NewsBytes

Studying Force in 3-D

Mechanical forces drive many processes in the human body, from organ and tissue formation during development, to stem cell differentiation, to wound healing. Until recently, scientists could only study these forces at the single cell level in two-dimensional experimental models. Now, researchers have developed a new tool and computer model to study forces generated by cells in 3-D contexts at a very small scale.

"This is the first time we have been able to measure three-dimensional forces in very small structures or with a small number of cells," says **Christopher Chen, PhD**, bioengineering professor at the University of Pennsylvania and senior author of the work that appeared in a June 2009 issue of *Proceedings of the National Academy of Sciences*.

Chen's group measured forces exerted by human and mouse fibroblasts, a type of cell that is abundant in connective tissue in the body. Fibroblasts secrete proteins to form an extra-cellular matrix that binds cells together into tissues. "All cells aside from blood cells are adhered to this matrix, sort of like a carpeting that they're embedded in, and they're pulling against that matrix," Chen says. "When they feel those forces, there's a fair amount of data to suggest that they change their behavior."

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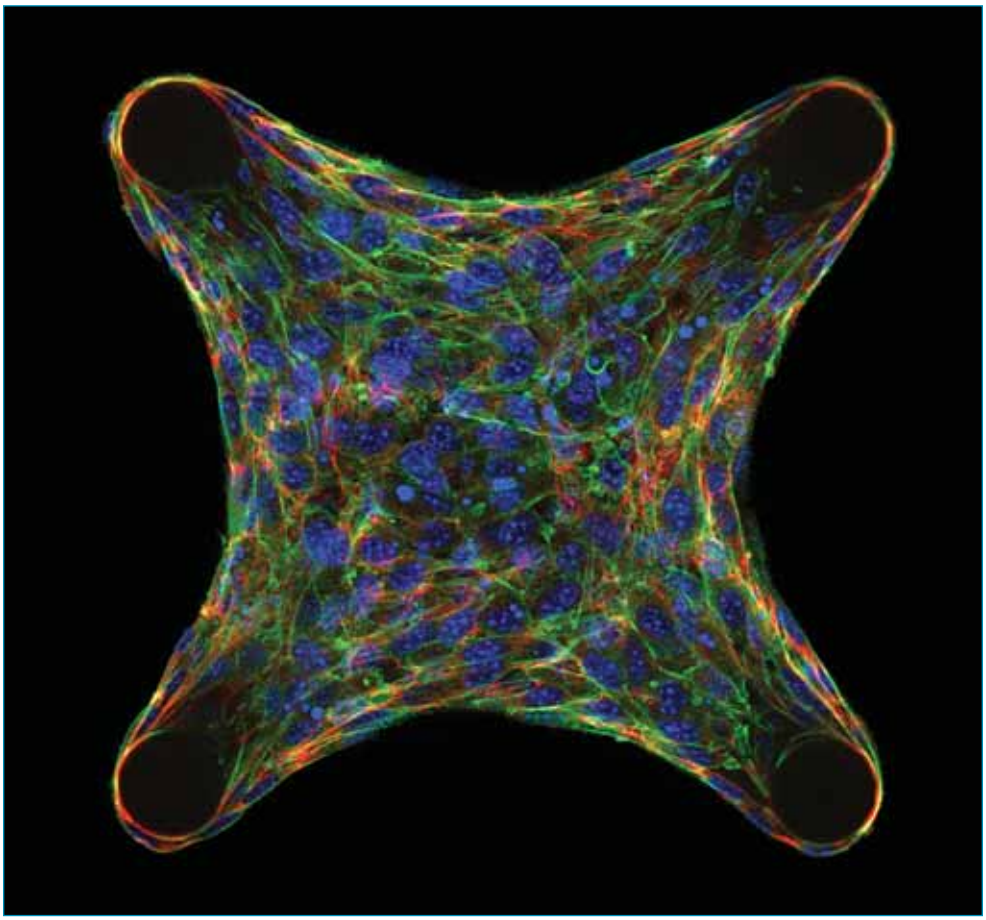

Fluorescent image of fibroblast cells embedded in a collagen matrix suspended between four small rods. Chen's study measured the force exerted by these cells using sensors at the small rods, and used computational models to predict the patterns of force throughout the microtissue. Cell nuclei are shown in blue, the cytoskeleton protein actin in green, and structural matrix proteins in red. Image courtesy of Wesley R. Legant.

