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GREEN VACCINES

DELIVERING DRUGS WITH PLANTS, DR. HENRY DANIELL AIMS TO SAVE LIVES

OPPOSITE: Henry Daniell, PhD, joined Penn Dental Medicine this June as Professor, Departments of Biochemistry and Pathology, and Director of Translational Research. Pictured in his lab where therapeutic proteins are introduced into plant cells and the specially engineered plants are grown, harvested, and encapsulated. This plant-based system has been shown to be effective in producing and delivering vaccines, insulin, and in the treatment of autoimmune disorders.

YOU MIGHT SAY that Dr. Henry Daniell, who recently joined the faculty of Penn Dental Medicine, has a green thumb. The plants he grows, however, are far from your garden variety greens. Packed with human genes, his specially engineered plants offer a novel platform for producing and delivering pharmaceuticals.

Dr. Daniell, a plant molecular biologist, pursued basic science research after earning a biochemistry Ph.D. from Madurai Kamaraj University in his native India. Yet he began to think more expansively about his work upon recognizing what he perceived as a human-rights injustice: the sky-high costs of medications taken for chronic or lengthy illnesses. Drugs costs soar in part due to complex production and delivery systems, which may involve fermentation, purification, sterilization, injection, and cold storage and transportation.

“Interferon, a common cancer drug, for example, costs \$30,000 to \$40,000 for a four-month treatment, and a third of the global population earns \$2 or less a day,” says Dr. Daniell. “To me, there is something morally not right about that. If you have something



that saves lives, you have an obligation to make it available to everyone. We won't solve the high cost of pharmaceuticals unless we solve the cost of production.”

That's why, when Dr. Daniell got his first faculty position two decades ago, he formulated an idea for new ways of making proteins for human therapeutics: producing them in plant cells.

His outside-the-box thinking has since turned lettuce leaves into drug-delivery systems, with results that have the potential to make disease treatment and prevention affordable and accessible to a global population—and, possibly, even more effective. Now, as Professor, Departments of



Biochemistry and Pathology, and Director of Translational Research at Penn Dental Medicine, Dr. Daniell is working to take his plant-based medicine platform from the lab to the clinic, and to begin saving lives.

A NEW MODEL FOR MEDICINE

Dr. Daniell arrived at Penn Dental Medicine this past June from a position at the University of Central Florida (UCF). There, he established a reputation as a path-breaker and attained the title of Pegasus Professor, the institution's most prestigious honor given to faculty members, for "outstanding accomplishments in teaching, research, and service." He was also the only Board of Trustees Chair in UCF's medical school and is a foreign member of Italy's National Academy of Sciences, only the 14th American to be inducted. (The first was Penn founder Benjamin Franklin, inducted in 1786.)

These honors stem from a simple, if innovative, idea. "Our concept is to grow a plant containing a therapeutic protein, put the plant material in a capsule, and deliver it orally," explains Dr. Daniell.

Over the last decade, Dr. Daniell and colleagues have produced several hundred proteins of pharmaceutical interest—candidates for insertion into plants that could then be encapsulated and easily administered. In experiments in model organisms, they've had great success at tackling some of medicine's most daunting conditions. In 2012, Dr. Daniell and colleagues published a paper in the *Journal of Plant Biotechnology* describing the creation of lettuce plants engineered to express a protein that stimulates the pancreas to produce insulin. Mice fed capsules of the freeze-dried plant material produced insulin and had normal sugar levels in their blood and urine—the appearance of a functional cure for diabetes. What's more, the capsules could be stored at room temperature for up to 15 months and retain their potency. Similarly, a 2010 publication in the National Academy of Sciences' journal, *PNAS*, demonstrated the ability to block severe immune reaction and death in hemophilic mice. Dr. Daniell's lab has also developed oral vaccines against polio, cholera, malaria, tuberculosis, anthrax, and plague.

"We've shown that we can deliver vaccines, we can deliver insulin for diabetes, and we can even use this to treat autoimmune disorders by teaching the immune system to tolerate certain drugs that can cause severe immune reactions," he says.

Dr. Daniell's technique also has the potential to navigate the blood-retina barrier as well as the blood-brain barrier—a critical step in treating neurological diseases like Alzheimer's. In fact, Dr. Daniell's lab has had promising findings related to the plaque formed in Alzheimer's brains. And his approach to manufacturing insulin boasts an advance over currently available insulin as it provides the complete, three chain protein; current formulations are missing the C chain.

The path from idea to application, however, was not simple or quick. Dr. Daniell and colleagues have methodically developed and tested each step of the system of plant-based therapeutics: from developing proteins of interest, to designing molecular tags that direct the proteins across epithelial barriers to reach the bloodstream or immune system, to introducing those proteins into a plant cell (via a "gene gun" specially designed by DuPont to operate using helium gas), and finally to growing, harvesting, freeze-drying, powdering, and packaging the engineered plants in capsules that can be easily administered and easily stored.

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ABOVE: The encapsulated form makes drugs produced in this way easy to administer and shelf-stable, easing distribution to remote areas of the world. Capsules can be stored for up to 15 months.

OPPOSITE: Proteins are introduced into plant cells via a "gene gun," which operates using helium gas. Dr. Jin Su of the Daniell's lab prepares to place a lettuce leaf into the device.



