

CEM COMMUNIQUÉ

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THE CENTER FOR
ENGINEERING IN
MEDICINE

Massachusetts General
Hospital 55 Fruit Street,
GRB 1401

Boston, MA 02114

Tel: (617) 726-3474

Fax: (617) 573-9471

Cemmail@sbi.org



Biomedical Engineering in Boston

Biomedical engineering is a dynamic and expanding field of engineering, which has the potential to make important advances in biomedical research and health care. The unique combination of basic biomedical sciences together with engineering and physical science generates an intersection where many powerful techniques and tools can be used to solve today's pressing medical problems. The CEM, a center-of-excellence in biomedical engineering research and education, is such an intersection, where, on a daily basis, physicians, scientists, and engineers strive to explore the mysteries of life and to develop diagnostic and therapeutic strategies of the future. Based at the MGH, but with affiliated faculty and laboratories at most of the other Harvard Teaching Hospitals, the CEM looks toward (1) creating a structured framework for cooperative research and educational activities of bioengineers at Harvard; (2) providing outstanding training opportunities in bioengineering and quantitative sciences to physicians, engineers, and scientists at all academic levels; and (3) building a mechanism for the advancement of the discipline of bioengineering and its practitioners within the Harvard community and beyond.

The research programs of the CEM faculty cover a broad spectrum of focus areas in bioengineering, all of which are marked by multidisciplinary collaborations and clinical relevance. Current research areas include: Applied Immunology, BioMEMS, BioNano Robotics, Bio-Preservation, Genomics and Proteomics, Metabolic Engineering, Stem Cell Bioengineering, Tissue Engineering, and Functional Imaging.

One noteworthy trend appears to be blossoming as a result of the CEM's highly innovative and rigorous environment. Over the last seven years, more than 25 former graduate students and postdoctoral fellows have secured high profile academic faculty positions, in part, as a result of their experience at the CEM. This remarkable trend together with the impact of the research performed with the CEM, bodes extremely well for the continued prominence of this dynamic organization.

In this issue Mehmet Toner, Professor of Surgery at Harvard Medical School, and Director of the Microsystems Bioengineering Laboratory discusses the BioMEMS capabilities of the CEM.

Tissue Engineering Profile

Padma Rajagopalan Ph.D.

Dr. Padma Rajagopalan joined the Liver group as a Research Associate in July 2002. She obtained her undergraduate degree at the Indian Institute of Technology, Kharagpur and her Ph.D. from Brown University working in the field of polymer synthesis and modification. Her post-doctoral research at the Department of Polymer Science and Engineering at the University of Massachusetts, Amherst and her subsequent research at General Electric Co. focused on polymer surface-modification and nano-composite materials. She returned to academic research at the Department of Biomedical Engineering at Boston University where she studied cell-substratum interactions. The focus of her research was to quantify the contractile forces exerted by fibroblasts and measure cell motility on chemical and mechanical gradient substrata. She joined the CEM to pursue her interests in tissue engineering. Her current research projects include the design of polymer scaffolds for building multi-cellular constructs and studying the effect of *in vitro* thermal stress on the detoxification pathways in hepatocytes.



Profile continued Bioartificial Liver

Chronic liver failure is a significant cause of fatality in the United States. Bioartificial livers consisting of functional hepatocytes can provide temporary support to patients with fulminant liver failure and save lives of patients awaiting orthotopic liver transplantation. The development of an extracorporeal bioartificial liver is a major focus of the liver group at the CEM. The ability to support a large cell mass without substrate limitations, and the ability to elicit and maintain maximum function of hepatocytes are some of the challenges faced in the development of a viable liver-assist device.

Polymer Scaffolds for Multi-cellular Architectures

The optimal function of a bioartificial liver

depends on the ability to maintain hepatocytes in an environment that resembles conditions *in vivo*. It is well known from previous *in vitro* studies that co cultivation of hepatocytes and nonparenchymal cells is critical for maintaining the phenotype of hepatocytes. To enhance hepatocyte function in liver-assist devices our goal is to build multi-cellular constructs that mimic the structures of tissue *in vivo*. These constructs consist of layers of hepatocytes and nonparenchymal cells (e.g. fibroblasts, endothelial cells) with ultra-thin polymer layers between adjacent cell layers. Polymer layer deposition is accomplished by taking advantage of the chemistry and electrostatic charge on hepatocyte surfaces. Preliminary results indicate that the functional capability of hepatocytes can be enhanced when cultured in multi-cellular layers.