The measurement tool Chen's group constructed contains two tiny cantilevers connected to a sensor with a collagen gel between them. Chen's group then put fibroblasts into the collagen goo. When fibroblasts hit collagen, they contract and reorganize the collagen fibers, Chen says, and this results in a mix of collagen and cells suspended between the cantilever rods, like a hammock. The scientists then measured the force from that contraction. They also varied conditions in the set up, such as the thickness of the collagen and the stiffness of the cantilever springs, and looked at how the cells reacted. The stiffer the springs, the more the fibroblasts contracted. And the more contractile forces the cells encountered, the more extra-cellular matrix they pumped out. In the body, this reaction to force is useful. For example in wound healing, the fibroblasts sense the tension from the wound edges pulling apart and secrete more matrix to form scar tissue.

Chen and his group then constructed a computational model to better understand the distribution of force within the collagen mass. They found that the points in the structure where their model predicted the highest stress correlated with the most production of extracellular matrix. The model can also be useful for predicting forces in more complicated geometrical structures, more like those found in the body, Chen says.

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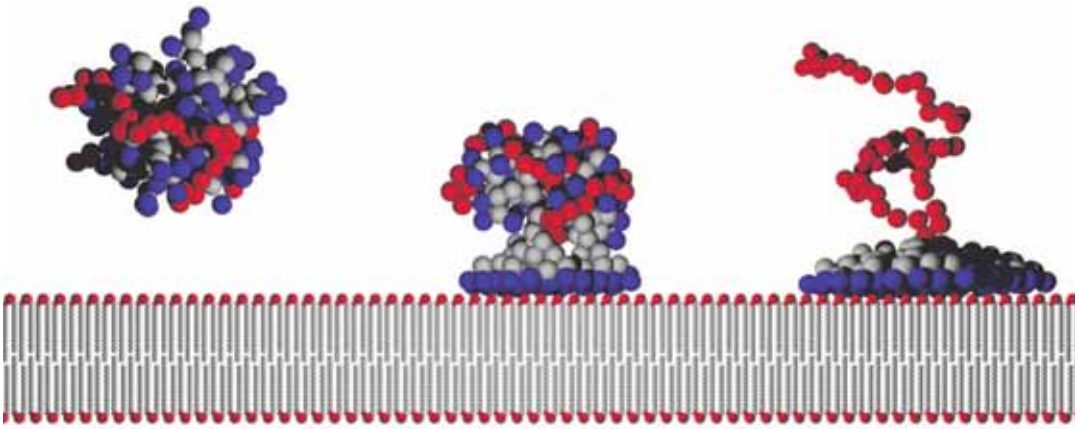


Snapshots of a simulation of a dendrimer-DNA complex arriving and docking at a model cell membrane. Reprinted with permission from The Journal of Chemical Physics *130, 155101, 2009. Copyright 2009, American Institute of Physics.*

"There's a growing appreciation of how important mechanical forces are for many biological processes ranging from directing stem cell differentiation, to tissue formation, to how cells respond to drugs," says **Ali Khademhosseini, PhD**, an assistant professor of medicine at Harvard and MIT. "This work generates a powerful model that can be used for many different applications. There's a lot of scientific follow-up as well as many potential technological and engineering advances that can come out of it."

—By Rachel Tompa, PhD

Modeling A Gene Therapy Delivery Vehicle

Gene therapy to correct inherited illnesses hinges on successful delivery of DNA into a person's cells. Most gene therapists work with viruses to ferry their DNA cargo. Yet the body tends to fight even disarmed viruses that should be harmless. As an alternative, researchers have devised dendrimers, branched molecules whose endings can be tailored to package DNA. Now, in the first molecular-level simulation of a gene therapy vector in action, researchers have simulated a dendrimer docking at a model cell surface and shown how long it can hold on to its DNA cargo.

