MAPK Signaling in 3-D Hydroxyapatite Scaffolds with Low-Intensity Ultrasound

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Background: Hydroxyapatite (HA) ceramics have been utilized as bone replacements and surface enhancers for improved bone-implant integration. Three-dimensional (3-D) scaffolds have found an ever increasing role as in-vitro platforms for detailed cell investigations, as well as their clinical use in regenerative orthopedics [Arinzeh 05, Uemura 03]. Low-intensity ultrasound has been applied to simulate osteogenesis during fracture healing [Nolte 01, Warden 00]. Additionally, the mitogen-activated protein kinase (MAPK) pathways couple environmental stimuli with genetic regulation. **Statement of Purpose:** To investigate the behavior of MAPK mediated stress signals in response to 3-D HA scaffolds with applied therapeutic low-intensity ultrasound.

Methods: Two dimensional (2-D) HA discs were hydraulically pressed from powder while 3-D HA scaffolds were fabricated by coating reticulated polyurethane templates (EN Murray, Denver CO) with ceramic slurry containing HA powder, 3% (v/v) polyvinyl alcohol and 1% carboxymethylcellulose binders, 3% ammonium polyacrylate dispersant and 3% dimethylformamide drying agent. Samples were sintered to 1230°C for 4 h. Characterization by micro-CT and SEM confirmed scaffold interconnectivity with SEM photographs shown in Fig. 1. Histomorphometry confirmed micro-CT scaffold porosity value of 80%.

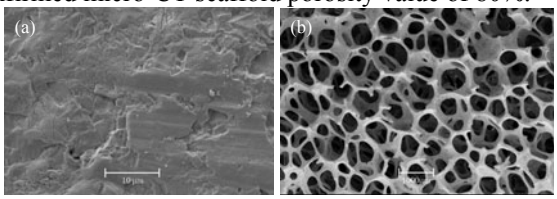

Fig. 1. SEM photographs of (a) 2-D HA surface, and (b) 3-D HA interconnected scaffold.

Intracellular phospho-activation of signaling proteins were evaluated using an osteoblast precursor, human embryonic palatal mesenchyme cell line (HEPM, ATCC, Manassas VA). Cells were seeded on tissue culture plastic, disc samples or scaffolds (2 x 10^4 cells/sample) and cultured for 24 h in growth media consisting of Dulbecco's modified eagle medium (DMEM) with 7% (v/v) FBS, 1% (v/v) PSA at 37°C with 5% CO2. Low-intensity therapeutic ultrasound (Exogen, Smith & Nephew, Memphis TN) was applied for 20 min with 1 h recovery to identify changes in the MAPK cascade and anti-apoptosis signal. Phosphospecific antibody cell ELISA (PACE) was utilized on the MAPK targets; extracellular signal-regulated kinase (ERK1/2 p44/42), stress activated protein kinases (JNK1/2) and P38, and the anti-apoptosis regulator protein kinase B (PKB/AKT). Briefly, samples were fixed in 3.7% formalin, peroxidase neutralized in 0.6% hydrogen peroxide, blocked in 10% FGS before incubated in primary phospho-antibodies overnight (Cell Signaling Tech., Danvers MA). Secondary antibody was HRP linked for colorimetric TMB chromagen detection.

Results and Discussion:

Stimulation of osteoblast precursors was performed in environments simulating trabecular bone. Application of ultrasound was sufficient to activate pERK on tissue culture plastic to a similar level seen on HA surfaces and scaffolds (Fig. 2a). Culture in 3-D stimulated the stress signals pP38 and pJNK1/2 significantly higher than using ultrasound alone (Fig. 2c and d), and corresponded with an increase in the anti-apoptosis signal pPKB (Fig. 2d).

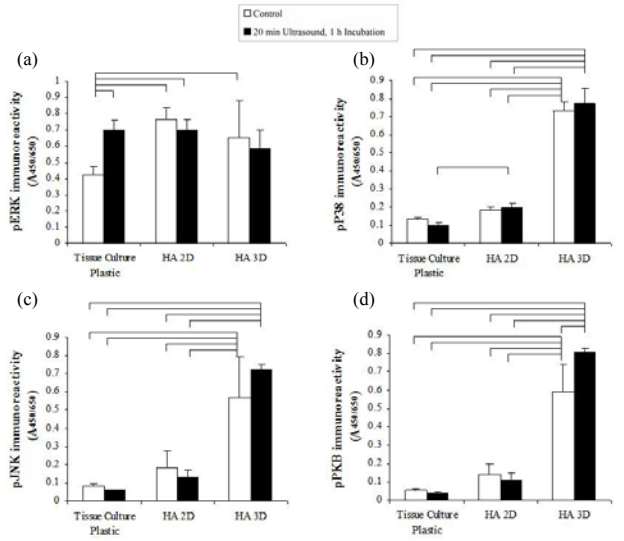

Fig. 2. PACE immunoreactivity of tissue culture plastic, 2-D and 3-D HA in the absence or presence of 20 min ultrasound treatment testing for (a) pERK1/2, (b) pP38, (c) pJNK1/2, and (d) pPKB. The data shown are the mean ± SD. ⌐¬ denotes $P<0.05$ by multiple comparison Tukey test.

Conclusions: While therapeutic ultrasound can increase the pERK level, it does not exceed the activation when cultured on HA substrates. Ultrasound also improved the 3-D pSAPK and pPKB responses, however, culture in scaffolds greatly increased the stress responses when compared to 2-D surfaces and should be considered as basal levels. These responses may be of a transitory nature and clinical ultrasound may find use with sustaining high MAPK activation rather than generating the signal. Future work in temporal based signal propagations is warranted.

References: Arinzeh TL. Biomaterials 2005;26:3631-38. Uemura T. Biomaterials 2003;24:2277-86. Nolte PA. J Trauma 2001;51:693-703. Warden SJ. Calcif Tissue Int 2000;66:157-63. Widmann C. Physiol Rev 1999;79:143-80. **Support:** NIH RO1AR46581.