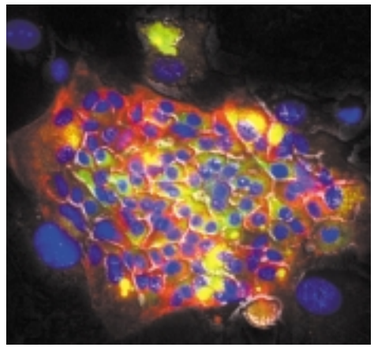
KEYWORDS • Cell Biology • Stem Cells • Medicine

Researchers Seek to Harness the Power of Stem Cells

Scientists strive to understand and exploit the power of stem cells in the hope of advancing regenerative medicine and developing next-generation therapeutics.



Isolation of human liver progenitor cells cultured *in vitro*. The blue and green colors correspond to staining with antibodies (green alpha-feto protein, red cytokeratin 19, blue DAPI counterstain for nuclei). (Image: Eric Lagasse)

Ask scientists in the field of stem cell (SC) research why they are interested in this type of work, and the answer is always "the potential of SCs." Indeed, the capacity of these unspecialized cells to divide and generate copies of themselves and specialized cell types, like blood and muscle, is the basis for their potential to shape regenerative medicine and yield curative treatments.

The road to these breakthroughs, however, requires the harnessing of SCs, which cannot fully occur until SCs and their behavior in the body are better understood. This lack of understanding also prevents scientists from comparing the potential of embryonic SCs (ESCs) to that of adult SCs (ASCs)—found in a tissue or organ in an adult body.

As to the limitations of the field of SC research, scientists agree that there are none. But there are challenges hampering the research. These hurdles include funding, time-consuming research, and the need for more trained scientists.

More people power

"The promise of human ESC (hESC) research is the hope of some day using hESCs to understand exactly what molecular switches drive differentiation into one specific cell type," says James Battey, Chairman of the Stem Cell Task Force at the National Institutes of Health (NIH), Bethesda, Md.

The rate-limiting step in this process, however, is the number of "well-trained scientists who are comfortable culturing and handling SC lines and other SC resources," says Battey. "That human resource is the most important target for me in trying to embrace the opportunity afforded by the availability of hESCs for research."

But with increasing funding support from private sources, as well as federal and state governments, Battey expects the number of scientists working in this field to grow. "Generally, science follows money," says Battey. "And money is appearing in a substantial way."

At the state level, the increase in funding is driven by "the growing appreciation of SC research, which in five to 10 years is going to create a biotech revolution similar to that of recombinant DNA and PCR (polymerase chain reaction)," says Battey. This, in turn, will benefit the states by generating both high-paying jobs and taxes from these companies.

The other rate-limiting issue for the hESC field, according to Battey, is culture conditions. Most of the time—with only a few exceptions—hESCs are cultured in the presence of mouse feeder cells. These feeder cells, which cover the bottom of a culture dish, supply nutrients and a sticky platform for the inner cell mass of hESCs. The problem is that microorganisms in this feeder layer may be transferred to human cells. "These ill-defined biologicals are going to pose safety issues for the Food and Drug Administration (FDA) when the time comes to move out of the basic science phase and into clinical trials with differentiated versions of these cells," says Battey.

As to the question of whether ESCs or ASCs hold more promise? Currently "it's unanswerable for the big spectrum of degenerative disorders," says Battey. Thus, he believes that the NIH and other funding agencies would be well-served by continuing support for both types of SC research. This is evident in the $24.3 million and $203.3 million the NIH spent on hESC and human non-ESC, respectively, in 2004. The discrepancy in the amount of money spent is not a measure of the NIH's enthusiasm for one type of SC research, however. It simply reflects the fact that "work on non-ESCs has been around for more than 20 years," says Battey. "The field is more mature and has moved into the phase of clinical trials where more money is needed."

Going once, going twice…

Different cities in Calif., among them San Francisco and Emeryville, are competing over which will house the headquarters of the California Institute for Regenerative Medicine. Initially expected to house 50 employees, the facility will manage $3 billion in stem cell research over a 10-year period. As the bidding gets heated, some cities are supplementing the minimum requirements requested by the committee judging the different bids with fringe benefits, such as free limousines and furniture.

Direct epicardial injection of stem cells into the heart (Image: Amit Patel)

Labor-intensive research

Dan Kaufman, a member of the Stem Cell Institute at the Univ. of Minnesota, Minneapolis, recognizes the NIH's role in bolstering the field of hESC research. He feels that in the next five to six years, the field of hESC research will "take off due to the concerted effort by the NIH and other groups to provide courses to train people in this type of SC research."

