Naturally Inspired

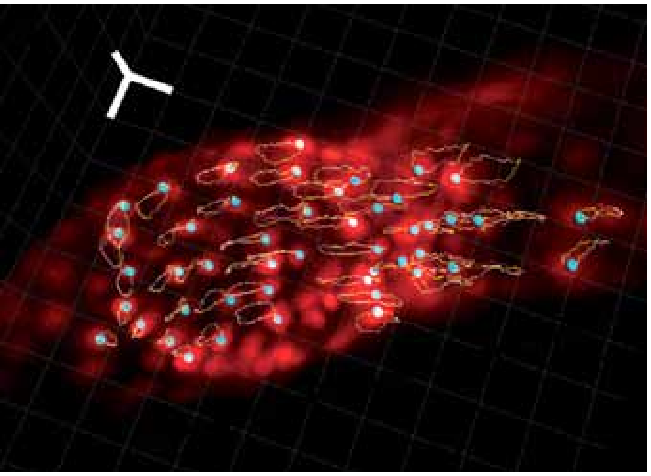
By Kimm Fesenmaier

In the Charyk Lab of Bioinspired Design, even an embryonic zebrafish heart can be an engineering muse. Here, Caltech engineers continue the time-honored tradition of teasing apart nature's tricks and borrowing the best bits.

When Mory Gharib (PhD '83) studies zebrafish, he sees straight to the heart of the matter. Literally. Since zebrafish embryos are almost transparent, it's quite possible to peer right through them. But when Gharib and his group started studying *Danio rerio* about a decade ago, they had a feeling there was more to its tiny pulsing heart than meets the eye.

Since then they've picked apart the properties of the fish's embryonic heart, and are now applying what they've learned to attack problems as diverse as ringing in the ears and overheated electronics. They've even developed the first pump built entirely from biological building blocks.

The striped, pinkie-sized zebrafish has become a mainstay for developmental biologists—it was one of the first animals to have its entire genome sequenced; it reproduces in large numbers and has a short life cycle; and since its see-through embryo develops outside the mother, the changes it undergoes as it matures are easy to follow. Gharib was introduced to this



An unlikely muse: The embryonic zebrafish heart is little more than a tiny tube of cells, but it gets the job done. This three-dimensional reconstruction shows fluorescently labeled heart-muscle cells and traces their movements over the course of two cardiac cycles. The Y-shaped scale bar indicates 20 microns in each direction.

little marvel by biologist Scott Fraser, director of the Rosen Bioengineering Center, whose confocal microscopy lab Gahrib uses. "We now have a very fruitful collaboration between the two groups," Gharib says.

Gharib is an aeronautical engineer, not a developmental biologist, so he's interested in species like the zebrafish for other reasons. He likes to steal their tricks, one-up them by enhancing what Mother Nature has accomplished, and find new applications where the tricks could come in handy.

"We can actually be more clever than nature," Gharib says. "We can get inspired by nature and use engineering to come up with better functions. Just look at 747s—they fly from LAX to La Guardia much more efficiently than any bird could." That's why Gharib runs the Charyk Laboratory for Bioinspired Design.

In the case of the zebrafish, the group homed in on a very early stage of the species' development, when its heart is little more than a short tube of cells. By studying the patterns of flow created in and by the tube, Gharib and his team determined that the action wasn't driven by peristalsis, as had widely been assumed. Peristalsis uses a traveling wave of contraction to move the fluid along—think squeezing a toothpaste tube from the bottom up to push the contents onto your brush. Instead, they found a much simpler mechanism, first described in 1954 by German cardiologist Gerhart Liebau and which the Gharib lab has dubbed "the impedance pump."

All you need for an impedance pump is a flexible, fluid-filled tube connected at both ends to less flexible tubes—even tubes made of the same material will suffice, as long as the walls are thick enough to make them substantially stiffer. The key is to make the transition abrupt, because the point where the wall suddenly becomes rigid acts as a reflector, bouncing the pressure wave back into the flexible section of the tube. By compressing the flexible section in just the right (off-center) location at just the right rhythm, the reflected and newly generated waves interfere constructively to create a pressure gradient that drives a flow through the system.

