

Australian  
Stem Cell  
Centre

## Collaborative Streams – Excellence in Australian Stem Cell Research

The four new ASCC Collaborative Streams are an innovative way of funding stem cell research in Australia. Each Stream consists of a network of high-calibre Australian scientists with internationally-recognised leaders who have agreed to work collaboratively to solve key questions in stem cell research and translation. The Collaborative Streams consist of 32 research modules from across Australia, built around four important themes of stem cell research: embryonic stem cells, adult stem cells, induced pluripotent stem cells (iPS cells) and bioreactors for the propagation of stem cells. Announced in July 2009, the Collaborative Streams are currently funded to June 2011.

### Collaborative Stream 1 – Bioreactors and Smart Surfaces for Stem Cell Propagation

The great promise of stem cells is that they will one day be used to replace a patient's diseased or damaged cells. It is essential that methods are developed for cells to be grown efficiently and economically in the laboratory in large enough numbers to support any cellular therapies that may become available.

#### S1: Bioreactors and Smart Surfaces for Stem Cell Propagation

*Stream Leader:*  
Prof Peter Gray (AIBN,  
University of Queensland)  
*Deputy Stream Leader:*  
A/Prof David Haylock (CSIRO)

Stem cells of all kinds respond to specific changes in their natural environment by dividing or by changing their patterns of gene expression so that they can carry out specific functions in the body. Bioreactor technology relies on smart surfaces, complex molecules and biologicals to approximate the natural environments suitable for cellular growth and expansion. The *Bioreactor and Smart Surfaces for Stem Cell Propagation* Collaborative Stream unites six currently-funded ASCC research groups with complementary expertise ranging from biomaterials, bioengineering and haematology to pluripotent stem cell biology. This Stream will design, test and validate artificial surfaces that recreate natural cell-cell and cell-surface interactions and can be mass-produced. In addition, the researchers will collaborate in the design, synthesis and testing of small molecules that can be directly added to media in the bioreactors that are used to grow particular types of stem cells and differentiated cells in large volumes.

#### Stream 1 Modules

**M1: Assessment of Smart Surfaces for Culture of Haemopoietic Stem Cells & Megakaryocytic Cells**  
*Module Leader:* A/Prof David Haylock (CSIRO)

**M2: AIBN Reactor Program**  
*Module Co-Leaders:*  
Prof Peter Gray, Prof Justin Cooper-White,  
A/Prof Ernst Wolvetang (AIBN, University of Queensland)

**M3: Functional Assessment of Ex Vivo Expanded**  
*Module Leader:* Prof A/Prof Susie Nilsson (CSIRO)

**M4: Production of Neutrophils**  
*Module Leader:* Prof Lars Nielsen  
(AIBN, University of Queensland)

**M5: Development of Pluripotency Reporter Lines for Facile Screening of Bioreactor Conditions**  
*Module Leader:* A/Prof Andrew Laslett (CSIRO)

**M6: Safe & Efficient Expansion of Genetically Stable hESC**  
*Module Leader:* A/Prof Ernst Wolvetang  
(AIBN, University of Queensland)

The research groups are located at the University of Queensland and CSIRO Victoria.

#### Case Study – Bioreactors – how do we generate cells in relevant numbers for future therapies?

Stem cells hold great promise, but before they can be used in patients, several technical and practical hurdles need to be overcome. Cells will need to be grown in large numbers and in controlled conditions using culture systems designed to expand populations of stem cells with uniform properties. This will allow controlled, reproducible differentiation of stem cells into selected mature cell types such as heart cells or blood cells. In addition, cells for use in humans need to be grown in conditions free from animal products and contaminants to ensure they are safe for transplantation.

Professors Justin Cooper-White, Peter Gray and Ernst Wolvetang are working together at the AIBN to overcome these hurdles. They have combined their multidisciplinary expertise in biomaterials, microfabrication, mammalian cell culture, bioprocess development and stem cell biology to focus on developing defined bioprocesses for the expansion of stem cells and the differentiated cells derived from them. Ultimately, these bioprocesses and the associated technology will be applicable to all stem cell researchers developing potential therapeutics based on stem cell technology.

