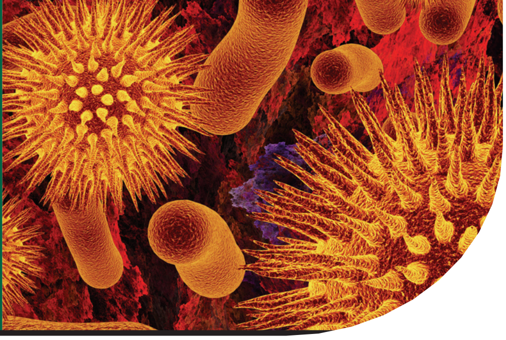

Stem Cell Promise - Research Brings Autograft Revolution Closer

Stem cells have shown the promise to revolutionise the treatment of many diseases, as noted by George Wolff in his book 'The Biotech Investor's Bible': "… The damaged brains of Alzheimer's disease patients may be restored. Severed spinal cords may be rejoined. Damaged organs may be rebuilt. Stem cells provide hope that this dream will become a reality." Professor Anthony Hollander, the ARC Professor of Rheumatology & Tissue Engineering in the Department of Cellular & Molecular Medicine at the University of Bristol, UK, is in the vanguard of this groundbreaking research area. His group has perfected stem cell culture protocols that provide the consistent starting material essential for all areas of their bioengineering research. Furthermore, their knowledge and facilities were instrumental in the first ever bioengineered tracheal graft.

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STEM CELLS SHOW THERAPEUTIC POTENTIAL

For Professor Hollander and his colleagues, stem cells provide the basis for their tissue engineering research. Much has been written and discussed on embryonic stem cells, but for Professor Hollander's group, the main focus has been on adult (somatic) stem cells. Prof Hollander commented, "Embryonic stem cells do have the potential to become every type of cell in the body, but they are very difficult to control fully – they form tumours relatively easily. Somatic stem cells do not possess the same breadth of differentiation capabilities as embryonic stem cells, but are more predictable and controllable." Importantly, somatic stem cells are found in a number of locations, such as the bone marrow, and can therefore be retrieved directly from patients. This means that it is possible for grafts to be grown from these cells and then re-implanted in the same patient – so called autologous grafts. This removes the need for immunosuppressive therapies to prevent rejection, thereby greatly increasing the chance of grafting success.

RESEARCH PROVIDES FOUNDATION FOR TISSUE REPLACEMENT

Bone marrow mesenchymal stem cells (BMSCs) harvested from the heads of femur bones are the major source of stem cells in Professor Hollander's lab. Dr Sally Dickinson, a research associate in the group explained, "Bone marrow mesenchymal stem cells are donated by patients undergoing hip replacement operations and are the perfect starting point for our research, as they are multipotent and can therefore form the major cell types involved in rheumatology applications."

The donated cells are suspended in a specialised stem cell culture medium formulated to promote the growth and differentiation of BMSCs. To remove any bone remnants, the cells are washed several times in the medium and fat is then removed by gently centrifuging the cells at

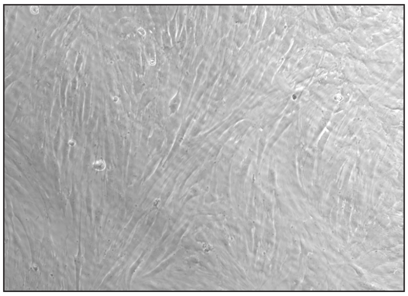

Figure 1. Adherent human adult bone marrow stem cells in culture. (Image courtesy of Dr Sally Dickinson, University of Bristol)

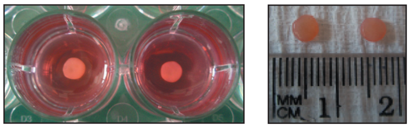

Figure 2. Tissue engineered cartilage produced from bone marrow stem cells (Macroscopic Appearance). (Images courtesy of Dr Sally Dickinson, University of Bristol).

1500 RPM for 5 min and recovering the cell pellet. Once the cells are clean and free from bone or fat, they are seeded in 175 cm^2 culture flasks at a density of 5-10 million cells. The cells are then placed in a CO_2 incubator (5% CO_2, 37 °C, 95% Humidity), with media changes after four days and then every other day until adherent cells have reached 90% confluence.

DIFFERENTIATION

Once successfully expanded, the BMSCs are further incubated in specially developed media to enable differentiation into either chondrogenic monolayers, osteogenic or adipogenic cultures. Alternatively, BMSCs can be added to polyglycolic acid (PGA) scaffolds and incubated for five weeks with regular media changes to create three-dimensional engineered cartilage. The majority of research work conducted in Prof Hollander's lab focuses on chondrogenic cultures, either monolayer or 3D, as these are the most important cell type for osteoarthritis applications.