Though the pump is simple, its fluid dynamics are complex. Thus the researchers in Gharib's lab built scaled-up pumps in order to tinker with their parameters and to discover the ins and outs of their function. The first mechanical pumps were centimeters in diameter—small, but still a hundred times larger than the zebrafish's tiny heart tube. Since then, they've been scaled back down. Some models were simple tubes, made with a two-centimeter length of latex tubing and a couple of glass capillaries. Others were more sophisticated, constructed from micromachined metals or silicone and built to lie flat on a tabletop.

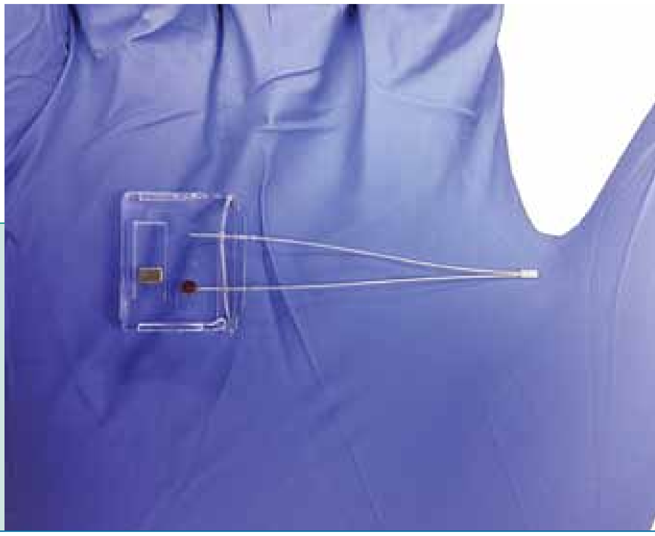
Since impedance pumps don't have valves, there are very few moving parts to break down. Some of the group's creations have managed to go through more than 200 million cycles, which is to say two weeks of continuous duty, without any degradation of performance.

The impedance pump's elegant simplicity is seductive. Clearly, it can operate on a very small scale—nature only needs a handful of cells to make one. "With something like peristalsis, you need to coordinate the motion of many different cells all contracting in succession. But with an impedance pump, all you need is a simple band that's continually beating at one frequency," says Derek Rinderknecht (PhD '08), senior research scientist in aerospace and a member of the Gharib lab. "This allows you to have a very simple way of moving fluid around at a very early stage in the fish's development."

The pumps are also astonishingly efficient. "Taking advantage of resonance allows you to have a much higher output for any given input," Rinderknecht says. "That means less energy is required."

Armed with a solid understanding of the physics at play, the researchers were ready to put their new tricks to work. "We knew we had a 'platform technology' that could hold value for many different areas of medicine," Gharib says. "These pumps could be useful pretty much anywhere you need localized, controlled delivery."

The inner ear, for example. A tiny, implantable pump could deliver a



A prototype impedance pump for treating chronic ringing in the ears nestles in Rinderknecht's gloved palm.

Right: In these frames from a video, water droplets do skateboarding tricks on a carbon-nanotube-coated surface.

Left: Grad student Hesham Azizgolshani watches the workings of a pump he made from biological building blocks.

steady, long-term trickle of medicine to help treat a variety of hearing disorders, and the Gharib team has partnered with pharmaceutical giant Sanofi to develop just such a device.

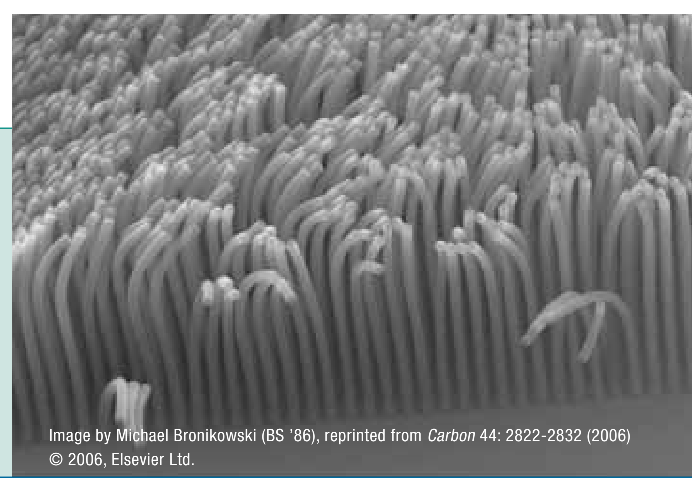
The pilot version is designed to alleviate ringing in the ears. Chronic tinnitus, to use the medical term, affects at least 20 million Americans, according to the National Institutes of Health. It's also becoming a common disability in the military, where loud explosions in close proximity are unfortunately a part of life. Drugs, devices to mask the "sound" of tinnitus, and neural stimulation have all been tried as remedies, with varying levels of success, but there is currently no cure. Part of the problem lies in the fact that tinnitus can spring from several causes. Some cases appear to originate in the inner ear; others in the nerves and brain regions associated with it.

