Schiller Saal 14.00–15.30

V 24

Stem Cells and Precursor Cells II

Stammzellen und Vorläuferzellen II

V 24-1

„Knock-in"-strategies to visualize committed precursor populations in vitro and in vivo

H. J. Fehling

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Since their successful isolation over 20 years ago, mouse embryonic stem cells (ES cells) have become an invaluable tool for the generation of increasingly complex mouse mutants thanks to their unique propensity to foster homologous recombination at high frequencies. More recently, another fascinating aspect of ES cell biology, namely their capacity to differentiate in vitro into a wide range of different cell types and tissues, has attracted hightened interest, and with the availability of human ES cells, the idea of generating defined tissues or cell populations for clinical applications has become a realistic perspective. By targeting "green fluroescent protein" (EGFP) into the "Brachyury" gene locus, we have generated an ES cell line allowing us to monitor – in vitro and in the mouse – the formation of mesoderm and its commitment to various cell populations derived thereof. Data will be presented demonstrating the usefullness of these targeted ES cells in order to optimise cell culture conditions for the derivation of particular cell lineages and to select against formation of teratocarcinomas.

V 24-3

Embryonic and adult endothelial progenitors for cancer gene therapy

Ch. Beltinger

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Tumors need vessels to grow. In addition to sprouting angiogenesis derived from existing vessels, de novo vasculogenesis from circulating endothelial progenitor cells (EPCs) play an, albeit controversial, role in tumor vessel formation. The ability of eEPCs to home to tumors has been exploited in preclinical studies with EPCs engineered to kill surrounding tumor cells. Both embryonic EPCs and adult EPCs derived from peripheral blood can serve as cellular vehicles. Since the clinical potential of this approach hinges on efficient homing, molecular mechanisms influencing the homing of EPCs will be discussed.

Mozart Saal 14.00–15.30

V 48

Surgical wounds

V 48-1

Povidone-iodine ointment: No delay of split skin graft healing

Povidon-Iod Salbe: Keine Verzögerung bei der Heilung von Spalthaut-Transplantaten

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Aim: In major burns, local treatment of the split skin graft after a burn injury is important to prevent serious infectious complications. Povidone-iodine has many clinical advantages like a broad anti-microbial spectrum without resistance development. However in vitro data suggest that povidone-iodine may be toxic to wound healing cells and therefore believed to cause a delay in wound healing. In view of this we evaluated the local effect of povidone-iodine on wound healing of freshly grafted burn wounds.

Methods: In this prospective study, comparable areas of the same patient were treated with povidone-iodine ointment 10 % (Braunol jodium zalf from B. Braun Switzerland) or with simple vaseline gauze. In a series of 10 major burn patients undergoing excision and split skin grafting, the total healing time, progression of healing and wound colonization (qualitative and quantitative bacteriology) were studied.

Results: The total wound healing time in both groups was 26.3 days. The 50 % healing time showed a statistically significant difference, p = 0.023 (table 1). In the second half of the wound-healing period, the number of colony forming units (CFU)was significantly reduced in the povidone-iodine group (table 2).

Table 1- V 48-1: Mean 50 % graft healing time in days.

Vaseline	Povidone iodine
10.1 ± 1.55	9.4 ± 1.41

Table 2 V 48-1: Bacterial colonization during different healing phases.

		Control group CFU/cm²	Povidone iodine CFU/cm²
0–25 % healing time	11	22.6 ± 3.3	17.3 ± 4.3
26–50 % healing time	23	18.0 ± 4.2	15.7 ± 3.9
51–75 % healing time	23	9.6 ± 2.5	4.0 ± 1.9
76–99 % healing time	23	4.0 ± 1.5	1.1 ± 0.7

Conclusions: The use of povidone-iodine 10 % did not prolong the wound healing process of the grafted burn area. The bacterial colonization of the povidone-iodine treated group was lower compared to the control group. No difference in pain perception was seen, between the area treated with povidone-iodine and a vaseline-gauze only.

Ziel: Bei größeren Verbrennungen ist eine lokale Behandlung eines Spalthaut-Transplantates wichtig, um ernsthafte Infektionen zu vermeiden.Povidon-Iod hat viele klinische Vorteile wie z. B. ein breites antimikrobielles Spektrum ohne eine Resistenz-Entwicklung. In-vitro- Daten schließen jedoch nicht aus, daß Povidon-Iod für die am Wundheilungsprozess beteiligten Zellen toxisch sein könnte, und wurde deshalb als Ursache für eine Verzögerung der Wundheilung angesehen. Vor diesem Hintergrund haben wir den lokalen Effekt von Povidon-Iod auf die Wundheilung von frisch transplantierten Verbrennungs-Wunden untersucht.

Methoden: In dieser prospektiven Studie sind vergleichbare Stellen eines Patienten mit Povidone-Iod Salbe -10 % (Braunol jodium zalf, B. Braun Medical AG, Schweiz) oder mit einfachen Vaseline-Kompressen behandelt worden. In einer Serie an 10, hauptsächlich von Verbrennungen betroffenen Patienten, bei denen eine Spalthaut-Transplantation vorgenommen wurde, wurden die Gesamtheilungszeit, der Fortschritt der Wundheilung, sowie der Wund-Kolonisierungsstatus (qualitative und quantitative Bakteriologie)erforscht.

