Summary

The treatment of recalcitrant lower extremity ulceration and decubitus wounds is a challenge to the healthcare provider, nursing service, decision makers, budget holders and wound dressers. Healing is not always achieved. The principles of wound healing management have not changed and include focus on control of infection, exudates, moisture and the underlying pathological condition (i.e. varicose veins, burns, arterial insufficiency with critical ischaemia, diabetes mellitus). The application of modern dressings, devices and VAC have facilitated wound healing. In the aged with assisted living, and poor predicted outcome with limited life expectancy, ulcers cannot always be healed, and just need to be dressed and the patient made comfortable. The clinician today has three topical biological approaches to treat intractable wound ulceration showing resistance to healing and conventional treatment. Anecdotal reports, case studies and data from open ended clinical trials are now available showing early outcomes. The three technologies directed at wound healing that have made an impact on wound care include:

- Topical autologous platelet-rich plasma (A-PRP).
- Topical autologous bone marrow mesenchymal stem cells (MSC) engineered on scaffolds (utilises the patient's bone marrow).
- Topical autologous adipose derived stromal/stem cells (ADSC) tissue engineered on matrices or biocomposite constructs in vitro. Adult stem cells of patient's own origin are used. Shown to be applicable to regenerative cardiology with potential to form cardiomyocytes.

In conclusion, topical biologicals such as PRP, marrow and adipose derived stem cells, have a definitive place in wound care management, together with conventional dressings and devices such as VAC.
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Platelet-Rich Plasma: Topical Application Of Platelet Gel And Growth Factors To Promote Chronic Wound Healing And Care

PRP is defined as, "Autogenous blood clot that contains a highly concentrated number of platelets". Activated platelets release important growth factors (GF) that include PDGF-BB, TGF-B1, VEGF, and EGF that initiate and modulate wound healing1. This has been verified by international haematologists. PDGF and TGF-beta play key roles in kick starting the wound healing process1. VEGF facilitates tissue granulation and collagen synthesis. EGF stimulates epithelialisation, remodeling and tissue healing. The topical application of autologous PRP and efficacy to heal and facilitate wound healing of chronic ulcers has been established in trials and published. In South Africa the product of choice amongst three, has been REGEN PRP (Regenlab, Mollen, Switzerland) because the process generates the highest platelet counts, optimal enrichment and most consistent release of GF needed to initiate the wound healing process. The REGEN blood collection tubes (and kits) are freely available in all major cities, and authorised for human use. PRP is safe to use, as it is autologous in nature. Studies at Stellenbosch by Du Toit et al and at Pretoria by Franz et al confirm the potential of PRP in clinical practice. Biomedical science that supports the use of PRP in wound healing includes; stimulation of cell proliferation and collagen production, and enhanced osteoblast and fibroblast proliferation in culture1. Dermal injection results in stimulation and proliferation of resident mesenchymal cells (MSC) which are of mesoderm origin and enhance the regenerative process. Published clinical trials show positive outcomes, including cost effective analysis, in the treatment of non healing diabetic foot ulcers with PRP1. Published works and evidence demonstrate that topical PRP has proven useful in the following clinical-situations:

Figure 1: Venous blood sample is centrifuged in special blood collection tubes designated for human use. Note buffy coat at separation of RBC and plasma. This layer above the buffy coat is PRP and is removed, then separated from PPP and activated with calcium chloride to enhance platelet gel formation and release of GF.

Figure 2: Centrifuges customised for the preparation of platelet-rich plasma (PRP).

Figure 3: Healed ankle varicose ulcer after repeated topical application of PRP and conventional occlusive dressings. The ulcer is still healed at 12 months. (Courtesy of Dr's Don du Toit and Wayne Kleintjes, The Specialist Forum 2008).
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Figure 4: Recurrent ankle varicose ulcer suitable for various preparations including: PRP, VAC, compressive occlusive dressing and sponge on ambulant basis, Aquacel®, Promogran®, L-Mesitran®, topica ASCC cell therapy transplantation and combination conventional occlusive dressings.

- Haemostatic properties relevant to cosmetic flap surgery, facelift and blepharoplasty.
- Lower extremity ulceration (traumatic, venous and diabetic).
- Dentistry: periodontal wound healing, bone grafting and bone healing.
- Diabetic foot ulceration.
- Together with fat grafting to improve contouring in aesthetic practice.
- Soft tissue sports injuries (muscles, fascia, tennis elbow, Achilles tendon injuries).

The preparation process of PRP is fairly standard and can easily be completed in a medical practitioner's side room: pre-donated 10ml peripheral venous blood is collected in two special Regen tubes (which render the best quality PRP). The blood is separated by centrifugation, the PRP aspirated off from the PPP and then activated with a small quantity of calcium chloride. This provides an enriched platelet gel or lysate that contains wound healing growth factors. Wound application follows. In summary, platelets and platelet gel initiate haemostasis and healing processes through facilitation of the wound healing cascade, concepts shown by proof of concept. Direct and indirect evidence from animal and human studies, indicate that the growth factors contained in PRP stimulate tissue regenerative processes, and are derived from activated platelets and not stemcells.

Figure 1 and 2 show generation of PRP and Figure 3 shows a healed chronic ulcer after multiple PRP topical applications. Figure 4 shows an ulcer base suitable for multiple treatments including ASCC.

