THE EFFECT OF OSCILLATORY FLUID FLOW ON HUMAN MARROW STROMAL CELL PROLIFERATION

Lidan You, Ying J. Li, Nikhil N. Batra, Christopher R. Jacobs

Department of Mechanical Engineering, Stanford University, Stanford, California 94305
Rehabilitation Research and Development Center, Palo Alto Health Care System,
Department of Veteran's Affairs, Palo Alto, California 94304

1. INTRUDUCTION

Mechanical loading is an important regulator of bone metabolism. However, the cellular level regulation of how bone cells respond to mechanical loading is incompletely understood. Changes in osteoblast number and metabolism are thought to be one mechanism by which mechanical disuse leads to decreased bone formation. Mechanical unloading has been associated with decreased osteoblast number [1-3]. One hypothesis is that the decrease in osteoblast number is partly due to a decrease in proliferation of osteoprogenitors [3]. This suggests that mechanical loading may affect osteoprogenitors, which is the subject of this paper. We examined the effects of oscillatory fluid flow (OFF), a cellular-level signal occurring in bone due to mechanical loading [4-6], on the proliferation of marrow stromal cells (MSCs), which contain osteoprogenitors [7-15].

We hypothesized that OFF, found to be a potent regulator of osteoblasts, may be an important regulator of MSCs. As bone tissue is loaded *in vivo*, the fluid in the tissue experiences a heterogeneous pressurization which results in fluid flow along pressure gradient. The fluid direction is reversed when the loading is removed, resulting in a dynamic oscillatory flow profile [5]. In the current study, the effect of OFF on MSCs proliferation was examined. We used BrdU incorporation as a measure of cell division, as MSC proliferation rate directly affects the numbers of osteoprogenitors available for recruitment into osteoblasts.

2. MATERIAL AND METHOD

Cell Culture: MSCs were obtained from BioWhittaker where they had been isolated from healthy human volunteers and cryopreserved. Cells were thawed and cultured at standard conditions of 37° C, 5% CO_2 and 95% humidity. Growth media consisted of DMEM, 10% Fetal Bovine Serum (FBS), 2% L-Glutamine, and 1% penicillin / streptomycin. Fresh media was added every 3-4 days. Cells were subcultured every 7 days using 0.05% Trypsin- 0.53 mM EDTA.

Oscillatory Fluid Flow (OFF): Cells were cultured on 0.01% poly-l-lysine coated glass slides. After 24 hours, cells were exposed to OFF in a parallel plate flow chamber, as described previously [5]. Briefly, cells were cultured on slides and placed against a polycarbonate manifold where a gasket was used to maintain a uniform gap between the two parallel plates. The flow channel geometry was 60x24x0.28 mm. Since the Reynolds number for flow rate used was below 50 and well within the laminar region, the shear stress on the cells could be calculated using Poiseuille's equations. The chamber was sealed and fluid flow was delivered to the manifold inlet by gastight Hamilton syringes mounted between electromechanical actuators (ElectroForce Actuator) from EnduraTec (Minnetonka, MN). The actuators were user controlled using Wintest software (EnduraTec) to determine the displacement, waveform, and frequency. Flow parameters were verified using a high-frequency ultrasonic flow meter (Transonic Systems Inc., Ithaca, NY.) .Cells were subjected to OFF at 1 Hz with peak shear stress of 10 dynes/cm^2 and perfused with media at 0.05 ml/min for 2 hours(20). Flow groups were exposed to OFF and no flow controls remained in the incubator.

Cell proliferation: Cell proliferation was assessed by the incorporation of 5-bromo-2'-deoxyuridine (BrdU), a thymidine analog (Absolute-S SBIP Cell Proliferation Assay Kit.) MSCs were cultured on coated glass slides to subconfluent levels (3 days) and subjected to OFF, as described above. Experimental groups were growth media with no flow and growth media with OFF. For all experimental groups, cells were collected 24 hours after flow and incubated with BrdU for 30 minutes, then photolysed and labelled. BrdU was then detected by an anti-BrdU antibody using standard immunohistochemical techniques. The fraction of BrdU-positive cells was observed visually and counted under microscope.

3. RESULT

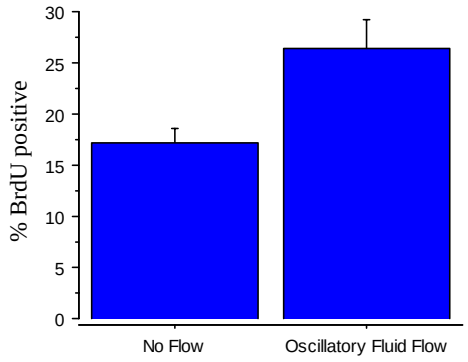

Fig. 1 The fraction of BrdU-positive cells for no flow group and oscillatory fluid flow group. The bars represent standard error of the mean.

