Novel Fabrication of PEEK Using Fused Strand Deposition Reduces Inflammatory Cytokines and Enhances MSC Differentiation in Vitro, Promoting Bone Growth and Implant Osseointegration Clinically

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DISCLOSURES: Dillon JT (N), Cohen DJ (N), Deng, J (N), Reith TM (3A-Curiteva, Inc.), Sabalewski EL (N), Pestov D (N), Jawad AY (N), Slosar PJ (3B-Curiteva, Inc.), Foley KT (3B-Curiteva, Inc.), Dryer RF (3B-Curiteva, Inc.), Bassett TD (3B-Curiteva, Inc.), Harrod CC (3B-Curiteva, Inc.), Bertino M (N), Erbe EM (3A-Curiteva, Inc.), Schwartz Z (N), Boyan BD (3C,5-Curiteva, Inc.; 5-Institut Straumann AG; 3C,5,8-Medtronic Inc.; 9-Pascal Medical Corp.; 3C,5-Spine Wave Inc.).

INTRODUCTION: Spinal fusion devices manufactured from polyether ether ketone (PEEK) by conventional machining, or by creating surface pores via salt leaching, often generate a fibrous interface rather than osseointegration of the implant with bone. Cellular studies indicate that bone marrow-derived stromal cells (MSCs) and macrophages respond to the osseointegration process in a surface-dependent manner. MSCs on titanium-aluminum-vanadium (Ti6Al4V) produce osteogenic and anti-inflammatory factors, while MSCs on smooth PEEK retain a fibroblastic, pro-inflammatory phenotype. Similarly, macrophages have been found to polarize into either regenerative or inflammatory states, depending on the substrate surface. We hypothesize that a fully porous architecture produced by fused strand deposition (FSD), a novel elevation of fused filament fabrication (FFF) additive manufacturing techniques, with or without a hydroxyapatite (HA) coating, would enhance the osteogenic response, reduce pro-inflammatory signaling, and increase anti-inflammatory signaling compared to solid PEEK constructs. To test this, we examined whether PEEK constructs fabricated using novel FSD fabrication of PEEK would facilitate osteogenesis in vitro and osseointegration in clinical cases.

METHODS: For the in vitro study, disks of solid PEEK (SP), FSD additively manufactured porous PEEK (PP), and FSD additively manufactured porous PEEK coated with HA (PP-HA) were fabricated using the same processing methods as for clinical implants. MicroCT quantification of volume and porosity parameters was performed. Surface wettability and characterization were evaluated via sessile water drop testing, scanning electron microscopy (SEM), laser confocal microscopy, micro-computed tomography (microCT), X-ray photoelectron spectroscopy (XPS), energy-dispersive X-ray (EDX) analysis, and atomic force microscopy (AFM). Human MSCs (23 yrs, F) and macrophages isolated from male C57BL/6 mice (6-8 weeks) were cultured on SP, PP, and PP-HA constructs, with hMSCs also cultured on TCPS to serve as an optical control (n=6). Macrophage isolation from murine femurs was approved under VCU IACUC. Cell attachment to constructs was visualized via laser confocal microscopy with MSCs treated with gold nanoparticles complexed with RBITC fluorophores. Osteogenic marker and local factor production were quantified on MSCs cultured on constructs for 7 days, using ELISAs and normalization to DNA content. Macrophages grown on PEEK constructs for 3 days, and polarization was further assessed for pro- and anti-inflammatory response via gene expression measured by RT PCR and protein production using ELISAs and normalization to DNA. Cervical spine cages fabricated via PEEK FSD were used in spinal fusion surgeries. CT images were assessed for successful fusion. All clinical trials received IRB approval.

