

**JOINT MEETING- WOUND HEALING SOCIETY AND  
EUROPEAN TISSUE REPAIR SOCIETY, IN PARTNERSHIP WITH  
AUSTRALIAN WOUND MANAGEMENT ASSOCIATION,  
AUSTRALASIAN WOUND AND TISSUE REPAIR SOCIETY AND  
JAPANESE SOCIETY FOR WOUND HEALING.**

**LIMOGES, FRANCE**

**AUGUST 25-29, 2009.**

**REPORT BY ASSOCIATE PROFESSOR MICHAEL WOODWARD**

This fifth joint meeting of the (USA) WHS and the ETRS, in conjunction with the other international partners including AWMA and AWTRS, was a high quality meeting highlighting the latest in basic tissue repair research and its clinical applications. Some 20 Australians attended, of a total of around 300 registrants, so we had a strong presence. Australians presented plenary lectures, invited and free papers and several posters- with one poster winning one of the three Young Investigator awards for best poster.

Limoges is a beautiful city in central France that many Australian tourists would probably not visit- to their loss. These joint meetings chose more out-of-the-way places, relying on the high quality program to attract intrepid travellers. Whilst one Australian did take three days to reach Limoges (broken planes, missed connections, the usual story) most found a flight from London or Paris that made it not too hard. Limoges is famous for porcelain and is a mediaeval half-timbered cathedral town on the Vienne river- a run or walk along the river banks, or a leisurely meal overlooking a beautiful 1300s stone bridge, made the work of conferencing even more rewarding.

The opening plenary on the first full day covered stem cells and Geoffrey Gurtner from Stanford reviewed the negative effect that diabetes has on the attraction of bone marrow endothelial precursor cells to ischaemic areas. This is mediated by reduced HIF-1 $\alpha$  binding to P400 but more importantly leads to less compensatory vascular growth in ischaemic areas, and ultimately to gangrene. So, diabetics may not just be predisposed to gangrene due to reduced vasculature- the diabetes itself may affect stem cell associated revascularization in the ischaemic area. Trials of vascular endothelial stem cells in diabetics are in progress, although they have proved disappointingly ineffective in other conditions such as the treatment of myocardial dysfunction in congestive cardiac failure. This failure may be due to using these stem cells too late, or infusing too few stem cells (which have few specific markers to identify them- finding them is more like trying to find a needle in a needle stack than a needle in a hay stack!).

His team are aiming to better identify these stem cells so they can be more accurately separated out and then infused in sufficient quantities.

Kenneth Liechty from Mississippi spoke in this stem cell session on mesenchymal (stromal) stem cells and diabetic wound healing. These stem cells increase growth and other factors and do seem to improve wound healing. Larger wounds appear to have more a reparative response, leading to scarring, than a regenerative response, and this may be influenced by increasing mesenchymal stem cells in the healing wound.

The next stem cell speaker, Pampee Young from Nashville, spoke of “super” mesenchymal stem cells that may further enhance their effectiveness in wound healing. An inhibitor of a specific pathway (wnt signalling), called sFRP2, seems to generate these “super” cells. Finally, Jean-Jacques Latiallade from Clamart, France, spoke of using mesenchymal stem cells in radiation burns after excision of necrotic tissue. He produced fabulous before and after photos from patients he has treated- they reflected France’s very international view and included subjects from Chile, Senegal and Tunisia. All improved after years of suffering prior to this therapy. However, these cells are not without risk- they are genetically unstable and can increase cancer risk.

It was the next session that even more clearly highlighted the depth of talent in the tissue repair community. These young investigators truly shone with the breadth of their knowledge and perspective. John Paul Tutela from New York explored the use of topical gene therapy to augment vascularity and improve diabetic wound healing. The master cell cycle regulator p53 is upregulated in diabetic wound healing. His team used topical gene therapy with p53 silencing RNA (siRNA) and found this augmented the vasculogenic cytokine profile and increased endothelial cell markers in diabetic wound healing- in short, this gene therapy may improve diabetic wound healing.