Effect of *in vitro* Heat Shock on Detoxification Capability of Hepatocytes

Heat shock preconditioning of livers has been shown to enhance ischemic tolerance. The induced heat shock or stress proteins are well known as cytoprotective proteins. In our studies we find that the detoxification capability of hepatocytes cultured in collagen double gel configuration is significantly enhanced upon exposure to heat shock for short periods for time. The activity of cytochrome P450 isoenzymes 1A1, 2B1 and 3A1 increased several-fold for hepatocytes exposed to 43°C for 1 hour in comparison to hepatocytes maintained at 37°C. Studies are currently underway to obtain a better understanding of the mechanisms by which heat-shock treatment modulates P-450 enzyme activity.

Microsystems Bioengineering

Mehmet Toner Ph.D.

The CEM microfabrication facility is now fully functional. The key capabilities include photolithography, soft lithography, surface chemistry and engineering, microfluidics, etching and micromachining, and computational modeling. Some of the most utilized capabilities are described below.

Photolithography

The facility is fully equipped to perform standard lithography using various mask and photo resist technologies. Chrome mask is utilized for most processing needs. Both negative and positive photo resists are available. For many applications, the epoxy-based photoresist SU-8 provides an excellent choice with resolution down to 0.2 μm . Figure 1 depicts a typical SU-8 after processing.

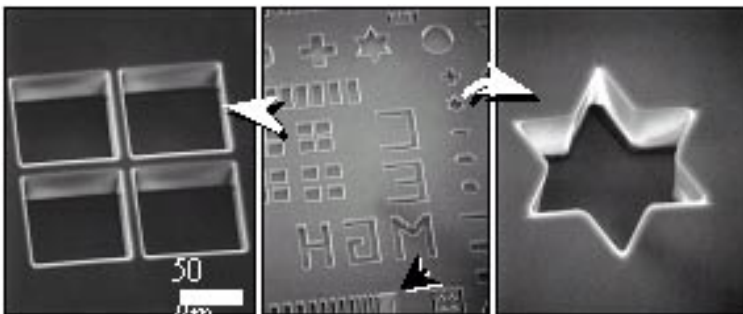


Figure 1. SEM images of a photolithographically patterned 53 μm thick SU-8 layer on a Si₃N₄-coated Si wafer.

Soft Lithography

Soft lithography is a technique that is based on the utilization of elastomeric stamps to create microstructures and/or microdevices. The technique is especially well-suited for biological applications because it can be applied to biologically compatible surfaces and most of the processing can be performed in a regular laboratory without cleanroom needs (except the manufacturing of the master.) Poly(dimethylsiloxane) (or "PDMS") stamps with complex micro-

structures can be fabricated from a given mask multiple times using replica molding. PDMS stamps can then be used in a variety of ways for microfabrication, including microcontact printing, microfluidic systems, and microstencils. Figure 2A depicts a negative replica of the mask shown in Figure 1. Figure 2B is a micro-stencil that can be used for protein and cell patterning.

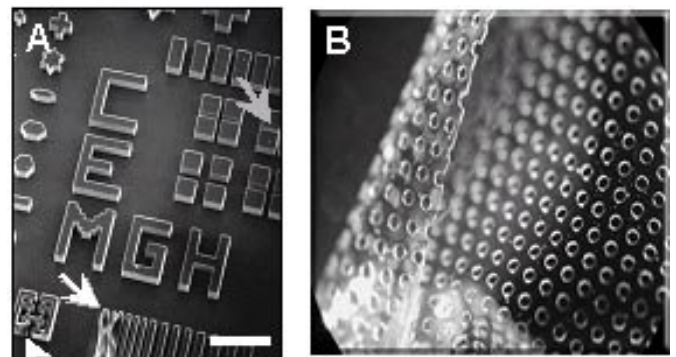


Figure 2. (A) SEM image of the PDMS replica of the sample in Fig. 1. (B) Micrograph of the PDMS stencil with a uniform distribution of 20 μm holes.

Rapid Prototyping

Many of the biologically relevant applications of BioMEMS require a quick turnaround time and inexpensive processing for testing of initial idea(s). Transparency mask technique provides an excellent alternative to conventional photolithography for such applications, whereby an actual microdevice can be prototyped within 24 hours for a cost of <\$40 for the transparency mask. The mask design can be printed onto a transparency sheet using high-resolution (5080 dpi) printers to create photoresist features with features down to 5 to 10 μm . Furthermore, this approach can be easily applied to large surface areas. Figure 3 shows the typical steps for rapid prototyping.