Rinderknecht is building an impedance pump that could be implanted in the mastoid bone, close to whichever tissues are affected. "The tinnitus project has been a good chance to go from a pump that works on a bench top to an implantable, proof-of-concept device," Rinderknecht says. "That's where we're heading right now. We're starting to lay the groundwork for eventually, hopefully, moving into some sort of clinical setting." The prototype version has just been completed, and now awaits testing.

The Gharib group has also found nonmedical applications. In the hot field of electronic cooling, for example, many researchers are looking for low-power ways to dissipate or redistribute the heat generated by the processors, circuits, and other components of our ever-shrinking electronics. The lab is now developing a circuit-board overlay, thin as a credit card, that would circulate a fluid over the board's electronic hot spots, carrying heat away to cooler areas.

"For electronic cooling, the unsteady flow that these pumps create could be a good thing," Rinderknecht says. "It can be used to move heat more uniformly into the fluid. It's almost like sloshing the contents of a cup in order to mix them up."

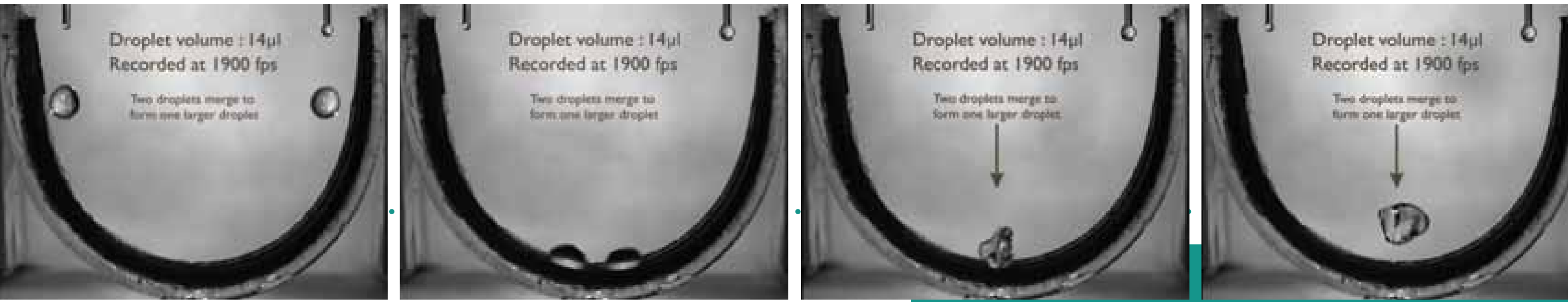
While these ventures may be efforts to one-up Mother Nature, another project could be described as an attempt to tune up Mother Nature—to fix something biological that isn't working properly. Toward that end, grad student Hesham Azizgolshani has actually built a "living" impedance pump using heart cells and intestinal tissue from rats. He strips the intestinal cells from the tissue, leaving a tubular scaffold that he repopulates with heart-muscle cells.



Right: An electron-microscope image of a carbon-nanotube array. Each tube is about 40-millionths of a meter long.

Far right: Manipulating the nanotubes' degree of hydrophobicity can create gradients across the array that will drive a fluid sample placed in the center into different wells for analysis. This chip is called "the Zen garden."

Image by Michael Bronikowski (BS '86), reprinted from *Carbon* 44: 2822-2832 (2006)



"We wanted to show that it's possible to make a pump fully out of biological materials," Azizgolshani says.

He plays a piece of video captured by a high-speed camera mounted on a stereomicroscope. Sure enough, the pump is pulsing, driven by the compression of a ring of cells around the tube. But this isn't enough, he says. "I can't just claim that I have made a pump. I have to verify and characterize its function."