"With our simple tinker toy model we're going to throw out a lot of information that is certainly important, but it gives us the basic physics," says Paul Welch.

The simulation rendered a quick but clear sketch of what happens at the cell membrane. "With our simple tinker toy model we're going to throw out a lot of information that is certainly important, but it gives us the basic physics," says **Paul Welch, PhD**, a materials physicist at Los Alamos National Laboratory and lead author on the study published in the April 2009 issue of *The Journal of Chemical Physics*.

In previous work, other researchers have modeled dendrimers interacting with membranes, but no one had simulated them transporting DNA.

Welch and his team created a molecular dynamics model of a dendrimer with an attached DNA strand. In their simulations, they let the dendrimer-DNA complex loose near a simple, planar membrane model to see whether it would bind or wander off. They found that both the propensity to bind and the duration of binding decreased in the presence of a more negatively charged membrane. There is a range of surface charges which allow binding for the optimal length of time—long enough for the complex to transit the membrane but not so long that the dendrimer retains a grip on the DNA after entry. In addition, the researchers found that big burly dendrimers are not necessarily the best delivery vehicles for DNA. In future simulations, Welch's team hopes to use a more realistic model of the membrane's lipid bilayer. Ideally, Welch says, the membrane would undulate, deform and perhaps form a little liposome (bubble) around the complex to pull it in, much as one would expect a membrane to behave in nature.

Ron Larson, PhD, a polymer physicist at the University of Michigan in Ann Arbor who models the use of dendrimers to poke holes in membranes to kill bacteria or deliver drugs, wonders whether the model should address possible interactions between the four bases of the DNA and the membrane. And he looks forward to experiments that would test the model. "People make these different particles by the seat of their pants and see how many go in," Larson says. "When things go wrong, they often don't know why. It's really helpful to have a theoretical model."

