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Effect of compressive force on human osteoblast-like cells and bone remodelling: An *in vitro* study

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ABSTRACT

Objective: The aim of this study was to determine the effect of continuous compressive force (CF) on expression by human alveolar bone-derived osteoblasts (HOBs) of some specific molecules involved in bone remodelling.

Design: HOBs were cultured with or without CF (control, 2.0, 4.0 g cm^{-2}) for 1, 3 and 7 days. Expression of alkaline phosphatase (ALP), type I collagen (Col I), osteopontin (OPN), osteocalcin (OCN), transcription factor Runx2, receptor activator of nuclear factor κB ligand (RANKL), osteoprotegerin (OPG) and prostaglandin E2 (PGE_2) was analysed by real-time-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA) and/or immunostaining.

Results: The results revealed that CF upregulated ALP and Col I expression at both messenger RNA (mRNA) and protein levels but did not affect expression of OPN and OCN mRNA. Runx2 mRNA was inhibited by CF, which also altered the expression of molecules involved in osteoclastogenesis, by enhancing RANKL expression and suppressing OPG expression. At 4.0 g cm^{-2} of CF, the expression of RANKL and PGE_2 was significantly upregulated.

Conclusion: The results suggest that initial application of CF on HOBs can simultaneously affect expression of markers related to both osteogenesis and osteoclastogenesis.

1. Introduction

During orthodontic tooth movement, the applied force generates the periodontal ligament (PDL) compression and tension sides. It is generally agreed that tension leads to bone formation, whereas compression causes bone resorption. Tooth movement towards the compression side is the result of remodelling of the alveolar bone and the PDL. There are some evidences supporting that the applied force, in fact, induces expression of molecules involved in both bone formation and resorption from the cells residing in the compression zone.[1,2]

Various cell types, that is, cells of the immune, vascular and nervous systems and bone cells are reported to participate in tissue remodelling during orthodontic tooth movement.[3,4]

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During bone remodelling, osteoblasts regulate bone formation and resorption. In the past decade, several research groups have investigated the interaction between osteoblasts and osteoclasts in the process of bone remodelling during orthodontic tooth movement. Our recent *in vitro* study has shown the effect of continuous compressive force (CF) on the expression of interleukin-6 (IL6) and chemokine CXCL8,[5] molecules reported to be involved in the inflammatory response and osteoclast activity during tooth movement.[6,7]

Osteoblasts are the bone-forming cells that secrete the bone matrix, which subsequently proceeds to mineralise extracellularly. In primary cultured osteoblasts, the developmental sequence of the osteoblast phenotype has been established to mirror the *in vivo* sequence, with the characteristic pattern of gene expression at different stages.[8] Type I collagen (Col I) and alkaline phosphatase (ALP) are observed at an early stage of cell differentiation. As the cultures progress towards the mineralisation phase, maximum levels of the non-collagenous proteins osteopontin (OPN) and osteocalcin (OCN) are expressed.[8]

ALP and Col I are commonly used as markers to monitor osteoblastic phenotypes under mechanical stress.[9,10] ALP is considered to reflect osteoblastic differentiation and new-bone formation,[9] whereas Col I is a major structural protein of bone matrix. Moreover, the sequential development of bone is mediated by the transcription factor Runx2. It has been demonstrated that CF enhanced expression of Runx2 and promoted the maturation of rat osteosarcoma (ROS 17/2.8) cells.[11]

As well as their role in bone formation, osteoblasts directly regulate osteoclastic activity during bone remodelling.[12] Osteoblastic lineage cells express receptor activator of nuclear factor κB ligand (RANKL), one of the key factors mediating osteoclastogenesis. This ligand triggers osteoclast formation and activity by binding to its specific receptor, RANK, on the surface of osteoclast precursors. Conversely, osteoblasts also produce a RANKL decoy receptor, osteoprotegerin (OPG). OPG is a secreted member of the tumour necrosis factor receptor family binding to RANKL, interrupting its interaction with RANK on osteoclast precursors and thus reducing osteoclast activity.[13] Accordingly, it is generally accepted that the ratio of RANKL to OPG controls the balance of bone formation and resorption. Moreover, it has been shown that the RANKL/OPG axis plays a crucial role in orthodontic tooth movement.[14,15]

Prostaglandins are ubiquitous lipid mediators which play an important role in physiological and pathological responses of bone. Among several subclasses of prostaglandins, prostaglandin E2 (PGE_2) has been reported to act as a potent stimulator of both bone resorption and formation[16,17] and detected at higher levels in the gingival crevicular fluid of patients with periodontitis[18] and those undergoing orthodontic treatment.[19]

The effect of mechanical stress on osteoblasts has been investigated in numerous *in vitro* studies. The results are, however, inconsistent, due to lack of conformity with respect to the types of osteoblasts used, the loading characteristics and the duration of application of the force.

In the present study, an *in vitro* model was used to clarify the role of primary osteoblasts derived from alveolar bone during orthodontic tooth movement. The aim of the study was to investigate the responses of primary human osteoblasts (HOBs) to CF of varying magnitude, by determining the expression of molecules involved in the process of bone formation (ALP, Col I, OPN and OCN), bone resorption (RANKL and OPG), an inflammatory mediator PGE_2 and osteoblast transcription factor Runx2.

2. Materials and methods

2.1. Subjects

The study protocol was approved by The National Committee for Research Ethics, Western Norway (225.05, dated 7 November 2005). Healthy non-smoking patients (aged 17–29 years), scheduled for removal of lower impacted molars, were invited to participate in the study. After informed consent was obtained, the surgery was performed and discarded mandibular alveolar bone specimens were harvested from the molar region.

2.2. Cell culture

The bone specimens were isolated and cultured as previously described.[5,20] Briefly, fresh alveolar bone pieces were washed vigorously in phosphate buffered saline (PBS) and the attached soft tissue and periosteum were scraped off. The specimens were then treated at 37 °C for 1 h in α-minimum essential medium (α-MEM, Gibco, Grand Island, NY, USA) containing type IV collagenase (1 mg ml^{-1}, Sigma–Aldrich, St. Louis, MO, USA). The cells were maintained in α-MEM containing 10% foetal calf serum (FCS) (PAA Laboratories, Pasching, Austria) and 1% penicillin/streptomycin solution (PAA Laboratories) in a humidified atmosphere of 95% air and 5% CO_2. HOBs migrating from the bone fragments after culture for 3–4 weeks were harvested and characterised using ALP staining and real-time polymerase chain reaction (RT-PCR), as previously described.[5,20] The cells used in the study were from passages 1–4 and were characterised by strong positive staining for ALP and expression of the bone-specific markers ALP, Col I, OPN, OCN and Runx2.

2.3. Alizarin Red staining

Alizarin Red staining was used to investigate the mineralisation potential of the characterised HOBs according to a previously described method by Li et al.[21] Briefly, cells were grown in 6-well plates (Nunclon Δ Surface, Nunc, Roskilde, Denmark) at a density of 200,000 cells per well. After reaching near-confluence, the culture medium was aspirated and replaced with osteogenic stimulatory medium containing 15% FCS, 50 μg ml^{-1} ascorbic acid, 10^{-8} M dexamethasone and 3.5 mM β-glycerophosphate (StemCell Technologies, Vancouver, BC, Canada) in α-MEM for 11 days. The cells were then rinsed with PBS, fixed with 4% paraformaldehyde in 0.1 M phosphate buffer at room temperature for 15 min and incubated with 2% Alizarin Red S powder (Sigma–Aldrich) dissolved in distilled water (pH 4.1). The cells were then washed five times with distilled water, followed by a 15-min rinse with PBS.