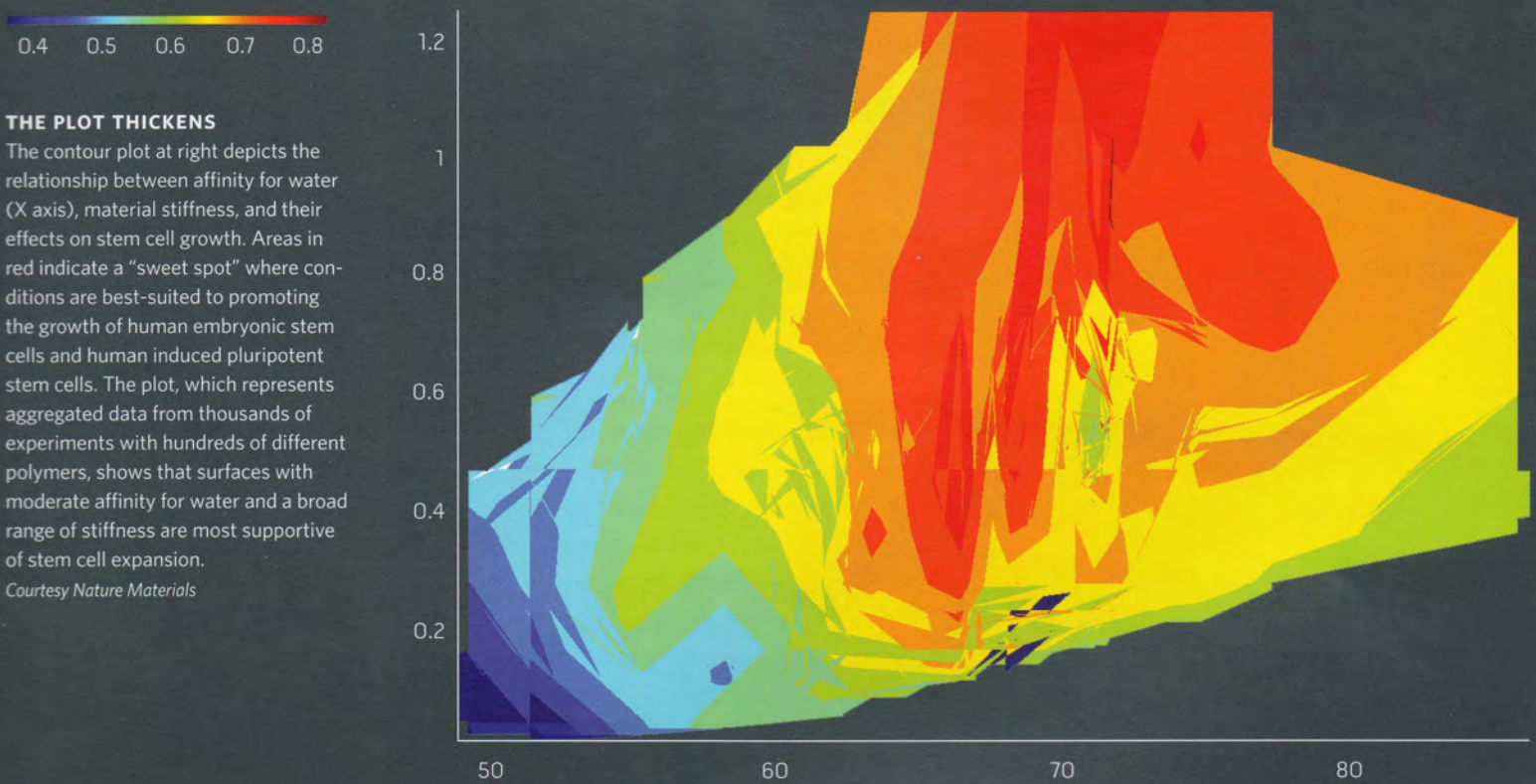
NEW GROWTH OPPORTUNITIES

A COLLABORATION IN BIOENGINEERING BRINGS STEM CELL CULTIVATION TO THE (SYNTHETIC) SURFACE

BY MATT FEARER



THE PLOT THICKENS
The contour plot at right depicts the relationship between affinity for water (X axis), material stiffness, and their effects on stem cell growth. Areas in red indicate a "sweet spot" where conditions are best-suited to promoting the growth of human embryonic stem cells and human induced pluripotent stem cells. The plot, which represents aggregated data from thousands of experiments with hundreds of different polymers, shows that surfaces with moderate affinity for water and a broad range of stiffness are most supportive of stem cell expansion.
Courtesy Nature Materials

The premise and the promise are often described in deceptively simple fashion.

Using human embryonic stem (ES) cells, possessed of pluripotency, or the unique ability to become virtually any cell type in the human body, scientists will one day generate new insulin-producing cells for type 1 diabetics, derive new neurons to treat patients with Parkinson's disease or spinal cord injuries, and even generate entirely new organs. And cellular "reprogramming"—wherein mature cells from various tissue types, such as skin or blood, are sent back to an ES cell-like state (the creation of induced pluripotent stem or iPS cells)—will overcome moral and ethical objections to traditional human ES cell research.

