

V 28

Poster Presentation

V 28-1

Acute effects of V.A.C.[®] therapy on muscle pressure-flow responses to sodium nitroprusside and phenylephrine in a rabbit model

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Introduction: Vacuum Assisted Closure[®] (V.A.C.[®]) Therapy is becoming a preferred treatment for wounds, however, investigations of the physiological mechanisms responsible for its efficacy are ongoing. In the present study, we tested the hypothesis that V.A.C.[®] Therapy applied to a cutaneous wound alters vascular regulation in the underlying tissue.

Methods: Seven New Zealand albino rabbits were deeply anesthetized with pentobarbital throughout these non-survival experiments. Normal body temperature and blood gas levels were maintained during the experiments. The animals were instrumented to measure mean arterial pressure (MAP), carotid blood flow and heart rate, and to mechanically control blood pressure. The experimental sites consisted of two dorsal full-thickness wounds. One site was treated with V.A.C.[®] Therapy (continuous -125 mmHg, GranuFoam[®] dressing and V.A.C.[®] Free-

dom, KCI, San Antonio, TX, USA), the other with saline-moistened gauze. Local perfusion in the muscle underlying each wound site was measured by laser Doppler flowmetry using probes placed on the muscle surface. To detect subtle effects of V.A.C.[®] Therapy, blood pressure was decreased progressively from baseline under control (vasonormal) conditions and after the systemic infusion of a vasodilator (sodium nitroprusside) and a vasoconstrictor (phenylephrine), respectively. The resulting pressure-flow curves for the two wound sites and the carotid were analyzed by repeated measures ANOVA.

Results: The pressure-flow relationships for the carotid and V.A.C.[®]-treated wound site showed 3 distinct curves for the dilated, control and constricted conditions, with the dilated curve shifted upwards and the constricted curve downwards relative to the control curve ($p < 0.05$). By contrast, the saline-gauze treated wound site failed to respond to the vasodilator - the dilated curve was indistinguishable from the control curve; only the constricted curve was shifted downward.

Summary: Unlike control wounds, V.A.C.[®]-treated wounds retained vascular responsiveness to vasoactive drugs similar to undisturbed carotid circulation. The results suggest that, in acute wounds, V.A.C.[®] Therapy preserves normal vascular regulation in the underlying muscle; in saline-gauze treated wounds, there was no apparent response to nitroprusside.

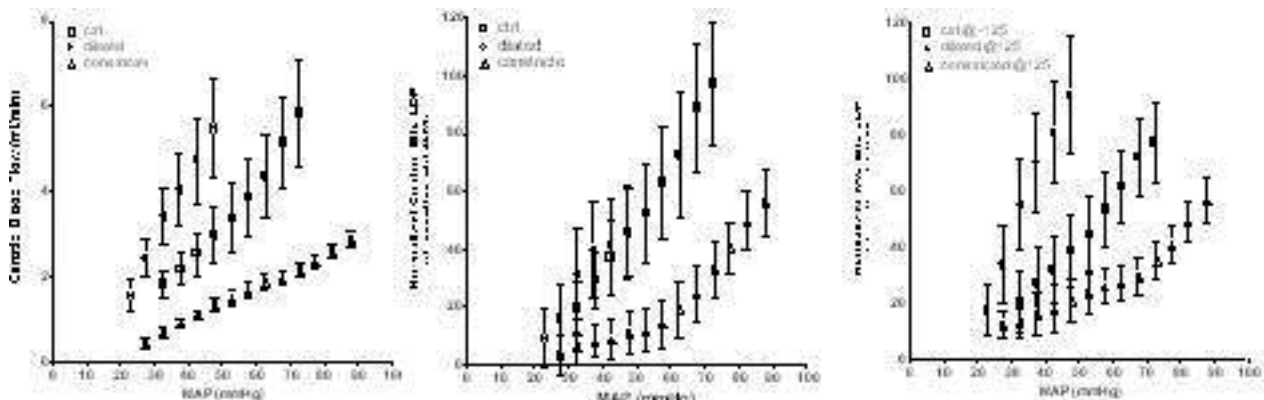


Figure 1 - V 28-1: Pressure-Flow Diagrams.

V 28-2

Biochemical differences in chronic and acute wounds: The role of the macromolecule environment

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The role of the macromolecule environment in chronic wounds has been the subject of many clinical studies. It has been reported that there are abundant biochemical differences between chronic and acute wound fluids. Predominantly the differences are seen in the levels of proteases present, in particular the matrix metalloproteinases, which are more prevalent in chronic wounds. It has been suggested that these factors attribute to a hostile wound environment that is detrimental to wound repair. The reasons for elevated levels of proteases are not well understood and it is still unknown whether this is due to a direct or indirect defect in protease regulation or inhibitor response. We hypothesize that an excess in serine proteases leads to the degradation of endogenous growth factors, which attributes to the delayed healing in chronic wounds. In this study, fluid from chronic and acute wounds were collected over a 24-hour period and samples were assessed for protease activity (specifically elastase and trypsin-like enzymes). The levels of the serine protease inhibitors and growth factors were also measured. We also evaluated the clinical model itself. The augmentation drainage acute fluid control was compared to an alternative model of wound healing in which fluid was harvested from skin graft donor site. A model which provides fluid that is more indicative of acute wound healing due to epidermal interactions. The results indicate that the serine proteases, predominantly elastase, were significantly elevated in the chronic wound fluids. Alpha-1-antitrypsin, the serpin designed to control these proteases, was not up regulated when compared to the acute controls. An increase in serine protease production without an increase in their serpins is thought to result in the reduction of growth factors measured. This work indicates that there is an imbalance in the ratio of inhibitor to enzyme, due to an up regulation of protease production in chronic wounds. We also conclude that misregulation of serine proteases may be responsible for the observed excessive degradation of the extracellular matrix and growth factors in these chronic wounds.

