The Winston Churchill Memorial Trust of Australia

The application of bio-engineered skin substitute for burn wound healing

Reported by Dr Zhe Li

2008 Churchill Fellow

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Signed: (Zhe Li) Date: July 26, 2009
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My sincere thanks also go to Concord Hospital administration, to Professor Peter Maitz, medical director of burns unit at Concord Hospital and to Ms Christine Parker, the co-chair of NSW severe burn service for their continuous interest and support in my fellowship study.

Special thanks to Professors Steve Boyce in Cincinnati, Dennis Orgill in Boston, Paul Van Zuijlen in Beverwijk, Harshad Navsaria in London, Odile Damour in Lyon for organizing my visit and the opportunities to visit their facilities.

Last but not the least, thanks to my family for the support and care through this fellowship study trip.
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1. TITLE

The application of bio-engineered skin substitute for burn wound healing

2. INTRODUCTION

Skin grafting remains the standard procedure in treating severe burns injury. Effective wound coverage and skin grafting in burns patients are critical for controlling infection, stopping body fluid loss and promoting wound healing. However, one of the major difficulties in treating patients with large, severe burns areas is that patients have very limited donor sites for skin harvesting.

Cultured epithelial Autografting (CEA) is a novel laboratory-based technology by which epidermal stem cells are isolated from a small skin biopsy (about 4 cm2) from patient, cultured into CEA sheets under sterile laboratory condition and then transplanted back to the same patient as an alternative for skin grafts in burn wound care. The benefits of CEA are many. It only requires a small skin biopsy to initiate the cultivation of CEA avoiding the creation of large donor site wound, which could compromise patient’s general well-being and wound healing process. The skin epidermal cells isolated from the biopsy can be cultivated to form expanded more than 500 times in area after about three weeks in skin culture laboratory. CEA is particularly suitable for patients with large burns. CEA sheets, containing living keratinocyte stem cells provide not only immediate coverage but also living cells and bio-factors for permanent wound closure.
Using CEA, we have achieved excellent results in healing burn wound with reasonable wound beds in Burns Units under NSW Severe Burn injury Service. But from time to time, CEA as substitute for skin graft also showed limitation in treating severe deep burns. Three weeks of CEA cultivation could be too long for some patients who need early excision of burnt tissues. Because CEA sheet is fragile and contains only the top layer of skin structure, unsatisfactory results were also observed clinically when CEA sheets alone were used for very deep burns involving dermal damage. Even the keratinocytes from CEA survive in deep burn wound, the quality of the regenerated skin are usually poor. Contraction and repeat breakdown of closed wound area occur often because of the lack of good dermal foundation to support and facilitate the growth and differentiation of keratinocytes.

Efforts have been made world wide to improve CEA technology and develop better skin substitutes by combined application of skin cell cultivation and engineered bio-scaffolds. The bio-scaffolds could be made of natural protein molecules or synthetic polymers that are biologically safe, compatible and degradable. The scaffolds could be used as carrier materials for quick delivery of skin cells to the wound, for immediate wound cover after early excision and to repair damaged wound beds for the subsequent application of cultured epidermal autografts. Or the bio-scaffold, with structures similar to human dermal extra cellular matrix, can be seeded with patient’s skin cells to engineer skin grafts with both epidermis and dermis comparable to normal skin under laboratory conditions. The research and development of tissue-engineered scaffold, dermal and
living skin substitutes could benefit the treatment of patients with severe deep burn wounds.

The aims of this fellowship study was to study the international experience on the research and application of engineered skin substitute as an alternative resource of skin graft for treating deep burn wound. Experience and knowledge gained from this project could help Skin Laboratory at Concord Hospital to develop its future service to Australian community.

3. EXECUTIVE SUMMARY

Project: The application of bio-engineered skin substitute for burn wound healing

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Burns injuries are still common in Australian community. The lack of healthy skin donor site remains a major issue in treating patient with severe and large burns injury. Delayed wound closure leads to serious complication including infection and death. Using epidermal progenitor cells isolated from a small skin biopsy, autologous epidermal grafts could be cultivated in laboratory and grafted for burn wound closure. But show it limitation in treating deep burns injuries with minimal dermal foundation. Skin tissue engineering could produce more reliable skin substitutes, a great alternative of human skin grafts for patient suffering from large and deep burns.