For his part, Kaufman is researching the beginning stages of blood development, looking at hESCs, to see how blood and related cell types arise. Specifically, his team is trying to determine which genes and proteins affect this development, using *in vitro* models to demonstrate how hematopoietic (blood-forming) cell differentiation occurs.

The knowledge gained by this research could have widespread applications. For instance, for transplantation therapies like hematopoietic cell transplants done for patients with blood cell cancers, this could be a novel source of hematopoietic SCs. There might also be applications in transfusion medicines, "so instead of getting red blood cells and platelets from the Red Cross, this would be a way to create them safely in the lab," says Kaufman.

The limiting factor in Kaufman's lab is the labor-intensive research. "People have to grow, monitor, and feed cells every day. A lot of observation is needed to determine what looks good and has the right phenotype," says Kaufman. Second to that is scaling up the number of SCs because this requires "specialized cytokines or growth factors that can be expensive in larger amounts."

Co-differentiation of SCs

Growth factors turned out to be key in Shahin Rafii's work at the Weill Cornell Medical College, New York, N.Y. As the director of the Ansary Stem Cell Center for Regenerative Medicine, Rafii is working on using SCs for organ regeneration and tumor targeting. Recently, he reported a breakthrough using growth factors, which include vascular endothelial growth factor-A (VEGF-A) and brain-derived neurotrophic factor (BDNF). These growth factors promoted the co-differentiation of a specific type of human fetal SCs simultaneously into muscle and blood vessel cells.

This co-differentiation is significant "because if you put SCs in the heart and you don't have blood vessels, the cells are going to die," says Rafii. However, "when the blood vessels co-develop with the tissue, engraftment is much more efficient."

According to Rafii, nature has done the job for researchers since the majority of SCs have the capacity to generate their own blood vessels. "It was just a matter of time before the growth factors and conditions needed to make SCs co-differentiate into blood vessels and tissue were discovered," says Rafii.

Translation of science

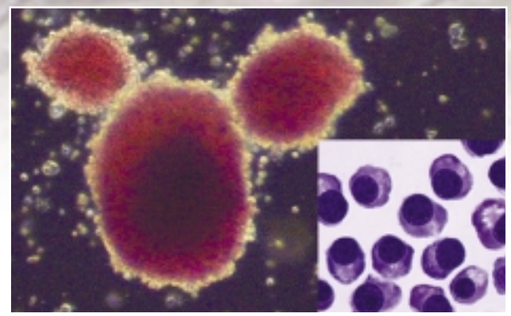
Traveling on a similar scientific path is Eric Lagasse, Director of the Cancer Stem Cell Center at the McGowan Institute for Regenerative Medicine, Pittsburgh, Pa. One of his main targets is liver disease, seeking to regenerate the liver by

SC research down under

Outside the U.S., stem cell (SC) research is being actively pursued in almost every corner of the world. At the Australian Stem Cell Centre, Victoria, researchers are focusing their efforts on both embryonic stem cells (ESCs) and adult stem cells (ASCs). "One field of inquiry impacts significantly on the other in combining to form a growing body of knowledge about the ways in which SCs behave and can be controlled," says Stephen Livesey, CSO at the Australian Stem Cell Centre.

This body of knowledge is still immature because ESC and ASC research are still in their infancy. "There is much work to be done, particularly in ESC research, before the most meaningful benefits will be realized," says Livesey. The arrival of such benefits could be hastened by the fact that ESC research—including derivation of new SC lines—is permitted in Australia. Regardless of the type of SC used, however, issues confronting Australian scientists are the same:

- Access to funding.
- The impact that legislative reviews may have on scientists' abilities to pursue research activities.
- Coordinating ESC and ASC cell research efforts.
- Translating research outcomes into therapeutic benefits.



Colonies of red blood cells derived from undifferentiated human embryonic stem cells. Insert: close-up of cells. (Image: Petter Woll and Dan Kaufman)

injecting liver SCs. He turned to SC therapy to address liver disease because "a SC is the mother of all cells," says Lagasse. And "instead of giving a drug that would just alter a certain function, you would replace the diseased cells with healthy ones, fixing the problem at its initiation."

With respect to the current challenges, Lagasse notes that funding remains a concern. "I am starting a lab and this costs a lot of money," says Lagasse. The other challenge he faces is translational—bringing to the bedside what has been feasible in animal models. This translation "is a slow and complex process. With the FDA and all the different regulations to follow, it will take 10 to 30 years to bring the technology into hospitals," says Lagasse.

SC mechanisms

Looking to provide therapies as well is Amit Patel, whose interest in SC research stems from having seen "so many cardiac patients that we couldn't help with surgery or with traditional medications." The director of Cardiac Stem Cell Therapies at the McGowan Institute for Regenerative Medicine, Pittsburgh, Pa., has been doing clinical SC research in patients for the past four years. He uses SCs derived from the bone marrow of patients' hipbones and injects them directly into the heart.