V 28-3

Bridging the gap in "the body of knowledge" as to the local treatment of difficult to heal wounds

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Aim: To date, medical lore has determined the use of dressings, topical agents or other local treatments for difficult to heal wounds. Since history gauze is the classical product used in local wound care. However, a profusion of sophisticated local wound care materials are winning ground. Whether this transition from classical to modern local wound care is based on strong and sufficient evidence, is to be questioned. Can level-one evidence from systematic reviews (SRs) bridge the gap in "the body of knowledge" about local wound management?

Methods: We searched the Cochrane Database of Systematic Reviews up to Issue 1, 2004 for SRs and protocols on dressings, topical agents or other local treatments for poorly healing wounds. We reviewed these SRs to find out how many Randomized Clinical Trials (RCTs) they comprised and what their implications were for treatment and research.

Results: We found 7 SRs, 1 on the local treatment of arterial leg ulcers, 2 on venous leg ulcers, 1 on diabetic foot ulcers, 1 on chronic wounds, 1 on surgical wounds healing by secondary intention and one on pressure sores. They comprised 1, 4, 22, 5, 2, 13 and 2 RCTs, respectively, involving 10 to 299 patients. Two SRs were not conclusive, 2 SRs were circumstantially conclusive, and 2 SRs were conclusive. The three conclusive SRs showed that hydrogel increases the healing rate of diabetic foot ulcers compared with gauze, compression increases ulcer-healing rate compared with no compression, and that there was no evidence of a benefit in using electromagnetic therapy to treat pressure sores. All conclusions were limited by the small size and poor methodology of the RCTs.

Conclusions: Even though SRs are available, evidence is strikingly scarce regarding local wound care for difficult to heal wounds, although this is a worldwide problem. In order to bridge the gap in "the body of knowledge" as to local wound care, the development and conduction of good methodological RCTs (which are the basis for SRs) is encouraged. The results of such trials might help to guide physicians and nurses in choosing the optimal wound care products.

V 28-4

The influence of β - and γ -radiation on the binding capacity of bovine collagen for PDGF-BB

Einfluss von β - und γ -Strahlung auf das Bindungsvermögen von bovinem Kollagen für PDGF-BB

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Introduction: Non-healing wounds lack essential growth factors e.g. platelet-derived growth factor (PDGF). This is due to an increased proteolytic degradation by proteases such as neutrophilic elastase and matrix metalloproteinases (MMPs). In order to support the normal wound healing process, the protection of growth factors is essential. As a previous study has shown, a wound dressing composed of bovine collagen type I (Suprasorb® C), is able to bind significant amounts of PDGF-BB and to release it successively afterwards. Since the binding, PDGF-BB is partly protected from proteolytic degradation. In this study, we investigated whether β - and γ -radiation modify the binding capacity of the collagen wound dressing for PDGF-BB. Radioactive radiation is a common way to sterilise wound dressings during the production process.

Material and Methods: Wound dressing samples were irradiated with β - or γ -radiation (20 kGy). Afterwards, the collagen sponge was cut to pieces with biopsy punches of 8 mm in diameter (corresponding to 0.5 cm²). Each sample was incubated up to 30 min at 37 °C in 1 mL PDGF-BB (1 ng/mL) solution. Non-irradiated Suprasorb® C obtained from the same lot served as control. Subsequent, the supernatants were collected and the wound dressing samples washed with PBS (+ 0.5 % BSA) for 1 h to recover bound PDGF-BB. The concentration of PDGF-BB in both the supernatants and the washing solutions was examined by ELISA (R & D Systems, Wiesbaden).

Results: Suprasorb® C binds already after 10 min significant amounts of PDGF-BB. Irradiated collagen samples exhibit nearly the same binding capacity for PDGF-BB after 1 h as the non-irradiated controls. After 1 h about 15 % of the bound growth factor could be recovered from Suprasorb® C, from the β -irradiated samples 40 percent, from the γ -irradiated 55 percent.

Discussion: The physical properties of Suprasorb® C such as porous structure and capillary activity enable the dressing to absorb large quantities of fluid. Furthermore, the collagen sponge absorbs substantial quantities of the platelet-derived growth factor (PDGF-BB). Despite the fact that β - or γ -irradiation partly destroy the three-dimensional structure of the collagen dressing as scanning electron micrographs reveal, radiation doses up to 20 kGy have no significant effect on its binding capacity for PDGF-BB. However, the bound growth factor was released more rapidly from the irradiated samples and is thus again vulnerable to proteolytic degradation.

Einführung: Die Wundflüssigkeit chronischer Wunden enthält erhöhte Konzentrationen entzündungsfördernder Proteasen,

die zum Abbau von Wachstumsfaktoren wie PDGF führen. Dies verhindert einen normalen Wundheilungsprozess. Der Schutz der Wachstumsfaktoren stellt somit einen therapeutischen Ansatz dar, das Milieu chronischer Wunden in Richtung einer fortschreitenden Wundheilung zu verbessern. In einer früheren Untersuchung konnten wir zeigen, dass der Kollagen-Wundverband Suprasorb® C PDGF-BB binden und sukzessive wieder abgeben kann. Dadurch wird PDGF teilweise vor einem proteolytischen Abbau geschützt. Die vorliegende Studie soll untersuchen, ob sich radioaktive Bestrahlung, die zur Sterilisation von Medizinprodukten verwendet wird, auf das Bindungsvermögen der Wundauflage für PDGF-BB auswirkt.

