Mendelson's Web

A microbiologist spins a tale of mutant bacteria

By RICHARD LIPKIN

Bending, arching, writhing in the dim light, the slender forms pause for a languid moment. The onlookers lean forward in their seats. Then, slowly, the entangled bodies resume their tortuous ballet. A hush falls over the room of intrigued scientists at the Boston Marriott. The speaker, a man whose broad-brimmed black hat hides a shock of white hair, clicks off the monitor of the time-lapse videotapes. "That," says Neil Mendelson, a molecular biologist at the University of Arizona in Tucson, "is *Bacillus subtilis* making macrofibers."

For more than 25 years, Mendelson has focused on this tube-shaped bacterium — which, at barely 4 micrometers long, ranks as one of the smallest material makers known. In 1975, Mendelson stumbled upon a mutant form of the bacterium, which divides end to end, budding out from its tips to form linked chains. With cylindrical bodies that twist like a screw, these bacteria also have a propensity for fashioning helical filaments. By twisting, coiling, and bending back on themselves, these colonies of bacteria create fibrous webs, eventually weaving themselves into what Mendelson calls "macrofibers" — strands that look like Lilliputian ropes.

By themselves, macrofibers grow only a few millimeters long. Yet Mendelson has learned to pull long, delicate "bacterial threads" from cultures rich in these filaments. With the dexterity of a classical musician (he plays the bass), Mendelson devised a way to tease strings from frothy webs. With a gentle tug, meter-long threads — composed of some 50,000 bacterial cells lined up end to end — emerge from as little as 10 milliliters of cultured cells.

Fascinated by the physical properties of these bacteria — their hearty cell walls made mostly from the two polymers peptidoglycan and teichoic acid — the biologist wondered: Why not try to make something from this material? After all, bacterial cell walls are strong, porous, flexible, and biodegradable. Tinkering further, he found that crystals would seed themselves and grow within the ethereal threads. With the soft structure of the fibers as a matrix, minerals evolved into crystalline networks, using the bacterial cell wall as a "structural backbone." Mendelson calls this new mineralized material "bionite."

The field of biomimetics — in which scientists emulate nature's own processes for fashioning high-quality compounds — is emerging with force. Increasingly, scientists are turning to biology for innovative ideas about how to make natural materials. From the nails on our fingers to the bones in our hands to the seashells on a beach, nature has proved to be an extraordinary material manufacturer. Slowly but surely, though, scientists are learning to imitate nature's tricks.

"Hidden in the current stream of chemical publications is mounting evidence of a quiet revolution," said Stephen Mann, a chemist at the University of Bath in England, in the Oct. 7 NATURE. Part of that revolution involves biomineralization, the process by which hard bones emerge from soft tissues. In bone, an organic gel forms a lattice in which mineral crystals take hold, spawning strong, light skeletons.

With the help of biomineralization techniques, scientists may be able to fashion substances with potential uses in electronics, magnetism, sensing devices, and so-called intelligent materials, which can adapt to their changing environment. "Where better to look for archetypes of these systems," Mann says, "than in the interactive, homeostatic milieu of biology?"

Using bacteria — which help digest the food we eat, decompose waste, and enrich soil — to make materials is largely virgin territory; mineralizing bacterial fibers is unique.

"People don't usually think of bacterial cells, especially their cell walls, for making materials or as models of material design, mainly because they are so small," Mendelson says. "But the bacterial cell wall is one of the most successful structures in the biological world. The question I'm interested in is this: Can we make this material into something, and if so, what would its properties be? And are there any material design principles that we can learn from these cells? I think the bacterial cell wall, and these macrofibers in particular, can provide a very useful model of a self-organizing, self-assembling system for a material in nature."

Bacteria make their cell walls from a curious material. Electrically active and physically resilient, the spongy, porous gel can shrink or swell, bend, twist, stretch, or shear, then resume its normal shape. An affinity for charged particles, Mendelson says, enables the walls to retain mineral deposits and form crystalline structures.

