[OP116] SEVERE HYPOXIA AND MALNUTRITION CAUSED BY MICROVESSEL OCCLUSION LEADING TO HUMAN HYPERTROPHIC SCAR REGRESSION

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Aim: This study aims to investigate whether hypoxia and malnutrition caused by microvessel occlusion in scar tissue play key roles to induce hypertrophic scar regression.

Method: In this study the tissue oxygen level was measured in proliferative and regressive hypertrophic scars before surgery. Resected scar tissue samples were processed to examine CD34, PCNA and HIF-1, in addition to analysis of apoptosis. Scar-derived fibroblasts were cultured *in vitro* with moderate and severe hypoxia and malnutrition to model condition of proliferative and regressive scar tissue. Cell viability, collagen production and apoptosis, as well as protein expression of HIF-1, VEGF, TGF-β_1, Bcl-2 and p53 were investigated post treatment.

Results: The results suggest that moderate hypoxia is present in proliferative scars and coincides with high PCNA and HIF-1 expression and a low percentage of apoptotic cells. In contrast, severe hypoxia was observed in regressive scars with less PCNA and HIF-1 expression and a higher number of apoptotic cells observed. The *in vitro* cell culture studies suggest that severe hypoxia and malnutrition resulted in significantly reduced cell viability and collagen production, as well as HIF-1, VEGF, TGF-β_1 and Bcl-2 protein expression when compared to control, however, P53 expression and cell apoptosis were increased.

Conclusion: This study suggests that the severe hypoxia and malnutrition caused by microvessel occlusion in scar tissue contributes to human hypertrophic scar regression.

[OP117] EFFICACY OF SILK FIBROIN AND HONEY BLENDED FILMS IN WOUND HEALING APPLICATION

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Aim: Wound healing is a process of reconstruction of wounded tissue. In mammals wound healing occurs mainly through fibrosis. So to achieve regenerative healing instead of fibrotic one we are developing wound dressing films through blending silk fibroin (8%wt/v) (SF) and honey (1% v/v) (H), as both materials are natural and demonstrate healing efficacy.

Method: SF and HSF 1% films were developed by drop casting technique. Further these membranes were physically and biologically characterized to understand the films features and its cellular bio-compatibility. For determining the effect of Silk fibroin and honey blended films on wound healing, mice were chosen as an animal model (5-6 weeks old). Full thickness wounds were created using 3mm punch biopsy on dorsal surface of mice. Then, mice were divided into 3 groups with 2 mice in each group (a) without treatment, (b) with SF film and, (c) with blended HSF 1% and dressed. Wound beds were observed at different time points by swept source optical coherence tomography (SS-OCT). Then, histo-pathological/histo-chemical findings before intervention and after intervention were correlated with SS-OCT findings.

Results / Discussion: The tensile strength of film was 15.5 ± 0.8 Mpa and elongation rate was 73.3 ± 0.6%. The films maintain its integrity and shape upon water immersion and swelling index was 4.3 ± 1.1. After 10 days of incubation in Phosphate buffer saline (PBS), films showed 7.5 ± 0.2 % of weight loss. Compared to neat silk fibroin film (SF), blended film of silk fibroin and 1% honey (1% SFH) showed better cell viability, attachment and proliferation of fibroblast cells. The SS-OCT observations when correlated with histo-pathological and histo-chemical attributes with 1% SFH demonstrated improved re-epithelialization and connective tissue matrix formation in comparison to SF and control groups.

Conclusion: The 1% SFH depicted reasonable performance in the context of regenerative healing potential.

[OP118] CORRELATED SWEPT SOURCE - OPTICAL COHERENCE TOMOGRAPHY AND HISTOPATHOLOGY OF CUTANEOUS WOUND HEALING UNDER CHARACTERIZED HONEY

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Aim: In non-invasive exploration of healing efficacy of characterized honey, full-thickness cutaneous wound in mice under topical application of honey has been studied using swept source-optical coherence tomography (SS-OCT) and histopathological evaluation.

Method: Swiss albino mice (n=8) with full thickness incisional dorsal wound beds (i.e. two wounds per mice between the 6th and 8th thoracic vertebrae symmetrically spaced 5 to 10 mm from the vertebral column formed) were imaged by SS-OCT before and after application of physico-chemically characterized honey for 16 days. Follow up wound margin biopsies were collected and processed for histo-pathological (H&E, PAS and VG staining), immuno-histochemical (Collagen III) examination and correlated with OCT image features.

