California Institute for Regenerative Medicine
Overview of Technologies Relevant to Stem Cell Research

Introduction

The following overview provides a high-level summary of new and existing technologies related to the purification, delivery, and imaging of human embryonic stem cells (hESC). It is designed to provide some background for our discussion and is not intended to be comprehensive or exhaustive.

I. Discussion of Technologies

As our understanding of stem cell science has evolved, so too have the technologies that make research in the field possible. This process often occurs in a mutually reinforcing manner, with science advancing technology and technology in turn advancing science. Given this, and the fact that CIRM will support the advancement of stem cell science, it seems there may also be a role for CIRM to play in supporting the development of new, stem-cell-related technologies.

A. Cell Labeling and Analysis

The process of cell identification and separation can proceed via two paths, positive selection, and negative selection. In positive selection, the cells of interest are labeled using an antibody, dye, or similar technique, and are then selected out from a larger population, with the remaining cells being discarded. In contrast, in negative selection it is the cells that are to be discarded which are labeled and selected out from a larger population, while the cells of interest are left behind. (1)

1. Antibodies

a. Antibody-based separation is a rapid cell separation procedure for the isolation of highly purified cells directly from a sample.
b. Many separation technologies rely on the high specificity of antibody recognition to a given antigen to tag cells bearing that antigen.
    i. The effectiveness of the technology relies on the identification of antibodies that are highly specific for the target cell population for positive selection and broadly specific for contaminating cell populations in the case of negative selections.
    ii. Oftentimes, these criteria are met only with more than one round of antibody selection, with positive and negative selection technologies often employed to achieve a desired subpopulation. (2)
c. Separation of an antibody tagged cell population is then achieved through a separation technology such as centrifugation, cell sorting, chromatography, or the use of magnets.

d. The advantage of negative selection antibody techniques is that desired cells are never labeled with antibody; positive sorting technique require that the cell be separated from the antibody before it can be used further. (3)

2. Nanodots / Quantum Dots

a. This separation method involves the use of fluorescent particles just 15 nanometers (or one-billionth of a meter) in diameter. These particles, called nanodots or quantum dots, have unique electronic and optical properties that make them easier to detect than conventional fluorescent tags used in biomedical research.

b. Following attachment of the particles, analysis of the intensity of the fluorescence signal (as well as other properties) can be used to identify and ultimately separate out the cells of interest. (4)

3. Fluorescent Dyes

a. Fluorescent dyes are one of the most common methods for labeling cells, either directly or indirectly.

b. Direct labeling methods, such as those that target DNA, can be used to stain the nuclei of living cells and to ultimately sort cells based on their DNA content.

c. Indirect methods, such as immunofluorescence, involve attaching a fluorescent marker to an antibody and using the complex to locate specific proteins on the cell surface. (5)

B. Cell Separation (analytical / small scale preparations)

1. Magnets

a. Antibodies reacting to specific cell surface markers can be bound to magnetic beads and used to specifically capture cells exhibiting the marker. This approach allows the selective enrichment of specific cell subpopulations by either negative or positive selection.

b. As this method is amenable for use in blood, tissue fluids, and culture medium, living cells can be recovered that can subsequently be subcultured. Cells positively labeled with and separated with magnetic nanoparticales can be further analyzed by flow cytometry.

c. Negative cell separation can also be used where undesired cell types are tagged using magnetic beads and removed from the desired cell population. (6)

2. Flow Cytometry (FCM) / Fluorescent Activated Cell Sorting (FACS)

a. Flow cytometry (FCM) is a general term that refers to a type of analysis based on detecting fluorescence levels of single cells. A specialized flow cytometer, a sorter, can sort cells depending on user defined parameters. Flow cytometry

is the current standard for high speed multi-parametric cell sorting and dispensing of objects from 10 to 1,500 microns in size.

i. Functional studies and sorting of larger cells / cell clusters (e.g., islets, embroid bodies) and small multicellular organisms (e.g., *C. elegans*, zebrafish, etc.) are now possible using large particle flow cytometry. The large bore fluidics and a gentle sorting mechanism allow use with live biological materials and sensitive chemistries.

b. FCM sorters can purify a very small subpopulation (1 cell out of 100,000) defined by criteria based of size and fluorescence properties and have a very low error rate. They typically require a minimum of 100,000 cells in the starting population to achieve a reasonable yield. (7)

c. The major disadvantages of FCM are limited throughput and loss of yield.