Macrofibers and threads arise when normal bacterial cell growth hits a glitch. Cell walls fail to cleave normally after cell division, and the bacteria link to form chains. As cells keep dividing, their cylindrical bodies warp, winding into helical coils — "like a twisted telephone cord," Mendelson says.

Each macrofiber goes through a primitive life cycle. A single cell spawns a chain, which winds into a double-stranded helix. That coil bends and twists into a four-stranded, then eight-stranded, then 16-stranded fiber, coalescing into tightly bundled, rope-like strands. A closed loop completes each end. Through experimental manipulation, Mendelson has grown different strains of the same bacterial species, controlling the degree of twist and whether the helix turns to the left or right.

"We're getting very close to a complete description, a robust model, of this unique self-assembly process," Mendelson says. "This model is important biologically because it explains a particular type of morphogenesis, whereby we may soon be able to control the biology, chemistry, and physics involved. Such models are very unusual in an organism this small."

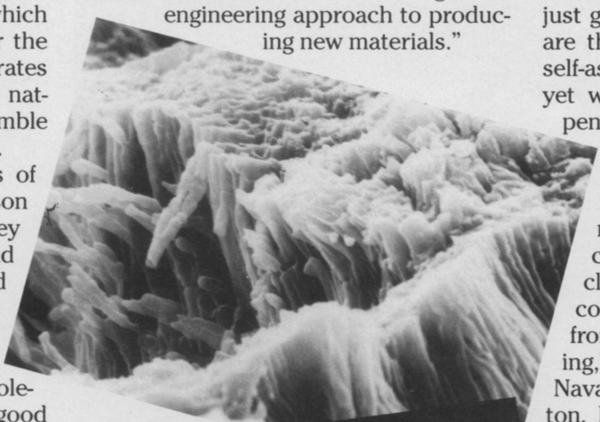
That nature repeatedly creates materials that twist and supercoil — from tiny proteins and DNA to the yarns, ropes, and cables spun from natural filaments — suggests that some fundamental process may affect how these diverse structures form. Such questions have brought mathematicians together with physical chemists to look at the "knot topology" of these biological shapes. If common physical processes do engender such related forms, then researchers as varied as cellular biologists and materials scientists may together find such knowledge valuable and illuminating.

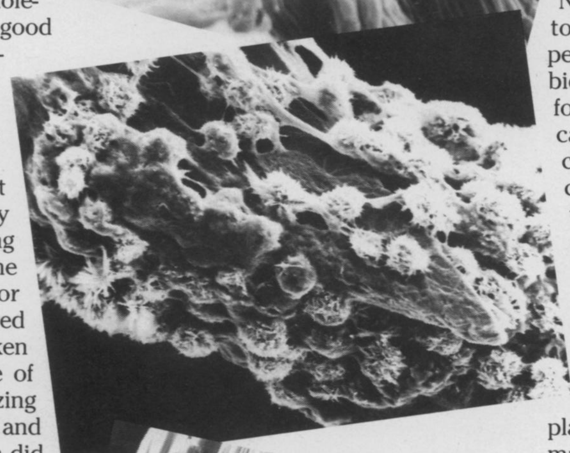
"The building plan for these bacterial fibers is unique in biology. It's a plan based entirely on the physics of twist and supercoiling," Mendelson adds. "That's an intriguing design strategy, one that can show us how nature self-assembles a complex material. Maybe it will provide a strategy for a material we can build. Exactly how that [material] might be used, it's too early to say."

From simple macrofiber filaments come the bacterial threads, which provide natural templates for the mineralization process that generates bionites. Made from the bacteria's natural casing, bionites vaguely resemble fiberglass in appearance and texture.

Struggling to measure the forces of twisting and supercoiling, Mendelson has dragged filaments through gooey fluids, studied time-lapse videos, and measured the folding, bending, and "snap-opening" motions of filaments as they wind and unwind. To see why the bacteria's gelatinous cell walls react so well to charged molecules — why they serve as such good seedbeds for some minerals — he soaked the bacterial webs in salts of calcium and iron. He found brittle precipitates clinging in bundles.