The Churchill fellowship gave me the opportunity to travel to international conference venue, top laboratories, hospitals and Universities in USA, UK, the Netherlands and France to meet with the world leaders in the areas of skin tissue engineering and wound healing. My fellowship started by attending the 41st conference of American Burns association in San Antonio and visiting USA Army Burns Centre and USA Army Institute of Surgical Research at Brooke Army Medical Center, San Antonio, Texas, USA. In addition, the highlights of my fellowship include meeting with the following leading scientists and surgeons in skin regeneration and wound:

- Professor Steve Boyce, Director of Skin Tissue Engineering Laboratory in Cincinnati Shriners Burns Hospital in Cincinnati, Ohio, USA
- Professor Denise Orgill, Chief surgeon, department of Plastic and Burn Surgery, Harvard Medical School and director of Tissue Engineering and Wound Healing Laboratory based at The Brigham and Women’s Hospital in Boston, Massachusetts, USA
• Professors Harshad Navsaria, professor of cell and tissue engineering at The centre for Cutaneou Research and Institute of Cell and Molecular Science, Barts and The London Queen Mary’s School of Medicine and Dentistry, University of London, London, UK

• Dr Simon Myers, Clinical Senior Lecturer in Burns & Plastic Surgery, Barts and The London Queen Mary’s School of Medicine and Dentistry, London, UK and Consultant Burn and Plastic Surgeon at Broomfield Hospital, London.

• Professors Edel O’Toole, Ian Mackenzie and David Kelsell at The centre for Cutaneou Research and Institute of Cell and Molecular Science, Barts and The London Queen Mary’s School of Medicine and Dentistry, London, UK

• Dr Paul Van Zuijlen, Specialist plastic and burns surgeon in Burns Centre of Red Cross Hospital at Beverwijk, The Netherlands

• Professor Esther Middelkoop, Director in Skin Regeneration and Wound Healing Laboratory, Association of Dutch Burn Centre at Beverwijk, The Netherlands

• Dr Welsly MJ Bodha, Director of Dutch Burns Foundation, at Beverwijk, The Netherlands

• Dr Ger Kropman, Director of Euroskin Bank at Beverwijk, the Netherlands.

• Professor Eric Dantzer, Director of Army burns Hospital in Toulon, France (meeting happened at 41st conference of American Burns association in San Antonio, Texas, USA)

• Professors Odile Damour, Director of Laboratoire des Substituts Cutanés at Hôpital Edouard Herriot, Lyon, France.
- Professor Fabienne Braye, Director of Severe Burns Centre at Hôpital Edouard Herriot, Lyon, France.
- Dr Celine Auxenfans, Dr Eric Venet of Keratinocyte Culture Laboratory at Hôpital Edouard Herriot, Lyon, France.

Engineered skin substitutes can be divided into two different categories, synthetic or living skin substitute. Synthetic skin substitute is usually a three-dimensional scaffold made of animal-proteins such as collagen and it does not contain living skin cells but may have a outer layer of silicone for temporary wound closure. The production and regulation of synthetic skin substitute or artificial skin is relatively easy to achieve. Some synthetic skin substitutes are already commercially available. However synthetic skin substitute is mainly used for repairing and regenerating wound bed. It requires further epidermal grafting for epithelialisation of the repaired wound surface. Engineered living skin substitute, with both epidermis and dermis comparable to normal skin, is produced by cultivation of living skin cells with dermal scaffold template and can be grafted to debrided burn wound in a one-step procedure.

The task of engineering living skin substitute is more challenging. However, the necessity and importance of developing engineered living skin substitutes for severe burns were agreed unanimously. It was widely accepted that while cultured epidermal autografts could play critical roles in saving life but engineered living skin grafts would improve the quality of wound healing in severe burns. Each laboratory and hospital I visited took different strategy and different biomaterials to generate the skin substitute. A
clinical trial by Prof Steve Boyce and his colleagues demonstrated that transplantation of engineered living skin substitutes led to quality healing comparable to conventional skin grafts. The valuable experience and knowledge were gained by discussing with those leading scientists and clinicians, visiting their facilities and directly observing the clinical outcomes following the transplantation of engineered living skin substitutes to patients.

Although living skin substitutes could be produced technically in many laboratories including ours at Concord Hospital, the development of a clinically suitable skin substitute is a lengthy and costly process full of challenges. The understandings of skin cell behaviors and cell culture systems need further refinement for engineering better skin substitute. Many factors have to be considered regarding to the bio-materials used for making skin substitute, for example its bio-compatibility, degradability and influence on skin cell proliferation and differentiation. There are hurdles in clinical trial and regulation to cross before its approval for clinical application. The efficacy and bio-safety of engineered skin substitute are consistently the focus of research and development in all institutions I visited. The safety and toxicity of each component used for making the skin substitute would be critical and multi-centre clinical trials are essential for meeting the standards and achieving the approval of regulation authority.