Ergebnisse: Die gesamte Wundheilungszeit war bei beiden Gruppen 26.3 Tage. Die 50 % - Heilungszeit zeigte einen statistisch signifikanten Unterschied, p = 0.023 (Tabelle 1). In der 2. Hälfte der Wundheilungsperiode reduzierte sich die Anzahl der koloniebildenden Einheiten (KBE)in der Povidon-Iod- Gruppe signifikant (Tabelle 2).

Diskussion: Der Gebrauch von Povidon-Iod 10 % verlängerte nicht den Wundheilungsprozess des transplantierten Wundgebietes. Die bakterielle Kolonisierung war in der Povidon-Iod-

Gruppe im Vergleich zur Kontrollgruppe niedriger. Es war kein Unterschied im Schmerzempfinden zwischen der mit Povidon-Iod und mit Vaseline-Gaze behandelten Wundflächen festzustellen.

(Korrekturvorschlag des Abstracts von Eva Linde)

V 48-2

L-Arginine suppletion improves blood flow in a free flap used for breast reconstruction

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Introduction: The free transverse rectus abdominis musculocutaneous flap (FTF) is a microvascular transplanted fat-skin flap used for breast reconstruction. Due to ischemia and reperfusion, there is a high percentage of partial flap loss (PFL). Arginine suppletion has shown to reduce PFL in experimental studies. This study was performed to investigate whether arginine suppletion reduces PFL in the FTF.

Methods: In this prospective study, twenty patients with a breast reconstruction using the FTF were included. Patient's demographics and flap data were gathered. Patients received either L-arginine-HCL (30 g/d) or iso-caloric L-alanine for five days. Blood was sampled for amino acids analysis by HPLC. Blood flow was measured in zone I (highest blood flow; closest to vascular pedicle) and IV (lowest blood flow; furthest from vascular pedicle) at standard time points using the laser doppler flowmetry (LDF, Perimed®). The time to peak (TP) and height of the peak (HP) were calculated. All data are displayed as mean ± SEM. LDF is presented in perfusion units (PU). The SPSS® program was used for statistical analysis with the Mann-Whitney or Chi-Square test.

Results: Due to technical problems, two patients were excluded. All patients' demographics and flap characteristics were similar between the groups. The arginine group showed a higher initial flow in zone I (19.1 ± 3.1 vs. 11.3 ± 0.8, $p < 0.05$) and zone IV (6.8 ± 1.3 vs. 4.5 ± 0.4, $p < 0.05$) compared to the placebo group. In addition, there was a significant difference in HP in the arginine group in zone IV (8.5 ± 0.8 vs. 6.5 ± 0.6, $p < 0.05$). Five out of ten patients in the control group had PFL (50 %) compared to one out of eight patients in the arginine group (13 %, $p = 0.09$).

Conclusions: Arginine improves blood flow of the FTF. Arginine reduces the risk of complications in microvascular tissue transfer.

V 48-3

The impact of localised warming on postoperative pain and wound healing after hernia surgery

A. Melling, D. Whetter, D. Leaper

University Hospital of North Tees, Professorial Unit of Surgery, Stockton-on-Tees, United Kingdom

Background: Warmth is an ancient remedy that has been used to reduce pain and aid healing. Pain relief and the prevention of infection following surgery remain an important challenge. The application of warmth directly to chronic wounds has been shown to aid healing and reduce pain. This study investigates the effect of warmth applied directly to the surgical wound and its impact on postoperative pain and wound healing.

Methods: Patients having inguinal hernia surgery were recruited on the day of surgery and randomised to receive either standard treatment or three days of local warming applied to the wound. Warming was applied for two hours immediately after surgery and then twice a day for three postoperative days. A disposable exothermic warming pad that was adhered to the wound dressing provided the warmth. Pain scores were recorded immediately after surgery and for the next three hours and then by the patients at home on a daily basis. Patients were asked to record the highest, lowest and current pain scores over the previous 24-hour period on a scale of 0-10. Patients were followed up at week one for a pain assessment and weeks two and six for blinded wound reviews.

Results: 144 patients were randomised to the study. The demographics of the two treatment groups were similar. The warmed patients reported significantly less pain three hours after surgery ($P < 0.05$) and also required less additional oral analgesia during their hospital stay. After discharge warmed patients reported lower pain scores over the next seven postoperative days ($P < 0.05$) and required less analgesia. The reduction in pain continued after warming stopped on day three. At week six 46 % of standard patients still had pain but only 35 % of the warmed patients. There were five wound infections (7.7 %) in the standard group and three in the warmed group (4.3 %) ($P = 0.4$).

Conclusions: The application of warmth directly to the surgical wound after inguinal hernia surgery reduces the patients experience of pain over the first seven days. In addition warming may reduce the amount of analgesia required as an inpatient and reduce the risk of surgical site infection.

V 48-4

Surgical site infection – a common and devastating complication following breast surgery

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Background: Breast surgery, usually following the diagnosis of cancer, is often a traumatic experience for the patient. Any wound healing complication is only likely to increase stress and may also lead to a delay in any supplementary treatment such as radiotherapy or chemotherapy. Breast surgery is classified as a clean procedure and as such should have a low rate of infection (< 5 %). However literature suggests that infection rates may be higher than expected and in addition the benefits of prophylactic antibiotics have not been proven.

Methods: In two randomised controlled trials patients having breast surgery have been followed up intensely to assess the rate of wound healing complication. Patients wounds have been observed by a trained wound care nurse, twice, over a six week period and patients interviewed about their wound healing experience. In addition, in one trial, patients were given a diary to document any wound healing complications.

