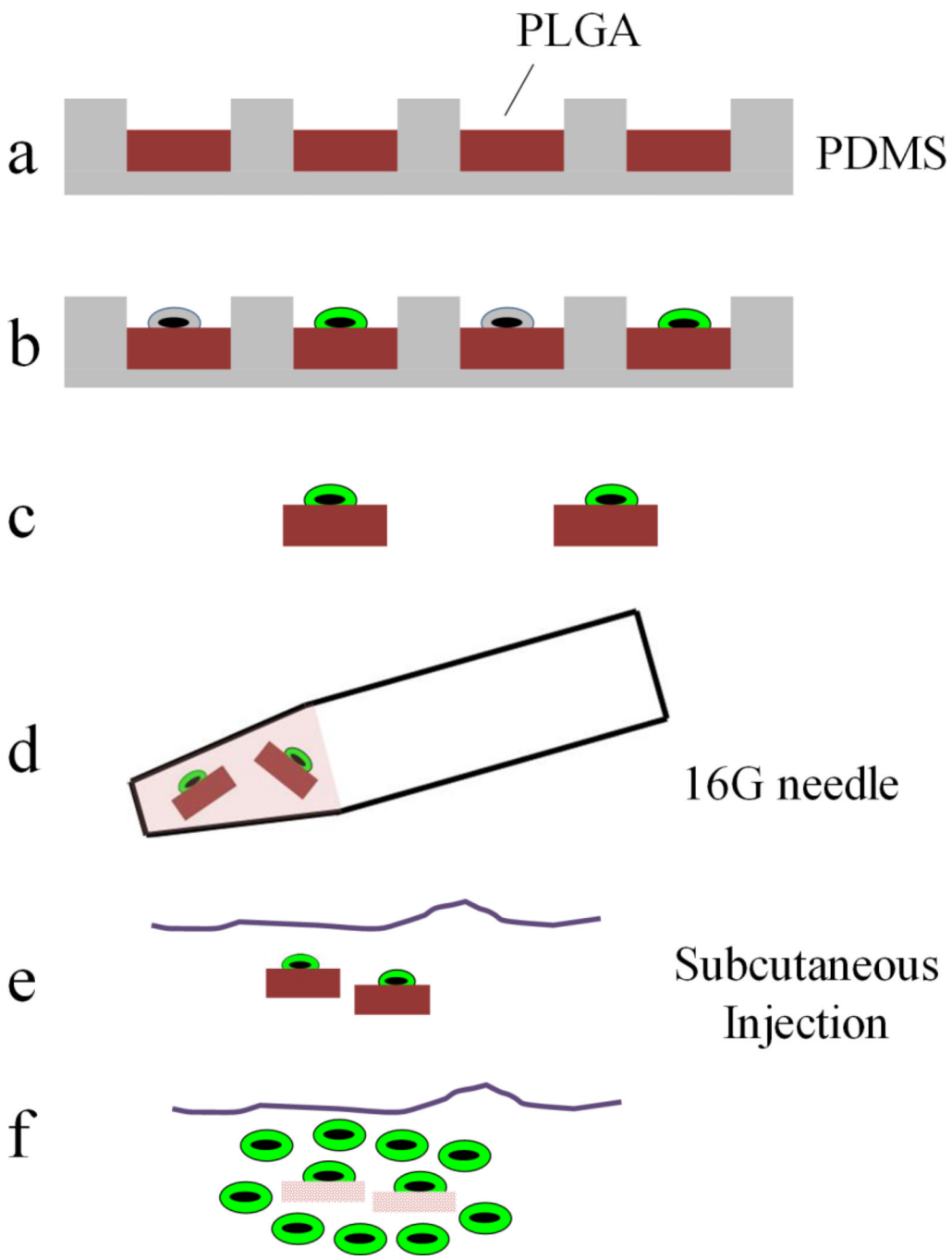

Fig. 1.