—By Roberta Friedman, PhD

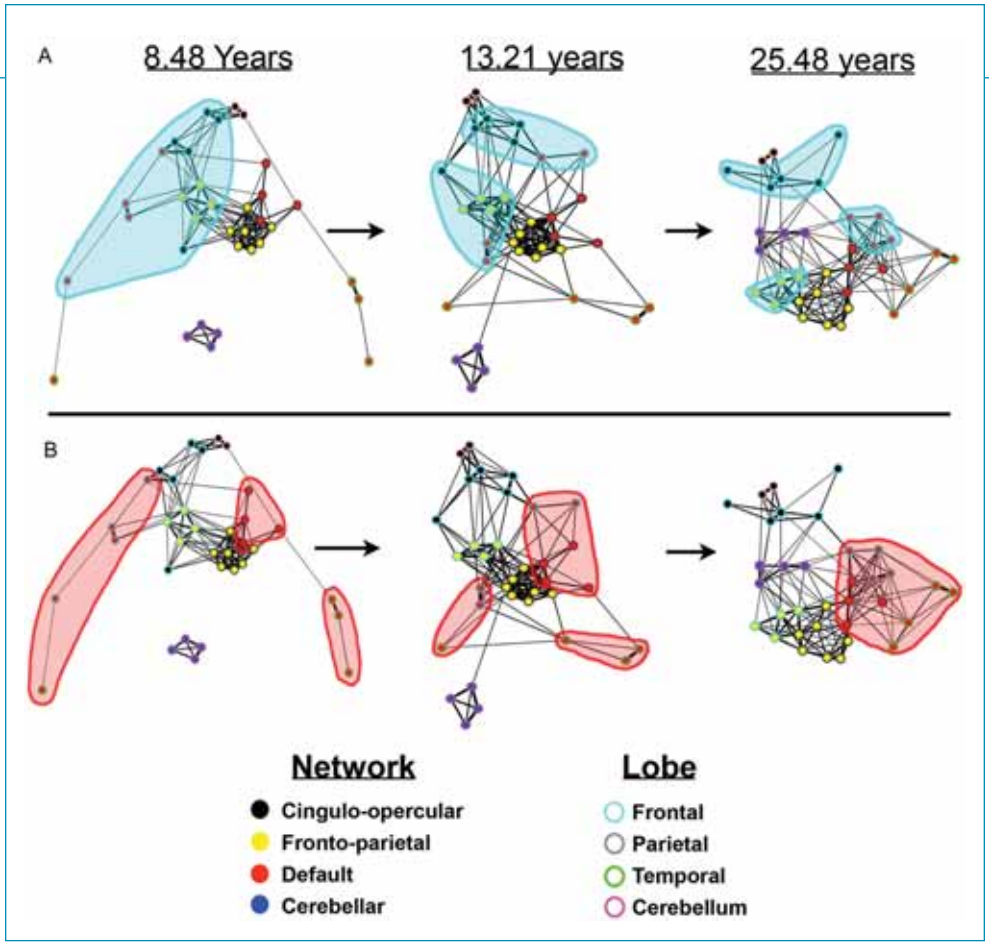
Different But Equal

Kids often claim they are just as smart—if not smarter—than their parents. Childish nonsense? Perhaps not, according to a recent study. It turns out that young children's brains are as efficient in solving information-processing tasks as their adult versions, despite being very differently organized. This finding could improve our understanding of normal brain development as well as of disorders such as autism and Tourette syndrome.

"Whether you are a kid or an adult your brain is organized in a pretty damn efficient way," says **Steven Peterson, PhD**, a neurophysiologist at the Washington University School of Medicine in St. Louis, and senior author of the study which appeared in the May 2009 issue of *PLoS Computational Biology*.

By seven years of age, a typical human brain has already attained 95 percent of its adult size and most of the wiring that connects neurons to each other is already in place. As the brain matures further, two things happen: its wires (axons) get better at transmitting signals and its unused junctions (synapses) are progressively trimmed out. Along with these physical changes, the brain changes the way it configures its various regions into functional networks during resting, reading, singing, walking, or other tasks. This phenomenon is a key to both normal and abnormal brain development, but until now it has been difficult to quantify.

In the new study, Petersen and his team used magnetic resonance imaging to study functional brain connectivity in a sample population of 210 subjects aged 7-31 years. When they cross-correlated the temporal activity of 34 key brain regions in each subject while resting, a clear pattern emerged: brain regions in children interacted mostly with their neighbors while those in adults enjoyed longer-range interactions. While this confirmed prior theories, further quantitative analyses turned up a surprise. Functional brain networks in both adult and child subjects proved to consist of tightly knit communities loosely linked to each other—both possessing a "small world" structure that typifies efficiently connected systems such as social networks and the Internet. Although these communities start off being spatially localized in children and grow more diffuse with maturity, measures of computational efficiency remain high throughout. "All of us were surprised when those numbers came out," says Petersen, who notes that these findings have now been replicated by Stanford University neuroscientist Vinod Menon's research team in the July 2009 issue of *PLoS Biology*.



This figure shows how the functional networks in the brain evolve during development. In each case, 34 key regions from four brain lobes are linked to each other based on mutual correlation into four functional networks. Regions are color-coded based on their anatomical locations (pastel rings) and functional network membership (solid dots). The top row shows how anatomically close regions—such as those from the frontal lobe, highlighted in turquoise—segregate into different functional networks with age. In contrast, the bottom row (the same data points) shows how anatomically distant regions integrate into a functional network—illustrated by the red-highlighted regions from the frontal, parietal, and temporal lobes, which integrate into the "default" network. Observe also how the cerebellar network (four blue dots with pink rings), initially isolated, gets integrated into the overall network with age. Reprinted from: Fair, DA, et al., Functional Brain Networks Develop from a "Local to Distributed" Organization,* PLoS Computational Biology *5(5): e1000381. doi:10.1371/journal.pcbi.1000381 (2009).

