Abstracts

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INDISCRIMINATE INHIBITION OF MATRIX METALLOPROTEINASES AND TUMOR NECROSIS-ALPHA CONVERTING ENZYME IMPAIRS ANASTOMOTIC HEALING IN EXPERIMENTALLY OBSTRUCTED COLON VIA DELAYED EPITHELIALIZATION

M. S. Ågren[1], M. Rehn[2], P.-M. Krarup[3], L. H. Christensen[4], J. B. Seidelin[5], I. Syk[2]
[1]Department of Surgery K and Copenhagen Wound Healing Center, Bispebjerg Hospital, Copenhagen, Denmark;
[2]Department of Surgery, Skåne University Hospital, Malmö, Sweden;
[3]Department of Surgery K, Bispebjerg Hospital, Copenhagen, Denmark;
[4]Department of Pathology, Bispebjerg Hospital, Copenhagen, Denmark;
[5]Department of Gastroenterology, Herlev Hospital, Herlev, Denmark

Emergency operations on obstructed colon are accompanied by increased risk of anastomotic insufficiency. Tissue-destructive matrix metalloproteinase (MMP) activity is elevated in obstructed colon and contributes to loss of suture-holding submucosal collagen. Our aim was to study the effect of the nonselective MMP and tumor necrosis-α (TNF-α) converting enzyme (TACE) inhibitor GM6001 (30 mg/kg) on anastomosis repair in an experimentally obstructed left colon. Partial obstruction of distal colon in male Sprague-Dawley rats was induced for four days, the obstructed colon segment resected day 0 and an end-to-end anastomosis constructed. Three days later the anastomoses were evaluated biomechanically and on day 7 for anastomotic leakage using predefined clinical criteria as well as by histopathology and immunohistochemical detection of α-smooth muscle actin and Ki-67. As opposed to the robust beneficial effect on anastomosis under uncomplicated conditions, breaking strengths were comparable in GM6001-treated rats (2.18 (1.50–2.48) N) and controls (2.10 (2.05–2.15) N) on day 3. On postoperative day 7, 7 of the 10 rats treated with GM6001 compared with 1 of 8 control rats developed intra-abdominal abscesses ($p = 0.006$). Impaired anastomotic healing with GM6001 treatment was also indicated histologically by the wider and minimally epithelialized wounds in the GM6001 group that were commonly covered with necroses on the luminal side and massively infiltrated with granulocytes. The abundance of myofibroblasts and proliferating cells was similar in the two groups. In vitro, GM6001 significantly ($p < 0.02$) delayed the repopulation of denuded monolayer of intestinal epithelial cells grown on type I collagen. Contrary to our expectations, nonselective MMP inhibition increased anastomotic complications following colon obstruction. This was possibly due to inhibition of TACE which is responsible for the release of TNF-α and epithelial mitogens resulting in delayed epithelialization.

STRIAE DISTENSAE (SD) OTHERWISE KNOWN AS STRETCH MARKS SIGNIFICANTLY IMPACT PATIENTS' QUALITY OF LIFE AND NECESSITATE DEVELOPMENT OF SPECIFIC PATIENT-REPORTED OUTCOME MEASURES

S. Al-himdani[1], J. Wilburn[2], S. Ud-din[1], S. McKenna[2], A. Bayat[1]
[1]Plastic and Reconstructive Surgery, University Hospital of South Manchester, Manchester, United Kingdom;
[2]Galen Research Ltd, Manchester, United Kingdom

