Osteoblast Response to Pulsed Electromagnetic Fields is Substrate Dependent

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Statement of Purpose: Osteogenesis in vivo occurs on a bone surface, however in vitro models to assess the mechanisms involved in the differentiation of osteoblasts use conventional cell culture methods, including a tissue culture polystyrene (plastic) substrate. We and others have shown the importance of substrate surface chemistry on osteoblast differentiation and their response to exogenous regulatory factors including estrogen, vitamin D metabolites, and BMP-2 [1], but it is not known if biophysical factors also regulate osteoblast behavior in a surface dependent manner. We recently showed that pulsed electromagnetic fields (PEMF) could enhance the osteogenic effects of BMP-2 on mesenchymal stem cells (MSCs) if the MSCs were grown on calcium phosphate surfaces rather than plastic [2]. While osteoblasts are sensitive to PEMF when cultured on plastic, the effects are not large [3], suggesting the possibility that their responses may be substrate dependent as well.

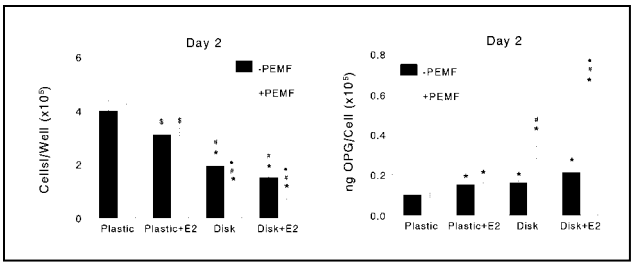
Pulsed electromagnetic fields (PEMF) are used clinically with the intent to stimulate osteoblasts and osteoprogenitor cells to become secretory osteoblasts that synthesize, secrete, and calcify osteoid. When PEMF is used to treat nonunions in bone, the goal is to achieve bony union via stimulation of bone formation. These observations suggest that production of osteogenic factors, including factors produced by osteoblasts that regulate osteoclastic bone resorption, might also be regulated by PEMF if the cells were cultured on a calcium phosphate substrate. To test this, human osteoblast-like cells were cultured on plastic or calcium phosphate disks and their response to PEMF determined as a function of decoy receptor osteoprotegerin (OPG) and RANK ligand (RANKL) production, both of which are associated with regulation of osteoclast differentiation.

Methods: Human osteoblast-like MG63 cells (male) and male normal human osteoblasts (NHOst cells) were grown in 24-well plates on tissue culture plastic or calcium phosphate disks (CaP, BD Bioscience, Inc.). PEMF was applied to confluent cultures via Helmholtz coils specially configured to two matched incubators using a signal that is used clinically (Biomet). Coils were activated for 8 hours per day in one incubator only. All experiments were conducted twice, alternating the active coil incubator to account for any variations that might exist. To determine if PEMF altered cell response to exogenous regulators of osteoblast function, confluent cultures of female SaOS-2 osteoblast-like cells were treated with 17-beta estradiol (E2). At harvest (days 0 [confluence], 2 and 4), conditioned media were examined for OPG and soluble RANKL using an ELISA assay kit. OPG and RANKL mRNAs were quantified by real time RT-PCR. In each experiment, each experimental variable was tested in six separate cultures. Data were analyzed by ANOVA and significant differences between groups determined using the Bonferroni modification of Student's t-test. $P<0.05$ was considered significant.

Results: At confluence the number of MG63 cells was reduced in cultures grown on CaP disks. Cells continued to grown on both surfaces but the increase was greater on CaP with time; PEMF had no effect on proliferation. OPG levels were increased at day-0 in media of cultures grown on CaP. Although OPG increased with time in the plastic cultures, it did not reach levels seen in CaP cultures. PEMF had no effect at day 2 but at day 4, PEMF increased OPG in the CaP cells. This was supported by an increase in OPG mRNA. No changes in sRANKL protein or RANKL mRNA were detected.

NHOst cells behaved in a similar manner to the MG63 cells. At all time points, cell number was reduced and OPG/cell was increased in cultures grown on CaP. However, no effect of PEMF was noted in either cell type on either surface. No changes in sRANKL protein or RANKL mRNA were detected.

SaOS-2 cells also exhibited decreased cell numbers when grown on CaP. Treatment with E2 or PEMF had no effect on confluent cultures, either separately or together. However, at 2-days post-confluence, E2 reduced cell number on plastic. PEMF did not alter cell number in the plastic cultures but it caused a decrease on CaP cultures and this was further reduced by E2 (left panel). When SaOS-2 cells were treated with E2 for 2 days, OPG was increased but PEMF had no effect in plastic cultures. In contrast, PEMF increased OPG in disk cultures and its effects were additive with those of E2 (right panel). At 4 days post-confluence PEMF treatment preserved the enhanced response to E_2 present in the CaP cultures at day-2.



Discussion: This study shows that when osteoblast-like cells are cultured on calcium phosphate substrates they respond to biophysical stimulation with decreased cell number and increased production of paracrine factors associated with reduced bone resorption like OPG. This was a consistent observation using two different osteoblast cell lines as well as normal human osteoblasts. RANKL was unaffected, indicating that the OPG/RANKL ratio was increased, further supporting a surface-dependent osteogenic effect of PEMF. Moreover, response of osteoblast-like cells to E2 was surface dependent and enhanced by PEMF, demonstrating that PEMF can modulate osteogenic responses to anabolic regulators of osteoblast function. These effects of PEMF would not be evident in models examining cells in traditional culture on plastic.

References

(1) Boyan et al., 2003 Eur Cells Materials 24:22-27.
(2) Boyan et al., 2006 SFB abstract.
(3) Lohmann et al., 2003 J Orthop Res 21:326-34

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