[EP139] CHARACTERIZATION OF A WOUND HEALING MODEL IN FARMED MINK (NEOVISON VISON)

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E-poster session: Basic Science 1

Aim: Currently, no scientifically substantiated guidelines exist for the assessment of cutaneous wounds in mink. The lack of criteria that can be used for supporting decision making regarding practical management of wounds is a problem to the mink farmers and the animals alike. The aim of the present study was to develop and characterize a wound healing model in mink with special emphasis on time perspectives of wound healing.

Method: Thirty male farmed American mink of brown colour type were subjected to an excisional wound model where square, 2x2 cm, full thickness wounds were created in the dorsal midline of the thorax under general anesthesia. The mink were euthanized after 1, 3, 7, 14, 30 and 60 days post wounding, after which macroscopic pathology and histopathology of wounds was evaluated together with degree of wound contraction.

Results / Discussion: The results show similar timing of events related to leukocyte infiltration, fibroblast proliferation, neovascularization, collagen deposition and reepithelialization of wound healing in mink as in other mammals. The wounds, however, were not completely healed after 60 days, which was found to be due to wound infection.

Conclusion: This is the first study to report the timing of events during cutaneous wound healing in the American mink. The results show that wound healing in mink follows the same timing of events as wound healing in other domestic mammals. Importantly, the results can be used to more accurately perform age assessment of wounds in forensic and other diagnostic cases.

[EP140] DETERMINATION OF THE BINDING CAPACITY OF A GEL-FOAM DRESSING FOR THE INFLAMMATORY MEDITAOR TNF-ALPHA

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Aim: The gel-foam dressing* combines a soft foam layer with a highly absorbing gel providing both, mechanical protection of the wound and absorption of excess wound exudate. An additional beneficial feature would be a direct influence on the inflammatory milieu, e.g. by reducing cytokines which are elevate in chronic wounds such as TNF-a. Here we investigated the binding capacity of the gel-foam dressing* for TNF-a in vitro.

Method: Dressing samples were cut into equal pieces (0.5cm^2), taken in a final volume of 1mL cytokine solution, and incubated up to 24h at 37°C. Supernatants were collected and stored at -20°C. Afterwards, samples were eluted in PBS for 1h at 37°C. TNF-α concentrations were determined by specific ELISA.

Results / Discussion: The gel-foam dressing* achieved a significant binding of TNF-α in the in vitro tests. Furthermore, binding of TNF-α by the gel-foam was confirmed to be irreversible.

Conclusion: The results suggest that the gel-foam dressing* should have an additional valuable effect on wound healing besides mechanical protection of the wound due to its ability to reduce the amount of specific inflammatory mediators.

*Sorbion aquafoam (sorbion GmbH & Co. KG)

[EP141] EXPERIMENTAL STUDY ON THE PH EFFECT OF POLYHEXANIDE CONTAINING WOUND CARE PRODUCTS

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Aim: Chronic wounds exhibit higher pH-values compared to acute wounds. It could be shown that the pH in chronic wounds most commonly has a range of 6.5 to 8.5. This alkalization is thought to be due to tissue necrosis and the presence of microorganisms. Therefore, establishing a low physiological pH might be a key factor to aid wound healing.

Methods: A study by Braunwarth et al. showed that the antimicrobial effect of polyhexanide (PHMB) containing wound dressings is pH-dependent, while silver-containing dressings possess a similar bacteriostatic effect over a pH-range of 5.5 to 9.0. To further investigate the influence of the pH on the activity of antimicrobial substances and wound dressings it is advantageous to determine microbial growth using microplate-laser-nephelometry (MLN).

Results / Discussion: *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibited an increasing sensitivity against polihexanide with rising pH. The increase of the efficacy of polihexanide can be explained by its polycationic nature. At physiological pH, the positively charged groups of polihexanide can bind rapidly to the negatively charged bacteria surface. This causes membrane damage and the death of the bacteria. In an alkaline solution the polycations become protonated, which leads to a higher charge density intensifying the binding to the bacterial surface.

