[OP001] NERVE GROWTH FACTOR AND S100A8/A9 IN EXUDATES FROM VENOUS LEG ULCERS ARE ASSOCIATED WITH WOUND PAIN STATUS

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Aim: To clarify the associations between biomolecules from venous leg ulcer (VLU) exudates and self-evaluated pain status to establish an objective evaluation method.

Method: Patients with VLUs participated in this cross-sectional observational study performed between April and October 2014 at two medical facilities. During participants' routine wound care, VLU exudate samples were collected after wound cleansing, and each patient self evaluated their pain status at each sample collection by using a 10-point numerical rating scale (present pain intensity) and short-form McGill Pain Questionnaire 2 (continuous pain, intermittent pain, neuropathic pain, affective descriptors, and total score). The concentrations of nerve growth factor (NGF) and S100A8/A9 in VLU exudates were measured using enzyme-linked immunosorbent assay kits. The association between the two protein levels standardized by wound areas and each pain status was evaluated using Spearman's correlation coefficient. The study protocol was approved by the ethical review board, and written informed consent was obtained from all participants.

Results / Discussion: Thirty samples collected from 13 participants were used for the analysis. NGF levels negatively correlated with continuous pain (ρ = -0.47, P = 0.01), intermittent pain (ρ = -0.48, P = 0.01), neuropathic pain (ρ = -0.51, P = 0.01), and total score (ρ = -0.46, P = 0.01). S100A8/A9 concentrations positively correlated with present pain intensity (ρ = 0.46, P = 0.03) and continuous pain (ρ = 0.48, P = 0.03).

Conclusion: NGF and S100A8/A9 levels in exudates from VLUs are associated with wound pain status. Thus, these two proteins may be useful for objective pain evaluations.

[OP002] ORAL SUPPLEMENTATION OF CHLORELLA REDUCED WOUND HEALING TIME IN MICE

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Aim: Patients nutritional status clearly influences wound occurrence and issue. The promise of healing time reduction with oral nutritional supplementation may be of interest in wound care. Preliminary studies have conferred to *Chlorella*, a green microalga rich in nutrients, potential benefits in the context of wound healing and burns when applied topically. The aim of the study was to investigate the effect of oral supplementation of green *Chlorella*[1] on wound healing in mice.

Method: At D0, 16 Skh-1 hairless mice were incised on both flanks (1 cm incision/mouse flank) and randomly assigned to 4 groups (n=4/group). Groups were orally supplemented (gastric intubation) with 125, 250 and 500mg/kg body weight of *Chlorella* or water (control), respectively, until complete healing. Wound healing was daily evaluated using a macroscopic score (wound length, width, bloating and visibility). In addition the duration for a complete lesion repair was measured.

Results / Discussion: At D0, no significant differences were observed between groups. From day 3, all *Chlorella* supplemented groups displayed a significant improvement of mean macroscopic healing scores compared to the control group. The strongest impacts were observed in the 2 higher dosage groups. Moreover *Chlorella*'s supplemented groups displayed a significant reduction of total wound healing duration, up to a 2.8 days reduction in the 250 mg/kg group ($p<0.001$).

Conclusion: Oral supplementation with *Chlorella* may reduce wound healing duration in mice. *Chlorella* may be of potential medical use in the treatment of wound healing.

[1] *algility*™ chlorella, ROQUETTE (Lestrem - France)

[OP003] THE STEM CELL POTENTIAL AND MULTIPOTENCY OF HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS VARY BY CELL DONOR AND ARE DIFFERENT FROM THOSE OF OTHER TYPES OF STEM CELL

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Aim: Human adipose tissue-derived mesenchymal stem cells (AT-MSCs) from various sites are applied in tissue engineering and cell therapy. The condition of AT-MSCs depends on the donor's age, body mass index (BMI), and gender.