The next speaker however was possibly the highlight of the conference- he did win one of the three Young Investigator paper prizes. Tero Jarvinen from Finland, working in Santa Barbara, used bacteriophages (viruses that attack bacteria) to deliver a library of- wait for it- a billion peptides to see which honed in on skin and tendon wounds. One of these peptides, CARSKNDC, has a predilection for early wound healing and was then combined with a therapeutic agent, Decorin, that inhibits TGF- $\beta$  and should prevent fibrosis, promoting instead tissue regeneration- not repair. He described this fusing of a target-seeking with a therapeutic agent as “synaptic” therapy- you heard it first here.

Ali Modarressi, from Lausanne, described how hypoxia impairs skin myofibroblast differentiation and function, a pertinent talk as the discoverer of this most important cell, Giulio Gabbiani, was honoured the next day in a lunchtime session. These cells cause both wound contraction and scarring, and are an obvious target of tissue repair research. Other speakers in the session discussed an injectable biodegradable polyurethane scaffold to assist wound repair and regeneration, and how mechanically stretching a wound may restore myofibroblast differentiation in hypoxia. Certainly mechanical factors and wound healing were a theme of the meeting (topical negative pressure, ultrasonic shock waves, electrical forces and others). In this way, wound healing sits somewhat apart from the rest of medicine. The beneficial effects of topical erythropoietin were also discussed, with a reduced wound healing time in diabetic rats.

Company/industry presence was low key, and the opposed (ie concurrent) session by the major sponsor, Systagenix (which started as the wound product division of Johnson and Johnson) was very poorly attended. They do have a portfolio of new wound products including a silver (“Silvercell”) but like most companies, rely on “before and after” case studies, rather than Randomised Controlled Trials (RCTs), to encourage use of their products. At lunch with their Vice President, the industry view was frankly presented to this attendee/reporter- “why would we spend hundreds of millions on good RCTs, as J & J did with Regranex, only to marginally improve uptake or to pave the way for a cheap generic to ride on our coat-tails”. A frank but very disturbing view. The other angle on this is setting up a trial that is bound to be positive- eg comparing the product with a superceded therapy such as guaze- a criticism of the Armstrong trial of negative pressure therapy that has partly fuelled enthusiasm for this approach.

The plenaries the following day discussed the molecular and cellular basis of tissue regeneration. The first talk was largely on the potential benefits of a very large cyclotron-fuelled laser microscope in Hamburg that should directly assess protein actions at the molecular level. Exciting, and potentially ground-breaking- watch this space. Mathias Schäfer from Zürich spoke of the transcription factor Nrf2 which protects cells against oxidative stress. Activation of this on the one hand protects cells from UVB cytotoxicity but on the other hand disturbs keratinocyte differentiation. And herein lies the rub- some potential therapies certainly look good, but potential toxicities need constant consideration. Don’t mess around with cellular processes with impunity! Sarah Herrick from Manchester spoke about fibroblasts and myofibroblasts in peritoneal pathology- especially post surgical adhesions and endometriosis. In these conditions, brand new fibrotic tissue is created and understanding this may lead to a range of anti-

fibrotic therapies that at the very least could be used by surgeons after abdominal treatments. Therapies that increase fibrinolytic activity are an obvious starting point.

