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ACS MEETING NEWS

MICROFABRICATION IN CELLULAR MILIEUS

New materials constructed among living cells may help unlock workings of neuronal systems

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JASON B. SHEAR LOVES TO MAKE movies. One of his clips shows a ball tethered to a post through a cable and swinging round and round. Another—a favorite of his three-year-old son's—captures little critters flitting about a surface while an Etch-a-Sketch-like line surreptitiously propagates, forming an enclosure that eventually corrals one of the hapless creatures

These scenes, created in labs at the University of Texas, Austin, where Shear is an associate professor of chemistry, demonstrate some of the components of analytical tools he envisions will help characterize dynamic living systems, such as neuronal networks. The ball and chain might be used to pin a single neuron and make it accessible to probes or experimental treatments. The Etch-a-Sketch-like line might be used to confine neuronal secretions so they can be measured accurately.

At the American Chemical Society's recent national meeting in Washington, D.C., Shear described his approach to understanding neurons during a Division of Analytical Chemistry session.

Although neurons can be characterized in various ways, such as by their electrical properties, Shear is going in a different direction: "We've taken the approach of microfabricating materials in the presence of cells so that we can observe how a cell is developing; measure materials it contains or releases; and try to alter development so that the cell is guided, for example, to make contact with another cell at a site we might find of interest," he said.

In Shear's lab, light is key to building microstructures in living systems without damaging the cells. Using proteins as monomers, Shear and his coworkers fabricate balls, chains, fences, tubes, wires, and more by photon-activated protein cross-linking. They aim to build on-site, at well-defined coordinates relative to living cells, and use the structures to carry sensors or development-modifying agents or to perform other functions.

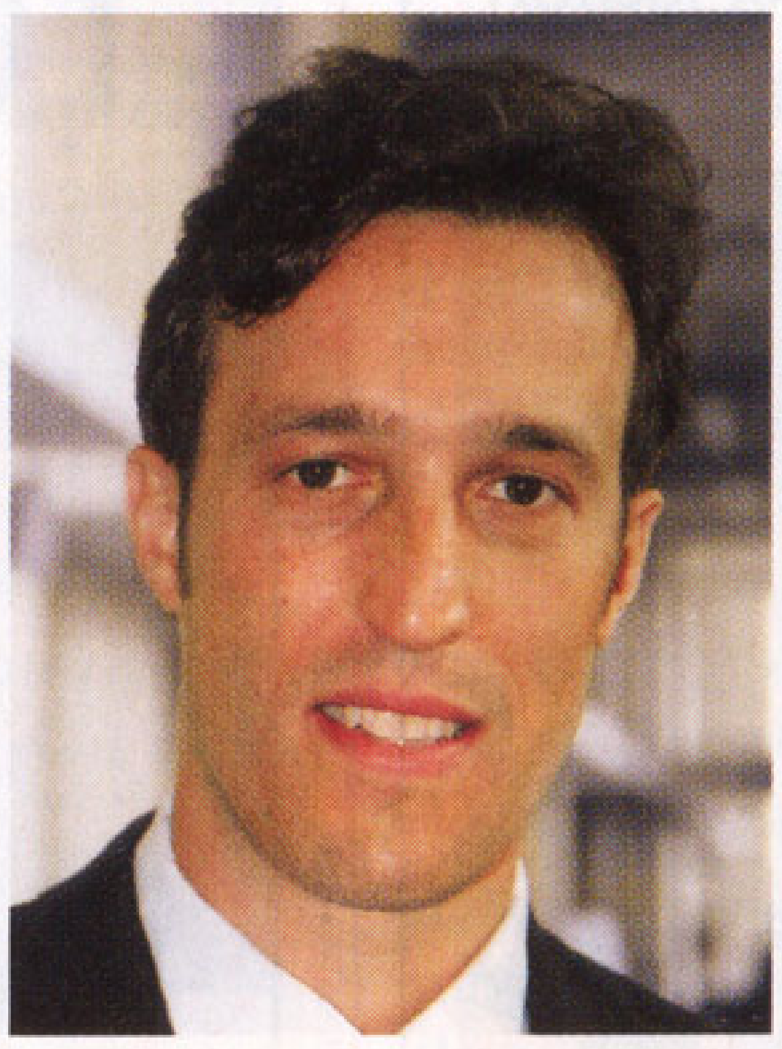


PHOTO BY MAUREEN ROUHI

Shear

The Etch-a-Sketch-like lines, for example, erect physical barriers to growing neurites, which are the extensions that neurons use to communicate. Shear and graduate students Bryan Kaehr and Richard Allen have forced a neurite to change direction after contact with a photofabricated wall, about 0.75 µm high, made of cross-linked bovine serum albumin deposited near a growing neuron. The neurite is redirected to form a contact with a neuron different from the one that would have been contacted had growth followed the original trajectory.