Striae distensae (SD) or cutaneous stretch marks, an extremely common form of dermal scarring, pose a significant psychological burden on patients' lives. Literature regarding the impact of SD on patients' quality of life (QoL) is scarce. We evaluated the effect of SD on quality of life through interviews in order to assist in the development of a patient-reported outcome measure (PROM). Recruitment of participants was undertaken at a university teaching hospital. Interviews were carried out, recorded, transcribed and analyzed in order to evaluate statements pertaining to the following categories: symptoms, activity limitations and QOL. A needs-based model was used to generate items initially. A total of 30 patients, 29 females and 1 male, participated in the interviews (aged 19–54, mean 33 years). On average 3 anatomical locations were affected of which the most common were the abdomen ($n = 18$) and hips ($n = 18$) followed by the thighs ($n = 15$), breasts ($n = 13$) and buttocks ($n = 6$). Half of patients rated their SD as moderately severe ($n = 15$). SD occurred on average at 18 years of age and were attributed to pregnancy (43%), weight gain (27%) and adolescent growth (23%). Identification, categorization and subcategorization of statements from interviews identified fundamental areas of impact. 670 statements were identified from interviews. Statements were categorized into: impairments (itching), activity limitations (swimming and holidays) and QoL (preoccupation, reduced socialization, relationships, self consciousness, restriction in choice of clothing, confidence and lack of control). This research identifies areas of substantial impact that have not been previously reported. QoL is significantly affected in these patients. As well as for the development of a SD-related PROM, outcomes from this study may allow for earlier therapeutic intervention targeting those patients with significant disturbances in their QoL.

DEVELOPMENT OF A PRECLINICAL MODEL OF THIRD-DEGREE BURN

M. Alexaline[1], M. Trouillas[2], M. Nivet[2], C. Thépenier[2], T. Leclerc[3], E. Bey[4], P. Saint-Blancard[5], J.-J. Lataillade[2]
[1]Research and Cell Therapy, CTSA / Celogos, Clamart / Paris, France;
[2]Research and Cell Therapy, CTSA (French Military Blood Transfusion Center), Clamart, France;
[3]CTB (Burn Treatment Unit), Percy Hospital, Clamart, France;
[4]Plastic Surgery Department, Percy Hospital, Clamart, France;
[5]Anatomopathology Department, Percy Hospital, Clamart, France

The skin, with its extensive ability of regeneration and healing serves as a protective barrier at the interface between the human body and the surrounding environment. The extensive damage caused by massive burns still constitutes a major surgical challenge for wound coverage and healing. For third degree burns over 70% of total body area, dermal allografts followed by cultured epidermal autograft (CEA) represents the actual lifesaving yet much perfectible gold standard therapy. But today this treatment remains unsatisfactory because the dermis is not taken into account and the quality of basement membrane is not satisfactory (Cuono et al., 1987; Neveux et al., 1995).
Our aim is to develop a murine preclinical model of burn injury reproducing the actual gold standard therapy for massive burn patient before CEA application. This model will allow us to evaluate several epidermal substitutes under development. We compared an epidermal substitute cultured on fibrin matrix (clotted human plasma) to a commercial CEA (Epicel®).
A third degree burn was applied to immunodepressed rats. After several days, burn was excised and replaced by human cryopreserved skin xenograft. Once engraftment was achieved, epidermal was abraded and several skin substitutes were tested. Healing process was next evaluated by planimetry, histology, and immunohistochemistry.
We have achieved the development of an animal model which can now be used as a powerful tool to improve skin substitute quality.

OPPOSING GROWTH FACTOR RELEASE TO CREATE DISTINCT STABLE TISSUE PATTERNING BETWEEN BONE AND CARTILAGE

E. Lippens, P. Arany, G. Huang, M. Darnell, D. J. Mooney
SEAS / Mooney Lab: Labarotory for Cell and Tissue Engineering, Harvard University, Cambridge, MA, United States

While significant progress has been made in integrating stem cell biology with scaffold-based techniques for the production of bioengineered single-tissue constructs, a remaining challenge is the recreation of stable tissue boundaries between juxtaposed tissues present in the human body like for example cartilage and bone in articulating joints.
Our hypothesis was that we can recreate spatially segregated bone and cartilage tissue formation by spatially and temporally controlled co-delivery of stimulatory and inhibitory factors from a multilayered PLG (poly(D,L-lactide-co-glycolide) scaffold. The stimulatory agents used in this study were BMP4 for bone induction and TGFbeta for cartilage induction, the inhibitory factors were respectively BMP and TGFbeta neutralizing antibodies.
As a second hypothesis we investigate the role of VEGF on the chondrocyte phenotype . We used our same scaffold system for the neutralization of VEGF in the cartilage layer to prevent chondrocyte hypertrophy and as such prevent endochondral ossification of the cartilage.
Multilayer scaffold designs were optimized by mathematical modeling, and the generation of spatially segregated morphogen gradients was validated in vitro and in vivo. Scaffolds seeded with mesenchymal stem cells demonstrated the production of juxtaposed cartilage and bone tissue, as evaluated by biochemical staining, immunohistochemistry for tissue-specific matrix proteins and μCT. The effects of VEFG and neutralizing VEGF on the chondrocyte phenotype both in cell culture and on the in vivo chick chorioallantoic membrane assay were evaluated with the same techniques.
Our data indicates that this systems allows segregated tissue formation with clear distinctions between bone and cartilage tissue in the scaffold.

