One-Upping Nature's Materials

Striving for designer substances that build themselves from individual molecules

By JESSICA GORMAN

Before he gets down to the nitty-gritty business of discussing protein structure, Shuguang Zhang teaches a little history to his bioengineering class at MIT.

Civilization's earliest materials were biological, Zhang tells them. People used skin, fur, and bone for making clothing and shelter and for hunting down food. Later, they used stone. Then, bronze. Then, iron. Eventually, society reached what Zhang calls the plastic age, and in the past 30 years, the silicon age.

It's a fair enough history lesson. When Zhang turns to the present, he tells his students that they live on the leading edge of a new era that harks back to the days of prehistory. He calls this the age of "designed biological materials."

In other words, civilization has come full circle . . . with a twist.

In the last decade, Zhang and many other researchers have sought a return to materials of the categories employed by living things, but they want to do one-better than nature. Biology's material inventions, taken as is, however, rarely hit the mark for high-tech applications. So, scientists often have tried to recreate or mimic the substances' good aspects in nonbiological materials, such as plastic or nylon.

In recent years, however, scientists have focused on designing small biological units that assemble themselves into complex structures. The researchers anticipate that these self-assembling materials might become degradable scaffolds for growing cells into custom-made tissues and organs. They could also serve as time-release devices for drugs encapsulated within them. Beyond biology and medicine, the materials might even lead to microscopic computers or other advanced applications.

One of the most enthusiastic proponents of this developing field is Zhang, who started his career as a card-carrying biologist. While studying biology in China in 1979, he read an article in SCIENCE NEWS about DNA research conducted by Alexander Rich at MIT (SN: 12/22&29/79, p. 420). Inspired, Zhang headed in 1980 to the United States, where he eventually wound up as a researcher in Rich's lab. Zhang's work with Rich led to the discovery of an unusual yeast protein, zuotin, one portion of which would self-assemble into a silk-like sheet (SN: 5/15/93, p. 316).

Since that finding, Zhang's work has come to center on materials. Last year, he organized the first self-assembling peptide conference, now a biannual event in Crete.

Zhang wants to build on the inherent benefits of biological substances, especially their compatibility with the human body for medical applications. There's plenty of incentive, he says.

The plastics and other artificial materials that are often used to help repair tissue or deliver drugs can cause an immune or inflammatory response, Zhang explains. And animal-derived natural materials, such as collagen, could transmit viruses or other pathogens, adds Todd C. Holmes of New York University.

Within the past few years, Zhang, Holmes, and their colleagues have designed many new self-assembling materials by determining which aspects of peptides, or protein fragments, are necessary for them to self-assemble into sheets, films, and other structures. The researchers have coaxed skin, liver, bone, and pancreatic cells to grow on many of the resulting materials.

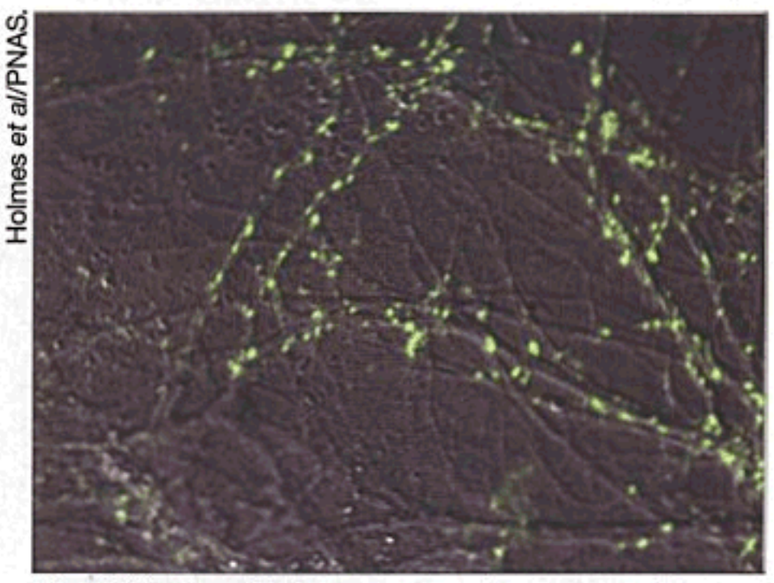
One of Zhang's biggest hopes is that researchers someday will be able to grow replacement tissues or organs from scratch on custom-made peptide foundations that will later dissolve harmlessly in the body.