A conceptual scheme to isolate and deliver adherent cells for direct animal implantation. (a) Biodegradable microraft array. (b) Cells plated on the array selectively attach to the surface of microrafts. (c) A microraft with targeted cells is dislodged from the array and collected. (d) The microraft is transferred using a syringe needle. (e) The microraft is implanted. (f) The implanted cells grow *in vivo* while the microrafts degrade into metabolizable small molecules.

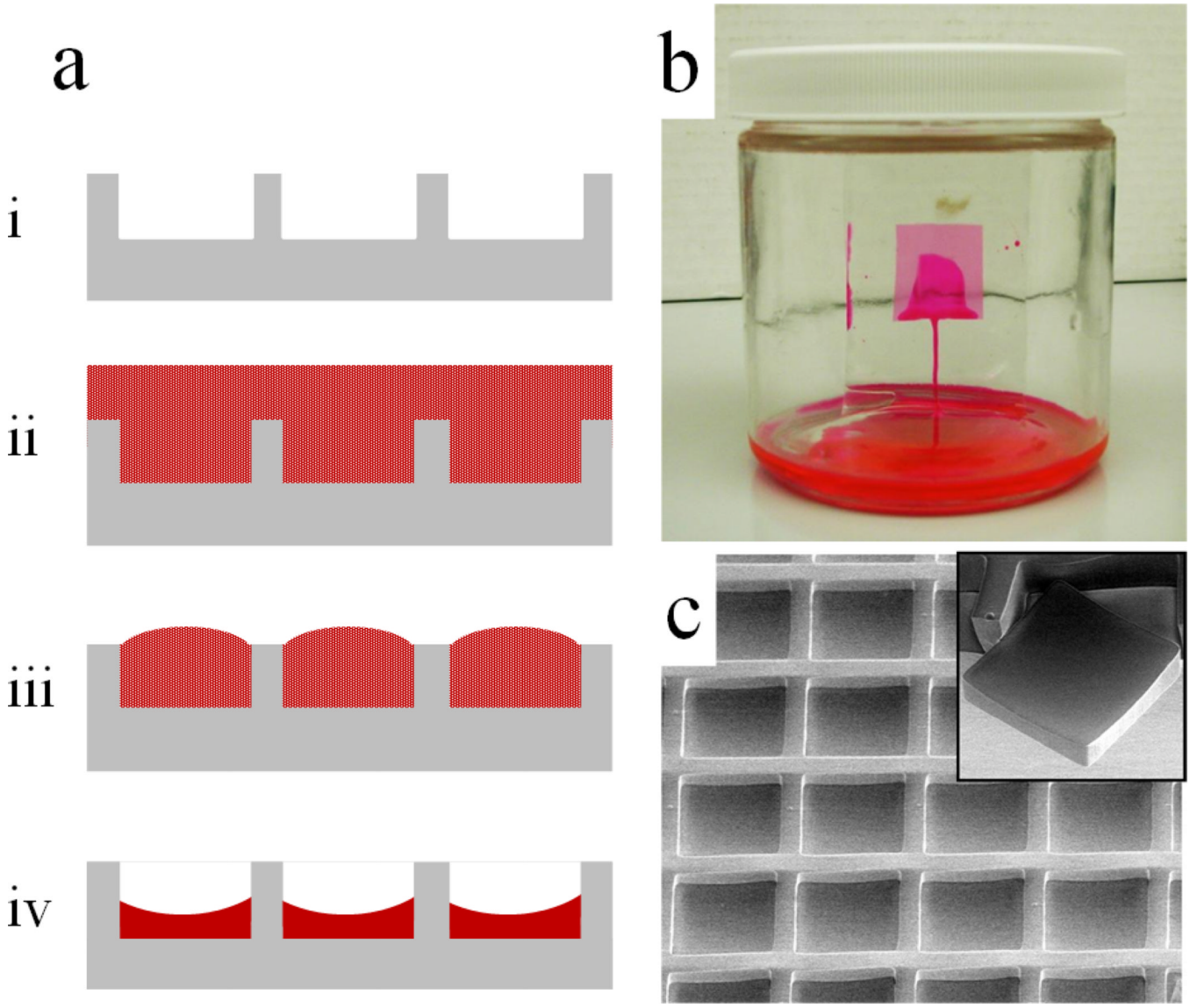

Fig. 2.

