



# Turning Skin Cells into Human Neurons



Institute for Stem Cell Biology and Regenerative Medicine

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Stanford creates nation's first stem cell PhD program



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Surgical dressing reduces scarring

## Human skin cells turn directly into neurons, skipping iPS stage

Human skin cells can be converted directly into functional neurons in a period of four to five weeks with the addition of just four proteins, according to a study by researchers at the Stanford University School of Medicine. The finding is significant because it bypasses the need to first create induced pluripotent stem cells, and may make it much easier to generate patient- or disease-specific neurons for study in a laboratory dish.

It may also circumvent a recently reported potential problem with iPS cells, in which laboratory mice rejected genetically identical iPS cells — seemingly on the basis of the proteins used to render them pluripotent.

The new research parallels that of the same Stanford group in 2010, which showed it was possible to change mouse skin cells directly into neurons with a similar combination of proteins. However, when done in human cells, the conversion of skin cells to neurons occurs less efficiently and more slowly.

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# Skin cells transformed directly into neurons

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“We are now much closer to being able to mimic brain or neurological diseases in the laboratory,” said Marius Wernig, MD, assistant professor of pathology and a member of Stanford’s Institute for Stem Cell Biology and Regenerative Medicine. “We may perhaps even be able to one day use these cells for human therapies.” Wernig is the senior author of the research, published online May 26 in *Nature*.

Postdoctoral scholars Zhiping Pang, PhD, Nan Yang, PhD, and graduate student Thomas Vierbuchen share first authorship of the paper.

Wernig’s laboratory collaborated with that of neuroscientist Thomas Sudhof, MD, the Avram Goldstein Professor in the School of Medicine, on the work.



Thomas Sudhof

After their success in laboratory mice — the results of which were published last year in *Nature* — the researchers applied a similar technique to human cells. They first showed that they could convert human embryonic stem cells to neurons by infecting them with a virus expressing the same combination of proteins: transcription factors called *Brn2*, *Ascl1* and *Myt1l*. They termed the treatment “BAM” for short. BAM treatment readily turned the embryonic stem cells into functional neurons within six days. It also worked on induced pluripotent stem cells.

So then the scientists moved to their big challenge: Could they do the same with human skin cells? In experiments using skin cells from fetuses and newborns, they found that BAM treatment caused these mature skin cells to look more like neurons, but that the resulting cells were unable to generate the electrical signals that neurons use to communicate with one another.

They wondered if there was a missing ingredient. Adding a fourth transcription factor called *NeuroD* proved to be the tipping point: The skin cells then transformed to functional neurons in the laboratory culture dish within about four to five weeks —

expressing electrical activity and even integrating into and interacting with mouse neurons grown on a laboratory dish.

Although about 20 percent of mouse skin cells can be transformed directly into neurons, only about 2 to 4 percent of human skin cells make functional neurons under the current culture conditions. And while the mouse cells accomplished their switch within just a few days, the human cells required several weeks and generated less-robust electrical signals than naturally derived neurons.

“Clearly mice and humans are different in significant ways,” said Wernig, who said that he and his colleagues are now working to optimize the technique and culture conditions to increase the efficiency and speed of the direct transformation.

The direct conversion of skin cells to neurons contrasts with similar research that first transforms skin cells to a pluripotent, or developmentally flexible, state and then coaxes them to become neurons or other specialized cells. A separate team of Stanford researchers recently used this technique to generate patient-specific neurons from a woman with Parkinson’s disease. However, that process is labor-intensive and relies on cell lines that may not fully reflect the cell-to-cell diversity that occurs in a natural population. Wernig emphasized that it is important to continue to explore both research techniques.

“The iPS cell approach is doable and has been shown to work,” said Wernig. “We need to keep working on both strategies. It’s possible that the best approach may vary depending on the disease or the type of research being done.”

