

## **Previous Research Projects: Restore Burn and Wound Research**

### **1. Burn Injury and Inflammation**

The earliest project that Restore undertook was a clinical study into how nerves regrow into a skin graft after burns surgery. Both clinical examination and histological biopsy showed that sensation was slow to recover and relatively deteriorated with greater skin graft thickness. Following this, work undertaken by Mr Michael Tyler, current Director of Clinical Studies at Restore, made an exciting discovery about the nature of the early burn wound. In a group of 22 adult human burn patients who had sustained scald injuries, clinical assessment was compared with laser Doppler measurement of blood flow and histological biopsies. Using the patient's own skin as a control, it was found that the patients who went on to scar had lost the ability for certain white cells within the blood (lymphocytes), but not other white cells (neutrophil leukocytes), to invade the wound. Leukocytes are particularly active in making a burn wound inflamed by the release of molecules called proteases. This was one of the first studies internationally that emphasised the role of inflammation and immunology in determining the outcome of wound healing and scar formation. Was it possible that lymphocytes kept leukocytes in check thus limiting inflammatory damage?

Given this hypothesis, the next stage in our clinical studies of burns looked at whether we could reduce the number of inflammatory signals that trigger neutrophil cells to invade. At this juncture, the use of negative pressure dressings had just been introduced for the treatment of chronic wounds and this seemed a direct way of removing potentially harmful soluble mediators. A negative pressure dressing is literally a vacuum that 'sucks' liquids away from the wound surface. In these studies, pioneered and then developed by fellows Paul Banwell, Titus Adams and Robert Caulfield, clinical studies of the application of KCI® vacuum dressings to burns in both children and adults were completed. This work introduced completely novel approaches that exemplify the ingenuity of Restore's strategy. When we wanted to know what was happening to the surface of the wound, we could sample this indirectly by a unique device for trapping wound fluid before it was contaminated by the vacuum machine. When we wanted to establish what was happening deeper within the skin, we were more direct in our approach to the problem.

With full ethical approval, we found that patients who had been burned were more than happy to allow biopsies of their wound. With no obvious benefit to themselves, they agreed to participate in the knowledge that any discovery might help subsequent patients. The key discovery of this period of work was that it was the balance of certain chemical mediators – metalloproteinases versus their inhibitors – that determined whether a scald burn would go on to scar previously uninjured skin.

Leading on from Michael Tyler's work, given that leukocytes were thought to be involved in the progression of a scald burn, a project was initiated to modulate the activity of these cells in fresh human burns. Again after meticulous ethical appraisal, research fellow James Murphy found that administration of an anti-inflammatory agent to burns patients limited the progression of the injury. However, as soon as the infusion was stopped, the burn again deepened. An exciting picture of common mechanisms and potential drug targets was developing.

### **2. Focus on clinical outcomes**

To illustrate that Restore does not just fund research related to acute care, burn wound healing and scarring, the next project successfully tackled a key issue in post-burn rehabilitation, that of clinical outcomes measurement. This would permit the better assessment of whether a patient graded their scarring as mild, moderate or severe, vital if treatments for burns are being compared. Research Fellow Stefan Cano was jointly supervised with the London School of Hygiene and Tropical Medicine to complete a PhD thesis. He successfully piloted and then validated a questionnaire that allowed individuals with scarring to quantitatively indicate how they subjectively progress with time. Stefan's career has centred on outcome measures since this time and Restore is fortunate to have his continued input as a collaborator and advisor.

### **3. Focus on Angiogenesis**

With time, the focus in the burn wound turned to angiogenesis (new blood vessel growth). Angiogenesis plays a key role in the early wound environment in both thermal and mechanical wounds. To develop competence in this area of science, Mrs Emma Hormbrey and Miss Charlotte Davies collected wound drain fluid from patients who were having surgery for breast cancer and compared this with equivalent fluid from those patients having

breast surgery for benign disease. Specifically, they looked at soluble chemical signals such as vascular endothelial growth factor (VEGF) which affect vessel growth. They found that the levels of such factors were enhanced in the breast cancer samples. Again, this project emphasises that our research is, wherever possible, based at the bedside and clinically relevant. Restore's projects invariably address models of human disease, usually with human subjects in the context of our carefully vetted trials.

