JOURNAL OF Applied Polymer SCIENCE

In Vivo Biocompatibility and Bioactivity of *In Situ* Synthesized Hydroxyapatite/Polyetheretherketone Composite Materials

Rui Ma,[1] Luqian Weng,[1] Xujin Bao,[2] Shenhua Song,[1] Yu Zhang[3]

[1]Department of Materials Science and Engineering, Shenzhen Graduate School, Harbin Institute of Technology, Shenzhen 518055, China

[2]Department of Materials, Loughborough University, Loughborough, Leicestershire LE11 3TU, United Kingdom

[3]Department of Biological Sciences, School of Life Sciences, Shenzhen University, Shenzhen 518060, China

Correspondence to: L. Weng (E-mail: marui067@gmail.com)

ABSTRACT: Hydroxyapatite/polyetheretherketone (HA/PEEK) composite materials were prepared via an *in situ* synthesis process in order to achieve strong bonding between PEEK matrix and hydroxyapatite fillers, and ultimately to improve the mechanical properties of the composites. In the study, the biocompatibility of the synthesized HA/PEEK materials was investigated by acute toxicity test, hemolytic test, sensitization test, pyrogen test, intradermal test, and toxicity assay test on animal tissue and cells for the purpose of examining the possible adverse effects of the residue organic chemicals from the *in situ* synthesis process. *In vivo* bioactivity of both lab-synthesized PEEK and HA/PEEK composites with various HA content was also studied. It is found that the *in situ* synthesized composite materials possess good biocompability without toxicity. Although the bioactivity of the material increases with HA content, the composite material with 5.6 vol % HA exhibits satisfactory bioactivity without compromising its excellent mechanical performance, which hints to a potential use as load-bearing orthopedic material. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: hydroxyapatite; polyetheretherketone; composites; *in-situ* synthesis; biocompability; *in vivo* bioactivity

Received 15 December 2011; accepted 19 April 2012; published online
DOI: 10.1002/app.37926

INTRODUCTION

The concept of bioactive fillers' reinforcing polymers, as bone analogue, was first introduced by Bonfield, and the hydroxyapatite/polyethylene composite biomaterial is one of the most clinically successful examples.[1] Other high performance polymers have been investigated as matrix material during last decade in order to further enhance the properties of composite materials.[2,3] Polyetheretherketone appears to be a suitable polymer, as biological implant, since it possesses both outstanding chemical stability and high mechanical performance.[4] Although the PEEK is biologically inert,[5] it is anticipated that, by incorporating bioactive HA fillers into the PEEK matrix, they could provide the bioactivity necessary for promoting bone regeneration and forming strong interfacial fixation between host tissue and implant.[6,7] Indeed, HA/PEEK composite materials fabricated by a compounding and injecting process are bioactive,[8] but the mechanical strength and ductility of the composite materials decreases substantially with increasing HA content because of the severe debonding between hydroxyapatite particles and PEEK matrix.[9] It is also found that the debonding deteriorates the fatigue properties of the composite materials.[10] Considering that the fatigue property of biomaterials is a most critical property for long term biological applications, such as implants, it is much needed to improve the reliability of the composites by improving the bonding between HA fillers and PEEK matrix.[11] Recently our research indicates that the strong bonding can be achieved in HA/PEEK composites synthesized by an *in situ* process, which leads to a substantial improvement on the mechanical properties of the materials. In the in-situ synthesis process of HA/PEEK composites, HA particles were mixed into PEEK oligomers with short molecule chains and low viscosity, and good wetting and contacting between HA fillers and PEEK oligomers were achieved. With continuing polymerization, the PEEK oligomers on the HA surfaces become polymeric molecules with long molecular chains, which firmly wrapped the HA partcles. The strong bonding probably is due to physical

