TIMP-3 is Differentially Regulated in Response to Mechanical Microenvironment in Cells Growing in Tissue Engineering Scaffolds

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Statement of Purpose: Since Yannas and Burke first introduced artificial skin in the form of a collagen-glycosaminoglycan (GAG) dermal replacement in 1980[1-3], the fields of biomaterials and medicine have worked toward perfecting a wound healing material that would offer immediate wound closure and quick and physiological wound repair. Little research has been done to determine how and in response to which stimuli these templates guide remodeling of the dermis. It is likely that the local mechanical environment of the wound site greatly influences the deposition and remodeling of the extracellular matrix during wound healing. To quantify these effects, we have looked at the expression of genes and their proteins known to be involved with wound repair in various biomaterial microenvironments both loaded and unloaded. Specifically, we focus here on Tissue inhibitor of metalloproteinase 3 (TIMP-3), a protein that inhibits the activity of several matrix degrading enzymes.

Methods: Bovine tendon type I collagen (Cat # C9879, Sigma, St. Louis, MO) was homogenized in a solution of 0.05 M acetic acid. The solution was then sonicated at 4 °C. A chondroitin-6-sulfate (Cat # C4384, Sigma, St. Louis, MO) solution (0.5 mg/ml) was added drop wise and sonicated at 4 °C to yield a milky white solution. The solution was poured into moulds and frozen stepwise, first at –20 °C and then at –80 °C. Meshes were lyophilized and heat crosslinked in a vacuum oven. Alternatively, two dimensional collagen-GAG was coated onto tissue culture plates (CC plates). WS-1 human fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA) containing fetal bovine serum (10%) and penicillin/streptomycin (1%). All cells were trypsinized (0.25% trypsin-EDTA) from TCPS plates, washed, spun down and resuspended at an approximate concentration of $1X10^6$ cells per 100 µl of media. The three dimensional collagen-GAG meshes were placed in 50mm radius TCPS plates, coated with a thing layer of 1% agarose gel to keep cells from migrating off of the mesh to the plate. Each mesh was seeded first with 1.5 x 10^6 cells and placed in the incubator for 15 minutes. Meshes were then seeded with another 1.5 $x10^6$ cells and incubated for 10 additional minutes. The wells with meshes were filled with 4.0 ml of media and returned to the incubator. Each CC and TCPS plate was seeded with approximately 2.5 x 10^6 cells. The cell-seeded CC and TCPS plates were incubated for time periods concomitant with loaded samples.

The mechanical loading involved application of a cyclic compressive load for 4 hours at a frequency of 0.1 Hz with a preload force of 1.0 N and amplitude of 100 µm. Loads were applied using a TA Instruments Q800 Dynamic mechanical analyzer. A 5% CO_2/air mixture and a temperature of 37˚C were maintained inside a sealed experimental chamber.

Results/Discussion: *Histology:* Representative meshes were fixed in 2% paraformaldehyde after cyclic compression in the modified DMA. The meshes were sectioned and stained using Masson's Trichrome. Under cyclic loading, at 1 hour, the cells have penetrated the three dimensional mesh but have not yet begun to contract it. Without loading, cell-cell interactions are dominant, and only the bottom layer of cells is attached directly to the mesh.

MTT Assay: Cell viability on collagen-GAG meshes produced in the laboratory when incubated at 37°C in 5% CO_2 in a sterile, humidity-controlled environment were compared to incubation in 5% CO_2/air at the same temperature in the sealed DMA chamber. The cell concentration for the DMA mesh was 1.0×10^6 cells/mL and the cell concentration for the incubator mesh was 8.5×10^5 cells/mL.

Gene Expression Studies of Cells Growing on Cyclically Loaded Meshes: We have demonstrated that gene expression events can be altered in cells growing on collagen-GAG meshes by changing applied loading histories. Reverse transcription (RT) was performed in conjunction with real-time polymerase chain reaction (PCR) to enable quantification of TIMP3 mRNA expression. For statistical purposes, experiments were performed in triplicate. TIMP3 expression is high in cells growing on TCPS substrates, lower in cells growing on collagen-GAG coated plates and lowest on cells growing in collagen-GAG meshes. Using real time qPCR, we saw the lowest expression of TIMP3 on the cyclically compression loaded collagen-GAG meshes. This suggests that inhibition of MMPs is the lowest in the loaded configuration for this experiment. These results make sense in light of the histological data showing much faster invasion of the cells into the loaded mesh.

ELISA Assay: The gene expression events were congruent with changes in protein expression for unloaded collagen meshes, collagen coated plates and untreated polystyrene plates. The results indicate that the mRNA expression levels correlate with the amount of protein expressed. TIMP3 protein is down regulated in the presence of the collagen-GAG, an effect that seems to be more pronounced in the presence of the 3D architecture.

Conclusions: In this work we have demonstrated that TIMP-3 gene and protein expression are downregulated in the presence of three dimensional tissue engineering scaffolds and in response to mechanical compression.

References: 1.Burke JF, Yannas IV, Quinby WC, Jr., Bondoc CC, Jung WK. Ann Surg 1981;194(4):413-28. 2.Yannas IV, Burke JF, Warpehoski M, Stasikelis P, Skrabut EM, Orgill D, Giard DJ. Trans Am Soc Artif Intern Organs 1981;27:19-23. 3.Yannas IV, Burke JF. J Biomed Mater Res 1980;14(1):65-81.