Improved Bioactivity and Bacteriostasis of PEEK Polymers by Addition of Silicon Nitride Particles

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Introduction: Biomaterials that support appositional bone ingrowth and concurrently resist bacterial adhesion and biofilm formation are advantageous for use in spinal fusion surgery. Although polyetheretherketone (PEEK) is widely used as an interbody spacer, the material has poor osteoconductive and bacteriostatic properties.[1, 2] In contrast, monolithic silicon nitride (Si_3N_4) has shown enhanced osteogenic and antimicrobial behavior.[3, 4] Therefore, it was hypothesized that a PEEK-Si_3N_4 composite might improve bone bonding while impeding biofilm formation.

Methods: A PEEK polymer (Otsuka Chemical) was melted and compounded with three different silicon nitride powders at 15 wt.%, including: (i) α-Si_3N_4; (ii) a liquid phase sintered (LPS) ß-Si_3N_4; and (iii) a β-SiYAlON mixture. These three ceramic powders had slightly different solubilities, polymorphic structures, and/or chemical compositions. Osteoconductivity was assessed by seeding specimens with 5 x 10^5/ml of SaOS-2 osteosarcoma cells within an osteogenic media for 7 days. Antibacterial behavior was determined by inoculating samples with 1 x 10^7 CFU/ml of *Staphylococcus epidermidis* (*S. epi.*) in a 1 x 10^8/ml brain heart infusion (BHI) agar culture for 24 h. After staining with PureBlu™ Hoechst 33342 or with DAPI and CFDA for SaOS-2 cell adhesion or bacterial presence, respectively, samples were examined with a confocal fluorescence microscope (BZ-X700; Keyence) using a 488 nm Krypton/Argon laser source. Images were also acquired using a FEG-SEM in secondary and backscattered modes on gold sputter-coated specimens (~20-30Å, 108auto, Cressington). Hydroxyapatite (HAp) deposition was measured using a laser microscope (VK-X200 K Series, Keyence). Raman spectra were collected for samples in backscattering mode using a triple monochromator (T-64000, Jobin-Yvon, Horiba Group) using a 532 nm excitation source (Nd:YVO_4 diode-pumped solid-state laser; SOC JUNO, Showa Optronics Co. Ltd.).

Results: PEEK composites with 15 wt.% α-Si_3N_4, LPS ß-Si_3N_4, or the β-SiYAlON mixture showed significantly greater SaOS-2 cell proliferation (>600%, $p<0.003$, *cf.*, Fig. 1(a)) and HAp deposition (>100%, $p<0.003$, *cf.*, Fig. 1(b)) when compared to monolithic PEEK. The largest increase in cell proliferation was for the β-SiYAlON composite, while the greatest amount of HAp was found on the LPS ß-Si_3N_4 composite. With respect to the bacterial tests, the composite containing the LPS ß-Si_3N_4 powder showed one order of magnitude reduction in adherent live bacteria ($p<0.003$, *cf.*, Fig. 1(c)) as compared to solid PEEK. It is interesting to note that the composite containing α-Si_3N_4 had the worst bacterial resistance (*i.e.*, ~100% higher than monolithic PEEK), suggesting that the bacteriostatic effectiveness of Si_3N_4 bioceramics is apparently dependent upon the presence of selective sintering additives, including yttria and alumina.

Discussion: Monolithic PEEK has been utilized as an orthopedic biomaterial since the 1980s.[5] However, because of its petroleum-based nature, it has neither effective bone apposition nor adequate bacteriostasis. Compounding PEEK with HAp particles or coating PEEK with HAp, titanium, or tantalum have been developed as potential solutions. Although improvements in osteoconductivity have been noted,[6] these composite devices still lack anti-microbial resistance. Compounding PEEK with Si_3N_4 represents a significant advancement due to its ability to provide both improved bone apposition and bacteriostatic behavior.

Significance/Clinical Relevance: The addition of 15 wt.% of specific Si_3N_4 powders to PEEK showed enhanced SaOS-2 cell adhesion, proliferation, and HAp deposition when compared to monolithic PEEK. These same composites also showed resistance to *S. epi.* adhesion and biofilm formation. PEEK/Si_3N_4 composites may improve spinal fusion outcomes and offer an advanced alternative to other PEEK-based composites used as interbody spacer devices, such as PEEK-hydroxyapatite and PEEK-titanium.

References: [1]K. Phan *et al.*, *J. Clinical Neurosci.*, **24** 138-140 (2016); [2]T. J. Webster, *et al.*, *Acta Biomater.*, **8** [12] 4447–4454 (2012); [3]R. M. Bock *et al.*, *J. Biomed. Mater. Res. Part B Appl. Biomater.*, 1–13 (2017); [4]R. M. Bock, *et al.*, *J. Biomed. Mater. Res. Part A*, **105** [5] 1521–1534 (2017); [5]S. M. Kurtz and J. M. Devine, *Biomaterials*, **28** [32] 4845–4869 (2007); [6] I.V. Panayotov, *et. al.*, *J. Mater. Sci. Mater. Med.*, **27** [7] (2016).

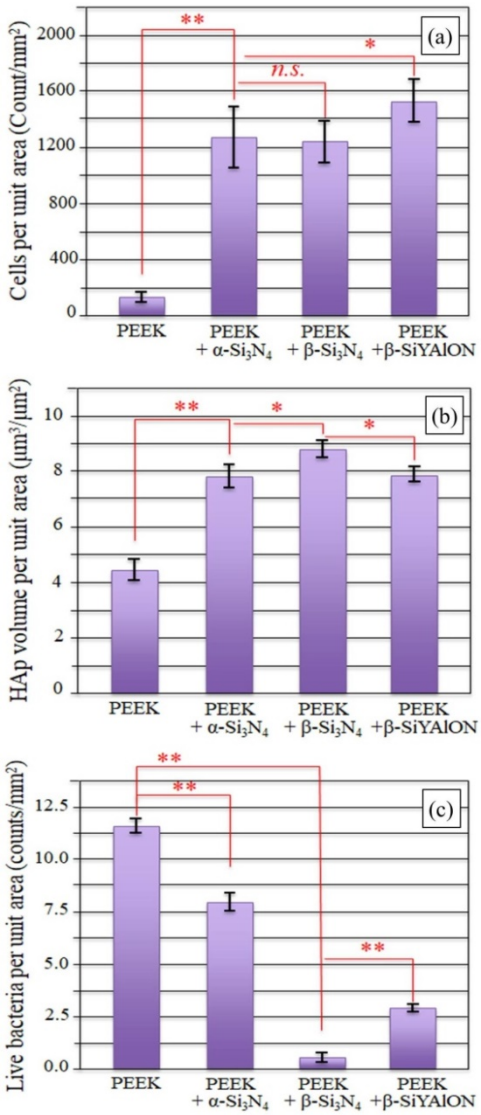

Figure 1. (a) Fluorescence microscopy evaluation of SaOS-2 cell adhesion; (b) 3D laser microscopy results for HAp deposition; and (c) CFDA/DAPI stain analysis for live *S. epi.* bacteria; n = 3 for each material and test; $^{**}p < 0.003$; $^{*}p < 0.05$.