Abstracts

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Listings are in alphabetical order according to the last name of the presenting author.

001

COMP - MODIFIER OF DERMAL ECM SUPRASTRUCTURE IN FIBROTIC REACTIONS?

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Cartilage oligomerix matrix protein (COMP) is an extracellular glycoprotein epxressed primarily in cartilage. Recently, microarray analysis demonstrated high COMP transcript levels in fibrotic skin lesions from patients with scleroderma, while no COMP was reported in healthy donor skin. We hypothesize that COMP would modify the supramolecular organization of skin ECM by virtue of its binding capability to collagen I and that this altered ECM would contribute to fibroblast activation and ECM production.
Enhanced COMP expression was confirmed in systemic as well as localized scleroderma skin lesions with varying intensity levels and distribution. Surprisingly, COMP was also found deposited in healthy skin in a linear pattern along the upper papillary dermis. Highly elevated COMP levels were further detected in the granulation tissue of non-healing ulcers and in the stroma surrounding basal cell carcinoma islets, but never in tumor areas. These observations suggest that enhanced COMP deposition could correlate with fibrotic skin reactions in general. Deposition close to epidermal keratinocytes might point to factors released from keratinocytes that stimulate COMP production in fibroblasts.
In vitro binding studies demonstrated that next to collagen I, COMP also binds to collagen XII, which decorates the surface of collagen I fibrils. Interestingly, collagen XII is found deposited in the same sub-epidermal region as COMP, pointing to the possibility of interacting also in vivo. By binding to collagen I and FACIT collagens, COMP might alter the density and packaging of the collagen network in skin.

002

GROWTH FACTOR PROFILE OF PLATELET-RICH FIBRIN

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Autologous platelet-rich fibrin (PRF®) is a platelet concentrate in a biomatrix of autologous fibrin prepared by an automated system (Vivostat A/S, Allerød, Denmark) used to promote soft and hard tissue repair. Although PRF stimulates proliferation of human fibroblasts and their collagen biosynthetic ability the factors responsible for these effects are unknown. The aim of this study was to quantify the growth factors fibroblast growth factor-2 (FGF-2), platelet-derived growth factor-AB (PDGF-AB), PDGF-BB, transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF), and the matrix metalloproteinase-9 (MMP-9) in PRF compared with baseline. Ten healthy volunteers, 5 females and 5 males, aged 31 to 55 years (39 ± 10 years, mean ± SD) donated blood for generation of PRF and serum. PRF was homogenized in T-PER® reagent using Ultra-Turrax®. Complete recovery of added growth factors could be demonstrated using this method. Growth factors and MMP-9 in the PRF extracts and sera were measured by enzyme-linked immunosorbent assays and gelatin zymography. Results were normalized to the total soluble protein content determined by the Bradford method. The order of abundance of growth factors in PRF was: TGF-β1 ($12{,}200 \pm 5{,}500$ pg/mg) > PDGF-AB > PDGF-BB > VEGF > FGF-2. The largest increase compared with baseline was found for FGF-2 (300-fold) followed by TGF-β1 (20-fold), PDGF-AB (10-fold), PDGF-BB (6-fold) and VEGF (5-fold). In contrast, MMP-9 was lowered ($p < 0.001$) more than 10-fold compared with serum, reflecting leukocyte depletion in PRF. This is the first study that successfully has quantified important growth factors in platelet-rich fibrin.

003

EFFECT OF CYCLIC MECHANICAL STIMULATION ON ENGINEREED TENDON SUBSTITUTES

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Mechanical stress plays a significant role in modulating cell behaviour and has driven the development of mechanical bioreactors for tissue engineering applications. Our aim was to investigate the effect of cyclic mechanical stimulation on human tenocytes seeded onto hyaluronic acid based scaffold. Human tenocytes were isolated from tendon samples obtained from hand lesions. Tenocytes were expanded in vitro and then seeded onto hyaluronic acid based scaffold. Tissue constructs were placed into a bioreactor, devised by the authors, able to reproduce a physiological mechanical traction. Controls were left untensioned. Histological, immunohistochemical, biomolecular, transmission and miscoscopic electron analyses were used to evaluate the results after 7, 14, and 21 days. Tenocytes adhered and proliferated within the biomaterial and produced main components of the extracellular matrix. Microscopically, cyclically tensioned samples showed parallel orientation of collagen fibers and spindle-shaped cell nuclei mimicking the morphology of normal tendons. Mechanostimulation resulted in significantly stronger and stiffer constructs compared to untensioned samples. Higher expression of matrix proteins such as collagen I and adhesion proteins such as integrin 1 and scleraxis (tendon specific markers) were found in cyclically tensioned samples. Human tenocytes were able to develop a structure similar to normal tendon if mechanical stress were applied in vitro. Mechanical traction was essential to maintain cellular phenotype, and enhanced cell proliferation and tenocytes longitudinal alignment. Duration, frequencies, and amplitude of loading directly influence cell behaviour in vitro. Understanding the physiological window for these parameters represents future challenges of research in the field of tendon tissue engineering.