Fabrication of an array of biodegradable microrafts by a drain coating process. (a) Schematic of the fabrication process. (i) A PDMS microwell array (shown in gray) is fabricated by standard soft lithography. (ii) A polymer solution (shown in dark red) is added to the PDMS microwell array. (iii) The dewetting of polymer solution from PDMS results in an isolated convex polymer solution in each well. (iv) Evaporation of solvent results in concave polymer microrafts inside each well. (b) Setup of the drain coating process. The PDMS microwell array is hung vertically in a glass bottle. To improve visualization in this example, PLGA #10 (40 wt% in GBL) was mixed with rhodamine B (0.001 wt% of polymer). (c) SEM image of an array of biodegradable microrafts (PLGA #10, 100 μm square, 20 μm inter-raft gap). Inset shows a close-up of a disrupted section of an array.

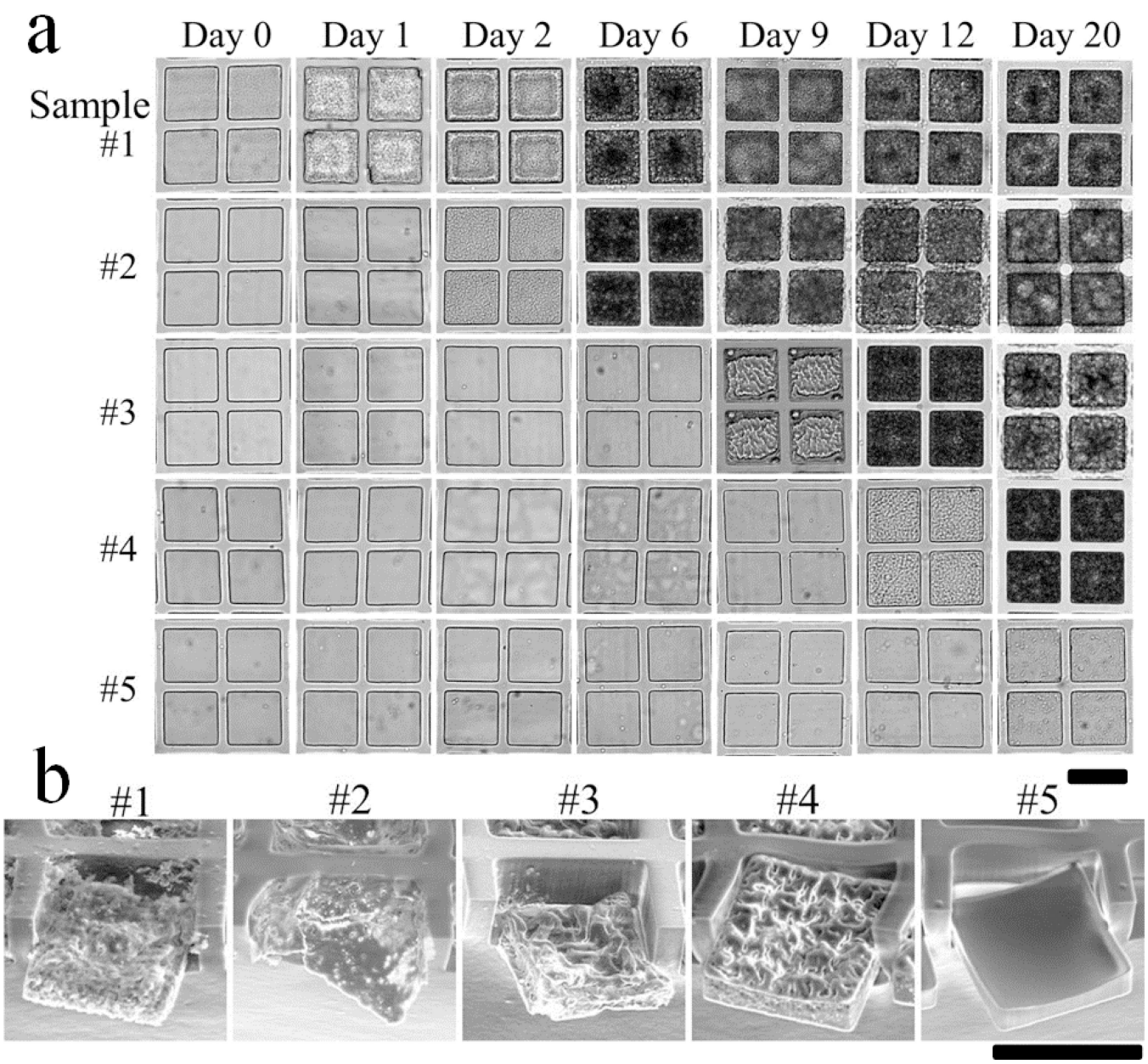

Fig. 3.

In vitro degradation of biodegradable microrafts made from PLGA #1–#5 in PBS at 37 °C. (a) Brightfield images of microrafts at different time points over a 20 day period. (b) SEM images of microrafts after 20 days in PBS at 37 °C. Scale bar is 100 μm.

