MECHANICAL STRESS STRONGLY INDUCE IL-6 PRODUCTION BY OSTEOBLASTS : A new *in vitro* 3D compression model

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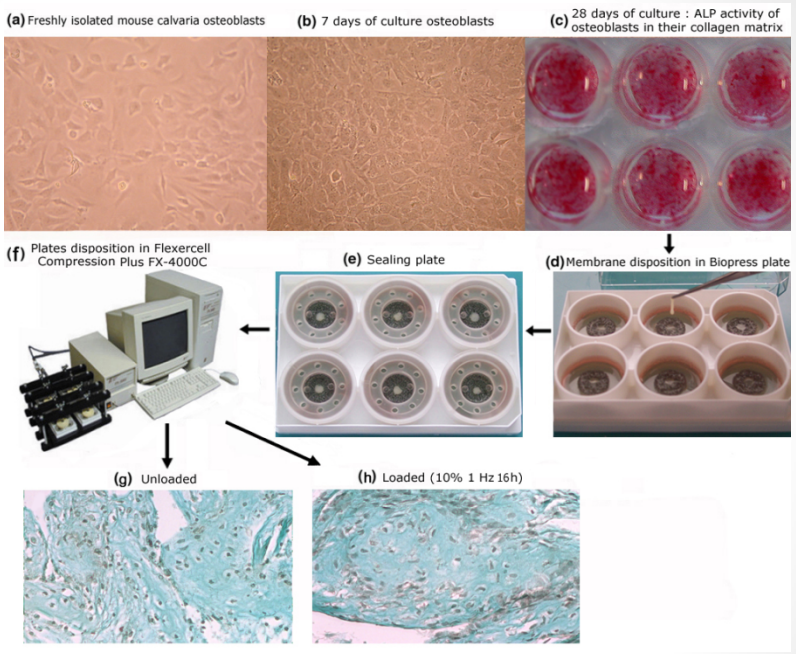
Objective. To study mechanical stress on a new model of osteoblast compression in their own-produced collagen matrix.

Methods. Primary calvaria osteoblasts were isolated from new born mice and cultured for 28 days in monolayer. At the end of this period, osteoblasts were embedded in their abundant and newly synthesized collagen matrix. This collagen membrane containing osteoblasts was then submitted to compression in Biopress Flexercell plates (6 to 10% compression at 1Hz frequency) during 1 to 8 h. The expression of 20 genes was investigated by real time RT-PCR. Interleukin (IL)-6, matrix metalloproteinase (MMP)-3 and prostaglandin (PG)E_2 were assayed in the culture medium by immunoassays.

Results. Mechanical stress highly increased IL-6 (36.2-fold) and COX-2 (6.6-fold) mRNA levels in osteoblasts. In parallel, increased amount of IL-6 (36.4-fold) and PGE_2 (35.6-fold) were found in the supernatant of loaded osteoblasts. This stimulation reached a maximum after 4 h of 10% compression, and was 4-fold more important than after a 6% compression. iNOS (11.6-fold), MMP-2 (1.95-fold), MMP-3 (1.98-fold), MMP-13 (1.49-fold), FOSB (4.1-fold), and vascular endothelial growth factor (VEGF, 3.1-fold) mRNA levels were also increased by compressive stress, while osteoprotegerin (OPG, 0.69-fold) and 15-hydroxyprostaglandin-dehydrogenase (15PGDH, 0.55-fold) were significantly decreased and COX-1, microsomial prostaglandin E synthase-1 (mPGES1), mPGES2, cPGES, COL1A1, osteocalcin, osteopontin and RUNX2 mRNA levels were unmodified.

Conclusions. These results demonstrate that IL-6 is a highly mechano-inductible gene in osteoblasts and suggest that IL-6 could be a key mediator of the mechanically-controlled bone remodelling.



3D-osteoblasts membrane and Flexercell apparatus used for compression application.
(a-c) *Calvarias were harvested from one litter of 6-day-old Swiss mice, enzymatically digested and osteoblasts plated in 12-wells plates and cultured for 28-days.* ***(c)*** *After 28 days of culture, osteoblasts were embedded in an extensive extracellular matrix and expressed membrane alkaline phosphatase (ALP);* ***(d)*** *Collagen membrane containing osteoblasts were unsticking. Two membranes were pooled and put into a Biopress 6-wells culture plate.* ***(e)*** *1.5 ml of media was added and each well was hermetically sealed with a specific cap.* ***(f)*** *The compression was applied by the Flexercell Compression Plus system. The cyclic compression was applied at the magnitude of 1.67 MPa (corresponding at 10%) and at the frequency of 1 Hz with a sinusoidal waveform. Microscopic view of light green/hematoxylin stained 3D-osteoblasts membrane in the control conditions* ***(g)*** *or after 16h of 10% loading* ***(h)*** *(magnification 40x).*