There was willingness of collaboration from each laboratory I visited. The international collaboration in addition to the knowledge and experience gained from my fellowship trip could provide great insight and guidance for Skin Laboratory at Concord Hospital to reshape its skin tissue engineering program and develop better clinical services in future.
4. FELLOWSHIP PROGRAM (22nd March to 11th May 2009)

Visited Institutions and people during my fellowship study

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| March 22 to March 28 | San Antonio, USA  | - Attending the 41st American Burns Conference  
- Meeting with international speakers in skin engineering and burn wound healing  
- Attending scientific seminars  
- Visited USA Army Burns Centre and USA Army Institute of Surgical Research at Brooke Army Medical Center |
| March 29 to April 1 | Cincinnati, USA   | - Professor Steve Boyce and his scientific team members  
- Skin Tissue Engineering Laboratory  
- Clinical and research facilities at Cincinnati Shriners Burns Hospital  
- Observing patients grafted with engineered living skin substitute at Cincinnati Shriners Burns Hospital  
- University of Cincinnati |
| April 1 to April 10 | Boston, USA       | - Professor Denise Orgill, Chief surgeon, Dept of Plastic & Reconstructive Surgery, Brigham and Women’s Hospital (BWH) and Harvard Medical School; Director of Tissue Engineering & Wound Healing Laboratory at BWH  
- Drs Douglas Helm, Michelle Ramirez and Pouya Dastouri at Tissue Engineering & Wound Healing Laboratory  
- Brigham Hospital Research Institute  
- Burns Unit of Brigham and Women’s Hospital |
| April 11 to April 19 | London, UK        | - Professors Harshad Nasvaria, Edel O’Toole, Ian Mackenzie Professor David Kelsell and Dr Simon Myers at the Centre for Cutaneous Research, Institute of Cell and Molecular Science (ICMS), Barts and The London Queen Mary’s School of Medicine and Dentistry  
- 60 min presentation as invited speaker at ICMS expert seminar |
| April 19 to April 26 | Beverwijk, Netherlands | - Dr Paul Van Zuijlen, Specialist Surgeon and Dr Pauline Verhaegen at Burns Centre of Red Cross Hospital  
- Dr Ger Kropman, Manager of Euro Skin Bank  
- Prof Esther Middelkoop, Director of Skin Regeneration & Wound Healing Laboratory, Association of Dutch Burn Centers  
- Dr Welsly MJ Bodha, Director of Dutch Burns Foundation  
- 60 min presentation as invited speaker at Red Cross Hospital |
| April 26 to May 11  | Lyon, France      | - Professors Odile Damour, Director of Laboratoire des Substituts Cutanés, Hôpital Edouard Herriot  
- Fabbine Braye, medical director of Severe Burns Centre, Hôpital Edouard Herriot  
- Dr Celine Auxenfans, Dr Eric Venet at Keratinocyte culture laboratory, Edouard Herriot Hospital  
- Presentation to Keratinocyte Culture Laboratory |
5. MAIN BODY

5.1. United States of America

➢ San Antonio, Texas

The first stop of my fellowship study trip was San Antonio, Texas, USA where I attended the 41st annual conference of American Burns association. The American Burn Association is a professional organization with the mission to improve the lives of everyone affected by burn injury through patient care, education, research and advocacy. It has more than 3,500 members in the United States, Canada, Europe, Asia, and Latin America.

About 1500 people including physicians, nurses, scientists, allied health workers attended the conference. The conference was an excellent venue to meet people from different countries, to share scientific knowledge and experience and to exchange opinions on clinical and scientific issues in burn care. At the conference, I met many people including Prof Steven Boyce and his team from Cincinnati; Prof Eric Dantzer from France; Prof Ghahary’s team from Canada; Prof Niann Dai and Prof Chun Hsieh from Taiwan; Prof Palmer Bessey from William Randolph Hearst Burn Centre and Prof Fiona Wood of Australia. About 234 people presented their works on the basic sciences and clinical issues of burn wound infection, wound healing, skin regeneration, skin substitutes and scar formation. I had the privilege to meet many internal speakers and attended their scientific presentations on exciting topics.
Engineered skin substitutes that consist of dermal and epidermal layers are very promising products to improve the current practices of deep burn wound healing. However, difficulties in obtaining autologous skin cells in a timely manner make this approach to be non-feasible. A collaborative study by Prof Ghahary of University of British Columbia and Prof Boyce and Prof Supp of University of Cincinnati showed that Indoleamine 2,3-dioxygenase (IDO) expression can improve the efficacy of non-autologous engineered skin substitutes for the purpose of wound healing by creating an immunosuppressive environment and that prevents rejection of the foreign tissue.