Additional Stanford researchers involved in the study include graduate student Austin Ostermeier; undergraduates Daniel Fuentes and Troy Yang; postdoctoral scholars Ami Citri, PhD, and Samuele Marro, PhD; and research manager Vittorio Sebastiano.

## Stanford creates first PhD program in stem cell science

Stanford University's Faculty Senate today approved the creation of what officials believe is the first PhD program devoted solely to stem cell science in the nation and, perhaps, the world. The new doctoral program in stem cell biology and regenerative medicine is also the first interdisciplinary doctoral program created by the School of Medicine in recent years.

School officials say the fact that the university is taking the rare step of creating a new doctoral program acknowledges the growing importance of stem cell research in the realm of biomedical science. The senate's initial approval of the program extends for five years.

"Stem cell biology is a distinct discipline that requires unique skills and includes a scope of knowledge and a skill set that is not covered by other disciplines," said Renee Reijo Pera, PhD, professor of obstetrics and gynecology and director of the new PhD program. Program leaders note that Stanford is among a small number of U.S. universities that have the necessary ingredients to create a doctoral program teaching the full range of stem cell science. They add that although a few other schools have recently established PhD programs involving stem cell biology, Stanford is the first to create a free-standing doctoral program dedicated solely to stem cell biology and regenerative medicine.

In particular, Stanford has received \$186 million during the past five years — more than any other institution in the state — from the California Institute for Regenerative Medicine, which was established by state voters in 2004 to advance stem cell research in the face of more-restrictive federal funding policies. The funds have enabled Stanford to build facilities such as the Lorry I. Lokey Stem Cell Research Building (believed to be the largest building in the nation dedicated to stem cell research), to develop educational outreach and tissue banking capabilities, and to recruit a number of renowned researchers and trainees from whom the new PhD students can



**Renee Reijo Pera**

learn both the science and ethics of human stem cell research.

Philip Pizzo, MD, dean of the School of Medicine, noted that the program will draw on expertise from throughout the university to "prepare tomorrow's leaders to make fundamental discoveries in how understanding the basic biology of stem cells can impact the study of human biology and disease." The program, he added, will also create new tools and technologies to repair and regenerate cells, organs and tissues.

"It is an exciting and rapidly changing new discipline and one where Stanford can certainly be a leader," Pizzo said.

CIRM president Alan Trounson, PhD, said the doctoral program "is unique in its interdisciplinary nature and focus on applying discoveries to treat disease. This program, along with CIRM's training and research support at Stanford, will prepare the next generation of scientists to become leaders in the search for new cures."

The creation of a doctoral program will also provide a boost to graduate students and accelerate the development of the field itself. "We are establishing an entirely new field that affects both life sciences and medicine," said Irving Weissman, MD, director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine and professor of pathology.

## Device gives “stunning” reduction in surgical scarring

Researchers at Stanford University have developed a special wound dressing that they report was able to significantly reduce scar tissue caused by incisions. Results of animal tests and of an early clinical trial of the dressing were “stunning,” said Michael Longaker, MD, MBA, the Deane P. and Louise Mitchell Professor at the School of Medicine and senior author of a study that details the findings. “It was a surprisingly effective treatment.”

The study was published online May 23 in the *Annals of Surgery*.

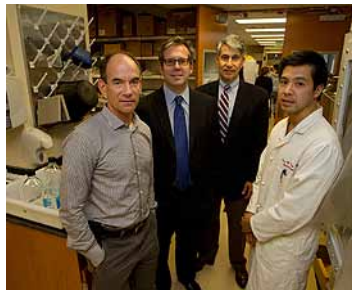
After sutures are removed, the edges of a healing incision are pulled in different directions by the taut,

surrounding skin, causing scar tissue to thicken and spread. The novel dressing, which the authors refer to as a “stress-shielding device,” eliminates this tension and hence a considerable amount of scarring.

“This work actually started 20 years ago when I was an intern at Massachusetts General Hospital,” said lead author Geoffrey Gurtner, MD, professor and associate chair of surgery. “I realized early on that we were not going to solve the problem of scarring with current surgical tools and techniques.”

