Bone 106 (2018) 194–203

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Pulsed electromagnetic fields inhibit human osteoclast formation and gene expression *via* osteoblasts

Zhiming He [a,1], Nagarajan Selvamurugan [b,1], Johanna Warshaw [a], Nicola C. Partridge [a,*]

[a] Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY, USA
[b] Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 31 May 2017
Revised 18 September 2017
Accepted 27 September 2017
Available online 28 September 2017

Keywords:
PEMF
Human osteoclasts
Human osteoblasts
Gene expression

ABSTRACT

Pulsed electromagnetic fields (PEMFs) can be effective in promoting the healing of delayed union or non-union fractures. We previously reported that PEMF (Spinal-Stim® by Orthofix, Inc., Lewisville, TX) stimulated proliferation, differentiation and mineralization of rat calvarial osteoblastic cells in culture. In the present work we investigated the effects of PEMF (Physio-Stim® by Orthofix, Inc., Lewisville, TX) on human bone marrow macrophages (hBMMs) differentiated to osteoclasts. PEMF had striking inhibitory effects on formation of osteoclasts from hBMMs from both younger and older women. There were significantly greater changes in gene expression as ascertained by RNAseq from cells from older women. Interestingly, all of the genes identified by RNAseq were upregulated, and all were genes of mesenchymal or osteoblastic cells and included members of the TGF-β signaling pathway and many extracellular matrix proteins, as well as RANKL and osteoprotegerin, indicating the mixed nature of these cultures. From these results, we suggest that PEMF can inhibit osteoclast formation *via* action on osteoblasts. Thus, PEMF may be very effective for bone mass maintenance in subjects with osteoporosis.

1. Introduction

Pulsed electromagnetic fields (PEMFs) may be clinically beneficial in the treatment of fracture healing especially in nonunions [1]. There is also evidence indicating that PEMF might be effective in the treatment of osteoporosis [2], aid in bone graft incorporation [3] and spinal fusion [4]. We previously reported that both BMP-2 and PEMF (Spinal-Stim® by Orthofix, Inc., Lewisville, TX) separately stimulated proliferation of rat primary calvarial osteoblastic cells and stimulated expression of early osteoblast differentiation in culture [5].

PEMF may also affect bone metabolism by decreasing bone resorption by osteoclasts [6,7]. It has been suggested that PEMF has the potential to accelerate apoptosis of osteoclasts derived from co-cultures of primary osteoblasts and bone marrow cells [8]. In a murine marrow co-culture system, PEMF regulates osteoclastogenesis, bone resorption, and concentrations of osteoprotegerin (OPG), receptor activator of nuclear factor kappa B ligand (RANKL), and macrophage colony-stimulating factor (M-CSF) [9]. In this study, we hypothesized that PEMF (Physio-Stim® by Orthofix, Inc., Lewisville, TX) would have inhibitory effects on human bone marrow macrophages (hBMMs) differentiated to osteoclasts. Thus, we investigated these effects and also determined the effects of PEMF on osteoclast numbers and gene expression from bone marrow cells of younger and older women.

2. Materials and methods

2.1. Experimental design

The experimental design is shown in Fig. 1. We purchased fresh bone marrow samples from Lonza (Walkersville, MD) who are only able to provide bone marrow samples for human females from 18 to 45 years old. In fact, we were provided with 13 samples from them ranging in ages from 18 to 36. For this reason, older samples (10 ranging in age from 50 to 86 years old) were left over tissue from surgical procedures at New York University Hospital for Joint Diseases. Since both groups of samples were de-identified, this is not considered Human Subjects Research by the New York University School of Medicine Institutional Review Board. Not all procedures were applied to all samples due either to quantity of the sample needed (e.g., gel electrophoresis) or cost (e.g., RNAseq analysis).

* Corresponding author at: Department of Basic Science and Craniofacial Biology, 345 East 24th St, 902A, New York University College of Dentistry, New York, NY 10010, USA.
E-mail address: ncp234@nyu.edu (N.C. Partridge).
[1] Contributed equally to the work.