The fraction of cell proliferation in oscillatory fluid flow group is 26 +/- 2.7%, which is significantly higher than the fraction of cell proliferation in no flow group, which is 17+/- 1.3% ($p<0.0005$)

4. DISCUSSION

Several studies on skeletal unloading in rats have found changes in marrow cell metabolism. Several studies have found that unloading decreased the proliferation of marrow cells [3, 16]. Our results suggest that MSCs are mechanosensitive. Loading induced OFF may upregulates osteoblasts number by increasing the proliferation rate of MSCs. Insights into MSC response to mechanical signals will enable better understanding and treatment of bone loss diseases such as osteoporosis and bone loss due to mechanical disuse. Existing studies have focused on osteoblasts, osteocytes and osteoclasts; whereas, mechanical loading may also have potent effects on progenitor cells. Another benefit of studying MSCs is that they may offer distinctive treatment options in gene therapy and tissue engineering. For example, one therapeutic proposal is to isolate MSCs, introduce genetic alterations and reintroduced them to patients [18]. MSCs are superior vehicles for gene delivery than mature osteoblasts since they are easier to isolate and have long-term self-renewal capabilities. Consequently, genetic changes to a small number of progenitor cells can be passed onto descendent cells. MSCs are also being considered for tissue engineering applications, where they are culture expanded ex-vivo and subsequently implanted [8].

REFERENCE:

1. Dehority W, Halloran BP, Bikle DD, Curren T, Kostenuik PJ, Wronski TJ, Shen Y, Rabkin B, Bouraoui A, Morey-Holton E 1999 Bone and hormonal changes induced by skeletal unloading in the mature male rat. Am J Physiol **276**(1 Pt 1)**:**E62-9.
2. Wronski TJ, Morey-Holton ER, Doty SB, Maese AC, Walsh CC 1987 Histomorphometric analysis of rat skeleton following spaceflight. Am J Physiol **252**(2 Pt 2)**:**R252-5.
3. Barou O, Palle S, Vico L, Alexandre C, Lafage-Proust MH 1998 Hindlimb unloading in rat decreases preosteoblast proliferation assessed in vivo with BrdU incorporation. Am J Physiol **274**(1 Pt 1)**:**E108-14.
4. Weinbaum S, Cowin SC, Zeng Y 1994 A model for the excitation of osteocytes by mechanical loading induced bone fluid shear stresses. J of Biomechanics **27**: 339-360.
5. Jacobs CR, Yellowley CE, Davis BR, Zhou Z, Cimbala JM, Donahue HJ 1998 Differential effect of steady versus oscillating flow on bone cells. J Biomech **31**(11)**:**969-76.
6. You J, Reilly GC, Zhen X, Yellowley CE, Chen Q, Donahue HJ, Jacobs CR 2001 Osteopontin gene regulation by oscillatory fluid flow via intracellular calcium mobilization and activation of mitogen-activated protein kinase in MC3T3-E1 osteoblasts. J Biol Chem **276**(16)**:**13365-71.
7. Friedenstein AJ, Chailakhyan RK, Gerasimov UV 1987 Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. Cell Tissue Kinet **20**(3)**:**263-72.
8. Caplan AI 1991 Mesenchymal stem cells. J Orthop Res **9**(5)**:**641-50.
9. Haynesworth SE, Goshima J, Goldberg VM, Caplan AI 1992 Characterization of cells with osteogenic potential from human marrow. Bone **13**(1)**:**81-8.
10. Pereira RF, Halford KW, O'Hara MD, Leeper DB, Sokolov BP, Pollard MD, Bagasra O, Prockop DJ 1995 Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. Proc Natl Acad Sci U S A **92**(11)**:**4857-61.
11. Prockop DJ 1997 Marrow stromal cells as stem cells for nonhematopoietic tissues. Science **276**(5309)**:**71-4.
12. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR 1999 Multilineage potential of adult human mesenchymal stem cells. Science **284**(5411)**:**143-7.
13. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP 1997 Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem **64**(2)**:**295-312.
14. Bruder SP, Jaiswal N, Haynesworth SE 1997 Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem **64**(2)**:**278-94.
15. Haynesworth SE, Baber MA, Caplan AI 1992 Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. Bone **13**(1)**:**69-80.
16. Keila S, Pitaru S, Grosskopf A, Weinreb M 1994 Bone marrow from mechanically unloaded rat bones expresses reduced osteogenic capacity in vitro. J Bone Miner Res **9**(3)**:**321-7.
17. Machwate M, Zerath E, Holy X, Hott M, Modrowski D, Malouvier A, Marie PJ 1993 Skeletal unloading in rat decreases proliferation of rat bone and marrow-derived osteoblastic cells. Am J Physiol **264**(5 Pt 1)**:**E790-9.
18. Mosca JD, Hendricks JK, Buyaner D, Davis-Sproul J, Chuang LC, Majumdar MK, Chopra R, Barry F, Murphy M, Thiede MA, Junker U, Rigg RJ, Forestell SP, Bohnlein E, Storb R, Sandmaier BM 2000 Mesenchymal stem cells as vehicles for gene delivery. Clin Orthop (379 Suppl)**:**S71-90.

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ACKNOWLEDGEMENTS:

This work was supported by NIH Grant AR 45989 and US Army Medical Research Award DAMD 17-98-1-8509.