## Collaborative Stream 2 – Reprogramming and Induction of Pluripotency

Reprogramming, of an adult cell to a pluripotent stem cell – referred to as an induced pluripotent stem (iPS) cell - has been one of the most significant advances of the past ten years. First described using adult mouse cells in 2006, iPS technology was quickly adapted for use in human stem cells. iPS cell technology provides scientists with a new method to investigate reprogramming, cell maintenance and differentiation. Ultimately, iPS cell technology will also be used to better understand disease and to develop possible treatments.

A number of Australian researchers, including those funded by ASCC, have already established iPS technology within their laboratories. The *Reprogramming and Induction of Pluripotency* Collaborative Stream will bring researchers across Victoria, Queensland, New South Wales and South Australia together to drive the development of iPS-cell based disease models and drug screening platforms. The researchers will investigate the molecular mechanisms involved in reprogramming and will apply these skills to the investigation of disease models – for example schizophrenia and dental disease.

The collaboration consists of eight modules, groups who will form a close liaison with the ASCC's core facilities, which will bank, analyse and distribute the iPS cells generated within the collaboration for use by researchers around Australia.

### S2: Reprogramming & Induction of Pluripotency

*Stream Leader:*  
A/Prof Ernst Wolvetang  
(AIBN, University of Queensland)  
*Deputy Stream Leader:*  
Dr Andrew Laslett (CSIRO)

### Stream 2 Modules

<p><b>M1: Novel Methods of Reprogramming</b> <i>Module Leader:</i> A/Prof Ernst Wolvetang (AIBN, University of Queensland)</p>
<p><b>M2: Novel Approaches for the Generation of Clinically Relevant iPS Cell Lines</b> <i>Module Leader:</i> Dr Paul Verma (Monash Institute of Medical Research)</p>
<p><b>M3: Are Human iPS Cells Equivalent to hESC?</b> <i>Module Leader:</i> Dr Andrew Laslett (CSIRO)</p>
<p><b>M4: Transcriptomic &amp; Epigenomic Analysis of Pluripotency &amp; iPS Utility</b> <i>Module Leader:</i> Prof Sean Grimmond (IMB, University of Queensland)</p>
<p><b>M5: iPS Cells as Models of Complex Brain Disorders</b> <i>Module Leader:</i> Prof Alan Mackay-Sim NCAS, Griffith University)</p>
<p><b>M6: Reprogramming Adult Cardiac Stem Cells to Pluripotent iPS-like Cells In Vitro Without Genetic Modification</b> <i>Module Leader:</i> Prof Richard Harvey (Victor Chang Cardiac Research Institute)</p>
<p><b>M7: Primitive iPS-derived MSC for Bone Repair</b> <i>Module Leader:</i> Prof Nick Fisk (University of Queensland Centre for Clinical Research)</p>
<p><b>M8: Characterisation &amp; Utilisation of iPS Cells for Dental Regeneration</b> <i>Module Co-Leaders:</i> Prof Mark Bartold (Colgate Australian Clinical Dental Research Centre, University of Adelaide) &amp; Prof Stan Gronthos (Hanson Institute)</p>

### Case Study – How collaboration using iPS cells will help us better understand schizophrenia

Professor Alan Mackay-Sim, Director of the National Centre for Adult Stem Cell Research at Griffith University has a long-standing research interest in neurological diseases. In this new collaboration he will work with Associate Professor Ernst Wolvetang in Queensland to generate iPS cells from patients with schizophrenia.

Schizophrenia, a medical condition affecting the normal functioning of the brain, arises from complex interplays between genes and the environment with multiple genes of small effect acting together.

The schizophrenia iPS cells will be compared with control cells, ultimately aiming to elucidate the differences in the many genes thought responsible for this disease. Increased understanding of these differences may help to develop better treatments for individuals with the disease.

