

Implants for teeth and bone

The present application claims priority from Australian provisional patent application no. 2023903710, filed on 17 November 2023, the entire contents of which are incorporated herein
5 by this reference.

Field

The present disclosure relates to implants for teeth and bone. The implants are prepared from a polymer of the poly (aryl ether ketone) family, preferably poly ether ketone (PEK), and are
10 activated with plasma to improve integration with teeth and bone.

Background

In the repair or replacement of teeth, it is often necessary to attach a dental implant to the underlying jawbone. This is usually accomplished by embedding a prosthesis such as a bridge,
15 crown or denture. Dental implants are typically made of titanium or zirconia.

Implants for bone are required under a range of situations, ranging from replacing a section of bone to correction of bone defects. The bone implant may act as an attachment point for a mechanical support and/or as a replacement for bone that has been removed due to a critical
20 defect created by disease or trauma. Bone defects can result for example from physical fracture, bone loss from trauma or from surgery to treat diseases such as cancer etc. In some cases, bone defects are so extensive that implantable bone substitutes are required to replace bone loss. Critical-sized bone defects, such as in the mandible, are those that are unable to self-repair without the assistance of implantable bone substitutes. These defects can either be segmental
25 or partial. A segmental defect results in loss of continuity of the host bone and therefore affects the ability to chew, which is not the case for a partial defect.

It would be advantageous for dental implants and bone implants to be able to achieve osseointegration, to improve the chances of success of the implant.
30

The reconstruction of critical-sized segmental defects in bone, following for example surgical removal of diseased or damaged tissue, is an ongoing challenge. Such defects are a common

problem in the treatment of benign and malignant tumours of the jaw and oral cavity. For oral cancer patients, there are additional challenges that are not often encountered in long bone defects, such as oral microflora, postoperative radiotherapy, and the need to dentally rehabilitate patients which involves placement of osseointegrated implants into the neomandible to support a dental prosthesis. Normal daily activities, such as chewing and swallowing, repetitively stress the structural integrity of mandibular implants, highlighting the importance of having sound biomechanical properties. Thus, implants designed for the repair of segmental defects in the mandible should be able to withstand bacterial contamination and multiaxial (occlusal) loading in a compromised tissue environment.

Autologous bone may be used to reconstruct bone defects, either as free grafts or as vascularized flaps; however, these bone implants require long and complex operations that are associated with substantial donor site morbidity and are unable to sufficiently replicate the complex 3D geometries of the facial skeleton. The complex geometry of bones makes them prime candidates for reconstruction using an appropriate biocompatible material which can be prepared easily, such as with 3D-printing.

Titanium is a 3D-printable implant material typically used for manufacture of plates and screws in craniofacial surgeries. It has advantages of being strong, malleable, non-toxic, and integrates with bone. However, titanium also has several drawbacks in the setting of radical cancer treatment where radiotherapy dose intensification and poorly suited mechanical properties can lead to plate failure. Furthermore, X-ray dose shielding may increase the risk of tumour recurrence or delay its detection due to imaging artefacts. Titanium also has an elastic modulus (110 GPa) several times higher than that of cortical (18-20 GPa) or cancellous bone (10-14 GPa). This modulus mismatch is problematic for titanium plates with high cantilever forces, such as those observed in the body of the mandible, where stress shielding and concentration may lead to bone resorption (osteolysis) and gradual screw loosening. This contrasts with other scenarios where axial loading of osseointegrated titanium is associated with favourable outcomes, such as dental implants.

Various polymeric biomaterials are being studied as alternatives to titanium. An advantage of some polymers is that they show deflection or strain when forces are applied that is comparable

to that which would be shown by natural fully formed bone were it to be placed in the same location. This degree of deflection or strain under load is a stimulation for bone to form and be retained. One drawback of polymeric materials is that they may lack sufficient stiffness to act as a suitable fixation to enable new bone to form in the region of the critical defect to form and remain in place while natural repair processes operate. A further drawback of polymeric materials is that they may be bioinert, and therefore fail to integrate fully with newly forming bone tissues in the region surrounding the defect by forming a strong adhesive interface with them that resists mechanical separation when forces are applied. Polymeric biomaterials used to repair bone or for use as a dental implant preferably possess one or more of the following properties:

- osteoinduction (a process that stimulates new bone formation),
- osteoconduction (formation of bone on the surface of a material), and
- osseointegration (a biological phenomenon resulting in the stable anchorage of an implant to the tooth or bone which can be quantified mechanically and/or by the degree of dental/bone-implant contact).

Thus there is a need in the art to provide a polymer-based dental or bone implant that overcomes one or more of the above disadvantages.

Summary

In a first aspect, there is provided a poly ether ketone (PEK) dental or bone implant, wherein at least a portion of the surface of the PEK implant has a water contact angle that permits osseointegration of the PEK implant.

In second aspect, there is provided a poly ether ketone (PEK) dental or bone implant, wherein at least a portion of the surface of the PEK implant has a water contact angle and bioactivity that are conducive to osseointegration of the PEK implant.

As used herein, “a water contact angle and bioactivity that are conducive to osseointegration” refers to a water contact angle and bioactivity that permits or promotes osseointegration. The

amount of osseointegration of an implant can be determined by, for example, calculating the ratio of bone area (BA) to total area (TA) in the zone 0 – 24 μm from the surface of an implant.

In a third aspect, there is provided a poly ether ketone (PEK) dental or bone implant, when
5 formed by additive manufacturing, wherein the surface of the implant has been activated with plasma immersion ion implantation (PIII).

In a fourth aspect, there is provided a method of preparing a poly ether ketone (PEK) dental or bone implant, the method comprising:

- 10 (a) forming the PEK implant from PEK; and
 (b) activating the PEK implant with plasma immersion ion implantation (PIII).

In a fifth aspect, there is provided a method of preparing a poly ether ketone (PEK) dental or bone implant, the method comprising:

- 15 (a) forming the PEK implant from PEK; and
 (b) activating the PEK implant with plasma immersion ion implantation (PIII); and
 (c) heating the PEK implant, then quenching the PEK implant, typically with a gas or liquid.

20 In a sixth aspect, there is provided a poly ether ketone (PEK) dental or bone implant, prepared by the method of the fourth or fifth aspects.

In a seventh aspect, there is provided a dental or bone implant, comprising:

- (a) a PEK dental or bone implant according to any one of first, second, third or sixth
25 aspects; and
 (b) a biologically active agent covalently linked to at least a portion of the surface of the implant.

In an eighth aspect, there is provided a dental or bone implant, comprising:

- 30 (a) a PEK dental or bone implant according to any one of the first, second, third or sixth aspects; and

(b) a biologically active agent covalently linked to at least a portion of the surface of the implant, wherein the biologically active agent is selected from the group consisting of: an antimicrobial peptide, an antibacterial agent, bone cells, encapsulated bone cells, bone precursor cells, encapsulated bone precursor cells, adipose tissue derived stem cells (ADSC's), a bone morphogenic protein or other cell differentiation agent, an anticancer drug, an anticancer targeting agent, an anticancer immunotherapy agent, a membrane receptor targeting agent, vascular cells, immune cells, a calcium phosphate substrate, a calcium phosphate ceramic, periosteum, or any combination thereof.

10

In a ninth aspect, there is provided a method of repairing and/or regenerating bone tissue, comprising implanting into a subject in need thereof a bone implant according to any one of the first, second, third, sixth, seventh or eighth aspects.

15 In a tenth aspect, there is provided a dental implant, comprising:

- (a) a PEK dental implant according to any one of the first, second, or fourth aspects; and
- (b) a dental composite cement bonded to at least a portion of the surface of the dental implant.

20 In an eleventh aspect, there is provided a method of increasing hydrophilicity of a poly ether ketone (PEK) dental or bone implant, comprising activating the implant with plasma immersion ion implantation (PIII).

In a twelfth aspect, there is provided a kit comprising:

- 25
- (a) poly ether ketone (PEK); and
 - (b) instructions for preparing a PEK dental or bone implant using the PEK as a feedstock in a 3D printer.

In a thirteenth aspect, there is provided a method of promoting bone growth, comprising:

- 30
- (a) contacting the PEK implant of any one of the first, second, third, sixth, seventh or eighth aspects with at least a portion of a cross-linked hydrogel matrix comprising stem cells, typically adipose tissue derived stem cells; and

- (b) incubating the PEK implant comprising the cross-linked hydrogel matrix and stem cells under conditions which promote bone growth.

Brief Description of the Drawings

- 5 Figure 1: (A) design of examples of mandible implants with one cylinder and either single or double fixation points; and (B) design of examples of maxillary implants with two cylinders and double fixation points.

- Figure 2: slice preview of an example of grommet implants generated by Simplify3D software. The preview demonstrates the printing layers and print orientation with respect to the build plate (101), removable raft (102), solid infill (103), and perimeter (104).
10

Figure 3: is an example of a mandibular implantation site: two PIII-PEEK-FFF and one PIII-PEK-SLS implants were placed on the left side and one titanium and one untreated PEEK-FFF implants were placed on the right side.

- Figure 4: an example of a maxillary implantation site: one double PIII-PEEK-FFF implant was placed on the left side and one double untreated PEEK-FFF implant was placed on the right side.
15

- Figure 5: histomorphometric analysis of a bone-implant contact (BIC) using an example histology image. The image was segmented to distinguish the implant (105), bone matrix (106) and the two zones of bone area to total area (BA/TA) quantification (107, 108). The two zones (107, 108) were selected for analysis based on their distance from the implant surface: 0-24 μm (107) and 24-80 μm (108).
20

Figure 6: representative (A,B) SEM and (C) AFM images of screw surfaces of PIII-PEEK-FFF. Image B is a high-magnification image of the area highlighted in the box in A.

- Figure 7: representative (A,B) SEM and (C) AFM images of screw surfaces of PIII-PEK-SLS. Image B is a high-magnification image of the area highlighted in the box in A.
25

Figure 8: representative images of a bone-implant interface in mandible implant groups at 8-, 10- and 12-week time points. The scale bar = 1000 μm . One representative implant from each group and the surrounding tissues stained with Goldner's trichrome are shown.

- Figure 9: quantitative analysis of the bone-implant contact (BIC) based on histology staining images in the mandible implant groups at 8-, 10- and 12-week time points. (A) shows results for the zone nearest the implant (0-24 μm), and (B) shows results for the zone further from the implant (24-80 μm). Results show mean $\pm$ standard error of the mean.
30

Figure 10: quantitative analysis of the bone-implant contact (BIC) based on histology staining images in the four types of mandible implants at all time points combined (Titanium $n = 5$; PIII-activated PEEK $n = 10$; Untreated PEEK $n = 5$; PIII-activated PEK $n = 5$). (A) shows results for the zone nearest the implant (0-24 μm), and (B) shows results for the zone further from the implant (24-80 μm). Results show mean $\pm$ standard error of the mean. * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$.

Figure 11: (a) CAD model images of the sheep mandible with a critical-sized partial defect along the inferior border of the ramus, and (b) show a transparent view of the sheep mandible showing the underlying cortical bone and teeth.

Figure 12: (a) 3D CAD model of a porous bone implant reconstructing a partial defect in a sheep mandible. The arrows correspond to the direction of force applied in finite element (FE) analysis to simulate the activation of the muscles of mastication. The grey triangle represents a constrained node to prevent translation in the z -direction. The black line on the condyle (between black triangles) represents a set of constrained nodes that are prevented from translating in the x -, y -, and z -directions and from rotating about the y - and z -axes. The black triangles point to constrained nodes distributed along the black line. Symmetrical boundary conditions are applied to the cross-sectional face of the mandible along its midline; (b) CAD model of the one-body implant with open anterior and posterior ends allowing tissue ingrowth and solid superior plate preventing tissue ingrowth from above; (c) the implant shown in (b) fastened to the mandible with six screws; (d) FE mesh view of the partial defect when the implant is transparent. The soft tissue layer between the tissue bone implant and host bone is 200 μm thick; and (e) The porous tissue section of the bone implant is assembled using Schwarz P-surface unit cells (shown here as $kk=0$ and $aa=2$).

Figure 13: an example of a FDM-PEEK bone implant with (a) isotropic view, (b) side view and (c) underside view; and SLS PEK implant with (d) isotropic view, (e) side view and (f) underside view.

Figure 14: schematic for the mechanical testing protocol for a sheep mandible bone implant: (a) section of sheep mandible with bone implant; (b) schematic of sheep mandible bone-implant system; and (c) schematic of reverse 3-point bending test.

Figure 15: microCT scanned images of FDM-PEEK mandibular implants in sheep 1-4. (A, B): lateral view of the bone ingrowth from each edge of the host mandibular bone at the bone implant contact surface for sheep 1 and 4, respectively; (C, D): medial view of the bone

ingrowth from each edge of the mandibular bone at the bone-implant contact surface for sheep 1 and 4, respectively; (E, F): images for sheep 2 and 3. Due to wound complications, there was no bone ingrowth into the bone implants which detached from the implantation site.

Figure 16: microCT scanned images of SLS-PEK mandibular implants in sheep 1-4. (A-D):

- 5 lateral view of the bone ingrowth from each edge of the host mandibular bone at the bone-implant contact surface for sheep 1, 2, 3 and 4, respectively; (E-H): medial view of the tissue ingrowth originating from each face of the host bone at the bone-implant interface for sheep 1, 2, 3 and 4, respectively.

Figure 17: an example of gyroid TPMS design application to a hollow cylinder of PAEK

- 10 material. A) smooth or graded intersection of gyroid TPMS with “solid” cylinder to minimise stress concentration at the interface between the gyroid and the outer cylinder; and B) biomimetic cortical-bone-like sheath of PAEK material on the cylinder to strengthen against the initiation of surface cracks that lead to bending failure.

Figure 18: shows a biomimetic design applied to an example cylinder with cleaning hole

- 15 principles applied to produce a cleaning hole with minimal stress concentration or failure prone local regions. A) An example location of a hole made on the cortical-like-sheath; B) all seven possible through-hole-axes shown for the first hole location; C) good quality through-hole with highly normal and circular intersection with the cortical-like-sheath (indicated), and bad quality through-holes with non-circular intersection with the cortical-like-sheath (indicated).

Figure 19: shows an example of a bone implant design with cleaning holes.

Figure 20: shows a comparison of water contact angle of untreated (UT) and PIII-activated (PIII) PEEK sheet, FDM printed PEEK and SLS printed PEK. PIII activation was conducted for at least 8 min at 10 kV. Shown are mean values and standard deviation ($n \leq 5$), the measurement for PIII-activated SLS PEK is 0. Above the graph are photographic images of water on the surface of a PEEK sheet (4 μ L), FDM PEEK sheet (1 μ L), or SLS PEK sheet (1 μ L).

- Figure 21: shows effect of Mel1 and Mel4 bound to PAEK specimens against *S. aureus*. Shown are the microbial counts in logarithms of the colony forming units (CFUs)/mL on PEEK sheet, FDM PEEK and SLS PEK surfaces with respect to untreated (UT) and PIII-activated surfaces with either no peptide, inactive peptide (Mel1) or active peptide (Mel4) immobilised to them. Shown are the mean values and standard deviation of 3 replicates.

Figure 22: is a graph showing Saos-2 cell adhesion to PAEK specimen measured using relative fluorescence intensity (gain setting 1522). UT: Untreated, PIII: Plasma Immersion

Ion Implantation activated. The mean value and standard deviation of 2 – 3 replicates are shown. Effect of UT/PIII and presence of Mel4 was tested with t-test within one substrate group.

5 Figure 23: schematic showing displacement of Mel4 from an untreated surface, versus retaining it on a PIII-activated surface.

Figure 24: shows schematic of a half tensile bar prior to bonding with a dental composite cement. Dimensions are in mm. The thickness of the specimens is 2.5mm.

Figure 25: shows areal roughness as measured by an Olympus 3D Laser Measuring Microscope. The 3D root mean square height (Sq) was calculated from the 3D height data.

10 Figure 26: shows maximum stress at failure of composite bonded SLS printed PEK and FDM printed PEEK specimens. Bond surfaces were either PIII-activated (PIII; dark grey) or untreated (UT; light grey) Significances comparing PIII and UT: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.001$.

Figure 27: shows orientations used during fabrication of test bars. The building direction is marked by arrows and the individual printed layers are marked as lines: A) X-orientation with Upper Skin (US) and Lower Skin (LS) marked; B) Y-orientation with bend test maximum tensile stress in-plane with layers; C) XY45-orientation at 45 degrees between X and Y orientations and with bend test maximum tensile stress in-plane with layers; and D) Z-orientation with bend test maximum tensile stress normal to layers.

20 Figure 28: shows a schematic of the annealing process used to increase the strain-to-failure of a printed polymer bone implant. The printed sample (A) is placed in the holder, then transferred into annealing oven (B), to a temperature controlled by thermocouple (C). Following a heating step, the sample is moved to the quenching zone in the air stream (D) using the sample holder (E).

25 Figure 29: shows three-point bend test setup for a Z – orientation sample (see D, Fig. 28). Two supporting cylinders and one loading cylinder apply the three points to bend. The downward pointing arrow marks the direction of applied load, and the two opposing arrows indicate the area of maximum tensile stress.

30 Figure 30: shows comparison of isolated and synergistic effects of new laser parameters “3” (WL_TE) against default parameters “EOS” (EOS_UD) and quenched against non-quenched samples, all grouped by printing orientation. The graphs show the effect of quenching on maximum strain before fracturing (A), maximum stress (B) and bending modulus (C) for the

three different printing orientations, XY45, Y, and Z. Significance at $p < 0.05$ (*); Significance at $p < 0.01$ (**); Significance at $p < 0.001$ (***); Significance at $p < 0.0001$ (****); non-significant (ns).

Figure 31: shows (A) strain, (B) stress, and (C) bending modulus for the combined effects of laser parameter “3” (COBL) combined with annealing versus test material built according to the manufacturer’s standard parameters without quenching (EOS). The graphs show the effect of laser parameter variants on maximum strain before fracturing, maximum stress and bending modulus for the three different printing orientations.

Figure 32: is a graph showing stress as a function of strain, comparing the unquenched and quenched 3D printed test samples printed using parameter “3”.

Figure 33: shows SEM image of the fractured face of one of the quenched bars after testing, the upper surface is the one placed in tension.

Figure 34: shows Micro-CT analysis of SLS printed PIII-PEK chambers with ADSC/GelMA 10 weeks post implantation. Micro-CT Images demonstrated mineralization for lower (A) chambers, indicative of newly formed bone tissues. Mineralisation corresponded to the interface of the scapular bone and the chamber construct. Less mineralisation was evident within upper (B) chambers. Quantitative analyses of mineralisation (C) corroborated qualitative assessments. * $p < 0.05$.

Figure 35: shows H&E staining of upper (A) and lower (B) SLS printed PIII-PEK chambers with ADSC/GelMA 10 weeks post implantation. Arrows indicate periosteum, circles indicate newly formed bone, and stars indicate undegraded GelMA hydrogel. Blood vessels (capillaries) are apparent, indicative of vascularisation. For the upper chamber, substantial new bone appeared to have formed both inside the chamber and at the interface of the periosteum. For the lower chamber, consistent with Micro-CT analysis, bone formation was apparent at the interface of the scapular bone and the chamber construct.

Detailed Description

Definitions

The following acronyms are used throughout to describe the invention. For clarity, these terms are defined below:

Acronym	Meaning
3D	three dimensions, three dimensional

AB	AlamarBlue
ADSC	adipose tissue derived stem cells
AFM	atomic force microscope, atomic force microscopy
ARS	Alizarin Red S
BA/TA ratio	bone area / total area ratio
BIC	bone-implant contact
CAD	computer-aided design
CFU	colony forming units
CT	computed tomography
E	Young's modulus, modulus
EDS	energy dispersive spectroscopy
FBS	foetal bovine serum
FDM	fused deposition modelling
FE	finite element
FFF	fused filament fabrication
GelMA	gelatine methacrylate
HMDS	hexamethyldisilazane
Mel1	an inactive peptide
Mel4	an antimicrobial peptide
MHB	Mueller Hinton broth
MRI	magnetic resonance imaging
PAEK	poly aryl ether ketone
PB	PrestoBlue
PBS	phosphate buffered saline
PEEK	poly ether ether ketone
PEI	polyetherimide
PEK	poly ether ketone
PIII, PI ³	plasma ion immersion implantation
SEM	scanning electron microscope, scanning electron microscopy
SLM	selective laser melting
SLS	selective laser sintering

TPMS	triply periodic minimal surface
------	---------------------------------

The present invention relates to dental and bone implants made from a polymer consisting of ether and ketone chemical groups wherein the sequence of the chemical groups is some repeating sequence of the two types of groups. In some embodiments, the polymer is a poly aryl ether ketone (PAEK), more preferably poly ether ketone (PEK), wherein at least a portion of the surface of the implant has a water contact angle that permits osseointegration of the implant. The polymer is typically fabricated into a customised shape using 3D printing. In some embodiments, at least a portion of the surface of the polymer is treated by ion bombardment, such as plasma, whereby it becomes activated and able to integrate with new natural bone growth by forming strong covalent linkages with at least some of the material components of natural bone and alternatively to form strong covalent linkages with adhesives and composite cements as used in dentistry.

