microScience Imaging

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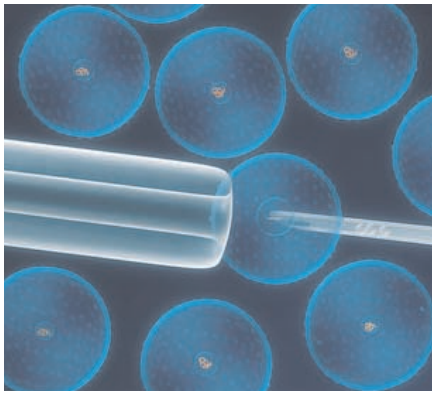
Heart Cells Grown in Lab Breakthrough

by Megan Ogilvie, *Health Reporter, The Toronto Star* (April 24, 2008)



In one of the Toronto laboratories on University Ave., a team of scientists has leapt over another hurdle in the pursuit of using human embryonic stem cells to repair damaged organs.

After careful coaxing, the team has grown the earliest form of human heart cells from embryonic stem cells and found a way to direct them into the three major cell types found in the human heart. These clusters of human heart cells, which beat in small dishes in the lab, are a feat experts say will accelerate the field of stem cell research in Canada and abroad.

The long-term hope is that the lab-created cells could be used to grow new heart tissue or repair heart muscle damaged during a heart attack. "Almost immediately, the new method of generating the heart cells can be used to make an unlimited supply for researchers to study cell processes and medical applications," says Gordon Keller, director of the McEwen Centre for Regenerative Medicine at the University Health Network in Toronto and lead author of the study.

"The ideal scenario would be that we could take these cells, freeze them away and thaw them and use them at will," he says. The manufactured heart cells likely will be used to study how the heart develops. That's something scientists know little about. Keller adds, "The cells could be used to test the effects of heart drugs, especially whether a drug is toxic to heart cells."

Click below to view a video of several hundred thousand human heart cells produced from human embryonic stem cells as seen in a Petri dish under a microscope. The cells were grown in a laboratory as scientists are researching a way to repair damaged heart tissue.
http://www.thestar.com

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The Objective

In this section, we like to highlight application-oriented stories of value to our readers.

Visit the Leica Microsystems website to view a **FREE WEBCAST** from **Prof. Dr. Jason Swedlow**, Welcome Trust Centre for Gene Regulation & Expression, College of Life Science, University of Dundee, entitled *"Life as a Voyeur of Cell Division."*

http://www.leica-microsystems.com

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Heart Cells

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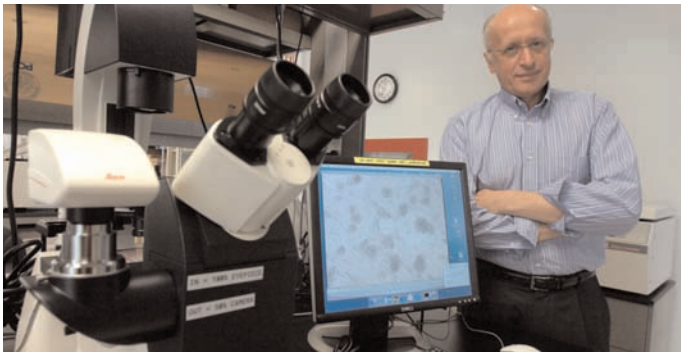
The team implanted the cells into mice and there was some evidence the cells could partially improve damaged heart tissue. But Keller says it is too soon to know whether this will work in humans.

Keller, one of the world's leading stem cell scientists, was lured to Toronto 18 months ago from his post as professor of gene and cell medicine at Mount Sinai School of Medicine in New York City. The move was considered a coup for Toronto. In January 2006, New York magazine named him one of six doctors the city couldn't afford to lose. In explaining his move, Keller credits Canada's less restrictive stance on human embryonic stem cell research. He says he was drawn to the city by the critical mass of world-class stem cells researchers with whom he could collaborate. The study, published in April 2008 in the journal *Nature*, is a medical first and outlines how human embryonic stem cells can be directed to develop into early-stage heart cells, known as heart progenitor cells.