https://doi.org/10.1016/j.bone.2017.09.020
8756-3282/

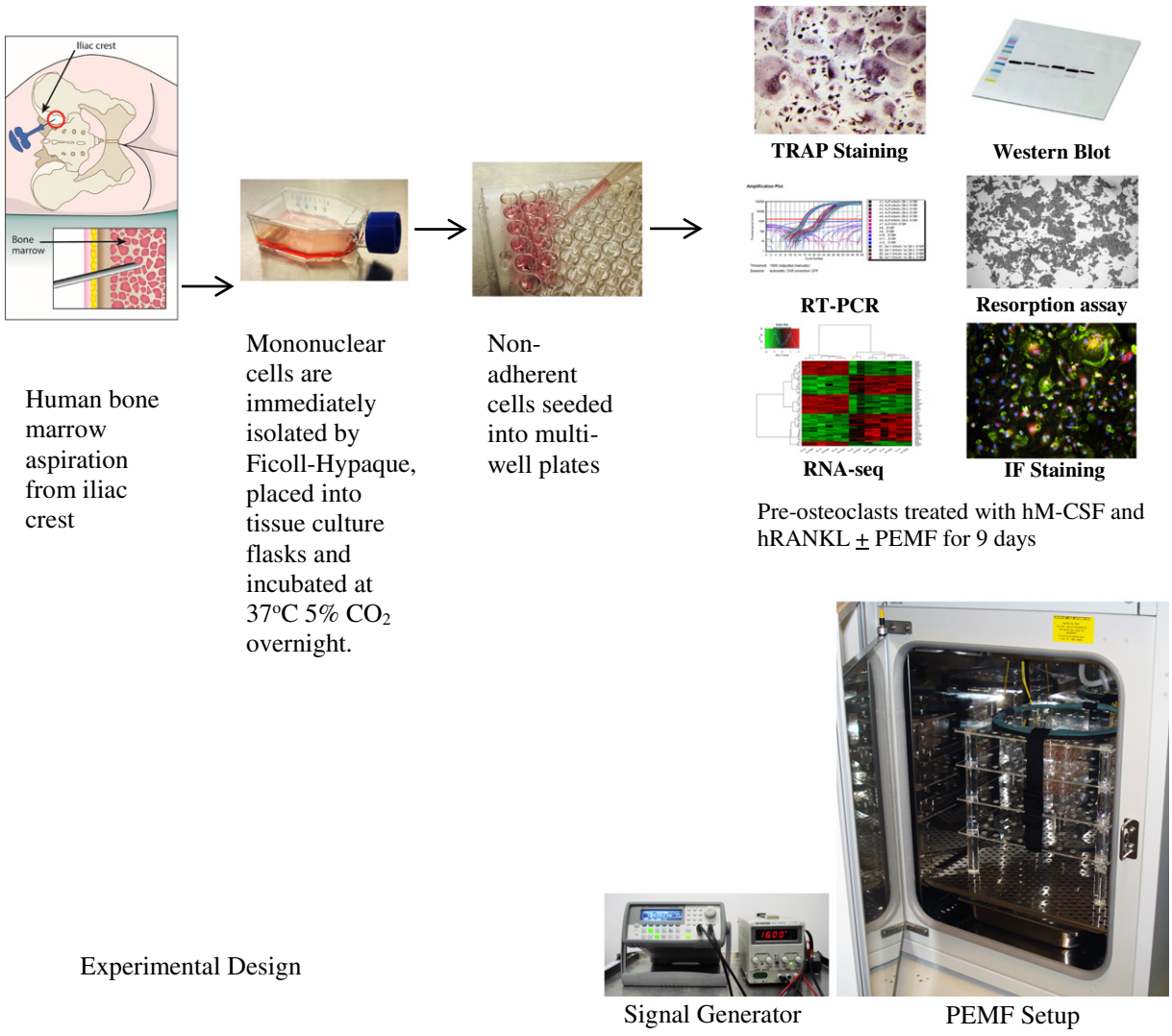

Fig. 1. Experimental design for collection of cells from female human iliac crest, isolation of non-adherent mononuclear cells, the treatment with hM-CSF and hRANKL ± PEMF and the parameters evaluated.

2.2. Cell culture

Fresh human bone marrows from 18 to 86-year-old women were used. Within 1 h of collection, human bone marrow samples were diluted 1:1 in Hank's Balanced Salt Solution (HBSS; GIBCO Laboratories, Grand Island, NY) containing 20 IU/mL of sodium heparin (Sigma Chemical Co., St. Louis, MO). The diluted bone marrow was layered over an equal volume of Ficoll-Paque Plus (GE Healthcare, Piscataway, NJ) and centrifuged at 400*g* for 40 min at 18 °C. The mononuclear cells at the interface layer were collected, washed three times with HBSS, re-suspended and seeded into a tissue culture flask and incubated at 37 °C in the presence of 5% CO_2 overnight. The medium used for culturing these cells was α-MEM (growth medium, Corning, Tewksbury, MA) containing 15% fetal bovine serum (FBS; GIBCO, Grand Island, NY), and Penicillin-Streptomycin (GIBCO, Grand Island, NY). Non-adherent cells (BMMs) from each subject were collected and placed in 24-well plates for RNA isolation (triplicate aliquots/wells for each sample and each treatment group) or 96-well plates (4 aliquots/wells for each sample and each treatment group) for TRAP staining or resorption assays at 1.5–3 × 10^5 or 3–4.8 × 10^4 cells/well, respectively, with or without PEMF exposure. Corning Osteo Assay Surface Multiple Well Plates (Corning, Lowell, MA) were used for the resorption assay. The cells were incubated at 37 °C in the presence of 5% CO_2.

2.3. PEMF exposure

The PEMF was generated as previously described [5] but was set to have similar waveform characteristics to a commercial, clinically-approved proprietary device (Physio-Stim). The specific differences from our previous publication [5] with Spinal-Stim was a burst frequency of 15 Hz, and a burst period of 67 ms compared with a burst frequency of 1.5 Hz and a burst period of 670 ms for Spinal-Stim. The induced magnetic field was vertical relative to the surface of the culture plates. The PEMF waveform was routinely checked for its consistency using a field probe and oscilloscope. The first PEMF exposure was initiated 24 h after seeding cells in wells (day 1) and continued daily at the same time for 4 h each day through the entire experiment. Control plates were placed in an identical incubator on Plexiglass shelves. The CO_2 concentration, humidity, and temperature of the control and treatment incubators (upper and lower chambers of the same double incubator) were identical and were not affected by the PEMF.

2.4. Osteoclast differentiation

Human BMMs were differentiated to osteoclasts by culturing for 9 days in osteoclast medium [α-MEM (Corning, Tewksbury, MA) containing 15% fetal bovine serum (FBS; GIBCO, Grand Island, NY),