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Fabrication of a hybrid microfluidic system incorporating both lithographically patterned microchannels and a 3D fiber-formed microfluidic network

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While fabrication of two-dimensional microfluidic systems has become a standardized, straightforward process, fabrication of true three dimensional microfluidic systems remains extremely challenging. There is an increasing demand, however, for three dimensional microfluidic architectures, particularly for tissue engineering applications, and several techniques have been developed to address this need. These techniques range from using complex techniques, such as solid freeform fabrication,^{[1]–[3]} to simpler approaches such as weaving^[4] or making use of multiple layers of lithographically patterned two dimensional fluidic structures. The multi-layer stacking technique, in which multiple layers of two dimensional microfluidic networks are assembled in a vertical stack with vertical connecting vias, has been adopted because it leverages the high-resolution capabilities of lithographic 2D microfluidics, requires no additional hardware, and is relatively straightforward. This technique has been used to form devices for applications such as organ assist devices,^{[5] [7]} microvascular networks and *in vitro* models in both biocompatible, nondegradable

materials^{[8]–[10]} and bioresorbable substrates,^{[11]–[14]} as well as for non-biomedical applications such as solving mathematical problems.^[15] Using this technique, the channel geometry in 2D is determined by the pattern, and the channel density in the third dimension is governed by the layer thickness, number of layers, and size of the vertical connecting pipe. Thus, formation of structures with high channel density in 2D can be easily achieved using the appropriate pattern, but to realize high channel densities in all three dimensions requires large numbers of very thin layers with extremely stringent layer-to-layer alignment techniques.^[16] For high density structures, stacking of many thin layers with high-precision alignment becomes tedious and impractical, and the risk of flow anomalies generated from layer misalignment is high. Recently, nontraditional techniques for fabricating high channel density 3D microfluidic networks have been developed (based on sacrificial melt-spun microfibers^[17] or electron discharge^[18]). These techniques enable the formation of capillary-bed-like structures with vessel orientations in all three dimensions, but forfeit control over the exact channel positions and sizes for scalability and inherent three dimensionality. Here we introduce a hybrid fabrication process that combines lithographically patterned channels with a sacrificial microfiber network to produce a channel architecture that retains a level of organization (due to the lithographically patterned structures) but has improved 3D channel density (due to the microchannel network formed using the microfibers) without the requirement for ultrathin layers or high-precision layer-to-layer alignment. Such a device offers the opportunity to expand the use of multi-layer stacking techniques towards applications requiring high channel density in all three dimensions.

Using the fabrication process outlined in Figure 1, we fabricated a 3D microfluidic network, produced using sacrificial melt-spun microfibers, integrated with a top and bottom lithographically patterned microfluidic system (illustrated in Figure 2a). The “sandwich” structure of the device is illustrated in the 3D reconstruction of microCT data (which has a resolving power just sufficient to visualize microfiber-formed channels) (Supplemental Video 1). Confocal microscopy images of a dye-filled device demonstrate that the patterned channels are confluent with the 3D microfiber-formed fluidic network (Supplemental Figure 1). This is reaffirmed by the observation that when a colored liquid is injected into the inlet patterned channels, the entire device becomes colored, suggesting smooth filling and fluidic continuity throughout the network. The colored liquid eventually exits through tubing attached to the outlet patterned channels in a manner expected for a fully confluent network (Supplemental Figure 2). Video fluorescence microscopy data indicated both flow in the lithographically patterned channels and in the 3D channel network (Supplemental Video 2), further demonstrating confluence.

A difference between the two device types is evident when the ability of the hybrid device to deliver dye to the device volume is compared to that of a device composed of two stacked lithographically patterned channel systems connected by a simple via (Figure 2). When both devices are exposed to the same flow of dye solution for the same time (resulting in diffusion of dye roughly 100 μm into the PDMS from all exposed channel surfaces), the device containing a 3D microfluidic network delivers dye throughout the device volume (as indicated by the cross-sectional images in Figures 2c, 2d, and 2e), whereas the device without the 3D microfluidic network only delivers dye in localized regions (Figure 2b). This is due to the fact that the device with the 3D microfluidic network contains a significantly larger channel surface area that is well distributed through the device material. If one assumes that the regular device was able to deliver soluble material throughout the entire device X-Y plane (85 mm²) and 100 μm in each vertical direction, the total volume addressed by this two-layer device is roughly 51 mm³ (ignoring the connecting large vertical pipe). This value is similar to what can be calculated by thresholding the cross-sectional image at the pixel value of 100 μm diffusion and using this percent coverage to determine the volume

addressed (60mm^3). However, if a similar calculation is made for hybrid devices, including the region containing the microfiber-based network (3mm thick), this value jumps to 268mm^3 . This roughly fivefold increase is obtained by accounting for 85% percent coverage as estimated by thresholding the image at a value that corresponds to $100\mu\text{m}$ diffusion distance. This improved ability to deliver soluble compounds throughout a volume is useful not only for tissue engineering applications, in which delivery of oxygen and nutrients to (and removal of waste from) interior cells in thick cell-laden scaffolds depends on a capillary bed-like vascular system, but also for applications such as gas or ion exchange with liquids.