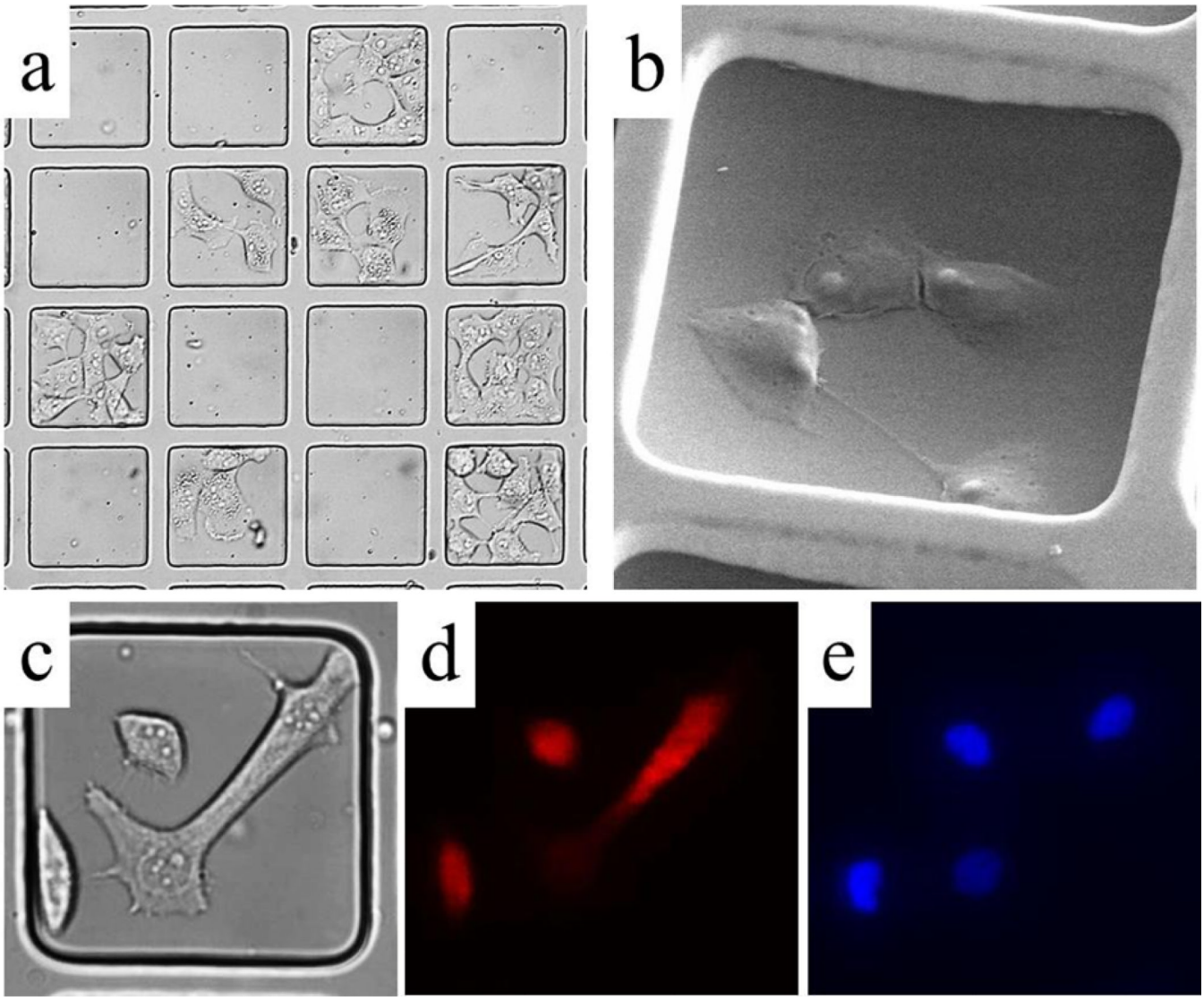

Fig. 4.

Cell culture and imaging on biodegradable microrafts made from PLGA #4. Brightfield (a) and SEM (b) images of H1299 cells growing on biodegradable microrafts after 48-h in culture. Brightfield (c) and fluorescence (d and e) images of four HeLa cells loaded with fluorescent dyes. Cytoplasmic staining utilized CellTracker Orange CMTMR (d), and nuclear staining was performed with Hoechst 33342 (e). The size of microrafts is 100 μm.