His results show that these injections benefit cardiac patients. Prior to these injections, the patients typically have less than 35% ejection fraction. (This ejection fraction—which determines the heart function—is at least 55% for an individual with a good heart). However, "within three months of the procedure, we're seeing a significant number of these patients return to greater than 50% ejection fraction," says Patel.

The challenge for Patel now is trying to figure out the mechanisms responsible for how these cells work. "So many people are using different cells and almost everyone shows some benefit. So the big question is whether these cells themselves are doing anything or recruiting other cells and substances that help the heart," says Patel. In other words, the injected cells may not become new blood vessels or new muscle. They could be acting as homing factors for other substances in the body to come to the heart and help repair it.

In five years, Patel hopes to "minimally invasively deliver the exact type of cell or cocktail of cells to patients before they become super sick, so we can act on this early on when patients are not at the end-stage of heart failure," says Patel. This will help other organs, such as the kidney and liver, from becoming affected by heart disease.

Locating better subpopulations

Lung therapy is at the center of Jay Kolls' work at the Univ. of Pittsburgh School of Medicine, Pa. The professor of pediatrics, immunology, and molecular genetics and biochemistry believes that researchers "need to think of new approaches, such as SC therapy." The reason is that "the prognosis for someone living with lung cancer in 2005 is essentially the same as it was 30 years ago," says Kolls.

Since the lung is too sophisticated in its immune response to different viral agents, the concept of using the patient's own cells became attractive to Kolls. Having helped initiate a program that examined populations of human bone marrow-derived ASC that had showed multipotency in different assays, Kolls decided to look at these cells' potential to differentiate into lung epithelial cells.

He adopted a system that involved taking different proportions of SCs and co-culturing them with adult human bronchial epithelial cells obtained from cystic fibrosis (CF) patients. "We found that the SCs have the capacity to differentiate into airway epithelial cells (AECs)," says Kolls. However, these cells were deficient in the transmembrane conductance regulator (CFTR) gene that channels chloride out of cells. This channeling is necessary because it influences the level of airway/surface liquid—which in CF is characterized by a low liquid level. Using a virus as a vector, the researcher then moved the CFTR gene into the SCs and established that the gene was expressed in the newly differentiated AECs.

"The main disadvantage right now is the low efficiency of the system and getting engraftment to the level that you would need to treat somebody with CF," says Kolls. "It's estimated that a 10% correction of the epithelial cells is needed and the engraftment efficiency in our culture is 2 to 3%." To remedy the situation, Kolls is hoping to find cellular subpopulations that will help increase that efficiency.

—Danielle Sidawi

>> Resources

Australian Stem Cell Centre Limited, 613-9271-1100, www.stemcellcentre.edu.au
Axon Instruments, 510-675-6200, www.axon.com
Chemicon International, Inc., 951-676-8080, www.chemicon.com
McGowan Institute for Regenerative Medicine, 412-235-5100, www.mirm.pitt.edu
National Institutes of Health, 301-496-5787, http://stemcells.nih.gov
RheoGene Inc., 610-650-8734, www.rheogene.com
Stem Cell Institute at the Univ. of Minnesota, 612-626-4916, www.stemcell.umn.edu
Univ. of Pittsburgh School of Medicine, 412-647-3555, www.upmc.com
ViaCell, Inc., 866-842-2355, www.viacellinc.com
Weill Cornell Medical College, 212-821-0560, www.med.cornell.edu
York Univ., 416-736-2100, www.yorku.ca

Emerging tools and technologies

Tools. Science follows money, according to the NIH's Battey, and invariably new tools will follow science. This is evident to Patrick Schneider, VP of R&D, Business Development at Chemicon International, Inc., Temecula, Calif., who sees "an increase in funding and interest in stem cell (SC) biology that will drive more investigators into the field." And as the number of SC investigators rises, "there will be an increased demand for new products that enable the derivation of new embryonic stem cells (ESCs), as well as the characterization and differentiation of new cell lines," says Schneider.

In response to that demand, the company recently launched two product lines, RESGRO and the PluriStem cell lines. RESGRO is a fully formulated media used to culture mouse ESCs, which removes differentiated cells from a mixed cell population. The PluriStem murine ESC lines, on the other hand, are used to make knockout or transgenic mice.

Catering to cell researchers as well is Axon Instruments, Union City, Calif., with its Axoporator 800A—the only single-cell electroporator currently on the market. "Other commercial devices electroporate cells in bulk and require much larger quantities of product to be delivered," says Al Walter, Product Manager at Axon. This tool, however, allows the user to target a cell of interest with a micropipette and then selectively electroporate it.