An even greater challenge: Could a peptide scaffolding inserted into the body direct recovery of an injured nervous system? The spinal cord and brain have notoriously resisted repair, but new substances, which the researchers refer to as biological materials, might pave the way for potential treatments.

The early signs are promising. Last summer, Zhang, Holmes, and their coworkers reported in the June 6 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES that self-assembling peptides do in fact support nerve cells, also known as neurons. What's more, the neurons' extensions, called neurites, which are necessary to neuronal function, also grow along the peptides. Synapses, the essential connections through which nerve cells communicate with one another, formed as well.

Fostering nerve cell growth wasn't easy. "Some neurons will die if you look at them sideways," says Holmes.

The body appears to tolerate the new peptide materials. When researchers injected the peptides into rats' leg muscles, they saw no immune or inflammatory response, Holmes says. Preliminary results from tests in which researchers injected the peptides into rat brains also have indicated that they are well tolerated, he adds.



Holmes *et al*/PNAS

Peptide scaffolds support nerve cells from a rat brain. The fluorescent-green dye indicates active connections between the cells.

The next step for the researchers is to determine if they can transplant structures made of these materials into animals, says Holmes, and then whether the peptides can help repair peripheral nervous system damage. Potential treatment of spinal cord or brain damage is a higher hurdle much further off, he says.

Robert Langer, an MIT polymer chemist who specializes in medical applications, notes that the extensive history of research and development in biological applications of polymer-based materials, such as plastics, will probably make it easier in the near future to continue using them. Still, he's optimistic about the work of researchers developing self-assembling

peptide-based materials. "I think they're very clever about engineering them to do different things," he says.

Researchers of diverse interests have been falling into the expanding interdisciplinary field that Zhang is so excited about. Princeton chemist Michael H. Hecht, for example, wanted to push nature's limits even before he entered the materials fray.

Over the past decade or so, Hecht's lab has designed proteins. "Why be limited by merely those proteins that are here on Earth, in humans and cows and yeast and bacteria?" Hecht asks. "Why not say, 'If I need a protein for a particular purpose, I'll just design one tailor-made for my purposes from scratch?'"

He feels the same way about the new peptide-based materials. "Hopefully, in the long run, one can engineer these or design them for your particular purpose, rather than settle for what nature's already done," he says.

About 3 years ago, Hecht was creating a large library of novel proteins when he caught the materials bug. He and his colleagues had one rule for their otherwise random collection of novel proteins: Peptide sequences must include alternating hydrophilic, or water-compatible, and hydrophobic, or water-avoiding, amino acids.

When the team finished making their "parallel universe of proteins," as Hecht calls it, electron microscopy pictures revealed that the new proteins self-assemble into fibers in water, regardless of their actual amino acid sequence. The peptides' hydrophobic amino acids were burying themselves on the inside of the fibers.

"The bottom line is that, despite great sequence differences, they all seemed to form these fibers," says Hecht, whose team published their results in the Sept. 28, 1999 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES. "So, that suggests that what they share in common is sufficient to predispose them to self-assemble into these fibers."