Results / Discussion: The follow up regenerative tissue from the wound margin was regularly examined for healing progression through recording pigmentation, hyper- /hypo lucid layers under OCT and correlated with histo-pathological findings. Pre-wounding intact epidermis with hair follicle was clearly visible both under OCT and histo-pathological images. At fourth day after wounding under honey intervention, wound bed showed thick inflammatory cellular infiltrations and scab tissue which accordingly associated with OCT findings. At eighth day after wounding, the control region showed less re-epithelialization and connective tissue formation in comparison to honey intervention group. On eighteenth day after wounding re-epithelialization and connective tissue formation were better in comparison to honey intervention.

Conclusion: Characteristic honey provided favorable bio-ambience for better wound healing through improved re-epithelialization and connective tissue formation which were confirmed by OCT, histo-pathology and immuno-histochemical findings.

[OP119] DEVELOPMENT OF AN IN VITRO WOUND MODEL OF BACTERIAL DIABETIC FOOT ULCER INFECTION TO EVALUATE NOVEL MATERIALS FOR INFECTION MANAGEMENT

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Aim: To establish *in vitro* a model for chronic diabetic foot ulcers contaminated with a *P. aeruginosa* biofilm, and demonstrate its utility in testing antimicrobial efficacy of a novel antibiotic–containing recrystallized calcium sulfate bead.

Method: Collagen gel matrices were prepared (35 mm in diameter), containing central 'voids' measuring 23 mm in diameter and 8 mm deep, bathed in simulated wound fluid (SWF). These were inoculated with *Pseudomonas aeruginosa* PA01 and incubated for one day. We tested for characteristics indicative of biofilm formation including histology, SEM and antimicrobial susceptibility changes. We finally used the model to test the effect of calcium sulfate beads* containing tobramycin on bacterial viability at various distances from the treatment site.

Results / Discussion: Histology confirmed the production of bacterial polysaccharide matrix and the presence of microcolonies within the matrix indicated growth of a biofilm. The biofilm was visualised within the collagen matrix by SEM. The biofilm also showed decreased susceptibility to antimicrobials (measured using e-test strips). Treatment of 1-day old biofilm with unloaded beads (control) resulted in in a bacterial density of $10^7 – 10^8$ CFU/ml. Treatment with tobramycin loaded beads resulted in eradication of all viable bacteria. Similar findings were obtained with a 3-day old biofilm.

Conclusion: We have successfully developed an *in vitro* collagen model for a diabetic foot ulcer biofilm, confirmed by histology, SEM and antimicrobial susceptibility changes. We used this model to demonstrate that antibiotics released from calcium sulfate beads may have antimicrobial efficacy up to 12 mm from the insertion site.

*Stimulan, Biocomposites Ltd

1. Werthen, M., Henriksson, L., Jensen, P.O., Sternberg, C., Givskov, M., Bjarnsholt, T. (2010). An in vitro model of bacterial infections in wounds and other soft tissues. APMIS 118:156-164.

[OP120] FEATURES OF THE WOUND PROCESS AFTER LYMPHADENECTOMY

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Aim: To determine the features of wound healing process and develop an algorithm of postoperative management of our patients.

Method: We generalized the experience of treatment and postoperative management of 286 patients undergoing axillary, inguinal and ilio-inguinal dissections.

In 137 patients lymphadenectomy was performed for melanoma metastases of melanoma in the lymph nodes. In 119 patients for metastases of squamous cell skin cancer and in 30 patients - for metastases in the lymph nodes of rare skin tumors, tumors of the internal organs and metastasis without identified primary hearth. Axillary dissection was performed in 155 cases, inguinal dissection - in 126 cases, and ilio-inguinal dissection - in 5 cases.

Results / Discussion: The longest terms of lymphorrhea were marked after inguinal and ilio-inguinal dissections. It ranged from 7 to 93 days (median 21 days). The terms of lymphorrhea after axillary dissection ranged from 5 to 34 days (median 12 days). The incidence of complications in patients undergoing inguinal and ilio-inguinal lymphadenectomy was 39.7% (52 patients) and after axillary dissection - 19.4% (30 patients).

Conclusion: 1. The extremely high percentage of complications indicates the need of further investigation and introduction of new treatment approaches into our clinical practice as well as the need of postoperative management of patients undergoing lymphadenectomy. 2. We created the proprietary algorithm of postoperative management of patients after lymphadenectomy