Bone Marrow Mesenchymal Stemcell Therapy (BMC) In Chronic Wound Healing

This approach also has application in regenerative medicine because the cell therapy contains adult stemcells. Fresh bone marrow and cultured preparations have been used in small clinical trials. It is possible to engineer these cells during a culture process onto artificial skin material as a composite graft.

Marrow mesenchymal cells have the potential to differentiate into various lineages including osteoblasts, chondroblasts, myoblasts and neural tissue and this concept is fairly well understood. Studies from Japan show that autologous BMC transplantation can be of value in the healing of chronic wounds. Fresh bone marrow delivers a low yield of stemcells and so the tissue regenerating ability is weak. Another drawback is that a lot of bone marrow aspirate is needed to render sufficient numbers of stemcells. Some units have been able to overcome this obstacle and have been able to treat intractable ulcers of the lower extremities with cultured autologous marrow mesenchymal cells engineered onto Pelmac constructs. Regarding the medical literature on the application of BMC, the following potential has been identified:

- Acceleration of wound healing.
- Subcutaneous tissue regeneration is possible as anti aging treatment.
- Topical application on decubitus ulcers and intractable skin ulceration in the aged, and enhancement of poor burn wound healing can be improved.
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BMC is potentially better than cultured fibroblasts, because the cell therapy of MBC origin contains fibroblasts, adipocytes and adult stemcells.

Adipose Derived Stemcells (ADSC): Role In Stimulating Wound Healing

From an embryological and developmental point of view, adipose tissue is derived from embryonic mesenchymal. ADSC "refers to the collective population of MSC and more committed adipose progenitors that are found within the stroma of adipose tissue" [1]. The cells express stemcell markers such as CD90+ and CD34+ amongst others, including vascular endothelial growth factor (VEGF). Embryonic cells are bypassed by the use of adult and autologous cells, thus negating controversial ethical issues. Mizuno of Japan, has shown this novel option for regenerative cell therapy. The same author defines stemcells as "Cells that are characterised by the ability to self-renew and to differentiate along multiple lineage pathways" [1]. In addition, post-natal ADSC can differentiate into many different cells and tissues of mesodermal origin. The adipose tissue is collected by lipo aspirate tissue biopsy, a process less invasive than a bone marrow aspirate. Some workers have indicated that fresh ADSC is safer than cultured ADSC's. Mizuno and others have indicated that ADSC's secrete potent GF such as VEGF, HGF, FGF-2, and FGF-16. Experimental work has shown that ADSC applied to skin ulcers can accelerate healing. Furthermore, in other laboratory studies, it has been shown that the combination of PRP and ADSC results in a synergistic effect. Figures 5-7, show ADSC in tissue culture. Figure 8 shows REGEN-KIT® for generation of PRP. Potential clinical-benefits of this important research include:

- Closure of resistant fistulas in Crohn's disease (clinical studies are in progress).
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- Treatment of trachea mediastinal fistulas.
- Management of intractable, radiation induced ulceration.
- Control of steroid refractory GVHD after bone marrow transplantation.
- Management of chronic heart failure by cell therapy (potential to differentiate into cardiac myocytes).

Adipose Derived Tissue Cell Culture Studies: Changes In CD34+ Expression

Adipose tissue in man consists of adipocytes and cells composing stromal vascular fraction (SVF). Freshly isolated SVF expresses CD34+, but this expression decreases during cell culture. At Stellenbosch, we support the findings of the Toulouse group that adipocytes and endothelial cells share a common progenitor. This research group has shown the potential of adipose lineage cells to differentiate into endothelial cell phenotype and contribute neovascularisation that can potentially abrogate ischaemia. The same bipotential progenitors may therefore differentiate into white adipocytes or de-differentiate into endothelial cells and microscopic networks. This adipose lineage cell plasticity may well have clinical application in therapeutic angiogenesis. Moon and co-workers, from Pusan National University have shown the value of adipose derived stem cells in stimulating postnatal neovascularisation in experimental models of hind-limb ischaemia.

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Figure 7: RBIO TECHNOLOGY ASC: Adipose derived stromal or stromal cells in tissue culture, showing early proliferation, cell migration and directionality. At this stage cells are CD34+. Preparation for topical cell therapy. University of Stellenbosch.

Figure 8: REGEN-KIT (Motion, Switzerland), available in South Africa for the generation of platelet-rich plasma.

Our research group have demonstrated PRP-enhanced cell culture motility and cell migration of epidermal cells (keratinocytes and dermal fibroblasts). The potential of PRP to stimulate cell proliferation of adipose derived stromal cells is currently part of further studies.

References

Others have demonstrated that adipose lineage cells release potent angiogenic factors such as monobutyryl, VEGF and leptin. These research endeavours could well have an impact on vascular occlusive disease in man.

Regarding refractory ulcer healing, our group postulate that the efficacy of adipose derived stemcell topical cell therapy, may well be enhanced by co-culture with PRP. Transforming Growth Factors are contained in platelets, but not all sub fractions. TGF beta1 and TGF beta2 stimulate cell replication. TGF beta3 may act as a “traffic control” that orchestrate and regulate dermal and epidermal cell motility and directionality during wound healing.