"We don't know if this is a functional synapse, but the connection persisted for at least 90 minutes," Shear said. The experiment indicates that "it's possible to influence how neurons grow and develop together." The demonstration, although rudimentary, lays the ground for probing how neurons would respond to other, more complex developmental cues, he suggested.

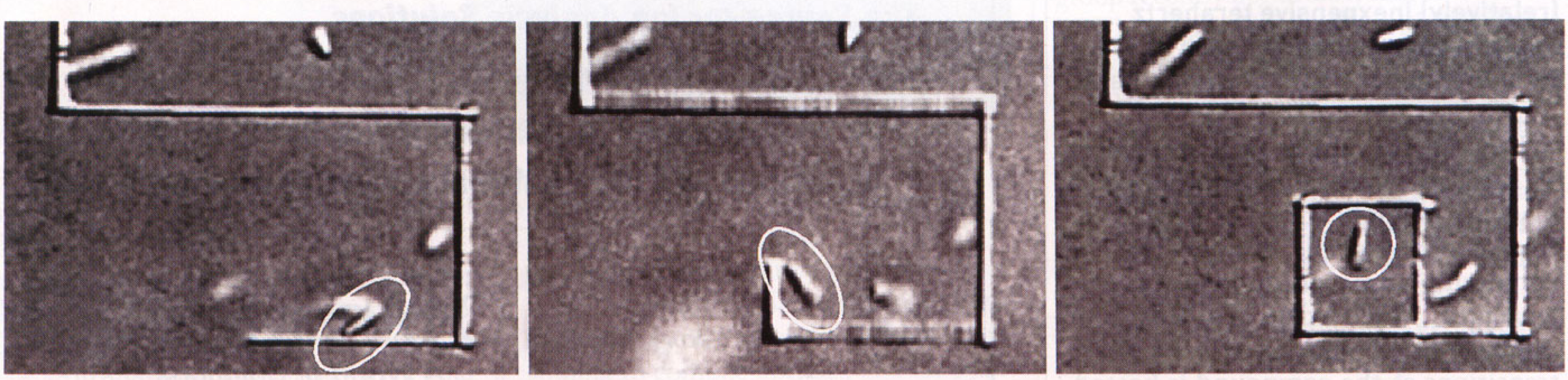
SHEAR'S GROUP also has been working on ways to use the photofabricated microstructures to make electronic materials.

"With a little bit of work, we think we'll be able to place electronic components within cellular environments," Shear said. "One might envision placing electrodes that could sense the release of electroactive components at single synapses."

In this area, Shear is collaborating with his electrochemist colleague Keith J. Stevenson. They and graduate students Ryan T. Hill and Jennifer L. Lyon have fabricated electronic components by depositing gold nanoparticles over cross-linked bovine heart cytochrome c.

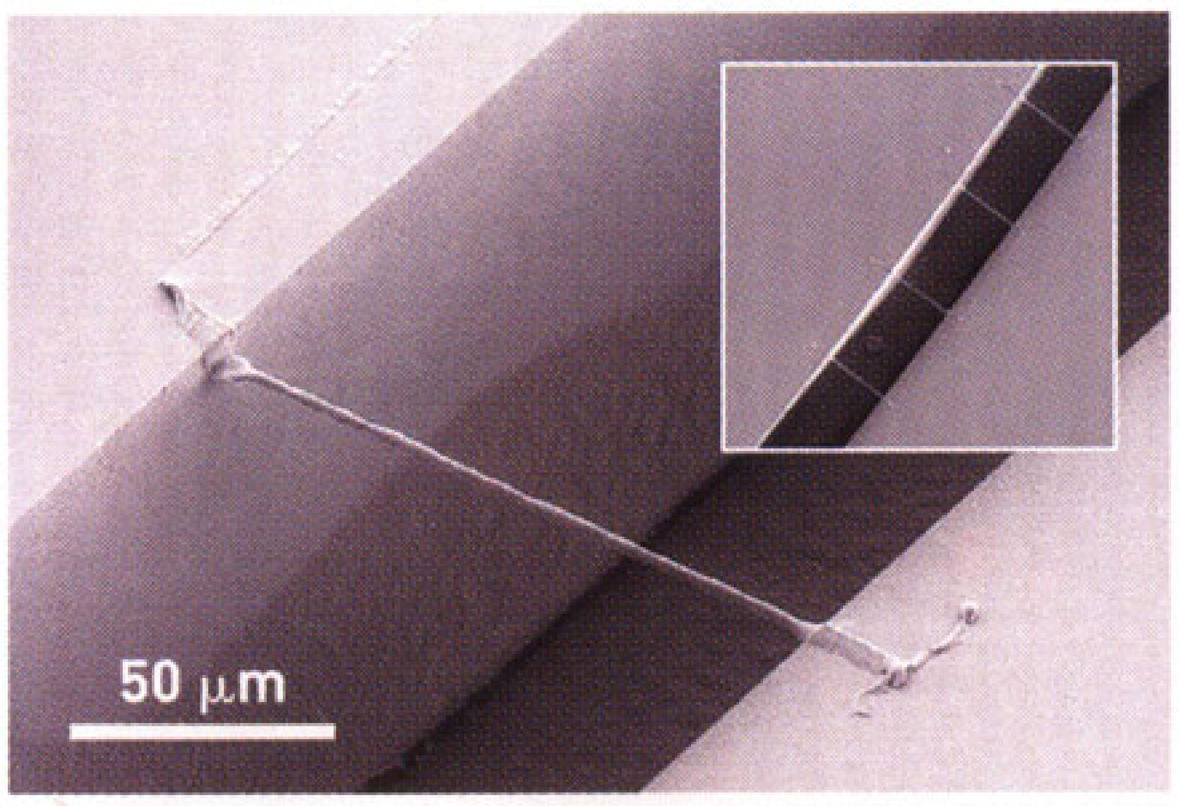
Bovine heart cytochrome c is positively charged under neutral conditions. To make the gold nanoparticles stick to the cross-linked matrix, the researchers coat the nanoparticles with a protein that would be negatively charged at neutral conditions. After the nanoparticles are deposited, they are allowed to grow. The result is a highly conductive surface over a biological support. "We can get conductivities that are not quite as high as those for gold wires but are still very good—much greater than what you can get with conductive polymers," Shear said.

