Hydroxyapatite-incorporation improves bone formation on endosseous PEEK implant in canine tibia

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Abstract
Background: Poly Ether Ether Ketone (PEEK) has been considered as a potential alternative material for endosseous dental implants, for its low elastic modulus, biocompatibility, and low cost in customized device manufacture. Hydroxyapatite-incorporation is supposed to improve the poor osseointegration of PEEK.

Methods: In the present study we analyzed the in vivo response of hydroxyapatite-incorporated PEEK (PEEK-HA) implants in canine tibia. PEEK-HA and PEEK implants were implanted and were examined 4 weeks and 12 weeks after implantation with radiology and histology. Commercial titanium dental implants served as controls.

Results: The ratio of bone volume to tissue volume of PEEK-HA implants was higher than that of PEEK implants 4 weeks after implantation in the μ-CT analysis. The bone implant contact of PEEK and PEEK-HA implants showed no statistical difference in the histological examination, but newly-formed bone around PEEK-HA implants showed more signs of mineralization than that around PEEK implants.

Conclusion: The study suggested that bone formation was improved with hydroxyapatite-incorporation in PEEK. Hydroxyapatite-incorporated PEEK implants may represent a potential material for endosseous dental implant.

Keywords
Poly Ether Ether Ketone, dental implant, osseointegration

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Introduction
Dental implant has been accepted as one of the best solutions for replacing missing teeth. For present, the majority of dental implants are made of titanium and its alloys. The advantages of titanium-based implant include good biocompatibility, corrosion resistance, and mechanical strength. High survival rates of titanium-based implant have been continuously demonstrated over last sixty years. Nevertheless, the limitations of the titanium implant are obvious. The high elastic modulus of titanium implant causes the stress-shielding effect, inhibiting bone regeneration while enhancing bone resorption. In addition, long-term clinical observation on peri-implantitis raised the concerns that the release of metal particles into the tissues may play a role in the pathogenesis of peri-implant diseases. A semicrystalline thermoplastic polymer, Poly Ether Ether Ketone (PEEK) is currently considered as one
of the potential alternative materials for dental implants. The elastic modulus of PEEK is close to that of the human trabecular bone, which is supposed to be able to alleviate stress-shielding effect; and PEEK is also free from metal particles to induce allergic reactions. PEEK has already been used widely as a biomaterial for implants in trauma and orthopedic applications. Clinical studies support that load-bearing medical devices made of PEEK offer promising outcomes even better than equivalent of metals or trabecular bone, which is supposed to be able to alleviate stress-shielding effect; and PEEK is also free from metal particles to induce allergic reactions.4 PEEK has already been used widely as a biomaterial for implants in trauma and orthopedic applications.5,6 Clinical studies support that load-bearing medical devices made of PEEK offer promising outcomes even better than equivalent of metals or prosthetic scheme.

The integration of implants with the surrounding bone, a process termed osseointegration, is pivotal for long-term clinical success in dental rehabilitation. Although PEEK may overcome some drawbacks of titanium implant, the poor osseointegration is the primary limitation for its application in long-term functional loading. Fibrous tissue encapsulation instead of direct bone contact occurs around PEEK implants more frequently than titanium implants.7 The phenomenon is mostly attributed to the chemical inertness and hydrophobic nature of PEEK. The bioinert surface of PEEK is related to reduced osteoblastic differentiation of progenitor cells and production of an inflammatory environment that favors cell death via apoptosis and necrosis.8 Long-term clinical observations indicate that the failure of bioinert implants involves non-stable implant fixation to the bone tissue.9

To improve the poor osseointegration of PEEK, incorporation of bioactive material has been investigated in many researches.10 Hydroxyapatite is a ceramic material with the calcium-phosphorus ratio similar to that of the natural bone, and its osteoconductive behavior has been well-documented in treatment of bone defect. Surface coating of hydroxyapatite on PEEK is proven to be able to enhance bone apposition around implants.11 Another approach is bulk incorporation of hydroxyapatite into the PEEK matrix. The composite exhibited enhanced osteogenesis in comparison with pure PEEK in vivo.12 And more importantly, while coating technologies can bring additional processing steps and costs, bulk incorporation allows the conventional rapid processing of PEEK material. The later approach may realize one of the dentists’ ideals that the dental implant is prepared bench-side with all parameters individualized to the anatomic condition and prosthetic scheme.