Without wishing to be bound by theory, it is believed that the implants have a surface bioactivity or chemical activity that permits penetration of body fluids to assist in the creation of strong biological linkages that permits osseointegration of the implant to bone. The surface activity also allows strong binding between the implant and dental adhesives or dental composite, for the attachment of surface finishes to prosthetic teeth that may also be made from PAEK.

20

It is advantageous to have a dental or bone implant with a modulus of elasticity, also known as Young's modulus, that matches the corresponding bone as closely as possible, to avoid stress shielding osteolysis that occurs with stiff materials such as titanium.

It is also advantageous for a dental or bone implant to have porosity of an appropriate size and geometry so that living cells can penetrate the structure. A network of pores that enable ingrowth and a surface roughness that encourages on-growth are also desirable properties of a prosthetic tooth or bone implant. Adequate ingrowth and on-growth with associated vascularisation will form tissue that is protected by the natural mechanisms of defence used by the body against infection.

30

It would be desirable for at least a portion of the surface of the dental or bone implant, both interior and exterior, to be wettable or hydrophilic to ensure it is receptive to the binding of biomolecules. It is also desirable that the biological on-growth and ingrowth should be strongly attached to the dental or bone implant so that regenerating bone is securely attached, preferably by strong linkages to regenerating bone or to adhesives, linkages that are in the form of covalent chemical bonds. Furthermore, immediately after insertion of an implant, there is an elevated risk of infection at the site. Infections impede the healing process and can jeopardise the success of the implant. One of the most serious complications of infection is biofilm formation and immune system evasion on the implant surface which is difficult to control with conventional antibiotics. To prevent or reduce biofilm formation, a biologically active agent such as an antimicrobial peptide may be attached to at least a portion of the surface of the PAEK polymer, following activation of the polymer with plasma ion immersion implantation (PIII). The attachment is preferably covalent in nature. Tethering of an antimicrobial to the surface by strong bonds, preferably covalent bonds, has advantages over controlled release into the surrounding tissues, not the least of which is that the antimicrobial action is restricted to the site and is therefore less likely to create unwanted toxicity elsewhere in the body.

Implants previously used for bone repair include implants prepared from autologous bone, and titanium implants, such as 3D-printable titanium implants. However, bone implants prepared from autologous bone suffer from a number of drawbacks such as not being easily sourced, not being easily customisable, and are liable for rejection. Although 3D-printable titanium implants address a number of these issues, the metallic nature is still problematic, especially where the bone repair is associated with cancer which requires subsequent radiotherapy. Further, the modulus of elasticity of titanium implants does not match the corresponding bones, which may lead to complications such as osteolysis.

Materials such as poly ether ether ketone (PEEK) have been used to produce bone implants. However, in its natural form, the surface of this material is not sufficiently receptive to the on-growth and ingrowth of cells, and therefore osseointegration is problematic.

The inventors have found that poly ether ketone (PEK) bone implants address a number of drawbacks of previously described bone implants and have surprisingly improved osseointegration compared to PEEK implants.

- 5 In one aspect, the present invention relates to a poly ether ketone (PEK) dental or bone implant, wherein at least a portion of the surface of the PEK implant has a water contact angle that permits osseointegration of the PEK implant.

10 Implants made of PEK and other similar polymers are typically borderline hydrophobic, meaning that the contact angle with water is closer to 90 degrees than to zero degrees due to the nature of their composition. It is believed that such hydrophobicity resists penetration by body fluids, which may result in insufficient osseointegration. In contrast, the PEK implants of the present invention have a very low water contact angle as a result of activation with PIII. Therefore, the surface of the PEK bone implant of the present invention is hydrophilic.

15

As used herein, “contact angle” refers to the angle between a liquid surface (taken as the tangent of the liquid surface) and a solid surface that the liquid sits on, where they meet. Contact angle quantifies the wettability of a solid surface by a liquid via the Young equation. The contact angle depends upon the medium above the free surface of the liquid, and the
20 nature of the liquid and solid in contact. It is independent of the inclination of solid to the liquid surface. It changes with surface tension and hence with the temperature and purity of the liquid. For the present invention, the contact angle between water and the polymer surface is used, that is, the water contact angle.

- 25 As used herein, a low water contact angle is less than about 45°, preferably less than about 30°, and more preferably less than about 25°. A very low water contact angle is less than about 20°, preferably less than about 15°, more preferably less than about 8°, still more preferably less than 4°.

- 30 In some embodiments, the contact angle may be measured by the sessile drop method. In the sessile drop method, a water droplet of known volume (e.g. 1 µL) is deposited on a surface (for example n = 6 or more) by a syringe. A photograph is taken within 1 minute after droplet

placement and contact angle is measured, such as with a droplet contact angle goniometer (Kruss, Germany) according to the manufacturer's instructions.

In some embodiments, the water contact angle of a PEK implant is in the range of from 0° to 20°, such as from 0° to 15°, 0° to 10°, 0° to 5°, 0° to 4°, 0° to 3°, 0° to 2°, 0° to 1°, 5° to 20°, 10° to 20°, or 15° to 20°. In some embodiments, the water contact angle of the PEK implant is about 0°, about 1°, about 2°, about 3°, about 4°, about 5°, about 6°, about 7°, about 8°, about 9°, about 10°, about 11°, about 12°, about 13°, about 14°, about 15°, about 16°, about 17°, about 18°, about 19° or about 20°. In some embodiments, the water contact angle of a PEK implant is about 0°.

The low water contact angle has been found to improve cell contact with the surface of the PEK implant and permit osseointegration of the PEK implant. Furthermore, the activation of the surface of the PEK implant using PIII also creates the possibility of strong adhesion of biological on-growth and in-growth to the implant that may assist with osseointegration. The low water contact angle also allows biological fluids including those containing bone cells and bone progenitor cells and other cells to penetrate into porous bone implants, so as to encourage the formation of blood vessels known collectively as "vasculature" to ensure that the cells remain in a healthy condition. Additional advantages of PEK over titanium are that PEK possesses bone-like density, stiffness, and radiolucency.

Activating an implant with PIII has been found to impart an ability of the implant surface to create covalent linkages with biologically derived molecules or biologically active agents that come into contact with the surface. This ability to form covalent linkages promotes strong adhesion of the tissue containing bone cells, or an added agent, to the surface of the implant. Exposure of cells and their extracellular medium to a PIII-activated bone implant allows mineralisation within and around the implant, mineralisation that is incorporated within the biological material produced by the cells and this matrix is strongly bonded to the implant. The covalent linkages attach the newly forming bone to the implant and enable strong integration of the newly formed bone to the implant in such a way as to resist loosening.

PIII activation is achieved by exposing a dental or bone implant to a plasma, which may be generated by a dielectric barrier discharge in a gas, such as nitrogen, under reduced pressure. The implant may be exposed to plasma at one or more different reduced pressures, sequentially, and at one or more different orientations, so that the surface receives energetic bombardment by ions and electrons from the plasma under different conditions and from different directions. An example of PIII activation has been described in WO 2021/072502. The implant may be exposed to plasma gas at a pressure in a range selected from about 10 mTorr to about 750 mTorr, such as from 10 mTorr to 350 mTorr, 200 mTorr to 500 mTorr, or 350 mTorr to 700 mTorr. In some embodiments, the implant is exposed to plasma in a gas at a pressure of about 350 mTorr. In other embodiments, the implant is exposed to plasma in a gas at a pressure of about 700 mTorr. In other embodiments, the implant is exposed to plasma in a gas at a pressure of about 350 mTorr, and then at a pressure of about 700 mTorr. In other embodiments, the implant is exposed to plasma in a gas at a pressure of about 350 mTorr, then at a pressure of about 700 mTorr, and then at a pressure of about 350 mTorr. 1 Torr is equivalent to about 1.33 mbar. In some embodiments, the gas is nitrogen gas.

Activation of a dental or bone implant may be achieved by exposing the dental or bone implant to plasma for a period of time in a range from about 1 minute to about 1 hour, such as from 1 minute to 10 minutes, 5 minutes to 30 minutes, or 10 minutes to 50 minutes. The dental or bone implant may be activated by exposing the dental or bone implant to plasma for a total period of time of about 1 minute, about 2 minutes, about 3 minutes, about 4 minutes, about 5 minutes, about 6 minutes, about 7 minutes, about 8 minutes, about 9 minutes, about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, about 50 minutes, about 55 minutes, or about 60 minutes. In some embodiments, the dental or bone implant may be activated by exposing the dental or bone implant to plasma for a total period of about 8 minutes. In other embodiments, the dental or bone implant may be activated by exposing the dental or bone implant to plasma for a total period of about 20 minutes. In other embodiments, the dental or bone implant may be activated by exposing the dental or bone implant to plasma for a total period of about 40 minutes. In the above embodiments, the implant may be exposed to plasma in a gas at one or more reduced pressures. In the above embodiments, the implant

may be exposed to plasma in a gas at one or more reduced pressures and at one or more different orientations.

Activation of a dental or bone implant may be achieved by exposing the dental or bone implant to plasma generated with a negative pulse in a range from about 1 kV to about 20 kV amplitude, such as from 1 kV to 10 kV, 5 kV to 15 kV, or 10 kV to 20 kV amplitude. The dental or bone implant may be activated by a plasma generated with a negative pulse with an amplitude of about 1 kV, about 2 kV, about 3 kV, about 4 kV, about 5 kV, about 6 kV, about 7 kV, about 8 kV, about 9 kV, about 10 kV, about 11 kV, about 12 kV, about 13 kV, about 14 kV, about 15 kV, about 16 kV, about 17 kV, about 18 kV, about 19 kV, or about 20 kV amplitude. In some embodiments, the dental or bone implant may be activated by a plasma generated with a negative pulse with an amplitude of about 10 kV.

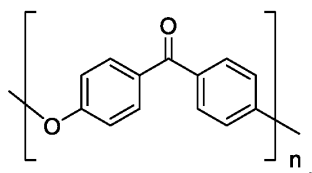
Activation of a dental or bone implant may be achieved by exposing the dental or bone implant to plasma generated with a pulse length in a range from about 1 μ s to about 100 μ s, such as from 1 μ s to 20 μ s, 5 μ s to 25 μ s, 10 μ s to 30 μ s, 10 μ s to 50 μ s, 20 μ s to 60 μ s, or 30 μ s to 70 μ s. The dental or bone implant may be activated by a plasma generated with a pulse length of about 1 μ s, about 5 μ s, about 10 μ s, about 15 μ s, about 20 μ s, about 25 μ s, about 30 μ s, about 35 μ s, about 40 μ s, about 45 μ s, about 50 μ s, about 55 μ s, about 60 μ s, about 65 μ s, about 70 μ s, about 75 μ s, about 80 μ s, about 85 μ s, about 90 μ s, about 95 μ s, or about 100 μ s. In some embodiments, the dental or bone implant may be activated by a plasma generated with a pulse length of about 20 μ s. In some embodiments, the dental or bone implant may be activated by a plasma generated with a pulse length of about 40 μ s.

Activation of a dental or bone implant may be achieved by exposing the dental or bone implant to plasma generated with a pulse repetition frequency in a range from about 200 Hz to about 2000 Hz, such as from about 200 Hz to about 1200 Hz, about 500 Hz to about 1500 Hz, or about 1000 Hz to about 2000 Hz. In some embodiments, the dental or bone implant may be activated by a plasma generated with a pulse repetition frequency of about 1000 Hz.

Other advantages of PEK over implant materials such as titanium are that it is relatively affordable, lightweight, radiolucent, has excellent mechanical properties, excellent chemical resistance under physiological conditions, and is non-metallic. Being non-metallic and of low

density means that the implant does not interfere with treatment processes (such as radiotherapy) or imaging or other analysis techniques (such as X-ray and MRI) that are typically seen with the types of metals used for bone implants.

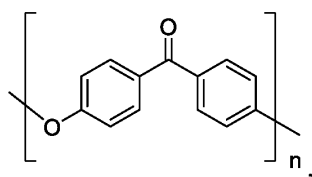
- 5 PEK has the following chemical structure:



wherein n represents the number of repeating units. The average molecular weight of the PEK is typically in the range of 5,000 to 200,000. For example, the average molecular weight of PEK may be within the range of from 5,000 to 50,000; 10,000 to 100,000; 20,000 to 150,000; or 30,000 to 200,000. In some embodiments, the average molecular weight of PEK may be about 5,000, about 10,000, about 15,000, about 20,000, about 25,000, about 30,000, about 35,000, about 40,000, about 45,000, about 50,000, about 55,000, about 60,000, about 65,000, about 70,000, about 75,000, about 80,000, about 85,000, about 90,000, about 95,000, about 100,000, about 150,000, or about 200,000.

15

The number of repeating units, n, for a PEK polymer of a known average molecular weight can be calculated by dividing the average molecular weight by the molar mass of the PEK repeating unit. The molar mass of the PEK repeating unit shown below is about 196.21. Accordingly, in some embodiments, PEK has the following chemical structure:



20

wherein n is from about 25 to about 1000. For example, n is from about 25 to about 250; about 50 to about 500; about 100 to about 750; or about 150 to about 1000. In some embodiments, n may be about 25, about 50, about 75, about 100, about 125, about 150, about 175, about 200, about 225, about 250, about 275, about 300, about 325, about 350, about 375, about 400, about 425, about 450, about 475, about 500, about 750, or about 1000.

25

The above polymer is readily prepared from simple starting materials, and following polymerisation is rigid and has excellent chemical resistance compared to other poly ether ketones. The preparation of PAEK polymers such as PEK has been described in Concise Polymeric Materials Encyclopedia (Ed. Joseph C. Salamone, 1999, CRC Press), and these
5 polymers are commercially available, such as from EOS Gmbh (<https://www.eos.info/polymer-solutions/polymer-materials>).

When prepared in the manner specified in some embodiments described herein, the PEK implant has a surface texture (roughness) that further assists in the attachment of cells and
10 integration of the implant into tooth or bone tissue. The surface of such implants has cavities or recesses, as well as protrusions, typically in the form of polymer grains bonded together but not fully melted together. Such cavities encourage the formation of mechanical interlocking that increases the mechanical strength of the interface between the PEK implant and the biologically-derived material.

15

Surface roughness is quantified by measuring the arithmetic average of the absolute values of surface height deviations measured from the mean plane, over a given line (profile roughness) or area (areal roughness). Areal roughness is a better measure of roughness, as it takes into account a larger data set. If the deviations from the mean plane are large, the surface is rough;
20 if they are small, the surface is smooth. Roughness can be measured using any means known in the art, such as by atomic force microscopy, laser microscopy, or optical interference microscopy. Areal roughness can be measured by calculating the squared mean height, Sq (the mean difference in height) over an area. Profile roughness can be measured by calculating the root mean square deviation (Rq) over a given line, to provide a RMS value (standard deviation
25 of the height distribution). A PEK implant with a “rough” surface can be considered to have an areal roughness of from about 10 μm to about 25 μm , such as from about 10 μm to about 20 μm , from about 15 μm to about 25 μm , or from about 15 μm to about 20 μm . A PEK implant with a “rough” surface can be considered to have a profile roughness of from about 10 μm to about 25 μm , such as from about 10 μm to about 20 μm , from about 15 μm to about 25 μm , or
30 from about 15 μm to about 20 μm .

A PEK implant with a “very rough” surface can be considered to have an areal roughness of from about 25 μm to about 50 μm , such as from about 25 μm to about 40 μm , from about 30 μm to about 50 μm , or from about 30 μm to about 40 μm . A PEK implant with a “very rough” surface can be considered to have a profile roughness of from about 30 μm to about 60 μm ,
5 such as from about 40 μm to about 60 μm , from about 30 μm to about 50 μm , or from about 40 μm to about 50 μm . In some embodiments, roughness is measured by laser microscopy according to ISO25178.

In some embodiments, at least a portion of the surface of the PEK implant has an areal
10 roughness in the range of from about 100 nm to about 50,000 nm (50 μm). In some embodiments, at least a portion of the surface of the PEK implant has a profile roughness in the range of from about 100 nm to about 50,000 nm (50 μm). In some embodiments, the areal or profile roughness may be within a range of from 100 nm to 200 nm, 130 nm to 150 nm, or 120 nm to 1,000 nm. In other embodiments, the roughness may be within a range of from 1
15 μm to 50 μm , such as from 5 μm to 25 μm , 15 μm to 30 μm , 15 μm to 20 μm , 25 μm to 50 μm , 30 μm to 45 μm , or 35 μm to 45 μm . In one embodiment, the surface has a saw-tooth structure to increase surface area, wherein the roughness of the surface with saw-tooth structure is in the range of from about 100 nm to about 300 nm. Without wishing to be bound by theory, it is believed that a high roughness assists with osseointegration owing to the effect of
20 mechanical interlocking. In some embodiments, the implant has an areal or profile roughness in the range of from 10 μm to 30 μm , such as from about 10 μm to about 20 μm , from about 15 μm to about 25 μm , or from about 15 μm to about 20 μm . In other embodiments, the implant has an areal or profile roughness in the range of from about 25 μm to about 60 μm , such as from about 25 μm to about 50 μm , from about 35 μm to about 60 μm , or from about 35 μm to
25 about 50 μm .

In some embodiments, the PEK implant permits osseointegration, wherein osseointegration of the PEK implant has occurred when the ratio of bone area to total area (BA/TA), in the zone 0 – 24 μm from the surface of the PEK implant, is from about 0.30 to 0.80 as measured from 8
30 weeks to 12 weeks from time of implantation. For example, the BA/TA ratio in the zone 0 – 24 μm from the surface of the PEK implant, may be in a range of from about 0.30 to about 0.60, or about 0.40 to about 0.70, or about 0.50 to about 0.80, as measured from 8 weeks to 12

- weeks from time of implantation. In some embodiments, the BA/TA ratio in the zone 0 – 24 μm from the surface of the PEK implant, is in a range of from about 0.60 to about 0.80, as measured from 8 weeks from time of implantation. In some embodiments, the BA/TA ratio in the zone 0 – 24 μm from the surface of the PEK implant, is in a range of from about 0.40 to about 0.60, as measured from 10 weeks from time of implantation. Advantageously, as described in the Examples, the BA/TA ratio for PEK is greater than for PEEK, as measured from 8 weeks to 12 weeks from time of implantation. Thus, the PEK implant of the invention exhibits greater osseointegration than the PEEK implant described herein.
- 10 In another aspect, the present invention relates to a poly ether ketone (PEK) dental or bone implant, when formed by additive manufacturing, wherein the surface of the implant has been activated with the plasma treatment process known as plasma immersion ion implantation (PIII).
- 15 As used herein, “activated” refers to modification of the surface functional groups of the polymer by exposure to plasma. The modification typically results in conversion of a hydrophobic polymer to a hydrophilic state. Without wishing to be bound by theory, it is believed that the modification is due to conversion of one or more functional group in the polymer into a group which is hydrophilic and/or functionalisable, for example, it has been
- 20 shown that carbon centred radicals and to a lesser extent oxygen centred radicals are created in the polymer, which could then be further functionalised by exposure to, for example, a biologically active agent or is otherwise hydrophilic in nature, allowing interaction with body fluids following insertion of the dental or bone implant.
- 25 As used herein, “additive manufacturing” refers to production of a three-dimensional product by depositing a material in layers. An example of additive manufacturing is 3D printing. The inventors have found that producing PEK implants by 3D printing results in surface features and/or surface chemistry that may assist in osseointegration of the implant. Implants made by methods other than additive manufacturing, such as injection moulding, do not contain the
- 30 same surface features compared to 3D-printed implants made by additive manufacturing. In addition, it is of great advantage to form a bone implant by additive manufacturing, since bones are not typically flat or straight, or the same size between individuals, meaning that a cast for

a polymer bone implant is typically useful only for the bone defect of one individual. By contrast, there is no need to prepare a cast of a bone implant for additive manufacturing. To prepare a PEK bone implant, the area requiring repair is scanned non-invasively by computed tomography to provide precise geometrical dimensions for the bone implant, as described in the Examples, before printing the bone implant on a 3D-printer. Activation with plasma immersion ion implantation to change the surface chemistry of a PEK dental or bone implant, is quick, simple and can be performed on any shape of bone implant. This technique can also be used to sterilise the implant, which would eliminate one step in the process of providing an implant to a patient awaiting surgery.