i. For reliable sorting, even on a high-speed sorter, the flow rate cannot exceed a few thousand cells / second.

ii. In addition, when pairs of cells go by too close together to distinguish, they must both be discarded.

iii. For a subpopulation which is 20% of the starting population, a high-speed sorter will yield less than 10^6 cells / hour. Many experiments require far more cells than this. (For a frame of reference, a single mouse can provide more than 10^8 lymphocytes, or 10^9 tumor cells.)

iv. Running the high-speed sorter all day gets expensive, and may pose problems in keeping the cells in best condition during such a long sort.

d. The term FACS (fluorescently activated cell sorting) is often used generically to refer to cell sorting; the term is actually a brand name coined by Becton, Dickinson and Company to refer to a specific set of their flow cytometry products. (8)

3. New Microfluidic Sorting Technologies

a. Microfluidic-based approaches may be particularly advantageous for "lab-on-a chip" applications where handling a small numbers of cells (10,000 – 100,000) with high yield is required. This feature is beneficial in applications utilizing precious cells such as primary and / or stem cells and / or where assay miniaturization is desired. (9).

b. Microfluidic cell sorters have utilized several methods for the active control of cell flow including diaelectric force switching and optical force switching.

i. Dielectric Force Switching

- In dielectric force switching, cells are labeled with specifically engineered dielectrophoretic tags, so that the differences in dielectric constants (a measure of a material's electrical conductivity) provide a basis for separation.
- The labeled cells are then passed through a multi-stage array of sorting chambers, an approach which enhances throughput, purity, and recovery.

ii. Optical Force Switching (9).
    - Optical forces are used as a switch to sort cells in a fluorescence activated microfluidic cell sorter with high purities and recovery rates; physiologic buffers such as cell culture medium could also be used. In a study done on mammalian cells, stress genes were not induced suggesting cell sorting by this technique should result in a viable cell population.
    - Throughputs are lower than conventional cytometer, but sorting cell populations of 1,000-280,000 cells can be achieved in less than an hour, a reasonable time for small cell number / high yield applications for which microfluidic devices are most suited

4. Nanotechnology

a. Microscale approaches are potentially powerful tools for stem cell biology because they can be used to control cell microenvironment interactions (e.g., between cells, between cells and the extracellular matrix, and between cells and soluble factors) and to miniaturize assays for high-throughput experimentation.

b. A number of novel microscale technologies have been developed than can control cell-microenvironmental interactions that provide control over spatial and temporal presentation of cues to cells.
    i. These tools include patterned co-cultures, microfluidics, and micro / nanopatterened substrates have been used to control ES cell differentiation in defined conditions and have led to novel culture conditions to regulate cell differentiation.

c. It is envisioned that the incorporation of patterning approaches can be used to direct cell behavior and induce stem cell differentiation to generate desired cell types. (10) (11)

B. Cell Delivery

1. Cell Encapsulation

a. Encapsulating cells in a biocompatible substance prior to implantation is one is one mechanism designed specifically to protect the cells after implantation.
    i. For example, it has been shown that using of polyethylene glycol to coat the surface of islets (clusters of cells containing insulin-producing cells) prior to implantation allows glucose and insulin to pass freely through the coating while preventing the body's immune system from destroying the islets.
    ii. The ability of insulin and glucose to permeate the cell coating allows the transplanted islet cells to regulate insulin levels in response to blood glucose concentration. (12)

2. **Scaffolds**
    a. Scaffolds are being used to target the location of cell delivery and manipulate the environment after delivery. (13)
    b. Researchers have developed scaffolds using fibrin (a form of the plasma protein fibrinogen) which are used as a "patch" to deliver cells to the appropriate location.
        i. Such a patch has been used to deliver autologous bone marrow stem cells to sites of myocardial infarction; this approach may serve as a therapeutic modality for myocardial repair.
        ii. The success of tissue engineering strategies for bone repair that involve injecting stem cells into biocompatible, 3-D fibrin matrices and then transplanting the scaffolds at the site of bone injury depend to a large extent on the concentration of the fibrinogen formulation used to prepare the scaffold and the structure of the clot that forms. (14)
    c. Increasingly, synthetic biomaterials are being developed as cellular scaffolds to address potential issues associated with purification, immunogenicity, and pathogen transmission and to allow greater control over materials properties and tissue responses. (15)

3. **Injection (16)**
    a. There are several strategies for the use of injection as a cell delivery modality in stem cell therapy.
    b. The cells can be delivered through coronary arteries, coronary veins, or peripheral veins.
    c. Alternatively, in the case of cardiac therapies, direct intramyocardial injection can be performed, using a surgical, transendocardial, or transvenous approach.

