Laser ablation and PDMS mold fabrication

1. Poly (methyl methacrylate) (PMMA) was purchased from Ithaca plastics Inc (Ithaca, NY). The UV laser micromachining system Resonetics Maestro 1000 (Resonetics, Nashua, NH) was used to fabricate high-aspect ratio holes in PMMA. The laser energy was stabilized at 50 mJ by using the energy stable function. A stainless sheet with 4 mm diameter circle was used as laser shutter. The laser pulse rate was set at 75PPS (pulse per second), and the pulse number was set to 1100. At these parameters, the depth of hole was estimated to be approximately 506 μm, which was later confirmed by confocal microscopy. The distance between rows and columns was set to be 200 μm for 25 holes/mm² density. To measure the depth of hole, a drilled PMMA sheet was coated with gold by a gold sputtering system (Polaron) for 30 min to generate detectable signals. The depth was measured by Wyko HD-3300 noncontact surface height measurement system (Veeco Instruments Inc, Tucson, AZ). A linear relationship was found between laser pulse number and hole depth (Figure 1). PDMS monomer and curing agent (Sylgard 184, Dow Corning, Midland, MI) were mixed at 7:1 ratio, and poured onto the PMMA with holes. After degassing to remove bubbles and ensure the PDMS prepolymer solution filled up the holes, PDMS was cured at room temperature overnight. After curing, the PDMS mold was slowly peeled off.

![Figure S-1 Measured depth of holes in PMMA vs. laser pulse number](image1)

![Figure S-2 (A) PMMA surface with holes. There are 25 holes in 1mm². Each hole has oval shape due to the melting effect of laser. The longer axis of the oval is about 200 μm, and the shorter axis is about 160 μm. (B) PDMS surface with villi structures (C) Alginate mold with holes (D) Collagen scaffold with villi structures](image2)
2. Fabrication of alginate mold and collagen/PEG-DA scaffold

For fabrication of an alginate mold, a PDMS stamp with villi structure was made first. An aluminum gasket rig was designed based on the previously reported method using a gasket for fabricating microfluidic channels in calcium alginate. It consists of a base frame (G1) with a recess (7 mm x 7 mm, 0.7 mm depth), a middle frame for holding PDMS (G2), and the top frame (G3). The three frames were secured with screws. The PDMS stamp was cured overnight at room temperature to avoid deformation of aluminum from heating. After curing, G1 was removed, and the PDMS villi structure (made from the PMMA mold) was glued on top of the cured PDMS. Uncured PDMS prepolymer solution was used as glue. The rig was left at room temperature overnight until the PDMS glue set. After the PDMS villi piece was fully glued, an aluminum gasket (G4) was secured on top of the PDMS stamp. G4, a square piece with a 10 mm by 10 mm hole, is used as a gasket for holding the alginate mold. Sterile-filtered 2.5% sodium alginate (10/60 sodium alginate, FMC biopolymer, Philadelphia, PA) was inserted into hole in G4. The top was covered with a polycarbonate membrane (G5, 8 μm pore size, 25 mm diameter, Fisher Scientific, Pittsburg, PA) and a perforated aluminum piece (G6) with 1mm diameter holes. An aluminum gasket (G7), which works as a reservoir for calcium chloride solution is secured on top, and 3 ml of 60 mM calcium chloride solution was inserted into the reservoir. After incubating at room temperature for 4 hours, a gasket with alginate mold (G4) was separated from other gasket pieces. Collagen or PEG-DA pre-gel solution (5 mg/ml final concentration in 0.1% acetic acid for collagen and 20% (w/v) for PEG-DA with 0.5% 2,2’-Azobis(2-methylpropionamide) dihydrochloride as a photoinitiator) was placed in the alginate mold. Collagen pre-gel solution was neutralized with 1M NaOH and kept in ice before the insertion. Collagen was gelled by raising the temperature to 37°C, and PEG-DA was polymerized by exposure to UV for 30 minutes in a UV crosslinker (Spectronics Corporation, Westbury, NY). The collagen was further crosslinked with 0.1% glutaraldehyde for 4 hours. After the gel was made, the alginate mold was dissolved using 60 mM EDTA solution for 3 hours at room temperature. The whole process is summarized in Figure 3.
Figure S-3. Summary of fabrication process. A PDMS stamp holds the PDMS villi structure on top, where sodium alginate is placed and cured with a calcium chloride solution. After curing alginate, collagen is inserted into the alginate mold and crosslinked. The sacrificial alginate mold is later dissolved with an EDTA solution.

3. Cell seeding and staining

After fabrication, collagen scaffold was incubated in 5% L-glutamic acid for 48 hours at room temperature to reduce the cytotoxicity of glutaraldehyde and restore the biocompatibility. Then the scaffold was washed in PBS three times, and incubated in PBS until cell seeding. Caco-2 cells were maintained in Dulbecco’s Modified Eagle’s Media (DMEM, Cellgro, Manassas, VA), with 10% FBS (Invitrogen, Carlsbad, CA) and 1X anti-biotic anti-mycotic (Invitrogen). After trypsinization, live cells were counted and cells were resuspended in fresh medium to the final concentration of $1 \times 10^5$–$5 \times 10^5$ cells/ml. A drop of cell suspension was placed on top of the collagen scaffold and incubated for 30 minutes before medium was added. After cell seeding, the collagen scaffold was maintained in a cell culture incubator with the medium changed every two days. Depending on the initial seeding density, cells will cover the collagen scaffold in 7–10 days. After the collagen scaffold was covered, cells were fixed with formaldehyde, washed with PBS, and then stained with Alexa Fluor 488 phalloidin (Invitrogen) and TO-PRO-3 (Invitrogen). Fluorescently labeled phalloidin is a high-affinity probe for F-actin and TO-PRO-3 is a nucleic acid stain. Confocal images were taken with Leica SP2 confocal microscope (Leica Microsystems, Bannockburn, IL) and 3-D image was rendered using Volocity (Perkinelmer, Waltham, MA). To maintain sterility, all gasket pieces were autoclaved prior to use and the gasket assembly and cell seeding was done in biosafety cabinet.

4. Confocal image of the collagen scaffold with and without cells

After cells covered the whole collagen villi surface, the size of the villi changed. The height of the villi was reduced to about 250 μm, which is about half of the original size (Figure S-4A). This change was not due to the instability of the collagen scaffold, as it remained intact while immersed in cell culture medium for three weeks (Figure S-4B).

Figure S-4 3-D rendered image of collagen scaffold (A) covered with cells and (B) without cells

References

Supplementary Material (ESI) for Lab on a Chip

This journal is © The Royal Society of Chemistry 2011