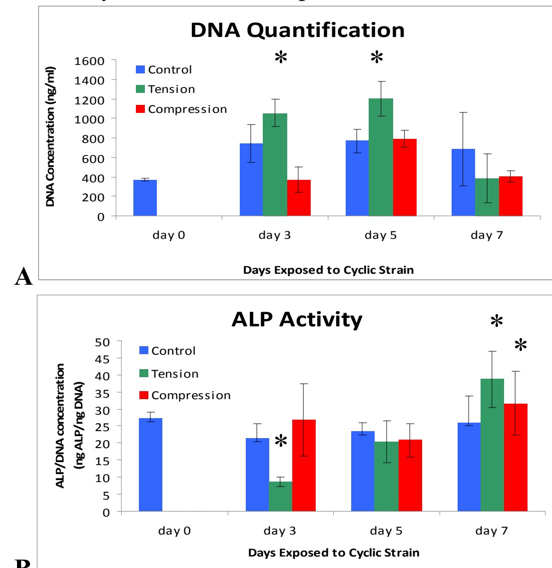
Response of Osteoblast Precursor Cells to Cyclic Compressive and Tensile Strain on a Titanium Substrate

Andrew P. Noblett, Warren O. Haggard, Joel D. Bumgardner
Department of Biomedical Engineering, University of Memphis, Memphis TN

Statement of Purpose: Orthopedic and dental implants are a billion dollar industry that improves the quality of life for thousands of patients each year. After implantation, most of these devices are subjected to cyclic loading and experience strain. Bone cells, as well as the other neighboring tissues, actively respond to loading at the bone-implant interface which influences osseointegration.[1] The response of osteoblasts, in particular, has been show to be dependent upon the substrate, magnitude of the applied strain, rate of loading, and the duration that the load is applied.[2-7] Many studies have been conducted to investigate specific responses, but few have been performed using titanium as the substrate substrate. Also most studies only examine the effect of tensile strain, but implanted devices are subjected to both tensile and compressive strains. In this study we wished to begin examining the response of osteoblast-like cells on titanium under both cyclic strain conditions.

Materials and Methods: Three commercially pure (cp) titanium plates were wet ground to 1200 grit SiC and then thoroughly cleaned with distilled water, tissue culture safe detergent, and ethanol. SonicSeal® slide wells were attached to the plates with a biocompatible silicone rubber sealant. SAOS2 cells, a human osteosarcoma line, were seeded into each well at a density of $5x10^4$ cells/cm^2 in growth media; McCoys 5A® supplemented with 10% FBS and 1% antibiotic/antimyocotic. After 24hrs the growth media was switched to mineralizing media, McCoys 5A® supplemented with 10% FBS, 1% antibiotic/antimyocotic, 50µg/ml ascorbic acid, and 10mM β−glycerophosphate. The plates were then subjected to cyclic strains of 900µε at a rate of 1Hz for 30minutes a day for 7 days with a custom built pneumatically controlled 4-point bend machine. One plate was strained under tension, another was strained under compression, and the third plate was not strained and served as the control. Cells were lysed with RNAase free water after 0, 3, 5, and 7 days of loading.
Cell proliferation was assessed by DNA quantification of the lysates using a Picogreen® assay kit Alkaline phosphatase (ALP) enzyme levels were also measured on the lysates with an assay kit. The ALP concentrations were normalized to DNA for analysis.

Results: Cells responded differently to cyclic tensile and compressive strains over the 7 days. According to DNA quantification, tensile strains resulted in higher cell proliferation than the control after days 3 and 5 days of loading. Compressive loading resulted in maximum DNA concentrations at day 3, but never reach higher values than those of the control plate. Both loading conditions seemed to inhibit proliferation by day 7.
ALP concentrations increased incrementally with additional days of cyclic tensile strain. No loading and compressive strains, on the other hand, resulted in relatively constant ALP expression.



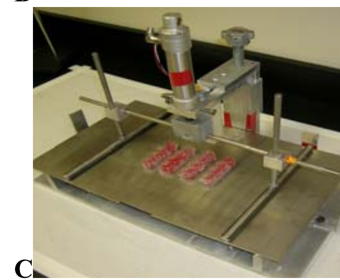

C

Figure1. DNA concentrations (A), ALP expression normalized to DNA (B), and a photograph of the cyclic straining device (C).
* significant difference from control ($p<0.05$)

Conclusions: Tensile and compressive cyclic strains do seem to affect osteoblast-like cells differently. The data suggests that tensile strains promote cell proliferation where as compressive strains had less effect on cell number. Previous studies have shown similar trends in DNA and ALP expression after cyclic tensile straining, but comparing different experiments is difficult due to varying cell lines, loading conditions, and experimental time points. Future work will examine the responses of cells to additional loading conditions and compare multiple cell lines.

References: 1. Zarb GA. Int Peri Res Dent. 1991;11:88-91. 2. Stanford CM. J Ortho Res 1995;13:664-70. 3. Winter LC. J Biomed Mater Res. 2003;67:1269. 4. Kaspar D. J Biomech. 2002;35:873. 5. Di Palma F. Biomaterials. 2005;26:4249. 6. Winter LC. Ann Biomed Eng. 2002;30:1242. 7. Rubin CT. Clin Orthop Relat Res. 1994;298:165-174.