Petersen and his colleagues, including pediatric neurologist **Bradley Schlaggar, MD, PhD**, have applied this methodology to study Tourette syndrome, a neurological disorder characterized by physical and vocal tics. Brain connectivity patterns in adolescent Tourette sufferers appear to lag by 2-3 years compared to normal, says Petersen. "The context we got from studying normal development allowed us to interpret what we observed in Tourette subjects."

"This study is incredibly innovative in providing original and direct evidence about how circuits are formed in the brain," says **Beatriz Luna, PhD**, a developmental psychologist at the

Functional brain networks in both adult and child subjects consist of tightly knit communities loosely linked to each other—both possessing a "small world" structure that typifies efficiently connected systems such as social networks and the Internet.

University of Pittsburgh Medical Center. Luna suggests that the method could next be applied to study subjects engaged in specific activities. **BJ Casey, PhD**, a neuroscientist at the Weill Medical College of Cornell University in Ithaca, New York, finds the study to be "a very novel characterization of neural system development" ideally suited to study developmental disorders such as autism. "It's going to drive a lot of research," she says.

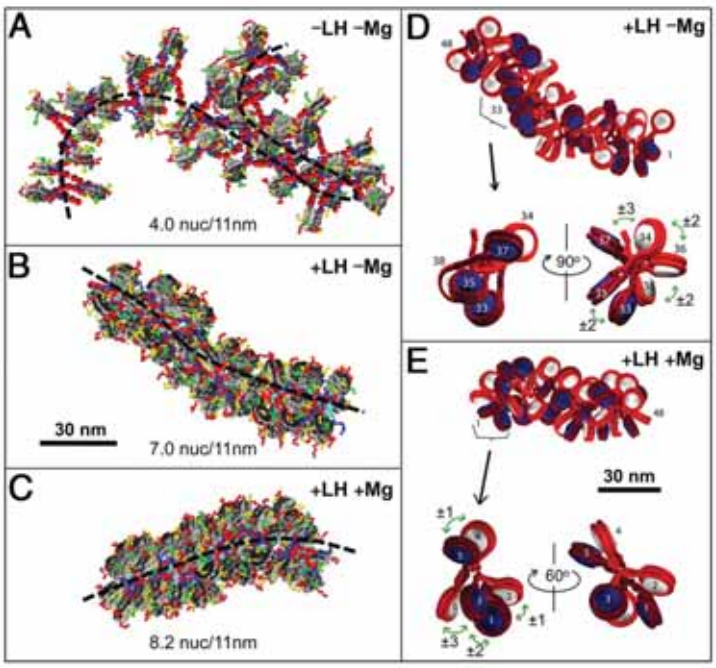
—By Chandra Shekhar, PhD

Chromatin Fiber: Zigzag or Solenoid?

Try packing a two-meter-long stretch of DNA into a cell nucleus just a few millionths of a meter thick—with key coding segments readily accessible. It's a seemingly impossible feat that eukaryotic cells routinely pull off by building a highly compact, fibrous mix of DNA and proteins called chromatin. Now a new study uses a combination of novel lab experiments and computer simulations to provide long-sought details about the structure of chromatin fibers.

"Our study appears to resolve a 30-year-old controversy about the structure of chromatin fiber," says **Gaurav Arya, PhD**, assistant professor of nanoengineering at the University of California, San Diego. The findings, published in the August 11 issue of *Proceedings of the National Academy of Sciences*, could improve our understanding of cell growth, differentiation, and cancer.

This much is generally accepted: Chromatin starts off as a series of nucleosomes—protein spindles wrapped with about a turn and a half of DNA—connected by stretches of linker DNA; this "beads on a string" structure then folds itself into stiff, compact fibers. What is debated is the interaction and arrangement of nucleosomes within this fiber.



Chromatin packing gets denser with the addition of linker histones (LH) and divalent ions (Mg) in this computational simulation (A-C) . In the close-ups at right, the cores of alternate nucleosomes have different coloring (white or blue) with red linkers for better visualization. The zigzag structure dominates at low ionic concentrations (D) but in the presence of magnesium chloride, several nucleosomes have bent linkers and the nucleosomes interact in more of a solenoid arrangement (E). Reprinted from Grigoryev, S, et al., Evidence for heteromorphic chromatin fibers from analysis of nucleosome interactions, Proceedings of the National Academy of Sciences, 106: 32:13317-13322 (2009).