Then, adding copper, he saw that copper deposits showed up only after the threads had dried. Retaining their sinewy, fiberglass feel, the threads took on a silvery, blackish, or greenish hue. Mendelson discovered that the minerals had indeed taken hold within the caged architecture of the bacteria's cell walls. Scrutinizing more closely, he observed that iron and calcium held more tenaciously than did the copper — further clues to the cell walls' properties. Whereas one iron bionite grew dark red and opaque, other versions brewed orange, translucent, cube-like crystals.





These scanning electron micrographs demonstrate three bionites. The top and bottom images show two types of KDP bionites: mineralized filaments, above, and hollow tube crystals, below. The middle image reveals a calcium and phosphate bionite with ball-like and platy crystals.

Mendelson

Since bacterial webs must be handled gingerly while forming, Mendelson concocted a way to grow them in funnels, draining one growth medium from below while adding another gently from above. By changing the ratios of calcium, phosphate, potassium, and other ingredients in the bacterial stew, both "platy" and "ball-like" crystals enmeshed themselves in the threads.

Intrigued by the idea that bionites might have unique nonlinear optical properties — such that they could prove useful in fiberoptics or microelectronics — Mendelson brewed a web in potassium dihydrophosphate. Drawing and incubating the airy strands, he watched "KDP bionites" emerge — long, hollow, tubular crystals protruding from the mineralized bacterial walls.

"These are very peculiar structures," he says. "It's just more evidence that from these tiny bacteria we can obtain macroscopic structures, exploiting both the cell's natural materials and a genetic engineering approach to producing new materials."

But what could one possibly do with such bacterial threads, mineralized or otherwise? "It's possible that the bionites could lead to some interesting applications," says Ilhan Aksay, a materials scientist at Princeton University. "For instance, this area of biomineralization may teach us how to make better ceramics. Today, most ceramics are made by packing powder very tightly — like a grocer packing apples." But for ceramics made of very small particles — less than 100 nanometers — scientists can't control the assembly process. "It will be hard to make better ceramics without biology, because biology uses the most efficient path," he adds.

Studies of biomineralization could also foster a deeper knowledge of how bones grow, says Aksay. For biomedical use, "this could be the most important outcome of biomineralization research. This whole area will be a big technology in the next century. There's more here than just great potential. It already exists. We are the proof. Our bones are made by self-assembly and biomineralization. And yet we barely understand how it happens."

The notion of using bacteria, which exist in such abundance and diversity, to make materials may be an idea whose time has come. "I think the general idea of cloning bacteria to excrete particular compounds, or making materials from cellular materials is very exciting," says Joel Schnur, a chemist at the Naval Research Laboratory in Washington, D.C. "There are some very clever people exploring the possibility of using biological templates and mineralization for a purpose. The big question is, How can we use this knowledge for rational control? Can we make things like biocompatible implants, or teeth, or better ceramics? In the long run, the answer is probably yes. But we're not there yet."

What if biomineralization could be a controlled process? A merger of genetic engineering and mineralization — in which genetically tailored organisms pop out sought-after templates — could spawn some remarkable materials. What better way to make a compound than to grow it in a controlled environment or to program tiny servants of nature to make it themselves?

Such dreams are not so far-fetched. "These are exploratory times in biomolecular materials," says Hagan Bayley, a biologist at the Worcester Foundation for Experimental Biology in Shrewsbury, Mass. "It's nice if you get a product. But it's important to keep basic research going to show us how nature makes material. In terms of basic science, Mendelson's work is very unique. As far as I know, he's really out on his own. But the knowledge coming out of this work really is new knowledge. Thousands of people are working on new superconductors. But only a few people are doing something like this."

"If people only think about applications, like making silk hats from bacteria, then research like this will become very stultified," says Bayley. "We don't always want to direct science by applications. Although we don't know exactly how this work will be applied, a lot of people have the gut feeling that something interesting will come of it." □