Keller is careful to point out this is not the first demonstration that human embryonic stem cells can make heart cells. Rather, his study is the first to isolate heart progenitor cells and map their development from embryonic stem cells to the early-stage heart cells and finally to the three major cells in the heart. "These cells are remarkable . . . in that they can make cardiomyocytes, the cells that actually contract, and they can also make cells that contribute to the blood vessels in the heart," says Keller.

"The study demonstrates there is a common cell making three different types of cardiac cells and that it is possible to develop a pure population of heart cells," says Michael Rudnicki, director of the Canadian Stem Cell Network at the University of Ottawa. The purity of the heart cell populations is important since a challenge in the field is keeping out cells that can turn into tumors. "This is a very big advance," says Rudnicki. "To do this with human embryonic stem cells, which are very difficult to work with, is a technical tour de force."

The study built on work Keller and his team have done with mouse embryonic stem cells. In March 2006, the team began to translate the mouse findings to the human system. They hoped to develop a method they could use to track the progression of embryonic stem cells into different heart cells. The team used two different human embryonic stem cell lines, from Singapore and Wisconsin. "Both are approved for use by the U.S. National Institutes of Health and by the Stem Cell Oversight Committee in Canada," says Keller.



Dr. Gordon Keller with his Leica Microsystems inverted microscope

Last year, a group of researchers led by Charles Murry, director of the Center of Cardiovascular Biology at the University of Washington in Seattle, showed it was possible to turn human embryonic stem cells into heart muscle cells. That was the first key finding in what Murry calls a multi-step process towards completely understanding how the heart develops.

"When we started our work," he says, "we added certain growth factors that control early stages of embryonic development on Day Zero and pulled out heart muscle cells on Day 14. What happened in between was a black box. The exact mechanism through which these cells came about were mysterious."

"But Keller identified the key middle step," he says, "the progenitor for the entire cardiovascular system." He adds that he and his students in Seattle will adapt the new method of heart cell generation immediately. Perhaps one of the finding's most exciting possibilities is using adult stem cells, rather than embryonic ones, to derive the progenitor heart cells.

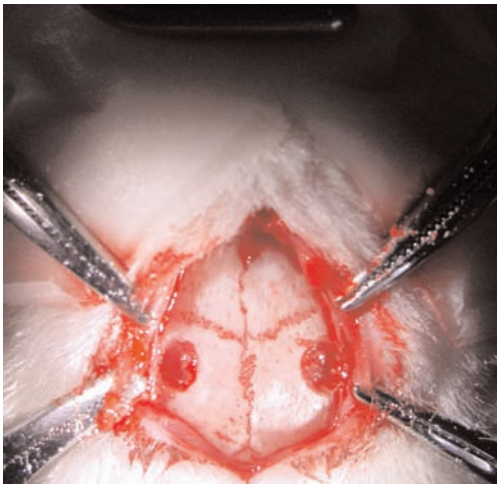
Last November, two research teams, from Japan and Wisconsin, found a way to turn adult skin cells into cells resembling human embryonic stem cells, called induced pluripotent stem cells. Using these cells, scientists could theoretically grow heart cells from a patient's own tissue, eliminating the challenges of organ rejection in tissue transplantation. "That," says Keller, "would allow scientists to study specific genetic abnormalities in patients, which, for example, may make them more predisposed to heart defects."