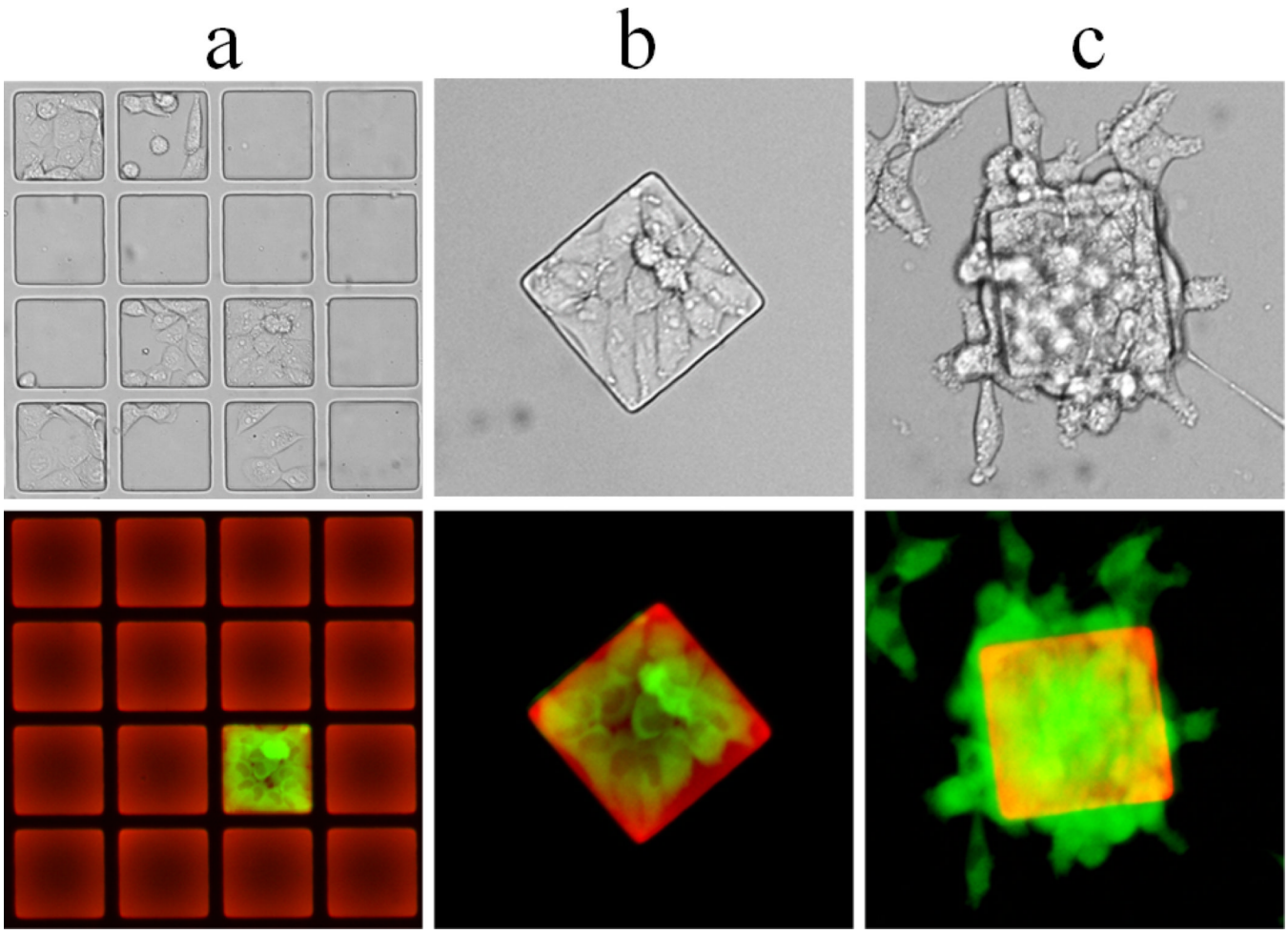

Fig. 5.

Cell sorting with biodegradable microrafts made from PLGA #3. To enhance visualization, PLGA #3 was conjugated with TF3 dye. (a) Brightfield (top) and fluorescence (bottom) images of a region of an array containing colonies of H1299 cells possessing fluorescent cytoplasm after 72-h culture. (b) Images of the colony seen in “a” 4 h after release and collection. (c) Images of the same colony 72 h after isolation. The fluorescent daughter cells are seen to be dividing and growing off the isolated microraft. The size of microrafts is 100 μm.