ANALYSIS

Several analytical techniques are used to assess BMSC cultures and their differentiation, including histological staining and real-time PCR. However, the bulk of the analyses on the engineered cartilage are carried out using enzyme-linked immunosorbant assays (ELISAs), since they provide quantitative biochemical measurements for key molecules such as collagen types I and II. All ELISAs in Professor Hollander's lab are analysed on a photometer.

A range of laboratory instruments are used in the research, many of which are from the Thermo Scientific Stem Cell Excellence portfolio.*

CLINICAL COLLABORATIONS LEAD TO PATIENT THERAPIES

As one of the foremost scientists in the rheumatology field and the development of chondrogenic cultures from BMSCs, Professor Hollander works closely with clinical teams to develop patient-specific cartilage autografts. These are generated by extracting cartilage cells (rather than BMSCs) from the patient and culturing them to provide autologous chondrocytes, which are then seeded into a three-dimensional biodegradable material (derived from the total esterification of hyaluronan with benzyl alcohol and constructed into a non-woven configuration). These engineered grafts are then placed at the site of the cartilage injury, often without the need to glue or suture them in place. Furthermore, the procedure does not necessitate open surgery since a mini-arthrotomy is usually sufficient. Once in place, the graft quickly integrates with the patient's existing tissues, providing good collagen composition and integration with the underlying bone. The autograft technique provides several distinct advantages, namely less stressful surgical procedures and perhaps more importantly, the lack of any immune response. This is a major advance over allografts, which require the use of powerful immune-suppressing drugs for extended periods post-transplant.

THE FIRST EVER BIO-ENGINEERED TRACHEAL GRAFT

Last summer, Professor Hollander received a request for help from a friend and colleague, Professor Martin Birchall, a surgical professor at the University of Bristol. A patient of

Professor Anthony Hollander, ARC Professor of Rheumatology & Tissue Engineering in the Department of Cellular & Molecular Medicine at the University of Bristol, UK. (Image courtesy of Dr Sally Dickinson, University of Bristol).

Dr Birchall's had suffered serious damage to her trachea as a result of contracting tuberculosis (TB). The patient, Claudia Castillo, was a young mother whose only chance of survival at the time was to have one lung removed, which would have seriously affected her quality of life and her ability to look after her children. After much discussion, Professor Hollander and his team very quickly set to work adapting their existing osteoarthritis-based protocols to enable Professor Birchall to grow a large population of chondrocytes derived from Ms Castillo's BMSCs. A section of human trachea was donated for use as a scaffold on which the new tissue could be grown. The trachea was stripped of the donor's cells, leaving a trunk of non-immunogenic connective tissue onto which the chondrocytes were seeded. This seeding process used a novel bio-reactor developed at the Politechnico di Milano, Italy, which provided the right environment for the cells to form the cartilaginous part of the trachea within four days of seeding.

The graft was then lined with epithelial cells and transplanted into Ms Castillo, who responded very quickly to the new airway section, without any sign of rejection (no antibodies to the graft were found). Subsequent biopsies have shown that the new section is fully integrated with the existing airway and is fully supplied with blood vessels. She is now able to live life as if she had not been struck down with TB, a result that would never have been possible if her lung had been removed.

DISCUSSION

Stem cell based-therapies have promised huge changes in the treatments of many diseases and disorders, but much research is still required to ensure safety and consistency before they can be applied more extensively. Prof Hollander and his colleagues at the Department of Cellular & Molecular Medicine at the University of Bristol have been investigating the fundamental principles governing the differentiation of bone marrow stem cells into chondrocytes – the source of cartilage. Through this research they aim to further improve the processes used to generate chondrocyte-based autografts, which have already started to prove their value in the treatment of cartilage damage. Throughout their pioneering research, Professor Hollander's team has come to rely on the dependability and functionality of a broad array of standard and advanced laboratory equipment specifically designed to provide the highest quality and reliability in the cell biology laboratory.

As a result of their dedicated work, Professor Hollander's team was able to take part in the amazing feat of the first ever bio-engineered tracheal graft. Their work has enabled Claudio Castillo to regain an amazing quality of life following a life-threatening condition while increasing the drive among researchers and clinicians to more expansive use of stem cell based therapies.

*Instruments used from this range include the Thermo Scientific Sorvall Legend RT-Plus centrifuge, the Thermo Scientific Cytoperm 2 CO_2 Incubator, and the Thermo Scientific Multiskan microplate reader. At every manipulation stage, the cells were handled within a Thermo Scientific Herasafe KS12 Type 2 Class 2 biological safety cabinet. Cell culture research also requires a large amount of manual pipetting which – if not done properly – can lead to inconsistencies and possibly repetitive strain injuries for the user. In Prof Hollander's lab, the group used Thermo Scientific Finnpipettes. Essential reagents, which contribute to the ongoing success of the lab, were stored in a Thermo Scientific Revco freezer, which provides ultra low temperature storage at (-86ºC).