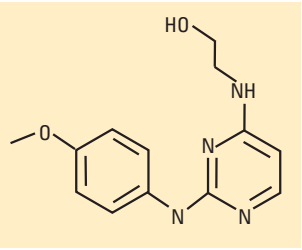


Cardiogenol C.

Stem cells have a heart

Researchers have uncovered a small synthetic molecule that efficiently induces mouse embryonic stem cells to form cardiac muscle cells, or cardiomyocytes. The finding by scientists at the Scripps Research Institute and the Genomics Institute of the Novartis Research Foundation (GNF) is an important step toward developing embryonic stem cell therapy for heart disease.

Embryonic stem cells can self-renew and differentiate into any number of specialized cells that make up a fully functioning organism. However, directing the differentiation process to a particular organ lineage, such as heart tissue, has been a major challenge.

"Known procedures for in vitro differentiation of embryonic stem cells into cardiomyocytes involve a poorly defined, inefficient, and relatively nonselective process, which yields less than 10% cardiomyocytes with the contamination of cells in other lineages," says Xu Wu, a graduate student at Scripps and lead author of the study.

However, Wu says, mammalian heart cells are, by and large, terminally differentiated, which means they can't regenerate to repair damaged tissue. Embryonic stem cells offer the promise of mending otherwise permanently damaged muscle tissue.

To find chemical tools to help direct cardiomyocyte differentiation, the Scripps/GNF researchers screened a combinatorial library of heterocyclic molecules (*J. Am. Chem. Soc.* **2004,** *126,* 1590–1591). Thirty-five of the library compounds upregulated two specific markers of cardiomyocytes—atrial natriuretic factor and myosin heavy-chain protein (MHC)—when screened against mouse embryonic carcinoma cells. The scientists then analyzed the effects of the four most potent of these hits against mouse stem cells.

A week after initial contact between the stem cells and the compounds, beating cardiac muscles were visible under a microscope. The researchers also observed a substantial presence of heart-specific proteins. Cardiogenol C, the most effective of the molecules, triggered MHC expression in 50% of the cells and the expression of three other cardiac muscle cell markers in more than 90% of the cells.

Furthermore, this molecule showed cellular toxicity only at concentrations greater than 25 µM, while its observed activity was established at 0.25 µM.

Determining precisely how cardiogenol C accomplishes its activity, the researchers say, will be the vital next step to revealing novel mechanisms involved in heart formation and to eventually developing therapies. "However," Wu says, "little is known about why cardiogenol C has those effects."

—DAVID FILMORE

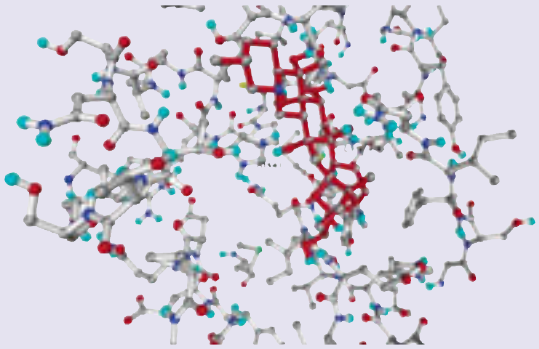
SARS in the sights

Researchers at Montclair State University and the University of Pennsylvania Medical School have identified a potential compound against severe acute respiratory syndrome (SARS). The discovery, the scientists say, is "a promising first step toward structure-aided design of nonpeptidyl anti-SARS agents."

The high fatality rate associated with SARS last spring, particularly among the elderly and very young, led researchers to aggressively pursue isolating the infectious agent, a novel human coronavirus. Recently, scientists isolated a viral protease, $3CL^{pro}$, from this coronavirus and solved its crystal structure.

The Montclair State/Penn team used this structure to model potential ligands from a database of compounds maintained by the National Cancer Institute (*J. Med. Chem.* **2004,** *47,* 1079–1080). They applied an algorithm to map each of the 1853 compounds onto a precomputed grid of the protease surface, focusing on a cysteine residue that forms part of the catalytic dyad of $3CL^{pro}$. They docked each compound 10 times to achieve low-energy structures and considered any structures of the same ligand with a root-mean-squared deviation of <0.5 Å to be part of a reproducible cluster of solutions. The researchers then ranked the compounds by docking energy and subsequently by cluster size—the latter being a measure of the number of docking solutions at a particular energy level.