Method: AT-MSCs from 66 human donors were analyzed, and the cells were sorted according to donor age (10–19 years: n=1, 20–29 years: n=5, 30–39 years: n=12, 40–49 years: n=22, 50–59 years: n=12, 60–69 years: n=9, and over 70 years: n=5); BMI (under 25 kg/m2, 25–30, and over 30); and gender (19 males and 48 females). Additionally, AT-MSCs were compared to bone marrow MSCs (BM-MSCs) and chorionic tissue-derived MSCs (CT-MSCs). We measured MSCs yield, growth rate, colony-forming units (CFUs), multipotency, and surface antigens.

Results / Discussion: AT-MSCs proliferation was greater in cells isolated from individuals less than 30 years of age compared to proliferation of AT-MSCs from those over 50 years old. BMI was correlated with osteogenic differentiation potency. Adipogenic differentiation was more strongly induced in cells isolated from donors less than 30 years of age compared to other age groups. Also, a BMI above 30 was associated with enhanced adipogenic differentiation compared to cells isolated from individuals with a BMI below 25. BM-MSCs were strongly induced to differentiate along both osteogenic and adipogenic, whereas AT-MSCs predominantly differentiated into the chondrogenic lineage.

Conclusion: The type of regeneration required and variations among potential donors must be carefully considered when selecting MSCs for use in applied tissue engineering or cell therapy.

[OP004] THE SECRETORY FACTORS OF HUMAN CHORION-DERIVED STEM CELLS ENHANCE ACTIVATION OF HUMAN FIBROBLASTS

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Aim: Wound healing remains a principal challenge in modern medical science. Chorion-dervied stem cells (CDSCs), isolated from human placenta, have largely been overlooked, and few studies on their potential in wound healing have been conducted. In this study, we investigated the functional characteristics of CDSCs compared with adipose-derived stem cells (ASCs) on human fibroblasts (HFs).

Method: We analyzed CDSCs by flow cytometry to confirm their mesenchymal stem cell (MSC) characteristics. We then evaluated the paracrine effects of CDSCs on human fibroblasts (HFs) in a co-culture system, and focused on fibroblast proliferation, migration and collagen synthesis. To explore the potential of CDSCs in wound healing, CDSCs and ASCs secreted factors was compared by using a cytokine antibody array.

Results / Discussion: CDSCs exhibited morphology similar to MSCs and expressed an MSC phenotype. HF proliferation and migration increased more than fivefold when co-cultured with CDSCs. Furthermore, Western blot and reverse transcription polymerase chain reaction (RT-PCR) analysis showed that expression of collagen type I and III in fibroblasts was up-regulated twofold when co-cultured with CDSCs. Cytokine array results of CDSC-conditioned medium (CM) and ASC-CM revealed the presence of growth factors known to influence wound healing, including IL-6, IL-8, MCP-1 and RANTES.

Conclusion: Our data demonstrated that CDSCs are functionally similar to ASCs, promote HF activation, and secrete growth factors that influence wound healing. Therefore, we suggest that CDSCs are potentially applicable in wound healing.

[OP005] EVALUATING THE EFFECT OF BIOPHOTONIC THERAPY ON PHYSICAL AND HISTOLOGIC PARAMETERS IN AN INCISIONAL RAT MODEL

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Aim and Introduction: The role of BioPhotonics has been evaluated in various skin models, but there is a true paucity of data evaluating the cohesive interaction between light, chromophores and cellular pathways.

Purpose of study was to evaluate the impact of BioPhotonic therapy on physical and histologic parameters using a rat incisional skin model.

Method: Forty-two male Wistar rats (250 g) were randomized into 4 groups: Saline (control); External Biophotonic therapy short-interval treatments (day 0,2,5,7); External BioPhotonic therapy long-interval treatments (Day 0,7,14,21); and internal BioPhotonic therapy long-interval treatments (Day 0,7,14,21). A single 6cm incision was created on the dorsal midline and consequently closed as per protocol. Treatment was applied topically immediately following closure for the externally treated rats and internally prior to closure for the internally treated group. Rats were assessed on a daily basis and sacrificed at days 14 and 28. Tensile strength was evaluated with a tensiometer and biopsy specimens were taken, to evaluate the effect of treatment on histologic parameters.