Among the reported PEEK composites that are available in clinical settings, commercial medical grade HA-incorporated PEEK, such as PEEK OPTIMA® HA Enhanced (Invibio Limited, Lancashire, United Kingdom) appear to be promising for endosseous dental implant, as there’re already certified products in spine fusion, fracture fixation and arthroplasty made of this material. However, the commercial PEEK composite has never been explored as dental implant systematically. It’s not clear whether incorporation of HA enhances bone formation over pure PEEK. And specific to the application scenario, the performance of HA-incorporated PEEK with conventional rapid processing has never been investigated quantitatively in comparison with titanium dental implant. The evidence in the existing reports does not support quantitative assessment on the possibility of clinical application of PEEK-based dental implant. The purpose of this investigation was to compare the in vivo response of commercial pure PEEK (PEEK) and HA-incorporated PEEK (PEEK-HA) in respect of bone formation. A standard titanium dental implants was used as control to evaluate the possible application of HA-incorporated PEEK as dental implants.

Materials and methods

Animals and ethics statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of Guangzhou Medical University. Animal cares were conducted in accordance with Standard Operating Procedures of the Experimental Animal Center of Guangzhou Medical University. Four hounds were used in the experiment. The animals were individually housed in steel cages under natural light-dark cycle at room temperature. The size of the cages was 90 cm × 100 cm × 100 cm. All the surgical procedures were performed under general anesthesia. Animals were euthanized with overdose pentobarbital in consistent with the recommendations of the American Veterinary Medical Association Guidelines on Euthanasia.

Endosseous implant preparation and characterization

Endosseous implants were fabricated with PEEK (PEEK OPTIMA® Natural, Invibio, Lancashire, UK) or HA-incorporated PEEK (PEEK OPTIMA® HA Enhanced, Invibio) (n = 6). The implants were milled to the size and shape of the control titanium implants using computer numerical control machining (SmartCNC500E, Jingdiao, Beijing, China). The surface was cleaned with distilled water. The implants were sterilized with autoclave before use. Commercial titanium implants (Duravit EV, 4.1 × 10 mm, B&B Dental, Bologna, Italy) served as the control (n = 4). The surface morphologies of the implants were examined via scanning electron microscopy (S3400N, Hitachi, Tokyo, Japan). The chemical composition of implant surface was evaluated via Energy dispersive spectroscopy (EDS).

Surgical procedures

General anesthesia was induced with ketamine (20 mg/kg) and maintained with 1% pentobarbital. After induction of anesthesia, both proximal tibias were shaved with a razor.
blade and disinfected with antiseptic iodine solution. Subcutaneous infiltration anesthesia (5 ml per incision of 2% lidocaine) was applied. A 6 cm-long incision was made through the skin and the muscles and periosteum were elevated to expose the bone. Implant osteotomy was performed using a dental implant surgical kit (B&B Dental) under sterile saline irrigation. The implants were placed with an inter-implant distance of 1 cm (Figure 1).

Postoperative pain management and prophylactic antibiotics were provided for 7 days. The animals were euthanized with anesthesia overdose before harvesting the samples.

Microcomputed tomography
The tibias were harvested 4 weeks or 12 weeks after implantation. The samples were scanned with microcomputed tomography (μ-CT) (SkyScan high-resolution μ-CT imaging system, Bruker, Kontich, Belgium) at 20 μm resolution, 80 kV, and 100 μA. An aluminum-copper filter was used to optimize the contrast. Volumetric reconstruction and analysis were conducted using the software packages NRecon, CTan, and CTvox (Bruker). Due to the overlap of radiographic density of trabecular bone and PEEK, only the bone formation in cortical bone was analyzed quantitatively. A threshold of 60–100 was chosen to segment out cortical bone, 10–30 for PEEK implants, and 110–255 for titanium implants. The tissue within 200 μm to the surface of implant in cortical bone was chosen to calculate the ratio of bone volume to tissue volume (BV/TV).

