

3D Tissue Culture in a Microfluidic Bioreactor Array

Tissue engineering products have huge potentials for prosthetic applications in therapy. Meanwhile, the technology developed in this vibrant field can also be utilized to create a highly *in vivo*-like microenvironments for *in vitro* applications, such as drug discovery and development, disease models, and personalized clinical drug testing in various cell-based platforms. For example, in drug discovery, cell-based assays are increasingly used for drug target validation and drug ADMET (absorption, distribution, metabolism, elimination and toxicity) studies because cells can give more representative responses to drugs than simple molecular assays and are easier to use in a high-throughput format than animals. However, there are intrinsic drawbacks with conventional *in vitro* cellular tests using 2-D cultures, which lack a 3-D scaffold to support cell growth and proper tissue function and thus are not able to mimic *in vivo* cellular conditions. There has been an increasing number of research works addressing this difference. However, a big challenge in 3D tissue culture is the mass transport barrier within the tissue construct, resulting in a limited nutrient supply and accumulated metabolic waste. Therefore, the authentic cellular response to the tested drug may be camouflaged by the limitations of a metabolic environment. This, however, could be alleviated via perfusion culture. For this purpose, the combination of tissue engineering and microfluidic techniques provides an effective strategy for such applications. In this project, a microfluidic bioreactor array has been developed for long-term culture.

Microfluidic Bioreactor Array

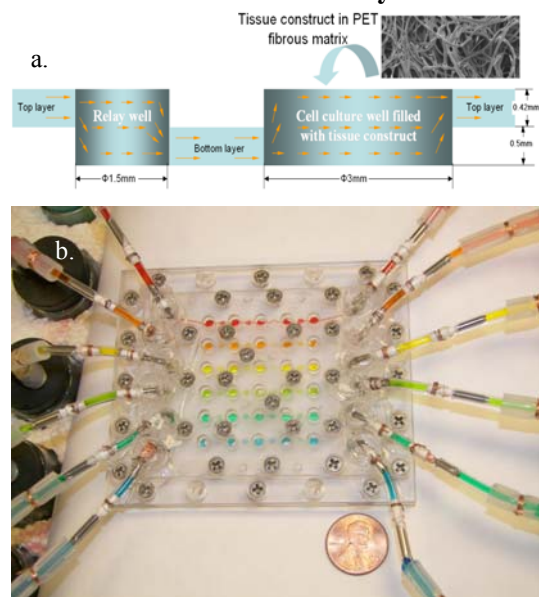


Figure 1. a) A drawing for the designed flow path formed by two layers of PDMS; b) Picture of a packaged microfluidic bioreactor array.

The mold of the device was fabricated by photolithography using a negative photoresist SU-8 on a yang.15@osu.edu

silicon wafer, and the PDMS pieces were made via replica molding. PDMS prepolymer and a curing agent were mixed in a ratio of 10:1 and poured onto the masters before polymerization on a 75°C hotplate for 2 hours. Then the PDMS layers were cut and peeled off of the wafers. The microfluidic bioreactor array had two layers made of poly(dimethylsiloxane) (PDMS) with features facing each other and in close contact to form a 3D fluidic path (Fig. 1a), which was packaged using frame-assisted assembly (Fig. 1b).

Perfusion Culture of Human Colon Cancer Cells

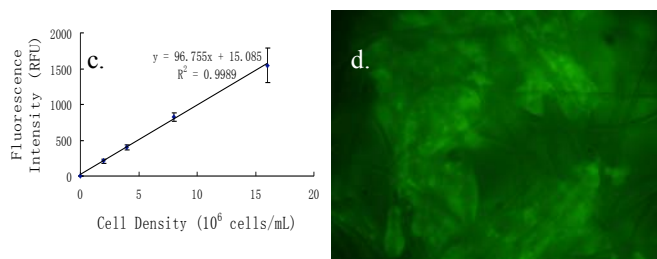
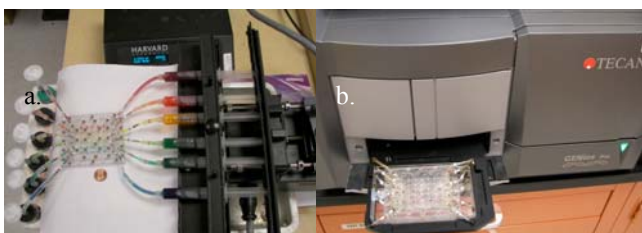


Figure 2. a. Perfusion experimental setup. b. Non-invasive fluorescence intensity measurement with microplate reader. c. Linear correlation between fluorescence intensity and cell density. d. In situ fluorescence microscopic picture of cells grown in 3D fibrous matrix.

A human colon cancer cell line, HT-29 (ATCC: HTB-38™), was maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco), supplemented with 10% fetal bovine serum (FBS, Gibco) in a cell culture incubator at 37°C with 5% CO₂. The HT-29 cells were transfected with PEGFP-N3 (Clontech) using Lipofectamine 2000 (Invitrogen), with which enhanced green fluorescent protein (EGFP) was expressed under the control of cytomegalovirus (CMV) promoter. Six 10mL syringes were autoclaved and filled with cell culture media, which were then seated on a syringe pump (PHD 2000, Harvard Apparatus) (Fig. 2a). PET fibrous scaffolds were seeded with a human colon cancer cell line, HT-29, and the device was packaged using the frame-assisted assembly method. The device was then connected to the six corresponding syringes on the pump, and a waste collector was connected to each line of culture. This process was carried out in a cell culture hood aseptically. Then the bubbles within the microchip were driven out by infusion at a flow rate of 100μL/min. The whole system was placed in a CO₂ cell culture incubator, and the perfusion was set at a rate

of 1.0 μ L/min. The cell proliferation was analyzed once a day with the specific plate definition scheme in the microplate reader (Fig. 2b, c). The perfusion culture was maintained for about 15 days. The *in situ* living tissue culture in the chip was accessible with a fluorescence microscope (Fig. 2d).