Results: In both studies infection rates after breast surgery were higher than expected. The vast majority of infections occurred after discharge and were managed by the primary healthcare team. In patients receiving standard treatment the rate of infection was above 15 % and it was clear that this was having a detrimental effect on patients quality of life. Wound healing in breast surgery was further complicated by high rates of seroma formation (a collection of serous fluid below the wound surface that usually requires aspiration), haematoma and flap necrosis.

Conclusions: Infection rates following breast surgery are high and its classification as clean surgery may be misleading. The high rate of infection identified was in part due to intense patient follow up. In addition the prevalence of other wound complications will have increased the risk of infection. The high risk of infection and the devastating impact of any wound healing complication suggest that patients having breast surgery should receive optimum treatment to prevent infection which may include appropriate prophylaxis, temperature management, optimum tissue perfusion, stable blood glucose levels and supplemental oxygen.

V 48-5

German wound surgeons 1450–1750

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Aim: During the early Middle Ages, Italy and France were at the forefront of developments in wound healing and wound management. At this time Germany lagged behind, as there was little formal education available for either physicians or surgeons. Most surgeons obtained training by apprenticeship to a barber-surgeon and had no university training. This paper will explore the beliefs and practices of German wound surgeons during the period 1450–1750, based on the writings of some of the surgeons of the period.

Methods: The databases of the Wellcome History of Medicine Library, London and the Centre for the History of Medicine, University of Birmingham were searched for references to German surgeons during 14th, 15th, 16th and 17th centuries. All sources in English were followed and reference lists searched for further references.

Results: The works of Heinrich von Pfolspeundt, Hieronymus Brunschwig (1450–1533), Hans von Gersdorff, Wilhelm Fabry (1560–1634), Johannes Scultetus (1595–1645), Matthäus Gottfried Purmann (1648–1721) and Lorenz Heister (1683–1758) were studied. Many of these surgeons had experience as military surgeons and therefore learnt wound management practices from others as they travelled with the armies they served. Their methods of managing gunshot wounds, head injuries and burns will be described as well as details of some of the dressings and bandages in use over the period. The encouragement of suppuration in gunshot wounds was advocated by both Gersdorff and Heister, but Gersdorff proposed the use of warm oil whilst Heister preferred honey. Scultetus proposed the use of a variety of materials for managing craniotomy wounds such as hemp, honey, wine and astringent oils. Fabry wrote the first book solely on burns including assessment, care and treatment of scarring and contractures.

Discussion: A limitation of the study was that only texts in English were used, however, it was still possible to obtain detailed information with the use of translations from the original German. Much of the body of work produced by the German Wound Surgeons provides detailed information, often well illustrated, of the care of wounds in Germany over a 300 year period.

Hall Köln, Bonn, Hamburg 14.00–15.30

V 54

Adhesion molecules, Cytokines and Chemokines

Adhäsionsmoleküle, Zytokine und Chemokine

V 54-1

Analysis of IL-6 and TNF α in wound fluids of diabetic foot ulcers: an objective reflection of inflammation and the state of healing

Analyse von IL-6 Cytokinen und *TNFα* in Wundflüssigkeite von diabetischen Fußulcera: Evaluation der Inflammation und des Status der Wundheilung

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The defects of wound healing on a cellular level in patients with diabetes mellitus can be explained by a dysfunction of granulocytes, particularly with regard to the release of mediators of wound healing and growth factors. Also the inflammatory reaction seems to be affected by the systemic disturbances of diabetes and changes of the cellular processes may be responsible. In this study we investigated cytokines (IL-6) and growth factors (TNF) in wound fluids in relation to wound size, wound healing duration bacterial load and systemic parameters of inflammation (crp, leukocyte count).

Methods: For this, 22 diabetic patients (10 men, 12 woman; mean age 67 ± 19 years; HbA1c 7,7 ± 1,4) with DFS (stage Wagner 2 and 3) were recruited. The wound fluids (wash out with 5 ml NaCl solution) were collected after changing the wound dressing and stored at -20 °C. In parallel, microbiological analyses of deep swabs were performed and correlated to cytokines and clinical staging.

Results: We found no differences in wound size, glycemic control duration of wound healing or patients age between Diabetic patients with and without positive microbiological result. We found a clear and significant correlation between TNFα and the wound size. Dependent on the bacterial load no differences were found for CRP or leukocytes. However TNFα (28 vs. 23 pg/ml; p = 0.012) and IL-6 (367 vs. 42 pg/ml; p = 0.03) were significantly increased, when wound were infected by significant germs. This was independent on the clinical stage of wound scoring.

Conclusions: The definition of the infected wound is still in discussion. Compared with the clinically established , but not very specific CRP, our study presents a strong correlation of TNFα and IL-6 with the microbiological state. Further studies are necessary to investigate the importance of this preliminary data. In addition we found a correlation of TNFα - as MMP stimulator - and the wound size. These data underlines our hypothesis of fundamental processes on the cellular and molecular level in the non healing wound of patients with diabetes mellitus.

