THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Report by KELLIE HAMILTON – 2001 Churchill Fellow

The SIR WILLIAM KILPATRICK CHURCHILL FELLOWSHIP to study techniques for the isolation and culture of autologous chondrocytes (cartilage cells) to be transplanted to repair deep cartilage defects

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INTRODUCTION

The ability of human articular cartilage to repair itself when damaged is very poor. Numbers of techniques to encourage repair have been attempted over the years, but with little success.
In 1994 a Swedish team of scientists developed a technique for isolating chondrocytes (cartilage cells) from biopsies of healthy cartilage. The biopsies are taken from patients with deep cartilage defects of the knee. The cells can be in special media thus resulting in the production of millions of cells from an initial few thousand. These cells can then be provided back to the surgeon for reimplantation into the lesion of that patient in a second operation. My Churchill Fellowship was to learn this technique.

First and foremost I would like to thank the family of Sir William Kilpatrick and also the Winston Churchill Memorial Trust of Australia. Without their finances and dedication to assisting Australians in achieving dreams to benefit Australians, this study would never have been achieved.
I would sincerely like to thank the staff at the Donor Tissue Bank of Victoria, in particular Lyn Ireland and Professor Stephen Cordner. Both Lyn and Professor Cordner encouraged me to apply for a Fellowship, and have supported me enormously throughout my career at the DTBV.
I could not have undertaken this study without the fantastic people and institutions I visited overseas:

- In Gothenburg, Sweden I would like to thank Jenny Goodwin, Catherine Bengtsson and Maria Andersson who taught me the technique for culturing chondroctyes, and provided me with a lot of laughs. Also in Sweden, Eva Sjögren-Jansson, Professor Anders Lindahl and Dr Mats Brittberg who arranged aspects of my visit. Thanks also to the other staff at the Research Centre for Endocrinology and Metabolism for making me feel so welcome.
- In Boston, Massachussetts (USA) I would like to thank Dr Julie Glowacki from Brigham and Women's Hospital, John Heffernan from Genzyme Biosurgery, and Dr Alan Grodzinsky and Dr Elliott Frank from the Centre for Biomechanical Engineering at Massachussetts Institute of Technology. These people all took time out of their busy schedules to discuss with me the research and development that they are undertaking into cartilage repair at their respective institutions.
- At LifeNet in Virginia Beach, Virginia (USA), I would like to thank Bud Brame, Scott Bottenfield, Rhonda Smith, Tim May and Mark VanAllman for showing me around their enormous facilities. One can only dream that we could ever be that big! The staff at LifeNet provided me with so much information to be able to bring home to improve our service to the community.
- At Wright Medical Technologies in Arlington, Tennessee (USA) I would like to thank Sam Wheeler, Kim Sevo, Cary Hagan, Kelly Richelsoph and Barbara Blum for preparing a fantastic tour of the facilities and a presentation for me.
- At Osteobiologics Inc. in San Antonio, Texas (USA) I would like to thank Gabriele Niederauer for discussing the research into creating a biodegradable scaffold for use in cartilage repair. I wish you well with the success of your product.

Final thanks must go to my husband Chris and my family who have supported me throughout and whom I love dearly.

EXECUTIVE SUMMARY

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PROJECT DESCRIPTION:

To study techniques for the isolation and culture of autologous chondrocytes (cartilage cells) to be transplanted to repair deep cartilage defects.