### Case Study – Investigating the use of iPS Cells to treat gum disease

Professor Mark Bartold of the University of Adelaide and Dr Stan Gronthos of the Hanson Institute, are leaders in the analysis of dental stem cells. Their current work is focused on sheep and pigs, whose teeth, like those of humans, suffer from periodontitis - inflammatory diseases of the bone, gums and tissues that can result in tooth loss.

Working with Dr Andrew Laslett at the Australian Stem Cell Centre in Melbourne, the Adelaide group will investigate the development of iPS cells from oral cells and investigate their usefulness for tissue regeneration around teeth and dental implants in sheep and pigs. These animal models, the researchers believe, are a stepping stone to one day translating the research into humans.

## Collaborative Stream 3 – Pluripotent Stem Cell Differentiation

Human embryonic stem (hES) cells and iPS cells (see Collaborative Stream 2) are highly prized by researchers because they are pluripotent, that is, they have the ability to turn into any cell type in the body. However it remains a major scientific challenge to reliably direct these pluripotent stem cells to differentiate into specific cell types such as blood, heart or kidney. Once these hurdles are overcome, it will be possible to create the large numbers of mature human cells needed for research into normal development, disease progression and, in the longer term, for clinical use as cell therapies.

The *Pluripotent Stem Cell Differentiation* collaboration brings together eight modules, including one previously unfunded by the ASCC, with extensive expertise in researching pluripotent stem cells and in guiding these cells to become specific cell types.

The immediate goals of this Collaborative Stream are to:

- efficiently generate human progenitor and mature cells to study both normal development and disease;
- generate reporter lines to facilitate this first goal and also for use in screening programs to identify small molecules replacements for expensive growth factors;
- provide mature cells of value to researchers and the pharmaceutical industry for testing drug efficacy and toxicity.

The long-term goal of the Stream is to generate large numbers of safe, mature cells for transplantation therapy.

This collaborative initiative involves researchers in Victoria and Queensland.

### S3: Pluripotent Stem Cell Differentiation Program

*Stream Leader:*  
Prof Andrew Elefanty (MISCL)  
*Deputy Stream Leader:*  
A/Prof Susie Nilsson (CSIRO)

### Stream 3 Modules

**M1: Haematopoietic Differentiation & Expansion of Human Pluripotent Stem Cells**  
*Module Leader:* Prof Andrew Elefanty (MISCL)

**M2: Genetic Modification of Pluripotent Stem Cells**  
*Module Leader:* Prof Ed Stanley (MISCL)

**M3: Characterisation of Respiratory & Thymic Epithelium Derived from the In Vitro Differentiation of Pluripotent Stem Cells**  
*Module Leader:* Prof Ed Stanley (MISCL)

**M4: Refining the Pluripotent Stem Cell Phenotype**  
*Module Leader:* Dr Andrew Laslett (CSIRO)

**M5: Regenerative Therapies for Renal Repair**  
*Module Leader:* Prof Melissa Little (IMB, University of Queensland)

**M6: Functional Assessment of HSC & HPC Derived from Directed Differentiation of hESC & iPS Cells**  
*Module Leader:* A/Prof Susie Nilsson (CSIRO)

**M7: Characterisation & Propagation of hES &/or iPS Cell Derived Haematopoietic Precursors**  
*Module Leader:* A/Prof David Haylock (CSIRO)

**M8: Stem Cell Derived Cardiomyocytes: Tools for Investigating Cardiac Disease**  
*Module Leader:* Dr David Elliott (MISCL)

### Case Study – Investigating the safety and potential of blood cells made from pluripotent cells

Associate Professor David Haylock of the ASCC has long been interested in the development of innovative cell therapies based on haematopoietic, or blood forming, stem cells. In this new collaboration, Associate Professor Haylock will apply his expertise in haematopoietic stem cells (HSCs) from adult sources such as bone marrow and cord blood, to understand whether haematopoietic cells made from embryonic stem cells and iPS cells are similar to their adult counterparts. He will collaborate with embryonic and haematopoietic stem cell experts to determine the genetic stability, safety and ability of haematopoietic stem cells generated in the laboratory and to grow these cells in large numbers with the ultimate view to using them in patients.