C. Imaging

When applied to stem cell science, imaging can be used in two ways. The first is an *in vitro* process, where imaging is used to study the behavior of stem cells in culture by focusing on single cells and / or cell-cell interactions. (17) The second is an *in vivo* process, where the noninvasive imaging of cells is used to follow cell migration and behavior following transplantation and to ascertain if cells are reaching their intended target, if they are still alive when they do, and if they functioning. (18)

1. **Confocal Imaging and Analysis (19)**
    a. Confocal microscopy of fluorescently labeled cells coupled with computerized digital imaging is a powerful technology for exploring biological event in cells *in vitro*.
    b. Current technology allows multi-dimensional imaging of five or more fluorescent probes in the same sample as well as real-time, single cell kinetic and end point imaging in living cells.

2. 3-D Embryonic Stem Cell GFP-Based Fluorescence Assay

a. Researchers have designed, built, and tested a high-throughput, real time, bioactivity assay based on the three-dimensional (3-D) culture of GFP-expressing embryonic stem cells.

b. This assay approach can increase the fluorescence signal to noise ratio by at least one order of magnitude as compared to conventional 2-D culture system.

c. This system has the potential for use as high-throughput biosensors, microbioreactor arrays for fast cell culture media development, cytotoxicity assays for drug screening and discovery, and the whole-process monitoring of embryonic stem cell proliferation and differentiation. (20)

3. Magnetic Resonance Imaging (MRI)

c. Image-guided Surgery (21)

i. Image-guided surgery systems use medical imagery to decrease the invasiveness of a procedure and to increase accuracy and safety and may have applications as a precise method to deliver stem cells for transplantation.

ii. Researchers have developed a guidance and visualization system which integrates data analysis and on-line guidance into the interventional MRI setting; use of this system enhances and speeds up tissue characterization and precise localization and targeting.

iii. To date, this tool has been used in numerous neurosurgical procedures. The system's flexible design will likely allow its expansion into other applications.

d. Functional Magnetic Resonance Imaging (fMRI) of the Brain (21)

i. fMRI is a relatively new technique that builds on the basic properties of MRI to measure quick and tiny metabolic changes, including in the active brain, thus providing not only an anatomical view of the brain, but a minute-to-minute recording of actual brain activity.

ii. This technology is now being used to study and compare the anatomy of the normal, diseased, and injured brain and to assess risks associated with surgery or other invasive treatments.

iii. An advantage of the fMRI technique is that it can be used to compare results from animal and human studies if and when new therapies for the treatment of stem cell based spinal injury can be tested on patients.

e. *In-Vivo* (Non-invasive) MRI

i. Given the relatively low toxicity of the label used, the ability to follow labeled cells for approximately 1 week after delivery, and the high spatial resolution that allows localization to the suborgan level, *in vivo* MRI is an attractive approach for determining the location and amount of cellular delivery following cellular transplants. (22)

ii. A major challenge in this area will be to combine the reporting of stem cell delivery with a noninvasive evaluation of stem cell migration, engraftment, and differentiation. (22)
iii. While *in vivo* MRI seems well suited to such a noninvasive approach, ultimately, combining multiple modalities may aid in a more complete evaluation of stem cell therapy. (23)

4. Photon Emission Tomography (PET)

a. PET produces images of the body by detecting the radiation emitted from substances injected into the body that are usually tagged with a radioactive atom that has a short decay time. (24)
b. As questions about the use of embryonic versus adult stem cells with respect to robustness and durability are addressed in animal transplantation models, continued advancements in noninvasive imaging technologies, such as PET, will allow these events to be observed in real time with reasonable resolution and without having to use large numbers of animals. (25)

5. Single Photon Emission Computer Tomography (SPECT) (26)

a. In SPECT, images are generated by using radioactive materials that emit single photons of a given energy. Images are captured at multiple positions by rotating the sensor around the subject; the three-dimensional distribution of radionuclides is then used to reconstruct the images.
b. SPECT can be used to observe biochemical and physiological processes, as well as the size and volume of structures.
c. Researchers from Seoul National University have shown that SPECT can be used to evaluate the effectiveness of stem cell therapy with ischemic or coronary heart disease.