Engineered dermal substitutes, a porous structure made of natural proteins were widely used in reconstructive surgery following severe burns. Dermal regeneration using the substitutes was therefore one of the hot topics at the conference. Constructive surgery of burn scars involving dermal substitute is usually completed by two surgical procedures. The first one removes the scars or contracted tissue and regenerate the dermal bed with the substitute. The second procedure of epidermal grafting is done in about two to three weeks when the neovascularisation is developed. However, Professor Eric Dantzer of Military Hospital Sainte Anne, Toulon, France grafted the dermal substitute and epidermal sheets in a single surgical step procedure for acute burn and post-burn reconstructive surgery. The technique reduced the number of surgical process, final healing time and the hospital stay. The one step procedure could also reduce the infection risk comparing to the two-step surgical procedures. There were studies examining the effects of topical negative pressure on the neovascularisation of
dermal regeneration template by Dr Yarrow of UK and how the application of dermal substitute affects the range of motion or patients’ functional outcome. Grafting of collagen/elastin dermal scaffold pre-seeded with fibroblasts exhibits enhanced capacity for keratinocyte promotion and basement membrane formation.

Burn wound infection is common while cultured keratinocytes demonstrated reduced expression of human beta-defensins (HBD), a family of antimicrobial proteins normally detected in skin. Dr Gibson from University of Wisconsin-Madison, Madison, WI, USA reported using non-viral strategy to enhance HBD expression and produces an antimicrobial human skin substitute.

There were studies examining the keratinocyte-fibroblast interaction, the effects of keratinocyte releasable factors on skin ECM or scar tissue modulation and efficacy of therapeutic agents for hypertrophic scar. There were also reports on the delivery of cultured keratinocytes for burn wound healing and the evidence of bone marrow cells participating in burn wound healing.

During my time in San Antonio, A guided tour was organized by the conference organizer to visit the USA Army Burns Centre and The U.S. Army Institute of Surgical Research (USAISR). The trip was an eye-opening experience. USAISR is part of the U.S. Army Medical Research and Materiel Command and is collocated with Brooke Army Medical Center. The USAISR is dedicated to both laboratory and clinical trauma research. The USA Army Burns Centre, a medical facility full of cutting-edge technology
in burn care provides state-of-the-art trauma, burn, and critical care to Department of Defense beneficiaries around the world and civilians in our trauma region; and provides Burn Special Medical Augmentation Response Teams to deliver world-class medical augmentation in burns, trauma triage, resuscitation, treatment, and evacuation. The USAISR is very strong in biomedical research; a new hemostasis bio-dressing material is engineered in the institute. The pain management research was very impressive.

At the conference, I also met the representatives of many companies dealing with wound care products at the conference. Many of them deal with cryopreserved allogenic human skin tissue that is often used as temporary coverage in burn. A couple of them produce synthetic dermal substitute. One company provides CEA cultivation service to burns patients but none of them provides engineered living skin substitute for burn wound healing.

➢ **Cincinnati, Ohio**

The Cincinnati Shriners Burns Hospital, part of Shriners Hospitals for Children - Cincinnati is a 30-bed pediatric burn hospital, research and teaching center and is one of the three hospitals specializing in burn care operated by Shriners Hospitals for Children in USA. The Shriners Hospitals have been leaders in burn care and research since opening in the mid-1960s. Treatment is provided for burn injuries and related scarring, along with physical and emotional rehabilitation. The hospital is verified as a burn center by the American College of Surgeons and the American Burn Association.
In Cincinnati, I visited Professor Steven Boyce and his team in Skin Tissue Engineering Laboratory at the hospital. Professor Steven Boyce is the director of Skin Tissue Engineering Laboratory and professor in Department of Surgery, University of Cincinnati College of Medicine. He is an expert in the development of human cell culture systems, wound healing with cultured human skin, skin regeneration with cultured cells and biopolymers, treatment of chronic wounds, control of infection and dermal irritation. He has invented the technology for a cultured skin substitute through nearly two decades of research. He has developed culture systems for skin substitutes that are used to treat burn wounds as a significant improvement to traditional split thickness skin grafting procedures. He characterized these cultures and the living skin substitute with noninvasive biophysical techniques. Dr. Boyce's expertise in skin restoration and his focus on research applications are critical cornerstones of the Skin Sciences Institute in University of Cincinnati. The Skin Sciences Institute is a unique, multidisciplinary group of scientists from The Children's Hospital Research Foundation, The Departments of Surgery and Dermatology, The Shriners Burns Institute, The College of Pharmacy, The College of Engineering and The College of Nursing. Skin Sciences Institute consolidates and integrates their regional strengths in the areas of skin research, evaluation, and clinical application.