### Case Study – Pluripotent stem cells as tools for research and biotechnology

Professor Ed Stanley is an expert in making stem cells glow in the dark. By genetically modifying human pluripotent stem cells so they fluoresce when certain genes are switched on or off, researchers are able to quickly see if stem cells have turned on the genetic differentiation pathways that lead to the generation of blood, pancreatic or cardiac cells. Recently, Professor Stanley and his collaborators have been able to modify ES cells to glow green when they turn into beating heart cells. This 'reporter-line' technology is a key tool to understanding how stem cells differentiate into other cell types.

## Collaborative Stream 4 – Adult Stem Cell Program

Bone marrow contains large numbers of haematopoietic or blood-forming stem cells which were first used clinically in bone marrow transplants in the 1950s and 1960s. Subsequently, the discovery of adult stem cells in other organs suggests that stem cells exist in all tissues of the body.

Adult stem cells are involved in the day-to-day maintenance of organs and tissues as well as repair of disease and injury. When required, adult stem cells emerge from their dormant state, divide, and differentiate into the appropriate cell types, which repopulate the organ. Adult haematopoietic stem cells from patients can be isolated in the laboratory and used in clinical procedures. Such an example is the delivery of healthy hematopoietic cells into a patient whose own bone marrow is either dysfunctional or has been destroyed by treatment for leukaemia or other types of cancer.

The *Adult Stem Cell* Collaborative Stream brings together world class adult stem researchers in Australia, who will share their knowledge and expertise in the biology of stem cells from a variety of organs - kidney, blood, thymus, heart, lung and brain. The Stream will also analyse the properties and roles of stem cells in disease, particularly cancer, where recent research has shown a possible role for stem cells in tumour formation.

The *Adult Stem Cell* collaboration consists of ten modules spanning Victoria, Queensland and New South Wales.

### S4: Adult Stem Cell

*Stream Leader:*  
Prof Richard Harvey (VCCRI)  
*Deputy Stream Leader:*  
Prof Melissa Little (IMB,  
University of Queensland)

### Stream 4 Modules

<p><b>M1: Endogenous Cardiac Stem Cells</b> <i>Module Leader:</i> Prof Richard Harvey (Victor Chang Cardiac Research Institute)</p>
<p><b>M2: Determining the Location, Origin &amp; Normal Function of Endogenous Renal MSCs &amp; their Relative Capacity to Elicit Renal Repair</b> <i>Module Leader:</i> Prof Melissa Little (IMB, University of Queensland)</p>
<p><b>M3: Endogenous Neural Stem Cells: Function &amp; Regulation</b> <i>Module Leader:</i> Prof Perry Bartlett (Queensland Brain Institute)</p>
<p><b>M4: Adult Lung Stem Cells</b> <i>Module Leader:</i> Prof Ivan Bertonecchio (ASCC)</p>
<p><b>M5: Adult Thymic Stem Cells</b> <i>Module Co-Leaders:</i> Prof Richard Boyd (MISCL) &amp; Dr Anne Chidgey (MISCL)</p>
<p><b>M6: Models of Human Breast Epithelial Stem Cell Function</b> <i>Module Co-Leaders:</i> A/Professors Jane Visvader &amp; Geoff Lindeman (WEHI)</p>
<p><b>M7: Haemopoietic Stem Cells &amp; Their Niche</b> <i>Module Leader:</i> A/Prof Susie Nilsson (CSIRO)</p>
<p><b>M8: Molecular Control of de novo Haemopoietic Stem Cell Generation</b> <i>Module Leader:</i> Prof Doug Hilton (WEHI)</p>
<p><b>M9: Transcriptional Regulation of Haemopoietic Stem Cells</b> <i>Module Leader:</i> Drs Tim Thomas &amp; Anna Voss (WEHI)</p>
<p><b>M10: Database of Adult Stem Cell Transcription</b> <i>Module Leader:</i> Prof Doug Hilton (WEHI) &amp; Dr Christine Wells (Griffith University)</p>

### Case Study – Stem cells in the heart

Heart attack, stroke and related diseases are the leading cause of death in the western world, with 300,000 Australians suffering from heart failure alone. The current optimal therapy for heart failure is heart transplantation, however donors are scarce.