10

In some embodiments, the PEK implant is prepared by sintering. A surface prepared by sintering may be referred to as a sintered surface. Typically, the sintering is selective laser sintering (SLS). SLS has the particular advantage of generating a rough surface, with the roughness being due, for example, to the size of the polymer particles that are fused together with this technique and/or density of packing of particles, which may be controlled by the printing parameters. It is believed that the greater roughness, along with the chemically modified surface due to activation with plasma immersion ion implantation, improves osseointegration of the PEK implant to a tooth or to bone.

15

In some embodiments, the PAEK polymer has a particle size in the range of from 10 μm to 200 μm in diameter, such as from 10 μm to 90 μm , 20 μm to 80 μm , 40 μm to 120 μm , or 50 μm to 200 μm in diameter. In some embodiments, the PAEK polymer, such as PEK, has a particle size of about 10 μm , about 20 μm , about 30 μm , about 40 μm , about 50 μm , about 60 μm , about 70 μm , about 80 μm , about 90 μm , about 100 μm , about 110 μm , about 120 μm , about 130 μm , about 140 μm , about 150 μm , about 160 μm , about 170 μm , about 180 μm , about 190 μm , or about 200 μm in diameter, or any combination thereof. In some embodiments, the PAEK is poly ether ketone (PEK). In one embodiment, the PAEK polymer is PEK, and the PEK polymer has a particle size of about 40 μm in diameter. In another embodiment, the PAEK polymer is PEK, and the PEK polymer has a particle size of about 60 μm in diameter.

25

30

In one embodiment, there is provided a PEK bone implant comprising sintered PEK, wherein the sintered PEK has a water contact angle and/or surface chemistry that permits osseointegration of the PEK bone implant to bone.

- 5 An advantageous property of a dental or bone implant is the ability of the dental or bone implant to bear load, especially in cases where an amount of tooth or bone material has been surgically removed so that the remaining bone no longer has sufficient load-bearing capability. The load bearing capability of the dental or bone implant would ideally offer adequate fixation to the tooth or bone to allow regrowth across the interfaces as well as
- 10 sufficient resistance to fracture induced by large stresses, especially suddenly applied stresses. These aspects are measured by a sufficiently high fracture strength and a sufficiently large strain to failure. The resistance to fracture in impact loadings is in part determined by the ability to absorb energy without failure by fracture. The energy absorbing properties are determined by high values of both fracture strength and strain to failure.

15

- Young's modulus (E , modulus) is a mechanical property that measures the tensile or compressive stiffness of a solid material when a force is applied lengthwise. It quantifies the relationship between tensile/compressive stress, σ (force per unit area) and axial strain ϵ (proportional deformation) in the linear elastic region of a material. Young's modulus may
- 20 be measured using linear regression of the stress/strain curve for a given sample, such as between strains of 0.05% and 0.25% strain. The stress/strain curve can be generated with the stress calculated by dividing the measured force by the nominal cross-sectional area of the sample, whilst strain can be calculated using the displacement of the crosshead with the distance between grips as the nominal gauge length, as per ISO 527.

25

- In some embodiments, the Young's modulus of a PEK implant is in the range of from about 3.7 GPa to about 5.0 GPa, for example, from about 3.8 GPa to about 4.2 GPa, or from about 3.9 GPa to about 4.5 GPa, or from about 4.0 GPa to about 4.7 GPa. In some embodiments, the Young's modulus of a PEK implant is about 3.7 GPa, about 3.8 GPa, about 3.9 GPa,
- 30 about 4.0 GPa, about 4.1 GPa, about 4.2 GPa, about 4.3 GPa, about 4.4 GPa, about 4.5 GPa, about 4.6 GPa, about 4.7 GPa, about 4.8 GPa, about 4.9 GPa, or about 5.0 GPa.

The inventors have found that the load bearing performance of the PEK bone implant can be improved by an annealing step which comprises a heating step followed by a rapid quench of its surface, typically accomplished by cooling in a fluid, such as a liquid or a flowing air stream. Although such a process has been described for toughening glass by inducing
5 compressive stress in the surface layers, it is not known for treating polymer bones or bone implants. The likely effect of this treatment on PEK is to encourage better bonding between the fused particles on the surface and the formation of an amorphous phase on the surface. “Amorphous” refers to the lack of crystalline order and is believed to be advantageous in increasing the toughness or fracture resistance of a material in cases where planes of
10 weakness are created between or within crystals. The effect of both of these changes is to suppress the initiation of cracks on the surface that could act as sites where cracks rapidly propagate throughout the structure to cause failure by brittle fracture.

Accordingly, in one embodiment, the PEK dental or bone implant has been annealed, and then
15 quenched, typically with a liquid or a gas, such as an air stream.

Annealing of the bone implant is a treatment which comprises heating the bone implant to a temperature, for example above its glass transition temperature, and then rapidly cooling the dental or bone implant. The rapid cooling step is herein referred to as quenching. Typically,
20 the bone implant is quenched with a gas, such as for example, a flowing air stream. It is believed that quenching serves two purposes, first to increase the content of the amorphous phase relative to the crystalline phase and potentially also to induce compressive stress in the surface layers. The amorphous phase has a greater strain to failure and also a greater fracture resistance and the compressive stress, when present, has the effect of reducing tensile stress in
25 the surface as a result of bending.

The strength of the PEK dental or bone implant can be improved by encasing the porous part of the bone implant in a strong, low porosity outer layer of PEK, imitating the design of the fracture resistant outer layer in human bone known as “cortical” bone, which encases the
30 weaker “trabecular” bone. When placed under a bending load, the tensile stress is maximum in the outer part of the bone implant, where the material can be subjected to large stresses and may produce large strains but without failure. The bending stress is accommodated in the

outer layer where initiation of a crack is unlikely, and the entire object is prevented from failing by fracture. This design also allows the bone contacting faces of the implant to be made porous and therefore conducive to the ingrowth of bone and potentially the development of blood vessels (vascularisation).

5

The outer cortical component may be treated as described above to increase its resistance to tensile fracture by controlling its crystallinity and stress level by annealing that portion of the bone implant. Such treatment increases strain to failure of the outer layer which prevents the formation of tensile cracking in the outer layer that could lead to failure of the whole object.

- 10 Crystallinity can also be controlled and reduced in the surface by treatment with ions from a plasma in the PIII process, the same process that has been described above for reducing the water contact angle and imparting covalent bonding capability.

- In one embodiment, the bone implant has a single gyroid triply periodic minimal surface structure. A single gyroid triply periodic minimal surface (TPMS) structure is a promising structure for designing additively manufactured porous structures for allowing porosity in bone implants while retaining as much strength as possible. It can be additively manufactured with relative ease, has a high surface to volume ratio, contains a single uniformly interconnected void space, the surface has uniform and consistent curvature, and has highly consistent stiffness modulus and strength across different loading orientations.
- 15
- 20

- The design of a load bearing bone-replacement implant using a TPMS structure requires some consideration. Where the TPMS structure is terminated to match an arbitrary anatomical geometry there exists opportunity for very poor-quality design subject to mechanical failure. The TPMS cells will be cut in some regions, creating solid sections of small cross-sectional area that are likely to fracture when loaded, especially under a bending load where the maximum tensile stress is located on the outermost surface. This can be seen in Fig. 17A where a cylinder has been used as a simple representation of a long bone and the gyroid TPMS is applied. Across the curvature of the cylinder, the gyroid terminates differently, resulting in regions of poor-quality mechanical design. The inventors have found that by including a cortical-bone-like sheath on the outer surfaces, where penetration for osseointegration is not required, such mechanical issues are resolved. This can be seen in Fig.
- 25
- 30

17B where the top and bottom of the cylinder are left open for bone to penetrate. The interface between the cortical-sheath and the TPMS structure is graded to provide a smooth transition free of stress concentrations in sharp corners. This cortical-like-sheath provides maximum strength in the regions of maximum stress during a bending load, and eliminates
5 the poorly terminated sections of the TPMS lattice.

Some consideration should be given to cleanability since the design is manufactured using Selective Laser Sintering (SLS), a 3D Printing technology using a powder precursor. SLS printing results with the manufactured parts embedded in a polymer powder cake. The parts
10 should preferably be cleaned of unsintered and semi-sintered polymer powder. For objects like these, this is often done through blasting the part with media such as glass bead or dry ice. These techniques rely on 'line of sight' for the blasting trajectory of the blasting media to reach the interior surfaces of the object. While the gyroid has favourable cleanability (see Fig. 18), thanks to seven axes of through-hole-clearance (where a woodpile structure for example
15 only has 3), the cortical-like sheath would make this bone implant difficult to clean unless suitable access holes are provided. A compromise must therefore be reached between the number and location of access holes and the fracture toughness of the structure. The inventors have addressed this problem by the addition of cleaning holes to the cortical sheath that make the structure cleanable, while minimising the mechanical weakness they impart on
20 the structure. The cleaning holes are typically designed so that they align with the inherent through-hole-axes of the TPMS lattice to allow 'line of sight' for blasting media to travel through the bone implant (see Fig. 18C).

Fig. 19 shows an example of a bone implant with cleaning holes. The principles for selecting
25 the position of the holes are as follows:

- a) Holes shall be aligned with one of the 7 through hole axes of the TPMS Gyroid lattice or similar TPMS structure.
- b) The first through hole axis chosen will be the one that most closely aligns with a normal to the outer surface of the cortical-like layer.
- 30 c) Where multiple options for the choice of axis in b are available, holes shall be selected to minimise the number of axes without a cleaning hole.

- d) Holes shall be circular (to minimise the stress concentration around the hole) and of a nominal diameter close to or matching the average or typical diameter of the through-hole void spaces of the TPMS lattice.
- e) Holes shall be spaced maximally apart to optimise cleaning while minimising stress concentration in a local region.

The method by which the holes are specified is related to crack initiation theory and stress concentration factors. The selected shape of a hole to reduce stress concentrations in an arbitrary direction for bending is a circular hole which is aligned to one of the through-hole-axes. However, depending on where the surface of the cortical sheath intersects with the TPMS there can be multiple through-hole-axes that could be used for cleaning hole alignment. Some of the through hole axes are undesirable for use at certain surface plane orientations as they create near tangency glancing holes resulting in thin weak features. The method by which the axis that is chosen for the circular cleaning hole is dependent on the nature of the surface where the hole exits the bone implant as well as surrounding holes. The axis that is most normal to the plane at the point of intersection of the axis on the surface is chosen to maximise the strength of the object. In addition, the holes have a nominal minimal spacing from one another to avoid large stress concentrations.

Preferably, the surface of the PEK bone implant has a low water contact angle, such as in the range of from 0° to about 20°. In this regard, the surface of the PEK bone implant is hydrophilic. It is believed that hydrophilicity of the surface improves osseointegration of the PEK bone implant to bone by allowing improved contact with biological fluids.

Contact angle may be measured using any method known in the art for measuring contact angle. For example, the water contact angle may be measured using the static sessile drop method, by evaluation of a photographic image of a sessile (fixed) water droplet of known volume on a sessile droplet contact angle goniometer, such as a Kruss GmbH Germany Drop Shape Analysis System DSA 10 Mk2. In some embodiments, the volume of the sessile water droplet is 4 µL. In other embodiments, the volume of the sessile water droplet is 1 µL.

In another aspect, the present invention relates to a method of preparing a poly ether ketone (PEK) dental or bone implant, the method comprising:

- (a) forming the PEK implant from PEK; and
- (b) activating the PEK implant with plasma immersion ion implantation (PIII).

5

Advantageously, the activation with plasma results in a hydrophilic surface, which permits osseointegration of the PEK dental or bone implant. The activated surface also advantageously allows covalent attachment of the PEK surface to other useful substances, such as biologically active agents, ceramics such as calcium phosphate ceramic, substrates, such as a calcium phosphate substrate, dental adhesives, and dental composite cement.

10

Typically, the PEK implant is formed by additive manufacturing. In one embodiment, the implant is formed using FFF, where the PAEK material is supplied as a filament. In another embodiment, the implant is formed using selective laser sintering (SLS) where the PAEK material is supplied as powder and sintered together using a laser beam.

15

PIII is typically carried out in an ionised gas under reduced pressure, or at two or more reduced pressures, sequentially, and uses an electric field to implant ions, typically nitrogen or argon or other inert gas ions, into the dental or bone implant structure, creating unpaired electrons (sometimes referred to as carbon-centred persistent free radical groups) that are capable of diffusion within the structure of PAEK. The PIII process creates a reservoir of buried free radicals that is capable of diffusion to the surface. This reservoir has beneficial effects for the PEK bone implant of the invention. Firstly, the presence of the free radicals on the surface of the polymer leads to a lowering of the surface energy which is manifested by a reduction in the water contact angle, increasing the wettability or hydrophilicity of the surface, and as a result of the size and depth of the reservoir created by PIII, this lowering of contact angle may remain for months and even years before the reservoir of persistent free radicals is exhausted. Secondly, the presence of free radicals within the PIII-activated structure that can diffuse to the surface enables the formation of covalent linkages to biological molecules that are in contact with the surface and present within the wetting layer of fluid on the surface.

20

25

30

Examples of gases used for ionisation in the PIII process include hydrogen, helium, nitrogen, oxygen, argon, ammonia, water vapour, methane, ethylene, acetylene, other hydrocarbons, or other inert gases, or other aminated or oxygenated hydrocarbons or any combination of the aforementioned gases and vapours.

5

In some embodiments, to obtain an effective PIII treatment of a porous structure such as a bone scaffold or dental implant containing provision for osseointegration, the PIII treatment is provided to the interior as well as exterior surfaces. For this purpose, a two stage or multiple stage process operated at various gas pressures below atmospheric pressure is preferred to allow the plasma to penetrate into the porosities. The inventors have generally found that higher pressures favour the penetration of the plasma into smaller porosities and hence the treatment of the surfaces of smaller porosities, while lower pressures generally promote more uniform treatments of the exterior surfaces.

Following exposure to plasma and ionised gas, the dental or bone implant can optionally be exposed to a functionalising gas such as oxygen, nitrogen, methane, acetylene, other hydrocarbons, or other aminated or oxygenated hydrocarbon gases or vapours any combination thereof to generate a range of functional groups on the surface. In addition the surface can be functionalised with any molecule that can be bound to the surface in a liquid phase incubation step, including molecules that perform some biological function such as infection control, permanent contact angle control, cell differentiation, cell signalling, cell attachment or cell spreading.

In some embodiments, the PIII activation is carried out as described in WO 2021/072502.

In some embodiments, the method further comprises:

annealing the PEK implant, comprising heating the implant, typically to a temperature above the glass transition temperature of PEK (about 150°C), and then quenching the implant, typically with a gas.

30

The implant may be annealed by heating to a temperature in the range of from 100°C to 360°C, such as from 150°C to 350°C, from 220°C to 345°C, from 280°C to 340°C, or from 290°C to

340°C. The implant may be annealed by heating to a temperature selected from about 100°C, about 110°C, about 120°C, about 130°C, about 140°C, about 150°C, about 160°C, about 170°C, about 180°C, about 190°C, about 200°C, about 210°C, about 220°C, about 230°C, about 240°C, about 250°C, about 260°C, about 270°C, about 280°C, about 290°C, about 5 300°C, about 310°C, about 320°C, about 330°C, about 340°C, about 350°C or about 360°C. In one embodiment, the implant is annealed by heating to a temperature of about 300°C. In another embodiment, the implant is annealed by heating to a temperature of about 330°C.

The implant may be annealed by heating to a temperature as stated above, for an amount of 10 time from 20 seconds to 300 seconds, such as from 100 seconds to 290 seconds, 220 seconds to 280 seconds, or 230 seconds to 260 seconds. The implant may be annealed by heating to a temperature as stated above, for an amount of time selected from about 20 seconds, about 30 seconds, about 40 seconds, about 50 seconds, about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds, about 120 seconds, about 15 130 seconds, about 140 seconds, about 150 seconds, about 160 seconds, about 170 seconds, about 180 seconds, about 190 seconds, about 200 seconds, about 210 seconds, about 220 seconds, about 230 seconds, about 240 seconds, about 250 seconds, about 260 seconds, about 270 seconds, about 280 seconds, about 290 seconds, or about 300 seconds. In some embodiments, the implant is annealed by heating for an amount of time of about 240 seconds.

20 Following heating, the implant is cooled, typically with a gas or liquid. In one embodiment, the implant is cooled with a gas. In one embodiment, the implant is cooled with a liquid. Typically, the cooling is rapid. The amount of time that the implant is cooled for is not particularly limited, as long as the implant reaches room temperature. In one embodiment, the 25 implant is cooled for about 50 seconds.

As discussed above, it is believed that annealing followed by rapid quenching with a fluid results in a tougher bone implant. In preferred embodiments, the annealing is performed before activating the PEK implant with PIII. In other embodiments, the annealing is performed after 30 activating the PEK implant with PIII.

The purpose of the gas is for cooling of the annealed implant. Consequently, the type of gas is not critical provided the gas is capable of cooling the annealed implant. In some embodiments, the gas is an air stream.

- 5 In other embodiments the cooling medium is a liquid rather than a gas.

Typically, the PEK used to prepare an implant, before 3D printing, is in the form of a powder. An advantage of using a powder is that the polymer does not need to be prepared as a fibre, which is required in some forms of additive manufacturing. A further advantage is that the
10 powder produces a rougher surface than a fibre following sintering, resulting in a rougher surface of the bone implant. The roughness of the surface will depend on the particle size, density of packing of particles which will be determined by the printing parameters, and any post-printing treatments such as polishing or heat processing (annealing) to encourage flowing together of the partially fused grains.

15

In another aspect, the present invention relates to a poly ether ketone (PEK) bone implant for bone repair, prepared by the method described herein.

In another aspect, the present invention relates to a method of repairing and/or regenerating
20 bone tissue, comprising implanting into a subject in need thereof a bone implant as described herein. In another aspect, the present invention relates to a method of anchoring a dental implant, comprising implanting into a subject in need thereof a dental implant as described herein. Advantages of using PEK, over PEEK, include improved osseointegration, as measured by BA/TA ratio in the zone 0 – 24 μm from the surface of the PEK implant, as
25 measured from 8 weeks to 12 weeks from time of implantation. Specifically, the BA/TA ratio for PEK when prepared by selective laser sintering, is greater than for PEEK when prepared by fused filament fabrication (FFF), as measured from 8 weeks to 12 weeks from time of implantation.

- 30 In some embodiments, the implant comprises a biologically active agent. In some embodiments, the biologically active agent is covalently linked to at least a portion of the surface of the implant after PIII activation and before being implanted. In some embodiments,

the biologically active agent is selected from the group consisting of: an antimicrobial peptide (for example, with bone cell adhesion promoting properties), an antibacterial agent, bone cells, encapsulated bone cells, bone precursor cells, encapsulated bone precursor cells, stem cells (e.g., adipose tissue derived stem cells (ADSC's)), a cell differentiation agent such as bone morphogenic protein (BMP), an anticancer drug, an anticancer targeting agent, an anticancer immunotherapy agent, a membrane receptor targeting agent, vascular cells, immune cells, a calcium phosphate substrate, a calcium phosphate ceramic, periosteum, or any combination thereof. Advantages of an antimicrobial or antibacterial growth promoting agent include greatly reduced bacterial count, and improved cell adhesion. In one embodiment, the biologically active agent is covalently linked to at least a portion of the surface of the implant, typically by means of incubation in a liquid medium containing the active agent. Without wishing to be bound by theory, it is believed that success of surface bound antimicrobial peptides may be due to the strength of the linkage between the peptide and the surface, since the Vroman effect will result in more or less rapid removal by displacement of the peptides when other biomolecules have a higher affinity for the surface. It is believed that prior activation of a PEK surface with PIII allows covalent linkages to form between the surface and a biologically active agent, which prevents displacement.