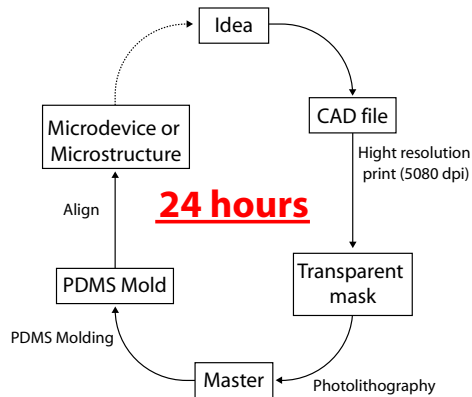


Figure 3. Steps in rapid prototyping

Surface Engineering

In order for the microfabricated surfaces to be “biologically-friendly”, such surfaces need to be modified by immobilization of various ligands or cell-active molecules. There is now an armamentarium of approaches to engineer surfaces with molecular-level control of its properties. Both photolithography and soft lithography approaches are utilized to create spatially defined patterns of biologically relevant surfaces, including self-assembled monolayers (SAMs) on gold, aminosilane SAM on silicon or glass, physisorbed proteins, and immobilized antibodies to cell surface proteins. “Inert” surfaces that do not promote binding of proteins and/or cells can also be made using poly(ethylene glycol) (PEG). The combination of adherent and nonadherent surfaces with micron scale control of their spatial distribution provides a versatile approach to surface engineering. Figure 4 shows examples of surfaces engineered with extracellular matrix proteins or cells.

Microfluidic Systems and Devices

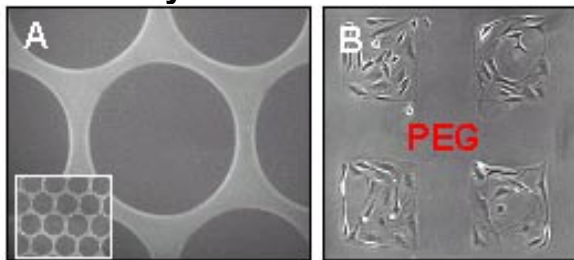
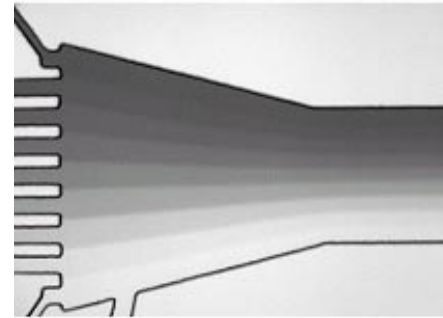


Figure 4. (A) Fluorescent staining of collagen immobilized onto glass substrate using aminosilane chemistry; (B) Keratinocytes patterned onto a fibronectin surface surrounded with PEG-coated nonadherent .

This is a very rich and rapidly growing aspect of BioMEMS. Due to its self-sealing, high oxygen permeability, and inert nature, PDMS has become the preferred mode of manufacturing microfluidic devices in research laboratories. The microstructured PDMS surface can be designed to form a network of channels on the areas where the stamp does not contact the surface. The microchannels can thus be used to deliver fluids to selected areas of a substrate. A very significant added benefit of using PDMS is the fact that it hermetically seals on most surfaces and thus can be applied to all sorts of substrates, including glass, plastics and biomaterials. It also provides the ability to create microfluidic devices to control the microenvironment of cells such as the shape and composition of chemoattractants to investigate chemotaxis. Figure 5 depicts a typical gradient within the cell chamber.



Cell Patterns and Microengineering of

Figure 5. Micrograph of a color dye entering the microchannel from nine ports on the left, each 20 μm . At the shown flow rate, there is no mixing due to laminar flow.

Tissue Units

With the application of microfabrication technology into biology, it is now possible to design surfaces that reproduce many of the critical aspects of the intricate architecture of tissues on a micrometer scale. Patterns of a single cell type individually or in microunits can be achieved by patterning cell attachment sites to specific micro-domains on the substrate. Cells can also be placed directly onto specific regions of a substrate using either microfluidic or microstencil lift-off techniques. Furthermore, using both photolithography and soft lithography mosaic patterns of multi-cellular systems can be easily engineered. Figure 6 shows micropatterned cells and tissues using various techniques.