The possibility of alternative therapies is driving the research of Professor Richard Harvey at the Victor Chang Cardiac Research Institute in Sydney. His team is working towards a better understanding of the stem cells that reside in the heart and how we might one day use these cells to help prevent or repair damage.

### Case Study – Using adult stem cells to understand breast cancer and other cancers

The epithelium is the membranous tissue that covers most internal and external surfaces of the body and its organs. Epithelial cancers such as breast, lung, colorectal and prostate account for 80% of human cancers. Breast cancer is of particular interest to Associate Professor's Jane Visvader and Geoff Lindeman of the Walter and Eliza Hall Institute in Melbourne. Their research is focused on cancerous stem cells in the breast, with the long-term goal of identifying tumour markers that could be used for more accurate diagnosis and prognosis, and for development of potential therapies for breast and other cancers.

## Types of Stem Cells – The Facts

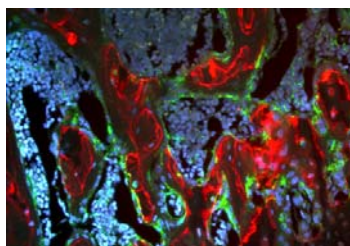
The body is made up of about 200 different kinds of specialised cells such as muscle cells, nerve cells, fat cells and skin cells. All specialised cells originate from stem cells. A stem cell is a cell that is not yet specialised. The process of specialisation is called differentiation and once the differentiation pathway of a stem cell has been decided, it can no longer become another type of cell. A stem cell that can become every type of cell in the body is called pluripotent whilst a stem cell that can become only some types of cells is called multipotent. Stem cells are found in the early embryo, the fetus, placenta, umbilical cord, and in many different tissues of the adult body.

### Adult Stem Cells

Adult stem cells are undifferentiated cells found in the tissues and organs of the body. They are capable of self-renewal. Their differentiation is mainly restricted to forming the cell types of that tissue or organ. The chief role of adult stem cells is to maintain and repair the tissue in which they are found. Skin stem cells, for example, give rise to new skin cells, ensuring that old or damaged skin cells are replenished.

It now appears that all tissues probably contain adult stem cells, but only in very small numbers. In each tissue, they are used to produce new mature cells as old ones die in the natural processes of senescence. They may also remain dormant until activated by disease or injury. Their small numbers make adult stem cells difficult to isolate but they have been successfully isolated from the brain, bone marrow, blood, muscle, skin, pancreas and liver. Most research has been carried out on haematopoietic (blood forming) stem cells isolated from bone marrow and blood.

Within the body, adult stem cells normally only generate the cell types of the tissue in which they are found. Haematopoietic stem cells, for example, are found in the bone marrow and give rise to the many types of cells found in the blood, including red and white blood cells and platelets. The existence of these types of stem cells has been known for a long time and bone marrow transplants containing such cells have been used for over 50 years to treat people with a variety of life-threatening disorders such as lymphomas, leukaemia and thalassemia.



Adult mouse bone marrow

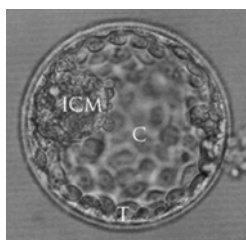
Although adult stem cells are less versatile than embryonic stem cells, their use in research is less topical as it does not involve the destruction of embryos. Their potential use for cell-based therapies is also attractive as it may be possible to use a patient's own stem cells to generate tissue for transplantation, thus avoiding problems with immune rejection.

One of the potential hurdles for the use of adult stem cells for transplants is their limited ability to generate different cell types. Recent experiments, however, have revealed that, under some experimental conditions, certain types of adult stem cells from one tissue may be able to colonise a completely different tissue. This phenomenon is called plasticity and some researchers believe that adult stem cells may, in the future, may be as useful as embryonic stem cells in generating tissue for transplants. A major focus of current research is the investigation of factors and conditions that control the differentiation of adult stem cells in the laboratory.

