In Vitro Osseogenesis at the Bone-Implant Interface Under Cyclic Tensile and Compressive Strains

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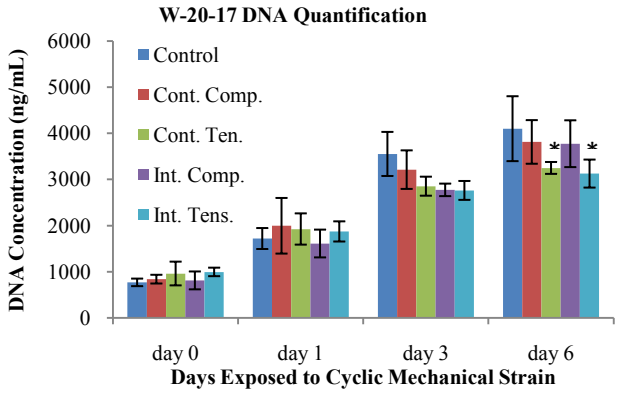
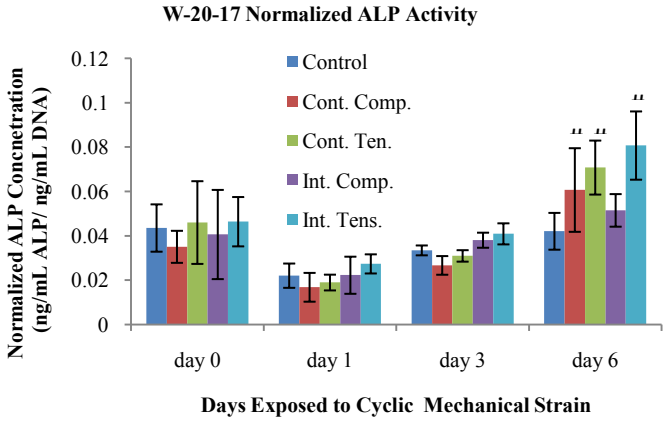
Statement of Purpose: Bone cells, as well as the other neighboring tissues, actively respond to loading at the bone-implant interface of dental and orthopedic and dental implants. This is clinically important because loading conditions on an implant effect local bone remodeling and can affect osseointegration[1]. Although osteocytes are thought by many to be bone's mechanosensors, osteoblasts are also very responsive to mechanical stimuli. Many studies have been conducted to investigate specific responses, but few have been performed using titanium as the 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: Five commercially pure (cp) titanium plates were wet ground to 1200 grit SiC and then thoroughly cleaned with distilled water and isopropanol. Bottomless culture dishes were then adhered to the surface with biocompatible silicone rubber sealant. W-20-17 cells, a murine stromal line, were seeded into each well at a density of $5x10^4$ cells/cm^2 in mineralizing media, McCoys 5A® supplemented with 10% FBS, 1% antibiotic/antimyocotic, 50□g/ml ascorbic acid, 10mM □□glycerophosphate, and 10nM dexamethasome. The plates were then subjected to cyclic strains of 800με at a rate of 1Hz for 30minutes a day for 6 days with a custom built pneumatically controlled 4-point bend machine. Plates were strained under continuous tension or compression or under intermittent tension or compression (15mins straining, 15mins rest, 15mins straining). The remaining plate was not strained and served as the unloaded control. Cells were lysed with DNAase free water after 0, 1, 3, and 6 days of straining. Cell proliferation was assessed by DNA quantification of the lysates using a Picogreen® assay kit. Total protein concentrations were assessed using the Pierce BCA protein assay kit and 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: The osteoblast precursors responded differently to the different strain conditions. Although the cells continued to proliferate on the titanium plates throughout the experiment, the level of proliferation was not significantly increased by exposure to cyclic mechanical strains. In fact, tensile strains resulted in the lowest overall proliferation. Total protein concentrations tracked DNA as expected. ALP concentrations were significantly increased by the daily doses of strain. Ironically tensile strains resulted in the largest tensile strains suggesting that the cells were differentiating in order to lay down more bone matrix and minimize the experienced tensile strains. Also there was no difference between continuous and intermittent strain cycles.





Conclusions: Tensile and compressive strains affect osteoblast precursors differently. Compressive strains elicit little response while tensile strains induced differentiation. 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 osteoclast-like cells, RAW264.7, to the conditioned media of the strained W-20-17 cells. Understanding the close nit relationship between osteoblasts and osteoclasts at the bone-implant interface may allow us to improve implant osseointegration and build better, longer lasting implants in the future.

Refferences: 1.Prendergast,P., et al. J Biomech,1997. **30**:p.539-548. 2.Winter, L., et al. Ann Biomed Eng, 2002. **30**: p. 1242. 3.Winter, L., et al. J Biomed Mater Res, 2003(67A): p. 1269-1275. 4.Kaspar, D., et al. Journal of Biomechanics, 2002. **35**: p. 873-80. 5.Neidlinger-Wilke, C., et al. J Ortho Res, 1994. **12**: p. 70.

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