The major part of this project was to visit the Department of Clinical Chemistry and Transfusion Medicine (Research Centre for Endocrinology and Metabolism) at **Sahlgrenska University Hospital** in Gothenburg, Sweden. A technique for the culture and preparation of chondrocytes for reimplantation, and the associated surgical procedure, were first developed at Sahlgrenska University Hospital by a team of scientists and surgeons. I had the opportunity to meet with two of the members of this team, **Professor Anders Lindahl** and **Dr Mats Brittberg**. Professor Lindahl runs the laboratory which processes the biopsies and cultures the cells for Sweden and neighbouring European countries. Dr Brittberg is an orthopaedic surgeon at **Kungsbacka Hospital**, south of Gothenburg. Four people in the laboratory provided the valuable information regarding the techniques for chondrocyte culturing and preparation for transplantation: **Jenny Goodwin, Catherine Bengtsson**, **Maria Andersson** and **Eva Sjögren-Jansson**. I had the fantastic opportunity to go down to Kungsbacka Hospital for one day to observe Dr Brittberg perform 3 cartilage operations. One of the operations was an autologous chondrocyte transplantation using cells prepared in the laboratory at Sahlgrenska University Hospital.

I then visited six institutions in the USA, each for 1 day. The first city in the USA visited was Boston, Massachusetts, where I met with three people who work in the field of cartilage repair. **Brigham and Womens Hospital** and **Massachusetts Institute of Technology** both have groups involved in research into cartilage and bone repair, while the third place visited, **Genzyme Biosurgery,** produces cultured cartilage cells for transplantation on a massive commercial level.

LifeNet, situated in Virginia Beach, Virginia is one of the largest not-for-profit tissue banks in the USA. LifeNet has often been treated as a benchmark for the Donor Tissue Bank of Victoria's services. My visit there was mainly to observe use of their clean room facilities since we have recently had one installed and will be likely to use it for the chondrocyte culturing programme. I was also able to observe a bone demineralizing procedure which we are soon to be implementing. Demineralized bone can be used for a number of applications. Recent research by **Dr Julie Glowacki** at Brigham and Womens Hospital, Boston has looked at using demineralized bone for cartilage repair.

Wright Medical Technology in Arlington, Tennessee is a company which manufactures medical-grade calcium sulphate for transplantation. Calcium sulphate is used in conjunction with demineralized bone from various tissue banks to provide a better healing option for certain orthopaedic procedures. This technology may be used to assist in healing the subchondral bone beneath a cartilage lesion. I visited this company to learn about the applications of their product to see if it is useful in Australia.

Osteobiologics Inc. is situated in San Antonio, Texas. I visited this facility to find out more information about, and to have a look at their product called IMMIX™ which is a biodegradable scaffold primarily for cartilage repair. Its layers mimic those of articular cartilage, and cultured

cartilage cells may be loaded into the scaffold for faster repair. The product is currently awaiting approval from the FDA in the USA, and should be available to surgeons in the near future.

Through my Churchill Fellowship, I have learned the procedure for chondrocyte culture from the people who developed the methodology. This information will be used to develop a culture laboratory at the Donor Tissue Bank of Victoria to provide a chondrocyte culturing service to orthopaedic surgeons around Australia. We are currently awaiting funding from various sources to begin the set up, before applying for approval from the Therapeutic Goods Administration (TGA) to begin providing the service. I have also learned the importance of sharing information between institutions. The people and companies I visited gave me a great deal of information which I can use at our Bank to better our operations and service to the community. At the same time I was able to provide them with information from our Bank which could assist them in their work. Some of the information which I have gathered while on my Fellowship has already been implemented in the day to day activities of the Donor Tissue Bank of Victoria, especially in the area of demineralised bone matrix production.

The Churchill Fellowship will result in the Donor Tissue Bank of Victoria providing a wider service to the community in need of tissue transplantation, while maintaining highest possible quality.