Conclusion: Our results showed that the efficacy of polihexanide was not affected regardless of which formulation was used, no significant differences between the tested antimicrobials* were observed at pH 7.0 to 9.0.

*Products tested: Lavasept, Prontosan, Suprasorb X + PHMB

[EP142] THE CONTACT LAYER TLC PROMOTES IN VITRO THE CLOSURE OF ARTIFICIAL WOUND IN KERATINOCYTE-BASED MODEL

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Aim: The aim of the wound healing process is to obtain wound closure. This is possible thanks to keratinocytes, which migrate and proliferate from the wound edges to the center in the final phase of wound healing (epithelialization). The objective of this study was to develop a new model and tools evaluating wound healing closure and then to apply this model to the evaluation of a contact layer with TLC commercially used for epithelialisation.

Method: The wound healing model consists in creating a circular wound by incubation of keratinocyte cell line, HaCat, around a cylindrical device during 3 days in a Petri dish. After pad removal, the tested dressing is placed on the cellular crown. Cell growth and migration in the cell-free area are then imaged directly into the incubator using the small lensless device which allows the real time follow-up of biological processes in a large field (25 mm^2). Finally, the culture dish is removed from the incubator and then scanned with a lensless-based microscope which allows the imaging of the whole dish surface.

Results / Discussion: Several parameters are obtained from the acquired pictures and scans such as the average speed of cells, the mean distance from the initial crown and the average area newly covered by cells. Based on these approaches, we observed that the contact layer with TLC statistically promotes keratinocytes migration compared to control.

Conclusion: These results confirm the ability of the contact layer with TLC to promote wound healing, especially in the epithelialisation stage.

[EP143] FISH SKIN ACELLULAR DERMAL MATRIX DERIVED FROM THE ATLANTIC COD (GADUS MORHUA) SUPPORTS CELL INGROWTH

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Aim: Acellular xenografts are native extra cellular matrices (ECM's) that have been decellularized, also referred to as acellular dermal matrices (ADMs) and have successfully been used to treat chronic wounds. The wound healing properties of the grafts have been attributed to their ability to act as support for cell migration and regeneration of tissue.1: Chronic wounds are indeed characterized by a lack of cell migration to the wound bed. 2: Our investigation focused on an acellular dermal graft from fish skin, in particular its ability to serve as support for fibroblast ingrowth.

Method: The structure of the fish skin was examined with scanning electron microscopy (SEM) to assess its suitability to support cell ingrowth. Further experiments focused on the interaction of cells with the graft, NIH 3T3 fibroblasts were seeded onto a definite area of decellularized fish skin pieces anchored in 96 well plates. Intact fish skin ADM was viewed with confocal microscopy after fluorescent labeling. Slices of Fish skin ADM dyed with hematoxylin and eosin (H&E) staining and embedded into paraffin mold were examined with light microscopy. Negative control was graft without cells.

Results: SEM images suggested that the structure of the fish skin ADM supports cell ingrowth. Following experiments using light-and confocal-microscopy revealed that NIH 3T3 fibroblasts can migrate into and grow within the fish skin ADM as well as on its surface.

Conclusion: The ability of fish skin ADM to support cell ingrowth indicates its suitability to aid tissue regeneration in the treatment of chronic wounds.

[EP144] THE EFFECT OF PH ON FIBROBLAST MIGRATION IN AN IN VITRO MODEL COMPARING CHRONIC WOUND AND NORMAL WOUND FIBROBLASTS

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E-poster session: Basic Science 1

Aim: Increasing evidence suggests that pH has a role to play in chronic wound healing. Measurements of chronic wounds such as venous leg ulcers show an increase in alkalinity with recordings of pH 7-9 compared to healthy skin that has a more acidic pH. The aim of this study was to assess the effect of pH on fibroblast wound closure.