6. Contrast Enhanced Nuclear Magnetic Resonance (NMR)

a. While most cellular transplantation techniques designed to repair damaged myocardial tissue require histologic analysis to determine cell status, the ability to label mesenchymal stem cells with a contrast medium and nuclear tracers makes contrast enhanced NMR a viable option for noninvasive, serial tracking and quantification of transplanted cells. (27)
b. Cardiac magnetic resonance imaging of this type is one of the most sensitive techniques available to assess spatial and temporal changes following local or systemic therapies; the availability of complementary techniques enables the integrated analysis of physiology, morphology, and metabolism in one setting. (28)

II. Potential Role for CIRM - Topics for Discussion

A. Making New, ESC-Related Technologies Known to the Research Community

1. As some of the newer ESC related technologies may not yet be well known to some researchers (particularly those new to the field) CIRM might consider ways to make such technologies better known to the research community.
2. CIRM might also support courses, perhaps in conjunction with the service cores discussed below, to educate and train researchers in the use of such technology.

B. Making ESC Related Technologies Available to the Research Community

1. CIRM might consider supporting researchers / institutions in their purchase of some of the equipment discussed above (e.g., flow cytometry machines) with the understanding that such equipment must be made available to as broad a segment of the research community as possible.
2. Alternately, CIRM might establish cores that offer researchers access to such technology (particularly larger scale technology, such as imaging equipment) on a service basis.

C. Supporting Refinement / Development of New Technologies

1. CIRM might consider how it could foster interdisciplinary discussions about technology gaps and possible solutions.
2. CIRM might support the refinement of existing technologies discussed above, including broadening their applications to stem cell science.
3. CIRM might also support for "proof of principle" type experiments that allow researchers to demonstrate the viability of new technologies, whether aimed at stem, cell purification, delivery, or imaging.

References

(1) http://www.stemcell.com/product_catalog/easysep.asp
(2) http://www.sciencemag.org/products/assays.dtl#Two
(3) http://www.stemcell.com/product_catalog/rosettesep.asp
(4) Nucleic Acids Research, February 11, 2004
(5) http://probes.invitrogen.com/handbook/sections/0805.html
(6) Methods Mol Biol. 2005;295:291-300
(7)http://www.dddmag.com/ShowPR.aspx?PUBCODE=016&ACCT=1600000100&ISSUE=0310&RELTYPE=PR&ORIGRELTYPE=INS&PRODCODE=00000000&PRODLETT=AF
(8) http://www.bio.umass.edu/micro/immunology/facs542/facsprin.htm#terminology
(9) Nature Biotechnology 23:83-87, 2005
(10) Dr. Ali Khademhosseini, Harvard / MIT Division of Health Sciences and Technology, www.triconference.com
(11) http://www.tissueeng.net/lab/papers/Khademhosseini%20NanoBio_CEP.PDF
(12) http://www.novocell.com/encapsulation.htm
(13) *Am J Physiol Heart Circ Physiol* 287: H501-H511, 2004
(14) Tissue Engineering, June 27, 2006, Volume 12, Number 6 www.liebertpub.com/ten
(15) Nature Biotechnology 23:47-55, 2005
(16) Nature Clinical Practice Cardiovascular Medicine (2006) 3, S110-S113
(17) http://www.snidd.org/home.htm
(18) http://www.rt-image.com/content=8004J05C4876908040B69A7644A080441
(19) http://www.bdbiosciences.com/pharmingen/products/display_product.php?keyID=99
(20) www.tri-conference.com/track6_STM.asp
(21) http://www.nbib.nih.gov/HealthEdu/Pubs/Discovery/HistPerspective
(22) PNAS, December 10, 2002, vol 99, no. 25, 16267-16272
(23) Radiology 2004;233:625-626
(24) http://science.howstuffworks.com/nuclear-medicine2.htm
(25) http://stemcells.nih.gov/info/scireport/chapter10.asp
(26) http://interactive.snm.org/index.cfm?PageID=4093&RPID=627
(27) Nature Clinical Practice Cardiovascular Medicine (2006) 3, S2-S7
(28) http://www.diagnosticimaging.com/molecularimagingoutlook/2003jun/09.jhtml