Results / Discussion: Use of the Klox BioPhotonic treatment system was able to modulate tensile strength and histology based on the site of application (internally vs. externally), as well as with frequency of application. More specifically, tensile strength was modulated without any effect on clinical parameters, and proliferation of macrophages can be quantitatively increased without any detriment to tensile strength.

Conclusion: The results of the present study supports the role of biophotonics in modulating the wound healing cascade to suit the end goal of therapy, either improved wound healing vs improved cutaneous scarring. The roles of TGF-β and various factors, as well as future directions will be presented.

[OP006] EFFECT OF BONE MARROW MESENCHYMAL STEM CELLS (MSCS) ON DIABETIC WOUND HEALING OF DIFFERENT MICE MODELS

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Object: To observe the promoting concrescence of diabetic wound effect of MSCs using two kinds of experimental diabetic animal models, genetically induced spontaneous diabetes models (BKS-DB mice) and experimentally induced non-spontaneous diabetes models (STZ –induced diabetic C57BL/6 mice).

Method: To detect cell surface antigens of culture expanded MSCs with flow cytometry and multipotential ability with differentiation experiment; To establish one of the diabetes mouse models with STZ intraperitoneal injection and to chose the C57BLKS/Nju spontaneous mutation diabetic mouse (BKS-DB mice) as the other; To establish two 6-mm full-thickness excision skin wounds on back of both diabetic models; To set four groups: STZ-PBS group vs STZ-MSCs group; BKS-PBS group vs BKS-MSCs group. To observe wound healing when MSCs cells were injected intradermally around the wound at four injection sites.

Results: Full-thickness excisional wound model was successfully established on STZ –induced diabetic C57BL/6 mice and BKS-DB mice; significantly promoted wound closure was found in both kinds of diabetic mice that with intradermal injection of MSCs cells.

Conclusion: MSCs enhanced wound healing of different diabetic model, but with different efficiency, which indicated that different Wound microenvironments of the two model exist.

[OP007] THE THERAPEUTIC EFFECTS OF BONE MARROW-DERIVED CELLS ON THE SURVIVAL AND WOUND HEALING IN MICE WITH RADIATION COMBINED BURN INJURY

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Aim: Combined radiation and burn injury (CRBI) is a pathophysiological condition due to ionizing irradiation combined with burn insults that complicate systemic responses and exacerbate the acute radiation syndrome. Ionizing irradiation can inhibit this effect by damaging bone marrow—a major source of hematopietic stem cells and mesenchymal stromal cells. In this study, we aim to investigate whether transplantation of bone marrow–derived cells is effective in the management of CRBI.

Methods: We established a working model for CRBI, in which mice received a single dose of 6Gy whole body radiation and followed by 2.5% III degree total-body surface skin burns. The synergistic effects of total body irradiation and burns on the mortality, bone marrow suppression and wound healing impairment was investigated. The engraftment of bone marrow-derived cells for hematopoiesis and wound healing was also studied using GFP-tagged cells.

Results: Our results showed that CRBI significantly aggravated mortality, hematopoiesis and delayed wound healing, while transplantation of bone marrow-derived cells significantly improved the survival and hematopoiesis in mice with CRBI. Transplantation of bone marrow-derived cells also accelerated the skin wound healing via promotion of angiogenesis and repairing cells, and improved healing quality via decreasing scar formation and increasing collagen deposition. Enhanced engraftment of GFP-positive bone marrow-derived cells was observed in the cutaneous wounded area in CRBI mice.

Conclusions: These results showed that CRBI led to a serious damage to hematopoiesis and wound healing, while bone marrow-derived cells provide new perspectives for management of the traumatic tissue injury combined with ionizing irradiation.