INTRODUCTION  Although osteocytes are thought to be the sensor cells of bone adaptation and to play an important role in the coordination of osteoclast and osteoblast activities [1], there are few studies demonstrating the direct influence of osteocytes on osteoblast functions in situ. In this study, we use a 3D co-culture system of primary osteocytes in their native trabecular bone tissue environment with seeded primary osteoblasts to study their interaction, as well as the effect of dynamic hydrostatic pressure. Peak hydrostatic pressures in bone pores are thought to be as high as 2.2 MPa [2], yet there are no studies that have examined the effect of dynamic hydrostatic pressure on osteocyte and osteoblast functions in situ. The specific aims of the current study are to examine the effect of 1) the presence of live osteocytes, and 2) dynamic hydrostatic pressure loading on osteoblast functions.

MATERIALS AND METHODS  Cylindrical trabecular bone cores (Ø5x4 mm) were harvested from metatarsal bones of 3-month-old calves under sterile conditions. The cores were cleaned of bone marrow and most surface cells with sterile PBS using a dental water pick. Half of the cores were devitalized by repeated freeze-thaw, resulting in an initial seeding density of 5x10⁵ cells/core. The cores were divided into four groups: 1) live cores (live osteocytes) with and 2) without hydrostatic pressure loading, and 3) devitalized cores with and 4) without hydrostatic pressure loading. All cores were cultured in αMEM with 10% fetal bovine serum and 1% penicillin/streptomycin.

Dynamic hydrostatic pressure loading was applied using a custom-feedback controlled bioreactor [3]. The cores were placed in sealed sterile plastic bags with 8ml of supplemented αMEM, then placed in the pressure chamber. Sham loaded samples were similarly sealed in plastic bags, but were not subjected to pressure loading. Loaded cores were subjected to a peak 3 MPa load at 0.33Hz with a triangle waveform for 1 hour per day. All experiments were performed at 37°C.

The cores were harvested on days 2, 8, 16, and 22 (n=3). At each time point, each core was vertically cut in half. One half of the cut cores were stained using a live/dead cell viability stain and imaged using a Olympus confocal microscope system. The specimens were then fixed in neutral buffered formalin. Half of each specimen was decalcified in buffered formic acid, paraffin embedded, and sectioned at 10µm thickness. Immunofluorescent staining was performed on these sections using an alkaline phosphatase (AlkP) primary antibody and FITC conjugated secondary antibody, then counterstained with propidium iodide nuclear stain. The other halves were stained with Goldner’s trichrome to determine the osteoid surface thickness. Immunofluorescent staining was performed on these sections using an alkaline phosphatase (AlkP) primary antibody and FITC conjugated secondary antibody, then counterstained with propidium iodide nuclear stain.

RESULTS  Live/dead staining confirmed that devitalized cores did not have live osteocytes, and that some osteocytes were alive in live cores at day 22.

A trend of increased osteoid surface per bone surface in live cores compared to devitalized cores, both subjected to pressure loading, was observed, and was significant at day 15 (p<0.04; Fig. 1). A similar trend was observed in cores subjected to sham loading. Also, there was a significant increase in Os/Bs between day 2 and 15 and 22 (p=0.02) for live cores with pressure loading, while the increase in devitalized cores was not significant. Although there was no significant difference between loading and sham within each time point, loading significantly increased Os/Bs in live cores between day 2 and 15 and 22 while there was an increase in sham only between day 2 and 22 (Fig. 2). Pressure loading did not significantly increase Os/Bs in devitalized cores. There was no difference in osteoid width between any groups or with time.

Alkaline phosphatase staining showed highest intensity and largest number of stained osteoblasts at day 15 for all groups. Both live and devitalized cores subjected to pressure loading showed increased AlkP staining at day 15, but were similar at all other times (Fig. 3). However, live cores did not show more AlkP staining than devitalized cores.

DISCUSSION  We have found that the presence of live osteocytes in a 3D trabecular explant culture seeded with osteoblasts increases the osteoid surface per bone surface. Also, dynamic hydrostatic pressure loading causes significant increases in Os/Bs with time only in live cores. Both suggest that osteocytes modulate osteoblast activity and may also transmit their response to the osteoblasts. However, neither the presence of osteocytes nor pressure loading affected osteoid width. These data suggest that the presence of live osteocytes and pressure loading increases osteoblast recruitment to the bone surface but not the function of each osteoblast. Pressure loading also increased alkaline phosphatase in both live and devitalized cores. Deformational loading of rat metatarsal cortical explants has been previously shown to increase AlkP and osteoblast recruitment [4], and intermittent hydrostatic pressure loading has been found to increase AlkP in osteoblast monolayers [5].

It would be of great interest to investigate the effect of dynamic deformational loading as well as osteocytes on osteoblast functions in our 3D explant culture, to elucidate mechanotransduction mechanisms in trabecular bone.

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