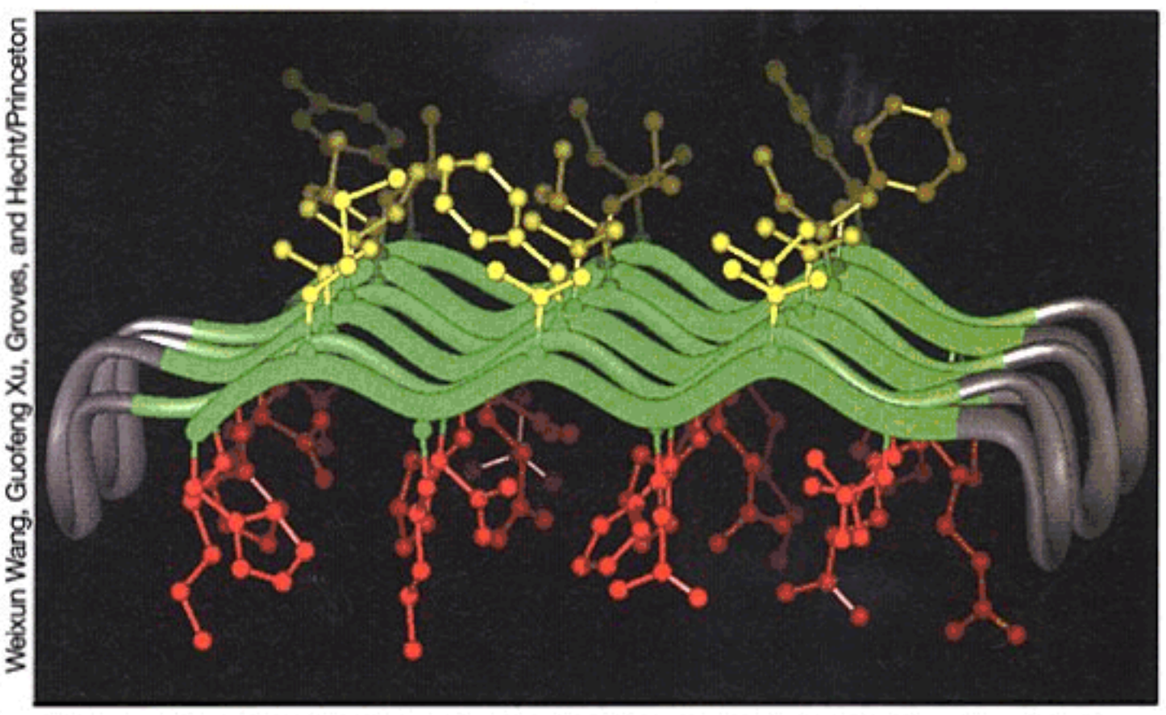
With the extensive new protein library in hand, a team led by Hecht and his Princeton colleague John T. Groves has used a common technique to create a variety of two-dimensional peptide sheets. In unpublished work, the team dissolved each protein in a solvent that they then layered on the surface of a small tub of water. As the solvent evaporated, the peptides organized themselves on the water's surface, with their hydrophobic amino acids sticking up and the hydrophilic ones down. Then, the team pushed the peptides together.

"All the molecules are now lined up, self-assembled into a monolayer on the surface," says Hecht. "They're all likely fit together like tiles on the floor."

In even more recent work, a team including Hecht, Ilhan A. Aksay, and Dudley A. Saville, all at Princeton, has taken single members of the new protein library and made a layer of each on graphite. In this case, graphite provides a surface for the hydrophobic parts of the peptides. Graphite, which has a hexagonal structure, seems to provide a template for the self-assembly of proteins on its surface, says Hecht. Atomic force microscopy images showed proteins assembled in the shape of stars of David, he says.

The motivation for that work comes from nature's own materials, Hecht adds. The abalone shell, for example, intersperses proteins between layers of inorganic minerals to create ultrahard substances (SN: 11/25/00, p. 350).

Jeffery W. Kelly of the Scripps Research Institute in La Jolla, Calif., entered biological materials research as an outgrowth of his lab's main focus: developing strategies for neurodegenerative diseases similar to Alzheimer's. In these diseases, abnormal protein in the brain self-assembles into plaques called amyloid fibrils. Kelly's team wanted to understand this process on a molecular level. "It got us to thinking that if peptides have a propensity to do this, maybe we could take advantage of this property," says Kelly.