Material und Methode: Proben des Wundverbandes wurden jeweils 20 kGy β bzw. γ -Strahlung ausgesetzt. Anschließend wurden aus diesen bestrahlten Proben Stücke einheitlicher Größe (je 0,5 cm²) ausgestanzt und mit jeweils 1 mL PDGF-BB-Lösung (1 ng/mL) bei 37 °C bis zu 30 Minuten inkubiert. Als Kontrolle diente unbestrahltes Suprasorb® C aus der identischen Charge. Die Konzentration des ungebundenen PDGF-BB wurde anschließend im Überstand mittels ELISA (R & D Systems, Wiesbaden) bestimmt. Nach der Inkubation wurde die Wundauflage mit jeweils 1 mL PBS (enthält 0,5 % BSA) 1 h gewaschen, um den gebundenen Wachstumsfaktor zu eluieren.

Ergebnisse: Suprasorb® C bindet bereits nach 10 Minuten eine signifikante Menge PDGF-BB. β - und γ -bestrahlte Kollagen-Proben weisen eine nahezu identische Bindungskapazität für den Wachstumsfaktor auf wie die unbestrahlte Kontrolle. Nach 1 h werden etwa 15 Prozent des gebundenen Wachstumsfaktors aus dem nativen Suprasorb® C eluiert, aus den bestrahlten Proben ca. 40 Prozent bei β -Bestrahlung; 55 Prozent bei γ -Bestrahlung.

Diskussion: Suprasorb® C ist ein Kollagenschwamm mit großporiger Struktur, die eine hohe Kapillaraktivität zur Folge hat. Diese Eigenschaft ermöglicht es Suprasorb® C neben großen Mengen Flüssigkeit auch Wachstumsfaktoren wie PDGF-BB zu binden. Durch die radioaktive Bestrahlung kommt es zu einer teilweisen Zerstörung der 3-dimensionalen Struktur der Wundauflage. Dadurch wird der gebundene Wachstumsfaktor schneller wieder freigesetzt als aus dem unbestrahlten Kollagenverband und kann durch Proteasen abgebaut werden. Bestrahlungsdosen bis 20 kGy haben keinen Einfluss auf die Bindungskapazität des Kollagen-Verbands für PDGF-BB.

V 28-5

A novel hydrogel technology for the detection of pH in chronic wounds

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Clinical evidence suggests that the pH of a chronic wound has important implications for the current state of the wound and an effective course of treatment. Daily studies, in this laboratory, of wound exudate have shown that bacterial infection is accompanied by a rapid rise in pH, of the order of 3 to 4 pH units, the wound becoming alkaline in nature. Greener and co-workers have reported that healing of such wounds requires a balance

between proteinase and inhibitor levels, which is very sensitive to pH, higher levels mitigating against rapid healing. There is therefore a clearly defined need for 'real-time' monitoring of wound pH, over the whole surface of the wound. This would enable a more informed diagnosis of the wound to be made. This technology is in the form of a strong, very flexible, hydrogel sheet, 2 mm thick, which changes colour as a function of pH. The hydrogel has a water content of higher than 90 % and a 'natural' pH of 4. When placed on the wound the hydrogel changes colour, within 10 minutes, to reflect the pH of the wound. This colour change is easily discernable by eye and is sensitive to within 0.2 pH units. The clinician therefore has immediate information on the state of the wound, over its total surface. The hydrogel sheet also has the advantage, because of its constitution, of being antibacterial in its own right and is capable, because of its high water content, of easily transmitting actives, such as anti-biotics and anti-septics to the wound surface, when these are painted on the outer hydrogel surface. Because of the high water content, the sheet is comfortable to wear for long periods, were it to be used in this manner.

V 28-6

Characterisation and potential reversal of the diabetic wound fibroblast phenotype

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Introduction: The failure of diabetic foot ulcers to heal is a major financial burden on health service providers and is a consequence of cellular dysfunction within the wound bed. One potential reason for these aberrant responses is due to the onset of cellular replicative senescence which is linked to telomere shortening. The aims of this investigation are to determine the senescent/wound healing status of diabetic wound fibroblasts (DFs) compared to patient-matched normal fibroblast (NFs) and to determine whether reversal of any distinct cellular responses can be achieved through expression of human telomerase (hTERT).

Methods: Fibroblasts were established from biopsies taken from the centre of non-healing diabetic wounds and patient matched, control skin after full ethical approval and informed patient consent. Senescence was assessed according to population doubling levels (PDL), cellular morphology and senescence associated μ -galactosidase (SA μ -Gal) activity. The wound healing processes investigated included cell attachment, cell spreading, extracellular matrix reorganisation (using fibroblast populated collagen lattices) and wound repopulation (monolayer scratch assay). Immortalised cells were by retroviral infection with hTERT.

Results: Compared to NF, after 35 population doublings, the DF demonstrated decreased growth rates, a senescent morphology (enlarged cells with evidence of actin stress fibres) but no differences in SA μ -Gal activity. With respect to wound healing responses whilst there were no differences in cell spreading

or wound repopulation between DF and NF, DF did demonstrate a 3 fold increase in their ability to adhere to both fibronectin and type I collagen ($p < 0.01$) and an increased ability to remodel their surrounding ECM environment ($p < 0.01$). Restoration of telomere length restored normal fibroblast morphology and restored the proliferative capacity of the DF hTERT cells (compared to mock transfected and DF). Furthermore, hTERT infection reversed the effects on cell attachment and ECM reorganisation observed for DF.

Discussion: These data suggest that the DF are phenotypically distinct from patient-matched NF and that the diabetic (possibly senescent) phenotype of DF can be reversed through the action of hTERT. It is envisaged that a successful reversal of cellular phenotypic will be critical in the development of alternative treatments and therapies for individuals with diabetes.