Method: Normal fibroblasts (NF) and Chronic Wound fibroblasts (CF), isolated from normal equine skin and chronic granulation tissue, were seeded in 6-well plates ($1x10^5$ cells/well). When cells reached 80-90% confluence cultures were scratched with a 200µl plastic pipette tip, gently washed with PBS and fed with 2mls of pH-adjusted media (6, 7.5 and 9). Scratched cultures were imaged to obtain an initial scratch area (time 0). Cell cultures were incubated at 37°C in a humidified 5% CO_2 incubator. Scratch images were taken at 24 hours and 48 hours before the assay was terminated. Images were analysed using the Image J software and percentage scratch area calculated for each time point.

Results / Discussion: NF migration was significantly diminished in pH6 conditions, with only 20% wound closure achieved after 48 hours compared to ~70% closure in pH7.5 and pH9 conditions. CF showed a different trend compared to the NF; wound closure was slowest in pH9 conditions with only 40% closure compared to 60% in pH6 and 90% closure in pH7.5 conditions.

Conclusion: pH does play a role in chronic wound healing and reducing the alkalinity of the wound could help improve cell migration, the rate of wound closure and successful wound healing.

[EP145] A PIONNEER APPROACH IN THE EVALUATION OF TISSUE REGENERATION AS SUPPORT IN TRANSLATIVE RESEARCH

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E-poster session: Basic Science 1

Aim: In wound healing, classical histopathology approach can be completed with immunohistochemistry and gene expression analyses, which are time consuming and expensive. In this study, we developed an alternative approach for an insightful histopathology analysis by setting up new quantitative and integrative metrics.

Method: The performance of a test and predicate wound healing product (polyester mesh impregnated with hydrocolloid particles) were compared in adult Wistar rats. Preserving the *Panniculus carnosus*, paravertebral dermo-epidermic wounds (2.5 x 2.5 cm) were created. The healing time was 21 days.

***Histopathologic metrics**:*

a) Quantification of the total collagen content and type I and III collagens by image analysis.

b) Quantification of cell ingrowth rate by image analysis.

c) Measurement of the granulation tissue growth rate (GTR) by integrating the two last parameters.

Results / Discussion: The quantitative results were consistent with the qualitative findings. Although the test material promoted slightly more collagen deposit and cell ingrowth, no significant statistical difference was observed between the predicate and test groups in terms of GTR. The collagen was similarly remodeled (collagen I/III ratio) in the two groups.

Conclusion: The investigated test article and predicate showed similar wound healing performance as demonstrated through this innovative quantitative measurement of the GTR. This methodology showed its value i) in performing multiple analyses of wound healing markers on the same sample and ii) in better discriminating the tissue components (cellular and extracellular) versus standard histology, in a fast and cost effective way.

[EP146] DEVELOPMENT AND EVALUATION OF MODIFIED HYALURONIC ACID MATERIALS FOR MMPS AND FREE RADICAL MODULATION

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E-poster session: Basic Science 1

Aim: It is well known that over excess, high levels of proteinases especially matrix metalloproteinases (MMPs) within the chronic wound environment stalled the healing process. Therefore, we aim to develop novel materials for advanced chronic wound dressing to regulate and inhibit MMPs. The HA based biomaterials ($MMPh^+$) was first developed and further evaluated their effectiveness to reduce the activities of MMP-3 and MMP-9, and radical scavenging ability via in vitro assays. Our findings suggest that the MMPh+ shows potential for providing a favorable environment in healing process.

Method: Hyaluronic acid immobilized with capped histidine as active binding sites were developed. The chemical structures, concentration of micelle formation value (CMC) with various grafting contents were characterized. The inhibitory effect of $MMPh^+$ on MMP-3 and MMP-9 were evaluated with In vitro assays. ABTS radical scavenging assay were used to compare the antioxidant activity.