In some embodiments, the antimicrobial peptide is Mel4.

In some embodiments, the biologically active agent is in the form of a hydrogel. In one embodiment, the hydrogel is gelatin methacrylate (GelMA).

In one embodiment, the implant comprises a hydrogel. In one embodiment, the hydrogel is a cross-linked hydrogel matrix. In one embodiment, the cross-linked hydrogel matrix comprises one or more biologically active agents. In one embodiment, the biologically active agent is a cell. In one embodiment, the biologically active agent is a stem cell. In one embodiment, the stem cell is an adipose tissue derived stem cell. In one embodiment, the adipose tissue derived stem cell is pre-differentiated to osteogenic lineage (i.e. osteoinduced) using osteogenesis differentiation medium prior to implantation to a subject.

In one embodiment, the hydrogel is cross-linked gelatin methacrylate.

As described in the examples, incorporation of cross-linked gelatin methacrylate and osteoinduced adipose tissue derived stem cells into PEK implants as described herein results in ectopic bone formation.

5

Accordingly, one aspect provides a method of promoting bone growth, comprising:

(a) contacting a PEK implant as described herein with at least a portion of a cross-linked hydrogel matrix comprising stem cells; and

10 (b) incubating the PEK implant comprising the cross-linked hydrogel matrix comprising stem cells under conditions that promote bone growth.

In one embodiment, the PEK implant is in contact with at least a portion of the cross-linked hydrogel matrix comprising stem cells.

15 In one embodiment, the PEK implant is coupled to at least a portion of the cross-linked hydrogel matrix comprising stem cells.

In one embodiment, the PEK implant is covalently coupled to at least a portion of the cross-linked hydrogel matrix comprising stem cells.

20

In one embodiment, the cross-linked hydrogel matrix is a cross-linked gelatin methacrylate matrix.

25 In one embodiment, the conditions that promote bone growth comprises implanting in a subject the PEK implant comprising the cross-linked hydrogel matrix and osteoinduced stem cells.

In one embodiment, implanting the PEK implant comprising the cross-linked hydrogel matrix and osteoinduced stem cells comprises arranging the implant in tissue such that the implant is in contact with bone or periosteum. Typically, the implant is in contact with bone or periosteum such that at least a portion of the cross-linked hydrogel matrix is in contact with bone or periosteum. In one embodiment, the implant is in contact with bone. In another embodiment, the implant is in contact with periosteum.

30

In one embodiment, the stem cells are adipose tissue derived stem cells.

In one embodiment, the PEK implant forms a chamber in which the cross-linked hydrogel
5 matrix is contained.

In another aspect, the present invention relates to a method of repairing a tooth, comprising implanting into a subject in need thereof a dental implant as described herein.

- 10 In another aspect, the present invention relates to a dental or bone implant, comprising:
- (a) a PEK dental or bone implant as described herein; and
 - (b) a biologically active agent covalently linked to at least a portion of the surface of the implant.
- 15 In some embodiments, the biologically active agent covalently linked to the surface of the implant comprises an agent selected from the group consisting of: an antimicrobial peptide, an antibacterial agent, bone cells, encapsulated bone cells, bone precursor cells, encapsulated bone precursor cells, adipose tissue derived stem cells (ADSC's), a bone morphogenic protein or other cell differentiation agent, an anticancer drug, an anticancer agent such as a targeting agent, an anticancer immunotherapy agent, a membrane receptor targeting agent, vascular cells, immune cells, a calcium phosphate substrate, a calcium phosphate ceramic, periosteum, or any combination thereof.
- 20

In another aspect, the present invention provides a dental implant, comprising:

- 25
- (a) a PEK dental implant as described herein; and
 - (b) a dental composite cement bonded to at least a portion of the surface of the dental implant.

In one embodiment, the dental composite cement is covalently linked to at least a portion of
30 the dental implant.

In another aspect, the present invention provides a dental implant, comprising:

- (a) a PEK dental implant as described herein; and
- (b) a dental composite cement that is covalently linked to a portion of the surface of a PEK implant, wherein the dental composite forms a functional coating on the surface of the PEK implant to increase the durability of the coated surface and improve the cosmetic appearance of the surface, such as where the implant functions as a tooth.

In another aspect, the present invention relates to a method of increasing hydrophilicity of a poly ether ketone (PEK) dental or bone implant, comprising activating the implant with plasma immersion ion implantation (PIII). In preferred embodiments of this method, at least a portion of the surface of the activated implant has a water contact angle in the range of from 0° to about 20°. Whilst there may be other chemical or physical methods of increasing hydrophilicity of a polymer surface, an advantage of using a plasma is that it is simple, quick, clean, and can be performed on a dental or bone implant of any shape. Other advantages of the plasma immersion ion implantation process are that it has a sterilizing action and is a non-contaminating dry process.

A further aspect provides a method of increasing the load bearing capability of a dental or bone implant formed from a poly aryl ether ketone polymer, the method comprising:

- (a) heating the dental or bone implant to a temperature approaching or above the glass transition temperature of the polymer; and
- (b) thereafter rapidly cooling the dental or bone implant.

In one embodiment, the polymer is PEEK. In another embodiment, the polymer is PEK.

In embodiments in which the polymer is PEK, the temperature to which the bone implant is heated is above approximately 300°C.

In one embodiment, the polymer is rapidly cooled by applying one or more gases. In one embodiment, the one or more gases is an air stream.

In another aspect, the present invention relates to a kit comprising:

- (a) poly ether ketone (PEK); and
- (b) instructions for preparing a PEK dental or bone implant using the PEK as a feedstock in a 3D printer.

The instructions may include detailed settings for the operational parameters of the 3D printer.

5

In some embodiments of the above kit, the PEK is in the form of a powder.

EXAMPLES

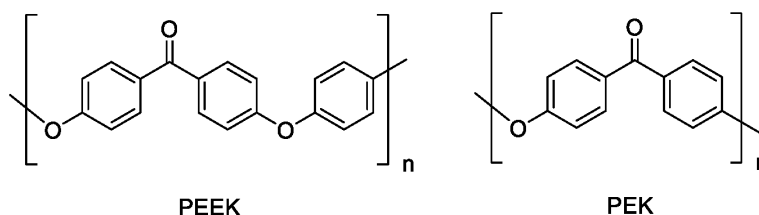
The present disclosure is further illustrated by the following non-limiting examples.

10

EXAMPLE 1 – Bone implants

The poly aryl ether ketone (PAEK) family is one type of polymer that possesses features that are amendable as a biomaterial, such as radiolucency, non-reactivity, and temperature resistance. Poly ether ether ketone (PEEK) and poly ether ketone (PEK) are two particular members of this family. Their structures are shown below:

15



PEEK has been used for custom implants for craniomaxillofacial reconstruction, but unlike titanium, it does not naturally osseointegrate or osteoinduce because it is hydrophobic and bioinert. Various physical and chemical surface modification strategies have been developed to make PEEK more hydrophilic. For example, plasma ion immersion implantation (PIII) activation is a treatment that increases the bioactivity by the provision of covalent linkages and increases the hydrophilicity of PEEK. Successful osseointegration of such an implant, within living bone, has been reported.

20

25

PEK and PEEK are non-resorbable polymers with excellent tensile strength, high modulus, chemical resistance, and thermal stability. Due to its non-toxicity and biocompatibility, PEEK has been used previously as an implant in the facial skeleton. PEEK and PEK cylindrical structures that were PIII-activated, were implanted into sheep mandibles and maxillae and compared to implants with the same design made of titanium as a positive control and with

untreated PEEK as a negative control. Bone-implant contact was evaluated through histological analyses of the PIII-activated, 3D-printed PEEK and PEK implants.

Defects of 5.6 mm in diameter were surgically created in the mandible and maxilla of a sheep. PIII-activated PEEK and PEK 5mm cylindrical implants manufactured using FFF and selective laser sintering (SLS), respectively, were implanted into the defects and compared with grade 23 titanium implants manufactured by selective laser melting as positive controls and untreated-PEEK implants as negative controls. Implant surface properties were characterized using scanning electron microscopy and atomic force microscopy. Osseointegration was evaluated qualitatively and quantitatively at 8-, 10-, and 12-weeks post-implantation by examining bone-implant contact histomorphometry on resin embedded bone tissue sections stained by Goldner's trichrome. Significantly improved osseointegration was demonstrated for both PIII-activated PEEK and PEK implants compared to that of the untreated controls. The results of this study suggest that PIII activation improves the osseointegration of FFF printed PEEK and that PIII-activated SLS printed PEK demonstrates similar osseointegration to 3D printed titanium.

Implant design

Mandible and maxilla implants were designed using 3dsMax 2020 (Autodesk, Inc San Francisco, California, U.S.) and polygonal modeling technique on an Alienware A51 v1.12 workstation (DELL Inc.) configured with Intel Core i7-9700 CPU 3.00 GHz, 8 Core(s), 64 GB RAM and NVIDIA GeForce RTX 2080 graphic card (NVIDIA Corporation, Santa Clara, California, U.S.).

Mandible implants comprise a single cylinder that is 5 mm in height and diameter and has nine grooves creating a 'saw-tooth' structure to increase surface area. These implants have either a single or double fixation site (Fig. 1A). Maxillary implants comprise two cylinders and two fixation sites (Fig. 1B). The right mandible and maxilla's digital implant models were produced using the mirror function after the left side of the mandible implant and maxillary implant were constructed in 3D.

Additive manufacturing of 3D PEEK implants

PEEK implants were prepared by FFF. FFF, also known as fused deposition modelling (FDM) involves feeding a continuous filament of a polymer onto a movable heated printer extruder head, which deposits the polymer in layers according to a computer-guided map onto a flat surface, to build up a three-dimensional structure.

5

The PEEK mandible and maxilla implants were designed using firmware v3.3.5 and fabricated via FFF on an AON-M.2 3D printer from AON3D, Montreal, Canada with 1.75 mm Thermax PEEK (batch 49-080620-06JV) filament produced by 3DXTech, Grand Rapids, Michigan. The nozzle was a 0.4 mm diameter E3D-V6 Nozzle X and a high temperature polyetherimide (PEI) build plate was used for adherence to the platform. The filament spool was dried for 8 hours at 120°C prior to printing and kept in a sealed filament box at 10% relative humidity during the printing process. Z calibration was performed prior to each print for inter-batch consistency. The designs were oriented with the flat base of the design in contact with a raft as shown in Fig. 2 to eliminate the need for support material and potential contamination to the implant from the build plate. PEEK implant designs were sliced for 3D printing with Simplify3D (V4.1.2) (Fig. 2). Key printing parameters are listed below in Table 1. To achieve good and consistent definition of the saw-tooth structures, perimeters of each of the layers were printed from inner to outer and the printing layer thicknesses were matched to the groove feature sizes. The PEEK was processed with high temperatures in the nozzle, platform, and chamber to target high polymer crystallinity as printed. This was confirmed by visual inspection of the implants opaque light-beige colour.

10

15

20

Table 1: Typical FFF PEEK printing parameters

Nozzle temperature	405°C
Platform temperature	170°C
Chamber temperature	120°C
Layer height	0.25 mm
Extrusion width	0.5 mm
Print speed	30 mm/s
Outline underspeed	70%
Shells	2
Infill percentage	100%

Infill pattern: raster angles	Rectilinear: 45°, -45°
Raft	3 layers of PEEK, 100% density

Additive manufacturing of 3D PEK implants

PEK implants were prepared by high temperature selective laser sintering (SLS). SLS involves selective fusing of a material such as PEK powder. After pre-heating an inert nitrogen gas-filled process chamber, the temperature of the powder is carefully controlled throughout the build process while a laser-system sinters selected parts of each successive layer of powder, which accumulate into a powder cake. After printing is complete, the sintered parts can be removed from the powder cake. Between the warm-up and cool-down phases, the usual procedure is as follows: a blade re-coater deposits new layers of powder onto the existing powder cake and the temperature of the new layer is monitored using a pyrometer and controlled using infrared heating irradiators directed at the top surface of the cake as well as heating elements in the building frame and platform. Once the temperature of the new layer is stable at the set point, two 50W CO₂ laser scanning systems expose and sinter cross-sections of parts in the top layer powder. Once the laser scanning is complete, the powder layer is subjected to a further period of infrared irradiation to finish the sintering process. Typical parameters for PEK bone implants for an EOS SLS printer (EOS P800 from EOS GmbH, Krailling, Germany) are summarised in Table 2:

Table 2: Typical SLS PEK printing parameters

Exchangeable frame temperature	343°C
Platform temperature	336°C
Chamber temperature	364°C
Base powder thickness before warmup	2.5 mm
Base powder thickness after warmup	6 mm
Layer thickness	0.12 mm
Post-sintering time	12 s
Beam offset	0.41 mm
Cool-down cycle	High-Temp Cooldown
Build Volume	Reduced build volume X (width) Reduced build volume Y (height) 350 mm × 230 mm

All printed implants were cooled with the high temperature cooldown cycle on the P800. Only after the process temperature had fallen below 60°C were the doors on the system opened. The

exchangeable frame was removed and transported to an unpacking stand where the implants could be removed from the powder cake.

The PEK polymer material used was EOS PEEK HP3. Designs for this work were printed with the cylinder axis aligned to the Z axis with the cylinder side facing downwards. Implants were sliced into layers with a layer thickness of 0.12 mm using Materialise Magics (software version 23.0.1.19) with the EOS build processor (version 1.2). The layer files were then assigned the EOS_dec laser exposure strategy from the PAEK1304_120_011 material set with the EOS PSW software (version 3.7). After the build process was completed, the implant was left to cool with the default high temperature cooldown routine until temperatures fell below 60°C, then removed from the powder cake and blasted clean with dry ice using a ColdJet MicroClean at 1.4 – 4.2 bar gauge pressure and a feed rate of 0.05 – 0.15 kg/min. A comparison of physical properties for PEEK and PEK is shown below:

Table 3: Physical properties of PEEK and PEK

Property	PEEK	PEK
Density (g.cm ³)	1.3	1.32
Hardness (Shore D)	87	90
Notched Izod Impact strength (Ft-lbf/inch)	74	59
Ultimate Tensile Strength (MPa)	100	115
Tensile Modulus (GPa)	3.7	3.7-4.0
Elongation at break (%)	50	20
Melting Temperature (C)	343	373
Glass Transition Temperature (C)	143	150
Particle size (µm)	-	60

Additive manufacturing of 3D titanium implants

Titanium mandible implants with double fixation sites were produced via a powder bed fusion technique referred to as selective laser melting (SLM). This technique is similar to SLS, but melts the material with a high-energy laser instead of sintering it. Grade 23 titanium alloy (CL41 Ti6Al4V-ELI, 20 – 63 µm, AP&C, Canada) powder was used in conjunction with a Concept Laser MLAB200R, (GE Additive, Boston, United States) metal additive

manufacturing system. STL files of the same geometry as the above implants were pre-processed using Materialise Magics software version 21 with Concept Laser MLAB200R build volume profile. Files were orientated with the axis of the implant cylinder aligned to the Z axis of build direction, but with the cylinder side facing upwards, as this provided the most reliable orientation for this printing technique to accurately recreate the saw-tooth structure of the implant. The base of the implant was offset by 3 mm from the build platform. Support structures were applied to adhere the implant to the build platform during processing as well as to conduct heat away from weld zones during printing. The combined model and support files were transferred to the Concept Laser MLAB200R workstation where slice and laser parameters were applied as per the manufacturer supplied material parameter file (Mlab 200R Titanium Ti6Al4V Base Parameter Profile 0012, GE Additive) with a layer height specified as 25 μm .

After printing, components were removed from the inert process chamber, excess powder removed, and parts manually separated from the build tray using side cutters. The roughened interface where the base of the implant met the support structure was cleaned and finished using 200-grit sandpaper on a belt finisher. Implant surfaces were further processed to ensure all loosely bound powder particles had been removed by manual media blasting ($\text{Ø}20\text{ }\mu\text{m}$ glass bead, Peenmatic 550, Iepco AG, Höri, Switzerland) at 2.0 bar gauge pressure.

20 PIII activation of PEEK and PEK implants

Both FFF printed PEEK and SLS printed PEK implants were activated by immersing the component in a dielectric barrier discharge plasma in nitrogen gas at two different pressures of 350 mTorr and 700 mTorr in such a manner that the surface of the component is capacitively coupled to the external electrode of the dielectric barrier discharge so that during the application of a high voltage pulse of negative polarity to the external electrode, the surface receives energetic bombardment by ions and electrons from the plasma. The discharge was excited by a high-voltage electrode consisting of a metal electrode covering the bottom and sides of a conical, borosilicate Erlenmeyer flask (250 ml, neck diameter 25 mm). Negative voltage pulses of 10 kV were applied to the external electrode using a RuP6 power supply (GBS Elektronik GmbH Germany). The pulse frequency was 1000 Hz, the pulse length was 40 μs and the total treatment time was 40 mins. All implants were steam sterilized before implantation into sheep mandible and maxilla.

Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) assessment of PIII activated PEEK and PEK implants

Implants were cut and sputter-coated with platinum and imaged using a TESCAN MIRA3 SEM (Tescan, Czech Republic). Surface roughness was examined with a Dimension Icon AFM (Bruker, Massachusetts, U.S) and shown as image Ra, the arithmetic average of the absolute values of the surface height deviations measured from the mean plane.

Implantation surgery

Six female sheep aged 7-8 years and weighing 70-80 kg were distributed in three groups with two sheep per group. Each group represented a defined timepoint (8-, 10- and 12-weeks post-implantation. Details are given in Table 4). The sheep were housed on straw bedding and fed a standard chaff and hay diet for a minimum of two weeks prior to surgery. All animals were deemed healthy on physical exam prior to surgery. All the procedures were performed with approval from the animal ethics committee (ethics approval number: 2020/1817) of the University of Sydney. Sheep were premedicated with 0.2 - 0.5 mg/kg methadone (Methodyne®, Jurox, Australia) and 0.2 - 0.5 mg/kg diazepam (Ilium diazepam, Troy, Australia) via a pre-placed intravenous (IV) canula. After general anaesthesia was induced with IV propofol (Propofol-Lipuro 1%, B. Braun Melsungen AG, Germany) (2 – 4 mg/kg) administration, the entire head region was shaved, and the surgical sites were sterilized with chlorhexidine. A 10 cm incision was made along the lower border of the right and left mandible. The masseter muscle was stripped off the lateral ramus of the mandible. A drill guide was used to create three full thickness osteotomies. Sequential dental drills to 5.6 mm diameter were used at low-speed under irrigation to create the osteotomies, which were 0.6 mm over-sized to prevent damage to the saw-tooth structure of the implant and to allow for any offset (Southern Implants Pty Ltd, 1 Albert Road, Irene, RSA). Two PIII activated PEEK-FFF implants (superior and middle) and a single PIII activated PEK-SLS implant (inferior) were placed passively in the left mandibular vertical ramus. A titanium implant (superior) and an untreated PEEK-FFF implant (inferior) were placed in the right mandible as control (Fig. 3). For the maxilla implantation, an incision was made along the zygomatic arch bilaterally and a similar procedure was carried out (Fig. 4). A PIII activated PEEK-FFF double implant was placed in the left maxilla and an untreated control PEEK-FFF double implant was placed in the right

maxilla as control. All implants were secured to the native bone using 2 mm x 6 mm titanium fixation screws. The implant location was confirmed using intraoperative cone beam CT (Siemens Artis Pheno, Siemens Healthcare GmbH, Erlangen, Germany). The wounds were closed in two layers using 4/0 absorbable suture composed of 90% glycolide and 10% L-lactide (Vicryl®, Johnson & Johnson, North Ryde, NSW, Australia) for deep tissue and 4/0 absorbable suture composed of Poliglecaprone 25 (Monocryl®, Johnson & Johnson, North Ryde, NSW, Australia) for skin. Betadine ointment was applied for dressing. One sheep from group 2 (the 10-week implantation group) was euthanized on the surgery day due to aspiration. Sheep from group 1, group 2, and group 3 were euthanized at the end of 8-, 10- and 12-weeks after the surgery, respectively. All samples were harvested at each timepoint and immediately stored in 10% neutral buffered formalin to fix the tissue for subsequent histological analysis.