V 28-7

Effect of V.A.C.[®] therapy on the expression of TNF- α , IL-1 β , MMP-3, MMP-9 and TIMP-1

Effekt der V.A.C.-Therapie auf die Expression von TNF- α , IL-1 β , MMP-3, MMP-9 und TIMP-1

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Introduction: Vacuum Assisted Closure (V.A.C.[®]) Therapy has gained increased acceptance and exhibited success in treating wounds. Some aspects of the biological mechanisms of action of this therapy are still unknown. The purpose of this study was to characterize changes in the biochemical composition of wound fluid collected from chronic pressure ulcer wounds in adults treated with V.A.C.[®] Therapy.

Methods: Eight patients with stage III or IV pressure ulcer patients were treated with V.A.C.[®] Therapy (KCI, San Antonio, TX). Therapy was delivered at continuous -125 mmHg using GranuFoam[®] dressing. The ages of these patients, who were primarily in home-health or in extended care, ranged between 39 and 79 years. Concentrations of inflammatory cytokines (IL-1 β , TNF- α), metalloproteinases (MMP-3, MMP-9), and tissue inhibitor of metalloproteinase (TIMP)-1 in wound fluids were measured at baseline and after 1 day, 3 days and 7 days of continuous V.A.C.[®] Therapy. Parametric or non-parametric 1-way ANOVA was used to analyze results; if differences were detected, student Newman-Keuls tests were used for multiple comparisons.

Results: At days 1, 3, and 7 of continuous V.A.C.[®] Therapy, MMP-3 levels and MMP-3 to TIMP-1 ratios were significantly and comparably lower than baseline ($p < 0.05$). MMP-9 levels significantly decreased from baseline at days 1 and 3 ($p < 0.05$). There were no significant differences in TIMP-1, TNF- α , IL-1 β at any

of the time points when compared to baseline.

Discussion: MMP levels, and perhaps more importantly, MMP to TIMP-1 ratios decreased with V.A.C.[®] Therapy. Decreases in these metrics have previously been shown to have prognostic value for healing (1). Other studies in pressure ulcer patients, who were in a hospital setting, showed decreases in only TNF- α (2). Future studies should be conducted with randomized control-treatment arms and should consider wound etiology, systemic confounders, and care setting.

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V 28-8

Wound fluid collection guidelines for V.A.C.[®] Therapy based studies

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Introduction: There is increasing interest in trying to better understand the physiological mechanism of Vacuum Assisted Closure[®] (V.A.C.[®]) Therapy on wound healing. Although collecting wound fluid (WF) through V.A.C.[®] dressing is an attractive approach, proteins have previously been shown to interact with synthetic polymers [1]. The goal of this work was to develop general recommendations for fluid collection for V.A.C.[®] Therapy studies with GranuFoam[®] dressing.

Methods: Results were analyzed from 3 separate previously published studies that assessed the effect of exposure of actual or simulated WF to foam dressing on molecules of interest. In the first study [2] WF from 5 pressure ulcer (PU) patients were collected from under an occlusive dressing and then incubated with GranuFoam[®] Dressing for 0 h, 1 h, and 5 h, at RTP. Concentrations of TGF- β 1 were then measured. In the second study, using similar techniques, WF from 5 PU patients were exposed to GranuFoam[®] Dressing and V.A.C.[®] tubing and the concentrations of proteases (MMP-2, MMP-3, MMP-9), inflammatory cytokines (TNF- α , IL1 α), and tissue inhibitor of metalloproteinases (TIMP-1, TIMP-2) were measured [3]. The third study [4] measured the effects of exposure of simulated WF (without proteases) to GranuFoam[®] Dressing on levels of angiogenin, VEGF, and FGF-2 for 0h and 3h at RTP. Unexposed time-paired WF was used as control in all studies.

Results and Discussion: TGF- β 1-concentration in WF from PUs decreased similarly with time at room temperature in both

unexposed and GranuFoam[®]-exposed groups. No other effects of time or materials exposure were detected. The results are consistent with findings of others, who showed protease concentrations were relatively stable compared with protease-sensitive growth factors [5]. From these data, some general recommendations can be made for fluid collection for V.A.C.[®] studies: wound fluid should be collected from the foam, or tubing close to the foam, as quickly as possible, particularly if protease sensitive molecules such as growth factors are being studied. If proteases are not being studied, use of protease inhibitors may be an alternative option.

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V 28-9

Human mesenchymal stem cells are successfully transfected with green fluorescent protein (GFP) DNA plasmid

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Human mesenchymal stem cells (hMSCs) are effective in regenerative medicine of cutaneous wound healing as well as mesenchymal lineage such as bone, cartilage, muscle, and fat. The authors previously reported that grafted hMSCs impregnated, immediately before application, with artificial skin substitutes accelerate the nude rat cutaneous wound healing as "non-mesenchymal lineage regeneration" and cranial and ectopic bone formation in the nude rat model as "mesenchymal lineage regeneration", however, the detail fate of the grafted hMSCs are unknown. In order to elucidate the cell kinetics, proliferation and differentiation, the hMSCs transfected with Green Fluorescent Protein (GFP) gene and the optimal condition were investigated. 1 μ g GFP DNA plasmid (pIRES-EGFP) was transfected with hMSCs in LIPOFECTAMINE 2000 at various conditions. The condition mediums with or without serum (fetal bovine serum) and incubation time at 3, 6, 9, 18, 24 or 48 hours were investigated. The GFP transfected-hMSCs are almost 100 % positive up to 3 passages of the 3-hour and 6-hour incubations, whereas the GFP transfected-hMSCs 18-hour and 24-hour incubations dropped to 10% efficiency at 3 passage. The GFP fluorescence disappeared by 6 passages. The proliferation curve was identical to control hMSCs at 1 passage. Therefore, the relatively shorter incubation time and younger passage cells may be used for further grafting experiments.

