

In Vitro 3D Bone Response to Fluid Flow-induced Shear Stress and Mechanical Strain

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INTRODUCTION

Mechanical loading is a critical factor in the bone remodeling process and subsequently the strength and structure of bone. Trabecular bone is subjected to a complex physical environment due to its complex microstructure. This results in bone cells, within the bone tissue, being exposed to various mechanical signals including fluid flow-induced shear stress and mechanical strain. Many studies have hypothesized that bone remodeling and mechanical loading can be correlated using mathematical relationships [1,2]. However, most studies have been performed in vivo where it is difficult to control for all variables or 2D in vitro where it is impractical to verify the mechanical loading effects on bone tissue. Also, a single type of loading mechanism (e.g., mechanical strain) is applied in each study making it difficult to compare different loading mechanisms. Therefore, in this study, we used two most popular types of loading mechanisms – fluid flow-induced shear stress and mechanical strain – to stimulate 3D bone formation. The tissue/cell biological responses were correlated to the 3D trabecular bone morphological parameters.

METHODS

Cylindrical trabecular bone scaffolds (diameter = 5 mm, length = 5 mm) were prepared from fresh bovine vertebrae using a diamond core drill and Isomet low speed diamond saw. The scaffolds were cleaned of bone marrow and cells using a water jet. The scaffolds were then micro-CT (Skyscan) scanned at 35 μ m resolution. Morphological parameters including bone volume fraction (BVF) and trabecular number (Tb.N) were quantified using the CTan software (Skyscan).

MC3T3-E1 pre-osteoblastic cells were then seeded on trabecular bone scaffolds in a sterile environment using a custom-built cell perfusion device. Cell-seeded bone scaffolds were cultured in complete medium (alpha-MEM with 10% FBS and 1% penicillin/streptomycin) and placed in an incubator (37C, 5% CO₂).

Mechanical loading (either oscillatory fluid flow (OFF)-induced shear stress or compressive force) was applied seven days after cell seeding. OFF was applied using a flow rate of 0.3 ml/min at 1/60 Hz frequency for 1 hr. Cyclic compressive force (CF) of 30 N was applied inside a sterile biochamber filled with complete medium at 1 Hz frequency for 30 min.

Immediately after loading, bone scaffolds were removed from the loading device and placed in 96-well plates with serum-free medium for 1 hr. Bone formation markers alkaline phosphatase (ALP) and prostaglandin E₂ (PGE₂) protein levels were quantified using Quantichrome ALP assay kit and PGE₂ ELISA kit, respectively. Using each

bone specimen, trabecular morphological parameters were linearly correlated with protein levels.

RESULTS AND DISCUSSION

In oscillatory fluid flow stimulated cells, ALP and PGE₂ release both increased with higher BVF (Figures 1 and 2). Furthermore, the linear correlations between ALP/PGE₂ and BVF were statistically significant ($p < 0.05$). However, in compressive force stimulated cells, ALP and PGE₂ were not linearly correlated with BVF ($p > 0.05$). Similar results were obtained for Tb.N (results not shown).

Results suggest that bone cells are more sensitive to fluid flow than mechanical strain. In general, higher BVF and Tb.N result in narrower spacing within bone tissue and thus higher fluid flow-induced shear stress. Therefore, these results indicate that bone remodeling occurs mostly through regulation of local fluid flow-induced shear stress and not mechanical strain.

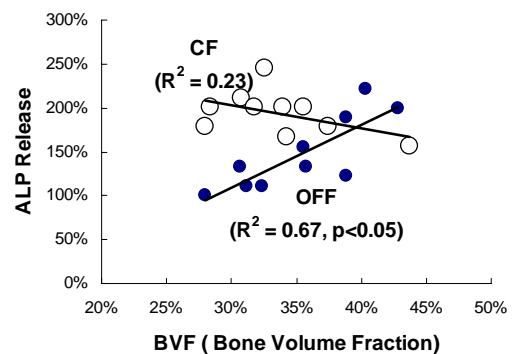


Figure 1: ALP protein release after oscillatory fluid flow (OFF) or compressive force (CF) loading linearly correlated with bone volume fraction (BVF).

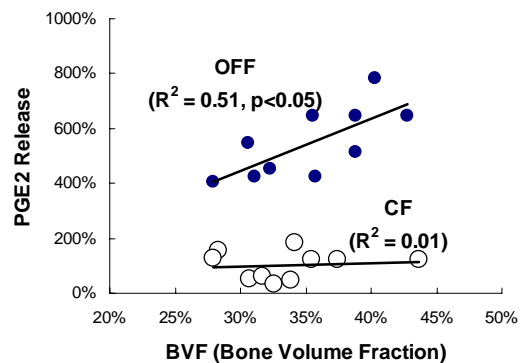


Figure 2: PGE₂ protein release after oscillatory fluid flow (OFF) or compressive force (CF) loading linearly correlated with bone volume fraction (BVF).

REFERENCES

1. Kim CH, et al. *J Bone Miner Res.* **18**:2116-2125, 2003.
2. Ruimerman R, et al. *J Biomech.* **38**:931-941, 2005.