#### **4. Focus on wound healing-partial thickness wounds as a model of the superficial burn**

2001 saw the inception of landmark work that has direct relevance to all wounds that scar. Professor Angus McGrouther, Director of Research, had previously noted that the deeper an injury through the skin - be it a burn, frostbite, mechanical injury or laser wound - the more likely it was that a scar would be the final common outcome. Research Fellow Patrick Gillespie used this information to propose a simple hypothesis - that there was a threshold depth within the skin below which a scar was the inevitable result of injury. He invented a mechanical device that injures the skin from full thickness to no thickness at all on a constant gradient over six centimetres. This clinical model, the Dermal Scratch (DS), was piloted in cadaveric human skin and found to successfully make a reproducible injury. This was essential as many studies in the past have been limited by models that are not entirely standardised in order to compare the effect of two treatments. Subsequently, the next two fellows Christopher Dunkin and Jonathon Pleat, advanced the model to a large scale clinical study of scarring in a human population over two years. They showed that the model produced two types of wound healing within close proximity: a deep cut wound that went on to scar and a superficial wound that did not scar. For the first time it was shown that human skin had to be injured to a depth of about 33% of its thickness before a scar was inevitable. The physical development of the DS scar after a highly stereotyped injury produced the first record of the natural history of a human scar as documented by photography (surface morphometry), ultrasound (deeper morphometry) and laser Doppler imaging (blood flow). This baseline dataset is invaluable for studying the effects of future therapeutic interventions on scarring - we know how healthy skin should heal.

The Dermal Scratch Model proved to be an immensely powerful paradigm of healing, analogous to a thermal injury, in which depth was found to be critical to scarring. As the patient acts as their own control at opposing ends of the cut, there is elimination of much of variability between individuals that can mask true changes. In collaboration with the Department of Biochemistry at Oxford University, this led Jonathon Pleat to successfully use the technique of proteomics to screen many thousands of proteins at the same time in samples from both ends of the DS injury. With ethical approval and fully consented breast reduction patients, he utilised the skin underneath the breast that is due for removal at operation in eight ladies, undertaking the DS injury the day before surgery. The protein differences between the two ends of the DS yielded 179 potential targets for antiscarring treatments. These are now the subject of a provisional patent and overall, this work was critically acclaimed with Chris Dunkin winning the Young Investigator Medal at the European Tissue Repair Society Conference in 2003 for the model and his histological analysis of cells that enter the acute wound.

#### **5. Study of the Genes Activated in the Burn Wound.**

In 2007 the Stoke Mandeville Burns and Reconstructive Surgery Research Trust was relaunched as Restore Burns and Wound Research. This was done not only to emphasise our unique clinical focus but also to emphasise that we are not a charity allied to a single body or institute. Appropriately, the first project to come to fruition in this period was an entirely new model of a thermal injury with an acute wound that was devised by Tze Kong. As with previous models, we targeted the clinical scenario of a burn in a human subject. Again, the skin due for excision in breast reduction patients proved to be an optimal site for study; fully consented subjects had a stereotyped contact burn made on the under surface of their breast at the start of their operation. This was done using an electrical device ('thermode') developed and engineered by Restore which delivered a precise and known energy to the skin contact area. At various time points during the operative procedure, the same skin was removed and snap frozen for comparison with similar skin from unburned areas. Tze screened messenger RNA - the precursors of proteins and key intracellular signals - using a gene array transcriptomic approach. In doing this, he revealed many hundreds of potential signals that are changed in the very earliest burn wound environment. Further, he took this work one stage further and looked at the molecular changes that occur with the clinical treatment of immediately cooling the same injury. This revealed many chemicals that are implicated in reducing the discomfort of the acute injury and which also may be involved in decreasing inflammation and scarring. This unique and powerful work is not yet published while we pursue intellectual property rights on our molecular discoveries.