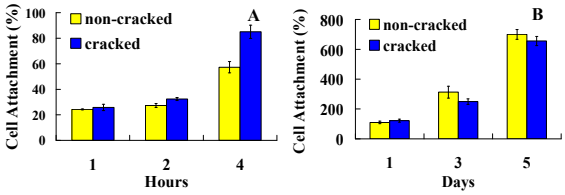
Microcracks Enhance OB Maturation *In Vitro*

Y. Shu, M.J. Baumann, E.D. Case, S. Meyer, M. Buczkowski, K. Tyler, R. Irwin, L.R. McCabe
Michigan State University, East Lansing, MI 48824

Statement of Purpose: Microcracks resulting from microdamage due to bone fatigue were found to trigger bone remodeling in vivo.[1, 2] The focus of our study is to determine the significance of microcracking on bone forming osteoblast (OB) cells with the intent of improving cell response and ultimate mineralization of bone tissue engineering scaffolds. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) comprises two-thirds of the inorganic component of bone and exhibits excellent biocompatibility,[3] making it an ideal substrate material in the study of microcracking on OB behavior. In our study, microcracks were introduced onto HA and MC3T3-E1 OB attachment, growth, alkaline phosphatase (AP) activity and maturation related gene expression (AP, type I collagen (COL I), osteocalcin (OC), and Runx2) were compared on cracked and non-cracked HA. OB morphology and mineralization were also characterized using the confocal laser scanning microscope (CLSM).

Methods: HA powders (4-6 μm) were pressed and sintered in air at 1360°C for 4 hours, followed by polishing to 0.5 μm. Microcracks were introduced to the polished surface using Vickers indentation in a 7×7 grid pattern at 2 mm intervals. OBs were seeded at an initial density of 11,320 cells/cm^2 for OB attachment at 1, 2 and 4 hours, and OB growth at 1, 3 and 5 days.[3] For evaluation of AP activity and maturation related gene expression (AP, Col I, OC, Runx2), OBs were seeded at an initial density of 20,000 cells/cm^2 and harvested at 21 days as previously described.[3] Rhodamine Phalloidin actin, Hoechst 33342 DNA and Xylenol Orange fluorescent dyes were used to observe OB morphology, distribution and mineralization in the CLSM. Three samples were used per time and all trials run in triplicate.

Results: Significant increases were found in OB attachment at 4 hours and AP activity and gene expression after 21 days on cracked HA specimens (Student's t-test $p<0.05$) (Figure 1-A, C and D). An increase in Col I expression was also detected at 21 days (data not shown). No significant differences were detected in OB growth (Figure 1-B) and OB morphology appeared similar between surfaces, spherical in shape, 1 hour after seeding. However, after 4 hours, OBs elongated in several directions and appeared to align with the crack structure (Figure 2-A, D). Similarly, after 21 days, mineral deposition appeared more concentrated on the cracked versus the non-crack areas (Figure 2-E, F).



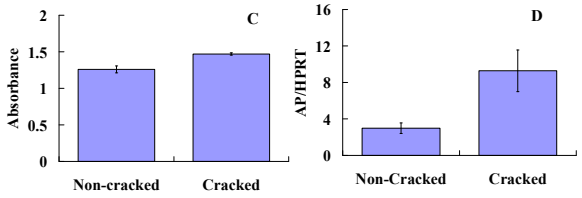


Figure 1. A) OB attachment at 1, 2 and 4 hours. B) OB growth at 1, 3 and 5 days. C) OBs AP activity detected at 21 days. D) Gene expression of AP on cracked and non-cracked HA specimens at 21 days. Three specimens were used for each condition and the experiments were run in triplicate. Error bars represent standard error.

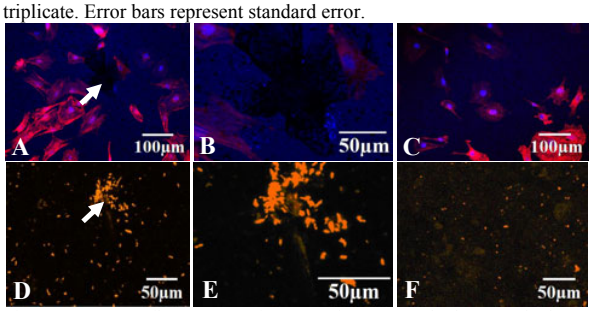


Figure 2. CLSM fluorescence images of OBs attached to cracked HA specimens (A) and non-cracked HA specimens at 4 hours (C). CLSM Z-stack maximum fluorescence intensity projections of OBs mineral depositions on cracked (D) and non-cracked (F) HA specimens at 21 days. White arrows represent the cracked sites. Figure (B) and (E) are enlarged images of indentation cracks from images (A) and (D), respectively.

Conclusions: Introducing microcracks significantly increased early OB attachment and enhanced OB maturation as shown by the increases in AP activity and AP expression. Early alignment of OB to microcracked regions could contribute to enhanced localized mineralization at 21 days, as represented by the focal fluorescence. Thus microcracks may improve the osteogenic potential of HA scaffolds *in vivo*. The mechanism(s) giving rise to enhanced bone formation at the crack sites will be further explored by analyzing the microenvironment and surface morphology in the vicinity of cracks. Protein secretion and *in vivo* studies are forthcoming.

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References:

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