One school of thought favors a spiral arrangement, or solenoid, in which successive nucleosomes interact and are connected with bent DNA linkers. Another school argues that DNA is too stiff to bend easily, and proposes instead a zigzag structure with straight linkers in which alternate nucleosomes interact. Until now, this issue could not be resolved because the available experimental techniques required the chromatin fiber to be unwrapped before it could be studied.

In the new work, researchers first used formaldehyde to create permanent cross-links between interacting nucleosomes. These interactions give rise to loops in the fiber when it is unwrapped under various conditions. Studying these loops under an electron microscope, the researchers found evidence to support the existence of the zigzag structure in the absence of divalent ions such as magnesium; in the presence of such ions, however, a fraction of nucleosomes switch to the solenoid motif.

The researchers then used a computational model developed by New York University researcher **Tamar Schlick, PhD**, to simulate the structure of chromatin fiber. The model confirmed the experimental results and added additional details: Without divalent ions present, the zigzag fiber packs about 7 nucleosomes per 11nm stretch; with divalent ions, about 20 percent of the linkers in the fiber bend, solenoid-style, and this helps the fiber accommodate about 8 nucleosomes per 11nm.

> "Our study appears to resolve a 30-year-old controversy about the structure of chromatin fiber," says Gaurav Arya.

"These results show that both the zigzag and solenoid topologies may be simultaneously present in chromatin fiber," says the study's lead experimentalist, **Sergei Grigoryev, PhD**, associate professor of biochemistry and molecular biology at Pennsylvania State University. "It's very exciting that we could show this using both computational and experimental techniques."

University of Wyoming molecular biologist **Jordanka Zlatanova, PhD**, who has been studying chromatin for more than 30 years, says the paper is an important contribution "because finally we seem to really understand what the

chromatin fiber structure is." It's also a major advance experimentally, she says, because it captures nucleosome interactions under physiological conditions. Further, no other group has been able to come up with a computational model that fits the native structure of chromatin so well, she says.

—By Chandra Shekhar, PhD

Predicting Cancer Treatment Success

No two cancer patients respond identically to treatment. Some will be cured while others will see their cancer return, and physicians are at a loss to explain why. Now, using MRI imaging researchers have developed a mathematical model of tumor growth that identifies two factors that are predictive of cervical cancer treatment success: responsiveness to radiation and the ability to clear dead cells.