Co-author Reinhold Dauskardt, PhD, professor of materials science and engineering in the School of Engineering, recalled a meeting he had with Gurtner that launched the effort to create a stress-shielding device. “We were talking about our respective research,” Dauskardt said. “Geoff had a lot of experience in wound healing and was thinking about factors that led to scarring. He said, ‘If only we could keep in check the mechanical forces acting on the wound.’ I had multiple programs on skin biomechanics and transdermal-drug delivery. I said, ‘I think I can do that.’”

Dauskardt and his colleagues created the dressing in



The research group

his lab. It is made of a thin and elastic silicone plastic that is stretched over the incision after sutures have been removed. The dressing sticks to the skin with the help of an adhesive. As it contracts, it provides uniform compression across the wound. Scar tissue, which is less flexible than regular skin, can cause functional problems, such as limiting motion. Hair does not grow in a scar, and it doesn't have sweat glands. In addition, scars do not look like regular skin: They are often raised and have a pinkish hue. Many people consider them unattractive. Yet they are an unavoidable side effect of surgery. Every year in the United States, more than 50 million incisions are created during operations.

Meanwhile, hundreds of millions of people already have scars that



The wound dressing

they would prefer to eliminate. Current scar-removal techniques, including surgical excision, steroid injections and laser therapy, are generally expensive, painful or simply not very effective, the authors say.

The researchers predicted the dressing will be used not only to reduce scarring from incisions, but also to make the surgical revision of existing scars a more appealing option; the second scar would be much less visible, if visible at all.

Norbert von der Groeben

This novel dressing, which Stanford researchers call a “stress-shielding device,” helps to eliminate tension that occurs after sutures are removed when the edges of a healing incision are pulled in different directions by the taut, surrounding skin, which causes scar tissue to thicken and spread.

Test on human patients who have undergone an abdominoplasty (“tummy-tuck”) procedure and used the device showed significant reduction in scar formation.

A longer version of this article can be found at <http://stemcell.stanford.edu>

## Scientists create neurons with symptoms of Parkinson's disease from patient's skin cells

Neurons have been derived from the skin of a woman with a genetic form of Parkinson's disease and have been shown to replicate some key features of the condition in a dish, say researchers at the Stanford University School of Medicine. The scientists hope to use the neurons to learn more about the disorder and to test possible treatments. Such a tool is critical because there are no good animal models for Parkinson's disease. It also validates the use of induced pluripotent stem cells, or iPS cells, to model various diseases.

"Now that we can see that these neurons exhibit some of the earliest signs of the disorder, we can begin to develop methods to screen for factors that might protect them," said Renee Reijo Pera, PhD, director of the Center for Human Embryonic Stem Cell Research and Education and co-senior author of the research.

"This work has the potential to remove a major bottleneck in Parkinson's disease research by allowing scientists to directly screen living human neurons with features of the disease," said William Langston, MD, of the Parkinson's Institute and Clinical Center in Sunnyvale, Calif., which was involved in the study. "Advances such as these highlight the importance of patients participating in medical research."

Associate professor of neurosurgery Theo Palmer, PhD, is the other senior author of this new paper; the research was conducted in the labs of both Palmer and Reijo Pera, who is also a professor of obstetrics & gynecology. Ha Nam Nguyen, a former research associate now at Johns Hopkins, along with graduate students Blake Byers and Branden Cord are joint first authors of the work.

"This is the first time that neurons from a Parkinson's disease patient have exhibited disease qualities in a petri dish," said Palmer. "And it provides hints of what to look for in patients who have different genetic mutations or where a cause has not been identified. By comparing neurons from patients with different forms of Parkinson's disease, we may find commonalities or

differences that will help to optimize future treatments for each patient."

Parkinson's disease is a neurodegenerative disorder that causes the gradual loss of a certain type of neuron in the central nervous system. Most cases of Parkinson's occur sporadically, but some (between 0.5 percent to about 8 percent) are caused by a genetic mutation.