Deep Brain Surgery in Animal Research

Reach a target in the brain from any approach angle with the Angle Two™ Stereotaxic Instrument

by Dr. Charles W. Scouten, Ph.D, *Innovation Specialist, Leica Microsystems*

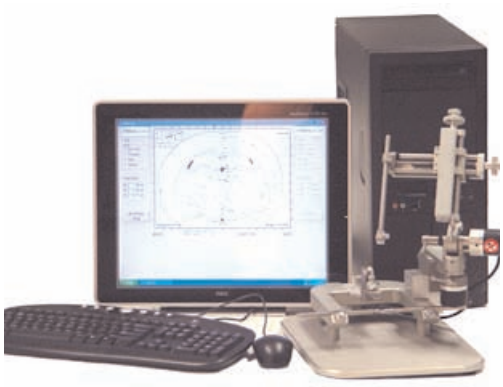
Leica Microsystems has introduced the FCM1000 for minimally invasive cellular imaging in vivo, including deep brain imaging. To image deep in brain, it is necessary to place an endoscope probe at the specific site in brain where the activity is of interest. Such activity might include movement of fluorescent labeled substances into or out of cells in that area, axonal sprouting after chemical or physical trauma, migration and fate of injected stem cells, micro vascularization changes, or measurements of cellular calcium content changes using fluorescence techniques.



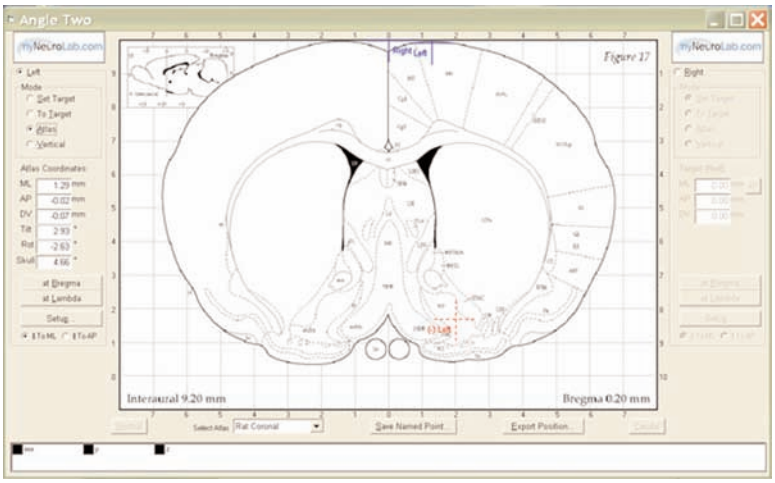
Intersections of Bregma and Lambda

Through the acquisition of Coretech Holdings LLC and MyNeuroLab.com LLC, Leica Microsystems now offers the Angle Two Stereotaxic Instrument to reach any target site in brain from any chosen angle accurately and consistently.

A stereotaxic instrument consists of a species-specific head holder mounted on a base plate and used to orient the animals' head in a defined position, and a manipulator with movement axes aligned with the head holder. The manipulator is used to move a probe to selected targets in the brain of that animal relative to chosen zero points, usually skull landmarks Bregma and Lambda, visible lines where skull plates have grown together. These points overlie the brain at consistent positions relative to brain structures in rodents (less reliably in primates or most other mammals). They are at a crossover of the midline suture, and anterior and posterior coronal sutures across the skull perpendicular to the midline.



Angle Two Stereotaxic Instrument



Angle Two screen

The Angle Two manipulator features linear encoders on the 3 linear axes, and rotary encoders on tilt and rotation movements, 5 instrumented axes in all. These connect to a computer, into which a target point in conventional atlas coordinates relative to Bregma and assuming "skull flat" (Bregma and Lambda at the same vertical coordinate) is entered either by typing, or by scrolling an onscreen atlas of coronal sections of the brain, and clicking on the desired target point with the mouse.

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Open Forum

by Ann Korsen, *Director of Sales and Marketing, Ultramicrotomy, Leica Microsystems*

How do I transfer samples between preparation instruments and analysis tools?

