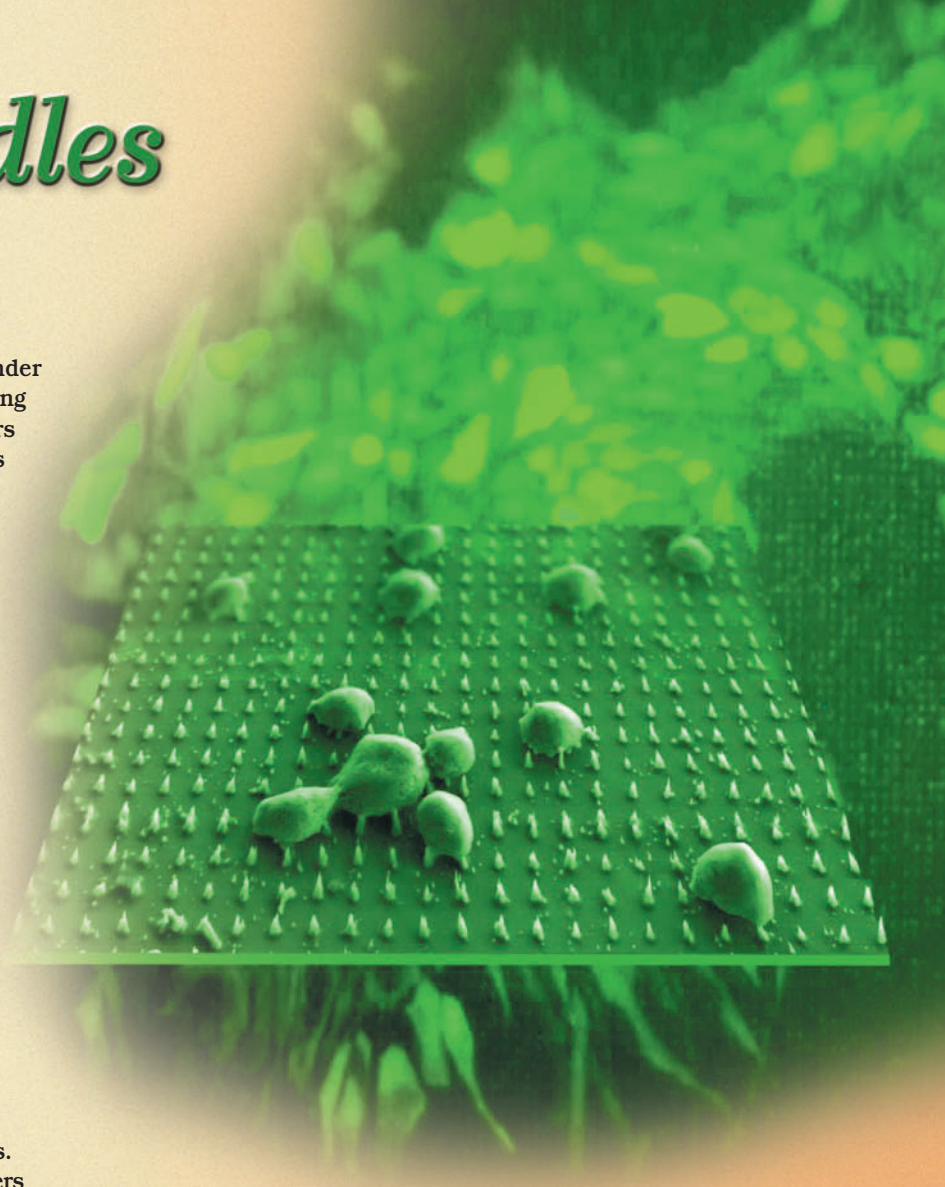
Nano-needles to Cells

An ORNL group is finding a way to get under your skin and coax your cells into pumping out a gene product that's good for you. The researchers hope to make a tiny patch dotted with painless nanofibers that would needle your cells into becoming a specialized protein factory.

Imagine you are diagnosed with an acute bacterial infection that, if left untreated, could cause a severe peptic ulcer and ultimately gastric cancer. You might be prescribed a course of antibiotics and told that if you miss even one dose, the treatment would be useless. What if you could simply wear a patch that coaxed your own cells, already intimately coupled with your circulatory system, to produce a blood-borne peptide that destroys the invading microbe? Ideally, you could wear the patch during treatment and remove it when the infection is eradicated, thus eliminating pills and the potential for missed doses.

This mode of gene therapy might be possible using a recently demonstrated drug and DNA delivery technique. Harnessing vertically aligned arrays of carbon nanofibers as parallel injectors, researchers in ORNL's Molecular Scale Engineering and Nanoscale Technologies (MENT) Group and Life Sciences Division have demonstrated massively parallel delivery of macromolecules to groups of mammalian cells. They have shown that living cells impaled by the nanofibers take up the small molecules of a fluorescent dye, propidium iodide, from the surrounding solution. Shortly after fiber penetration, however, the cells can reseal and exclude the dye. Larger molecules, such as gene-containing DNA, may be delivered by physically impaling cells upon DNA-modified nanofibers.



Living cells impaled on carbon nanofibers. In background a colony of cells penetrated earlier by DNA-modified nanofibers express green fluorescent protein.

"The DNA can either be adsorbed onto the nanofiber tips or linked by covalent bonding," says Tim McKnight, a researcher in the MENT Group in the Engineering Science and Technology Division. "In both cases, when fibers are introduced into cells, the delivered DNA can be expressed, even when it remains tethered to the nanofiber scaffold." In an ORNL seed-money-funded demonstration, expression resulted in the production of a green fluorescent protein within targeted cells.

An array of DNA-modified carbon nanofibers could prompt cells to produce a needed protein.

With this technique, the introduced genes can be tethered to the nanofiber scaffold, potentially allowing them to be withdrawn from the manipulated cells when the gene products are no longer required. Upon removal, the cells would revert to their normal behavior, and the temporary genetic change imparted to those cells could not be inherited.

"We are seeking funding to explore the use of this technique for controlled delivery of genes to tissue, including potential delivery through skin," says McKnight. "It could provide a whole new approach to gene therapy."

The aim of gene therapy has been *permanent* gene insertion to provide missing genetic machinery to cure a patient of a specific disease or deficiency. However, permanent insertion can cause undesired side effects, as recently reported for retroviral-based gene therapy patients in France. Problems arise when delivered genes insert into and disrupt other necessary genetic elements within the cell. As such, gene therapy normally is limited to patients with life-threatening conditions.

"The use of scaffolded genes might reduce the potential for unwanted insertion and provide a potential control mechanism to return patients to their original condition if necessary," McKnight says. Although this approach will not achieve permanent changes sought through conventional gene therapy, the controllable nature of fiber-mediated delivery may increase the safety and potential use of gene-based therapies to treat non-life-threatening conditions.