Osteoinductive Properties of Silicon Nitride, Alumina, and Titanium

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INTRODUCTION: Bone formation occurs by osteoinduction, a process that entails the recruitment of pluripotent immature cells and their stimulation into bone-forming cells, (*i.e.*, osteoblasts). In contrast, osteoconduction refers to bone growth on a surface, such as a porous titanium implant.[1] In implants designed to help spinal fusion, osteoinduction and osteoconduction are highly desirable properties. In an effort to improve the fusion characteristics of synthetic spinal spacer cages, calcium orthophosphates (CaP) and/or hydroxyapatite (HAp) are applied as coatings or they are admixed to form composites.[2] This *in vitro* study investigated the ability of three synthetic biomaterials to facilitate stem cell adhesion, proliferation, differentiation, and hydroxyapatite formation in the absence of CaP or HAp and it compared their relative osteoinductive effectiveness for use as spinal arthrodesis materials. The null hypothesis was that no differences in osteoinductivity would be manifest between different biomaterials.

METHODS: Dense Ø12.7mm x 1mm discs of silicon nitride (Si_3N_4, *MC*$^{2®}$, Amedica Corporation, Salt Lake City, UT), a titanium alloy (Ti6Al4V, ASTM F136), and a medical grade alumina (Al_2O_3, ASTM F-603), n=3 each, were ultrasonically cleaned and UV-sterilized prior to seeding each with 1 x 10^5/ml KUSA-A1 mesenchymal bone marrow stromal stem cells (JCRB Cell Bank, Osaka, Japan). Seeded discs were placed into well-plates with an osteogenic media (Dulbecco's modified Eagle medium (DMEM) and 10 wt.% fetal bovine serum, ascorbic acid, hydrocortisone, and β-Glycerophosphate). This media was refreshed twice weekly during a 14 day incubation period. Seeding of KUSA-A1 cells into empty well-plates with and without the osteogenic media served as positive and negative controls, respectively. After incubation, a 3D laser scanning microscope with a 150x objective lens at a numerical aperture of 0.9 (VK-X200, K series, Keyence, Osaka, Japan) was utilized to determine the volume of HAp formed on the biomaterials. *In situ* laser Raman microscopy (Raman-touch, Nanophoton, Osaka, Japan) was also utilized to quantify the amount of HAp by observing the relative intensities of the PO_4^{3-} and CO_3^{2-} bands at approximately 975 cm^{-1} and 1075 cm^{-1}, respectively. Collagen formation was analyzed using the CH_2 and CH_3 bands at approximately 2875, 2950, and 3000 cm^{-1}. To assess osteogenic activity, the concentration of Gla-osteocalcin (*i.e.,* γ-carboxyglutamic acid) was determined using a mouse high-sensitivity EIA assay kit (Takara Bio, Inc., Kusatsu, Japan) in accordance with manufacturer's instructions.

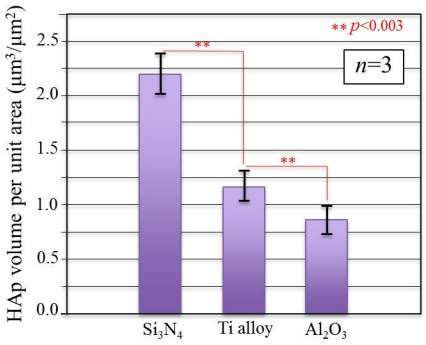

Figure 1 – HAp formation on tested biomaterials after 14 day incubation in an osteogenic media

RESULTS: As shown in Figures 1~3, mesenchymal cell proliferation and differentiation occurred in each of the well-plates containing the osteogenic media and the tested biomaterials. However, well-plates containing Si_3N_4 enhanced osteoinductivity by 80% and 160% over the Ti-alloy and Al_2O_3, respectively (*cf.* Figure 1). The Raman results of Figure 2 confirmed that HAp deposition on Si_3N_4 was significantly greater than either Al_2O_3 or the Ti-alloy in spite of the fact that collagen contents on the two biomaterials were essentially equivalent. Figure 3 presents the results for the Gla-osteocalcin assay. Consistent with HAp formation, it showed significantly larger amounts of osteocalcin in the Si_3N_4 well-plates than for the other biomaterials and control groups.

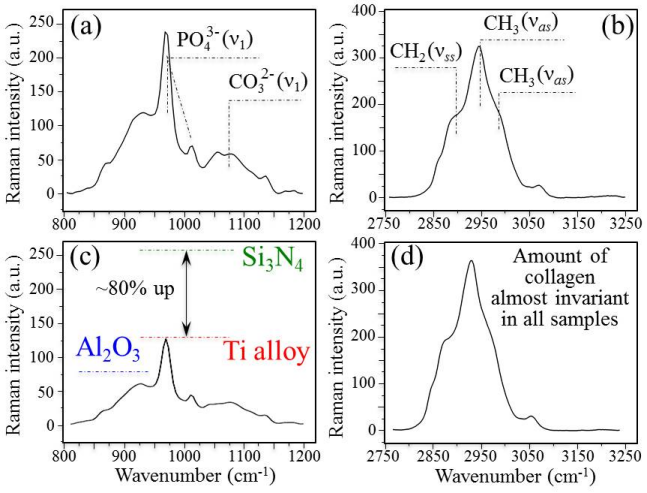

Figure 2. *In situ* Raman spectroscopy results using PO_4^{3-} and CO_3^{2-} bands to assess HAp formation on (a) Si_3N_4 and (c) Ti6Al4V; and corresponding CH_3 and CH_2 bands indicating collagen formation on (b) Si_3N and (d) Ti6Al4V.

DISCUSSION: Enhanced osteoconductivity for Si_3N_4 was reported in prior studies using SaOS-2 osteosarcoma cells.[3, 4] However, the current study is the first to suggest that Si_3N_4 is more effective in promoting bone marrow stem cell differentiation and HAp formation than either Al_2O_3 or Ti. Furthermore, since Gla-osteocalcin is produced by osteoblast cells and only binds with HAp,[5] its presence indicates that the KUSA-A1 cells were highly active, with significantly greater amounts of Gla-osteocalcin observed in well-plates containing Si_3N_4. This result suggests that Si_3N_4 is more effective in enhancing osteoinductivity than the other two biomaterials.

SIGNIFICANCE: Enhancement of osteoinductive activity and hydroxyapatite formation is an important objective in order to accelerate fusion of spinal segments subsequent to surgical intervention. Silicon nitride appears to be a more effective biomaterial than either Ti or Al_2O_3 in achieving this goal, and as such, its use may lead to improved patient outcomes, shorter recovery times, fewer comorbidities, and reduced cost.

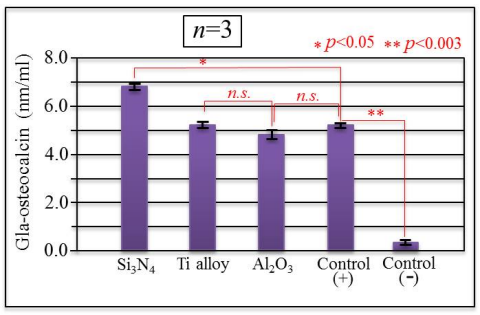

Figure 3. Gla-osteocalcin concentration within the well-plates of the biomaterials and controls.

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