Statistical analysis
All data were presented as mean ± SD. Statistical differences between two groups were assessed by unpaired t-test. Statistical differences among multiple groups were assessed by one-way ANOVA. Post multiple comparisons were performed using Tukey’s Test. The significance level was set at \( p < 0.05 \). Statistical analysis was conducted using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

Results
Surface characterization
The surface morphology of PEEK and HA-PEEK implants was determined using SEM. The surface of PEEK was relatively smooth (Figure 2(a)), in contrast with hydroxyapatite particles on the PEEK-HA implant (Figure 2(b)). The element analysis with EDS confirmed the existence of calcium and phosphorus on the surface of PEEK-HA implant (Figure 2(d)). The chemical composition of implant surface is shown in Table 1. The results indicated that the milling procedure retained the presence of hydroxyapatite on the implant surface.

Bone formation assessed with μ-CT
In the μ-CT images, the titanium control group showed close contact of bone and implant 4 weeks (Figure 3(a)) and 12 weeks (Figure 3(d)) after the implantation. Both PEEK (Figure 3(b)) and PEEK-HA (Figure 3(c)) implants showed clear margin with cortical bone 4 weeks after the implantation. No discernable resorptive activity could be noticed in the vicinity of implant. The stable state was the same in the images at 12 weeks (Figure 3(e) and (f)). The results suggested that PEEK and PEEK-HA support bone formation on the surface. However, the margin was not so clear in the trabecular bone. The part of implant in the trabecular bone was excluded in quantitative analysis.

Quantitative μCT analysis (Figure 6(a)) demonstrated that the BV/TV within 200 μm to the surface of implant in
Figure 2. Scanning electron microscopy (SEM) observation of implant surface: the surface of PEEK implant was relatively smooth (a), hydroxyapatite particles distributed homogeneously on the surface of PEEK-HA implant (b), calcium was not detectable on the surface of PEEK, and only trace mount of phosphorus was seen (c), and by contrast, calcium and phosphorus were detected on the surface of PEEK-HA (d).

Table 1. Element composition of implant surface. PEEK: milled implant of pure PEEK; PEEK-HA: milled implant of hydroxyapatite-incorporated PEEK.

| Sample     | Atomic composition/At.% |
|------------|-------------------------|
|            | C           | O           | P           | Ca          |
| PEEK       | 79.93 ± 3.23 | 19.00 ± 2.84 | 0.76 ± 0.31 | 0.29 ± 0.17 |
| PEEK-HA    | 76.20 ± 3.09 | 18.71 ± 3.99 | 4.02 ± 1.26 | 1.09 ± 0.34 |

Figure 3. μ-CT images of implants in tibia: implants were scanned 4 weeks (a, b, and c), and 12 weeks (d, e, and f) after implantation, titanium implants were well-osseointegrated in tibia radiologically (a and d), both PEEK (b and e), and PEEK-HA (c and f) implants showed clear margin in cortical bone. There was no identifiable bone resorption area between implant and bone. Induced trabeculae could also be observed in the medullary cavity. The contact between cortical bone and implants of PEEK and PEEK-HA showed no discernable alteration between the scans at 4 weeks and 12 weeks after implantation.
cortical bone was 81.3% in PEEK group 4 weeks postoperatively, 97.3% in PEEK-HA group and 97.8% in titanium group. Significant difference was found between the PEEK, PEEK-HA and titanium. And the difference originated mainly from the low BV/TV in PEEK group in comparison with PEEK-HA and titanium. The BV/TV value of PEEK group (93.9%) increased significantly 12 weeks after implantation, but was still slightly less than that of PEEK-HA (96.7%) and titanium (97.7%). The results suggested that bone formation is possible around both PEEK-HA and PEEK implants and takes place earlier around PEEK-HA implants than PEEK implants.

**Bone formation assessed with histology**

The implants were collected for undecalcified histological examination. Sections were stained with methylene blue-acid fuchsin, in which cell nucleus was stained in dark blue, fibrous tissue in blue, mature bone matrix in pink and newly-formed bone in dark pink.

