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Identification of Calcium in Osteoid as a Result of Load Profile Utilising Backscattered Electron Detection in Combination with X-Ray Microanalysis

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INTRODUCTION: The ability to identify calcification of tissue-engineered bone in relation to constructs allows for modelling of tissue development and also monitoring of load profile effects. The effects of chemical agonists, for example Bay K8644, which augment L-type voltage-operated calcium channel (VOCC) opening times, can also be investigated for their effects on mineralisation relative to controls. Field emission scanning electron microscopy (FESEM) can be utilised in backscattered electron (BSE) mode allowing for identification of medium-density materials, for instance calcium, in comparison to low-density organic materials, for example cells and extracellular matrix (ECM)[1&2]. Following identification of medium-density material, appearing in images as higher contrast entities, these areas can be investigated for their chemical composition using energy dispersive X-ray microanalysis (EDX).

METHODS: Primary bone cells from rat were seeded in silicon tubes with 2mm internal diameter and cultured statically for 3 days in supplemented DMEM. Subsequently, cell-seeded tubes were subjected to either load or static conditioning in the presence/absence of Bay K8644 at physiological agonist levels for a further 3 days. Mechanical conditioning was induced via a perfusion-compression bioreactor[3] where constructs were subjected to 1% strain for 1 hour per day at 37°C. Samples were then fixed utilising a standard chemical fixation protocol[4] without post-fixation with osmium tetroxide, dehydrated in an ethanol series and critically point dried (CPD) prior to mounting and coating with 10nm carbon. All imaging and analysis was conducted using a Hitachi S-4100 FESEM equipped with an Autrata-modified YAG single crystal scintillation BSE and Oxford ISIS EDX detector.

RESULTS: Complimentary BSE and EDX was utilised to identify calcium crystals in primary bone cells cultured in tubes, *Figure 1*. Loaded samples exhibited increased amounts of medium-density material distributed throughout, however positive calcium identification was only made in Bay-loaded samples. Loaded cells exhibited rougher morphologies in comparison to smooth, flat non-loaded cells. Furthermore, loaded cells appeared to alter cell-cell contacts resulting in cluster and group formations in comparison to static samples, where cells appeared to be contact inhibited.

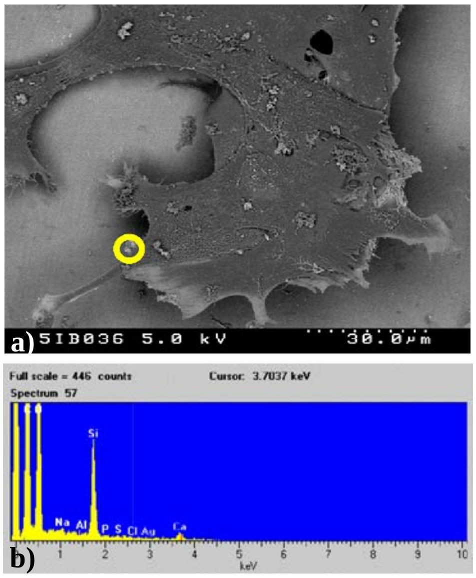

Fig. 1: BSE identification of medium-density materials, a). Highlighted area was then investigated using EDX, b), where calcium was observed to be a primary constituent (peak at 3.7037keV).

DISCUSSION & CONCLUSIONS: BSE imaging in combination with EDX analysis presents a good platform to study bone cell response and mineralization as a result of external mechanical and chemical cues. Furthermore, tubular scaffolds may be used to represent the loading profile found in a single pore of a porous scaffold.

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