Störungen der Wundheilung auf zellulärer Ebene bei Patienten mit einem Diabetes mellitus können als Dysfunktion der Granulozyten und darauf aufbauenden Störung der Freisetzung von Mediatoren der Wundheilung und Wachstumsfaktoren gesehen werden. Die inflammatorische Reaktion scheint bei Patienten mit einem Diabetes mellitus bereits auf systemischer Ebene gestört zu sein und Veränderungen auf zellulärer Ebene scheinen dafür verantwortlich zu sein. In dieser Studie untersuchten wir Zytokine (IL-6) und Wachstumsfaktoren (TNF) in Wundflüssigkeiten in Bezug auf die Wundgröße, der Wundheilungsdauer, der bakteriellen Last und systemischen Parametern der Inflammation (CRP, Leukozytenzahl).

Methoden: 22 Patienten (10 Männer, 12 Frauen, mittleres Alter 67 ± 19 Jahre, HbA1c 7,7 ± 1,4 %) mit einem diabetischen Fußsyndrom (Stadium Wagner 2 und 3) wurden in diese Studie eingeschlossen. Die Wundflüssigkeiten (Spüllösung mit 5 ml NaCl) wurden vor Wechsel des Wunddressings gesammelt und bei -20 ° C asserviert. Parallel dazu wurden mikrobiologische Analysen von diesen Abstrichen analysiert und mit dem Zytokin sowie dem klinischen Zustand in Korrelation gesetzt.

Ergebnisse: Wir fanden keine Unterschiede hinsichtlich der Wundgröße, der Stoffwechseleinstellung, der Wundheilungsdauer oder dem Patientenalter zwischen den Patienten mit einem Diabetes mellitus mit und ohne positiven mikrobiologischen Befund. Wir fanden eine klare und signifikante Korrelation zwischen TNFα und der Wundgröße. Abhängig von der bakteriellen Last waren keine Unterschiede hinsichtlich CRP oder Leukozytenzahl nachweisbar. Dennoch waren insbesondere TNFα (28 vs. 23 pg/ml; p = 0.012) und IL-6 (367 vs. 42 pg/ml; p = 0.03) signifikant erhöht, wenn eine Wunde von relevanten Keimen infiziert war. Dies war dabei unabhängig vom klinischen Stadium der Wunde.

Zusammenfassung: Die Definition einer infizierten Wunde ist weiterhin ein aktuelles Thema in der wissenschaftlichen Diskussion. Verglichen mit dem klinisch häufig angewendeten, aber nicht sehr spezifischen CRP-Wert zeigte unsere Studie eine klare Korrelation von TNFα und Interleukin-6 mit dem mikrobiologischen Status. Weitere und umfangreichere Studien sind notwendig, um die Wertigkeit dieser ersten Daten zu unterstützen. Weiterhin fanden wir eine Korrelation von TNFα (als Stimulator der Metallo-Matrixproteasen) und der Wundgröße. Diese Daten unterstüt-

zen unsere Hypothese eines fundamentalen systemischen Prozesses auf zellulärer und molekularer Ebene bei der schlecht heilenden Wunde von Patienten mit einem Diabetes mellitus.

V 54-2

TNFα polymorphism associated with increased susceptibility to venous leg ulceration

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Many venous leg ulcers can be attributed to factors that place people at high risk of deep vein thrombosis (DVT). While there is an increased prevalence of the factor V Leiden mutation in ulcer patients, evidence for the role of genetic predisposition to ulceration is limited. We hypothesize that functional polymorphisms in pro-inflammatory cytokine and matrix metalloproteinase (MMP) genes, or genes involved in their regulation, may be risk factors for the development of venous leg ulcers.

We conducted a case-control study comparing the frequency of gene polymorphisms in 181 patients with a history of confirmed venous leg ulceration and 181 age- and gender-matched healthy controls. Polymorphisms were selected on the basis of associations with other complex diseases. The tumour necrosis factor-α (TNFα) gene is located in the major histocompatibility complex (MHC) and 70 % of Caucasian individuals carrying the TNFα· -308*A allele also have part or all of the 8.1 ancestral haplotype associated with numerous immunopathological disorders. We therefore examined a polymorphism located in intron 10 of the BAT-1 (HLA-B-associated transcript I) gene used as a marker of this haplotype. Polymorphisms were genotyped using PCR-based methods.

The significant association of the TNF-α··-308*A allele (Table 1) with venous ulceration is a novel finding. For homozygous individuals the odds ratio was increased to 7.24 (95% C.I. 0.88-59.47, p=0.0319), indicating a gene dosage effect. Polymorphisms in the MMP-3 [5A/6A @ -1171], PAI-1 (plasminogen activator inhibitor-1) [4G/5G @ -675], IL-1ra (interleukin-1 receptor antagonist) [variable number of tandem repeats in intron 2] and MMP-2 [C/T @ –1306] were not associated with ulceration in this population. Since the 8.1 ancestral haplotype is carried by most TNF-α -308*A individuals, it is difficult to determine if TNF-α-308*A is the causal allele or a marker of a neighbouring susceptibility allele located in this part of the major histocompatibility complex.

Table1- V54–2.