085

RASCH-ANALYSIS OF THE PATIENT AND OBSERVER SCAR ASSESSMENT SCALE (POSAS) IN BURN SCARS

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The Patient and Observer Scar Assessment Scale (POSAS) is a questionnaire that was developed to assess scar quality. Although previous studies support the clinimetrical properties of the POSAS using the traditional methods, the scale has never been subjected to a more modern and stringent approach in the form of a Rasch analysis.
To examine the clinimetrical properties of the POSAS on burn scars setting using Rasch analysis.
A dataset of 1629 observer scores and 1427 patient scores of burn scars was used for this analysis. We performed standard Rasch analysis such as fit to the model and dimensionality of the POSAS.
The obtained item map showed some overlap of item difficulties. All items fitted the Rasch model except for *surface area* in the O-SAS and *pain* and *pruritus* in the P-SAS. Person-and item reliability of the O-SAS were 0.82 and 1.00 respectively and of the P-SAS were 0.77 and 1.00 respectively. The categories of the O-SAS are proper ordered but the categories of the P-SAS are disordered. Both the O-SAS and the P-SAS measure 1.7 items.
The POSAS performs well in the Rasch model and is unidimensional which means that the included items measure a single variable, in this case scar quality.

086

DISTANCE MAPPING AND FOURIER ANALYSIS: COLLAGEN BUNDLE MORPHOMETRY IN SKIN AND SCAR TISSUE

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Histopathological evaluations of fibrotic processes require characterization of collagen morphology in terms of features such as bundle orientation, thickness and spacing. There are currently no reliable and valid techniques for measuring bundle thickness and spacing. Hence two objective methods quantifying these aspects were tested for reliability and validity: the Fourier first-order maximum analysis and Distance Mapping.
Histological slides were constructed and imaged from 50 scar and 50 healthy skin biopsies with an analysis by two observers allowing the inter-observer reliability to be determined via the Intraclass Correlation Coefficient (ICC). To assess validity, the image properties of 120 computer generated images (CGIs) with fixed bundle thicknesses and spacings were compared with the outcomes of the Fourier first-order maximum and Distance Mapping.
The inter-observer reliability for the Fourier first-order maximum and Distance Mapping, calculated by the ICC were above 0.72 and 0.89, respectively. The correlation between Fourier analysis and the CGIs was 0.87, with an ICC of 0.83. The correlation between the Bundle Thickness or Bundle Spacing of Distance Mapping versus the CGIs was 0.94 or 0.91 with an ICC of 0.52 or 0.58, respectively.
The reliability of both measurement methods was good. Only one observer was necessary for a reliable measurement. The validity of the Fourier analysis was good. For Distance Mapping, in spite of a high correlation with the CGIs, an intermediate agreement was found. In the future, both methods can be used for morphometry of skin and scar tissue, but also for the quantitative evaluation of other fibrotic processes.

087

A RANDOMIZED CONTROLLED TRIAL USING A SKIN STRETCHING DEVICE IN BURN SCAR RECONSTRUCTION

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Burn survivors are frequently faced with disfiguring scars and impaired function, which necessitates reconstructive surgery, such as scar excision. In this multi-centre RCT we investigated if a larger burn scar can be excised using a skin stretcher for wound closure compared to excision of a scar and closure without stretching the skin (serial excision). This could allow the surgeon to excise a scar in a one-step procedure instead of two or more steps for serial excision.
Two methods for excision of burn scars were compared: closure by skin stretching (group 1) and serial excision (group 2). Surface area of the scar and wound, postoperative pain scores (VAT-scores) and complications were registered.
Up to now, 23 patients were recruited and analyzed: 13 patients were randomized for skin stretching and 10 for serial excision. The mean surface area of the wounds closed by skin stretching (155 cm^2, range 41–280 cm^2) was significantly larger than those closed by serial excision (91 cm^2, range 23–149 cm^2). The VAT-scores did not differ significantly between the groups. One patient in group 1 and one patient in group 2 experienced partial wound dehiscence.
In burn scar reconstructions we demonstrated that significantly larger wounds can be closed using a skin stretcher compared to serial excision, without an increased risk on wound dehiscence. No significant differences between the treatment groups were found for VAT-scores and complication rates. In the future skin stretching may play a role in large burn scar excisions, but possibly also in closure of other types of wounds.

088

BONE MARROW-DERIVED CELLS IN SKIN WOUND HEALING

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Wounded skin recruits progenitor cells, which repair the tissue defect. These cells are derived from stem cells in several niches in the skin. In addition, bone marrow-derived cells are recruited and contribute to wound repair. We hypothesized that larger wounds recruit more cells from the bone marrow. Wild type rats were lethally irradiated and transplanted with bone marrow cells from GFP-transgenic rats. Seven weeks later, 4, 10, and 20-mm wounds were created. The wound tissue was harvested after 14 days. The number of GFP-positive cells in the wounds and the adjacent tissues was determined. Bone marrow-derived myofibroblasts, activated fibroblasts, and macrophages were also quantified. The recruitment of bone marrow-derived cells ($23 \pm 11\%$) was found to be independent of wound size. Similar numbers of GFP-positive cells were also detected in non-wounded adjacent tissue ($29 \pm 11\%$). Therefore we conclude that bone marrow-derived cells are not preferentially recruited to skin wounds. Furthermore, wound size plays no pivotal role in the recruitment of bone marrow-derived cells.