Researchers at the Parkinson's Institute collected skin cells from a 60-year-old woman with a genetic form of Parkinson's. The members of the study team reasoned that they would have better chance of replicating the signs of the disorder with her cells rather than the cells of someone with the sporadic form.

Byers coaxed the iPS cells from the patient to develop into the type of neurons that die off in Parkinson's disease. At first, the neurons looked and acted normally: They were able to generate electrical signals, they produced and secreted a messaging molecule called dopamine, and their gene expression profiles over time mimicked those of neurons created from "normal" iPS cells.

However, after about 30 to 60 days of culture, the neurons from the Parkinson's patient began to express higher levels of genes for proteins needed to deal with oxidative stress and churned out elevated levels of a protein involved in abnormal clumps of protein called Lewy bodies that are found in the neurons of people with Parkinson's and Alzheimer's disorders. Oxidative stress has been previously associated with Parkinson's disease.

The researchers are now planning to begin testing various compounds to see if they can protect the neurons. They are also investigating whether they can see similar signs of disease in iPS-cell-derived neurons from patients with the non-genetic form of the disorder.

A longer version of this article can be found at <http://stemcell.stanford.edu>

**CIRM  
NEWS**

News and Information from the  
California Institute for Regenerative Medicine

## Scientists receive \$5.7 million in new funding from state stem cell agency

Four scientists at the Stanford University School of Medicine have been awarded a total of \$5.7 million by the state stem cell agency to investigate the basic mechanisms of stem cell biology, cellular plasticity and differentiation.

The awards, which were announced today, were part of \$37.7 million distributed to 27 investigators from nine institutions by the California Institute for Regenerative Medicine in the third round of the agency's Basic Biology Awards.

Stanford scientists who each received \$1.42 million Basic Biology Awards include:

**Michael Clarke, MD**, the Karel H. and Avice N. Beekhuis Professor in Cancer Biology, to study the role of a gene involved in the self-renewal of stem cells in Down syndrome and cancer.

**Renee Reijo Pera, PhD**, professor of obstetrics and gynecology and the director of Stanford's Center for Human Embryonic Stem Cell Research and Education, to correlate time-lapse studies and single-cell molecular analysis to better understand human embryo development.

**Joseph Wu, MD, PhD**, associate professor of cardiovascular medicine and of radiology, to use induced pluripotent stem cells to study the molecular basis of familial hypertrophic cardiomyopathy, a leading cause of cardiac death in young people.

**Joanna Wysocka, PhD**, assistant professor of developmental biology and of chemical and systems biology, to study how non-coding genetic regulatory regions called enhancers rapidly switch on the expression of genes to induce stem cell differentiation.

With these grants, Stanford has now received a total of about \$192 million from CIRM — more than any other institution.

In addition, the organization's governing board also voted to award \$25 million to Geron Corp., based in Menlo Park, Calif., to fund the company's ongoing FDA-approved clinical trial of the use of neural support cells derived from human embryonic stem cells to repair damage from spinal cord injury. This is the first human clinical trial of a stem cell-derived therapy funded by the agency.

In January, Stanford and Santa Clara Valley Medical Center became the third site approved to participate in Geron's phase-1 clinical trial of the cells. The first patient was treated in October 2010 at the Shepherd Center in Atlanta; Stanford has not yet treated a patient. Up to 10 patients will be enrolled during the first phase of the trial at seven sites nationwide.

"Supporting the Geron trial is a landmark step for CIRM," said board chair Robert Klein in a statement issued by the institute. "However, we must remember that there will be successes and interim failures as human trials proceed through the refinements necessary to achieve a successful human therapy. ... When the people of California voted for Proposition 71, they did so with the hope of seeing new therapies for disabling diseases like Alzheimer's disease, Parkinson's disease, diabetes and other chronic diseases and injuries. By funding this trial, CIRM is taking a major step toward making that hope a reality."