After a perfusion culture of nearly 350 hours, the device was dismantled and the culture in individual wells was characterized with scanning electron microscopy (SEM). A high density of cells filled the void space of the fibrous matrix, which can be seen from a top view of the tissue construct (Fig. 3a). Inside the matrix, a cross-sectional view also indicates that high density cells are grown throughout the whole depth of the tissue construct (Fig. 3b). In addition, the colon cancer cells in the 3D matrices exhibits a round shape, which is considered a close resemblance of the *in vivo* morphology of solid tumor cells (Fig. 3c). Even ECM proteins secreted from the tumor cells seem to be present around the cells (Fig. 3d).

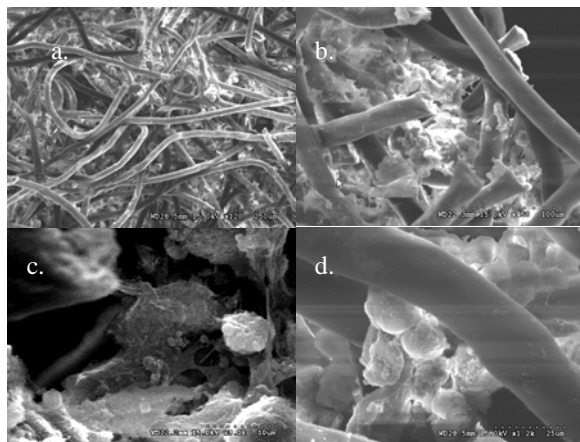


Figure 3. SEM pictures showing the overall high density of cells and the individual cellular morphology in the PET fibrous matrix after long-term perfusion culture in the microfluidic bioreactor array.

Electrospun Nanofibers for Cell Attachment

The PET fibrous matrices showed great capability as tissue engineering scaffolds for high-density 3D culture. However, it does not exactly resemble the realistic ECM *in vivo*. The ECM fibrous matrix has features at several a scale of hundred nanometers. An electrospun nanofiber matrix bears extreme similarity in dimension and morphology compared with *in vivo* ECM. In contrast, a nonwoven PET matrix has micrometer scale fibers, which are even larger than mammalian cells. Therefore, in order to create a highly *in vivo*-like microenvironment, the nanoscale features of real ECM should not be overlooked.

An electrospun polycaprolactone (PCL) nanofiber matrix was supplied by Jed Johnson in Dr. Lannutti's lab from the Nanofiber core technology group in NSEC. In addition, we had samples of electrospun polyamide nanofiber matrix from Donaldson Company, Inc. HT-29-EGFP cells were seeded at a series of concentrations onto the PCL, polyamide and PET matrices. Linear correlation could be obtained between

cell number and fluorescence intensity (Fig. 4a). A cell attachment efficiency study was conducted 2 hours after seeding by measuring the fraction of fluorescence intensity emitted from the cells on the matrices with respect to their initial values (Fig. 4b).

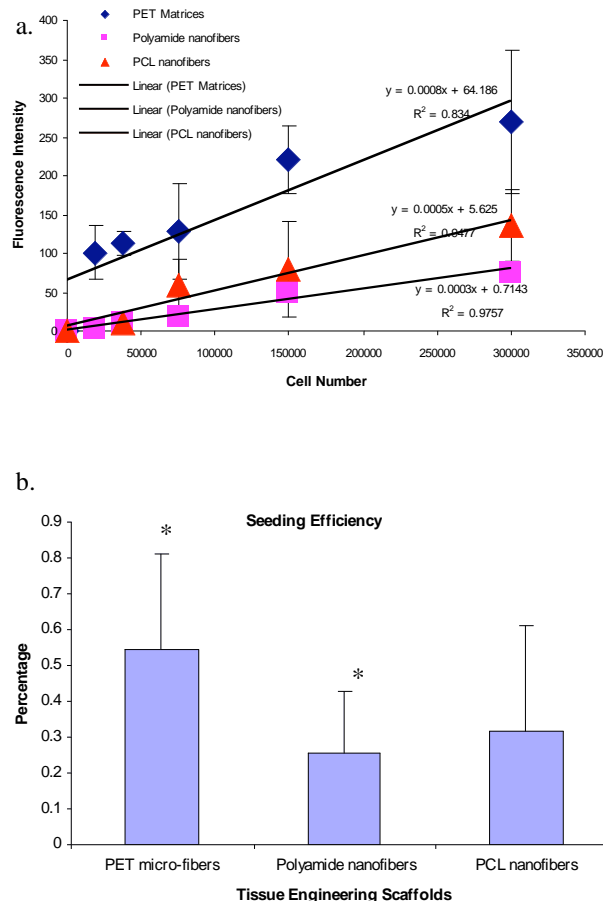


Figure 4. a) The PET microfiber matrix, polyamide nanofiber matrix and PCL nanofiber matrix all exhibit a linear correlation between fluorescence intensity and seeded HT-29-EGFP cell number; b) Comparison of seeding efficiency. The stars indicate p value < 0.05.

Based on these experiments, a challenge has been identified with electrospun nanofibers. Seeded cells form a monolayer on the matrix, and 3D infiltration will take a long time (up to months) to occur because of the biodegradable material used for the matrix. However, this 2D growth is not suitable for the microfluidic bioreactor array. It is suggested that multilayering electrospinning and mixing electrospinning be used for mesoscopic spatial designs of nano- and microfiber meshes for tissue engineering in future work.