The final talk of this session was inspirational and potentially groundbreaking. Colin McCaig from Aberdeen presented data on the electrical control of wound healing. The skin is vertically electrically charged (100mv) and a wound affects this, essentially creating a horizontal gradient that attracts cells to the wound- unless they have a competing gradient such as a growth factor gradient, although that always follows, rather than precedes, an electrical gradient after wounding. The cells affected by an electrical gradient include inflammatory, epithelial and nerve cells. Induced nerve cell sprouting is clearly of vital importance to recovery after neurological injuries and is relevant to skin injuries, where we know sensory nerves themselves have an effect on wound healing. He showed superb videos that demonstrated cells migrating along electrical gradients and turning around when that gradient was reversed. Certain drugs can affect this gradient, and this may lead to specific wound healing therapies. The gradient is dependent on ATP and modifiers of this are potential therapies. More directly, dressings with embedded electrodes are very promising (this was covered in detail at the subsequent company session- the product is called WoundEL) but more trials are needed before this probably expensive therapy is shown to be safe and effective in humans. The 24 trials to date, individually, are unconvincing, but meta-analyses, in diabetes-related ulcers (N= 591) look more convincing. This approach has shown a healing rate averaging 5 times that of sham therapy. Those who want to jump the gun should be aware that the recommended treatment is one hour of electrical therapy, in total, each day- but only if the peri-wound transcutaneous pressure of O<sub>2</sub> is at least 20mmHg. To the company's credit, a large RCT is planned.

The next day's plenaries tackled the vital issue of nervous system repair. The vital role of the Schwann cell was discussed by the first speaker, Piotr Topiloko, working in Paris. These intriguing cells sprout towards denervated regions, secondarily attracting nerve cells (neurones) to the region. The whole issue of neuronal sprouting was elegantly discovered many decades ago by a Spaniard, Roman Y Caval, whose work remains pivotal. He showed that when a nerve is cut, axonal neurones attempt to reconnect with the distal neural bundles, but may well enter the wrong one, and indeed many simply fail to find their way across the gap. This process of neural regeneration was given a clinical dimension by Robert Ouvrier (OK- you thought French?- actually, an invited Aussie from the University of Sydney). He described a young child who suffered a congenital cyst that

destroyed a segment of her third cranial nerve- the oculomotor nerve. This left her with a dilated pupil, a drooped eyelid (ptosis) and an eye that was directed down and outwards. When the cyst was removed the brilliant neurosurgeon reconnected the nerve ends, and sprouting promptly began. There was a remarkable degree of recovery- the neurones could not resist this empty distal nerve, but they were often misdirected. So, whilst the patient could now move the eye better, and the ptosis largely resolved, attempting to move the eye caused unwanted eyelid elevation. The pupil never recovered, suggesting a differential of recovery potential. A great deal of work is required to translate these observations into better neural therapies, but the journey has clearly begun towards this last horizon of wound regeneration- brain and spinal injury repair. Our stroke patients, brain tumour sufferers, quads and paras are keenly waiting.

I had the pleasure of opening the talks at one of the next concurrent sessions, tackling the issue of transcutaneous oxygen tension at various temperatures as a predictor of wound healing. This will hopefully be published soon, but our group believes  $tcpO_2$  at  $39^\circ$  is a predictor of wounding,  $tcpO_2$  at  $44^\circ$  a predictor of wound healing and that the ratio of these is a good predictor of delayed healing.

The following talk was on the important issue of calciphylaxis- better described as uraemic small vessel arteriopathy. Histology should show calcium in the small vessels, intimal hyperplasia and vessel thrombosis/occlusion- but you need a good biopsy section and a good lab to confirm this. Subcutaneous calcification is **not** the same process. The condition can be fatal, usually through scratching, infection and sepsis. The clinical diagnosis requires pain, itchiness, a raised lesion, ulceration then (if untreated) gangrene. Therapies include increasing dialysis frequency or using drugs (bisphosphonates, Cinacalcet, sodium thiosulphate (well known to photo enthusiasts), analgesia and antibiotics). Surgical parathyroidectomy, to lower calcium, is not effective in most cases. Skin grafting and amputation are surgical approaches. Hyperbaric oxygen can also be very effective, with healing rates of 30-90 %. Indeed, failure to respond to these approaches is a predictor of a high risk of mortality, up to 65% in some series.