The biomaterials can be used in various ways. Positioning the conducting structures to make contact with or merely be near neurons would help studies of electrical field ef-


COURTESY OF JASON SHEAR

MOVIE CLIP **One of Shear's movies shows the photofabrication of a protein microstructure in the midst of live bacteria so that it eventually encloses one bacterium (encircled in the images). The diameter of the protein "line" is less than 0.5 µm. The time elapsed from the left frame to the right frame is two seconds.**

fects on neuron growth. On the other hand, "if instead of growing conductive structures, we simply use the proteins coating the nanoparticles, we would create well-defined, chemically reactive regions within a cell culture environment," he added.



COURTESY OF JASON SHEAR

HIGH WIRE **A cable photofabricated from bovine serum albumin bridges two coverslips. Structures such as the series of parallel cables shown in the inset can be used to guide the growth of neurons in three dimensions.**

Shear's group has demonstrated this concept with horseradish peroxidase-coated gold nanoparticles deposited on cross-linked bovine heart cytochrome c. In the setup, horseradish peroxidase converts a fluorogenic molecule to a fluorescent molecule in the presence of hydrogen peroxide. With a working system, "you can see streaks of fluorescence coming off, which we can measure in various ways," Shear said. The ability to deliver particular enzymes to different microstructures could be used to influence neuronal development through enzyme-catalyzed production of neuroactive compounds or to detect neurotransmitters, he explained.

TAKING ADVANTAGE of the avidin-biotin binding leads to a more general approach to creating chemically reactive regions, according to Shear. This receptor-ligand binding is one of the strongest known. A cross-linked matrix fabricated from avidin that retains the ability to bind biotin would allow deposition of almost anything into the biomaterial as long as it is attached to biotin. Shear; Allen; and Rex Nielsen, another graduate student in Shear's group, have used the avidin-biotin association to generate chemical gradients in cell cultures and to assemble catalytically active nanoreactors.

At the ACS meeting session, the session chair—Jonathan V. Sweedler of the University of Illinois, Urbana-Champaign—introduced Shear's talk not by the title Shear submitted but by an alternative of his own: Shear Fantasy. "The idea of building dynamic structures around living cells as the cells grow and respond to their environment is something I would not have thought possible," he explained. Shear is "several steps ahead of what other people are doing."

Research in his lab does involve "a bit of fantasizing," Shear admitted in response to Sweedler's play on words. But what's really exciting is what's ahead, when researchers start using the techniques created in his lab to understand the characteristics of neuronal systems in ways that have not been possible before.

Already, Shear is collaborating with research groups to characterize well-defined neuronal circuits whose growth is guided by techniques his group developed. One goal is to explore use of the Shear route to biomaterials to develop neuroprosthetics.

"Where a nerve is severed, you'd like to regain functionality," Shear told C&EN. "We believe the capabilities to fabricate well-defined physical, chemical, and electronic materials in situ allows us to envision ways to devise reconnections that haven't been possible." ■