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Intracellular TGFβ Signaling is Potentiated by Substrate Stiffness

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Introduction: Pulmonary fibrosis (PF) is an incurable, progressive scarring disease with 50% of patients dying within 2–3 years after diagnosis, which is driven through the activity and persistence of activated fibroblasts, called myofibroblasts. TGFβ (transforming growth factor beta) has been shown to be sufficient to drive myofibroblastic differentiation and has been shown to be activated in a stiffness-dependent fashion from latent depots in the extracellular matrix. However, how intracellular TGFβ signaling is affected by substrate stiffness is not understood. We hypothesize that intracellular stress inhibits MAN1, an inner nuclear membrane integral protein and known inhibitor of SMADs, TGFβ's downstream signaling partners, thus potentiating TGFβ signaling on stiff substrates.

Results: Here, we show that nuclear stress, nuclear and cell morphology are a function of substrate stiffness (even when exposed to TGFβ). Human dermal fibroblasts, plated on polyacrylamide gels of different elastic moduli, presented significant changes in cellular and nuclear morphology over time. Moreover, using a SMAD-driven luciferase, we see that substrate stiffness is associated with increased TGFβ signaling. Currently we are assaying this phenotype's dependence on cellular contractility, MAN1 expression, and the association of SMADs with MAN1.

Discussion and Conclusion: This study explores a novel mechanism of TGFB regulation, thus providing new guidance for biomaterial design and new therapeutic avenues.

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Laminin-based Skin Substitutes in a Burn Animal Model

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Available treatments for skin regeneration are insufficient for promoting healing. The current study has aimed to produce a cutaneous substitute uniting mesenchymal stem cells, keratinocytes, and a PDLLA biomaterial constructed by electrospinning to use in *nude* mice. Six groups were tested: (1) only PDLLA; (2) only PDLLA/Lam, a hydrolyzed scaffold with the binding of laminin; (3) PDLLA with cells; (4) PDLLA/Lam with cells (n = 6/group) and (5) animals injured without scaffolds (lesion control group) and (6) healthy control group (n = 4/group). All the animals had 1 cm^2 defect performed on their backs, removing all the skin. The biomaterials(or scaffolds) were implanted in the mice for up to 9 days. Part of the defect was taken for histology and another for gene expression. Group 2 presented the best appearance with the softest wound. Gene expression analysis showed a considerable increase of TGFβ1 expression, increased VEGF and balance of the BAX/Bcl-2 ratio for the biomaterial groups when compared to the lesion group. Histological analysis showed well-formed tissue in the groups where the biomaterials and biomaterials plus cells were used. In some animals, in which biomaterials and cells were used, the epidermis was formed throughout the length of the wound. In conclusion, these biomaterials are capable of providing support for the growth of cells, indicating that they can be suitable biomaterials for use in tissue engineering.

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Human Embryonic-like Extracellular Matrix and Antimicrobial Peptides Scaffolds for Chronic Wound Treatment

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Hypoxia-induced mulipotent stem cells secrete a human extracellular matrix (hECM) that contains components associated with stem cell niches and scarless healing including laminins, decorin, hyaluronic acid, SPARC, tenascin, fibronectin, and fibrillin-2. hECM is manufactured reproducibly under GMP conditions and has been shown to support proliferation of hESCs and MSCs, without stimulating human dendritic cell activation. *Ex-vivo* assays have shown the ability of the hECM to significantly reduce inflammation by down regulating interleukins and a variety of metalloproteases while upregulating genes for new matrix production. CAM assays have also demonstrated the pro-angiogenic properties of the hECM. The high incidence of infections seen with severe thermal burns and chronic wounds led us to test the hECM along with antimicrobial peptides (AMPs), a potential alternative to antibiotics that may overcome antibiotic resistance problems. AMPs are short, positively charged peptides found in the innate immunity of hundreds of species, and demonstrate broad spectrum antimicrobial activity even against antibiotic resistant strains of bacteria. AMPs can be modified to bind selectively to collagen. hECM/AMP solutions were prepared and lyophilized to create flexible wafer-like scaffolds that liquify when in contact with the wound bed. The scaffolds have shown antimicrobial effectiveness *in vitro*, and the ability to induce angiogenesis in the CAM model, and are being studied in large full-thickness wounds in mice.

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Adoptive Peripheral Blood Vascular Stem Cell Therapy For Patients With Non-healing Diabetic Wounds

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Purpose: The efficacy of autologous endothelial progenitor cells (EPC) i.e., CD34+ cells therapy is limited since its function and numbers are impaired in diabetic patients. Therefore, we have recently disclosed a newly developed serum free *Ex Vivo* expansion system called Quantity and Quality Control Culture System (QQc) using peripheral blood mononuclear cells (PbMNC) to potentiate the vasculogenic property of diabetic EPCs for enhanced vasculogenesis. The purpose of this study is to evaluate the efficacy of QQc with PbMNCs (MNC-QQc) as a new therapeutic agent for diabetic wound healing.

Methods: PbMNCs were isolated from diabetic patients and healthy volunteers. Then underwent QQc for 7 days. The vascular function of QQ cells pre- or post QQc was evaluated with EPC colony forming assay (EPC-CFA), FACS, and qRT-PCR. For *in vivo* study,