Results / Discussion: The synthesized $MMPh^+$ are water soluble white to pale yellow powder or granules. It was found that the degree of modification was in the range of 17%~55% determined by NMR. The CMC values of $MMPh^+$ copolymers were from 0.10~0.18 mg/ml. $MMPh^+$ successfully reduces both MMP-3 and MMP-9 activities ranging from 14.04% to 86.25% with dose response effects. In addition, the scavenging ability of MMPh+ on ABTS free radical was shown and correlated well with increasing concentration. At the dose of 0.5%, $MMPh^+$ exhibited the scavenging activity above 50%.

Conclusion: $MMPh^+$, which counter-regulates the damaging effects of not only excessive proteinase including MMP-3 and MMP-9 , but also free radicals often found in wound bed and skin, may be considered as competent active ingredient for wound management.

[EP147] NOVEL SILVER NANOPARTICLE CONTAINING CELLULOSE FIBRES AND THEIR USE IN ANTIMICROBIAL WOUND DRESSINGS

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Aim: This research aims to develop a novel method of producing cellulose fibres impregnated with silver nanoparticles (AgNP fibres), which can be used to form antimicrobial wound dressings.

Method: The AgNP fibres were produced using cellulose as a nanoreactor, forming AgNPs in situ, on all surfaces of the fibres. Blending the AgNP fibres with gel forming fibres produced absorbent wound dressings with high antimicrobial activity and excellent wet strength. Antimicrobial efficacy was established according to Thomas & McCubbin (2005). Physical/mechanical properties were determined by standard laboratory techniques. Characterisation of the AgNP fibres and dressings was performed using SEM/TEM and UV-Visible Spectrophotometry. The total silver content of the dressings was determined using elemental analysis*.

Results / Discussion: SEM/TEM analysis of the fibres confirmed the presence of AgNPs on both interior and exterior surfaces, having an average diameter of 20-40 nm. The silver content of the fibres was 20-25% w/w. When the AgNP fibres were blended with gel forming fibres, the resulting dressing showed excellent absorbency (25g/g), retention (≥50%), wet strength (5N/cm) and it provided sustained bactericidal activity over seven days. Preliminary antimicrobial efficacy studies with *S.Aureus* and *P.aeruginosa* returned a log reduction ≥4.0 over the full seven day period, with no viable bacteria recovered within 24 hours.

Conclusion: AgNPs were successfully incorporated into cellulose fibres at high concentration. Blending with gel forming fibres produced highly absorbent wound dressings with excellent wet strength. These dressings show the ability to control common wound pathogens due to their sustained bactericidal activity.

*Microwave Plasma Atomic Emission Spectrometer (MP-AES).

[EP148] CHARACTERISTIC HONEY DILUTION PROTECTS KERATINOCYTE FROM HYPOXIC ASSAULTS DURING WOUND HEALING

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Aim: The aim of the present study was to examine the role of characteristic honey dilution to overcome the hypoxic situation which arises during epidermal wound healing. The altered morphological and molecular expression of keratinocytes was correlated with bio-electrical impedance behaviour in order to explore favourable bio-ambience in hypoxic wound healing.

Method: Honey dilutions were examined for *in vitro* keratinocyte cell viability under hypoxic conditions to select optimum dilution. Further, wound healing efficacy of selective honey dilution under hypoxic condition were evaluated by scratch assay and its cyto-morphological as well as cardinal molecular features *viz.* E-cadherin and p63 were observed by immuno-staining and correlated with electrical impedance changes recorded by electrical cell-substrate impedance sensing device.

Results / Discussion: The bio-impact of honey dilutions for wound healing under hypoxic condition was comparatively better for 0.1 % compared to control and other dilutions in respect to healing rate, cardinal molecular expressions and cellular electrical impedance behaviour.

Conclusion: Honey dilution reduced oxidative stress induced by hypoxia during *in vitro* wound healing as demonstrated by cellular, molecular and electrical attributes.