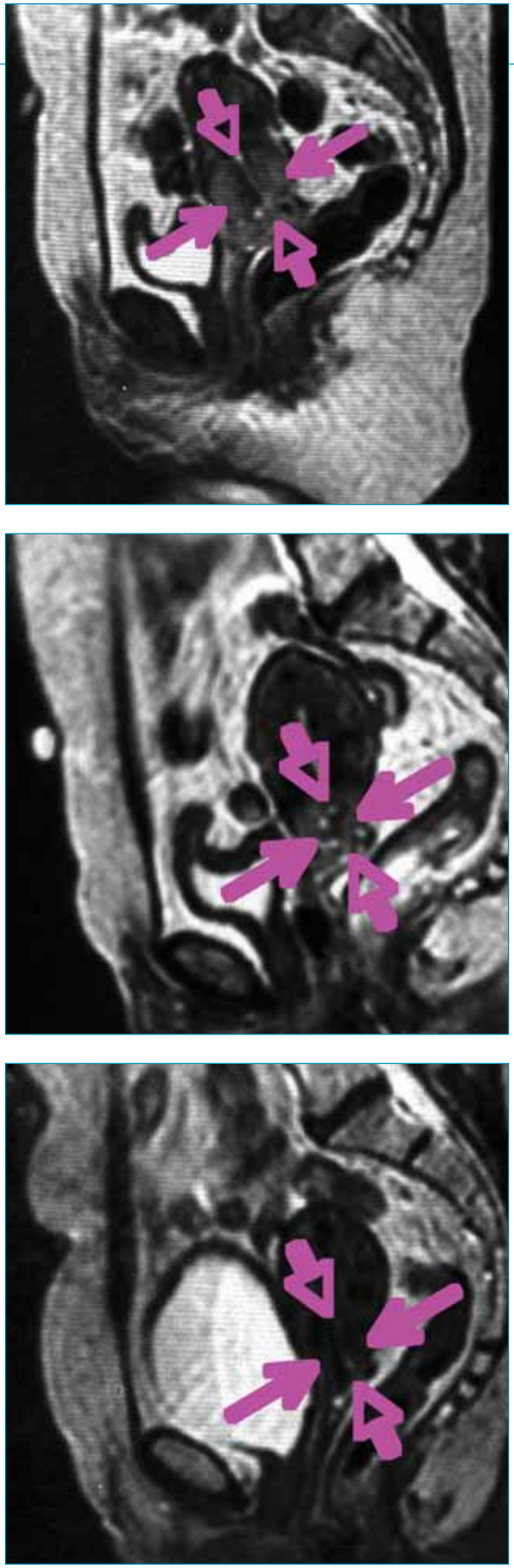
"This work gives us strategies to find out early on if the tumor does not respond to cancer therapy … and to adjust treatment to increase the chance of cure," says, **Nina Mayr, MD**, radiation oncologist at Ohio State University and principal investigator of the study. The work was presented at the annual meeting of the American Association of Physicists in Medicine.

Currently, "little is known about the underlying biological mechanisms that govern the tumor response to radiation therapy," says **Zhibin Huang, PhD**, a postdoctoral researcher at Ohio State University and lead author of the study. "We wanted to see if this imaging technology could find some early indications of the outcome."

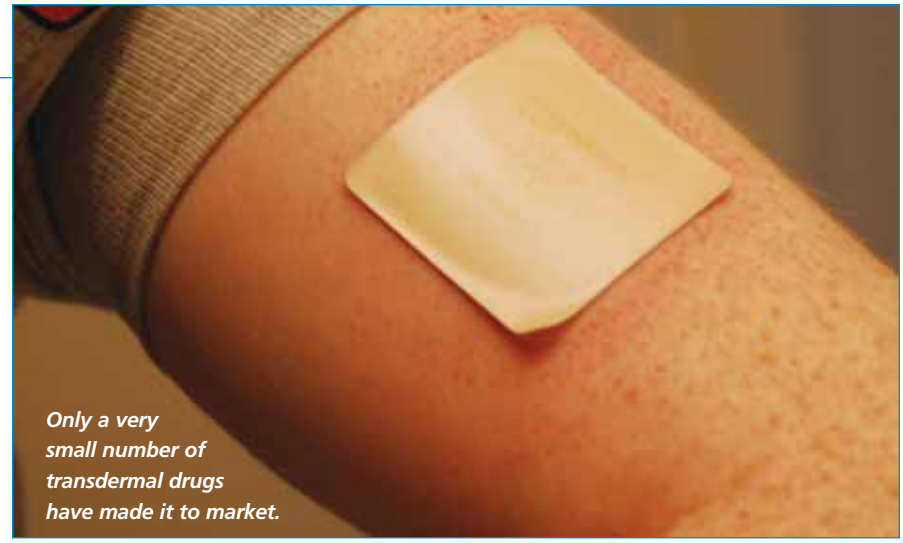
The research group, headed by **Jian Z. Wang, PhD**, medical physicist and the director of the Radiation Response Modeling Program at the Ohio State University, followed 80 women with various stages of cervical cancer—with tumors ranging from the size of a cherry to the size of a grapefruit. All of the patients received MRI scans before, during and after radiation therapy—the standard treatment for cervical cancer. With these scans, the researchers could measure the change in tumor volume over the course of the cancer therapy.