Resistance testing of the two device types reveals an important distinction between the resulting flow behaviors of these two approaches (Figure 3). The conventional microfluidic stacked device provides a relatively low fluid mechanical resistance, due to the large vertical pipes connecting the two layers. The measured values of flow resistance in the conventional device ($\sim 0.5\text{ psi min mL}^{-1}$) compare well with the value for a simulated two-layer microchannel network ($0.2\text{ psi min mL}^{-1}$) when resistance of the interconnects and tubing of approximately $0.3\text{ psi min mL}^{-1}$ is added to the network simulation value. Moreover, these values of device flow resistance are similar to those measured in lower mass flaps used for reconstructive surgery^[19] (such as forearm flaps, with an average of $\sim 0.7\text{ psi min mL}^{-1}$, and both fibula osteocutaneous and anterolateral thigh flaps, with an average of $\sim 0.3\text{ psi min mL}^{-1}$ [20]). By contrast, the hybrid device exhibits a much higher resistance (over one order of magnitude higher, ranging from $12\text{--}27\text{ psi min mL}^{-1}$) to flow, principally because the large number of vertically connecting channels have very small diameters and do not provide the same fluidic conductance as larger pipes. The smallest channel in the conventional devices has a cross-section of $100\mu\text{m} \times 50\mu\text{m}$, whereas the smallest channel diameter in the hybrid devices is roughly $10\mu\text{m}$, similar to that of a natural capillary. In addition to the much higher resistance values, there is a significant variation between the flow behaviors of the two hybrid devices measured (roughly twofold). Possible explanations for the high variability include the fact that the microfiber network architecture is not identical between hybrid devices, and that differences exist between the volume of sacrificial material, the density of fibers, and the interfaces between fibers and the lithographic pattern. Thus, while the hybrid devices are able to deliver soluble compounds to a much larger volume than the regular multilayer devices due to their increased channel density, this ability comes with the cost of higher fluidic resistance and more significant device-to-device variation. An important aspect of future optimization of the processing conditions for these hybrid devices will be to realize networks with lower fluidic resistance and improved interconnectivity.

In summary, we have integrated lithographically patterned microfluidic channels and a 3D microfluidic network formed using sacrificial microfibers to form a multilayer stacked microfluidic device containing a high density of channels in all three dimensions. These hybrid devices offer an improved ability to deliver soluble material to the device volume but, as expected, exhibit a larger fluid resistance due to the tortuous microfluidic network. These results suggest that an optimized combination of conventional planar microfluidics and 3D microchannels generated using a hybrid approach may be necessary to achieve both the volumetric delivery of nutrients and oxygen and the flow properties required for replication of organ vasculature and organ function.

Experimental

Device Fabrication

Devices were formed in polydimethylsiloxane (PDMS), an elastomer commonly used for microfluidic structures. The templates used to form the lithographically patterned channels

were made from conapoxy (Conapoxy FR-1080, Cytec Industries) by mixing the resin and hardener in a 3:2 ratio by volume, and casting into previously fabricated PDMS molds. After degassing to remove any bubbles, the conapoxy was cured at a temperature of 120°C for approximately 16 hours, and then at 180°C for 2 hours to optimize the physical properties of the mold. After curing, the conapoxy templates were cooled, removed from their PDMS molds, and coated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma Aldrich).

The conapoxy templates were exposed to an oxygen plasma treatment at a pressure of 250 mTorr and a power of 100 Watts for 30 seconds (March Plasma Systems, San Francisco, CA) and then coated with a thin film of sucrose using a spin coater (Headway Research Inc, Garland, TX), with a 10% sucrose solution at a spin rate of 1000 rpm for 30 seconds. This layer allowed the microfibers to adhere to the template surface. The microfiber network was produced using a commercially available cotton candy machine (The Helman group CCM-505) modified with variac control over the temperature and extractor head speed. Store-bought sugar was used to form microfibers, which were sandwiched between two sucrose-coated conapoxy molds under approximately ~50% RH humidity (in dry weather, the devices were briefly placed in a humid incubator to render the sucrose film sticky). PDMS (Sylgard 184, Dow Corning) was mixed with a resin/curing agent ratio of 10:1, degassed, and slowly poured into the area between the conapoxy molds. The PDMS was allowed to wet the templates and microfibers, and when it had fully filled the space between the two templates, the templates were squeezed together to within a separation distance determined by microscope slides used as spacers. The system was allowed to cure for 24 hours at room temperature. When the PDMS had cured, the templates were removed and the device volume of PDMS was cut out of the PDMS slab. To remove the microfibers, the device was placed in a stirred warm water bath for about 2 days. Finally, the device was assembled by using an oxygen plasma process to adhere solid slabs of PDMS to the top and bottom of the device, punching holes and attaching tubing to the reservoirs, and coating the entire device in several layers of PDMS to seal any exposed microchannels.