Gene Polymorphism	Controls Carrier frequency (%)	Ulcer Patients Carrier frequency (%)	Odds Ratio (Significance)	95 % C.I.
TNF-α G-308A	22.6	43.1	2.586 (p = 0,00003)	1.639–4.079
BAT-1 (HLA-B associated transcript I) -/C in intron 10	16.3	28.8	2.083 (p= 0,00549)	1.234–3.515

V 54-3

S-Nitrosoglutathione-containing hydrogel accelerates rat cutaneous wound repair

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To investigate the participation of nitric oxide (NO) on rat cutaneous wound repair, a NO donor (S-nitrosoglutathione, GSNO), incorporated in a hydrogel, was topically applied on excisional wounds. Wistar rats (n = 10) were separated in individual cages. GSNO (100 mM) was incorporated in F127 hydrogels. In the first day (0 d) a full-thickness excisional wound was done in the back of each animal, and the GSNO-containing hydrogel was topically applied onto wound bed daily up to 5 days post-wounding. In control group, hydrogel without GSNO was topically applied onto wound bed during the same time. The animals were sacrificed 21 days after wounding. Wound contraction and re-epithelialization were evaluated. Blood pressure was measured in the beginning and at the end of the experiment. After euthanasia, lesion and adjacent normal skin were formol-fixed and paraffin-embedded for histological analysis. Sections were stained with hematoxylin-eosin or Sirius red. The blood pressure of control and GSNO groups was equivalent at the beginning and at the end of the experiment. The wound contractions at the 14th and 21st days after wounding were greater in the GSNO group than in the control group (p = 0.02; p = 0.04, respectively). Fourteen days after wounding the re-epithelialized wound area was higher in the GSNO group (+77 %) than in the control group (p = 0.03). A higher amount of inflammatory cells was observed in the superficial and deep areas of the granulation tissue of the control group, compared to GSNO group. In control animals, thin red-yellow collagen fibers, occasionally distributed perpendicularly to the surface, were observed. In superficial wound area of GSNO group, thin yellow-greenish and red-yellow collagen fibers were distributed parallel to the surface while in deep wound area, thick red-yellow collagen fibers were arranged parallel to surface. Our results showed that the application of GSNO-containing hydrogel during early phases of wound repair accelerates wound closure and wound re-epithelialization and affects granulation tissue organization during rat cutaneous wound healing.

V 54-4

Hyperbaric oxygen enhances wound lactate production in rats

Hyperbarischer Sauerstoff verstärkt die Laktatproduktion in Ratten

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[2] Department of Surgery, University of California, San Francisco, San Francisco, United States of America.

Introduction: Healing wounds are characterized by high lactate levels in a relatively hypoxic microenvironment. In the past, the shift to an anaerobic energy metabolism was thought to be the main source for increased lactate concentration. Recently, it has been discovered that lactate is also generated by an NADPH-linked oxygenase using oxygen as one of its substrates, a mechanism called aerobic glycolysis. We hypothesize that hyperbaric oxygen therapy by increasing oxygen availability enhances wound lactate production.

Methods: Four wire mesh wound cylinders (n = 142) were implanted underneath the dorsal skin in each of 36 male Sprague-Dawley rats (312+11 g). Animals were randomized to 2 groups: The first group (n = 18) received 100 % oxygen with 2.1 ATA for 90 minutes twice a day for a total of 7 days. The second group (n = 18) was treated with 21 % oxygen with 1 ATA for the same time schedule. Hyperbaric treatments were administered in a hyperbaric chamber designed for small animals (Model 100; Western Hyperbaric Services, Union City, California). Wound fluid from the cylinders was aspirated between the two treatment cycles at day 2 and 5 and analysed for lactate using a lactate analyser (model 2700; Yellow springs Instruments, Ohio). Additionally, at day ten (after two days of treatment with 21 % oxygen in 1ATA in both groups) lactate concentrations in wound fluid were measured again. Data are expressed as mean + SD. A p-value < 0.05 was considered significant.

Results: Wound lactate concentration was significantly increased both in control and hyperbaric rats at day 5 and day 10 compared to day 2(controls 4.5 + 1, 5.9 + 0.8, 6.7 + 1.2 mmol/l; p = 0.04 and hyperbarics 4.4 + 1.2, 6.5 + 1.3, 8.2 + 1.3 mmol/l; p = 0.01). At day 2 and day 5 there was no significant difference in lactate levels between control and hyperbaric rats (p > 0.05). However, at day ten lactate concentration was significantly higher in the hyperbaric group compared to the control group (6.7+1.2 vs. 8.2+1.3 mmol/l; p=0.01).

Conclusions: Hyperbaric oxygen therapy enhances lactate production in wounds. This investigation provides evidence for the mechanism of hyperbaric oxygen action since lactate is known to stimulate collagen production and angiogenesis.

V 54-5

Ischemia-reperfusion, apoptosis, and hyperthermia preconditioning

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Aim: To investigate the role of apoptosis as a cell death mechanism following ischemia-reperfusion in a rat latissimus dorsi muscle flap model. To study the effects of hyperthermia preconditioning on apoptosis.

Methods: 50 male adult Sprague-Dawley rats were divided in three groups. 10- CONTROLS- latissimus dorsi muscle flap based only on its vascular pedicle, no ischemia reperfusion; 20- Ischemia Reperfusion (IR)- latissimus dorsi muscle flap elevation, 4 hr ischemia, 24 hr reperfusion; 20 HYPERTHERMIA preconditioning- hyperthermia treatment for 1 hr 30 min in a controlled hyperthermia chamber 24 hr before surgery, followed by same surgical and Ischemia-reperfusion protocol as IR group. At the end of the surgical and IR protocol, operated muscle and its contra lateral counterpart (nonoperated normal muscle) were harvested. Half the muscle was extracted for HSP 72 analysis with HeatXpress (Stressgen Corporation). The other half was fixed in formalin and paraffin sections were prepared for Apoptosis analysis with fluorescent TUNEL assay (Roche Applied Biosciences, USA). Apoptotic index (Ratio of apoptotic nuclei and total nuclei in a unit area) was calculated with image analysis of digital photos.