V 28-10

Evaluation of a new fibronectin based dressing in the treatment of venous leg ulcers

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A randomized, double-blind phase II study assessed the combination of high compression therapy, with the addition of homologous human plasma fibronectin in the treatment of venous leg ulcers. In this efficacy and safety study, the fibronectin was applied topically as a solid lyophilized disc in two concentrations (80 & 160 µg/mm²) and compared to a lyophilized Calcium Alginate disc. Patients were randomized into three groups: calcium alginate alone, 80 and 160 µg/mm² of fibronectin (Dermalink-FN, Biogentis, Montreal, Canada). Statistical analyses were performed on predefined populations: the ITT population, all randomized patients and all patients who received 75% of the study material. A safety analysis was performed, for efficacy, on all enrolled subjects who received at least one dose of the study material. Statistical analyses were also performed on all population subgroups.

Results: In the ITT population, baseline characteristics were significantly different for age of ulcer, history of venous ligation and venous stripping. Safety analysis for total population showed no significant difference between treatment arms. Efficacy analyses of the population taken as a whole demonstrated similar 12 week ulcer healing in all three groups. The other observation to note was the high healing rate of patients with compression and calcium alginate. A 54 % healing rate in this control population is higher than the rates found in other venous leg ulcer studies. Subgroup analyses showed a trend for fibronectin to accelerate the healing of older and larger ulcers. In this hard to heal population, the incidence of complete wound closure was approximately 2-fold higher with both concentrations fibronectin when compared to calcium alginate alone (53 % vs 25 %).

Given the heterogeneous distribution in relation to patients having undergone venous stripping, efficacy analyses were performed without these patients to see their influence on the overall results. No influence was observed. Additional studies are required to confirm the trend observed.

V 28-11

Platelet gel accelerates wound healing in vitro

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Aim: Platelet gel has been used clinically to help heal surgical as well as chronic wounds. However, evidence of success has been anecdotal and prospective randomized studies have yet to be conducted. These in vitro studies will serve to establish a scientific basis for the efficacy of platelet gel in wound healing.

Methods: Platelet-rich plasma (PRP) containing elevated counts of viable platelets and WBCs was obtained from the blood of healthy human donors using the Medtronic® Magellan® System. PRP was combined with thrombin to form platelet gel. Cell Proliferation & Migration: Normal human dermal fibroblasts (NHDF), human microvascular endothelial cells (HMVEC) and human keratinocytes (NHEK) were cultured in wells either directly in contact with APG, or in the presence of serum expressed by the gel. Cell proliferation was assessed over 96 hr using a proliferation assay that identifies viable cells. Cell migration was assessed at 4 hr using a chemotaxis assay in the Boyden chamber.

Angiogenesis: A section of freshly harvested bovine aorta was placed on a fibrin gel and incubated in the presence of culture media for one week. The media was supplemented with 10 % serum expressed by the platelet gel, 10 % fetal bovine serum, or was not supplemented. Angiogenesis was assessed by the number of vessels radiating from the aortic section and the length of vessel growth.

Results: Proliferation of fibroblasts, endothelial cells and keratinocytes was enhanced in the presence of platelet gel. Cell migration was markedly increased when serum from the platelet gel was used as the chemoattractant. Serum from the platelet gel enhanced the outgrowth of many vessels from a section of bovine aorta.

Discussion: Wound healing is tightly regulated by a number of growth factors and cytokines. The release of a natural cocktail of growth factors and cytokines at supra-physiologic levels by platelets and WBCs in platelet gel accelerate the healing process in vitro by enhancing cell proliferation, stimulating cell migration and causing angiogenesis.

V 28-12

Enhanced epidermal migration and wound closure by autologous platelet gel in a porcine model

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Aim: Platelet concentrate, also known as platelet-rich plasma (PRP), is used increasingly in the form of autologous platelet gel (APG) to accelerate wound healing and to help heal difficult wounds. The goal of these porcine experiments was to show that APG accelerates acute cutaneous wound healing by way of accelerated epithelialization and wound shrinkage.

Methods: Deep partial thickness study: Wounds (0.6 mm depth) were created on the paravertebral and thoracic area of 6 pigs. Wounds were either treated with APG and covered with a polyurethane dressing, were treated only with a polyurethane dressing, or were left untreated. Wounds were excised from each treatment group on days 4 through 10. Each intact specimen was assessed for full epidermal continuity using a well-established salt-split technique. The number of wounds completely epithelialized was divided by the number of wounds sampled per day. The percentage of wounds healed for each day was compared between test groups. Full thickness study: Wounds were created using a punch (17 mm diameter) on each flank of a neutered pig. On the treatment side, wounds were treated with APG and covered with a polyurethane dressing. On the control side, wounds were treated with saline covered with polyurethane dressing. Wounds were graded as "healed" or "non-healed" on days 14 and 28. The size of the wounds was measured using digital photography and a computer imaging system. Platelet rich plasma was prepared using the Medtronic® Magellan® System, a fully automated, easy-to-use, tabletop centrifuge device. APG was created by combining PRP with autologous porcine thrombin produced using the Magellan® ASD Kit.

Results: Wounds treated with APG enhanced the rate of epithelialization in the partial thickness study; 50 % more specimens were completely epithelialized in the APG group as compared to the polyurethane dressing group on day 6. In the full thickness study, 75 % of the APG group was healed compared to only 25 % in the control group on day 14. Also, the mean size of wounds treated with APG was statistically smaller than contralateral wounds treated with saline.

Discussion: APG accelerates healing of acute cutaneous wounds by enhancing epithelialization and wound closure.

V 28-13

Dynamic effects of serum from burned rats on the gene expression in rat bone mesenchymal stem cells

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Aim: To investigate the effects of serum from burned rats on the gene expression in cultured rat mesenchymal stem cells (BMSCs).