### Embryonic Stem Cells

Embryonic stem cells can replicate and generate every cell type of the body.

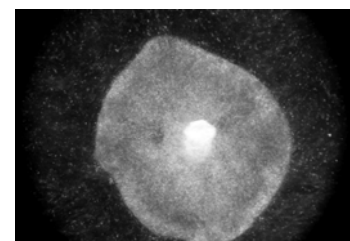
Human embryonic stem cells are derived from human blastocysts (early stage embryos) that are five to seven days old. These blastocysts are donated with consent from patients who have completed their infertility treatment specifically for research. At this stage of development the blastocyst is a hollow ball of about 150 cells and no bigger than a pinhead. Within the blastocyst, next to a large internal cavity (C), is a small group of approximately 30 cells called the inner cell mass (ICM). The outer layer is the trophectoderm (T).



Human Blastocyst

The ICM cells are able to develop into any type of cell in our body and can contribute to all the cells and tissues of the adult organism. These types of cells are called pluripotent and it is this pluripotency that makes them of interest to researchers. Embryonic stem cells are isolated

from the blastocyst when the inner cell mass is removed and cultured in the laboratory. During this process the blastocyst is destroyed.

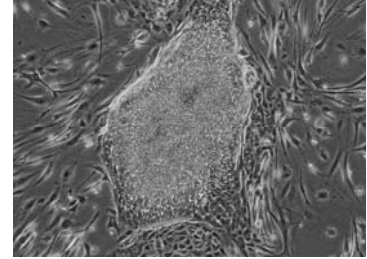


Human embryonic stem cell colony

Once the cells have been isolated they can be grown continuously in a laboratory culture dish that contains a nutrient-rich culture medium. As the stem cells divide and spread over the surface of the dish some are removed to populate fresh subcultures to form a stem cell line. Because these cells have the ability to keep dividing (self-renewing), large numbers of embryonic stem cells can be grown in the laboratory and also frozen for future use. Therefore, established HESC lines can be maintained in laboratories for research, shared between researchers and ultimately used in cell-based therapies.

### Induced Pluripotent Stem Cells (iPS)

In November 2007, a significant development occurred when scientists announced they had developed a new technology to cause mature human cells to resemble pluripotent stem cells similar in many ways to hESCs. These reprogrammed cells are referred to as induced pluripotent stem (iPS) cells.



Human iPS Cells

Initially iPS cells were generated using viruses to genetically engineer mature cells to achieve a pluripotent status. The purpose of the virus was to insert reprogramming genes into a cell such as a skin cell and then culture the cells in the laboratory for 4-5 weeks after which a small number of iPS cells begin to appear. However technologies for reprogramming cells are moving very quickly and researchers are now investigating the use of new methods that do not remain in the cells causing permanent and potentially harmful changes. These new technologies currently utilise different types of non-integrating viruses and chemicals and small molecules.

This technology allows scientists a new method of creating diseased cells for research by using mature cells from a patient with a genetic disease, such as Huntington's disease, and turning those cells into iPS cells. Such disease-specific stem cells may enable disease investigation and drug development offer a unique opportunity to recreate both normal and diseased human tissue formation in the laboratory. iPS technology also has the potential to produce genetically identical "patient specific" embryonic stem cell-like lines that would be recognised as "self" and not rejected by the patient they were made from.

Whilst the discovery of iPS cells is a significant breakthrough in the field of reprogramming, the use of iPS cells in the clinic is many years away - if it occurs at all - as several significant hurdles need to be overcome. It is still unclear how genetically stable or safe iPS cells will be for potential clinical use. More research needs to be done into induced pluripotent stem cells to discover if they will offer the same equivalent research value as embryonic stem cells. Having only recently discovered these cells, scientists are yet to confirm if iPS cells have the ability to divide and remain chromosomally stable like embryonic stem cells over a long period of time.

For more information see the Stem Cell Fact Sheets on the ASCC's website at [www.stemcellcentre.edu.au](http://www.stemcellcentre.edu.au)