Australia's AWTRS secretary, Rachael Murray, spoke about macrophages and inflammation in adult wounds. The mucosa is spared this response, and heals without scarring. If we could also reduce the number of macrophages in burns we could achieve a similar (beneficial) response- but how to do this? Anti-integrins, which counteract adhesion proteins, look promising, and are already in use in other conditions, but need more work in wounds.

Drugs such as tetanus and botulinum toxin affect macrophages as does Clostridium toxin. Another approach would be to inhibit macrophage migration, rather than numbers- but all these need more work.

The impact of the almost ubiquitous *Pseudomonas aeruginosa* on healing in burns patients was covered by Boekema Bkhl from the Netherlands. Some 475 burns patients were included in this retrospective case controlled analysis. 48 had *Pseudomonas* and almost half of these, 23, had wound healing issues. They compared these with those with *Pseudomonas* and no wound healing problems, and those with no such bug. It was an interesting study, but led to no immediate management strategies. It would seem prudent to avoid those factors that are associated with *Pseudomonas*-associated wound healing problems such as a greater proportion of full-thickness wounds, inhalational burns, a poor response to topical silver and longer ICU stays- but I defy potential burns victims to select such a path!

Another Australian, Leila Cuttle from the Royal Brisbane Children's, trumped the session with a superb distillation of her thesis on burns first-aid. Whilst this required additional in- depth dissection at dinner that night, overlooking the Vienne, the presentation was superb and extremely informative. She challenged all the dogma, even to the above-the-call extent of reputedly providing her own saliva, to show what really works with burns first aid and wound healing. She reviewed the history of burns first-aid, which includes animal faeces (still used in some parts of Africa), breast milk, onions and even flour. She showed from elegant experiments that burned more than a few pigs that we need to treat new burns with running cold, but not iced, water for 10-20 minutes (up to 3 hours after the wound, but the earlier the better), avoid tea tree oil/hydrogel ("Burnsaid") and also avoid saliva or Aloe Vera (although these may reduce pain) and avoid other evaporative/spray therapy. Her work has changed the first-aid approach of the Queensland ambulance service- surely that is more than most PhDs achieve!

Born, from Germany (in association with Philips, the light company) reviewed phototherapy and plasmatherapy approaches to wound healing. It was clear that many industries are keen to apply their expertise to wound healing, and some such as a cosmetics company that was a major sponsor (Oréal) were looking for a new technology to give them the edge. The applications of new generation light therapies to wound healing are inspiring, but need more outcome evidence. It is nearly convincing however that the new blue-light therapies are not toxic (compared with UV

therapies) and may improve wound healing through increasing nitrous oxide and/or reducing fibroblast proliferation.

The final day began with a plenary from an invited speaker to Perth next year, Paul Martin- and he is to be missed only with full knowledge of what you are missing! What a superbly knowledgeable and eloquent speaker- and to be respected even as a teetotaler! He reviewed inflammation and regeneration and confessed that the first signal from the wounded epithelium that attracts macrophages was actually discovered by another group, using an experimental model that was far less eloquent than his group's! This mysterious molecule is no less than H<sub>2</sub>O<sub>2</sub> -yes, hydrogen peroxide. This does however lead to a range of therapeutic options that could move wound repair to wound regeneration, the holy grail of the meeting.

Ken Muneoka from New Orleans then showed that even humans are salamanders, to a degree. These amphibians can regenerate limbs, and we can regenerate digit tips- as long as the wound is quite distal. The grail here is to regenerate from more proximal injuries, and indeed to regenerate joints. BMP (a metalloprotease) is integral to this process but timing, dose and boundaries of effectiveness need more work. The P2 and P3 fibroblasts act differently in this response with the P3 group being more interested in nail regeneration. The regenerative entity here is called a blastema, and the cells found in this come mainly from the bone marrow- some form connective tissue and some form fibroblasts.