Table 4: Location and number of implants at different timepoints

	PIII- activated PEEK (n = 20)	PIII- activated PEK (n = 5)	Untreated PEEK (n = 15)	Titanium (n = 5)
Group 1 (8 weeks)				
Mandible (ramus)				
Superior	2	0	0	2
Middle	2	0	0	0
Inferior	0	2	2	0
Maxilla (zygoma)	4	0	4	0
Group 2 (10 weeks)				
Mandible (ramus)				
Superior	1	0	0	1
Middle	1	0	0	0
Inferior	0	1	1	0
Maxilla (zygoma)	2	0	2	0
Group 3 (12 weeks)				
Mandible (ramus)				
Superior	2	0	0	2
Middle	2	0	0	0

Inferior	0	2	2	0
Maxilla (zygoma)	4	0	4	0

Histology

Resin embedding and sectioning

All sheep explant samples were trimmed with an EXAKT precision band saw to remove excess bone tissues around the plates of the implants, leaving 2-5 mm of peripheral bone tissue around each individual implant in preparation for analysis using resin histology. Samples were dehydrated with increasing grades of ethanol and then infiltrated and embedded in a Technovit 9100 methyl methacrylate system (Kulzer GmbH, Wehrheim, Germany) without decalcification. Resin ground sections were obtained at around 50 µm on standard microscope slides using an EXAKT cutting and grinding system (EXAKT Advanced Technologies GmbH, Norderstedt, Germany).

Goldner's trichrome stain

Ground sections were stained using Goldner's trichrome. Briefly, the sections were stained with Weigert's haematoxylin (Merck, Bayswater, VIC, Australia) for 25 min then washed and immersed in acid Fuchsin-Ponceau working solution Fuchsin (Merck, Bayswater, VIC, Australia) for 10 min. Following washes in 1% acetic acid, the sections were stained with tungstophosphoric acid - orange G Fuchsin solution (Merck, Bayswater, VIC, Australia) for 20 min and light green solution for 15 min. After air drying, the sections were cleared in xylene and mounted for imaging.

Imaging

Imaging of implants was performed with a Zeiss Observer 7 microscope (Carl Zeiss Microscopy, Germany) at × 5 magnification via the brightfield tile acquisition mode. The tiled images of whole implants were stitched and exported as TIFF-format for further analysis.

Histomorphometry of bone-implant contact analysis

Bone-implant contact (BIC) was measured by histomorphometry image-processing of the histology images using a custom code (MATLAB 2021a). Bone and implant regions were segmented using manual thresholding in the L*a*b* colour space. Measurements were

conducted at the implant thread (or saw-tooth) by masking and excluding the implant head and shank. Osseointegration was quantified by the bone area (BA) to total area (TA) ratio (BA/TA) for two regions within 24 μm and 80 μm of the implant thread. Fig. 5 provides a diagrammatic representation of this process showing the histological micrograph, segmentation, and 0-24 μm and 24-80 μm regions.

Statistical analysis

Statistical analysis was performed using Stata Statistical Software: Release 17 (StataCorp LLC, College Station TX, USA) and GraphPad Prism 9 software (GraphPad Software Inc, San Diego CA, USA). The differences in BA/TA data obtained by histology image quantification between implant groups (PIII-activated PEEK n = 20; PIII-activated PEK n = 5; Untreated PEEK n = 15; titanium n = 5) were compared using one-way ANOVA (Tukey's multiple comparisons test) and multivariable analyses of all 45 implants combined were performed using random effects modelling to account for correlated data adjusting for the effect of time and location. All analyses were two sided and $p < 0.05$ was considered statistically significant.

Results

SEM and AFM

Both PIII activated PEEK-FFF and PEK-SLS implants were imaged with SEM in the saw-tooth regions. The PIII-PEEK-FFF implant demonstrated a smooth surface topography (Figs 6A, 6B) when compared to the PIII-PEK-SLS implant (Figs 7A, 7B). In Fig. 6A, the FFF print layers are clearly visible. In contrast, the PIII-PEK-SLS implant had a more distinguishable saw-tooth structure (Figs 7A, 7C). Consistent with the SEM images, the surface roughness of the PIII-PEK-SLS implants was higher ($R_a = 139 \text{ nm}$) compared to the PIII-PEEK-FFF implant ($R_a = 46 \text{ nm}$) (compare Figs 6C and 7C).

Histology stains and BIC analysis

Fig. 8 (mandible) shows the histology of the implants and surrounding tissues. Both PIII-PEEK-FFF and PIII-PEK-SLS groups showed good bone-implant contact, similar to the titanium positive control. In contrast, the untreated PEEK-FFF negative control had more connective tissue present on the implant surface. PIII-PEEK-FFF implants in the maxilla

demonstrated similar results (results not shown). The BA/TA results are summarised in Fig. 9A and 9B for the mandible implants. In the mandible, the plasma-activated PEEK-FFF and PEK-SLS implants demonstrated comparable BA/TA to that of the titanium implants at all time points in both 0-24 μm (Fig. 9A) and 24-80 μm (Fig. 9B) zones where the untreated control PEEK-FFF group showed lower BA/TA compared to all other implants in both zones.

In the maxilla, the plasma-activated PEEK-FFF implants showed higher BA/TA compared to the untreated control PEEK-FFF implant control group at all time points in both 0-24 μm and 24-80 μm zones (data not shown).

Combining all time points there was no significant difference in BA/TA between the mandibular PIII-PEEK-FFF and titanium implants; in contrast, the untreated control PEEK-FFF implants showed significantly lower BA/TA compared to the other three types of implant (Fig. 10). In the maxilla, PIII-PEEK-FFF implants showed significantly higher BA/TA compared to untreated control PEEK-FFF implants group in the 24-80 μm zone (data not shown).

On multivariable random effects analysis, after adjusting for the effect of the location and time, the PIII-PEK-SLS implant had significantly higher mean BA/TA ratio compared to the untreated control PEEK-FFF implant in the 0-24 μm zone ($b = 0.42$, $p < 0.001$) and 24-80 μm zone ($b = 0.27$, $p = 0.001$). Similar estimates were found for the titanium positive control implant in the 0-24 μm zone ($b = 0.34$, $p < 0.001$) and 24-80 μm zone ($b = 0.28$, $p = 0.001$). The PIII-PEEK-FFF implant also had significantly higher mean BA/TA ratio compared to the untreated control PEEK-FFF implant in the 0-24 μm zone ($b = 0.14$, $p = 0.001$) and 24-80 μm zone ($b = 0.22$, $p < 0.001$), however, this was surprisingly less than that observed for PIII-PEK-SLS, given the similarity in chemical constitution of these two polymers.

Discussion

Bone contact with the implant surface is important for the long-term success of implanted prostheses, especially in load-bearing maxillofacial reconstruction. The above work was conducted with the aim of evaluating the *in vivo* osseointegration properties of surgically implanted plasma-activated PEEK and PEK cylindrical implants. Although the implants had

a saw-tooth surface structure, it is considered that any surface with a roughness on the micrometre scale, caused by cavities and protrusions, would allow in-growth into the surface of the implant by cells and/or tissue. Without wishing to be bound by theory, it is believed that implant hydrophilicity, covalent linkages with biological material and/or surface topology may all play an important role in osseointegration. The macro and micro surface roughness can enhance the attachment of bone tissues onto implant surfaces.

Osseointegration was evaluated qualitatively and quantitatively using histological analysis of BIC in two zones adjacent to the saw-tooth surface, namely 0-24 μm and 24-80 μm . As shown by the BIC analysis, plasma activated PEEK-FFF and PEK-SLS demonstrated superior osseointegration compared to untreated control PEEK-FFF implants. SEM and AFM examination revealed the PEK-SLS has increased macro and micro surface roughness compared to PEEK-FFF implants, enabling better interlocking with the bone at its surface, resulting in significantly better BIC.

Qualitative assessment of the Goldner's trichome stained mandibular bone-implant tissue sections from PIII-activated PEEK-FFF and PEK-SLS experimental groups suggested that the intersectional space between the implant surface and the surrounding bone was richer in mineralized bone tissue towards the end point of the study. In contrast, in the untreated control PEEK-FFF group, the space was filled predominantly with collagen-rich connective tissues. These qualitative observations suggest better integration between the implant and bone in the experimental groups compared to the untreated negative controls, and similar to that observed in the titanium positive control. These findings suggest that osseointegration is facilitated by PIII-treatment of the polymer implant's surface. Although untreated control PEEK-FFF is hydrophobic, it is believed that high energy PIII-treatment modifies the surface structure of the material long-term by creating free radicals that form covalent bonds with adjacent proteins, thus enhancing hydrophilicity and enabling cellular attachment, migration, and tissue growth.

Whilst there are other surface modification strategies, including wet chemistry and titanium coating, these techniques require further intensive research, as they suffer from drawbacks such as toxicity of debonded nanoparticles and long-term failure from delamination. In

contrast, the PIII surface modification technique does not require wet chemistry, simplifying the bioengineering process and facilitating clinical translation.

Quantitative analysis of the BIC showed comparable osseointegration of the SLS-printed PEK implants compared to titanium implants in each zone (0-24 μm and 24-80 μm) at each time point (8-, 10-, and 12-weeks) of the study. Furthermore, the PIII-activated FFF-printed PEEK implants showed significantly better osseointegration than the untreated FFF-printed PEEK implants, which revealed a lower BIC compared to all other implants tested across all time points in both BIC zones. These results were consistent in both the mandible and maxilla, suggesting enhanced osseointegration mediated by plasma treatment.

Both qualitative and quantitative histological evaluations for the above work showed better mineralization and bone-implant contact for PIII-activated PEEK-FFF and PEK-SLS implants, suggesting the efficiency of PIII surface modification for improved osseointegration.

This work clearly demonstrates that PIII-treatment improves the osseointegration of FFF-printed PEEK, and it is believed that the superior performance of the SLS printed PEK implants was due to the PIII treatment, the surface topology, and/or physical characteristics of the bone implant design including the properties of the single gyroid triply periodic minimal surface structure.

The results from this study suggest that PIII-treatment enhances the bioactivity of FFF printed PEEK implants and that SLS printed PEK implants demonstrate similar or superior osseointegration to 3D printed titanium implants of the same design. Different additive manufacturing techniques contribute to better surface topology resulting in better tissue adhesion, thereby enhancing the integration between the implant and the surrounding tissues. These outcomes are an important step towards the generation of patient-specific bone substitutes that can replicate the complex craniofacial skeleton using additive manufacturing and better match the mechanical properties of bone than that of titanium.

EXAMPLE 2 – Bone implants

This work compared 3D printed implants made from PEEK and PEK, and their use as bone implants for bone repair in sheep models.

Animal Model

- 5 A sheep model was selected based on their similar body weight, size, and bone microarchitecture to humans. Bone remodelling and bone turn-over in adult sheep (> 6 years old) is reported to be similar to adult humans. Sheep are also relatively cost effective because of their compliance, easier husbandry, and social acceptance. This provides a desirable animal model for the preclinical assessment of bone implant prototypes before trialling in
- 10 humans. Ruminants have less anterior mandibular loading compared to carnivores / omnivores, thus reducing the risk of mandibular fracture.

- In this study, some challenges were encountered with using an ovine model. These include the presence of large molar teeth that extend close to the lower border of the mandible and
- 15 increase risks of anaesthesia due to the four-stomach configuration of small ruminants. Sheep re-masticate their food along with the fermentation; hence the rumen is usually very full. Regurgitation of rumen contents can occur leading to pulmonary aspiration. In addition, sheep are social and, therefore, need to be maintained in groups, which increases costs. Finally, there is minimal soft tissue overlying the inferior aspect of the mandible. This makes
- 20 surgical access to the mandible easier but provides little protection of the implant if the wound dehisces leading to implant exposure and infection. This problem was experienced in some subjects, particularly when implant fit was suboptimal because the surgical team were attempting to avoid affecting the mandibular molar teeth and the inferior alveolar artery, potentially causing excessive tension on the wound closure. This highlights the importance of
- 25 careful preoperative planning and meticulous surgical technique.

Surgical defect

- The interim experimental model described above requires that the surgical defect created should not repair without the assistance of an implantable bone substitute (i.e., critical sized),
- 30 have minimal perturbation of the mandible biomechanics when it is under physiological load (so it unlikely to impair nutritional intake), and have minimal risk of mechanical failure of the mandible during mastication. To satisfy the requirements of the interim experimental model,

a partial defect is created along the inferior border of the ramus of the sheep mandible (see Fig. 11). The surgical defect removes the cortical shell and a portion of trabecular bone but does not interfere with the inferior alveolar artery or dental structures.

5 Implants

PEEK and PEK were 3D printed as a porous construct using FDM and SLS techniques, respectively; and then activated using PIII. Following creation of a critical-sized bone defect on each side of the sheep mandibles, SLS-PEK was implanted to the left jawbone defect while FDM-PEEK was implanted to the right side for a comparative study.

10

Duration of implantation

The present work allowed a 13-week period for the SLS-PEK and FDM-PEEK to be implanted in the sheep for new bone healing before being harvested. The bone implant was harvested following euthanasia with 1 cm of bone attached to the anterior and posterior ends of the bone implant to allow for assessment of the mechanical stability and property of the host bone-implant interface. The bone implant was not left in situ within the mandible because mechanical testing would reflect the structural integrity of the residual mandible, hence the bone superior to the bone implant was removed so that it will not influence biomechanical tests.

20

Implant evaluation

Following harvest, the bone implants were assessed with micro-computed tomography (microCT) and mechanical testing. Mechanical tests were designed to evaluate the structural integrity of both the bone implants for osteoconduction and the topology, tensile and shear strength of the bone-implant interface for the purposes of osseointegration. Both FDM-PEEK and SLS-PEK bone implants were tested with a reverse three-point bending test to measure the overall stiffness and flexural rigidity of the implant-bone structure. The following sections describe the fabrication process, PIII treatment, sheep implantation, bone implant harvesting, microCT analysis, and mechanical testing protocol.

30

Sheep

Four female Dorset-cross sheep (aged 6-8 years) were purchased and acclimatized by Laboratory Animal Services at Charles Perkins Centre, University of Sydney. All the protocols relevant to the present research were approved by Animal Research Ethics Committee, The University of Sydney (2021/2004). The animals were housed in pairs to
5 prevent anxiety and distress.

Bone implant Materials

PEEK fibre and PEK powder were used to fabricate the porous bone implant constructs which were then implanted to the mandibular defects. PEEK was purchased from AON3D,
10 Grand Rapids, Michigan, while PEK was purchased from EOS GmbH, Krailling, Germany, and both were used as received.

Mechanical tests

Some mechanical tests were conducted using a commercially available universal testing
15 machine (UTM; Instron 5567) located at Materials Testing Lab at School of Aerospace, Mechanical, and Mechatronic Engineering (AMME), The University of Sydney. The machine was equipped with 10 kN load cell and operated in a uniaxial loading mode. All mechanical tests were performed on fresh samples (without being fixed or frozen) to avoid the potential deviation caused by these factors.

20

MicroCT

MILabs U-CT system (MILabs, Houten, The Netherlands) at Sydney Imaging, one of the Core Research Facilities at The University of Sydney was used to scan the harvested mandibular implants.

25

Experimental Protocol

The scaffold-based implant is a one-body structure, which consists of a bone implant that fills the volume of the defect with external 'wings' (flanges) that are fastened to the external surface of the bone with screws (see Figs 12 and 13). The pore structure is a 3D array of
30 periodic unit cells. The pore size of the bone implant is 1 mm. The flange is 2 mm thick. To avoid drilling, and risking cracking of the flange, the flange has six prefabricated holes of diameter 2.0 mm for screws to be inserted through.

The pathways of bone ingrowth from the surrounding host bone into the bone implant are controlled to enable clear visualization of ingrowth from only the anterior and posterior faces of the defect. Tissue ingrowth from the anterior and posterior faces of the defect is permitted via porosities but ingrowth is prevented from entering through the superior side of the host bone by a solid plate along the top of the bone implant. The implant CAD models designed for the right-hand side of the hemimandible are mirrored to generate those for the left-hand side.

10 Bone implant fabrication

FDM PEEK

FDM-PEEK bone implants were fabricated using an AON-M.2 3D printer (firmware v3.3.5), AON3D, Montreal, Canada with 1.75 mm Thermax PEEK (batch 49-080620-06JV) filament which was produced by 3DXTech, Grand Rapids, Michigan. The nozzle used for the fabrication was an E3D plated copper volcano nozzle with a modified 0.2 mm diameter. A high-temperature polyetherimide (PEI) build plate was used for adherence to the platform. Before the printing process, the filament spool was dried at 120°C for 12 hours and it was stored in a Polymaker PolyBox at 10% relative humidity during the printing process. Prior to printing each bone implant construct, the printer was left to thermally equilibrate for 4 hours. Z-axis calibration was set to be 0.02 mm to ensure printing accuracy and consistency.

Simplify 3D (V4.1.2) was used to create 3D-print slices using the fabrication parameters shown in Table 5. The 3D CAD model of the implant is oriented with the superior edges of the flanges parallel to the printing base to achieve reliable geometry of the fine Schwarz P-surface bone implant structures. A layer of raft material is added between the base plate and the superior edges of the implant to avoid contact of the implant with the PEI platform. Breakaway support was manually added to the flanges and flat base for simple post-processing. The flanges were printed with higher nozzle temperatures and lower speeds to obtain better strength, and the Schwarz P-surface bone implant was printed with lower nozzle temperatures and higher speeds to obtain the sophisticated structural topology, particularly in the overhanging regions. After printing, the FDM PEEK mandible implants were annealed to complete the crystallization process. The implants are placed in an oven and the temperature

steadily increased to 200°C, held at this temperature for two hours before steadily decreasing the temperature back to room temperature.

Table 5: FDM PEEK 3D Printing Parameters

Nozzle temperature – Flange	420°C
Nozzle temperature – Schwarz P	370°C
Platform temperature	120°C
Chamber temperature	120°C
Layer height – Flange	0.07 mm
Layer height – Schwarz P	0.12 mm
Extrusion width	0.25 mm
Print speed – Flange	4 mm/s
Print speed – Schwarz P	7 mm/s
Outline underspeed	70%
Shells	2
Infill percentage	100%
Infill pattern: raster angles	Rectilinear: 45°, -45°
Raft	2 layers of PEEK, 100% density

5

SLS PEK

SLS-PEK mandibular implants were fabricated by an EOS P800, a high temperature SLS polymer 3D printer from EOS GmbH, Krailling, Germany. Materialise Magics (V25.0) with EOS build processor (V1.2) was used to orient and slice the implants into printing layers (thickness: 0.12 mm). The implants are oriented at 45° with respect to the x-, y- and z- build plate axes to obtain the most consistent strut and hole geometry possible for the bone implant. The parts were built with the EOS_UD laser exposure strategy from the PAEK1304_120_011 material set with the EOS PSW software (version 3.8). After the build process was completed, the cake was left to cool with the default high temperature cooldown routine until the temperatures fell below 60°C, the implants were then removed from the powder cake and blasted until clean with dry ice using a ColdJet MicroClean at 1.8 bar gauge pressure and a feed rate of 0.15 kg /min. Pictures of exemplary fabricated FDM-PEEK and SLS-PEK bone implants are shown in Fig. 13.

10

15

Table 6: SLS PEK 3D Printing Parameters

Layer thickness	0.12 mm
Build area configuration	230 mm x 350 mm
Base layers	2.5 + 6 mm
Top layers	3 mm
Total build height	58.803 mm
Process chamber temperature	364°C
Building platform temperature	336°C
Exchangeable frame temperature	343°C
Post sintering time	12 s
Beam offset	0.41 mm

PIII-treatment for PEEK and PEK bone implants.

Plasma immersion ion implantation (PIII) was used to enhance biocompatibility,

- 5 hydrophilicity, and adhesion strength of living tissue to the bone implants. PEEK and PEK bone implant specimens manufactured by FDM and SLS 3D printing techniques, respectively were placed on a PEEK mesh elevated 5 mm off the bottom of an Erlenmeyer flask (borosilicate, 250 ml volume, 25 mm diameter neck). The flask was evacuated to a pressure of less than 8.9×10^{-5} mbar then the specimens were submerged in pure nitrogen gas at either
- 10 0.463 (low pressure) or 0.934 (high pressure) mbar.

