Upregulation of Osteoblastic Activity by Si-Y-N-O Phase Chemistry

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Introduction: It is becoming increasingly clear that the osteogenic capability of any biomaterial is governed by a number of critical surface properties such as its chemical composition, wettability, electrical charge, and topography. While development of an effective arthrodesis device requires concurrent optimization of all important properties,[1] one factor that has heretofore remained unreported is the effect of surface phase chemistry on osteoblastic activity. Prior work suggested that the crystalline phases present at the surface of a silicon nitride (Si_3N_4) bioceramic may, in fact, upregulate osteoblastic activity.[1] In this study fluorescence microscopy, coupled with high-resolution and nano-analytical electron microscopy, were utilized to examine the bioactivity of Si-Y-O-N phases present on the surface of Si_3N_4.

Materials and Methods: Pre-cultured SaOS-2 osteoblast cells at a concentration of 5 x 10^5 cells/ml were seeded onto sterile polished nitrogen-annealed Si_3N_4 discs[1] (∅12.7 x 1 mm, *MC*$^{2®}$, Amedica Corporation) in an osteogenic medium consisting of DMEM supplemented with about 50 μg/mL ascorbic acid, 10 mM β-glycerol phosphate, 100 mM hydrocortisone, and 10% fetal bovine calf serum. The samples were incubated for up to 7 days at 37°C with two medium replenishments. Cell metabolic activity was monitored *in situ* optically and by nitric oxide production using a dye, DAF-2(NO) (diamino-fluorescein-2 diacetate; Goryo Chemical, Inc., Sapporo, Japan). Deacetylation and esterification of this dye inside the SaOS-2 cells allowed NO detection by a confocal fluorescence microscope (BZ X710, Keyence, Japan). Transmission electron microscopy (TEM) images were acquired from focused ion beam (FIB)-prepared samples using a Hitachi HF-3300 TEM (300 kV). Scanning transmission electron microscopy (STEM) images were recorded using a Nion UltraSTEM 100 (60 kV). STEM high-angle annular dark-field (HAADF) imaging and energy dispersive X-ray spectroscopy (EDS) analyses were performed on a JEOL JEM2200FS (200 kV) equipped with a third-order CEOS aberration corrector and a Bruker XFlash silicon drift detector. Statistical differences were evaluated using a one-way ANOVA test. A p-value < 0.05 was deemed to be significant.

Results: Fig. 1(a)~(b) show optical and fluorescence images of SaOS-2 cell proliferation after 24 h exposure to the Si_3N_4 surface. Results of the time-course study in Fig. 1(c) indicated a gradual proliferation of cells, which corresponded to an increase in the concentration of NO. The role of NO, and specifically its effects on osteogenesis, are well known.[2] NO regulates cellular metabolism and its slow release stimulates osteoblast proliferation and differentiation *in vitro*.[3, 4] When produced at low concentrations directly by osteoblasts, NO is responsible for regulating the *in vivo* functions of both osteoblasts and osteoclasts. Fig. 2(a)~(b) show bright-field STEM cross-section images of the Si_3N_4/cell interface. In Fig 2(a) the underlying Si_3N_4 microstructure is covered by an intergranular glass phase (IGP) of high-yttrium-content. Deposition of cell-derived hydroxyapatite (HAp) occurred directly onto this IGP layer. In Fig. 2(b) the partially-crystalline HAp was appositionally coherent with two yttrium-silicate phases (*i.e.*, Y_2SiO_5 and $Y_2Si_2O_7$) at the atomic scale. Although rapid electron damage of the HAp was observed, the EDS analyses suggested a Ca/P ratio of ~1.44, along with the incorporation of Si and possibly N.

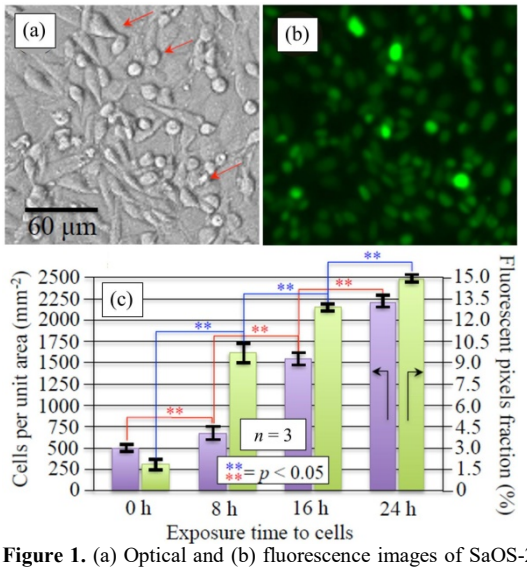

Figure 1. (a) Optical and (b) fluorescence images of SaOS-2 cell proliferation on Si_3N_4 at 24 h. The green dye indicates the level of metabolic activity as measured by NO production. The average cells/unit area and NO concentration as a function of exposure time are shown in (c).

Discussion: This study revealed some intriguing new aspects regarding the relationship between crystal chemistry and the bioactivity of quaternary Y-Si oxynitrides. The availability of nitrogen at the cell/substrate interface synergistically stimulated osteoblast metabolism and the release of $(SiO_4)^{4-}$ tetrahedra into the local biological microenvironment, providing the building blocks for enhanced apatite formation.

Significance/Clinical Relevance: This study demonstrates that optimization of bioactive materials requires a multivariate approach, which not only accounts for enhancements of topography, hydrophilicity, electrical charge, and physical chemistry, but also includes phase composition.

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References: [1]R. M. Bock *et al.*, *Acta Biomater.*, 26, 318-330 (2015); [2]M. Saura *et al.*, *Sci. World J.*, 10, 624-632 (2010); [3]D. M. Evans and S. H. Ralston, *J. Bone Miner. Res.*, 11 [3] 300-305, (1996); [4]H. MacPherson *et al., Bone*, 24 [3], 179-185 (2016).

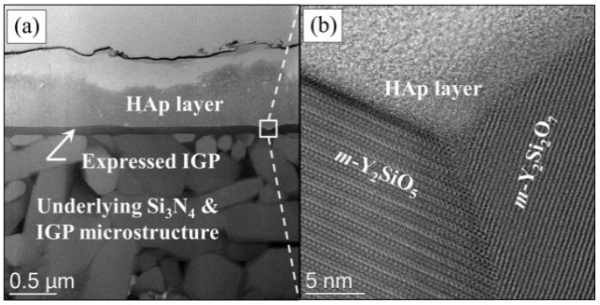

Figure 2. High resolution TEM images of HAp growth on a Si_3N_4 substrate. (a) Cross-sectional view showing the Si_3N_4 and IGP microstructure, expressed IGP, and HAp layers; and (b) Coherent apposition of the HAp to two yttrium silicate phases at the atomic scale.