Kazuo Kishi from Tokyo then reviewed foetal mice tissue regeneration, introducing the audience to the evocative sonic hedgehog gene. Yes, the Y-generation are now exerting their values and naming genes! This important gene (as, no doubt, is the generation doing this wild naming) determines tissue differentiation and foetal organ development. It is not expressed in tissue regeneration, but is expressed in tissue repair where it seems to be associated with scar formation.

The following session this reporter attended dealt with impaired healing in diabetes. Sylvie Meaume (yes, she is French, from Ivry-sur-Seine) noted that poor healing in diabetic wounds is related to the size, depth and duration- but not the site- of the wound. A thorough assessment and the use of evidence-based practice (eg total cast offloading) predict wound healing, not surprisingly. The extent of peripheral vascular disease and the presence of neuropathy are also predictors of impaired wound healing, as are general and social health and the level of activities. Depression is a poor prognostic sign, but age alone is not. Following amputation of a diabetes-related foot

ulcer, healing (69% at 20 weeks) was predicted by the number and grade of wounds, the development of infection and the use (versus non-use) of adjuvant therapy. She reviewed her **MAID** scale that predicts healing after debridement:

- **Multiple ulcers**
- **Area**
- **Impaired pedal pulses**
- **Duration**

There were other very useful talks on the molecular basis of impaired wound healing in diabetics (Sabrine Eming from Cologne) and the role bacteria play in poor healing (David Thomas from Cardiff). Sabrine noted that you can modify the influences of bacteria even without changing (elevated) sugar levels- eg by using hyperbaric oxygen or platelet- derived growth factors. Several drug therapies, not specifically targeting the sugar level, may also be effective. David spoke of the importance of anaerobes in diabetes- related ulcers (DFUs), pointing out that wound swabs usually only isolate 20% of the organisms present. In wounds that are not clinically infected, a swab is as good as a biopsy but if you need to know the bug (eg an infected wound) you need a Bx and PCR. This approach reveals *Pseudomonas* in virtually all diabetes- related ulcers, and suggests combinations (eg with *Staph aureus*) rather than single organisms mostly contribute to poor healing. He also pointed out, unsurprisingly, that aged fibroblasts are less effective than young fibroblasts in wound healing, demonstrating a replicative senescence that may be related to telomeric shortening, oxidative stress (his choice from the possible causes) or other factors. He also touched on biofilms, well known to this audience, and the role that polymers may have in attacking biofilms. Like other speakers, he has formed a company, “CICA”, that builds on his diabetes and infection expertise. Jan Apelqvist, from Malmö in Sweden, reviewed best practice in the treatment of diabetic foot ulcers, starting with the diabetes explosion. Did you know that already 70% of myocardial infarcts are in diabetics? Or that every 30 seconds there is a lower limb amputation from diabetes- yes, around 20 in the time you have spent reading this so far. Some 70% of all lower limb amputations are for diabetics with 85% preceded by a foot ulcer and 50% related to infection. Over 4 million people per year develop a diabetic foot ulcer which greatly increases health care costs- the later can be used to justify setting up a multidisciplinary diabetes foot ulcer service, at least for the more expensive after-the-horse-has-bolted inpatients. The observational Eurodiale study, using over 1,00 subjects from 14 European centres of excellence in diabetes care, has provided useful observational data- eg healing rates of 70%, without amputation, in DFUs. Neuroischaemic ulcers are prone to infection and have worse outcomes