- A custom-built pulsed power supply (ANSTO PI3) (Australian Nuclear Science and Technology Organisation) was used to apply negative pulses of 10 kV amplitude and 20 μ s pulse length with a pulse repetition frequency of 1000 Hz. The negative pulses were applied
- 15 to an exterior copper foil electrode covering the bottom and sides of the Erlenmeyer flask to excite the dielectric barrier discharge inside the flask. The PIII treatment regime consisted of 4 min treatment at a low pressure, 2 min at a high pressure, and followed by another 4 min at a low pressure. The specimens were then turned over to present a new orientation to the plasma, the flask was re-evacuated, and the treatment procedure was repeated. This regime
- 20 was designed to deliver uniform ion penetration of interior and exterior surfaces. Total treatment time was 20 min. After the PIII treatment, the bone implants were steam sterilized by an autoclave at 134°C for 5 min at an absolute pressure of 3077 mbar.

Ovine mandibular implantation

The surgeries to implant the bone implants to the mandibular defects were conducted at Hybrid Theatre at Charles Perkins Centre, University of Sydney. The sheep underwent general anesthesia and were laid prone on the operating table. The mandibular region was shaved and prepared with povidone iodine. Then, a 15 cm long incision at the lower border of right and left hemimandible was made to expose the mandibular bone and the masseter muscle with attached periosteum was partially stripped.

To create the surgical defect with the desired dimensions, a cutting guide, based on the 3D CAD model of the sampled sheep mandible (see Fig. 12d), was designed and 3D printed using a resin base material. In each of the mandibles, the guide was fixed to the inferior border of the ramus with titanium screws and both reciprocating and oscillating saws with 1 mm blade width were used to create an inverted 'U' shaped defect across the full thickness of the host bone measuring 20 mm in length and 12 mm in height. The PIII activated SLS-PEK and FDM-PEEK bone implants were placed into the left and right mandibular defects, respectively, and fastened using six titanium screws (2 mm x 6 mm).

The bone implants were covered with masseter muscle and attached periosteum before the wound was closed in two layers using 4-0 Vicryl for deep tissue and 4-0 Monocryl for skin. Antiseptic ointment was applied to dress the sutured wounds.

Sheep recovery

Following the implantation surgery, the anesthesia was reversed. The veterinary team carried out regular assessments on the following parameters: demeanour, vitals (temperature and heart rate), wound appearance (swelling, discharge, bleeding, dehiscence), tissue health (swelling, bleeding, formation of pus showing infection), hydration and regular pain assessments. The assessments were performed multiple times per day during the acute post-operative period; and the frequency was reduced as the wound healed and recovered. Multimodal post-operative analgesia was also administered by the veterinary team and a sheep pain scoring system was used regularly for sheep pain assessment.

Sheep euthanasia and bone implant harvesting

Upon conclusion of implantation period (13 weeks), the sheep were sedated and euthanized. The previous wound was re-opened to remove the wrapping periosteum and expose the mandibular implants. A bone saw was used to excise the bone implant and attached bone (1 cm on each side). A small amount of bone was left on the superior aspect of the bone implant to prevent damage to the bone implant during removal, and immediately soaked in saline and stored at 4°C before being delivered to microCT scanner and the mechanical testing laboratory.

MicroCT scan

MicroCT scanning was conducted on each mandibular bone implant harvested from the sheep using MILabs U-CT system applying accurate scan mode, single energy, and ultra-focus magnification. Each scan ran for 75 ms exposure time with 469 mGy dose and 0.25 step angle (rotational degree), taking approximately seven minutes to complete a full scan. After each scan, the specimen channel was carefully sterilized, and the specimen was immediately re-soaked in saline stored in an icebox. MILabTM (MILabs, Houten, The Netherlands) and Imalytics PreclinicalTM software (Medilumine, Montreal, Quebec, Canada) were used for reconstruction and bone morphology analysis, respectively.

Reverse three-point bending test (Stiffness)

Mechanical testing for characterizing structural integrity plays a key role in determining a successful restoration of a load-bearing bone implant. A mechanical test was conducted to evaluate the extent of osseointegration after the FDM-PEEK and SLS-PEK bone implants had been removed after the 13-week period. The mechanical test was a reverse three-point bending test (Figs 14a-c).

This test was aimed to non-destructively assess the elastic / structural stiffness of the host bone implant system. By carefully controlling the applied load, the mechanical response of the bone-implant system remained in the linear-elastic region. The detailed testing protocol is outlined below.

(1) The specimen was placed horizontally on two supporting fixtures installed on the testing platform of Intron 5567.

(2) The crosshead was lowered slowly using a control panel to create a gentle contact between the load cell and implant bottom surface. The contact area was positioned on the centre of the bone implant.

(3) Fine adjustment was required to ensure appropriate specimen contact, whilst minimising excessive compressive load locally.

(4) A downward displacement was applied by using the loading cell (10 kN) at a velocity of 1 mm/min and the maximum displacement was set as 1 mm. This value was selected to ensure that the present test was non-destructive and carried out within the elastic range of the implant materials. Both compressive force (N) and displacement (mm) for each specimen were automatically recorded by the Intron system.

(5) The test was stopped at the downward displacement of 1 mm and the crosshead returned to its original position automatically. Then the tested specimen was recycled and preserved in saline.

(6) The bending stiffness (flexural rigidity) was calculated from the slope of the force-displacement curves measured.

Results

None of the mandibles fractured during the 13-week period, which confirmed computer modelling predictions (via finite element analysis) that the site of implantation was at minimal risk of mechanical failure under physiological load. Results of the reverse three-point bending test are summarised in Table 7.

Table 7: Results of reverse three-point bending test conducted on left (SLS-PEK) and right (FDM-PEEK) mandibular bone implants after 13 weeks

	Sample	Stiffness (N/mm)
Sheep 1	Left	248.0
	Right	425.7
Sheep 2	Left	299.8
	Right	160.2
Sheep 3	Left	252.2
	Right	2.56
Sheep 4	Left	554.3
	Right	239.0

Average	Left	338.6
	Right	206.9

Discussion

Reverse three-point bending test results are summarised in Table 7. The slope of the load-displacement curve (not shown) obtained from the reverse bending test can be divided into two stages. In the beginning of loading, the slopes were relatively flat, which indicates low stiffness due to the presence of soft tissues in the host bone–implant interface. Since the soft tissues have a much lower elastic modulus than bony tissues, it provided additional flexibility to the host bone-implant system.

With the increase of load, the slope of the curve became steeper as the soft tissues reached its maximum flexibility and the load was transferred from soft tissues to the bony tissues. Therefore, the slope of the later region was considered as the flexural rigidity (bending stiffness) for the bone-implant system. In general, a higher level of stiffness indicates a better osseointegration between the host bone and bone implant because of bone-implant contact and bone ingrowth inside the bone implant. Surprisingly, the SLS-PEK bone implants implanted in the left-hand side of the mandibles exhibited a higher average stiffness than the FDM-PEEK bone implants implanted in the right-hand sides, despite the anomalous results for Sheep 1. This observation indicates a better loading capability of SLS-PEK than FDM-PEEK in resisting the bending deformation and maintaining the structural stiffness, and also better osseointegration.

Evaluation of osteoconduction using microCT

Mandibular bone samples with bone implants (FDM printed PEEK and SLS printed PEK) were microCT scanned to evaluate bone ingrowth from the anterior and posterior edges of the native bone towards the center of the implant.

FDM-PEEK bone implants in the right mandible of Sheep 1 and Sheep 4 showed moderate bone ingrowth from the edges towards the bone implant interior (Figs 15A-D). Unfortunately, Sheep 2 developed wound dehiscence and infection associated with both the FDM-PEEK and SLS-PEK bone implants, and Sheep 3 developed wound dehiscence and infection associated with the FDM-PEEK bone implant (Figs 15E-F).

SLS-PEK bone implants in the left mandible demonstrated moderate bone ingrowth in Sheep 1 (Figs 16A and 16E) and Sheep 2 (Figs 16B and 16F), and substantial bone ingrowth from the edges of the defect site in Sheep 3 (Figs 16C and 16G) and Sheep 4 (Figs 16D and 16H).

- 5 The mean bone volume measured within the SLS-PEK bone implants was $3.048 \mu\text{m}^3$ compared to $1.640 \mu\text{m}^3$ in the FDM-PEEK bone implants (statistical comparisons not made).

Summary

- The present work reports a detailed preclinical trial protocol on the design, manufacture,
10 implantation, procurement, and *ex vivo* assessment of the bone implant prototypes used for reconstructing segmental defects in the mandible. A partial mandibular defect model designed to have minimal effect on the sheep's physiology is proposed as an interim *in vivo* model to assess implant performance before scaling up to a segmental defect model. CT-based FE modelling is used to evaluate the location and extent of bone removed so as to have
15 minimal perturbation of the sheep mandible biomechanics and have minimal risk of the bone implant-host bone construct failing mechanically. This is important for animal welfare and to avoid early termination of experiments so that meaningful results can be obtained.

- To fabricate the one-body bone implants, PEEK and PEK polymers were 3D printed using
20 FDM and SLS techniques, respectively. Before implantation, all the bone implants were plasma activated to improve their biocompatibility and wettability to enhance the bone implant-host bone interactions. Following sheep euthanasia, the suitability of SLS-PEK and FDM-PEEK bone implants for future experiments of segmental mandibular defect repair were investigated by mechanical tests and microCT characterizations. Results indicate that
25 SLS-PEK bone implants exhibited superior mechanical performance, osseointegration, and osteoconduction, compared to FDM-PEEK counterparts.

- The mechanical protocol consisted of a reverse three-point bending test to characterize the overall stiffness of bone implant–host bone system. It was found that the SLS-PEK bone
30 implants exhibited a higher average stiffness than the FDM-PEEK counterparts. The preliminary results demonstrate that SLS-PEK is a promising material to fabricate bone implant implants that enhance bone implant contact and ingrowth in jawbone defects. The

microCT scan analyses supported the results from the reverse three-point bending tests, showing encouraging new bone growth at the bone implant-bone interface and inside the lattice structure of the SLS-PEK bone implants.

5 **EXAMPLE 3 – Coating experiments**

SLS printed objects have a considerably rougher surface than FDM printed ones due to the presence of partially sintered powder grains that protrude from the median plane, creating cavities and consequently surface porosity. The protection of such rough surfaces from microbial infection presents a serious challenge as does the lesser biocompatibility of PAEK
10 polymers compared to titanium.

Coating of an antimicrobial peptide onto a bone implant was tested. Mel4 was used as a representative for a growth promoting agent. Mel4 has been described to have broad spectrum antimicrobial activity including against antibiotic-resistant strains of
15 *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as being active against fungi. To the best of the inventor's knowledge, Mel4 has not been used on PEK. Three different levels of roughness were used in this study: a very smooth PEEK sheet, a semismooth FDM printed PEEK surface and a rough SLS printed PEK surface.

20 **Sample Preparation**

Specimens of polymer were cut with a 6mm hole punch from sheets that were either as purchased or in house manufactured by 3D printing. PEEK sheet specimens were cut from semicrystalline PEEK film with a 250 µm thickness (Aptiv sheet®, Victrex manufacturing Ltd, Thornton Lancashire UK). The sheets have a very smooth, optically reflective surface
25 finish. FDM PEEK discs were cut from sheets prepared by an AON 3D printer with PEEK filament. The surface finish was smooth on the print bed-facing side, whereas the top side shows tracks left by the 4 mm printing nozzle. SLS PEK specimens were cut from SLS printed PEK sheets that were prepared by selective laser sintering (SLS) using the EOS EOSINT system (EOS GmbH Germany). One side of the SLS PEK sheets showed a very
30 rough surface, typical for SLS printed materials due to use of PEK powder grains, which have a diameter of about 40 µm. Dry ice blasting was carried out before cutting of discs to

remove un-sintered, loose particles after SLS printing. Polymer disc specimens were either used as is (untreated) or activated with PIII, as described above.

Contact angle measurements

- 5 Wettability is considered to be an important measure for biocompatibility. Without wishing to be bound by theory, it is believed that hydrophobic surfaces can denature adhered host proteins; this may cause exposure of unnatural epitopes, leading to an unwanted immune response.
- 10 Contact angle measurements were performed for PEEK sheet specimens by evaluation of a photographic image of a sessile 4 μ L water droplet. Contact angle measurements for FDM PEEK and SLS PEK were evaluated by photographic image of a sessile 1 μ L water droplet. Contact angles were measured using a sessile droplet contact angle goniometer (Kruss, Germany) according to the manufacturer's instructions.

15

Peptide Immobilisation

- Active peptide Mel4 and inactive peptide Mel1 were sourced from Auspep Melbourne, Australia. Peptide immobilisation was carried out by overnight incubation at room temperature of untreated and PIII-activated PAEK specimens in a 2 mg/mL peptide in
- 20 phosphate buffered saline (PBS) or PBS only under mild agitation. Subsequently, the discs were washed 3 times with PBS to remove excess peptides followed by 3 washes with distilled water. Then, the specimens were air dried and sterilized by steam autoclaving.

Antimicrobial Studies

- 25 The antibacterial activity of Mel4-coated PAEK discs was analysed by growing bacteria directly on them. Briefly, an overnight culture of *S. aureus* (SA 38) was diluted in Mueller Hinton broth (MHB) media to a final concentration of 10⁵ cells/mL. The bacterial suspension was then added to the either PIII-activated or untreated discs that either had no peptide, Mel1 or Mel4 immobilised to them. The discs were then incubated in a humidified chamber for 24
- 30 hours at 37°C. After incubation the media was discarded and the surfaces of the discs were washed thoroughly with PBS. After the final wash the discs were submerged in PBS and vortexed vigorously to resuspend the strongly attached bacteria in the PBS. Serial logarithmic

dilutions of the obtained PBS solution were prepared and subsequently plated on agar plates. After 24 hours of incubation the number of bacterial colonies growing on them was counted. The number of colony forming units (CFU) per mL of the undiluted PBS solution was determined by multiplying the results with appropriate dilution factors.

- 5 Statistical analysis was performed to test the effectiveness of the Mel4 when physically adsorbed and covalently linked against the infectious agent.

Cell Culture

- Osteosarcoma cell line Saos-2 was used here as a model for bone-implant interactions. The cell line is known for its ability to differentiate from an early osteoblast like cell to a late osteoblast like cell when a preferable substrate is present. Saos-2 cells were purchased from the American Type Culture Collection. The basal growth media consisted of McCoy's 5A medium (Gibco/Life Technologies, Australia) supplemented with 15% foetal bovine serum (FBS; Gibco/Life Technologies, Australia). The cells were cultured under standard culture conditions at 37°C in a humidified atmosphere with 5% CO₂.

Cell Adhesion

- Saos-2 cell adhesion *in vitro* is an important indicator for the performance of the substrate material *in vivo*. Saos-2 cell adhesion was assessed by AlamarBlue (AB; Invitrogen/Life Technologies Australia) assay conducted according to the manufacturer's instructions. AB is a resazurin-based cell substrate, its fluorescent metabolite is used as a measure of cell viability and by that of cell number. For the cell adhesion test, Saos-2 cells were seeded onto wetted PAEK specimen containing either immobilised Mel4 or no peptide. The cells were seeded at a density of 1×10^5 cells/cm² in 96-well plates in serum free cell culture medium. Serum free medium was chosen to ensure the effect of Mel4 is not obstructed by other proteins and peptides in solution. The cells were allowed to adhere to the specimen for 3 hours before careful washing with FBS containing media to remove unbound and loosely attached cells. Then the cells were cultured for a further 48 h to allow them to fully adhere to the specimen. On the day of the AB assay, the PAEK discs with the attached cells were transferred into wells of new 96-well plates and incubated with 120 µL of 10% AB in culture medium and incubated for 2h. The fluorescent signal of 100 µL was measured with a

CLARIOstar microplate reader (BMG LABTECH) at an excitation wavelength of 545–20 nm and emission wavelengths of 600–40 nm.

Mineralisation

- 5 Another tool for the assessment of biocompatibility of biomaterials is the degree of cellular and acellular mineralisation. Surface properties are considered to be highly influential and may lead to the inhibition or promotion of acellular mineral deposition and nucleation. They can also cause cells contacting the material to produce fewer or more factors important for biomineralization. Mineral deposits can be stained with Alizarin Red S (ARS), a nonspecific
- 10 calcium dye, and quantified by subsequent elution under acidic conditions. The absorbance of the eluent is proportional to the amount of calcium in the sample. The effect of immobilised Mel4 and the effect of plasma treatment status on Saos-2 cell mediated calcium deposition was determined. Saos-2 cells were seeded onto the specimen at a density of 1×10^4 cell/cm² and incubated for 35 days in culture under standard culture conditions with biweekly growth
- 15 medium changes. The initial proliferation was monitored by AB assay as described above. On the final day the specimens were moved to a new culture vessel and a PrestoBlue (PB, ThermoFisher Scientific, Australia) assay was conducted according to the manufacturers instructions to measure the viability of the final cell population. The cell number on each sample was estimated based on a PB calibration curve with known cell numbers (quadratic
- 20 curve fit, $R^2 = 1$). Then the specimens were fixed in 4% paraformaldehyde in PBS for 20 mins. For the ARS procedure the cells were washed briefly with distilled water before adding a 2% ARS/H₂O solution (Sigma Aldrich) to the cells. After 15 min the ARS solution was removed, and the specimens were washed several times with distilled water until the water ran clear. ARS dye was eluted with 200 μ L of a 10% acetic acid, 10% methanol water
- 25 solution for 45 min under gentle orbital shaking. The absorption at 425 nm of 180 μ L of the eluant was measured with a CLARIOstar microplate reader. Each total ARS signal was normalised to the cell number of the respective specimen.

Cytotoxicity of unbound Mel4

- 30 Cytotoxicity of short-term exposure with free Mel4 was assessed by AB assay. In brief, Saos-2 cells were seeded at a density of 8×10^4 cells/cm² into culture vessels. The following day growth medium was replaced with media containing Mel4 in graded concentrations up to 3

mg/mL Mel4 peptide in culture medium and incubated for either 24 h or 72 h. Then the culture medium was removed and an AB assay performed as described above.

Scanning Electron Microscopy (SEM)

- 5 Visualization of the bacterial cell and bone cell interaction with PAEK substrates gives an insight into the mode of attachment and cannot be adequately determined by light microscopy due to opacity of the PAEK polymers. Electron microscopy offers a good solution to this issue but also enables the composition of any phosphate mineralization to be determined by energy dispersive spectroscopy (EDS). For SEM preparation, the specimens containing cells
- 10 were fixed with 2.5% glutaraldehyde and dehydrated in graded ethanol before critical point drying of the samples in either hexamethyldisilazane (HMDS, used for the bone cells) or a critical point dryer (used for bacterial cell analysis). Surfaces of the bone cell containing samples were imaged with a PhenomXL (ThermoFischer Scientific) using a backscattered electron detector and an incident electron voltage of 5 kV and air pressure of 60 mPa. The
- 15 bacterial cells specimens were sputter coated with 30 nm of platinum before imaging. A spot size of 3 and an accelerating voltage of 5 kV were used.

Results

Surface Characterisation

- 20 The results from measuring contact angle are shown in Table 8.

Table 8. Contact angle measurements for PEEK sheet, FDM PEEK and SLS PEK.

PEEK sheet		FDM PEEK		SLS PEK	
Untreated	Activated with PIII	Untreated	Activated with PIII	Untreated	Activated with PIII
81.8	13.1	94.3	13.0	98.2	0.0
84.9	18.3	83.3	26.0	130.9	0.0
86.5	17.3	86.6	15.7	123.7	0.0
87.2	16.4	107.9	12.3	99.8	0.0
87.5	18.5	115.7	11.4	115.0	0.0
79.0	16.9	96.4	0.0	123.6	0.0
		98.1	0.0	129.3	

			0.0	125.7	
			0.0		
			0.0		
			10.8		
Average					
84.5	16.8	97.5	8.1	118.3	0.0

Contact angle reduction of water on PEEK sheet, FDM PEEK and SLS PEK was observed after a standard PIII treatment (Fig. 20). Water contact angle for PIII-activated SLS PEK was 0, whilst that for PIII-activated FDM PEEK was about 8.1°.

5

Antimicrobial Effect of Mel4

Fig. 21 shows the results for the antimicrobial study of Mel4 against *S. aureus* on all surfaces. Overall, Mel4 gave a bacterial count reduction of 1.8 log on untreated surfaces and 3.7 log on PIII activated surfaces, respectively. While the control inactive peptide Mel1 increased microbial count by 0.2 log potentially due to physicochemical surface changes that the peptide coating facilitates that are beneficial for bacterial adhesion and/or increased presence of interaction sites for bacterial cell surface proteins. PIII treatment alone or with Mel1 did no significant change to bacterial adhesion. The PEK material led to an overall increase on bacterial adhesion when no Mel4 was present. This is probably due to the increased roughness that can trap bacteria and increases the surface area of the PEK specimens. The greater surface roughness has not impeded the antimicrobial action of the Mel4, which has reduced the microbial count to the same level as for the smoother PEEK surfaces.