In cortical bone, bone formation on the surface of titanium implants was continuous and fibrous tissue was seen rarely (Figure 4(a) and (d)). The osseointegration of titanium implant was evident. New bone formation could be seen around the PEEK (Figure 4(b) and (e)) and PEEK-HA implants (Figure 4(c) and (f)). But fibrous tissue interfered with the direct bone-implant contact more frequently on PEEK and PEEK-HA than on titanium. The sign of mineralization is noticed that the pink stain became much lighter and closer to the adjacent naïve bone 12 weeks after implantation when it’s compared with that at 4 weeks. Meanwhile, the cellular nucleus also decreased in the newly-formed bone tissue around PEEK-HA. The similar alteration was also noticed around titanium implants, but not around PEEK implants. The results suggested that the surface of PEEK and PEEK-HA allows direct contact with bone despite of fibrous response and that the surface of PEEK-HA implant may provide better condition for bone maturation than PEEK. The BIC of titanium, PEEK and PEEK-HA implants was 66.5%, 58.1%, and 57.3% 4 weeks after implantation, respectively. There was no significant difference in BIC among the groups. Twelve weeks after implantation, the BIC of titanium implants remained nearly unchanged as 69.7%, but the BIC of PEEK and
PEEK-HA implants reduced to 50.2% and 51.5%, respectively (Figure 6(b)).

In medullary cavity, trabeculae could be observed around each type of implants (Figure 5). Much of the surface of titanium implants was encapsulated with bone (Figure 5(a) and (d)). The bone formed on the PEEK and PEEK-HA implants 4 weeks after implantation (Figure 5(b) and (c)) but the bone formation reduced 12 weeks after implantation (Figure 5(e) and (f)). Four weeks after implantation, 27.1% surface of PEEK implants and 23.9% surface...
of PEEK-HA implants was estimated to have direct contact with induced trabeculae. However, the PEEK (0.4%) and PEEK-HA (1.8%) implants almost lost the bone contact in the samples with 12-week implantation in medullary cavity. The BIC of titanium implants was 50.3% and showed no significant alteration over time. The results suggested that the surface of PEEK and PEEK-HA might be suboptimal for long-term bone apposition in medullary cavity.

**Discussion**

Despite the many advantages of PEEK as implantable material, the osseointegration is the major concern for its possible medical application. The present study focused on the in vivo bone response to HA-incorporated PEEK (PEEK-HA) using radiological and histological methods. Our results supported an improvement of bone formation on HA-incorporated PEEK.

Radiology is one of the common ways to assess osseointegration non-invasively. The biocompatibility of PEEK-HA and PEEK in bone tissue could be viewed from the radiographs directly in the study. Both cortical and trabecular bone contacted closely with the surface of implants. No signs of bone resorption were seen around the PEEK and PEEK-HA implants. This was in accordance with earlier study on the same material, in which µCT radiographs did not reveal any adverse bony reactions in terms of resorption.18 But in the quantitative analysis, neither PEEK nor PEEK-HA implants could be segmented out reliably from the background of trabecular bone, due to the similarity of radiographic density. So, the quantitative analysis of bone to tissue volume ratio (BV/TV) around implants only included the part in the cortical bone. The PEEK-HA implants exhibited similar ability to support bone formation with titanium implants. But the BV/TV of PEEK implants was significantly less than those around PEEK-HA and titanium 4 weeks after implantation. An earlier study reported similar results that the effect of HA-incorporation was significant with respect to early bone formation.19 Overall, the present radiological results supported that HA-incorporation facilitates bone formation around PEEK-HA in the early phase in comparison with pure PEEK.

Histology is the standard method to evaluate bone growth on bioactive material. In the present study, the undecalcified sections were stained with methylene blue/acid fuchsin to distinguish fibrous and osseous tissue. To be comparable with the results from µCT, the histological data was interpreted as two parts: in cortical bone and in medullary cavity. In the cortical bone, all the materials allowed direct bone-implant contact (BIC). The BIC of PEEK-HA and PEEK didn’t differ significantly. But as a traditional bone substitution material, HA particles promote mineralization of bone matrix by positively modulating the expression of bone-specific markers and enhancing calcified matrix deposition during osteogenic differentiation.20 Thanks to the methylene blue/acid fuchsin stain in the study, bone around PEEK-HA was shown to be more mineralized. These results suggested that PEEK-HA offered more suitable environment for the maturation of bone in the vicinity than pure PEEK.

