Assessing osteogenic potential: effects of selected biomaterials on gene expression profiles of human osteoblasts

Experimental scheme: human primary and immortalized osteoblasts (OB) were plated 30,000 per well directly on plastic or onto three different titanium discs (namely XPEED, RBM, MACHINE) in triplicates. After 2 days of culture the regular media (10% FBS) was replaced with a standard media supporting OB function containing vitamin C, beta-glycerophosphate and dexamethasone in 20% FBS. Two days later cells were collected, total RNA extracted and OB-specific transcripts detected by quantitative real-time RT-PCR with specific primers.

Culture conditions (duration, cell density, administration and doses of trophic factors) were set based on preliminary studies to optimize the detection of experimental effects.

Gene expression levels are corrected by mRNA levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) upon verification of its stable expression in basal and treated conditions, as a *bona fide* housekeeping control gene.

In all graphs, expression levels are conventionally indicated as fold changes with respect to untreated conditions in the OB cell line. The immortalized line is indicated as "OB line", whereas primary *ex vivo* expanded OBs are referred to as "primary OB 1" and "primary OB 2".

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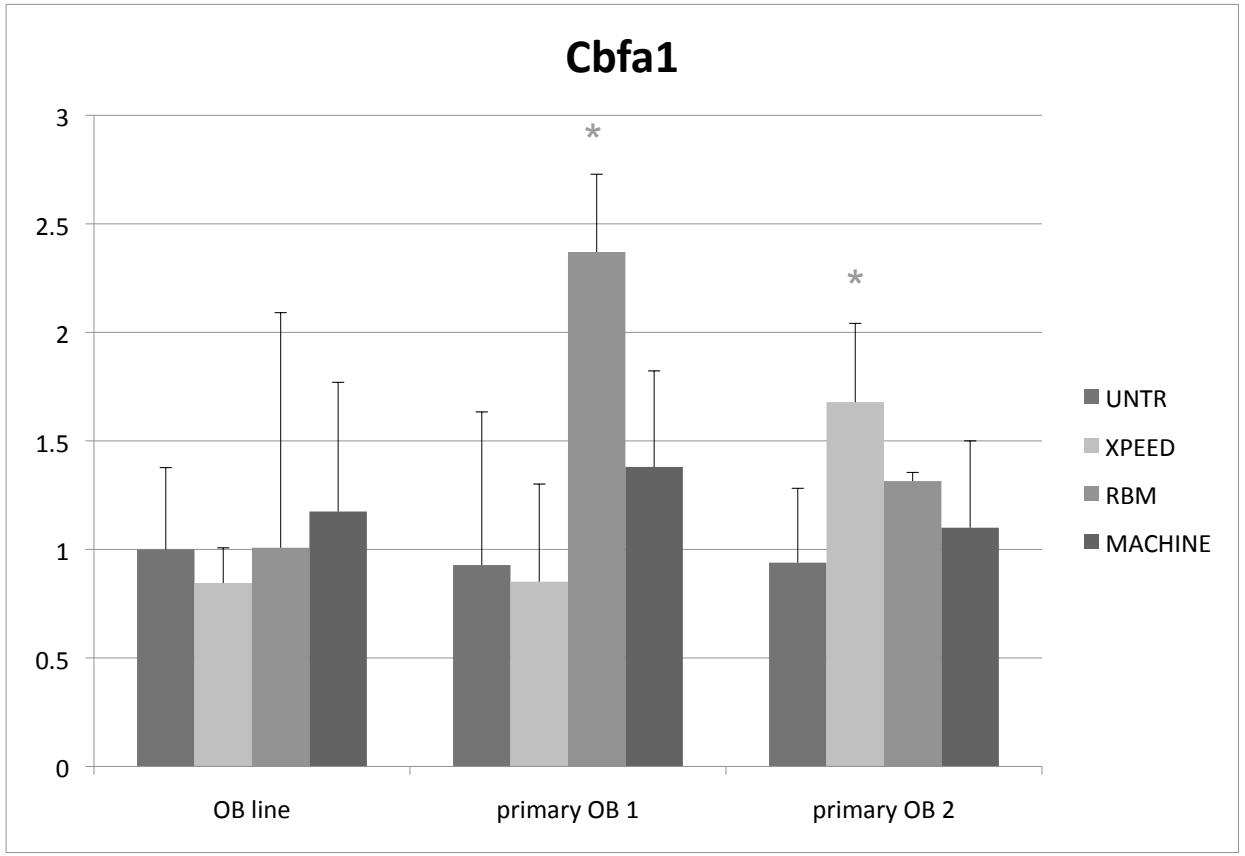
Differentiation: Cbfa1/Runx2



CBFA1 (Runx2) is a transcription factor key for OB differentiation and skeletal morphogenesis. Its levels are comparable in all cellular models, certifying their OB identity. The data show that Cbfa1 levels are not reduced by exposure to the biomaterials. Two materials further increase Cbfa1 is selectively in primary, but not in immortalized OBs.