During my time in his department, Professor Boyce and I had thorough discussion and exchanged views on different aspects of skin regeneration ranging from cell culture medium and cell growth to bio-scaffolding, living skin substitute and wound healing. We both introduced our work and strategies in treating severe burns with cultured skin cells
and skin substitutes. Prof Boyce was such a knowledgeable scholar and great mentor. He expressed his willingness to collaborate and help with our work. He guided me through the key departments of the burns hospital, research facilities, University of Cincinnati, and most importantly his skin tissue engineering laboratory. I met with his scientists in the laboratory, and had the opportunity to observe their work. The clinical laboratory was composed of several sections that formed a production line of their own culture medium system, engineered porous collagen scaffold, skin substitute cultivation and quality control. The skin substitute he has invented is an autologous living skin sheet (about 8x8cm²). It is composed of both epidermis and dermis with the structure and similar to normal skin. But it does not contain melanocytes, skin glands and blood vessels. After many years of research, a clinical trial of the skin substitute for deep burn wound healing was on the way in the burns hospital when I was there. I was very lucky to see one of their patients, a child who was grafted with the engineered living skin substitutes just about 10 days earlier due to severe burns of large area. The clinical outcome was surprising! The grafted skin substitutes were all taken and the large burn wound was fully closed. The closed wound was smooth and shining showing high quality of healing that was comparable to conventional skin graft. In the trial, the patient would be followed up to determine the long-term benefit of the skin substitutes. As Prof Boyce introduced, the primary results from follow-up observation were very promising. The skin substitute grafted area was as good as the control area grafted with conventional skin graft. The only drawback was that the skin substitute grafted area did not have pigmentation.
According University of Cincinnati news, Professor Boyce and colleagues just received $1.3 million to further develop and commercialize engineered skin substitutes for burn injury repairs as part of the newly formed Armed Forces Institute of Regenerative Medicine (AFIRM). Prof Boyce is currently carrying out pre-clinical studies focused on regulating pigmentation issue and growing blood vessels in engineered skin grafts. Adding blood vessels to the engineered skin grafts could enhance the speed of healing process and then minimize scarring.

**Boston, Massachusetts**

Boston is a city full of world-class universities, medical centers and research institutions. In Boston, I visited Professor Denise Orgill and his team at Brigham and Women’s Hospital (BWH). BWH is academically affiliated and directly adjacent to Harvard Medical School. Together with Massachusetts General Hospital, they are the largest healthcare providers in Massachusetts. Over the last ten years, BWH has been one of the largest non-university recipients of research funding from the National Institutes of Health. There are many world-first milestones in the clinical and research history of BWH. In 1990, Dr Joseph Murray, a professor of surgery together with Drs. William Murphy, George Whipple and George Minot of BWH was awarded the Nobel Prize for the first successful human kidney transplantation from one identical twin to another in 1954 and the subsequent development of immunosuppressive drugs.

The Burn Center at BWH is American Burn Association and American College of Surgeons-certified Level I burn and trauma center. It is one of the few burn centers in USA where plastic surgeons are in charge of the burn patients highlighting their
commitment in achieving the highest level of excellence in functional and esthetic reconstruction for burn patients.