Bone cell adhesion

Adhesion of Saos-2 cells to PAEK specimen was increased with the presence of Mel4 and with PIII on all polymeric substrates (Fig. 22). All available data for all surface types combined, equally weighted, and normalised to untreated demonstrate an increase of cell adhesion by 32% with the presence of Mel4 and an increase by 32% with PIII treatment. Covalently linked Mel4 on the PIII-activated surfaces increases cell adhesion by 88% which indicates a synergistic rather than an additive effect.

25

Mineralisation

The effect of Mel4 on mineralisation capabilities of Saos-2 cells PAEK specimen was investigated by culturing cells on the polymeric specimens for five weeks. Presence of mineral was determined at the end of the experiment. Fig. 21 shows the mineralisation signal per 1 million cells to account for the different surface areas produced by different roughness'. Overall, Mel4 reduced mineral deposition by about 34%, whilst PIII treatment increases it by 20%. There is potentially also a synergistic effect between the two factors. The displacement of proteins from a surface by more strongly binding protein is an effect previously described as the "Vroman" effect.

Discussion

The significant and larger reduction in microbial load on surfaces of all roughness levels when they are given a PIII treatment followed by incubation in Mel4 solution is remarkable and shows that even very rough surfaces can be protected from infection. Without PIII, the surfaces incubated with Mel4 had a reduction in microbial load compared to surfaces without Mel4, but significantly less than the reduction in load shown by surfaces that have received both the prior PIII treatment as well as the peptide. This highly significant effect has two main origins, one associated with the nature of the coverage of the surface enabled by PIII, the other associated with the effect of the surface on the conformation and orientation of the peptide as well as successive removal of Mel4 due to the Vroman effect.

The provision of a hydrophilic PIII-activated surface with a dense coverage of covalent binding sites is believed to result in a type of monolayer coverage of protein that is quite distinct from the three-dimensional type of geometry on untreated surfaces. It is proposed that the effect of the PIII treatment is the result of both increased hydrophilicity and covalent linkages. Hydrophilicity allows the peptide to retain its native conformation and present hydrophilic motifs (potentially, also the antibacterial motifs) to the solution rather than develop conformations and orientations that present hydrophobic motifs while the antimicrobial hydrophilic motifs are obscured. The reduction in contact angle introduced by PIII treatment of the surfaces has an additional advantage of allowing increased penetration by liquid, enabling the peptide to cover the interior surfaces of recesses in the material.

The ability to form covalent linkages on the surface with Mel4 is believed to prevent infection by resisting the attempts of the microbe to engage with the surface. As illustrated in Fig. 23, the microbe (large structure) is able to displace Mel4 (small structures) from the untreated surface and attach to the surface, while on the PIII-activated surface, the firmly attached monolayer of Mel4 kills the microbe.

The effect of the envisaged monolayer of Mel4 is not, however, unfriendly to attaching cells, which in our study are Saos-2 bone cell analogues. It appears that Mel4 in fact encourages adhesion of the Saos-2 cells while suppressing mineralization induced by the cells (although not significantly). This effect may be more apparent on the untreated surface while causing a smaller also non-significant decrease in mineralization on the PIII activated surface. In the application of a bone implant *in vivo*, it is important that the friendly growth by bone cells and to some extent inflammatory cells of the patient will exceed the attachment by microbes at early times after the insertion of a PIII-Mel4 treated implant. The proliferation of inflammatory cells in particular can be rapid and effective in providing protection from infection. The intention of the Mel4 treatment is to maintain the dominance of “friendly cells” over infective agents until the overgrowth of the surface is complete and the role of the prevention of infection is taken over by the immune system of the host.

20 **EXAMPLE 4 – Dental implants and adhesive bonding promoted by PIII**

Polymers in the PAEK family, including PEEK, PEK and PEKK, are promising materials for dental implants because of their relatively good match to the mechanical properties of teeth and bone. Dental implants are used to anchor a dental prosthesis such as a bridge, a crown, or a denture to the bone of the jaw or skull. The dental implant must have good adhesion to the dental prosthesis, via a dental composite cement.

The ability to form engineered structures to match the mechanical properties of dentin, trabecular and cortical bone when combined with a bioinertness would enable these PAEK dental implants to be implanted without adverse reactions by the immune system.

30 In this work, adhesion of a PAEK dental implant to a dental composite cement was studied. Currently there is a relatively weak bond to dental composite cements when the surface of the

PAEK polymer is smooth. A minimum bond strength of 17-20 MPa is likely to be needed to resist contraction forces of resin composite materials, for enamel and dentin. Without surface treatment, PAEK polymers have a relatively low surface energy and inert surface chemistry that gives a poor bond strength with dental composite cement. PIII-activated polymers were tested to see if adhesion of a PEK dental implant to a polymeric dental composite cement could be improved. For the purposes of understanding the role of surface roughness three different surface morphologies for SLS PEK were studied. Two of these (very rough and rough) are controlled by the printing conditions, while the other is milled to a smooth finish. In addition to the SLS PEK, included is FFF PEEK with a smooth milled finish to control for surface chemistry and compare PEK to PEEK.

Materials and methods

Implants for testing are shown in Fig. 24. Pull-off strength of the polymer composite was tested from the 3D printed implant using a simple tensile test. The half pieces are subjected to PIII treatment, the composite is applied to the mating surfaces of each half before curing, then samples were tested to failure under tension.

PEEK and PEK implants were prepared as described above. The PEK HP3 powder is roughly spherical in morphology with a mean particle diameter of 60µm. All milled FFF PEEK and SLS PEK samples had the bonding surface milled flat by removing 0.5 mm of material with a HSS cutter. Samples were PIII-activated, as described above.

Roughness assessment

3D roughness of representative samples was characterised using an Olympus LEXT OLS5100 3D Measuring Laser Microscope. From the 3D Map, the areal roughness assessment was carried out according to ISO25178.

Bonding with dental composite cement

Monobond Plus primer was applied to both surfaces before bonding with a 2 mm thick layer of Multilink Automix. The bonding was performed in a 3D printed jig to hold the samples in position. Teflon tape was applied to prevent bonding to the jig while curing. The curing was

performed with a UV light. All bonding steps were carried out at 24°C. After bonding, small amounts of excess composite were removed with fine grit sandpaper.

Tensile testing

- 5 Tensile testing was performed according to ISO 527 on a TMA-WDW-10E Universal Testing Machine from Test Machines Australia (Melbourne, Australia). The test machine utilises a 10 kN load cell calibrated and verified to Class 0.5 ISO 376. The samples were secured with wedge grip fixtures and a crosshead speed of 0.5 mm per minute which corresponds to a nominal strain rate of 0.9% per minute for the nominal distance between grips of 54 mm. All
- 10 samples were tested to failure at 24°C. The force at break of each bar was recorded to calculate the adhesive stress at failure after fracture surface analysis. The Young's modulus in tension of the bars were measured using linear regression of the stress/strain curve between strains of 0.05% and 0.25% strain. The stress/strain curve was generated with the stress calculated by dividing the measured force by the nominal cross-sectional area of the bars and
- 15 the nominal strain calculated using the displacement of the crosshead with the distance between grips as the nominal gauge length as per ISO 527.

SEM fracture surface analysis

- The fracture surfaces were examined in a scanning electron microscope (Phenom XL,
- 20 ThermoFisher) to obtain the bonded area for each sample tested.

Results

Roughness assessment

- A roughness assessment was obtained using optical interference microscopy using an
- 25 Olympus 3D measuring laser microscope. Fig. 25 shows the areal roughness for the following surfaces: milled FFF PEEK; milled SLS PEK which appeared to have a similar smoothness, except for occasional places where the milling was not able to remove the deepest pits in the sample; rough SLS PEK; and very rough SLS PEK. The roughness of the latter two samples was achieved by adjusting the print parameters.

30

Tensile Testing

The tensile test results presented in Fig. 26 are corrected for the actual area of contact deduced by viewing the fractured surface in an SEM. It is the actual area of contact that is subjected to the tensile force. The separation was mainly at the composite/polymer interface, but for the PIII surfaces, there is evidence that the separation was at least partly by failure in the composite and not at the interface. By estimating the actual area of contact for each sample, it was possible to find the best estimate of the separation stress. Making the correction reduced the standard deviation of the measurements. The results show that both high roughness and PIII treatment correlate positively with the stress required to separate the two parts of the test sample. Without wishing to be bound by theory, it is proposed that the increase in stress required to separate the two parts of the test sample is the result of an increase in bonding strength at the interface which is, in turn, the result of covalent linkages being formed between the activated PIII treated surface and the molecules of the dental composite, more specifically between the PIII treated surface and the molecules of the resin portion of the composite.

15

EXAMPLE 5 – Toughening experiments

High strain-to-failure is a key property of a material that is desirable for applications that require resistance to fracture under sudden impacts. The large strain can absorb the energy of an impact and dissipate it without harm. In orthopaedic load-bearing implants for example, such materials are valuable as they enable a bone implant to resist failure under the sudden loads experienced in falls and sudden stresses imposed by muscles. Plasticity is a property of natural bone that enables it to resist fracture. This property is largely imparted by collagen and other proteins incorporated into the bone structure. It would be desirable to provide an orthopaedic implant material, such as PEK, with plasticity properties approaching those of collagen. An implant containing regenerating bone in a patient, with such properties, will be suitable for long term use. This removes the need to extract temporary support structures used for fixation of regenerating bone, such as titanium plates or rods. Low strain-to-failure is associated with brittleness and occurs in the elderly through the loss of the protein component of bones and therefore a relatively increased mineral content.

30

The presence of crystallinity in polymers reimposes 3D order that may cause brittle failure at crystalline grain boundaries. Described herein is work to optimise parameters to increase fracture toughness.

- 5 There are three main parameters that are useful to characterize the mechanical performance of an implant: the strain-to-failure, Young's modulus, and fracture strength (defined as the stress at fracture). The strain-to-failure is the linear, shear or bending strain at which failure occurs. This feature is especially important in resisting bone fracture and is compromised in disease states of bone such as osteoporosis associated with aging such as excessive
- 10 mineral/protein (e.g. collagen) ratio. The Young's modulus measures the resistance to linear extension or compression. In an implant, this modulus should be comparable to that of bone to give adequate fixation during bone regeneration. The modulus should not be excessively large, otherwise stress shielding osteolysis may ultimately lead to loosening and failure. The fracture strength measures the stress, for example a bending stress, that initiates fracture and
- 15 is important, along with strain-to-failure, in providing an adequate level of fracture resistance.

- Explored herein was the possibility of modifying printing parameters and using an annealing procedure to improve the mechanical performance of 3D printed PEK materials prepared by SLS. For the annealing work, test pieces were subjected to heat treatments where the
- 20 temperature is raised and then reduced quickly in a manner similar to that used for toughening glass. As for glass, toughening was achieved using a heating cycle in which the item is heated to a maximum temperature and the surface is rapidly cooled by passing a cool air stream over it to create a compressively stressed exterior layer that inhibits the formation of cracks. Until now, it has not been clear that such a mechanism would operate for polymer
- 25 objects, and in addition there was a possibility of crystallinity changes that may also determine the outcome of an annealing process. To evaluate the changes from a microscopic perspective, the fracture surfaces were studied using scanning electron microscopy (SEM).

Materials

- 30 The material used for manufacturing test samples was virgin poly-ether-ketone (PEK; commercially labelled as PEEK HP3) powder, supplied by EOS GmbH (Krailing, Germany). PEEK HP3 powder has an average particle diameter of 60 μm , manufactured by EOS

specifically for use in the High Temperature Selective Laser Sintering (HT-SLS) machine, the P800. The powder was stored in 45% humidity at 22°C prior to printing. The solid form of CO₂ (Dry Ice) was used for blasting and was supplied in block form by ColdJet (Moorebank, Australia).

5

Sample dimensions and print orientation

Rectangular prisms for three-point bend testing were printed, with nominal dimensions of 80 × 25 × 2 mm. The samples were printed in different orientations to determine the level of mechanical heterogeneity of the SLS PEK following printing. Fig. 27 depicts the orientations in which the test samples were printed. The orientations are comprised of the part rotation with respect to the build direction as well as print surface that experiences tension during the three-point bend test, as discussed below.

1. Lower Skin (LS) refers to the bottom surface of the part as printed and is the first layers of each part to be printed.
- 15 2. Upper Skin (US) refers to the top surface of the part as printed and is the last layers of each part to be printed.
3. X orientation (X) tests the pure LS and US surfaces.
4. Y orientation (Y) tests the surface built on the vertical sides of the parts. During three-point bending of these bars, the maximum tensile stress is in-plane with the fabricated layers, pulling along the layer planes.
- 20 5. Z orientation (Z) is similar to Y-orientation except that during three-point bending of these bars the maximum tensile stress is normal to the printed layer plane, pulling them apart along their plane of weakness.
6. XY45 orientation (XY45) is rotated halfway between X and Y orientations and tests the practical LS and US surfaces. This orientation is the practically chosen printing orientation to balance between build height and large fluctuations of laser exposure duration per layer that can cause SLS printing issues when compared to the other orientations.
- 25

30 Laser parameters

Non-standard laser parameters were employed in this study to try to improve the mechanical performance of the printed PEK samples. The sets of laser parameters are shown in Table 9

in order of increasing energy density. The set labelled “EOS” was fabricated using EOS standard settings, which are proprietary, whilst the remaining columns show settings developed for this project. It was observed by inspection that the fracture initiation sites of earlier tested samples were approximately 0.8 mm from the surface. Another laser contour was employed to further sinter the powder in this region of the parts.

Table 9. Laser printing parameters used to create the test specimens. NU indicates the parameter is not used, X indicates the value is proprietary IP of EOS.

Parameter	EOS	1	2	3
Contour1				
Power (W)	X	8.5	8.5	8.5
Speed (mm/s)		1000	1000	1000
Beam offset (mm)		0	0	0
Contour2	X			
Power (W)		8.5	8.5	8.5
Speed (mm/s)		1000	1500	1000
Beam offset (mm)		0	0	0
Contour3	X	NU		
Power (W)			8.5	8.5
Speed (mm/s)			1500	1000
Beam offset (mm)			0.15	0.15
Contour total energy density mJ/mm ²	X	17.00	19.83	25.50
Upskin/Downskin hatching total energy density mJ/mm ²	X	32.16	32.16	32.16

Annealing

Thermal processing (annealing with subsequent quenching) was achieved through use of a house-fabricated furnace. Samples are loaded into a holder which can be transported between two treatment areas of the system, as shown in Fig. 28. The sample is transported into the first treatment area, a furnace preheated to a set temperature (heat soak temperature). After the heat soak time has elapsed, the sample is transported to the cooling area for rapid quenching. The cooling area functions by blasting the sample with a high flow of cool dry air for a set time. Parameters for two exemplary annealing/quenching processes are summarised below.

Table 10. Exemplary annealing parameters used in this work.

	1	2
--	---	---

Heat soak temperature (°C)	300	330
Heat soak time (s)	240	240
Quench time (s)	50	50
Quench air temperature (°C)	20-25	20-25

Mechanical testing

- All specimens were tested mechanically on a TMA-10 Universal Test Machine (Test Machines Australia, Melbourne Australia), which was equipped with a servo-electric crosshead and a 10 kN load cell. All specimens were measured with NATA (National Association of Testing Authorities, Australia) accredited calipers before testing and relevant dimensions entered into the test software FastTest for calculation of stress, strain, and Young's modulus.
- The three-point bend testing was performed according to ISO178. The span was 65 mm, the radius of the supports was 5 mm, displacement speed was 5 mm/min and the entrance load was 1 N (Fig. 29). Elongation was recorded via measurement of the crosshead displacement.

Scanning electron microscopy

- Test bars, with and without annealing/quenching, were taken from the same treatment group after testing and their fracture faces were examined using a scanning electron microscope (SEM).

Statistical analysis

- The statistical significance of the differences in observed properties was tested using one tailed equal variance paired t tests. Random pairing of samples was used where each sample within the same experimental replicate was paired with a sample for which only one parameter was different.

Results

Mechanical testing

- Fig. 30 shows results of the mechanical testing for the different test samples, comparing different print orientation (XY45-, Y- or Z-orientations), laser parameters ("EOS" versus parameter "3" from Table 9), and whether an annealing (quenching) procedure was used or not. The results show that quenching significantly increases the fracture strain for all

printing orientations, but only increases strength for the z orientation. The quenching decreased bending modulus for the y and z orientations. The new laser parameters (parameter “3”) increased the modulus for both quenched and unquenched, y and z orientations, and increased the maximum stress for both the unquenched and quenched y and z orientations.

5

Fig. 31 shows the effect of changing the print parameters according to the program shown in Table 9. The results show that both of the new parameter sets, labelled “2” and “3”, result in increases in strain at fracture, maximum stress at fracture and Young’s modulus above the standard EOS parameter set. The quenched results included here show the further increases to fracture strain and maximum stress, and decrease of Young’s modulus, when compared to the default EOS manufacturers parameter set. The new parameter sets and annealing leads to increases in fracture strain, maximum stress at fracture for all orientations and an increase in bending modulus for the z orientation.

Fig. 32 shows a comparison of the stress-strain relation for quenched and unquenched samples, demonstrating a large increase in bending strain at failure that results from quenching. This strain is fully recovered after the stress is removed, as confirmed by the flatness of fractured portions (not shown). The strain appears to be fully recovered within less than a second after the stress is removed by the fracture of the sample. This behaviour is consistent with the property of superelasticity. In addition, the absorbed energy per unit volume of the material, also known as the toughness, can be calculated by integrating the area under the stress-strain curve. The quenched sample has 200% greater toughness than the non-quenched sample.

Scanning electron micrographs of the fractured surface of one of the samples are shown in Fig. 33. The upper surface in the image was the one placed in compression during fracture testing and shows a clear separation line between a surface layer and the interior material. This surface layer is modified in stress and/or crystallinity in such a way that it resists the formation of cracks, suggesting that quenching has imparted a resistance to cracking in the surface layer. Without wishing to be bound by theory, it is believed that the separated surface layer protects the entire object by resisting initiation of cracks that would otherwise rapidly propagate into the sample interior.

30

Discussion

The results show that the annealing / quenching process significantly increases the strain-to-failure of SLS printed PEK parts. This process enables useful gains in the strain-to-failure without substantially affecting the maximum stress sustained by the printed object. Without wishing to be bound by theory, it is believed there are two possible reasons for this improvement. One reason is the formation of an amorphous surface layer that has no grain boundaries within it. Grain boundaries in a crystalline or semicrystalline material represent lines of weakness. The other is the presence of compressive stress in the surface layer that reduces the degree of tension in the immediate surface to initiate cracks. The reason for improvement is not likely to be a result of re-melting of powder grains at the surface of the parts for two reasons: firstly, SEM images show the extent of granularity of top and bottom surface is not changed by the annealing / quenching process, and secondly the temperature used by the annealing / quenching process does not exceed the temperature used during the manufacturing process. The observed increase in strain to failure is most likely the result of reduced opportunity for surface crack initiation by one or both of the above mechanisms. It is possible that amorphisation of the surface layer has been caused by annealing / quenching, which is supported by the reduction in Young's modulus. The Young's modulus should not be affected by the presence of compressive stress in the surface layers provided the material is still within the linear elastic region.

It is notable that the air quenching process used does not cause warping of the printed parts, compared with water quenching described previously. The annealing process also can also be carried out at any time after printing using a specialised quenching oven. This last improvement gives advantages of practicality and manufacturing ease when compared to the prior art where personnel must be ready and waiting to quench the parts by interrupting the building process, that may take anywhere from 4 to 36 hours.

In summary, oven annealing with rapid cooling causes fully recoverable strain-to-failure to reach more than 5%, an improvement over as-manufactured SLS PEK which has a strain of about 3.2% at failure, demonstrating potential for load bearing orthopaedic bone implants, which is particularly important for patients requiring bone replacement. The mechanism for

the improvement in fracture resistance is attributed to an amorphous skin or layer created on the bone implant surface, that may also be under compressive stress.