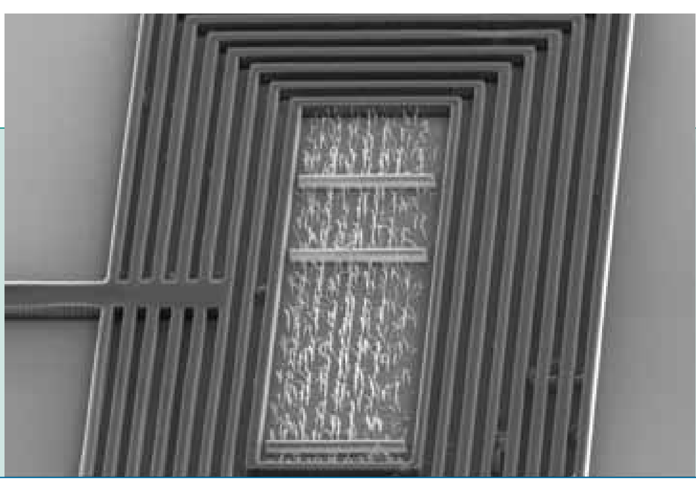
He starts a second video; this one features silver-coated glass particles suspended in the fluid within the tube. The particles slosh back and forth, so it's hard to tell if they are actually *flowing*. But when Azizgolshani plays the video in fast forward, a net left-to-right flow is apparent.

"We started from a biological system, we learned from it, and after we felt we were confident and comfortable in our understanding, we decided it was time to try to make our own biological pump," Azizgolshani says. "I think one of the beauties of this project is that we are closing that circle."

Currently, this pump is only a proof of concept. In his best effort so far, Azizgolshani has managed to keep a pump going in a bioreactor for two weeks. But eventually, durable, long-lasting pumps made from a patient's own cells could be implanted into human veins. This would assist diabetics, for example, who often suffer from poor circulation, and who would benefit greatly from "booster pumps" that would help move blood back to the heart from the peripheries.

"Instead of trying to get the body to accept our man-made materials, which can easily be rejected, the goal is to use the biological materials that are available to us," Azizgolshani says. "The possibilities are limitless." E&S

Morteza "Mory" Gharib is the Liepmann Professor of Aeronautics and professor of bioinspired engineering. He joined the faculty at Caltech in 1992, and he currently serves as one of the Institute's vice provosts. The Caltech-Sanofi nanopump project is funded by a Sponsored Research Agreement under the Caltech-Sanofi Alliance Framework. The nanotube work is supported by the Charyk Foundation and the Fletcher Jones Foundation.



SUPER-HYDRO-PHOBIC-LOTUS-ACTING-NANO-TUBULES

Zebrafish aren't Gharib's only muses. There's also the lotus leaf, which is exceptionally good at repelling water. Many of Gharib's students are working with carbon nanotubes, minuscule rolled-up graphite lattices that look, at the atomic level, like hollow bundles of molecular chicken wire. The superlatives fly when scientists talk about these tubes—it's not uncommon to hear them described as the strongest material ever made, or the most promising. But the descriptor grad student Adrianus Aria (MS '08) has focused on is "most hydrophobic."

Some arrays of carbon nanotubes are more water-repellant than others, and Aria has found out why. It's all in how you grow them—if their surfaces get slightly oxidized, the oxygen atoms attract water molecules. So Aria developed a recipe for cooking the arrays in a vacuum chamber to get rid of any stray oxygen atoms. The result? Superhydrophobic nanotubes.

Like the flower itself, the lotus effect lasts only a few days. But if Aria can figure out a way to make the effect more or less permanent, the potential applications are enormous. A ship with a nanotube-coated hull, for example, would knife through the water with almost no drag at all, consuming far less fuel and perhaps carrying much more cargo.

Aria can also make the nanotubes *less* hydrophobic, by treating them with ultraviolet light in the presence of air. "That means I can control the wetting properties," he says. That could come in very handy if, for example, you want to package a water-soluble drug in a waterproof container in order to ensure delivery to the target organ.

A video of water droplets bouncing off Aria's nanotube arrays or rolling down their hydrophobic surfaces—during one segment, it's hard not to envision skateboard champs in a half-pipe—recently showed up on YouTube. Within about two months, it had garnered 400,000 hits. Gharib smiles as he plays it. "Sometimes we just like to have fun, too."