Dr Denise Orgill is a professor of surgery of Harvard Medical School, the chief plastic surgeon and former Director of Burn Centre at BWH. He is also the director of tissue engineering & wound healing laboratory at BWH that conducts clinical and basic science research. He is very successful academics with exciting collaborations with scientists from Brigham and Women's Hospital, MIT, Children's Hospital of Boston, and the Joslin Diabetes Center to develop better technologies to treat wounds. His early research was more focused on the dermal regeneration. He has extensive experience with the use of collagen-GAG matrices to induce regeneration in skin. He holds many scientific inventions and patents in wound healing, nerve and skin tissue regeneration. He was involved in developing a Collagen-Glycosaminoglycan Matrix or dermal regeneration substitute for Skin Regeneration. Nowadays the dermal regeneration substitute is widely used for acute and constructive burn wound surgery. The dermal substitute, also called artificial skin is a bi-layer membrane system for skin replacement. The dermal replacement layer is made of a porous matrix of collagen and a glycosaminoglycan for the infiltration of fibroblasts, macrophages, lymphocytes, and capillaries derived from the wound bed. The temporary epidermal substitute layer is made of silicone and functions to close the wound, control moisture loss from the wound and control infection. The temporary silicone layer has to be replaced with epidermal autograft in two to three weeks to achieve permanent wound closure.
To interview him was such an inspirational and learning experience. During our meeting, he made interesting suggestions for experimental study of the application of the dermal substitute in combination of cultured keratinocytes for skin regeneration in burn wound under laboratory condition.

I also spent quite some time with his research team members Drs Douglas Helm, Pouya Dastouri, George, Michelle Ramirez in tissue engineering & wound healing laboratory at BWH and scientists in Biomedical research Institute at BWH. Each of them introduced their research projects and progresses; and some demonstrated their techniques and equipment used for the studies.

Their strategy on tissue regeneration and wound healing is innovative. Their research has been focused on the stimulation of complex wound heal by using a combination of methods including artificial extracellular matrices, growth factors and stem cells and mechanical forces. The connection with Prof Orgill and his research team will be a great asset for our research and development of skin substitute at Concord Hospital, Sydney.

5.2. United Kingdom

In London, I visited and interviewed Professor Harshad Nasvaria in Centre for Cutaneous Research, the largest cutaneous research centre in Europe. The centre is one the eight centers in the Institute of Cell and Molecular Science (ICMS) of Barts and the London School of Medicine and Dentistry in Whitechapel, London. ICMS is newly built at a cost of £45m and is the largest of the six institutes that make up Barts and The London
School of Medicine and Dentistry to deliver excellence in all aspects of research, teaching and clinical service in accordance with the overall mission of the School of Medicine.

Centre for Cutaneous Research (CCR) is composed of the academic unit of Dermatology and the Cancer Research UK, Skin Tumour Laboratory. Research within the Centre is currently organized into distinct programs which bring together a critical mass of clinical and non-clinical researchers interested in bio-molecular mechanisms of keratinocyte biology, wound healing and tissue engineering, epithelial-mesenchymal interactions and skin development, the roles of viral oncogenesis and developmental genes in non-melanoma skin cancer as well as the genetics of syndromic and non-syndromic hereditary and inflammatory skin diseases.

Dr Harshad Navsaria is the Professor in Cell and Tissue Engineering at the Centre for Cutaneous Research, Institute of Cell and Molecular Science, Barts and London School of Medicine and Dentistry, Queen Mary, University of London. Within the School, he is the Academic Dean and chairs the School Board. He is a founder member of the European Tissue Engineering Society and serves as a consultant to St. Andrew’s Burns Centre, Broomfield Hospital to direct and supervise research activities including clinical application of tissue engineered skin for burn patients. He has served as a board member on the scientific advisory committee for Purdue University, USA on tissue engineering. He was recently invited by the British Ambassador to Paraguay and
Dominican Republic to give a series of lectures on advances in science for improved burn treatment and is currently establishing a skin bank in Asuncion.

He has over 20 years of experience in the field of keratinocyte biology and tissue engineering. The expertise of his group is based around the biology of cultured keratinocytes and the application of tissue engineered skin in animal models and clinical patients. It has led on to the development of complex three-dimensional skin models (based on organotypical cultures), which allow the interactions of various cell types with extracellular matrix proteins. He has contributed to the development of novel dermal substitutes, Laserskin and Hyalograft, for clinical application in burns patients. Laserskin and Hyalograft are hyaluronan-based membrane rather than collagen-based scaffolds, they do not have silicone layer. They could be used for keratinocyte delivery to heal burn wound. His other activities have included the clinical application of cultured keratinocytes in the treatment of chronic and acute burns wounds; development of a porcine chamber model to study skin substitutes, animal and human studies of post-transplantation survival of cultured allogenic keratinocytes and fibroblasts, development of pre-confluent delivery systems for grafting, and transplantation of hair follicles in tissue engineered skin matrices. Other work includes the identification of novel keratin gene mutations associated with skin pathologies and the roles of mesenchymal signaling molecules in keratinocyte stem cell differentiation.