Within the lowest-energy dockings, they found 10 compounds that showed clustering of 5 or more structures, and they evaluated these ligands for desirable chemical properties. Only one looked promising: sabadinine, an herbal remedy isolated from the lily plant. The researchers tested the compound in vitro to see how it affected the replication of a SARS-type coronavirus, mouse hepatitis virus.



Targeting SARS. Researchers identified sabadinine (red) as a potential viral protease-binding agent. (Adapted with permission from Toney, J. H.; et al. *J. Med. Chem.* **2004,** *47,* 1079–1080.)

Although they did not see any inhibition in the sabadinine-treated sample, the scientists plan to test the compound against other coronaviruses and isolated $3CL^{pro}$.

—RANDALL C. WILLIS

PURDUE AGRICULTURAL COMMUNICATIONS/TOM CAMPBELL

Easy preparation. Purdue University graduate student Tom Huang assembles a new microfluidic chip by placing a thin layer of a flexible polymer on a glass microscope slide.

Microfluidics: Cheaper and easier

As part of an ongoing project sponsored by the U.S. Department of Agriculture, researchers at Purdue University have developed a new method for assembling microfluidic chips using poly(dimethylsiloxane) (PDMS) and basic laboratory materials, such as thin glass fibers, tweezers, and microscope slides.

Microfluidics accounts for a substantial market for drug discovery and clinical diagnostic applications. However, the cost of the technology can be prohibitive for scientists, particularly in nonindustrial settings. Traditionally, chips have been fabricated with photolithography, which uses X-rays or UV to form a pattern on a silicon or glass wafer that is then etched by washing the wafer with a variety of solvents. The process is time-consuming, often requiring weeks from design to manufacture, and it is expensive.

In the new approach developed by the Purdue team, a chip can be developed in hours in a typical laboratory setting, with inexpensive components.

"This development democratizes the preparation of microfluidic biochips," says Michael Ladisch, a professor at Purdue who developed the method with his graduate student, Tom Huang.

A chip is assembled by placing a fine fiber, approximately one-tenth the width of a human hair, on a glass slide and covering it with a square of PDMS. The polymer flexes slightly over the fiber, creating two small channels running the length of the fiber on either side. Applying a small amount of pressure sticks the PDMS to the glass.

"The chemical properties of the PDMS allow it to stick to the glass slide with enough strength to form a tight seal, which permits liquids to be pumped through the channel," Ladisch says. Additionally, he says, the small size of the channels allows researchers to use minimal amounts of costly or difficult-to-obtain experimental reagents.

In a proof-of-concept study, the scientists demonstrated that by manipulating the properties of materials coating the fiber, specific proteins could be separated from a mixed solution (*AIChE J.* **2003,** November). "We can control the chemistry inside the channel to determine what flows through, what sticks; and in that way, we can separate things out," Huang says.

According to the team, the speed and simplicity of the method give researchers the flexibility to experiment with the conception and construction of microchips for their focused needs.

—KIMBERLY S. CLEAVES

FDA: More with less?

Pressure on the FDA to maintain suitable levels of staff expertise to meet increasing demands will grow this year, according to the *Outlook 2004* report from the Tufts Center for the Study of Drug Development. The nonprofit research group says there will be limited funds available for new hires as experienced staff retire or change jobs.

"There has been talk over the past couple of years of the 'sweatshop' atmosphere and the strain from the PDUFA deadlines," says Christopher-Paul Milne, assistant director of the Tufts Center. PDUFA (the Prescription Drug User Fee Act) provides the FDA with the authority to collect fees from companies submitting New Drug Applications (NDAs) so that the agency can meet accelerated review time standards.