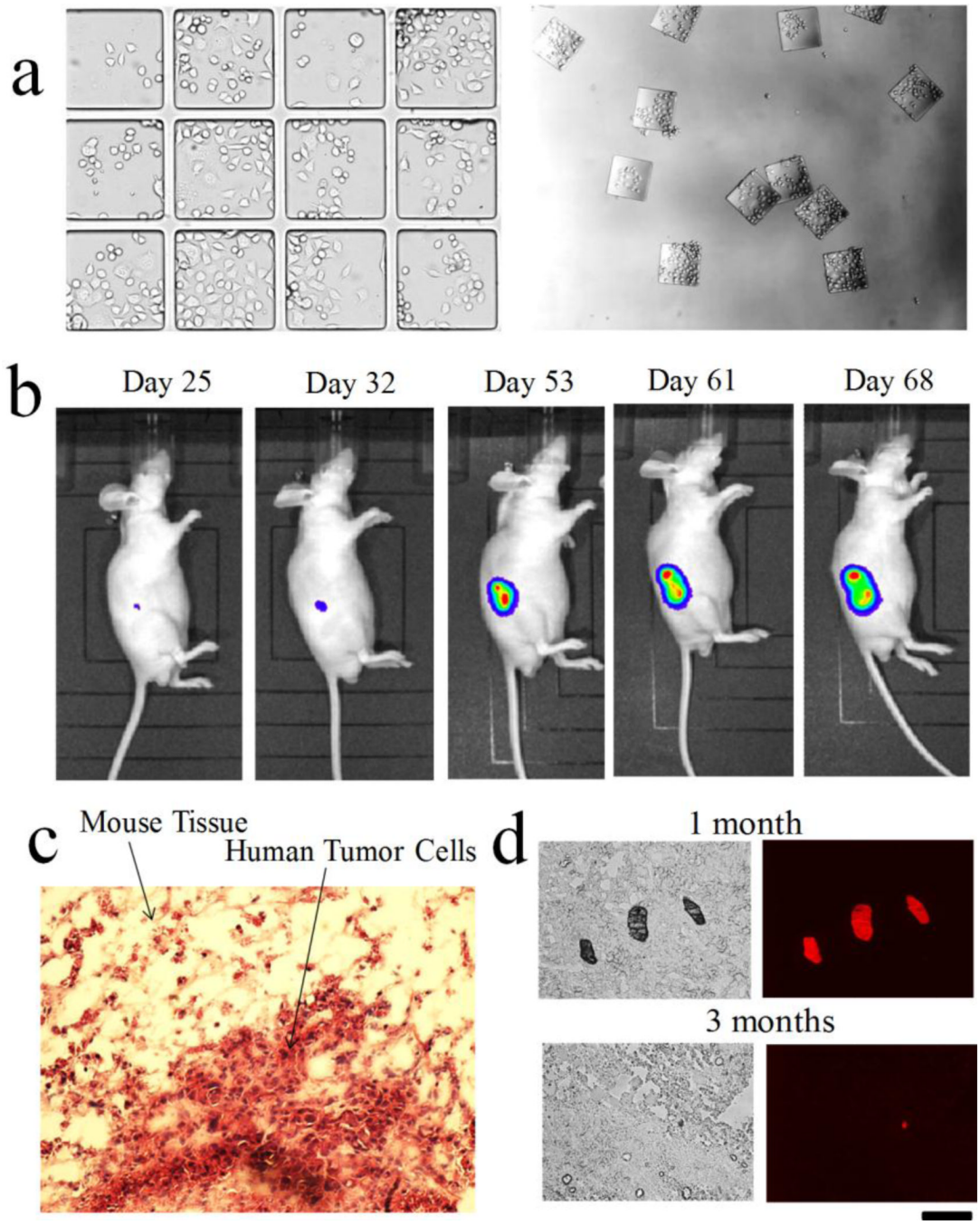

Fig. 6.

Implantation of AsPc-1-Luc cells in mice using biodegradable microrafts (PLGA #3). The PLGA #3 was conjugated with TF3 dye. (a) AsPC-1-Luc cells on PLGA microrafts. Left: AsPc-1-Luc cells cultured on the array for 48 h. Right: microrafts possessing cells were released from the array and collected in a separate Petri dish. The size of the microrafts was 200 μm. (b) *In vivo* imaging of xenograft tumor growth in a mouse after transplantation of 500 microrafts. (c) Photomicrographs of an H&E stained tissue section. The tumor was harvested from a mouse 3 months after implantation. A tumor mass at the lower of the image was surrounded by subcutaneous tissue displaying invading tumor cells and inflammatory cell infiltrate (100×). (d) Microscopic imaging of tissue sections harvested

from tumors at 1 month (left) and 3 months (right) post-implantation. The remnants of PLGA microrafts were discerned by both brightfield and fluorescence imaging. Scale bar in (d) = 200 μm.