At the time of my visit, Prof Navsaria’s work was focused mainly on research and development. His interest in Skin tissue engineering was on the application of stem cell
technology and the identification of stem cells. His research group in collaboration with others was studying epithelial/mesenchymal interactions in different skin pathologies including cancer and wound healing with an attempt to understand the distinction between regeneration and repair of damaged skin. He aimed to create the "perfect in vitro skin" containing all the cell types and mesenchymal components for clinical transplantation.

In ICMS, I also met with Professor Ian Mackenzie, Professor Edel O'Toole and Professor David Kelsell. Professor Mackenzie's research interest is epithelial stem cells and his group investigates various aspects of the normal and pathological behaviour of epithelial stem cells in relation to cell renewal, tissue engineering and cancer. Professor Edel O'Toole works on Keratinocyte Migration and Invasion. She studies the role of keratins in cell signaling, mechanisms involved in keratinocyte migration and skin cancer invasion, and genetics of disorders of keratinisation and atopic eczema. Professor David Kelsell's research group examines genetically inherited skin disease identifying the genetic and molecular mechanisms underlying epidermal disease. His studies have identified the important role of proteins involved in the regulation and formation of epidermal cell junctions specifically gap junctions, adherent junctions and desmosomal junctions.

During my visit, I was invited to give a 60min presentation on cultured skin cells for burn wound management at the ICMS Expert Seminar. There was a huge interest in our work at Concord Hospital. I met with Dr. Simon Myers, a plastic and burn surgeon with a PhD
degree from London University in keratinocyte growth and differentiation in cutaneous wound healing and cultured keratinocyte grafting. He was appointed as Clinical Senior Lecturer at Queen Mary & Westfield College, and Honorary Consultant Plastic Surgeon to the St. Bartholomew's and The London NHS Trust, and Honorary Consultant Burn and Plastic Surgeon at Broomfield Hospital. He had worked as a Burn Lead Consultant at the Chelsea & Westminster Burn Centre, achieving designation for that service in the National designation process. He became medical director of the Stephen Kirby Skin Bank, and established collaboration between the burn service, the Children's Fire and Burn Trust, and the London Fire Brigade aimed at burn prevention. There was long discussion among Prof Navsaria, Dr Myers and I regarding future collaboration and training by staff exchange.

5.3. The Netherlands

Beverwijk is located about 20 kilometers northwest of Amsterdam, it hubs some excellent hospital and research institutions of The Netherlands for burns injury services including burns centre of Red Cross Hospital, Dutch Burns Foundation, Euroskin Bank and Skin Regeneration & Wound Healing Laboratory, Association of Dutch Burn Centre.

I was privileged to be able to spend a lot of time in each of those institutions meeting with specialist surgeons in plastic and burn care, scientists and heads of institutions. In Red Cross Hospital, I joined my host, Dr Paul Van Zuijlen, a specialist burns and plastic surgeon and his team to observe their clinical and surgical work in the burns centre. Dr
Dr Paul Van Zuijlen is an excellent surgeon with PhD research experience in the application of Matriderm, another new version of dermal substitute containing elastin that is different from Laserskin and hyaograft or the dermal regeneration template invented in Boston. It does not have silicone as our layer, neither. Because of its elastin content, there were speculations that wound grafted with Matriderm could be better in scar contraction. Dr Zuijlen and his team, including Dr Pauline Verhaegen, are following up some patients grafted years ago to confirm the long-term outcome. His group is also trialing a novel technique, skin stretching for scar releasing and constructive surgery.

During my time at Beverwijk, I had meetings with Dr Welsly MJ Bodha, the director of Dutch Burns Foundation. The foundation was started in 1971 as a two-person crusade but has grown into a professional organization of 65 people. Only since 2006, The Foundation has raised over €9,000,000 to support clinical care and scientific research, life quality improvement, skin bank service, and burns prevention programs in the Netherlands.