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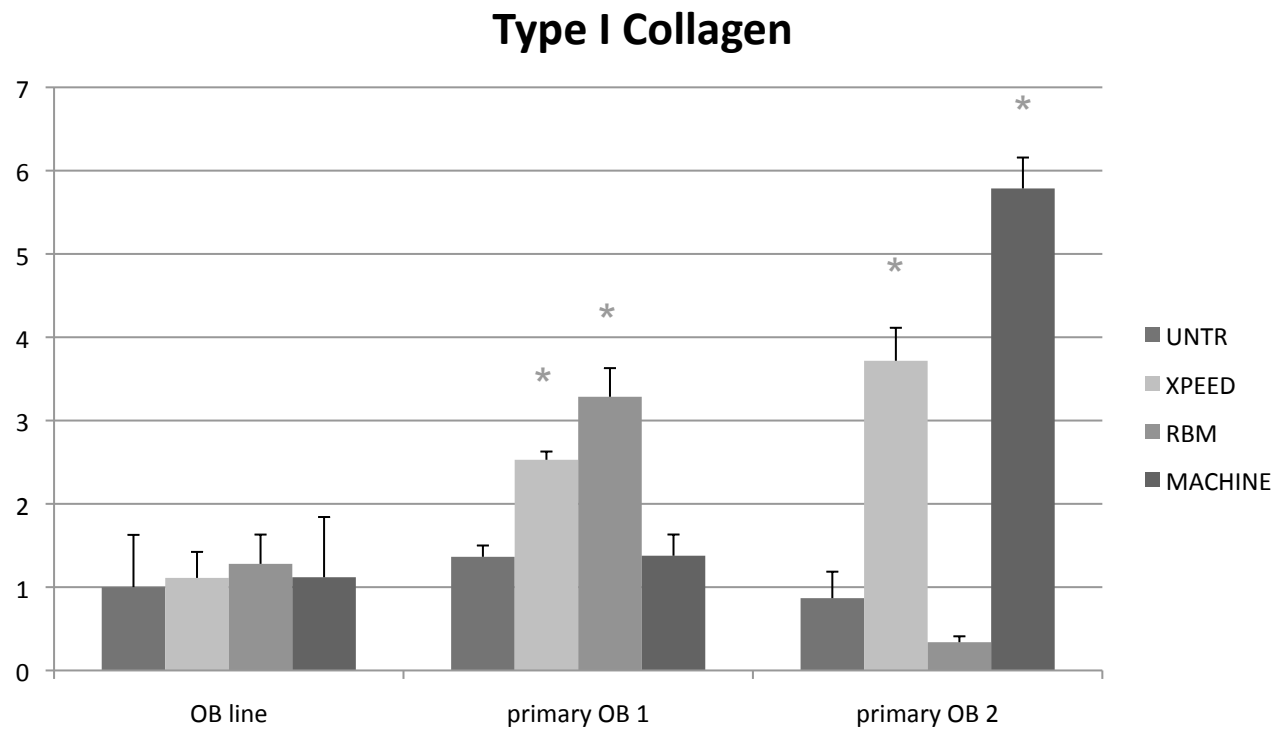
Organic Matrix: Type I Collagen



Type I Collagen is the main organic component of bone. Its expression is comparable in the three cell models. While its expression remains unaltered in the immortalized OB cell line, the biomaterials adopted seem capable to substantially increase Collagen I production in primary OBs, with XPEED appearing more consistent in this effect.