PHOTODISC

PDUFA III, the most recent renewal of the law that extends user fees through September 2007, added more benchmarks for quicker FDA review. This—in addition to an expanded counterterrorism mission, increasingly complex new product applications, and pressure to better harmonize U.S. and European drug regulations—has substantially increased the workload, the Tufts Center reports. These burdens, Milne says, add to other sources of employee discontent. Specifically, he points to employees reacting negatively, even resigning, after therapeutic biologic review responsibility was moved from the Center for Biologics Evaluation and Research (CBER) to the Center for Drug Evaluation and Research (CDER) in September 2002.

"Supposedly, 30% of senior review staff in CDER/CBER are eligible for retirement over the next five years," Milne says. "If you have a lot of things going on [that cause discontent], that is going to increase the pressure for people to jump ship."

Initially, the FDA projected receiving $1.25 billion from PDUFA III fees through 2007 that could be used to add 367 full-time review staff employees, but, according to Milne, "PDUFA revenues are anticipated to be below expectations." This results from steady declines in new product applications submitted by companies, a trend that has been ongoing over the past several years.

However, some of the FDA's performance numbers are improving. For instance, the median total approval time for CDER's priority NDAs went from 19.1 months in 2002 to 7.7 months in 2003. And the agency has committed itself to an across-the-board 10.5% reduction in review time.

"That is an ambitious goal," Milne says. "I think the FDA is trying really hard, but I think they are going to have a difficult time."

—DAVID FILMORE

ELI LILLY AND CO.

Lilly's Gaynor sees RNA-derived therapeutics as an exciting area.

Lilly joins Sirna in RNAi

Sirna Therapeutics is entering into an 18-month collaboration with Eli Lilly to develop novel RNA interference (RNAi) cancer therapeutics. The joint project will investigate Sirna's proprietary small interfering RNAs (siRNAs) against Lilly oncology targets.

"This collaboration represents a significant step forward in Sirna's development of RNAi-based therapeutics and is our first alliance with a major pharmaceutical company," says Howard Robin, president and CEO of Sirna. "We chose Lilly as a partner based on Lilly's extensive expertise in developing novel oncology compounds."

Overall, it is the second major strategic alliance that has been announced between any RNAi therapeutic-focused company and a leading pharmaceutical firm. In September 2003, Alnylam Holding Co. (consisting of recently merged Alnylam Pharmaceuticals and Ribopharma) and Merck announced a multiyear collaboration to develop therapeutics for a range of diseases using Alnylam's RNAi technology and Merck targets.

The RNAi mechanism, in which 20–25-nucleotide-long siRNAs induce the degradation of target RNA with the help of cellular protein machinery, is already widely exploited as a gene-silencing tool for functional genomics studies. RNAi products supplied for this purpose by companies like Ambion and Invitrogen are a growing market, estimated at $38 million in 2003, according to Front Line Strategic Consulting. Drugs based on the technology are not expected to reach the FDA approval stage for at least another 5–10 years, but a lot of enthusiasm has been generated.

"The field of RNA-derived therapeutics is one of the most exciting to be explored in the last 20 years, one that has the promise of improving medical practice in areas of great unmet medical need," says Richard Gaynor, vice president of cancer research at Lilly.

This agreement highlights a substantial turnaround for Sirna, which a year and a half ago was called Ribozyme Pharmaceuticals (founded on the basis of the science of Thomas Cech, a 1989 Nobel laureate in chemistry), a faltering company that had not produced ribozyme-based therapy after 10 years of research. Its newfound focus on RNAi was triggered by an April 2003 $48 million venture capital investment.

—DAVID FILMORE

Nonprofit drug development

The world's first nonprofit pharmaceutical company is less than a year away from seeking approval in India for its first medicine. The Institute for OneWorld Health announced its progress in developing paromomycin, an off-patent medicine, to cure the deadly parasitic disease visceral leishmaniasis (VL) in January at the World Economic Forum in Davos, Switzerland.

"OneWorld Health and our partners are dedicated to developing new therapies for neglected infectious diseases that are affordable and meet international standards of quality," says founder and CEO Victoria Hale. Instead of concentrating on discovering new drugs, the company is focused on uncovering the most promising drug and vaccine candidates that have been abandoned by for-profit companies or rediscovering new uses for already existing drugs.