Table 1

List of biodegradable poly(lactic-co-glycolic acid) tested in this study

Sample #	Lactic: Glycolic	i.v. (dl/g)	MW	Vendor/Catalog#	Comment
1	70:30 *	0.2	5,000	Polysciences/16587	Fast degradation
2	50:50	0.16–0.24	7,000–17,000	Sigma Aldrich/719897	Fast degradation
3	50:50	0.32–0.44	24,000–38,000	Sigma Aldrich/719870	Intermediate degradation
4	65:35	0.32–0.44	24,000–38,000	Sigma Aldrich/719862	Intermediate degradation
5	75:25	0.50–0.65	65,000	Polysciences/23988-5	Slow degradation
6	90:10	0.15–0.30	10,000	Polysciences/19076-5	Slow degradation
7	100:0	0.35–0.45	20,000–30,000	Polysciences/16585	Slow degradation
8	100:0	0.15–0.30	15,000	Polysciences/26969-66-4	Slow degradation
9	100:0	0.2		Purac/PDL02	Slow degradation
10	100:0	0.5		Purac/PDL05	Slow degradation
11	0:100	1.0–2.0	> 100,000	Polysciences/06525	Insoluble in GBL
12	100:0 *	1.3–1.6	80,000–100,000	Polysciences/18402	Insoluble in GBL
13	100:0	4.5		Purac/PDL45	High viscosity

* indicates L-lactic acid. Others are D,L-lactic acid