In addition to offering an injection-free alternative to taking medicines—a bonus for diabetics who might otherwise face 60,000 injections of insulin in their lifetime—this shelf-stable system also addresses a shortcoming in current vaccine production. Because vaccines contain pathogens—even if they are killed or inactivated—they must be refrigerated to ensure the organisms don’t reproduce and pose threats to individuals receiving the vaccine.

“Many vaccines are based on ‘killed’ or inactivated viruses, but there’s no such thing as one hundred percent killed,” says Dr. Daniell.

Dr. Daniell’s system, in contrast, uses only proteins from pathogens, rather than the entire organism, to “teach” the immune

system to recognize them as dangerous. Thus the required “cold chain” of refrigeration is eliminated, saving a step that increases the cost of vaccines and makes them difficult to transport to remote areas in developing nations where electrical connections are scarce.

PLANT ADVANTAGES

Plants have several properties that lend themselves well to producing biomedical molecules and carrying them into the body. First, plant cells are totipotent; in other words, all the different tissues of a plant can be grown starting from a single cell in a culture dish. This characteristic enables scientists to make modifications to one plant cell and, from that, grow a plant in which every cell has those modifications.

Plant cells also have fibrous walls made of cellulose, which cannot be broken down by human enzymes, but can be degraded by

the microbes that reside in the gut. This feature enables therapeutic proteins produced inside plant cells to survive the trip through the digestive system until reaching the intestines, where they can be released to disseminate into the bloodstream.

In addition, because plants are commonly consumed foods, most people are not allergic to them as they might be to some synthetically produced drugs, or those based on egg proteins, to which a substantial number of individuals are sensitive.

And finally, plants can be easily grown. Dr. Daniell has reported that just an acre of genetically modified tobacco plants, for example, could produce enough anthrax vaccine to immunize every person in the United States.

GREENVACCINES

THINKING BIG

Dr. Daniell's work has attracted attention from multiple funding bodies, including the National Institutes of Health, the Juvenile Diabetes Research Foundation, and the Bill and Melinda Gates Foundation. The Gates Foundation grant, awarded in 2011, has the specific aim of eradicating polio, once and for all. Though the disease was mostly wiped out thanks to an effective vaccination campaign in the latter half of the 20th century, localized outbreaks have prevented it from disappearing completely. The vaccine that Daniell is working on is aimed at offering protection against multiple strains of polio, and would be free of the refrigeration cold chain, easing the process of delivery to those areas of the world where the disease is still present.

"Gates wants me to develop a booster vaccine that will offer protection against multiple serotypes of the polio virus," says Dr. Daniell. "We have all the key players in place to move this to the clinic."

Pharmaceutical companies are similarly interested in the great potential of Dr. Daniell's work, which has generated more than 150 patents.

150 patents generated
by Dr. Daniell's work



ABOVE: A lettuce leaf is placed on a plate and inserted into the "gene gun" to introduce the therapeutic protein into the plant cells.

ABOVE RIGHT: Members for the Daniell Lab, left to right, standing: Dr. Man (Mandy) Zhou, CAMB doctoral student Mark Yarmarkovich, Dr. Andrew Devine, Dr. Kwangchul Kwon, and Dr. Jin Su, front, seated: Dr. Henry Daniell and Dr. Hui-Ting Chan (Marianne).



Collaborative work with Novo Nordisk and Bayer are among the tally of his lab's projects, which will be supported by a greenhouse erected in Penn's South Bank campus in Gray's Ferry, where plants harboring vaccine or drug proteins can grow and be harvested.

The applications of his platform are nearly limitless, but several could push forward dental medicine and practice. Dr. Daniell's lab has already made monoclonal antibodies against streptococcus mutants that cause dental caries. He and colleagues have also worked on designing antimicrobial peptides to insert in plant cells that could destroy bacteria, including oral pathogens. Such an approach could destroy oral infections without raising the risk of developing antibiotic resistance because the peptide's attack strategy—physically poking holes in a bacterial membrane—cannot be avoided by mutation.

Penn Dental Medicine is well positioned for Dr. Daniell to take advantage of interdisciplinary collaborations. Within the School, he is exploring collaborations with Dr. Anh Le, Norman Vine Endowed Professor of Oral Rehabilitation and Chair of the Department of Oral Surgery/Pharmacology, to work on vaccines to suppress oral tumors. A partnership is also in the works with Dr. Elisabeth Barton, Associate Professor in the Department of Anatomy and Cell Biology, on a project tied to multiple sclerosis therapies.

Cross-school partnerships are likewise developing. One project, for instance, will involve Penn's Perelman School of Medicine with an aim of developing a vaccine to protect hemophilia patients from developing immune reactions to injected blood clotting factors. In addition, Dr. Daniell has been invited to join Penn's graduate programs in immunology and in gene therapy and vaccines. And he is in talks with the Penn Medicine's Global Health Programs to get involved with work related to tuberculosis.

"I'm quite excited because the rationale for me to move here is that hospitals and clinics are in place for the technology to be validated by moving to clinical trials," he said. "President Amy Gutmann's mantra about integrating knowledge was also very attractive to me. Many universities have these brick walls around their disciplines. But if I were to work in isolation, I'd never get to the finish line."

At its core, Dr. Daniell's work aims to improve the human condition with the same energized sense of innovation that led to the tech boom.

"Unlike the fantastic inventions that we see in things like cell phones and IT and so on, medicine hasn't had the same types of leaps and bounds of progress," says Dr. Daniell. "I see a lot of room for improvement." ■

—By Katherine Unger Baillie