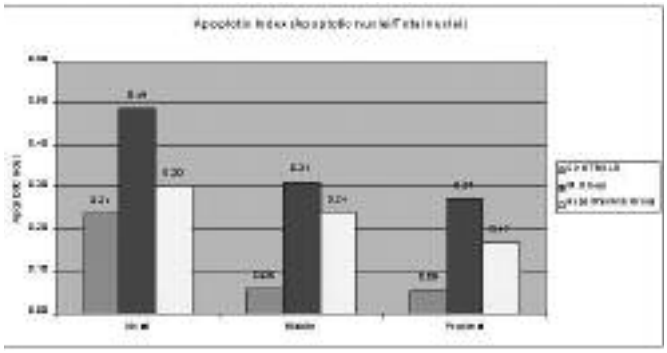

Figure 1- V 54-5.

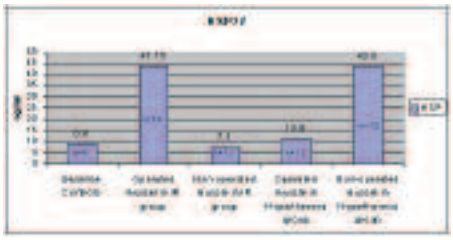

Figure 2- V 54-5.

Tabelle 1 V 54–5.

	Distal third	Middle third	Proximal third
Controls	0.24	0.06	0.05
Ischemia Reperfusion	0.49	0.31	0.27
Hyperthermia Reperfusion	0.30	0.24	0.17

Results: Two sided Student´s t-test was utilised for statistical analysis. Apoptotic index: Distal third of muscle demonstrated higher apoptotic index than proximal and middle third in all groups ($p < 0.05$). CONTROL group showed lower apoptotic index in all regions when compared to IR ($p < 0.05$) and Hyperthermia preconditioning groups. Animals in IR group demonstrated higher apoptotic index in all regions when compared to hyperthermia preconditioning group and the difference was statistically significant in distal third of muscle ($p < 0.05$). Hsp 72 levels were appx 4 fold higher in hyperthermia preconditioning group when compared to unoperated muscle in IR and control groups.

Conclusions: Apoptosis is up regulated following ischemia reperfusion injury. Its role is most prominent in the distal third of the muscle. Hyperthermia preconditioning protects against cell death following reperfusion injury and is most significant in the distal third of the muscle. Future studies looking in to molecular pathways of apoptosis induction and regulation in Ischemia-Reperfusion events are warranted. We are currently studying role of caspase-3 inhibitor in regulation of apoptosis in this model.

V 54-6

Ex-vivo cytokine analyses of wound exsudates reflect the inflammatory status in chronic ulcers

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Critical colonisation and infection are reasons for delayed wound healing. Decisions made for an antiinfective management are usually based on clinical signs. However, skin grafting studies have shown that using clinical signs of infection - regardless of bacterial burden and bacterial species - as a basis for decisions on treatment modalities lead to a high percentage of graft loss. Therefore, the evaluation of new markers for determining the inflammatory status of wounds is necessessary.

Methods: A total of 44 chronic ulcers (26 venous and 18 arterial) were assessed in terms of wound size (cm3), wound age (months), bacterial load - assessed by calibrated loop technique -, bacterial species and classification according to a mono- or polymicrobial infection. Furthermore, protein, IL-6 and TNF· concentrations of diluted wound exsudates were analyzed, and serum CRP and LBP levels measured.

Results: A good correlation was found between IL-6 and TNF· levels of wound exsudates. It was noticed that IL-6 was more sensitive than TNF· in predicting polymicrobial load. Among cultured bacterial species, Pseudomonas were related to the highest IL-6 levels, whereas a polymicrobial load has the most significance with regard to wound size. Compared to venous ulcers, arterial ulcers presented higher IL-6 levels, an increased ulcer volume and a longer history of delayed healing. CRP and LBP were not significantly related to any of the investigated parameters.

Conclusions: Increased IL-6 in wound fluids objectively reflects a polymicrobial and elevated bacterial burden. IL-6 is distinctively released with regard to bacterial species and the ulcer pathogenesis. In conclusion, IL-6 might be a useful tool for objective assessment and monitoring of wound bioburden and the inflammatory status of chronic ulcers.

TR 4.3.11-13 14.00–15.30

V 55
Wound infection and wound cleansing II
Wundinfektion und Wundreinigung II

V 55-1

E-Nose in diagnostic of wound pathogens

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Aim: Electronic noses (e-noses) that are capable to recognise the features of the odours have been successfully introduced in specific areas in practise, e.g. evaluation of foodstuff quality, detection of hazardous gases and even diagnostics of certain diseases. We demonstrate the capabilities of an e-nose in monitoring of the clinical infection of skin injuries. In this paper we describe the work carried out in Lithuania at Vilnius Semiconductor Physics Institute and Kaunas University of Medicine.