RESULTS: PP and PP-HA disks fabricated by FSD displayed similar trabecular-like 3D architecture and porosity. Wettability was significantly increased for PP-HA disks. Microroughness was greater for PP-HA than for PP constructs. The elemental surface composition for SP and PP was similar, while PP-HA constructs displayed higher oxygen, calcium, and phosphorus. XRD analysis confirmed the PEEK composition of all constructs, and EDX analysis showed the calcium and phosphorus present in PP-HA constructs. AFM showed similar hardness for SP and PP constructs, which were higher than PP-HA. Human MSCs adhered to all PEEK disks, and microCT and LSM imaging showed that cells migrated internally into the FSD constructs. MSCs synthesized osteoblast proteins in a surface-dependent manner. DNA content for all constructs was reduced compared to TCPS. All PEEK constructs increased the levels of osteocalcin (OCN), osteoprotegerin (OPG), and vascular endothelial growth factor 165 (VEGF) compared to TCPS. PP constructs produced higher levels of osteopontin (OPN) and OPG than SP or PP+HA. VEGF levels for PP+HA were increased compared to both SP and PP. Macrophages cultured on PP and PP-HA showed reduced expression of pro-inflammatory cytokines, inducible nitric oxide synthase (iNOS), tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and interleukin-1β (IL-1β) compared to SP. Macrophages cultured on PP had elevated expression of anti-inflammatory cytokine mannose receptor C-1 (Mrc1) relative to SP, while both PP and PP+HA expression levels of TGFβ1 were elevated compared to SP. PP and PP+HA protein levels of VEGF were higher than SP. Macrophages cultured on porous PEEK constructs did not exhibit a distinct M1/M2 polarization phenotype. Clinical correlation in four human patients implanted with PP-HA devices—each combined with a different bone graft material—showed consistent achievement of spinal fusion.

DISCUSSION: These findings demonstrate that porous PEEK implants fabricated using FSD support an osteogenic response by MSCs in contrast to machined PEEK. The trabecular-like architecture of the FSD constructs promoted a pro-regenerative immune profile, which polarized macrophages from a pro-inflammatory phenotype to an anti-inflammatory phenotype. At the same time, the HA coating further enhanced angiogenic signaling. The effect on both cell types enhanced the osseointegration of the implant in bone. The clinical use of the implant for spinal fusion surgery supports the in vitro data, indicating its success in inducing osseointegration and promoting spine fusion.

SIGNIFICANCE/CLINICAL RELEVANCE: The novel, additively manufactured, trabecular-like porous PEEK generated by FSD enhances osteogenic differentiation, promotes a pro-regenerative immune response, and promotes bone growth and implant osseointegration. Clinically, these implants can be successfully used for spine fusion surgery.

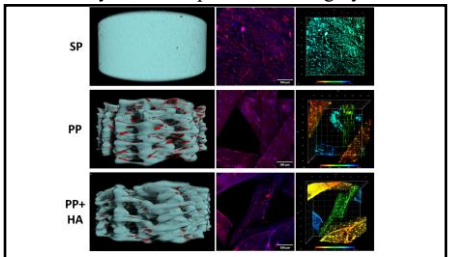

Figure 1: hMSCs attach to and migrate throughout porous PEEK constructs. Fluorophore-labeled hMSCs were seeded onto disks and visualized via microCT and LSM (left to right).

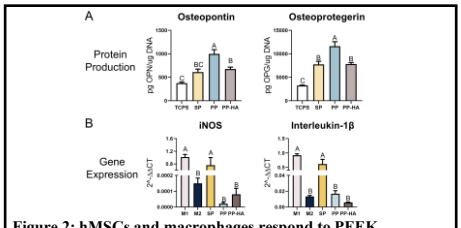

Figure 2: hMSCs and macrophages respond to PEEK constructs in a surface-dependent manner. hMSC cultured for 14 days and evaluated for OPN and OPG protein production (**A**). Macrophages cultured for 3 days and evaluated for gene expression for iNOS and IL-1β (**B**).

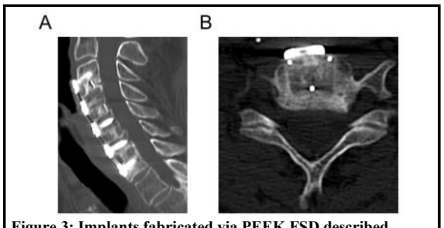

Figure 3: Implants fabricated via PEEK FSD described above support bone through-growth and fusion in vivo. Lateral (**A**) and axial (**B**) CT images of a spinal fusion patient who received PP+HA cervical spine cage. Demineralized bone matrix used to pack into the bone graft window.