A series of breakthroughs over the past three years have strengthened the belief that ES and iPS cells are heralding a new era in regenerative medicine and disease modeling. iPS cells have been used successfully to treat animal models of Parkinson's disease and sickle-cell anemia. Dopamine-producing neurons have been derived from iPS cells created from skin cells of Parkinson's patients. Methods of generating iPS cells have become vastly safer and more reliable, and techniques for inserting genes into iPS and ES cells' genomes (an essential step for repairing mutations in genetic disorders) have achieved unprecedented precision.

And yet, lost amid the excitement that accompanies each technical advance is one very sobering reality: human pluripotent stem cells are extremely difficult to grow. Moreover, in order to conduct research in support of the cells' use in human therapeutics, scientists need millions of ES and iPS cells at their disposal—in part because even the best gene targeting approaches that yield cells with sought-after genetic modifications are still remarkably inefficient.

"Our ability to manipulate these cells is relatively poor in terms of numbers," says Krishanu Saha, a chemical engineer by training who is conducting his postdoctoral research in the lab of Whitehead Institute Member Rudolf Jaenisch. Saha notes that even in the Jaenisch lab, which has been pioneering novel approaches to gene targeting in pluripotent stem cells, success occurs in approximately one in 1,000,000 human cells. That one cell then needs to be coaxed to grow into colonies of identical cells, and therein lies another issue.

"Human ES cells typically die as single cells," Saha says. "We wanted to determine a way to help dissociated (single) cells grow."

Saha, Jaenisch, and collaborators from the labs of MIT Professors Robert Langer and Daniel Anderson set out to improve the state of cell culturing art, focusing specifically on the surfaces lining tissue-culture dishes. Current methods have relied on coating such dishes with a protein gel layer and a layer of mouse cells or a mixture of related proteins meant to support ES cell growth. Such approaches can promote growth when sufficiently large numbers of cells are placed in the dish but have been largely unsuccessful in attempts to grow colonies from a single cell. Further complicating matters, the use of non-human cells and other materials means cells cultured in this manner would likely trigger an adverse immune response were they eventually used to treat human patients.

ENGINEERED FOR SUCCESS Chemical engineer Krishanu Saha, a postdoctoral researcher in the lab of Whitehead Member Rudolf Jaenisch, helped develop synthetic surfaces designed to support stem cell growth. *Photos: Matt Fearer*

Saha and colleagues, including Ying Mei, a postdoctoral researcher in chemical engineering at MIT, and Maya Mitalipova, Director of Whitehead's Human Stem Cell Facility, sought to devise a surface optimized to support stem cell growth without the concomitant use of non-human products. The group created roughly 500 synthetic polymers designed with varying degrees of stiffness, roughness, and "wettability" or affinity for water. They also experimented with embedding certain proteins and plastic-like components into the surfaces, and instead of mouse cells and proteins, they coated the materials with a human protein known as vitronectin. Each polymer was tested, in high-throughput fashion, for its ability to support and maintain growth of human ES and iPS cells while preventing any differentiation or maturation of the cells.

In a triumph of chemical engineering, biology, and materials science, the researchers created a surface capable of fostering colonized growth of millions of human ES and iPS cells from a single cell. During their research, whose findings were recently published in the journal *Nature Materials*, the scientists observed that cells grown on this surface survived in an undifferentiated state for more than three months. They concluded that the surfaces most conducive to cellular growth are those with high levels of acrylate, moderate affinity for water, and a coating of vitronectin to encourage cell adhesion. Somewhat surprisingly, surface stiffness and roughness appeared to play little role in mediating cell growth.

"We spent a long time characterizing how stiff these things should be, and in the end, it just wasn't that important," says Saha. "What is important is that this work starts to define characteristics that allow us to more predictably design substrates to promote human ES cell growth."

Jaenisch sees considerable promise in this systematic approach to surface design.

"Growing human ES cells is very labor intensive and costly," Jaenisch says. "To be able to have materials that would allow us to replace mouse feeder cells is very interesting, and to do it more cheaply would be great."

Jaenisch says these surfaces are undergoing more testing in his lab to ensure they are able to continue to support growth and maintain the cells' viability over longer periods of time. Full-scale adoption in the Jaenisch lab and other stem cell facilities would likely require a partnership with a company or companies capable of mass producing these surfaces. Jaenisch and Saha report that negotiations in pursuit of such an arrangement are now under way.

In the meantime, Saha continues to try to refine the methodology with the hope of introducing additional improvements to the synthetic surfaces.

"We could probably push it further," he says. "The best polymers right now are at 30% efficiency. It's not unreasonable to think that could be doubled."

Saha and MIT materials scientist Anderson believe the approach could be adapted to develop growth substrates for other cell types.

"It's definitely a question we're looking at," says Anderson. "Can one develop plastics that can then drive special differentiation events? You would start with the stem cell state and create medically relevant cell lines."

In such a scenario, one could envision a surface uniquely engineered, for example, to grow neural progenitor cells or functionally specific neurons.

"There are a lot of important cell types," Anderson adds. "We certainly haven't exhausted all possible surfaces."