The ability of the Axoporator to target individual cells is advantageous because only the small region of cell membrane beneath the micropipette tip is affected. As a result, "cells are more likely to recover completely from the electroporation and multiple electroporations on the same cell are possible," says Walter.

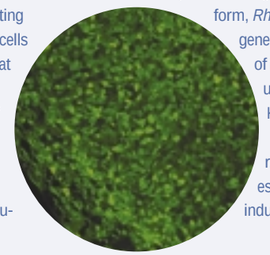
Two-channel chemical cytometry. During all fundamental biological processes, such as tissue regeneration by SCs, heterogeneous cell populations are generated. To elucidate their molecular mechanisms, the chemical differences between individual cells within heterogeneous cell populations need to be studied. "The extreme complexity of those molecular mechanisms requires that multi-component chemical analysis of single cells be performed," says Sergey Krylov, associate professor in the dept. of chemistry at York Univ., Toronto, Ontario, Canada. "This analysis can be performed only by chemical cytometry."

Working toward multiplexing chemical cytometry, a research team led by Krylov developed a two-channel chemical cytometry technique. "The novelty is in this method's ability to facilitate studies of symmetry/asymmetry of cell division/development, which cannot be done with one-channel chemical cytometry without introducing waiting-period associated biases," says Krylov. "The analysis of one cell can take a long time, and during that time the second daughter cell can enter a new stage in its development."

The two-channel chemical cytometer has two capillaries. It also features a laser and electrophoresis power supply to ensure that the variations in laser intensity and high voltage do not contribute to the differences in electropherograms from individual cells. Two cells are simultaneously injected into two capillaries, then lysed, before their contents are separated and detected under identical conditions. "Daughter cells can be sampled for analysis at different times past division, thus allowing us to observe the development of asymmetry for the daughter cells, which were born identical in a symmetric division," says Krylov.

Selective manufacturing. In the eyes of Marc Beer, President and CEO at ViaCell, Inc., Cambridge, Mass., the biggest challenge for scientists involved in SC work is manufacturing. "One of the rate-limiting issues in the SC area is being able to manufacture primary cells in pharmaceutical grade purity," says Beer. To overcome that challenge, the company is trying to leverage its ability to manufacture SCs to offer a commercial-scale availability of umbilical cord and adult SCs (ASCs).

ViaCell's scalable manufacturing process, *Selective Amplification*, applies proprietary growth factors in culture then separates out differentiated cells during a 14-day manufacturing process. "We understand through our analysis of cell cycle and cell culture systems that differentiation happens in day 4 to day 7 timeframe of the manufacturing process," says Beer. It is during that timeframe that scientists at ViaCell apply antibodies to bind to the differentiated cells, before pulling them out of the culture through a magnet technology.



Stained human embryonic stem cell colony with Chemicon's Tra-1-60 monoclonal antibody, MAB4360. (Image: Jeremy Crook, ES Cell International Pte Ltd)

Through concurrent repeated cycles of growth and purification, the vast majority of differentiated cells is extracted. "We believe it is the differentiated cells that put off their own cytokines and cause the other undifferentiated cells to become differentiated," says Beer. "So if we pick out the vast majority of differentiated cells, the result is a purified cell population."

Gene technology platform. At RheoGene Inc., Norristown, Pa., researchers developed two technology platforms: a gene targeting platform and an inducible gene regulation platform. The first platform involves recombinase enzymes that target a gene to a particular location in a cell. The second platform, *RheoSwitch* technology, allows the turning on and off of transgene expression, regulating the level of expression in a cell. "Both of these are designed to increase the precision and reduce the uncertainty of transgene expression in a SC," says Lorraine Keller, VP of marketing and communications at RheoGene.

The RheoSwitch technology is based upon a protein receptor that binds the promoter region of the gene of interest. That protein receptor is turned on when a small molecule inducer is introduced. "So when the inducer is there, the protein is turned on. When the inducer is taken away, the protein is turned off," says Keller.

Also featuring a small molecule inducer is the company's safety switch technology. This technology reflects RheoGene's interest in working on technologies that allow the elimination of therapeutic SCs in the body. This would be necessary if potential malignancy is indicated or the cells are no longer needed.

The fate of SCs in the body is a general concern in the field. This is mainly due to the cells' potential to proliferate without control. "It's not so much of a problem with ASCs," says Keller. "But when you think about putting cells into patients, and regulatory approval, these kinds of safety mechanisms are very important." Thus, to use SCs effectively as therapeutics, the expression of the gene introduced needs to be controlled over a long period of time.