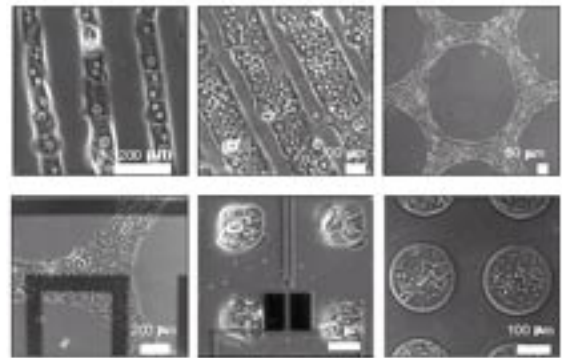


Figure 6. (A) Hepatocytes patterned as a single-cell line on polystyrene culture dish using microfluidic immobilization of collagen onto the surface. (B) Hepatocytes housed in PDMS microchannels of 200 μm (top view). (C) Hepatocytes patterned onto a biodegradable polymer (PMMA) using direct microfluidic seeding of cells onto the substrate. (D) Hepatocytes placed onto a glass substrate and gold electrodes. (E) Hepatocytes localized in an array onto a silicon substrate containing a gold sensor. (F)

Microtextured Three-Dimensional Structures

Microfabrication can be used to create three-dimensional templates for cell and tissue culture. A combination of microchip making, polymer casting, and cross-linking techniques are utilized to create microtextured basement membrane analogues. Figure 7 shows tissue engineering of living skin substitutes with controlled rete ridges to mimic the *in vivo* structure and also to develop a cosmetically acceptable skin substitute.



Figure 7. Microfabricated basement membrane analogue. Left panel shows microfabricated basement membrane analogue integrated onto a dermal equivalent. Middle panel shows the detail of the microfabricated membrane at high magnification. The thickness of the membrane is about 10 to 20 μm and the height is 300 μm . Right panel shows the skin equivalent after keratinocytes are grown on the microfabricated membrane and differentiated by bringing to the air-liquid interface during culture.

CEM Excursions

The Center for Exploration of the Mountains (the “other” CEM) was very busy in 2002. We organized several day hikes, week-long backpacking trips in remote areas, a ski trip, and a sea kayaking trip. Again this year we offer various activities that will suit the novice as well as the experienced alike. Anybody of any age can join in. In the past we’ve had participants as young as 3 years old and groups ranging from 3 to 20 people! For those who have no outdoor experience at all, we especially recommend the Catskills trip in May, which will initiate you to car camping and day hiking. Below is the trip schedule for the remainder of 2003. As usual, a sign-up sheet will be posted, and you may always e-mail the contact person. François.



DATE TRIP SUMMARY AND CONTACT E-MAIL

May. 3-5 Hiking

Shenandoah National Park, Virginia.
Beat the summer crowds. Stay in a cabin or shelter.
Sumatis@eden.rutgers.edu

May 17-18 Camping

Catskills, NY.
Day hikes while staying at a modern (hot showers) campground. This is for beginners...
Fberthia@sbi.org

June 7-10 Backpacking

Adirondack State Park, NY. The High Peaks Region Never disappoints those who like it strenuous.
Vitolo@rci.rutgers.edu

July 11-14 Sea kayaking

Bay of Fundy Natl. Park, NB, Canada.
Impressive tidal phenomena and marine life.
Fberthia@sbi.org

Aug. 16-17 Backpacking

Kilkenny Traverse, Pilot Range, NH.
Never gone backpacking? This easy weekend trip is for you!
Atilles@pol.net

Aug. 30- Sept. 7 Backpacking

Mahoosuc Range, NH. (Alternate: Saddleback Range, ME).
Thru-hike part of the Appalachian Trail.
Vitolo@rci.rutgers.edu

Oct. 4-6 Hiking

Smokey Mountains National Park, NC.
Day hike(s) to explore the park after the BMES meeting.
Sumatis@eden.rutgers.edu

Oct. 10-13 Annual Crag Camp Reunion

White Mountains, NH.
Day hikes in the Presidential Range. Stay in cabin near treeline.
Vitolo@rci.rutgers.edu

TO SUBSCRIBE OR PUBLISH HERE CONTACT:
MATTHEW ROSINSKI PhD
mrosinski@hms.harvard.edu
617-371-4915