Many analysis techniques require the transfer of specimens from a preparation chamber, such as a cryoultramicrotome or a, freeze fracture instrument, into a system such as an SEM, FIB, AFM SIMS or XPS chamber. This process requires a contamination-free, well-defined environment, which may include protective gas, high vacuum or low temperature. The Leica VCT100 transfer system is designed with this criteria in mind. The sample holders used for transfer are compatible with an integrated stage, which can be installed in any ultra high vacuum system or glove box and can be used for both room temperature and cryo applications. An air lock mounts the transfer shuttle to any SEM, FIB, AFM, SIMS or XPS chamber. Preparation and analysis can be performed in separate locations.

Cryo SEM has been established as the surface analysis method of choice for wet (soft, condensed, and hydrated) and beam-sensitive specimens. Cryogenic specimen preparation is faster than conventional preparation techniques like chemical fixation with subsequent dehydration and critical point drying. With the right experimental set-up the entire specimen preparation process from object extraction to immobilization (freezing), surface preparation, coating, and imaging can be done in less than one hour.

A typical preparation procedure starts with cryo fixation of the specimen using techniques such as high pressure freezing with the Leica EM PACT 2 or HPM100 high pressure freezer or plunge freezing with the Leica EM CPC, depending on the nature of the specimen. The specimen is then transferred to a preparation unit (Leica SCD500, MED020 or BAF060) using the Leica VCT100 shuttle. In the preparation unit the specimen can be fractured, etched, and coated. The specimen is then transferred, and protected under high vacuum and by a cold trap, onto the cryo stage in the SEM. For the actual analysis the shuttle detaches from the SEM.

Cryo SEM advantages include:

- Physical preparation (no artifacts as from chemical treatment)
- No handling with toxic reagents
- Sole preparation technique for many applications
- Beam damage reduction due to low temperature during imaging

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Your Educational Resource

Leica Microsystems will exhibit and hold tutorials at booth #430 during the **Microscopy & Microanalysis (M&M)** Exhibition at the Albuquerque Convention Center, August 3-7, 2008.
Email **ann.korsen@leica-microsystems.com** to register for the following tutorials:

Monday, August 4, 2008

How to optimize specimen preparation time of Hard Materials: Target X polishing, Ion mill

The Leica Target X cutting and polishing system for the production of high-quality flat surfaces suitable for preparation of many industrial samples such as LEDs, solder joints, microprocessors, PCBs, and board components will be demonstrated and discussed.

Tuesday, August 5, 2008

Cryo ultramicrotomy for CEMOVIS and TEMOVIS (with Diatome US)

We will discuss the important steps in the preparation of ultrathin cryosections of frozen hydrated samples, including the ultramicrotome environment, trimming, the antistatic device, sectioning, and section pick-up. We will also show video presentations of protocols and new developments.

Wednesday, August 6, 2008

Correlative microscopy: How to prepare samples for LM to EM live cell imaging.

There is an increased need to prepare a sample for both LM and EM techniques. This tutorial will discuss the latest advances in correlative microscopy utilizing high pressure freezers, which allow the same sample to be visualized by LM and EM for tomography, cryosectioning or immunolabeling.

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October 7-16, 2008

Optical Microscopy & Imaging in the Biomedical Sciences

Directors: Colin Izzard, State University of New York, Albany, and Robert Hard, University at Buffalo

http://www.mbl.edu/education/courses/special_topics/om.html

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Special Announcement

Leica Microsystems provides the world's most comprehensive product portfolio for electron, light, and confocal microscopy sample preparation with the acquisition of Bal-Tec AG. With this acquisition, Leica Microsystems is the only company able to cover the entire sample preparation process in the microscopy laboratory for both biological and materials sample preparation.

The former Bal-Tec AG, based in Liechtenstein and now part of the Leica Microsystems family, is an important manufacturer of both mechanical and cryo sample preparation equipment for Scanning Electron Microscopy and Transmission Electron Microscopy. Products such as the EM VCT100 Vacuum Cryo Transfer system for SEM, which provides sample transfer from preparation equipment to analysis systems for EM, and the EM HPM100 High Pressure Freezing unit, are now available from Leica Microsystems.