The team developed a mathematical model to fit the tumor volume data from the MRI scans and, using this model, identified two factors that correlated with the likelihood of a patient's cancer returning. The first is the patient's radiation sensitivity—essentially, the percentage of the cells that survived the radiation dose. The higher this number, the worse the outcome. More specifically,

"This work gives us strategies to find out early on if the tumor does not respond to cancer therapy … and to adjust treatment to increase the chance of cure," says Nina Mayr.



Ohio State University researchers used magnetic resonance imaging and a mathematical model to predict cancer recurrence. These images show decreasing tumor volume over a 5 week radiation course in a patient who was alive and cancer free 9 years later. Photo Credit: Dr. William Yuh and Dr. Nina Mayr.



Only a very small number of transdermal drugs have made it to market.

if radiation killed 30 percent or more of a woman's tumor cells during each day of treatment, then she is 33 percent more likely to be cancer-free than a woman whose tumor is more resistant to the radiation. The second factor is how quickly the dead cells are removed from the tumor area. For example, if it takes more than 22 days to clear the dead tumor cells after treatment, then that woman is nearly four times as likely to have her cancer return later on compared to a woman whose body clears the dead cells more quickly. When these two factors indicate that the cancer is likely to return, alternative treatments may be suggested for the patient. "Maybe we can use a more aggressive intervention instead," Wang says.

This is a very active area of research, says **William Small, Jr, MD**, professor of radiation oncology at Northwestern University Medical School. This kind of modeling could potentially be applied to other types of cancers treated with radiation. "It is very important to try to identify outcomes with surrogate markers," he says. "Doing so could allow us to finish clinical trials much quicker and dramatically improve our ability to test new therapies."

— By Liz Savage

A Multi-scale Model of Drug Delivery Through the Skin

Medicinal patches applied to the skin are an attractive route for drug delivery since they can release medicine slowly into the bloodstream and avoid being metabolized by the digestive system. Yet only a handful of medications have ever made it to market in patch form, largely because the stratum corneum, the skin's top layer, acts as a barrier. A new multi-scale computational model describing how chemicals move through that layer could help change that, opening the door to development of patches for a wider variety of drugs. The work was published in the June 2009 *Annals of Biomedical Engineering*,

"We believe that by separating the contributions from different levels of scale, the model can provide better insight about the barrier properties of the skin," says **Jee Rim, PhD**, a postdoctoral researcher at the University of California, Los Angeles, who did the research in collaboration with pharmaceutical company Alza while he was at Stanford.

Rim's model began on the micro level, with a molecular dynamics simulation of how a drug molecule diffuses along the middle of lipid bilayers like those in the stratum corneum. Since the lipid bilayers actually weave around impermeable cells called corneocytes, the next step was to consider the path the drug must take to avoid these cells and modify the diffusion coefficient (determined by the molecular dynamics simulations) to account for the behavior. The final step zoomed out to an even larger scale, modeling the effects of boundaries between the patch, the stratum corneum, and the rest of the epidermis.

"One of the insights gained from the study is that the stratum corneum acts as a regulating layer," says Rim. The slow journey of molecules through the lipid labyrinth seems to be the main reason drugs delivered via patch keep such a stable concentration in the blood.

Experiments with cadaver skin showed similar diffusion parameters to the ones calculated by the model. Drugs moved somewhat slower through the skin samples than the model, which the authors think may be due to water content. "[A future goal] is to consider the effect of water more carefully," says Rim. Other areas to pursue include studying in more detail how penetration enhancers, like oleic acid, enhance diffusion.

"This is a good example of how you can take calculations from a molecular level up into a more macro level and apply them to a practical drug delivery problem," says **Gerald Kasting, PhD**, of the University of Cincinnati. He cautions that the model uses assumptions about the stratum corneum that have been challenged—including the idea that the corneocytes are impermeable to drugs, and that the lipid bilayers contain enough cross-connections that they can be treated as isotropic. Despite those criticisms, he says, "I think this is a wave of the future. As we remove some of the limiting assumptions made in the analysis, some very useful results are going to emerge."

—By Beth Skwarecki □

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