

## READER'S FORUM

Hou J, Chen Y, Meng X, Shi C, Li C, Chen Y, et al.

**Compressive force regulates ephrinB2 and EphB4 in osteoblasts and osteoclasts contributing to alveolar bone resorption during experimental tooth movement.**

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I appreciate the authors who presented this informative and well-written article. Nevertheless, I have a few questions regarding the methods utilized in this study, which are listed below.

**Q1.** In this study, force-loading plates were subjected to cyclic uniaxial compressive strain via a four-point bending system at 0.5 Hz, in order to simulate a cellular condition in the compressive area during orthodontic tooth movement. However, I am not sure that the experimental conditions represent the actual condition during orthodontic tooth movement. Why did the authors use cyclic force? Orthodontic tooth movement normally occurs under unidirectional and static pressure applied on the periodontal ligament (PDL), although the PDL is frequently disrupted by forces arising due to mastication or from the tongue. Cyclic force may generate fluid flow over cell layers, which can affect cellular response through, for example, bending of primary cilia.<sup>1</sup> Moreover, it is not certain whether cells in the traditionally-designated compressive area are under compressive stress, since a recent well-designed finite element study reported that tensile stress surpassed compressive stress in this area.<sup>2</sup>

**Q2.** RAW264.7 cells are monocytes/macrophages

and they differentiate into TRAP-positive multinucleated cells after 3 to 4 days of exposure to RANKL, a strong osteoclastogenic stimulus.<sup>3</sup> In this study, to test the effect of compressive stress on the osteoclastic differentiation of RAW cells, quantitative real-time PCR for osteoclast marker genes was performed after short-term application (less than 4 hours) of compressive stress on RAW cells. Why did the authors not count the number of TRAP-positive multinucleated cells after application of compressive stress for a longer duration? This question is important because mRNA levels of NFATc1, CTR, and ephrinB2 were not different between experimental and control groups after application of compressive stress for 4 hours. Likewise, ST2 cells are bone marrow-derived stromal cells that require osteogenic stimuli such as ascorbic acid<sup>4</sup> or bone morphogenetic protein<sup>5</sup> to differentiate into osteoblast-like cells. However, these osteogenic stimuli were not present in the culture media for ST2 cells used in this study. The authors conclude that compressive force can inhibit osteoblast differentiation, but it is not sure whether ST2 cells would differentiate into osteoblasts even in the control group.

**Q3.** The authors performed only hematoxylin and eosin (H&E) staining to identify osteoblasts and osteoclasts in the compressive area during orthodontic tooth movement, and concluded that no osteoblasts were found in that area. How did the authors identify osteoblasts and osteoclasts using H&E stain? Osteoblast-like cells may be identified by toluidine blue staining or alkaline phosphatase staining, although these techniques are less effective than TRAP staining for osteoclasts.

Questioned by

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Thank you for your valuable questions and my answers are as follows.

**A1.** The response in tooth-support tissues to applied forces is very complicated. Orthodontic forces affect alveolar bone, blood flow in PDL, and extracellular matrix, leading to not only mechanical strain but also hypoxia and release of cytokines in these tissues. These effects, either physical or biochemical in nature, are frequently intertwined. So it is quite difficult for *in vitro* studies to simulate the condition *in vivo* exactly. The aim of our work is to investigate the roles of various factors, including mechanical force (compressive and tensile), hypoxia, and cytokines. The present study is only part of our whole work, which indicates that mechanical force at least partly contributes to remodeling during tooth movement.

**A2 and A3.** Owing to the great difference between the condition *in vivo* and *in vitro*, the characteristics of forces applied to cells in these two conditions cannot be the same. The force produced *in vitro* is substrate strain in nature and cells change their shapes as soon as the surrounding extracellular matrix is strained. During this phase, mechanical forces are transmitted by the cytoskeleton directly to the nucleus. However, the strained cells continue to react by attempting to gain their normal shapes, which could be achieved by detachment and reattachment of the cells to their extracellular matrix during the late phase, leading to the less response of cells.<sup>6</sup> So the cyclic stress is needed to maintain the response of cells. In addition, only early response of cells to mechanical force was investigated in our study, since the later cellular products are expressed, the more cells are influenced.<sup>7</sup>

In our work, ST2 cells grown in osteogenic medium (containing 10<sup>-8</sup> M dexamethasone, 50 mg/L of ascorbic acid, and 10 mM  $\beta$ -glycerol phosphate sodium) is also used and similar results were achieved. What's more, H&E stain is a routine method for us to identify osteoblast and osteoclast.

Replied by

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