I visited Euro Skin Bank (ESK), the largest non-profit and non-government organization in Europe providing glycerol-preserved allogenic skin to many burns patients for wound coverage and supporting research in burns treatment. The ESK processes and delivers over 2,000,000 m² of donor skin to countries in Europe and elsewhere. Dr Ger Kropman, the ESK manager, showed me around observing the process of donor skin preparation, preservation, and quality assurance. He is willing to provide help and training if we need to set up similar service at Concord Hospital.
I also visited Professor Ester Middelkoop, the research director of the Skin Regeneration & Wound Healing Laboratory, Association of Dutch Burn Centre. She is one of the top scientists in skin tissue engineering and burn wound healing. Her team is in the process of developing cultured skin substitute for burn. Some clinical trials are on the way to delivery cultured keratinocytes using dermal substitute. Dr Magda Ulrich, a senior scientist in the research lab who showed me her excellent work on skin regeneration under laboratory condition. Several other scientists introduced their research projects on wound infection and *in vitro* skin models for various research projects.

I was invited to give a 60 min presentation about my experience on cultured skin for wound healing. The talk was well received. We exchanged our views and shared our knowledge and experience in burn wound managements using cultured skin cells and dermal substitute. There are lots potentials and willingness for scientific collaboration using our combined expertise.

### 5.4. France

The last stop of my fellowship study was Hôpital Edouard Herriot, Lyon, France. The hospital hosts a severe burns centre and also the only skin culture facility for burns patients in France. I had chance to chat to Professor Fabienne Braye, the medical director of burns centre and Professor Odile Damour, the director of Laboratoire des
Substituts Cutanés in the hospital; I also met with Dr Celine Auxenfans, Dr Eric Venet of Keratinocyte culture Laboratory.

Professor Odile Damour is a scientific expert in cultured skin substitute and wound healing. Her group is divided into two teams, one for research and another for clinical service. She started to culture epithelial autograft (CEA) for wound care in 1988. Her group has made great effort to develop the dermal scaffold and skin substitute but so far they have been applied to patients due to lack of clinical trial. Currently they are severe burns with CEA, a strategy similar to what we have been doing at Concord Hospital. But in addition, her laboratory also cultures and cryopreserves allogenic epithelial grafts as bio-dressings to provide immediate wound coverage and facilitate wound healing. Their tissue culture service is part of the hospital tissue bank facilities which provides services of bone, cornea and stem cell banking. In total they have three clean rooms for different services. I was given the opportunity to visit their clean room facility for cultured epithelial graft production and attached laboratories. I had meeting with Drs Celine Auxenfans, Eric Venet of Keratinocyte culture Laboratory, who gave a slide presentation of their impressive work. In return, I also presented my data to their laboratory. The discussion and comparison of the difference between our cell culture system and strategy of using cultured for burn wound care were benefiting for improving our service.
6. CONCLUSIONS

This fellowship gave a precious opportunity to witness the frontier advance and initiate the connections with internationally renowned leaders and institutions in skin tissue engineering and wound healing.

There is a worldwide urgency to develop bio-engineered skin substitute for burns and reconstructive patients. Some synthetic dermal substitutes are developed and used clinically for burn wound healing but development in the application of engineered autologous living skin substitute for permanent is behind. Although living skin substitutes could be produced technically in many laboratories including ours at Concord Hospital, the development of a clinically suitable skin substitute is a lengthy and costly process full of challenges. Living skin substitutes already demonstrated promising outcome in preliminary trial in Cincinnati. There are still hurdles to cross in clinical trial and regulation before the final translation from laboratory to clinical setting. A collaborative and multi-disciplinary strategy are used in those institutions to improve our understandings of skin cell behaviors, stem cells, biomaterials and cell culture systems to refine the engineering technology for producing better living skin substitute identical to natural human skin.
7. RECOMMENDATIONS

1. The international collaboration in addition to the knowledge and experience gained from my fellowship trip are great assets for Skin Laboratory at Concord Hospital. There was willingness of collaboration and training from those institutions I visited. We use the international experience to guide and reshape our skin tissue engineering program and to develop better clinical services for Australia community.

2. The Knowledge and experience from this study are dissected for the modification of our current skin isolation and cultivation protocols, systems and facility.

3. A regional network or body should be established to consolidate and integrate our strengths in the areas of skin biology, stem cells, skin diseases, biomaterials, skin tissue engineering, and wound healing and clinical application. The multi-disciplinary strategy could facilitate the collaboration of fundamental research and technology development, boost our capacity for competitive funding, improving our knowledge on skin regeneration and wound healing, and speed up the process of living skin development.

4. The efficacy and bio-safety of engineered living skin substitute are the focus of research and development. The safety and toxicity of each component used for making the skin substitute would be scrutinized through the development and multi-centre clinical trials are essential for meeting the standards of health safety and regulation authority.