Methods: Bone marrow was extracted from sacrificed Wistar rats and BMSCs were then incubated in F-12 medium in the presence of normal rat serum (group N) or serum harvested from burned rats after injury for 3 days (group B) after burn injury for 24 hours and 72h, respectively. Total RNA was extracted from all groups. The mRNA was isolated. The Oligo microarrays containing 5705 genes were used to compare the differences of gene expression between two groups based on the different time for which the cells were incubated.

Results: There were four genes which differentially expressed in two groups. In comparison with group N, the expression of steroid sensitive gene 1 was decreased, but that of fibroblast growth factor 4, dihydroxyacetonephosphate acyltransferase and a EST, which is moderately similar to Bmp2-inducible kinase, were increased in group B.

Discussion: Serum from burned rats can change the gene expression of BMSCs, which may play the key role in wound repair.

V 28-14

The animal experiment of polyacrylamide hydrogel (amazingel) migrates in the muscle fissure

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Aim: To show if injected polyacrylamide hydrogel (PAHG) (amazingel) could migrate automatically in muscle fissure of rabbit body, and providing evidence for clinical application of PAHG.

Methods: The 10 ml PAHG was injected into the gluteus muscles or the gluteus muscle fissures of 2 groups of rabbits (6/group), respectively. Then, (1) high frequency ultrasound doppler was used to examine and observe the distribution of the injected PAHG (amazingel) 1/3/6 months postoperation. (2) the rabbit was killed and the PAHG (amazingel) was dissected and observed 1/3/6 months postoperation.

Results: (1) The ultrasound results showed the PAHG (amazingel) in gluteus muscles was enveloped by fiber membrane 1/3/6 months postoperation, and in gluteus muscle fissures it was not enveloped and also migrated into the lower limb, and

both of the dark liquid area were monomorphous. (2) The dissected results showed PAHG samples of both groups were transparent, the PAHG (amazingel) in gluteus muscles was enveloped by thin layer of capsule and looked like ellipse 1/3/6 months postoperation, but the PAHG (amazingel) in gluteus muscle fissures looked like ellipse or irregular shape and noun capsule was formed, and also the samples could not migrate into muscles, just due to gravity migrate along fissures and across popliteal fossa to fissure of tendo calcaneus of foot from up to down.

Discussion: PAHG (amazingel) which is injected into the gluteus muscles can form thin and slight layer of capsule membrane, is restricted and can not migrate into muscles, but PAHG (amazingel) which is injected into the gluteus muscles fissure can migrate and fluid to the fissure of tendo calcaneus of foot from superior to inferior due to gravity, and the envelopment can not be formed. Suggesting that it is necessary to pay attention not to inject PAHG to loose muscle fissures in human body to prevent migrate.

V 28-15

Retroviral marking with green fluorescent protein identifies sprayed cultured autologous keratinocytes in porcine wounds

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Introduction: The variable success of cultured keratinocyte sheets has precipitated the development of alternative methods of delivering cultured cells to the wound bed. One such method is to spray a suspension of cells in combination with a widely meshed skin graft. Previous studies have demonstrated an improvement in epithelialization, however the cells in these experiments were not cultured and not labelled so it is impossible to say whether the cells were responsible for improved epithelialization.

Aim: The labelling of cells in vitro prior to application will help to answer this. In this study cells were GFP labelled in order to establish their contribution to the process of epithelialisation.

Material and Methods: An established porcine model was used to study the effects of sprayed cultured keratinocyte suspensions in combination with widely meshed autograft. The cells underwent retroviral labelling in culture with Green Fluorescent Protein (GFP) in order to identify them on the wound bed.

Results: Photographic assessment of the wounds demonstrated an increased rate of epithelialization and also less con-

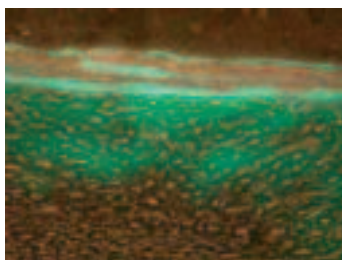


Figure 1 - V 28-15: GFP labelled cells forming layered epithelium.

tracture of the mesh pattern in wounds sprayed with cultured cells. Histological analysis of the wounds identified GFP labelled cells forming epithelial layers by day 10. The epithelium and basement membrane was also more developed in this group.

Conclusions: This method successfully identifies cultured keratinocytes, demonstrates their survival and ability to form an epithelial layer in between the meshed skin.

V 28-16

The effect of pressure profiling using topical negative pressure therapy in a non-wounded skin model

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Introduction: Topical Negative Pressure (TNP) is a non-pharmacological therapeutic modality that is an important adjunct in the armamentarium for acute and chronic wound management. It involves the application of a controlled negative pressure across a wound using an open-pore foam dressing interface. Experimental evidence suggests intermittent negative pressure confers an additional benefit over continuous negative pressure protocols. We investigated the effect of cycled pressure profiling produced on dermal blood flow in a non-wounded skin model and compared this to standard pressure protocols.

Methods: Ten (n = 10) volunteers were recruited to the study. TNP foam dressings were applied to the dorsum of the foot and blood flow and temperature measured. Blood flow was measured using a Perimed PF 5010 laser Doppler with a silicone wafer probe (dimensions 1cm by 1cm, thickness 2mm) with temperature being simultaneously recorded using a Datalogger 1000 thermister (probe 2mm by 2mm). Both probes were placed in contact with the skin in a central position beneath the polyurethane foam. Measurement of blood flow began when the temperature underneath the foam reached a steady state. Three TNP protocols were utilised:

- A: 125 mmHg continuous sub-atmospheric pressure.
- B: 125 mmHg intermittent sub-atmospheric pressure (5 minutes on: 2 minutes off).
- C: Pressure cycling between 150 mmHg and 100 mmHg sub-atmospheric pressure (3 minutes at 150 mmHg; 3 minutes at 100 mmHg).