Weixun Wang, Guofeng Xu, Groves, and Hecht/Princeton

Molecular model of a newly designed protein at an air-water interface shows a hydrophobic face that contacts the air above and a hydrophilic one touching the water below.

His group has now designed several different biological materials from their basic components. To direct the product's shape, the scientists place one or two synthetic amino acids which aren't found in biology, in a string of natural ones. The somewhat-artificial peptide is called a peptidomimetic.

In the June 7 JOURNAL OF THE AMERICAN CHEMICAL SOCIETY (JACS), Kelly's group reported using a peptidomimetic to create a variety of structures. The process starts with the peptidomimetic molecules, which spontaneously assemble in a solution to become a structure that can be seen with a microscope or even the naked eye.

The shapes that Kelly's group created include tubes, ribbons, and fibrils, which play a role in diseases related to Alzheimer's, says Kelly. The researchers can control which shape they get by manipulating solution conditions such as temperature, pH, and salt concentration.

Like Hecht, Kelly is interested in creating self-assembling structures that incorporate inorganic crystals. As a first step, Kelly and his colleagues formed peptidomimetic monolayers on water. These monolayers could then provide a surface on which crystals of cadmium sulfide grow, the researchers reported in the Aug. 11, 1999 JACS.

Kelly first approached the mineral work, he says, because he was interested in human bone growth, which occurs at protein surfaces. "We rationalized that we could do a similar thing . . . . and we prove in this JACS paper that you can in fact do that," he says.

"However . . . bone growth in humans is incredibly complicated, and it involves lots of different cells, and it's probably naïve to think that you could just inject a material like this and achieve bone growth," Kelly stresses. "Although, stranger things have happened."

Biologist Susan L. Lindquist at the University of Chicago grew interested in materials research while she was studying a yeast protein similar to the self-assembling amyloid proteins of neurodegenerative diseases.

"When [the yeast protein] changes shape, it attracts other proteins," Lindquist says. "It's sort of like flypaper." It forms aggregates that are highly ordered. Lindquist and her colleagues described their investigation into the molecular basis of this conformation change in the Aug. 25 SCIENCE.

By looking at the yeast protein in isolation from other proteins, Lindquist found also that it has properties that could be useful to materials researchers. The protein forms very tough fibers that resist disruption by detergents or other agents. "And unlike most other amyloids, which have the tendency to clump up on top of each other and stick, this one seems to form very beautiful, nice fibers that aren't all that sticky," she says.

In the last year or so, Lindquist's yeast protein has become the centerpiece of a University of Chicago research project, that includes two chemists and two physicists. Chemist Norbert F. Scherer leads the effort, which aims to build self-assembling nanoscale devices using altered versions of the yeast protein.

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"We're attempting to do things like put metal-binding groups on them and molecules of certain light-harvesting and -release properties," says Lindquist. "We're aiming toward taking advantage of biomaterials for nanofabrication with the idea that evolution has really honed the properties of this protein for millions of years already."

"It's given us some properties that are really quite wonderful," she says. "Then, we can go in with deliberate genetic manipulations and change it further . . . for our benefit."

Nevertheless, adds Lindquist, "we still have a long way to go before we can build a miniature computer chip out of proteins." (SN: 11/25/00, p. 350).

Zhang used to think it was a joke when someone called him a materials scientist. No longer. He's happy he has diverged from molecular biology's well-worn path. "It's better to take a hike, where you make discoveries, than to drive on the freeway," he philosophizes.

However, Zhang makes sure that he doesn't hike alone. He's collaborating with biologists, chemists, and engineers around the world to work on as many applications of the new materials as possible. "Now, I think the only limit is the limit of our imagination," he says. □