Methods: Our present study deals with the relationship between the features of the headspace volatile compounds detected by the e-nose and the type of pathogen. In the tests, we mainly used the most common wound pathogen – s.aureus. *S.aureus* was cultivated in Mueller-Hinton agar gel medium for 24 hours. Fourty-six samples of *S.aureus* in Mueller-Hinton agar gel medium (research group) and ten samples of pure Mueller-Hinton agar gel (control group) were analysed. The headspace air was analysed by the e-nose. Original graphical images based on the response of the e-nose were composed for the featuring of the sample smell.

Results: A response of the e-nose sensors was detected for all the samples of the cultures. It is demonstrated that the artificial features composed of the sensor signals are dependent on the contents of the culture. Presence of *S.aureus* correlated with the specific features of the e-nose outputs. It is also recognised that the e-nose outputs are dependent on the age of the culture.

Conclusions: We conclude that the e-nose technology is promising in the rapid or instant diagnosis of the wound infection and pathogen identification. In this early stage of the study it is necessary to determine the effectiveness of the recognition/prediction of different pathogens by the e-nose. The result of these developments might offer a number of significant advantages over existing technologies.

V 55-2

Antibacterial activity of the topical anaesthetic lidocaine/prilocaine cream (EMLA®)

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Aim: To test the effects of EMLA-cream on typical wound pathogenic bacteria in vitro. EMLA-cream contains lidocaine 2.5 % and prilocaine 2.5 %. The cream is well suited for topical anaesthesia in wound debridement. Local anaesthetics have antibacterial properties, but little is known of the combination of lidocaine and prilocaine (1).

Methods: 5 clinical isolates and one ATCC reference strain of *Pseudomonas aeruginosa* (PA), *Eschericia coli* (EC), *Streptococcus pyogenes* (SP), *Staphylococcus aureus* (SA) and a reference strain of methicilline resistant *Staphylococcus aureus* were tested. All isolates were incubated on blood agar (PA in serum broth) over night. Cultures were diluted in saline to obtain inoculums of 10^6 bacteria/ml. Seven tubes were prepared for each isolate, containing 100 mg EMLA and 100 microliters (Ìl) of bacterial suspension. After vortexing, the tubes were incubated for 0,1,2,3,4,6 or 24 hours at 35 °C (the streptococci in CO_2-rich atmosphere). 1 ml of 0.4 % Tween 20 in Mueller-Hinton broth was added to solubilize the cream. Three tenfold dilutions were prepared and samples of 100 Ìl of the 10^{-3}, 10^{-2} and 10^{-1} dilutions were plated onto agar. After incubation for 24 hours, colony-forming units (CFU) were counted and time-kill-curves were constructed. Saline controls were recorded.

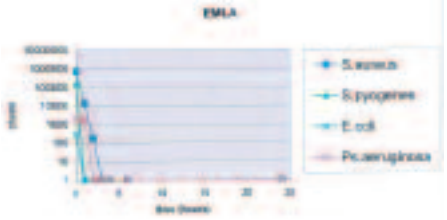

Figure 1 V 54-2.

Results: When exposed to the EMLA solution, there was a very rapid and significant reduction of all strains within the first hour ($p < 0.01$). SP and EC were almost immediately killed ($p < 0.05$), PA was killed within 2 hours ($p < 0.01$) and SA was killed within 3 hours ($p < 0.01$). A test with Tween 20 alone as growth control showed no antibacterial effect towards any of the tested bacteria (not shown).

Conclusions: As a positive side effect to the analgesic effect, EMLA is a powerful antibacterial cream, which may reduce the risk of infectious spread and bacteraemia when used for debridement of wounds.

References:

1: **Miller MA, Shelley WB.** Antibacterial Properties of Lidocaine on Bacteria isolated from Dermal Lesions. Arch Dermatol 1985;121:1157–9.

V 55-3

Use of Dermacyn®, a new antiseptic agent, for the local tretament of diabetic foot ulcers

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Introduction: Infection is, with peripheral vascular disease, the most important prognostic factor for the risk of amputation in diabetic foot. Use of antibiotic therapy, surgical treatment of deep infection, antiseptic dressing is mandator. Local antiseptic agents are widely used but there are few data about their efficacy.

Aim of the study: The evaluation of effectiveness of a new antiseptic agent, Dermacyn, a superoxydized water, as local treatment of infected diabetic foot ulcers. The global treatment was comprehensive of general antibiotic therapy, surgery, weight bearing relief.

Materials and methods: 220 consecutive diabetic patients have been enrolled for the study from November 2004 to March 2005. Were enrolled patients with stage II/III B-C-D ulcers using the Texas University Classification (T.U.C.). The localization of the lesion was below the ankle on the dorsal or plantar surface of the foot. 110 patients were treated in the Dermacyn group (D) and 110 in the control group (C) in wich iodopovidone dressing was used. The two groups were matched for age, duration of diabetes, class of ulceration. All the patients with peripheral vascular disease were referred for the revascularization using endovascular technique or by-pass surgery. All patients were treated surgically. In patients with T.U.C. III B/D lesion surgical treatment of bone infection was carried out (esostectomy-minor amputations).At the time of the enrollment and every month microbiological specimens were taken until surgical closure treatment. Local treatment was carried out daily using gauze with Dermacyn (D group) or gauze with iodopovidone (C group).

Results: All the patients had clinically and microbiologically infected ulcers. The mean follow up was 94.8 days (range 35–210). The mean number of microbiological agents was 3.2 in D group and 3.3 in C group. At the surgical time 75 % of ulcer in the D group had a negative microbiological specimen vs 48 % of ulcer in the C group ($p < 0.005$). No adverse effects were noted in the D group while 15 patients showed local reaction to iodopovidone in the C group.