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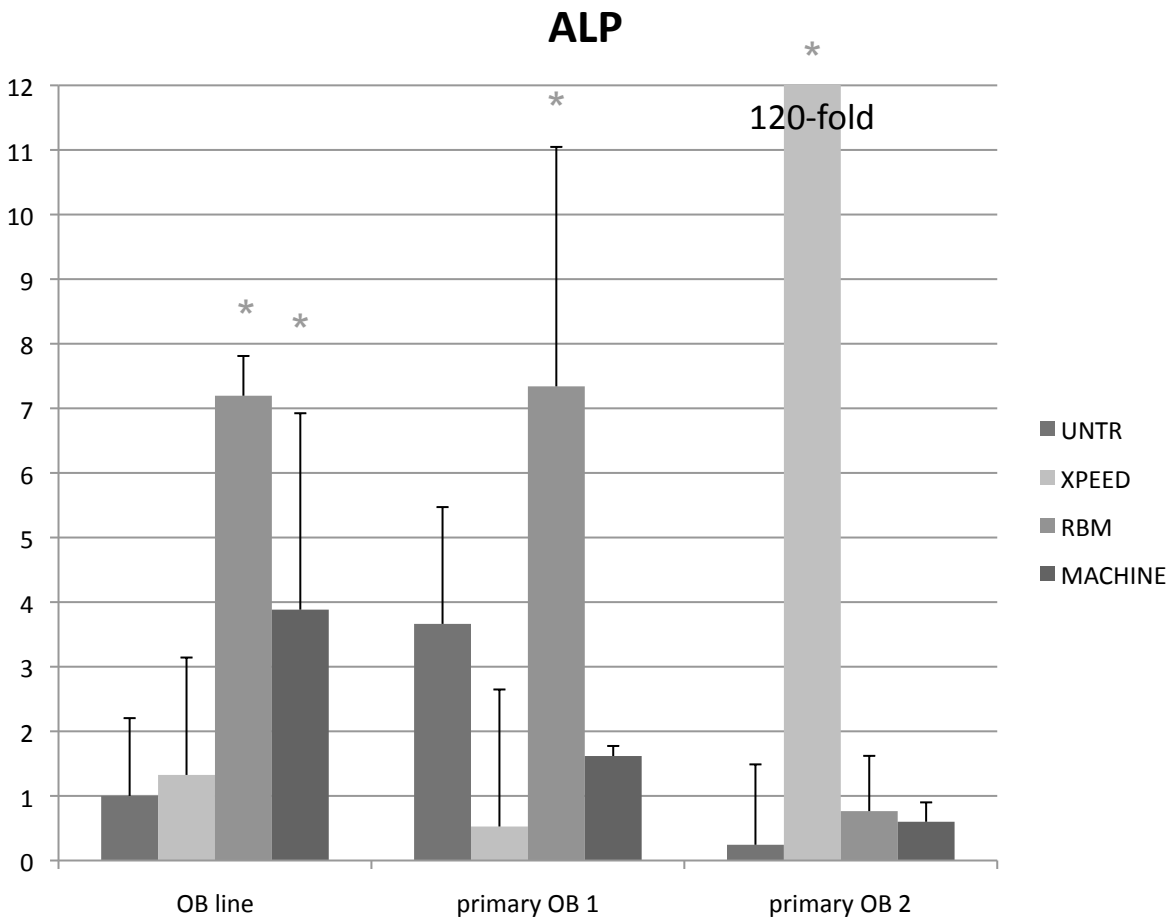
Organic Matrix: Alkaline Phosphatase



Alkaline Phosphatase (ALP) is a classical marker of OB activity in culture supernatants as well as in peripheral blood. RBM increases ALP expression in all three culture models (7, 2 and 3 times respectively). XPEED produced a striking increase (>29-fold over the control OB line, 120 times over its own baseline) only in one OB line.

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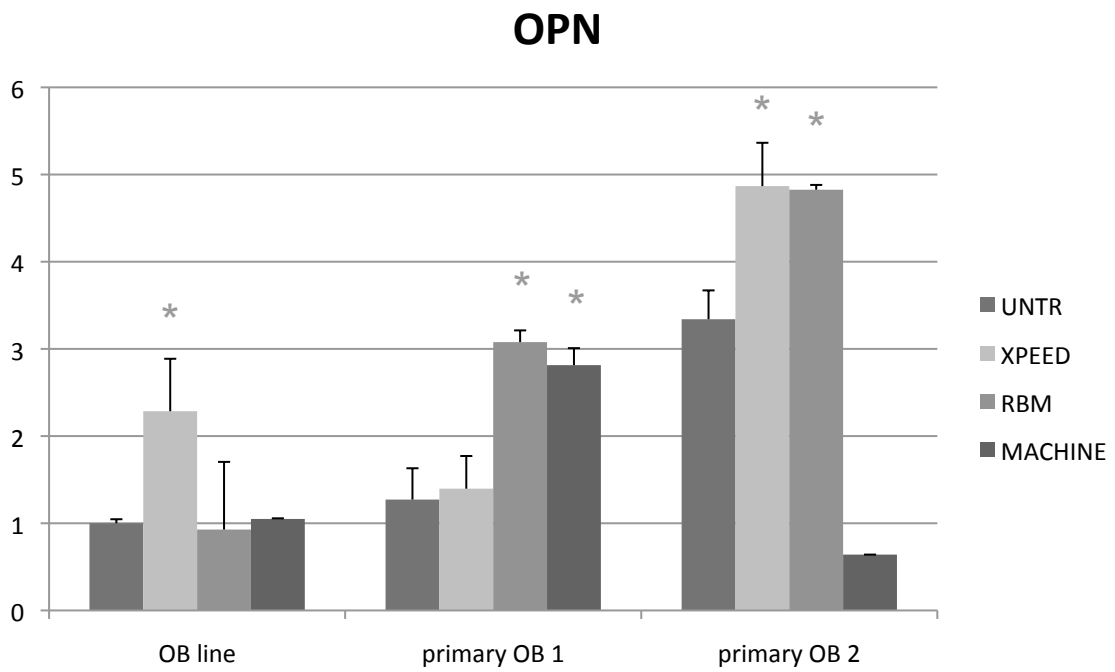
Organic Matrix and Remodeling: Osteopontin