CHANGES IN MOLECULAR AND MORPHOLOGICAL CHARACTERISTICS ASSOCIATED WITH WOUND CONTRACTION AND CLOSURE IN DIABETIC MICE – A SYSTEMS BIOLOGY APPROACH

P. Liu[1], X. T. Wang[1], C. McKeever[1], J. Tang[1], L. Bowden[2], H. Byrne[2], P. Maini[2]
[1]Plastic Surgery, Rhode Island Hospital/Brown Medical School, Providence, RI, USA;
[2]Mathematics, Oxford University, Oxford, United Kingdom

We are employing a systems biology approach to develop a predictive in silico model of diabetic wound healing. This implies that examining one pathway or component of the wound healing process, as is commonly done in the reductionist, Cartesian method of scientific enquiry most of us were raised in, has not be that useful in developing effective therapeutics for problem wounds. Accurate mathematical descriptions of healing, on the other hand, integrate multiple components or inputs important in the wound healing cascade. Running a computer simulation of a healing wound with parameters including blood supply, tissue oxygenation, bacterial colonization, diabetic severity, macrophage density, etc., may enable potential therapies to be screened without the need for hugely expensive clinical trials. As a first step in developing what will be a multiple partial differential equation model of wound healing, we attempt to quantify the processes involved in diabetic wound healing using an animal model. We can then perturb the process by varying critical components of the process (e.g. introduce more oxygen via hyperbaric O_2 therapy) and calibrate the model according to the effects on the rate of wound healing.
In 75 diabetic (Db/Db) mice, we quantified the contributions of epithelialization and contraction in wound closure. Normoglycemic littermates served as controls. 8 mm paired wounds over scapulae were created under aseptic conditions. Occlusive dressings were secured with cyanoacrylate. Animals were sacrificed daily and wounds sectioned/sampled. Measurements of epithelial gaps and dermal gaps using Image J software (NIH, Bethesda, MD) served to quantify unhealed epidermal wounds and the amount of wound contraction that had occured. Molecular analysis of potential key modulators of wound closure were compared between the diabetic and control animals. We will show how this data may be useful in developing an accurate, predictive model of diabetic wound healing.

SKIN PROGENITOR CELLS CONTRIBUTE TO SKIN FIBROSIS

S. Liu[1], Y. Herault[2], G. Pavlovic[2], A. Leask[1]
[1]Dentistry, University of Western Ontario, London, Ontario, Canada;
[2]Institut Clinique de la Souris – ICS, Mouse Clinical Institute – MCI, Illkirch Cedex, France

The origin of the cells that contribute to skin fibrosis is unclear. Herein, we assess the contribution of sox2-expressing skin progenitor cells to bleomycin-induced skin fibrosis. In response to bleomycin, sox2-positive/a-smooth muscle actin-positive cells are recruited to fibrotic tissue. Conditional CCN2 knockout mice in which CCN2 is deleted in sox2-expressing cells exhibit resistance to bleomycin-induced skin fibrosis. Collectively, these results indicate that CCN2 is required for the recruitment of progenitor cells and that CCN2-expressing progenitor cells are essential for bleomycin-induced skin fibrosis. Lineage tracing using mice in which a tamoxifen-dependent cre recombinase is expressed under the control of the sox2 promoter confirms that progenitor cells are recruited to the fibrotic lesion in response to bleomycin, but not in CCN2-knockout mice. These data indicate that sox2-positive skin progenitor cells are required for bleomycin-induced skin fibrosis and that CCN2 is required for their recruitment. Targeting stem cell recruitment of CCN2 may therefore represent useful targets in combating fibrotic skin disease.