Results: Mean and standard deviations for the three profile settings were for

- A: 21.4 ± 11.4 Perfusion units
- B: 23.4 ± 12 Perfusion units
- C: 17.7 ± 9 Perfusion units

No statistically significant differences in blood flow could be demonstrated between the three pressure profiles investigated (p = 0.69- using a one way ANOVA test).

Conclusions: These results, in an unwounded skin model, suggest continuous cycled therapy offers no additional benefit over existing protocols. Clinical studies should be conducted to investigate this treatment strategy in more detail.

V 28-17

Wound healing analysis and measurement by means of colour segmentation

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Introduction: Treatment of skin wounds costs the European Health Services, millions of Euros each year. Therefore it is vital to ensure the most appropriate treatment is employed for each individual wound. In order to assess whether a given treatment is working effectively clinicians require quantitative feedback on the healing progress. Using an algorithm, pixel values of a wound photograph can be analysed according to red granulation tissue, yellow fibrin and black necrotic tissue.

Methods: Pictures were then analysed using the Wound Healing Analysing Tool (WHAT). The WHAT allows assessment of the following parameters: Wound area, wound perimeter and elective distances (measured in ml and ml²), area of granulation, fibrin and necrosis (presented in pixels, ml² and percentage of wound area). The analysis algorithm was created after colour histograms of ~100 typical wound photographs were statistically evaluated. To verify the correct segmentation into the wound components, biopsies from wound sites where taken in 45 different wound cases and histologically examined. 12,500 cases were analysed until 05/2005.

Results: Morphological analysis of biopsies confirmed agreement with WHAT output on the identification of necrosis, granulation or fibrin tissue. In general, accuracy of 80–95 % was recorded, depending on basic procedures in photography. Some complicated wounds may require manual correction due to non typical structures affecting colour separating algorithms. By using the WHAT tool, we have been able to reduce infection rate by 30 % and length of stay in patients with wound infection by 3.4 days.

Conclusions: Digital wound photography can present a simple but effective method of clinical data storage, and moreover provide objective wound assessment. Bridging the gap between electronic engineering and patient care may also reduce therapy time and save resources and cost. This new tool for the assessment of wound healing can provide objective proof clinical studies and can contribute to reducing wound healing time.

V 28-18

Is the measurement of transcutaneous oxygen tension (tcpO₂) in daily routine capable of predicting long-term outcome of ischemic wounds?

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Introduction: Adequate tissue oxygenation is crucial for wound healing. However, quantitative assessment of peripheral microcirculation is still a severe diagnostic challenge. We evaluated the impact of transcutaneous tissue oxygen tension (tcpO₂) measurement in daily routine on treatment and outcome of ischemic foot ulcers.

Methods: Patients with ischemic foot ulcers were prospectively followed up in an outpatient wound care clinic. Ischemia was defined as absent pedal pulses and a decreased ankle/brachial index (ABI). TcpO₂ measurements were performed at the dorsum of the forefoot in a standardized setting. Clinical endpoints were categorized as healing, i. e. complete epithelization, or amputation. Life-table analysis was performed using the Kaplan-Meier method. Results are given as mean + SD. Differences between groups were calculated by Mann-Whitney U test or Pearson Chi² test where a p < 0.05 was considered significant.

Results: In total, 327 patients (69.5 + 10 years) were enrolled in this study. Mean wound area was 8 + 17.45 cm². Mean tcpO₂ was calculated to be 29.6 + 18.74 mmHg. 67 % of the patients were of male and 33 % of female gender. Mean time of follow-up was 153 + 175 days. Patients were highly comparable for baseline characteristics such as wound size, wound grading and comorbidity. Probability of healing correlated significantly with tcpO₂ levels. Wounds associated with a low tcpO₂ demonstrated a significantly lower probability of healing (p = 0.002) and higher infection rates (p = 0.032). In addition, amputation rates were significantly increased in wounds with tcpO₂ < 40 mmHg. However, there were no differences in major amputation rates.

Conclusions: Assessment of tcpO₂ in daily routine is capable of predicting long-term outcome in ischemic foot ulcers. In specialized wound care centers, this non-invasive and quantitative method should be included in the standard vascular diagnostic protocol in choosing the appropriate treatment schedule.

Table 1- V 28-20.

	n-patients	Wound duration (days)	Bone Involvement	Age (years)	p-Healing (after 365 days)
All	1549	444	18 %	68 + 0,3	65 %
Diabetic	607	213	29 %	68	71 %
Venous	455	799	2 %	69	67 %
Ischemic	209	318	23 %	72	65 %
Other	278	462	17 %	61	69 %

V 28-19

Indications that lactate released by oxygenated inflammatory cells directs healing

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Background: Strong evidence indicates that lactate accumulation exerts a compelling directive force in the mechanism of wound healing. It is known to drive wounds, more particularly macrophages, to release a variety of cytokines and growth factors. It stimulates collagen gene transcription, collagen hydroxylation and deposition, vegf release and angiogenesis, vasomotor relaxation, and cell replication. With lactate as the stimulus, all of these occur only in the presence of oxygen. A dilemma has arisen due to the old concept that lactate is only a product of hypoxia. An alternative source of aerobic lactate production is the NADPH-linked oxidase of phagocytes, phox. Its reaction is: glucose + oxygen → lactate + superoxide.

Aims: To demonstrate that aerobic macrophages release significant lactate in wounds.

Methods: RAW cells were cultured in oxygenated cultures with varying amounts of glucose, lipopolysaccharide, and in the presence of inhibitors of phox. Diphenyliodonium (DPI), an inhibitor of phox, was injected into wounds and PO₂ was measured.