Leica Microsystems' product range includes biological sample preparation instruments for TEM, where Leica Microsystems is the market leader. The Leica EM UC6 Ultramicrotome with FC6 Cryo attachment and the EM TP Tissue Processor established Leica Microsystems as the premier EM sample preparation instrument provider. Leica Microsystems recently entered the SEM and solid-state sample preparation market with the launch of the Leica EM TXP Target Preparation instrument.

Please contact Leica Microsystems to discuss your specific sample preparation needs.

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Upcoming Events

For more events, visit: **http://www.leica-microsystems.us**
(click on Company, then Events)

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For You

New Investigator Program
Starting a new lab? Beginning a new research project? If so, you could qualify for thousands of dollars in discounts on Leica Microsystems widefield microscope, stereomicroscope, digital camera, and imaging software products.

Demonstration Microscopes & Accessories starting at 25% Discount
Some of our best imaging equipment is available at sizeable discounts. All of the demonstration equipment mentioned here carries Leica Microsystems' full warranty and is available on a first come, first served basis. Currently available is a completely configured fixed cell, deconvolution microscope with automated z-drive (DM6000 B model). In addition, we have a large assortment of single objectives, filter cubes, prisms, and accessories available starting at 25% off of list price!

FREE Fluorescence Imaging Software
Receive a **FREE** copy of our ease-to-use AF6000 E fluorescence software package (a value of $2,400) when you purchase a fully configured Leica Research Upright, Inverted, or Stereofluorescence microscope with digital camera. Leica software guides users through experiments with a workflow-based design to allow users to be proficient very quickly and reduce time spent on training.

Ultimate Precision Trade-up Offer
Leica Microsystems is pleased to offer a trade-up discount on select EM and LM specimen preparation instruments: the Leica EM UC6 Ultramicrotome; Leica HPM100 and EM PACT High-pressure Freezers; Leica MED020, SD500, and SCD005/SCD050 Sputter-coat Devices; and the Leica CPD030 Critical Point Drying system. This offer is only valid on orders received from **August 13, 2008** through **December 31, 2008**.

Contact Leica Microsystems today at 800-248-0123 or email microscience.imaging@leica-microsystems.com to find out how these Special Offers can improve your research and save you money.

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Technology Fast Track

(continued from page 3)

The animal is then anesthetized and installed in the head holder, the skull landmarks exposed, and the manipulator tilted and/or rotated to any chosen complex angle. Touching the probe tip to Bregma, and clicking an onscreen button, informs the computer as to where Bregma is in the tilted and rotated and non-orthogonal coordinates, and enables it to instantly calculate how far to move along each linear axes to reach the chosen target, given the manipulator tilt and rotation. The atlas display on screen shows where the probe tip is now above or in brain as the operator moves it toward the target. The user can see what structures are being traversed as the probe moves toward the target.

Adjusting the animal's head to skull flat is normally a trial and error and time consuming process. Very small errors in head tilt give large errors in position reached, especially if the probe is being lowered to a position deep in brain. The Angle Two includes the Virtual Skull Flat™ feature. Touching the probe to Lambda, and clicking a button on screen, after showing the computer where Bregma is located, enables the computer to calculate the degree of head tilt, and how that alters the target position. The path to the target is then recalculated given the head tilt, and a mathematically correct target position presented. It is no longer necessary to achieve actual skull flat adjustment.

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Click on "Find Your Local Sales Representative"

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Editorial Staff

Editor-in-Chief:	Molly Baker
Managing Editors:	Pam Jandura, Lon Nelson, Dr. Charles W. Scouten, Ann Korsen
Graphic Design:	M.N. Kennedy
Special Thanks:	Dr. Jason Swedlow, Torstar Syndication Services

Note: *We are interested in your comments and thoughts about the newsletter. Please feel free to email your comments to:* ***microscience.imaging@leica-microsystems.com***