In medullary cavity, bone tissue is not normally present. No primary bone-implant contact immediately after implant insertion exists. Bone formation relies heavily on the superior osteoinductivity of implant surface.21 Rough surface and porous structure that facilitate cell adhesion out-perform smooth surface in terms of bone on growth.22 It was demonstrated that topography has a central role in determining osteoconductivity of PEEK.23 In this study, the surfaces of PEEK and PEEK-HA implants was resulted from machining directly and didn’t undergo any treatment. But the titanium implants were commercial ones and the surface was treated with sand blasting and acid etching (SLA) as stated by the manufacturer. SLA has been proven to be able to generate osteoconductive surface.24 As a result, the histological result was not surprising that the BIC of titanium implants in medullary cavity was significantly higher than those of PEEK-HA and PEEK. As all the BIC was formed after implantation in medullary cavity, it seemed reasonable that the BIC of all types of implants in medullary cavity was lower than that in cortical bone. But more disturbing was the descending trend of BIC of PEEK-HA over time. This raised the concern on the bonding stability of HA-particles exposed on the surface of PEEK-HA implant with machining.25

Most previous studies support the agreement between osteogenesis analyzes from µCT and histology.26 In this study, the part of implants in cortical bone was analyzed with radiology and histology quantitatively. Both µCT and histological measures demonstrated that PEEK-HA resulted in bone morphology and ongrowth similar to titanium. But discrepancy existed in the observation on PEEK implants, which exhibited relatively low B/V in µCT but comparable BIC with PEEK-HA and titanium in histology. The possible explanation might be that the thin fibrous encapsulation could not be viewed directly with the resolution of radiological image that µCT could achieve. While histological section enables direct observation of the tissue types on the implant surface. Moreover, µCT analysis offered stereoscopic measurement. While ground bone section is a destructive process and only allows single plane view, which was the plane along the implant length axis in this study.27 The methodological difference was aggravated in this study with a relatively small sample size, noting that the standard deviations were quite significant in the histological analysis of implant in cortical bone (Figure 6(b)). However, the discrepancy of PEEK implants did not undermine the result that PEEK-HA and titanium exhibited comparable bone formation. Since pure PEEK
displayed inferior ability to osseointegrate compared to titanium in several earlier investigations, the current results supported that HA-incorporation improved bone formation on PEEK.

The clinically oriented definition of osseointegration is described as a process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained in bone during functional loading. The biomechanical stability is one of the essential aspects to assess osseointegration. In the present study, the rigid fixation of the implants was failed to evaluate, as the biomechanical tests such as implant pullout was missing. But considering the structural difference of tibia and jaws, the biomechanical test would be more relevant in our experiment in mandible and maxillary bones. Another limitation of this study is the animal model could not simulate the environment of oral cavity where dental implants serve. Any single piece of dental implant interacts with alveolar bone, soft tissue and microorganism in oral cavity. The osseointegration under functional loading, biocompatibility to soft tissue, biofilm accumulation is still remained to be investigated to answer the question whether PEEK-HA is a suitable material for dental implant.

Conclusion

The present study investigated the histological and radiological manifestation of hydroxyapatite-incorporated PEEK implants in tibia and revealed inspiring results. The bone formation around hydroxyapatite-incorporated PEEK was improved over pure PEEK, but functional loading of clinical interest is necessary to clarify the significance of the improvement with hydroxyapatite-incorporation. Conventional milling of hydroxyapatite-incorporated PEEK preserved hydroxyapatite particles on the surface, which allowed bone formation and mineralization similar to SLA titanium surface, but the osteoconductivity assessed in medullary cavity was reduced. The study suggested hydroxyapatite-incorporated as a potential material for endosseous dental implant, but the material processing optimized for PEEK composite is required in future.

Declaration of conflicting interests

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