than purely neuropathic ulcers. The most important issue however is recurrence, as indeed it is with all healed chronic ulcers, but reassuringly recurrent DFUs last for a shorter time than their first episode. Nevertheless, "once a DFU, always a tendency to recur". He bemoaned that most new antibiotics are trialled simply to demonstrate non-inferiority with existing products- we need new antibiotics that are more effective than what we currently have. We have few guidelines for antibiotic use- which ones, what route, how long, etc. He noted that in the USA infected DFUs are frequently treated first up with amputation, whereas antibiotics are given a fair go first in Europe. He reviewed revascularization approaches and noted that these double the healing rate. Finally, he reviewed new drugs to enhance healing in DFUs- these include statins, antiplatelet agents, anticoagulants, vasoactive drugs, prostanoids and even gene therapies (esp VEGF) and stem cells. There is also HBO. These approaches should be used early, he argued- before near-amputation. He was not a believer in "new, you beaut" dressings as a saviour in DFUs. Indeed, he noted that we only have 70 RCTs of topical ulcer therapies, for any aetiology, of a total 2,000 wound dressing publications- and studies pre 2003 generally used non-modern therapies as comparators so should be regarded cautiously. Comparator groups without a 20-30% healing rate within 12-24 weeks are an unrepresentative comparator in RCTS, he said. He did concede we needed better outcome markers- eg biomarkers such as Growth factors and Proteases- and not just % wound healing.

In the final plenary session, Dorothy Fogerty from Nashville covered the prevalence of pressure ulcers (PUs) in USA hospitals, and the main risk factors (skin factors, systemic factors and infection). Old age seemed to be an independent risk factor, with age over 75 increasing PU risk by 12- fold. The incidence of PUs in USA elderly inpatients is about 17% - a figure we can surely improve on, even without the funding incentives and disincentives that seem to drive USA healthcare. She was followed by Denis Barritault from Paris, a recently retired professor who has put all his eggs into the basket of a matrix therapy, RGTA, which he markets as CACIPLIQ20, and which is a heparin mimetic. It seems simple, but he provided a cogent rationale, as professors usually do, and some evidence to back it up, such as rat trephine holes healing. The product is administered as a solution applied to a sponge then placed on the wound twice weekly. We keenly await RCTs to support the marketing push, although two non-controlled one to two month trials in humans with ulcers of over 6 months duration and poor vascular supply have been promising. 80% achieved 30% healing at one month and by 3 months 80% had fully healed. There is also a major application of the product in ophthalmology, where it is called

“CACICOL”. In one study, 4/5 refractory corneal ulcers healed within a month.

Robert Kirsner from Miami, which now has the largest medical school in the USA, finished the conference by picking up on the theme of the final speaker and indeed all the basic scientists. We have science, but where are the evidence-supported practical therapies? He noted that some 30-40% of patients are treated outside of existing evidence and some 20-25% of ulcer care is either not needed or potentially dangerous. He reviewed what drives medical practice- training, experience, evidence, rules of thumb and algorithms. He also gave great examples of the biases that influence our practice- availability, sunk cost (“I have it so I’ll damn well use it”), gamblers fallacy (“it worked on the last 2 so it will work on the next”), and a natural bias against clearly beneficial therapies that carry risk- esp if that risk gets a great deal of attention. Clinicians are more willing to support a therapy for a patient if potential patients are assessed with positive add-on points for good health than if they have to subtract points, and thus potentially not be eligible for a therapy, for bad health. Finally, he said that when there are multiple alternative choices, clinicians are more likely to choose none of them! He concluded by reviewing the guidelines that the USA Wound Healing Society has formulated. These cover most of the clinical scenarios we face and the evidence has been assessed well, but perhaps not to the NHMRC standards we are using with our venous leg ulcer guidelines. One major difference is that the WHS allows 2 concordant animal studies to support a high-level (human) recommendation, as long as there is also at least one supportive trial in humans. Also, the WHS allows a therapy with good evidence for one condition to be recommended in a related condition- ie evidence in venous ulcers supports therapies in other leg ulcers.

So, in conclusion, this was a very worthwhile conference and I doubt any attendees, or indeed readers of this report, will fail to identify with at least some relevant material. The challenges are clear- we need more RCTs, not just lab evidence, to support our therapies and we also need to better understand, and combat, the biases that deter us from incorporating evidence- supported practices. Over to you!