Osteopontin (OPN, also referred to as Secreted Phosphoprotein, SPP, or Bone Sialoprotein-1, BSP-1) is an organic component of bone. Along with structural functions, OPN is thought to play a role in remodeling by promoting osteoclast adhesion. Results vary from line to line, but the three biomaterials demonstrate the ability to increase OPN secretion. Among them, RBM appears more consistent as it increases OPN in primary OBs, including when OPN expression is already high.

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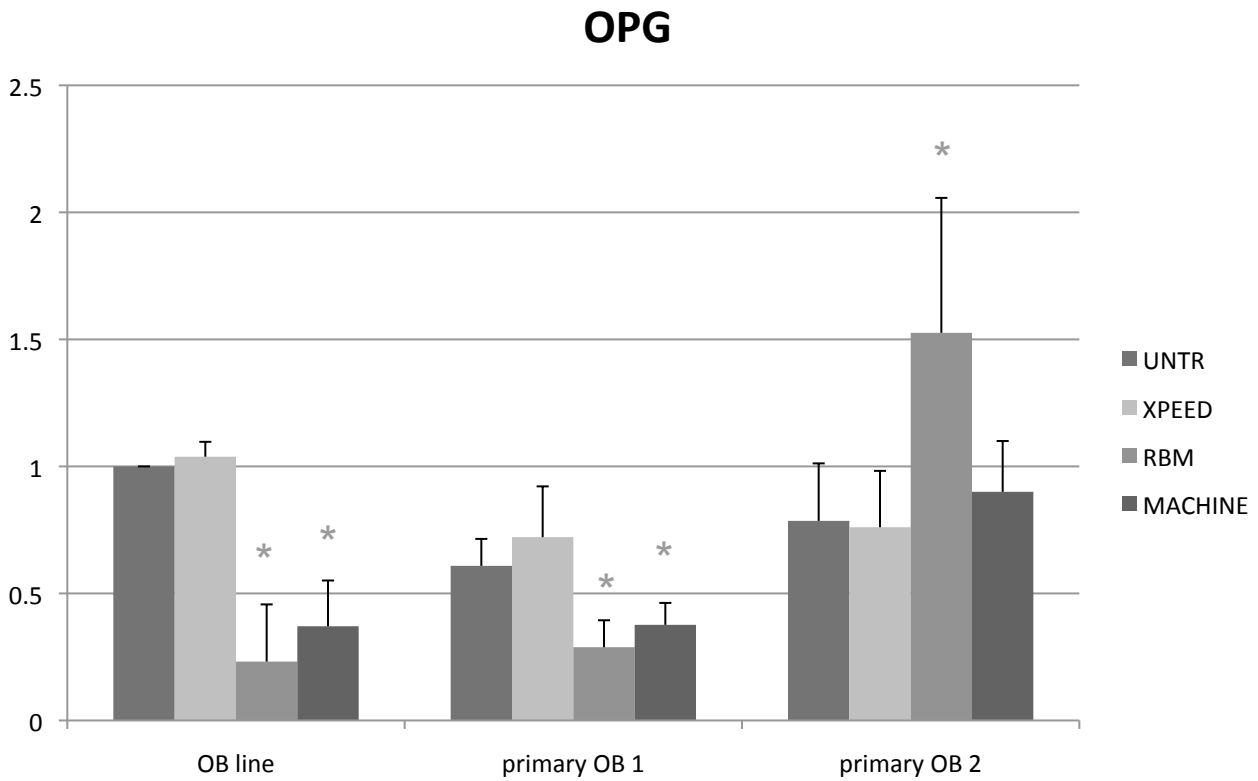
Remodeling: Osteoprotegerin



Osteoprotegerin (OPG) acts a decoy receptor for RANKL, thereby inhibiting osteoclast differentiation and activity. Its expression is inhibited by 2 biomaterials (RBM and MACHINE) in 2 out of three OB cell lines. One biomaterial (RBM) increases OPG expression in one primary OB line.

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