Results: RAW cells synthesize and release lactate in aerobic conditions. Their lactate production is increased several-fold by lipopolysaccharide activation. Inhibition of phox by DPI reduces it by about 80 % from the activated level. Added glucose increases lactate production about 2 fold. Added oxygen does not change lactate production of unactivated leukocytes though it profoundly affects the quantity of superoxide synthesized. Apocynin reduces oxygen consumption in wounds implying that it will inhibit lactate production there as well.

Conclusions and discussion: Macrophages are a significant source of aerobic lactate production in wounds by activation of the NADPH-linked oxidase of phagocytes. It is a dominant feature of wounds accounting for resistance to infection and production of growth factors/cytokines. What are the clinical consequences of these findings to diabetics? Can accumulated lactate reduce immunity by feed-back inhibition on the above equation? Can lactate also act as a homing signal for stem cells? See Aslam et al this meeting).

V 28-20

Impact factors for healing: an analysis on 4000 prospectively documented chronic wounds

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Introduction: We founded 12 years ago an interdisciplinary wound care center in the department of general surgery. The cooperation started with the departments of vascular surgery, radiology, internal medicine. Now we have an extended network also with several home care organizations. Treatment is performed according to a comprehensive wound care protocol. Since 8 years, treatment is documented within a special wound documentation system and we are now able to analyze our outcome on the basis of this prospectively collected database:

Results: Since 1998 we documented the course of 1549 patients with 4067 wounds. There were several patients with more than one wound, so we defined according to an algorithm (wound-duration - grading and wound size) a primary wound. There were 607 patients with diabetic, 455 patients with venous, 209 patients with ischemic wounds, 278 patients had wounds of other etiologies. Table 1 shows characteristics of wounds with different etiologies and the probability of healing within one year, calculated according to Kaplan Maier analysis (Tab 1).

Independently to the etiology we calculated the impact of several factors by Cox regression analysis (Tab 2).

Conclusions: Etiology is an important factor for healing, but compliance, wound size, age and diabetes have an impact on the healing of chronic wounds, independently of the underlining disease.

Table 2- V 28-20.

	Exp (B)	Significance
Age (< > 70 years)	0.765	0.05
Ischemia (yes/no)	0.978	0.88
Diabetes (yes/no)	1.523	0.01
Wound duration (< > 1 year)	0.771	0.18
Compliance (good/bad)	2.204	0.0001
Bone involvement (yes/no)	0.749	0.09
Wound infection (yes/no)	1.186	0.23
Wound size (< 1; 1-5; > 5 cm)	0.864	0.0001

V 28-21

Healing of chronic lower extremity wounds in the elderly*C. Wicke, S. Coerper, M. Witte, S. Beckert, A. Königsrainer*

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Introduction: In recent years insight has been gained into the impact of aging on cellular and tissue responses, resulting from impaired cytokine signal transduction, unchecked inflammation, an altered balance of protein synthesis and degradation, and subsequent downstream effects on the rate and quality of the wound healing response. Most of this information, however, results from animal studies. Little attention has been focused on assessing the healing capacity of the elderly in a clinical setting. The aim of this study was to determine key factors influencing the healing of chronic wounds in a population of patients with lower extremity chronic wounds treated in a specialized wound care center.

Methods: The study was a 5-year epidemiological evaluation of standardized data collected regularly with a computerized wound documentation system. Wound care followed an interdisciplinary, standardized, comprehensive wound care protocol. Data were sampled from February 1999 to January 2004. Only one leading wound per patient was part of the statistical analysis. The primary objective was to determine the frequency of wound closure in dependence of the patient's age.

Results: A total of 1,158 wounds in 1,158 patients were included into the statistical analysis. The clinical outcome was successful wound closure in 54.5 %, a still open wound in 45.5 %, and an amputation in 2.2 % of the cases. The frequency of wound closure was statistically significantly lower in older patients than in younger patients ($p < 0.001$). At the final assessment the cut-off point of ≥ 70 years resulted in a statistically significant odds ratio of 1.770 for the number of closed wounds in favor of the younger patients. For patients whose wounds closed, the velocity of wound closure was not affected by patient age.

Conclusions: The present study demonstrates a strong relationship between patient age and the frequency of wound closure in patients with chronic lower extremity wounds. The share of closed wounds is estimated to decrease by 23 % for the older patients compared to the younger patients. Underlying diseases, compliance, wound age, initial wound size, presence of infection and number of documented wounds per patient are also related to the frequency of closure of chronic wounds.

V 28-22

The ability of ORC/collagen containing silver to reduce bioburden and retain dermal cell viability*S. J. Gregory, D. Campbell, B. M. Cullen*

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Infection is a common cause of delayed healing. Historically, silver containing therapies have been used as topical antiseptics to help manage bioburden. However, they may also be cytotoxic to important cell types such as dermal fibroblasts. When overt clinical infection is present cytotoxicity is acceptable as the greater need is bacterial balance. Conversely, when clinical signs of wound infection are questionable, it is difficult to have correct diagnosis and determine appropriate use of topical antimicrobials. In these cases, a product, which addresses bioburden and retains cell viability, could help reduce the incidence of wound infection without impacting rate of healing. In this study, we investigated the effect of silver ions on human dermal fibroblasts. Extracts of silver dressings, including an ORC/collagen matrix containing 1 % silver-ORC, were assessed for their effects on cell viability. Antimicrobial activity was assessed using clinically isolated vancomycin-resistant Enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) in the Log₁₀ reduction test. Results show that while ORC/collagen matrix containing silver effectively kills important wound pathogens, it is not harmful to host dermal cells. This in vitro work suggests that this biomaterial, in contrast to other silver containing dressings, is not cytotoxic but still capable of controlling wound pathogens.