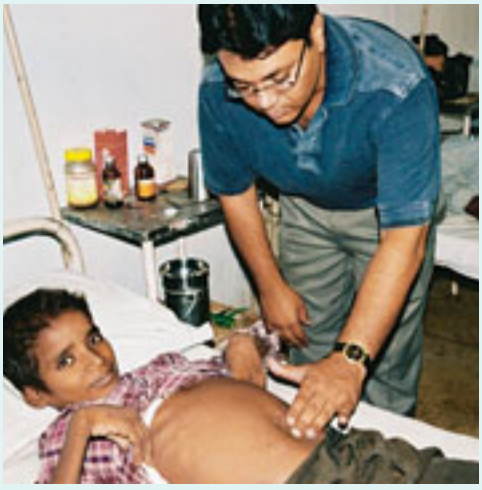
Paromomycin, for instance, was originally approved by the FDA as a broad-spectrum antibiotic. The World Health Organization (WHO) secured a license for the injectable form of the drug and initiated trials for VL treatment in the 1980s, but it didn't have the funds to see them through. Now, with the help of over $4 million in grants from the Bill & Melinda Gates Foundation, OneWorld Health is collaborating with WHO on a 670-patient Phase III clinical trial in Bihar, India.

The trial, according to Hale, is showing very positive results. Previous studies indicated the drug to be equivalent to a therapeutic vaccine, in which patients cured of the disease developed a lifelong immunity to the VL parasite.

VL (also known as kala-azar or black fever) is transmitted by the bite of a sand fly and infects approximately 1.5 million people worldwide. India, where as many as 200,000 victims die each year from the disease, carries the highest burden.

Performing the trials in India also offers the combined advantage of high clinical standards and substantially lower development costs than Western nations. According to the Confederation of Indian Industry *(www.ciionline.org)*, clinical trials cost several hundred million dollars less in India than elsewhere. This savings will be vital to OneWorld's goal of keeping the sale price of the drug to $1 a day for a three-week treatment.

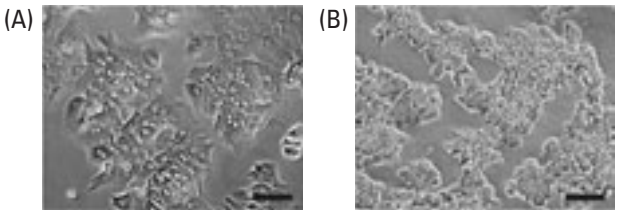
THE INSTITUTE FOR ONEWORLD HEALTH



A doctor examines the enlarged spleen of a child suffering from late-stage visceral leishmaniasis.

In addition to paromomycin, OneWorld's drug pipeline includes advanced treatments for schistosomiasis and childhood diarrheal disease, as well as potential treatments for malaria and Chagas disease.

—DAVID FILMORE



Living and dying. Healthy hepatocytes show clearly defined cells and nuclei (A), but cells exposed to oxidized CdSe quantum dots have died (B). (Used with permission from Derfus, A. M.; et al. *Nano Lett.* **2004,** *4,* 11–18.)

Quantum dot toxicity?

Cadmium selenide (CdSe) quantum dots, used as fluorescent labels, may be toxic to some cell lines. However, Sangeeta Bhatia and colleagues at the University of California at San Diego (UCSD) have shown that the toxicity of CdSe quantum dots depends on how they were prepared.

Nanometer-scale CdSe quantum dots have become popular alternatives to fluorescent dyes for biological imaging because they produce bright fluorescence, broad UV excitation, narrow emission, and high photostability. Although many groups have used quantum dots in vitro and in vivo, questions have been raised about their safety.

The UCSD researchers noted that although previous in vitro labeling studies have not shown significant toxicity, the cell lines used in these experiments were not sensitive to heavy metals or were exposed to only short-term quantum dot labeling. To probe the cytotoxicity question directly, Bhatia and coworkers labeled hepatocyte cells from rats with CdSe quantum dots, because cadmium can potentially accumulate in the liver and damage it (*Nano Lett.* **2004,** *4,* 11–18).