V 55-4

Methicilin-resistent Staphylococcus aureus *– problem in health care*

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Introduction: At present, MRSA infection is a grave burden for health-care systems; it is associated with considerable morbidity and mortality and poses economic problems. It is our intention to draw attention by our work to the economic differences in the treatment of that infection.

Methods: The share of MRSA in all nosocomial staphylococcus infections is high globally and MRSA infection stands high among all nosocomial infections. The Czech Republic reports the incidence of 3–10 %. According to the latest data from the National Reference Laboratory for Antibiotics, the Czech Republic exceeded in 2004 the 10 % boundary. MRSA was identified in 2003 at the 1st Surgical Department in two patients only, whereas 27 patients were affected in 2004. Nine of them had local defects and the infection was verified in the other patients in hemocultures, from nasal vents, etc. s. Figure 1. Numbers of Patients with General and Topical Signs of MRSA

Results: The basic measure in persons with suspected MRSA infection of the skin and soft tissues is the incision and drainage of abscesses. Surgical and topical therapy can result in eradication, especially in case dressing with silver ions or a superoxidated disinfection solution are used. The price of those products is much lower than prices of daily doses of antibiotics. We attempted to assess the financial costs of defects treatment in our patients. Success of the therapy is high.

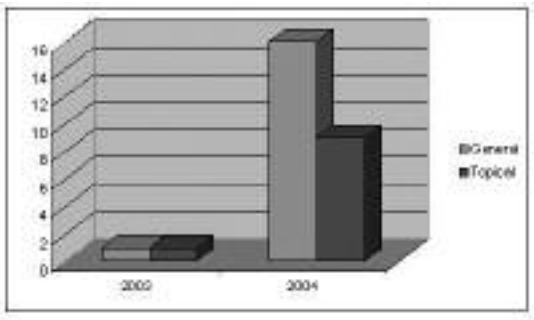

Figure 1 V 54-4.

Table 1 V 54-4.

Age	Number of Patients
up to 30 years	3
31–40	3
41–50	2
51–60	6
61–70	5
71–80	6
over 80 years	2

Conclusions: Undoubtedly, the incidence of MRSA infections will grow. It is absolutely necessary to take preventive measures. It is always good to differentiate between general infections and topical infections and select an optimal therapy. The economic requirements for the treatment of MRSA are evident. It is a good idea to consider the benefits of topical treatment, especially when economic aspects are taken into account because those aspects are reflected more and more in the daily routine of health-care professionals.

V 55-5

The development of an experimental in vitro model to study the interaction of microorganisms with human skin

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Aim: Candida albicans is a member of the normal oral microflora of humans and in suitably debilitated individuals may be associated with infection and impaired wound healing. The specific objective of this study was to examine tissue invasion and gene expression by specific strains of *C. albicans* in an in vitro model of human skin, using reconstituted human epithelium (RHE; SkinEthic Laboratories).

Methods: Candida albicans isolates (n = 19) were recovered from a range of oral conditions, including oral candidosis, oral cancer and healthy mucosa. Standard inocula of these isolates were used to infect the RHE. Infected tissues were incubated for 24 h and then one half of the tissue was fixed in paraformaldehyde. The remaining portion was retained in RNA-Later (Ambion) solution at -20 °C. RHE tissue sections (20 µm) were then stained with concanavalin A-Alexa 594 conjugate and examined by confocal laser scanning microscopy (CLSM). RT-PCR was used to assess expression of putative candidal virulence genes (secreted aspartyl proteinases and phospholipases) using RNA extracted from the frozen tissue.

Results: CLSM identified several infection types for different isolates, with variations evident in tissue colonisation, depth of invasion and morphology. Highly invasive strains were associated with CHC origin, hyphal presence and extensive colonisation of the tissue surface. The expression of proteinase and phospholipase genes was detected with all isolates, with no obvious correlation evident with infection type.

Conclusions: The RHE offers a suitable and reliable in vitro model to study the interaction between the skin and microorganisms. This model is amenable to CLSM and genetic analysis and may be of future use in studies into the role of bacteria in impaired human wound healing.

V 55-6

Retrospective evaluation of versajet as a debridement instrument

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Study aims: The primary aim of the study was to evaluate the safety and efficacy of Versajet (Trademark of Smith and Nephew} on the basis of clinical experience at the University of Medicine and Dentistry of New Jersey (UMDNJ) over a 12-month period in 2003. A secondary goal was to evaluate the potential cost savings associated with Versajet.

Methods: Retrospective case review including all patients who had an excisional debridement during 2003 and for whom Versajet was explicitly used. As a control, a sample of patients whose wound was debrided by conventional surgical methods was selected. Wound types were matched as closely as possible with the wounds in the Versajet group.

Results and conclusions: The number of debridement procedures per wound was lower in the Versajet group (on average 1.2 versus 1.9 with conventional surgical methods; p = 0.002). Debridement time was similar in the two groups. The observed differences in procedure time are not statistically significant. The pooled mean is 64.7 minutes. Cost savings with Versajet. The resource saving associated with the use of Versajet in this study was approximately $ 1,941 per patient. Thus, over a year the saving could exceed $ 200,000.

TR 4.4.20 14.00–15.30

W 4
Outcome Database