SCAFFOLD MEDIATED pH CHANGE IN THE ENGINEERED MICROENVIRONMENT IMPACTS THE MESENCHYMAL STEM CELLS MEDIATED OSTEOGENESIS

D. Logeart[1], L.-E. Monfoulet[1], P. Becquart[1], K. Vandamme[2], M. Bourguignon[1], D. Marchat[3], H. Petite[1]
[1]B2OA – CNRS / Paris Diderot University, Paris, France;
[2]KU Leuven & University Hospitals Leuven, Leuven, Belgium;
[3]Center for Biomedical and Healthcare Engineering, Saint-Etienne, France

Although the application of bioactive glasses (BG) as bone substitutes is expanding, information regarding the osteogenic potential of human bone marrow derived stromal cells (hBMSC) in combination with BG scaffolds is lacking in the tissue engineering (TE) field. We provided evidence that bone constructs prepared from granules of 45S5BG combined with hBMSC failed to form de novo bone tissue in contrast to those prepared with either hydroxyapatite / tricalcium phosphates (HA/TCP) or coral ceramics that displayed great and consistent ectopic bone. Such result was then correlated with various aspects of the effects of the scaffold materials tested on hBMSC functions pertinent to bone tissue formation. Particular attention was given to the pH within the constructs and its effect on hBMSC function; indeed, pH value measured in BG constructs after explantation was around 8.0, i.e. 0.4 to 0.5 unit more alkaline than in the HA/TCP- or coral- constructs. Results emphasized that the in vitro hBMSC osteogenic differentiation was not significantly affected at moderate external alkaline pH (≤ 7.90) but was dramatically inhibited at higher pH. Altogether, these findings provided evidence that 45S5 BG are not necessary proper scaffolds for TE, most likely due to the alkalinization of the 45S5 microenvironment that affects adversely the osteogenic differentiation of precursor cells. Controlling the shifts of pH in the local engineered extracellular environment is a critical issue for the development of bioactive TE scaffolds.

CHARACTERIZATION OF POLY (L-LYSINE) DENDRIGRAFTS AS BIOSYNTHETIC SUBSTRATES FOR TISSUE ENGINEERING

C. Lorion[1], C. Faye[2], B. Maret[3], M. Ramonda[4], T. Trimaille[1], T. Régnier[2], J. Sohier[1], P. Sommer[1], R. Debret[1]
[1]IBCP – LBTI UMR5305, CNRS, Lyon, France;
[2]COLCOM, Clapiers, France;
[3]IBMM – CNRS UMR5247, University of Montpellier / COLCOM, Montpellier, France;
[4]IBMM – CNRS UMR5247, University of Montpellier, Montpellier, France

Poly (L-lysine) dendrigrafts (DGL) are arborescent biosynthetic polymers of regular and controlled structures. Indeed, DGL synthesis follows a multi-generational scheme (DGL-G1 . . . DGL-Gn). The synthesis being carried out in water as unique solvent ("green chemistry"), DGL are environmentally friendly. They have specific properties such as biocompatibility, bactericidal, fungicidal and non-immunogenicity. Furthermore, DGL high surface density of NH2 functions can be easily modified and therefore appear as a powerful tool for the functionalization of hydrophobic polymers in the context of tissue engineering. However, DGL have never been evaluated as substrate for cell culture. Accordingly, the aim of this study was to investigate cell behavior when cultured on various DGL-coated surfaces.
Firstly, surface morphology of DGL was observed by atomic force microscopy (AFM). Then, human dermal fibroblasts interactions with DGL was studied by cell proliferation assay (Upti Blue) and adhesion test according to Percoll method. Fibroblasts phenotype was observed by immunofluorescence while variation of protein expression was evaluated by Western blot and zymography. Finally, fibroblasts behavior on functionalized poly-(lactic-co-glycolic acid) (PLGA) matrices was studied.
Our results showed that adhesion of fibroblasts was increased by 20% on DGL as compared to linear poly (L-Lysine) (PLL). In addition, protein expression of integrin α5 subunit was increased after 48 h of culture on DGL, in comparison to plastic or collagen I and PLL-coated surfaces. Furthermore, the presence of DGL did not lead to overexpression or activation of matrix metalloproteinases 2 and 9. Finally, fibroblasts adhesion was increased on PLGA matrices functionalized with DGL.
Overall, these features make DGL promising candidates for the design and functionalization of biomaterials in tissue engineering.