Although the quantum dots did not initially show cytotoxicity, they began to kill cells after the dots were exposed to air for 30 min before being solubilized. In general, quantum dots that were substantially oxidized by UV light or exposure to air killed significant numbers of cells. The researchers suggest that oxidation of the quantum dots produces free Cd^{2+}. This was confirmed by determining the Cd^{2+} ion concentrations in solutions of the various quantum dots.

Although careful preparation could reduce the chances of toxicity, the use of quantum dots in vivo might be difficult. There have been previous observations of quantum dots being removed from the bloodstream of mice by macrophages, which attempt to destroy foreign objects by H_2O_2 oxidation. The researchers did observe free Cd^{2+} formation upon exposure of the quantum dots to H_2O_2.

The investigators proposed and tested several solutions to the possible toxicity problem. Capping the dots with ZnO effectively prevented free Cd^{2+} formation upon exposure to air but not to UV. They are looking at other inorganic and organic caps as well.

Bhatia and colleagues stress that in vivo use of CdSe quantum dots must be critically examined because cadmium release may occur over time and still cause cell damage or death.

—MICHAEL J. FELTON

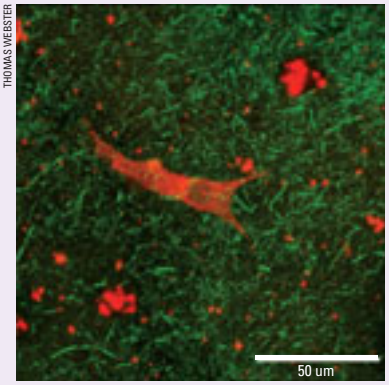
Nanofiber brain probes

Carbon nanofiber-based structures have better properties than currently used materials for brain probes and implants, according to recent experiments by scientists at Purdue University.

Silicon probes are commonly used for studying neurological function and disease, and research is continuing on their use in applying electrical signals to restore damaged areas of the brain. However, the body generally regards these conventional probes as foreign invaders. As a result, the probes become encapsulated with nonconductive glial scar tissue, which prevents them from making good electrical contact with brain cells.

The Purdue researchers have developed semiconducting carbon nanofibers, or nanotubes, which are polycarbonate urethane composites with nanoscale bumps that mimic features found on the surfaces of natural brain proteins and tissues, to reduce the formation of scar tissue (*Nanotechnol.* **2004,** *15,* 48–54). Specifically, they pressed carbon nanofiber/plastic composites into disk shapes that could be placed in liquid suspensions in Petri plates.

To assess the performance of these nanofiber probes, the scientists placed the disks in a suspension of astrocytes, the glial cells that produce the scar tissue. They then used fluorescence microscopy to count how many dyed astrocytes adhered to the probe compared with another nanofiber-based structure that did not contain the small brain tissue-like features. After an hour, nearly half as many astrocytes attached to the nanofiber composites containing the small features as to the materials that did not. The experiment was repeated for an additional two weeks while the nanofibers were left in the cell suspension, and yielded similar results.



Confocal image of neurons on a nanofiber/polycarbonate urethane (2:98%) composite.

"These astrocytes can't make scar tissue unless they can adhere to the probe," says Thomas Webster, a professor of biomedical engineering and lead author of the study. "Fewer astrocytes adhering to the nanotubes means less scar tissue will be produced."

The team further noted that increasing the concentration of nanofibers in the plastic composite led to a decrease in the number of astrocytes that attached to the probe.

The researchers also immersed the disks in a suspension with neurons and found that the nanoscale-featured probes stimulated neurons to grow 60% more neurites, or fingerlike extensions needed to regenerate brain activity in damaged regions, than more conventional probes. They also caused less scar tissue.

Because of the nanotubes' interesting electronic properties and the advantageous behavior observed in these experiments, the researchers believe that nanotubes hold great promise for replacing conventional silicon implants.

—KIMBERLY S. CLEAVES ■