

Primary Human Bone Cells from Older Patients Do Not Respond In Culture at Continuum Level *In Vivo* Strain Magnitudes

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INTRODUCTION

Many features of a strain history (e.g., magnitude, frequency, duration, duty cycle) influence bone adaptation, although their inter-relationships and relative importance are unknown. To explore these inter-relationships we developed a culture system allowing substrate deformation in the range of 100-3000 microstrain, 0.5-30 Hz, any programmable wave form, and any number of cycles or duty cycles (Bottlang et al., 1998). With the initial studies we intended to determine whether primary human bone-like cells responded to two specific stimuli similar to those causing *in vivo* responses.

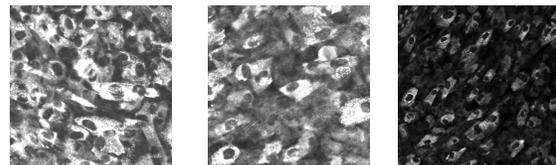
PROCEDURES

Primary human bone-like cultures (Robey, 1985; Zaharias, 1998) were obtained from hip arthroplasty procedures performed for osteoporotic fracture (n=8) or osteoarthritis (n=5). All patients were post-menopausal females older than 60. Cultures (96,000 cell/cm²) were strained in rectangular optically clear silastic wells. Three periods of axial substratum strain (1000 μ -strain, sine wave, 1 Hz or 20 Hz, 10,000 cycles) were provided every 24 hr using the 4-point bending, computer-controlled device (Bottlang, et al. 1998).

We used a semiquantitative immunolabeling confocal approach to ascertain levels of

bone-related proteins: Type I collagen (Coll), osteopontin (OPN), bone specific alkaline phosphatase (AP), osteonectin (OCN), bone sialoprotein (BSP), and a negative control antibody (*Drosophila* even-skipped protein, 3C10). Two cultures were assessed per protein per patient, using ten random data images (10x) per culture. We used NIH Image to calibrate and subtract background. Pixel density/image was calculated using a gray scale value (0-256) and averaged for semi-quantitative comparisons. We additionally determined AP activity and AP mRNA copy number using a cRNA RT-PCR approach. Strained cultures were compared to unstrained from the same donor and culture at the same time. Kruskal-Wallis analysis was performed on average percent in signal intensity of strained versus unstrained cultures. We presumed an $\alpha = 0.05$ as the level of significance.

RESULTS

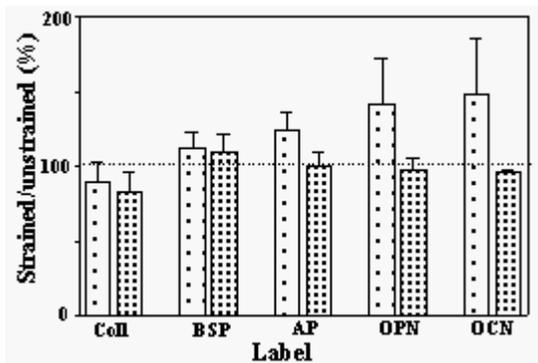


Coll

AP

BSP

All cultures qualitatively exhibited high levels of bone related proteins in most cells compared to negative controls (see above).



Strain did not alter ($p > 0.05$ in all cases) levels of bone-related protein levels, enzyme activity, or steady state copy number per cell in response to strain (standard errors of the mean; shaded bars osteoporotic cells).

We observed no differences ($p > 0.05$) in confocal imaging, AP activity, AP mRNA levels, or cellular proliferation at 1 versus 20 Hz.

DISCUSSION

Many authors have reported responses of primary and transformed bone-like cells to various strain regimens, although in all but perhaps one study the low frequency (1 Hz) strains were well above that believed physiological for bone as a tissue (i.e., over 3000 microstrain peak). Given higher frequency regimens appear to affect bone formation *in vivo*, we also used one higher frequency (20 Hz) regimen (Rubin, 1994). Only Brighton et al. (1991) have demonstrated responses below 3000 microstrain (1 Hz) and these were in newborn rat calvarial osteoblasts at 400 microstrain. The failure of our bone-like cells to respond to two strain regimens anticipated to evoke responses could be owing to a number of factors, including use of: 1.) primary human and not transformed cells; 2.) cells from older human females not on estrogens; 3.) low strain magnitudes. Perilacunar strains are as much as 10-15

times peak continuum (e.g., 1000-3000 microstrain) determined levels (Nicoletta et al., 1998a&b). Thus, "sensing" cells could be those experiencing 5-20,000 microstrain (below fracture levels).

SUMMARY

This data and that from the literature showing responses at much higher strain magnitudes are consistent with the notion that sensing cells require higher levels of strain than generally appreciated, although we cannot rule out lack of responsiveness of older primary human bone-like cells.

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