PROGRAMME

2001

May 21st – June 17th	Sahlgrenska University Hospital (Gothenburg, SWEDEN) *-Professor Anders Lindahl*
July 3rd	Brigham and Women's Hospital (Boston, MA, USA) *-Dr Julie Glowacki*
July 5th	Genzyme Biosurgery (Boston, MA, USA) *- Mr John Heffernan*
July 6th	Massachusetts Institute of Technology (Boston, MA, USA) *- Dr Elliott Frank*
July 13th	LifeNet (Virginia Beach, VA, USA) *- Mr Bud Brame*
July 16th	Wright Medical Technology (Arlington, TN, USA) *- Mr Sam Wheeler*
July 18th	Osteobiologics Inc. (San Antonio, TX, USA) *- Dr Gabriele Niederauer*

MAIN BODY

Gothenburg, SWEDEN

First and foremost, my Churchill Fellowship has enabled me to learn the procedures to independently culture chondrocytes for reimplantation to repair deep cartilage defects. These techniques were learnt at Sahlgrenska University Hospital in Gothenburg, Sweden over a 4 week period. During this time, amongst many other things I have learnt the following:

- Receiving cartilage biopsies from hospitals, and preparing the biopsies for culture
- Isolation of chondrocytes by digestion
- Setting up of isolated chondrocytes into primary culture vessels
- Sterility testing of cultures
- Media preparation for different steps of culture
- Media changing while maintaining sterility
- Thawing of cells for repassaging
- Trypsination of cells to remove from culture vessels
- Assessment of viability of a culture
- Freezing of cells for long-term or short-term storage
- Preparation of chondrocytes for dispatch to hospitals
- Clean-room maintenance including environmental monitoring
- Basic cell-culture research
- Performing cell counts on a culture

I also had the opportunity to observe 3 cartilage related operations at Kungsbacka Hospital during my time in Gothenburg. Two of these operations were standard arthroscopic procedures on knees where the internal structures of the knee are examined via a small camera inserted through a small hole, and debris and scar tissue are removed. The third operation was an autologous (from ones own body) chondrocyte transplantation. Cells had been taken from the patient's knee in a previous operation. These cells were then cultured in the laboratory in Gothenburg to increase the number of viable cells before being sent back for transplantation. It was an extremely valuable experience to see the entire procedure from start to finish. My experience in the laboratory in Gothenburg has given me the knowledge to be able to begin culturing procedures at the Donor Tissue Bank of Victoria immediately following the purchase of appropriate equipment. We will require a licence from the TGA (Therapeutic Goods Administration) to begin supplying the cultured cells to hospitals. However, the information gathered on this trip will expedite that process.

Boston, USA

The first city visited in the USA was Boston, Massachusetts. I visited three institutes that perform different research into cartilage repair. Dr Julie Glowacki from Brigham and Women's Hospital has been performing research into bone and cartilage repair for her whole career. Some of her most recent work has involved the use of dermal fibroblasts from skin and demineralised bone for cartilage production. Demineralized bone forms cartilage when injected subcutaneously. Through a process called 'endochondral ossification', this cartilage then transforms into bone. Dr Glowacki is performing research into identifying conditions at the cartilage formation stage that favour cartilage proliferation instead of bone formation. Demineralised bone is a product which the Donor Tissue Bank of Victoria will soon be providing to Australian surgeons, so the research that Dr Glowacki is performing is fascinating.

Genzyme Biosurgery was the second place visited in Boston, and is a commercial company specialising in tissue repair products. The main product which I was interested in seeing was Carticel (autologous cultured chondrocytes). However, I was also able to gather some

information about Epicel (autologous cultured keratinocytes) also. Genzyme performs the same culturing techniques on chondrocytes as I had learnt in Sweden, but on an enormous scale, being able to provide the service to Western Europe and the entire USA. I was able to tour the facility and learn about their clean-room techniques, and more importantly, learn how Genzyme standardised their production to comply with FDA (Federal Drug Administration) regulations, as the Donor Tissue Bank of Victoria will have to do the same with Australia's TGA.

