Stress Preconditioning on Preosteoblastic Cells for Bone Tissue Engineering

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Statement of Purpose: Successful creation of engineered bone tissue is often limited by insufficient cellular proliferation and formation of extracellular matrix. Stress conditioning protocols using thermal [1] and tensile stress have been shown to induce up-regulation of molecular chaperones called heat shock proteins (HSPs). These proteins have been linked to enhancing cell proliferation and collagen synthesis which is critical for formation of the extracellular matrix. Therefore, identification of effective thermal and tensile stress preconditioning protocols that enhance HSP expression could substantially advance development of replacement tissues for bone. Our objective was to identify thermal and tensile preconditioning protocols that enhance HSP and bone-related protein expression while minimizing cellular injury for ultimate use in engineering of bone tissue.

Methods: MC3T3-E1 cell line (American Type Culture Collection, Manassas, VA), a mouse preosteoblastic cell line was cultured as a monolayer with media composed of αMEM (Mediatech, Inc., Manassas, VA) including 10% FBS and 1% Penicillin-Streptomysin. Thermal stress was applied by submerging the cells in a 44° C water bath for heating duration of 2-10 minutes. Cells were returned to a 37°C CO_2 incubator following heating to permit induction of proteins. The Flexcell® Tension Plus™ System (Flexcell International Coporation, Hillsborough, NC) was utilized to apply cyclic tensile stress conditioning protocols of 3% elongation and 0.2 Hz (10s on/10s off).

After applying stress, cells were post-incubated for several time dutations (4, 16 hours, etc). Following both thermal and tensile stress, HSP and bone related proteins were measured with innunofluorescence and western blot. Antibodies for immunofluorescence were chosen to detect several HSPs and bone-related marker proteins: anti-HSP27, anti-HSP47, anti-HSP70, anti-ALP, anti-Collagen type I, anti-osteopondin, and anti-osteocalcin. Identical antibodies for HSP and collagen type I were used for western blot. Real time RT-PCR RNA was isolated to measure gene expression. cDNA were polymerized and analyzed in 7300 Real-Time PCR Systems (Applied Biosystems, Foster City, CA). Gene Expression Assay (Applied Biosystems, Foster City, CA) was used as a primer; the primers for specific genes of HSP27, HSP47, HSP70, MMP9, Collagen type I, and OCN were used.

Results: Thermal stress induced the expression of HSP 70 and Collagen type I with higher increases associated with longer heating durations. Minimal increases in HSP 27 and insignificant changes in HSP47 occurred following heating as shown by the immunofluorescence and western blot data (Fig. 1.). However, HSP 27, 47, and 70 gene expression increased following cyclic tensile stress conditioning of 3% elongation for 1 hr (not shown in the abstract).

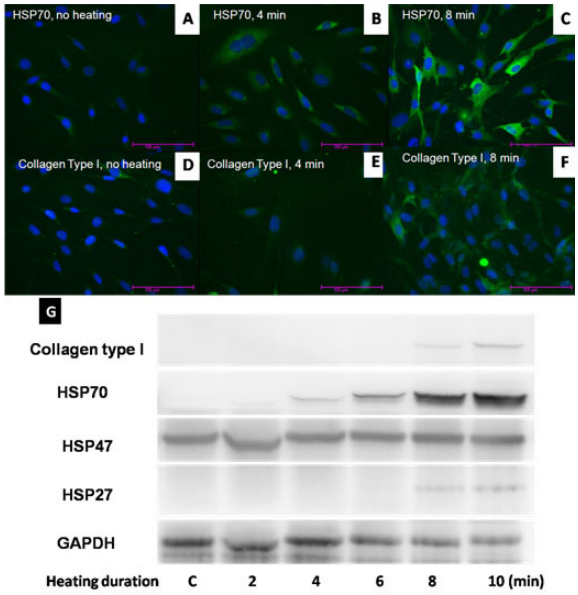

Figure 1. MC3T3-E1 cell response following heating at 44°C. Immunofluorescence staining (post-incubation: 16 h) shown in A-F for HSP 70, (A-C) and Collagen type I, (D-F) with no heating,(A, D) and following 4 min heating, (B, E); 8 min heating, (C, F). Western blot (post-incubation: 24 h) is measured in part G.

Conclusions: This study determined effective thermal and tensile stress conditioning protocols to induce HSP expression and bone-related proteins (e.g., collagen type I, osteocalcin, alkaline phosphatase, osteopondin) for bone tissue engineering. In conclusion, HSP70 and collagen type I increased dramatically following thermal stress with no significant difference in HSP 47 expression. Tensile stress induced significant increases in HSP27, 47, and 70. In the future, we will focus on optimizing the stress protocols by adding growth factors or comparing different stress conditions (thermal, shear, and tensile alone or in combination) to enhance the expression of bone-related proteins and HSPs. Finally, utilization of these stress conditioning protocols applied through bioreactor technology will provide a method for enhancing development of tissue engineered bone tissue through enhanced extracellular matrix formation.

References:

1. Rylander MN, et al., J Biomech Eng, 2005;**127**(5): 751-7.