EXAMPLE 6: 3D SLS printed PIII-PEK/Hydrogel combination for ectopic bone formation

6.1 SLS printing PEK chambers

Polyether ketone (PEK; commercially labelled as PEEK HP3) powder (EOS GmbH, Krailling, Germany) was SLS printed using an EOS GmbH P800 (Krailling, Germany) 3D printer system. The powder was stored in 45% humidity at 22°C until layer-by-layer sintering. SLS required pre-heating the inert nitrogen gas-filled process chamber of the printer, with the temperature of the powder cake carefully controlled throughout the build process whereby a laser-system sinters selected parts of each successive layer of powder added to the cake. Building parameters PEK chamber constructs are listed in Table 1. After printing was complete the cake was left to cool with the default high temperature cooldown routine until the temperatures fell below 60°C. PEK chambers were removed from the powder cake and blasted with the solid form of CO₂ (Dry Ice) using a ColdJet MicroClean (ColdJet, Moorebank, Australia) at 1.8 bar gauge pressure and a feed rate of 0.15 kg /min.

Table 11. The parameters for printing PEK chambers using the EOS SLS P800 3D printer system.

Layer thickness (mm)	0.12
Temperature set points (°C)	
Process chamber	364
Building platform	336
Exchangeable frame	343
Post-sintering time (s)	12
Beam offset (mm)	0.41
Cool-down cycle	High-Temp Cooldown
Build Volume	Reduced build volume X (width) Reduced build volume Y(height) 350mm x 230mm
Base powder thickness before warmup (mm)	2.5

Base powder thickness after warmup (mm)	6
---	---

6.2 PIII-treatment of PEK chambers

Plasma immersion ion implantation (PIII) was used to enhance printed PEK hydrophilicity, and ensuing adhesion and incorporation of 3D printed ADSC-laden GelMA hydrogel

5 (ADSC/GelMA) to the PEK chambers. In brief, the PEK chambers were placed on a PEK mesh elevated 5 mm off the bottom of an Erlenmeyer flask (borosilicate, 250 ml volume, 25 mm diameter neck), followed by evacuation of the flask to at least 8.9×10^{-5} mbar, and immersion of the chambers in pure nitrogen gas at either 0.463 (low pressure) or 0.934 (high pressure) mbar.

10

A custom-built pulsed power supply (ANSTO PI³) was used to apply negative pulses of 10 kV amplitude and 20 μ s pulse length with a pulse repetition frequency of 1000 Hz. The negative pulses were applied to an exterior copper foil electrode covering the bottom and sides of the Erlenmeyer flask to excite discharge of the dielectric barrier inside the flask. The

15 PIII treatment regime consisted of 4 min treatment at a low pressure, 2 min at a high pressure, and followed by another 4 min at a low pressure. The specimens were then turned over to present a new orientation to the plasma, the flask was re-evacuated, and the treatment procedure was repeated. This regime was designed to deliver uniform ion penetration of interior and exterior surfaces. Total treatment time was 20 min. After the PIII treatment, the

20 chambers were steam sterilized by an autoclave at 134°C for 5 min at an absolute pressure of 3077 mbar.

6.3 Surgical consumables and instrumentation

Surgical consumables and instrumentation for adipose tissue collection and PIII-PEK-

25 ADSC/GelMA chamber implantation and harvesting included sterile drapes, surgical gowns, surgical gloves, kidney dishes, gallipots, jugs, suction tubing, diathermy pencils, insulated diathermy tips, bipolar forceps, quivers, light handle covers, sponges, gauzes, #15 and #20 scalpel blades, marking pens, 20 ml, 10 ml and 5 ml syringes, blunt 10 g needles for irrigation tips, titanium vessel ligation clips (small and medium), sutures (3/0 Vicryl, 2/0 silk,

30 4/0 Monocryl – Ethicon, Johnson and Johnson, Bridgewater, New Jersey, USA), shavers, normal saline, chlorhexidine and povidone iodine antiseptic, drill bits (1.5 mm x 8), screws

(2.0 mm x 176), fissure burs, reciprocating and oscillating saw blades, 3D printed cutting guides for mandibles, Reynolds dissecting scissors, Mayo suture scissors, Gilles forceps, Debakey forceps, Senn-Miller retractors, Langenbeck retractors, malleable retractors, scalpel handles, haemostats (small and medium), rulers, periosteal elevators, vessel clip applicators (small and medium), screwdriver handles and tips, Frazier (ENT) sucker tips, Electric Pen Drive surgical drills and saw handpieces (DePuy Synthes, Johnson and Johnson, Warsaw, Indiana, USA), dental drill handpieces (Nouvag, Gallerstrasse, Goldach, Switzerland), a Diathermy Valleylab console (Medtronic, Parkway, Minneapolis, Minnesota, USA), an Electric Pen Drive motor (Depuy Synthes, Johnson and Johnson, Warsaw, Indiana, USA), and Motor System MD-11 (Nouvag, Gallerstrasse, Goldach, Switzerland).

6.4 Subscapular adipose tissue collection and ADSC isolation, culture, and encapsulation in GelMA hydrogel

Surgery to collect subscapular adipose tissue involved collection of approximately 50 g of tissue from an anesthetized sheep using a scalpel and placement of the tissue in PBS containing 5% penicillin/streptomycin (P/S) on ice, ahead of storage within a refrigerator at 4°C. The collected tissue was subsequently washed several times with PBS containing 5% P/S and debris removed, followed by transfer to a sterile tissue culture flask with 1:1 (w/v) 0.075% collagenase IV (Sigma) in Dulbecco's modified Eagle medium (DMEM) containing 2% P/S. The tissue was then minced using a scalpel and pipetted up and down several times with a serological pipette, followed by incubation for 2 hr at 37°C and gentle agitation every 5 min and pipetting up and down several times every 30 min using a 25 ml pipette. After digestion, an equal volume of DMEM containing 20% fetal bovine serum (FBS) and 2% P/S was added to neutralize enzyme activity, followed by centrifugation at 2000 rpm (760 x g) for 10 min. The fat layer was subsequently removed from the tube and the collagenase solution aspirated, followed by resuspension of the pellet in an equal volume of DMEM containing 1%P/S. Samples were again centrifuged at 2000 rpm (760 x g) for 10 min and the supernatant aspirated without disturbing the cell pellet. The pellet was resuspended in DMEM with 10%FBS and 1%P/S, and then filtered using a 70 µm nylon cell strainer. Cells were counted and seeded at a minimum cell density of 10×10^3 cells per cm^2 in tissue culture flasks in 10%FBS, 1%P/S and 10ng/ml bFGF in DMEM. Nonadherent cells were removed after 72–96 h culture, with medium changed every 3-4 days. Once cells reached 90% confluence,

adherent cells were harvested by trypsinization (0.05% trypsin-EDTA) before encapsulation within GelMA (LunaGel™, Gelomics Pty Ltd, Brisbane, Australia) containing lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) to obtain a final concentration of $3-6 \times 10^6$ ADSCs/mL. LunaGel was prepared as per the manufacturer's guidelines. Briefly, 2X LunaGel ECM solution (derived from porcine skin gelatin, type A) was incubated at 37°C until liquid. One vial of LAP was reconstituted in 1ml sterile Dulbecco's phosphate buffered saline (DPBS) and mixed with 5ml 2X LunaGel solution.

6.5 3D bioprinting, culture and differentiation of ADSC-laden hydrogel into PEK

chambers

ADSC/GelMA was 3D printed using a CELLINK BIOX6 bioprinter (CELLINK, Gothenburg, Sweden) into PEK chambers, with the print-head and print-bed temperatures set to 24°C and 4 °C, respectively. Crosslinking was performed after printing every second layer using a 405 nm photocuring toolhead for 90 sec, with 20 layers printed in total per PIII-PEK-ADSC/GelMA chamber construct. Constructs were then cultured in 10%FBS, 1%P/S and 10ng/ml bFGF in DMEM for 7 days in 6 well culture plates at 37°C in a 5% CO₂ humidified incubator for further cell expansion in situ. To predifferentiate cells in constructs to osteogenic lineage, culture media was replaced with osteogenesis differentiation medium and incubated for an additional 7 days before transplantation to sheep.

6.6 PIII-PEK-ADSC/GelMA chamber implantation

Surgery to implant the PIII-PEK-ADSC/GelMA chambers was conducted in the Hybrid Theatre at the Charles Perkins Centre, The University of Sydney. Adult sheep (70-80 kg) were housed on straw bedding and fed a standard chaff and hay diet for a minimum of two weeks prior to surgery. All animals were deemed healthy on physical exam prior to surgery. At the time of surgery, all sheep underwent general anaesthesia and laid prone on the operating table. The scapular region was shaved and treated with povidone iodine. A 20 cm long incision was made to expose the entire scapular and its covering fascia and musculature. A further incision was made to the covering muscle (infraspinatus and teres major) to expose the scapula. The periosteal branch of the circumflex scapular artery and vein supplying the lateral border of the scapula were identified and isolated. Afterwards, the periosteum at tip of the scapula was incised, followed by elevation of the periosteum off the scapula towards the

vascular pedicle using a periosteal elevator; taking care not to damage the periosteum or vascular supply. The periosteum was elevated from the entire external surface of the scapula. The PIII-PEK-ADSC/GelMA implants were positioned as two layers (bi-layered) that were back to back, with the openings of the chambers either face-down (Lower Chambers) or face-up (Upper Chambers) so that the ADSC/GelMA directly contacted the scapula bone or periosteum, respectively. The PIII-PEK-ADSC/GelMA implants were fixed to the scapula with titanium screws. The implants were covered with the muscle and the wound was closed using 4-0 Vicryl and 4-0 Monocryl, respectively. Antiseptic ointment was applied to dress the sutured wounds.

6.7 Sheep recovery

Following the implantation surgery, the anesthesia was reversed according to the Laboratory Animal Services Anaesthetic and Post-operative Care Sheep standard operating procedure (SOP) (SOP-OTH_11_Anaesthetic recovery and post-op care_sheep_20180301). The Laboratory Animal Services veterinary team regularly monitored the demeanour, vitals (temperature and heart rate), wound (swelling, discharge, bleeding, dehiscence) and tissue (swelling, bleeding, formation of pus showing infection) health, hydration, and pain using a sheep pain scoring system. If necessary, multimodal post-operative analgesia was administered, in line with Laboratory Animal Services Anaesthetic and Post-operative Care Sheep SOP (SOP-OTH_11_Anaesthetic recovery and post-op care_sheep_20180301).

6.8 Sheep euthanasia and PII-PEK-ADSC/GelMA implant harvesting

At the conclusion of the implantation period (10 weeks), the sheep were sedated and euthanized according to the SOP for Euthanasia and Humane Killing of Pigs and Sheep from Laboratory Animal Services at Charles Perkin Centre, The University of Sydney (SOP-EUT_03_LAS Euthanasia and Humane Killing Sheep Pigs_20170713). The implantation wound was re-opened to expose the implants. Once the implants were harvested, they were immediately soaked in saline and stored at 4°C before being delivered to microCT scanner followed by processing for histological analyses.

6.9 Micro-CT scanning

MicroCT scanning was conducted on each PIII-PEK-ADSC/GelMA construct harvested from the sheep using MILabs U-CT system (MILabs, Houten, The Netherlands) at Sydney

Imaging, The University of Sydney, by applying accurate scan mode, single energy, and ultra-focus magnification (Fig. 34). Each scan ran for 75 ms exposure time with 469 mGy dose and 0.25 step angle (rotational degree), taking approximately seven minutes to complete a full scan. After each scan, the specimen channel was carefully sterilized, and the specimen
5 was immediately re-soaked in saline stored in an icebox. MILabTM (MILabs, Houten, The Netherlands) and Imalytics PreclinicalTM software (Medilumine, Montreal, Quebec, Canada) were used for reconstruction and bone morphology analysis, respectively.

6.10 Histology

10 One third of each PIII-PEK-ADSC/GelMA construct was embedded into paraffin on the TES Valida[®] Modular Paraffin Embedding Center, sectioned at 4 μ m thickness with Leica RM2235 Rotary Microtome, and mounted on glass slides for staining with Sakura Tissue-Tek Prisma automated H&E staining machine. Tissue microanatomy including cellular composition and osteogenesis was evaluated qualitatively based on the microscopical presence or absence of
15 mineralised tissue formation, cartilage, and foreign body reaction (Fig. 35).

6.11 Statistical analysis

A t-test was performed to compare mineralization of constructs with and without ADSCs, imaged by micro-CT. $p < 0.05$ was considered statistically significant (Fig. 34C).

20

Results

As shown in Figure 34, microCT analysis demonstrated mineralization for lower (A) chambers, indicative of newly formed bone tissues. Mineralisation corresponded to the interface of the scapular bone and the chamber construct. Less mineralisation was evident
25 within upper (B) chambers.

Histological assessment of bioreactor chambers (Figure 35) revealed that substantial new bone formed both inside an upper chamber and at the interface of the periosteum. For a lower chamber, consistent with Micro-CT analysis, bone formation was apparent at the interface of
30 the scapular bone and the chamber construct.

Conclusions

1. Bioreactor chambers formed from sintered and PIII activated PEK (3D SLS printed PIII-PEK) can be used for ectopic bone formation.
2. It is envisaged that in vitro osteoinduced ADSC-laden hydrogel filled 3D SLS printed PIII-PEK bioreactor chambers for ectopic bone formation can be used to repair
5 segmental mandibular (and other) bone defects;
3. scapular bone and scapular periosteum show osteoinductive potential on in vitro osteoinduced ADSC-laden hydrogel filled 3D SLS printed PIII-PEK bioreactor chambers.

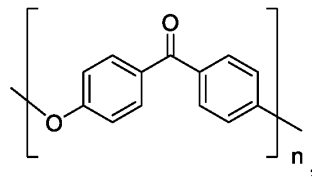
- 10 It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

- In the claims which follow and in the preceding description of the invention, except where
15 the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

CLAIMS:

1. A poly ether ketone (PEK) dental or bone implant, wherein at least a portion of the surface of the PEK implant has a water contact angle that permits osseointegration of the PEK implant.

2. The PEK implant according to claim 1, wherein PEK has the following structure:



wherein n is from about 25 to about 1000.

10

3. The PEK implant according to claim 1 or 2, wherein at least a portion of the surface of the implant has a roughness in the range of from about 100 nm to about 50000 nm.
4. The PEK implant according to claim 3, wherein osseointegration of the PEK implant has occurred when the ratio of bone area to total area (BA/TA), in the zone 0 – 24 μm from the surface of the PEK implant, is from about 0.30 to 0.80 as measured from 8 weeks to 12 weeks from time of implantation.
5. A poly ether ketone (PEK) dental or bone implant, when formed by additive manufacturing, wherein the surface of the implant has been activated with plasma immersion ion implantation (PIII).
6. The PEK implant according to claim 5, wherein the additive manufacturing is selective laser sintering.
7. The PEK implant according to claim 5 or 6, wherein the PEK implant has been annealed, then quenched with a gas.

25

8. The PEK implant according to any one of claims 1-7, wherein the water contact angle is in the range of from 0° to about 20°.
9. A method of preparing a poly ether ketone (PEK) dental or bone implant, the method comprising:
5 (a) forming the PEK implant from PEK; and
(b) activating the PEK implant with plasma immersion ion implantation (PIII).
10. The method according to claim 9, further comprising:
10 (c) annealing the PEK implant, then quenching the PEK implant with a gas.
11. The method according to claim 10, wherein the annealing is performed before activating the PEK implant with PIII.
12. The method according to any one of claims 9-11, wherein the PEK is in the form of a powder.
15
13. The method according to any one of claims 9-12, wherein the PEK implant is formed by additive manufacturing.
20
14. The method according to claim 13, wherein the additive manufacturing is selective laser sintering.
15. A poly ether ketone (PEK) dental or bone implant, prepared by the method of any one of claims 9-14.
25
16. A method of repairing and/or regenerating bone tissue, comprising implanting into a subject in need thereof a bone implant according to any one of claims 1-8 or 15.
17. The method according to claim 16, wherein the bone implant comprises a biologically active agent.
30

18. The method of claim 17, wherein the biologically active agent is covalently linked to at least a portion of the surface of the implant before being implanted.
19. The method of claim 17 or 18, wherein the biologically active agent is selected from the group consisting of: an antimicrobial peptide, an antibacterial agent, bone cells, encapsulated bone cells, bone precursor cells, encapsulated bone precursor cells, adipose tissue derived stem cells (ADSC's), a bone morphogenic protein or other cell differentiation agent, an anticancer drug, an anticancer targeting agent, an anticancer immunotherapy agent, a membrane receptor targeting agent, vascular cells, immune cells, a calcium phosphate substrate, a calcium phosphate ceramic, periosteum, or any combination thereof.
20. The method according to any one of claims 16 to 19, wherein the implant comprises a hydrogel.
21. The method of claim 20, wherein the hydrogel is a cross-linked hydrogel matrix.
22. The method of claim 21, wherein the cross-linked hydrogel matrix comprises a cell.
23. The method of claim 22, wherein the cell is a stem cell.
24. The method of claim 23, wherein the stem cell is an adipose tissue derived stem.
25. The method of any one of claims 20 to 24, wherein the hydrogel is cross-linked gelatin methacrylate (GelMA).
26. The method according to claim 19, wherein the antimicrobial peptide is Mel4.
27. A dental or bone implant, comprising:
- (a) a PEK dental or bone implant according to any one of claims 1-8 or 15; and
 - (b) a biologically active agent covalently linked to at least a portion of the surface of the implant, wherein the biologically active agent is selected from the group

consisting of: an antimicrobial peptide, an antibacterial agent, bone cells, encapsulated bone cells, bone precursor cells, encapsulated bone precursor cells, adipose tissue derived stem cells (ADSC's), a bone morphogenic protein or other cell differentiation agent, an anticancer drug, an anticancer targeting agent, an anticancer immunotherapy agent, a membrane receptor targeting agent, vascular cells, immune cells, a calcium phosphate substrate, a calcium phosphate ceramic, periosteum, or any combination thereof.

28. A dental implant, comprising:
- 10 (a) a PEK dental implant according to any one of claims 1-8 or 15; and
(b) a dental composite cement bonded to at least a portion of the surface of the dental implant.
29. The dental implant of claim 28, wherein the dental composite cement is covalently
15 linked to at least a portion of the dental implant.
30. The dental implant of claim 28 or 29, wherein the dental composite forms a functional coating on the surface of the PEK implant to increase the durability of the coated surface and improve the cosmetic appearance of the surface.
- 20 31. The dental implant of any one of claims 28 to 30, wherein the implant functions as a tooth.
32. A method of increasing hydrophilicity of a poly ether ketone (PEK) dental or bone
25 implant, comprising activating the implant with plasma immersion ion implantation (PIII).
33. The method according to claim 32, wherein at least a portion of the surface of the activated implant has a water contact angle in the range of from 0° to about 20°.
- 30 34. A kit comprising:
- (a) poly ether ketone (PEK); and

- (b) instructions for preparing a PEK dental or bone implant using the PEK as a feedstock in a 3D printer.
35. The kit according to claim 34, wherein the PEK is in the form of a powder.
- 5
36. A method of promoting bone growth, comprising:
- (a) contacting the PEK implant of any one of claims 1 to 8 or 15 with at least a portion of a cross-linked hydrogel matrix comprising stem cells; and
- (b) incubating the PEK implant comprising the cross-linked hydrogel matrix and stem cells under conditions that promote bone growth.
- 10
37. The method of claim 36, wherein the cross-linked hydrogel matrix is a cross-linked gelatin methacrylate matrix.
- 15
38. The method of claim 36 or 37, wherein the conditions that promote bone growth comprise implanting in a subject the PEK implant comprising the cross-linked hydrogel matrix and osteoinduced adipose tissue derived stem cells.
- 20
39. The method of claim 38, wherein implanting comprises arranging the implant in tissue such that the implant is in contact with bone or periosteum.
40. The method of claim 39, wherein the implant is in contact with bone or periosteum such that at least a portion of the cross-linked hydrogel matrix is in contact with bone or periosteum.
- 25
41. The method of any one of claims 36 to 40, wherein the PEK implant forms a chamber in which the cross-linked hydrogel matrix is contained.
- 30
42. The method of any one of claims 36 to 41, wherein at least a portion of the cross-linked hydrogel matrix is coupled to the PEK implant.

43. The method of claim 42, wherein the cross-linked hydrogel matrix is covalently coupled to the PEK implant.
44. The method of any one of claims 36 to 43, wherein the stem cells are adipose tissue derived stem cells.
- 5