Device Imaging

The devices were imaged using several techniques to observe the channel geometry and verify flow through the channel system. Micro-computed tomography (microCT) imaging (X-Tek HMXST225) was used to obtain low magnification 3D image data of the channel system. To verify that the channels could be filled, a solution of blue food coloring was introduced into one of the lithographically patterned channel systems, observed to flow through this system, then through the channels produced using the microfibers, then through the other patterned channel system, finally exiting through an outlet port. Channels filled with fluorescein dye were imaged using a Zeiss 510 confocal microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany), and the image stack was reconstructed in 3D using Volocity (PerkinElmer, Waltham, MA). To observe flow, a solution of fluorescently labeled polystyrene spheres (FluoSpheres, Invitrogen) was introduced into a separate device and imaged using video fluorescence microscopy (Zeiss Axiovert 200m).

To demonstrate the improved channel density and ability to deliver small molecules via diffusion from the channel system into the device volume, 150 μ M Rhodamine B solution was flowed through a filled device at a rate of 15 μ L/min for 45 minutes (using a New Era Pump Systems NE-300 syringe pump). The device was then rinsed in a water bath, cooled with liquid nitrogen, sectioned with a cryomicrotome (Leica CM1900), and rinsed with water a second time. Images of the cross-sections were taken with a fluorescence microscope, and indicated that dye diffused roughly 100 μ m into the PDMS during this exposure. A device was fabricated with a single large channel connecting the top and bottom patterned channels instead of the 3D microfluidic network, and exposed to dye under identical conditions.

Measurement of Fluidic Resistance

To determine fluidic resistance, the pressure across the network was measured as a function of flow rate using DI water. Pressure was measured using a Honeywell flow-through pressure transducer (Honeywell, Morristown, NJ) and an Omega DP25B digital meter (Omega, Stamford, CT). A Harvard syringe pump (Ultra, Holliston MA) with a 5mL syringe was used to perfuse the devices at constant flow rates with the device outlet kept at atmospheric pressure. This test was performed at flow rates ranging from 0.1 to 1mL/min in 0.1 mL/min steps in random order, with each flow rate being tested at least twice. The measured pressure was then divided by the flow rate to calculate the network resistance.

Simulations

A model of the vascular network was built in SimHydraulics (Mathworks, Natick, MA) using resistive tube elements to represent the fluid channels. To simplify the model, it was built in layers: first a capillary bed (consisting of the smallest channel network) was modeled using dimensions from the pattern CAD file. The calculated resistance of the capillary bed was used to calculate the dimensions of a resistive pipe with the equivalent resistance characteristics; this equivalent pipe represented the capillary bed in the next level of the model. Using the calculated resistance for each capillary bed, the overall resistance of the network, comprising the series-parallel combination of beds with parallel side channels, was calculated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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((Supporting Information is available online from Wiley InterScience or from the author)).

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Figure 1.

Illustration of hybrid device assembly process. A) A cone epoxy mold is patterned from a silicone master. B) The cone epoxy mold is coated with a thin layer of sugar. C) A melt-spun sugar microfiber network is adhered to the sugar layer on the cone epoxy mold. D) A second sugar-coated cone epoxy mold is placed on top of the microfiber network. E) The space between the two cone epoxy molds is infiltrated with uncured PDMS. F) When the PDMS has cured, the cone epoxy molds are removed. G) The device is placed in a water bath to remove all sugar structures. H) The top and bottom of the channel system are sealed using flat slabs of PDMS.

Figure 2.

A) Illustration of hybrid device architecture, showing two lithographically patterned channel systems (green) connected by a 3D microfluidic network (blue) formed with sacrificial microfibers. B-D) Fluorescence microscopy images of cross-sections of devices that have been exposed to Rhodamine B dye for 45 minutes. B) A conventional two-layer microfluidic device. C) A hybrid microfluidic device containing both conventionally patterned channels and a microfiber-formed 3D channel network. D,E) Higher magnification images of a hybrid device.

Figure 3.

Plot of fluidic resistance vs flow rate for several microfluidic devices. A point is missing for one of the hybrid devices because this device burst after acquiring data at 1 mL/min . The simulated device resistance dataset does not take into account interconnect resistance.