Massachusetts Institute of Technology was the third visit in Boston. One of the laboratories within this world famous institution is the Centre for Biomedical Engineering, where for many years the scientists and engineers have been studying the effects of trauma on the *cell constituents.* I met with Dr Elliott Frank who discussed with me some of the research being performed there, such as the effects of dynamic compression and static compression on the *insides* of the cartilage cells. These compressive forces are common everyday forces exerted upon articular cartilage, with much reference to the knee. The research at the Centre has involved the use of small discs of cartilage taken from femoral condyles (the upper part of the knee), compressing these, then using immunohistochemical and molecular biological techniques to determine which genes are switched on and what products the cell is producing. The most current work being performed involves the development of peptide gels in which the chondrocytes can divide and proliferate, producing cartilage matrix. The gel can then be injected into a lesion, but due to its lack of weight-bearing properties it can not yet be considered as an alternative treatment.

Virginia Beach, USA

I then travelled to Virginia Beach, Virginia to visit LifeNet, one of the largest not-for-profit tissue banks in the world. LifeNet are a much larger version of the Donor Tissue Bank of Victoria in that they process and distribute more than one tissue type for transplantation purposes – bone and related musculoskeletal tissue as well as heart valves (DTBV also retrieves, processes and distributes donor skin). Many tissue banks around the world specialize in one tissue type, for example, bone only. I met with Bud Brame who is in charge of the musculoskeletal program at LifeNet. I was led into their C100 facility where I was introduced to Rhonda Smith, the demineralization technician. Bone demineralization is a process for which the Donor Tissue Bank of Victoria is currently undergoing approval by the TGA. Demineralized bone has two main advantages over normal allograft bone. Firstly, the demineralization process releases proteins which promote bone growth and healing. Secondly, the recipient's own cells do not need to remove the mineral content from the graft before depositing new bone which leads to faster healing. Demineralized bone is being used in many different applications such as dental implantation and in conjunction with other products to fill cavities caused by the removal of bone tumours. The potential of demineralized bone for treatment of other types of lesions is enormous, as being investigated by Dr Julie Glowacki at Brigham & Women's Hospital. The process observed at LifeNet is a streamlined version of the current process used at the Donor Tissue Bank of Victoria, which will allow us to produce much more product in a shorter time-frame in the future. The experience of seeing how a tissue bank with over 300 staff (compared to our 12) operates was amazing!

Memphis, USA

Memphis, Tennessee was my next port of call. I visited Wright Medical Technologies in Arlington, 20 miles outside of Memphis. Wright Medical Technologies (WMT) is a company specializing in orthopaedic repair products of all different types, including prosthetic hip components, knee components, fingers, and calcium sulphate. Medical grade calcium sulphate is used in conjunction with demineralized bone matrix to produce new bone tissue. The calcium sulphate is osteoconductive, meaning that it will provide the framework for repair.

Demineralized bone is mildly osteoconductive, but more importantly osteoinductive. This means that demineralized bone can stimulate the transformation of osteoprogenitor cells (pre-bone cells) and subsequent bone healing. The combination of demineralized bone and calcium sulphate can be used to heal the damaged subchondral bone plate beneath cartilage lesions, particularly in the knee, as well as having dozens of different applications to heal bone throughout the body. I was given a tour of one of WMT's 3 plants situated in Arlington, which contained the equipment for production of the many prosthetic implants available, and also many pieces of mechanical testing equipment used for repetitive load and wear testing on all prostheses.

San Antonio, USA

My final destination was San Antonio, Texas where I visited Dr Gabriele Niederauer at OsteoBiologics Inc. This company is awaiting FDA approval for a biodegradable scaffolding which they have created to mimic cartilage matrix, allowing chondrocyte infiltration and subsequent cartilage matrix production and healing. This product called IMMIX™ is made from a polylactic/polyglycolic compound and is available in different sized plugs and shapes, which can be loaded with cultured autologous chondrocytes and/or growth factors prior to implant. The material is designed to degrade within 7 months following implantation, by which time the patient's chondrocytes will have produced new matrix. One benefit of this product is that a periosteal flap does not have to be removed from the patient's lower leg, preventing another potential site for infection. The staff at OsteoBiologics Inc. have also recently completed work on a new surgical instrument which accurately measures the compressive strength of cartilage *in vivo*, allowing surgeons to gain a better understanding of the health of a patient's cartilage during an arthroscopic procedure. This product has undergone the necessary trials and will very shortly be available on the market where it is hoped it will revolutionize the way that surgeons diagnose and treat damaged cartilage. The visit was extremely interesting in terms of learning what manufactured products are on the market which work in conjunction with autologous cultured chondrocytes.

CONCLUSIONS

It is proposed that the information I have obtained from my Churchill Fellowship will be used to develop a chondrocyte culturing laboratory at the Donor Tissue Bank of Victoria. The Donor Tissue Bank of Victoria will be required to apply for a licence from the Therapeutic Goods Administration to be able to provide this service to surgeons Australia-wide. The knowledge gained from the Fellowship will also open up a field of research possibilities for potential students affiliated with the Bank. The fact that the autologous cultured chondrocytes can be successfully transplanted without any other supporting materials means that the Bank does not need to rely on any other parties except orthopaedic surgeons, which we already have a good relationship with through our allograft tissue service. The Donor Tissue Bank of Victoria will need to purchase a few new pieces of scientific equipment to begin the culture laboratory. After this I will begin training current DTBV staff in the techniques of autologous chondrocyte culture which I have learnt in Sweden. The DTBV have recently employed a new scientist who has a background in culturing cells of many different types, which will be very useful both in the service to the surgeons and also for cell culture research.

The technique for demineralization of bone observed at LifeNet will be employed at the DTBV as soon as relevant equipment is purchased for this process. This process will streamline our current method which has the same principles but takes a lot longer to perform. This method will enable the DTBV to be able to produce a lot more demineralized bone matrix in a much shorter period of time. A training session for staff at the DTBV has already been organised for later in the year so that everyone will be taught the procedures.

RECOMMENDATIONS

To ensure that the autologous chondrocyte culturing laboratory at the DTBV is successful, I will do my best to gather information to assist us in obtaining a licence from the Therapeutic Goods Administration to begin producing and supplying cells to orthopaedic surgeons. The DTBV will continue its good relationship with our user surgeons in Victoria and wider Australia, which will assist in the success of the laboratory and subsequent success of the transplantations and benefits to patients. With my continuing involvement in the voluntary support and education group, Friends of the Donor Tissue Bank of Victoria, I will continue speaking to community groups and educating the public about our services. If people know that there is treatment available in Australia for cartilage damage in the knee then it will get people talking, thus promoting the process. This could also attract funding from research foundations for purchasing equipment etc.

Promotion of organ and tissue donation should have a higher priority in Australia. The voluntary members of the Friends of the Donor Tissue Bank of Victoria and the staff members of the Bank itself are committed to providing information and knowledge about the benefits of tissue donation to community groups such as Rotary clubs, schools and Legacy groups etc, but we cannot do it alone. While I was in the USA as part of my fellowship, I noted how much was being done to increase awareness of both organ and tissue donation. Banners across roads, advertisements on the television and advertisements on public transport were being used to increase public awareness and bring the topic into the open. The community in general is very aware of the issues because there is always information available. One idea which the USA has employed in recent years is cause marketing, whereby transplantation agencies across the country are turning to integrated marketing communications strategies and strategic partnerships to help achieve their goals. An example of such a partnership is the Government offering tax deductions to companies that sponsor advertising for organ and tissue donation. This benefits all involved as the donation of organs and tissues saves much money from the health budget of a government, the company gets tax breaks, and the donation rate will increase. This is an idea which should be raised here because it is embarrassing that Australia has one of the lowest organ and tissue donation rates in the western world. As a community it is in our best interests to improve this.

Encouragement of secondary school students to continue in science related disciplines is also a high priority. We have seen a decline in the number of students enrolled in Science in the last few years and this is only going to get worse without encouragement and financial support. Australia has the potential to be a world leader in medical research.