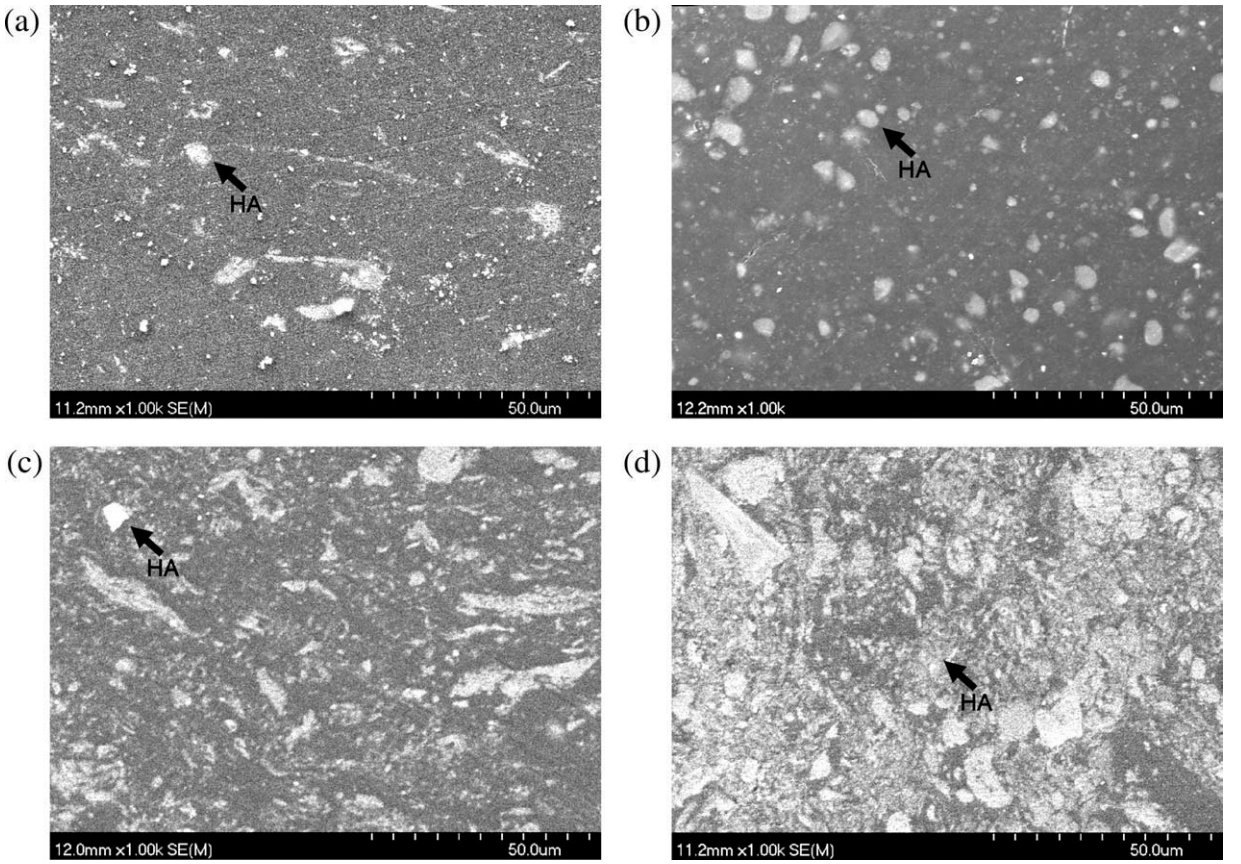

Figure 1. Polished cross-section SEM images of HA/PEEK composites by *in situ* polymerization with different HA content: (a) 2.6 vol % HA/PEEK,(b) 5.6 vol % HA/PEEK, (c) 13.4 vol % HA/PEEK, (d) 23.9 vol % HA/PEEK.

factors, such as the molecule chain wrapping and the interlock effect of PEEK molecules on the HA surface, rather than chemical factors since it is shown that the chemical bonding between HA fillers and PEEK is weak.[12] However, the possible adverse affects on the biocompatibility of materials, caused by the residue organic components in the synthesis process, are not yet clear.

On the other hand, although the *in vivo* bioactivity of HA/PEEK composites with 40 vol % HA has been briefly investigated in previous studies,[13–16] the high HA content significantly compromises the mechanical performance of the composites due to the severe HA agglomeration in the PEEK matrix, which results in more defects and stress concentration, and consequently decreases the tensile strength. Therefore, it is important to have the knowledge of the relationship between HA content and *in vivo* bioactivity of the composites in order to balance their mechanical properties and bioactivity.

This study performed a series of *in vivo* animal tests, such as acute toxicity test, hemolytic test, pyrogen and intradermal stimulus and sensitivity test to the *in situ* synthesized HA/PEEK composites to examine the biocompatibility and toxicity. Subsequently the *in vivo* bioactivity of the composites with various HA contents is investigated by virtue of Sprague Dawley (SD) rats as the testing host.

EXPERIMENTAL

Preparation of the Samples

Di-terbutyl peroxide, p-dihydroxybenzene and sulfobenzide were charged in a four-necked flask equipped with a reflux condenser. The mixture was heated to 180°C under constantly stirring under a pure nitrogen atmosphere. At 180°C, K_2CO_3 and Na_2CO_3 were added, and the mixture was further heated to 320°C. Subsequently, HA powders were introduced with different contents to the mixtures, and the mixtures were held for 3 h at 320°C before cooling. The composite materials were harvested by washing the mixtures with deionized water and acetone, and dried in vacuum at 140°C for 24 h. The powders of PEEK composites with different HA contents were compacted under a pressure of 50 MPa for 8 min, then heated from room temperature to 330°C at a rate of 15°C/min, and finally the samples were cooled down to room temperature at a rate of 10°C/min.

The HA contents in the final composite products were 2.6, 5.6, 13.4, and 23.9 vol %, respectively, determined by the weight loss of the samples burned at 700°C in air for 2 h. Microstructures of the composites with various HA contents, observed by scanning electron microscopy (SEM) (Titachi S-4700 microscope), are shown in Figure 1, exhibiting that HA fillers are firmly embedded in the PEEK matrix.

Table I. Comparisons of Hemolytic Ratio of the Samples

Tested materials	Number of samples	Hemolysis HR/%
HA/PEEK composites	7	1.8 ± 0.9
Ox bone	7	132.3 ± 0.86
Physiological saline	7	0 ± 0.9
Distilled water	7	100 ± 0.6

Biocompatibility of the Composite Materials

SD rats and New Zealand rabbits were used for biological tests in the study. The test protocols were strictly followed and the animals were cared for in adequate facilities that safeguard their well-beings throughout the experiments. Owing to the fact that the 5.6 vol %HA/PEEK composite possesses acceptable mechanical properties for implantation,[12] it is only considered for the tests of biocompatibility.

Acute Toxicity Test. The suspensions (1 g/mL) of the sterilized 5.6 vol %HA/PEEK composite powder and fresh ox bone powder were prepared respectively by dispersing the powders in physiological saline. Sixty SD rats with the weight of 17–22 g each were divided into three groups for the tests of the HA/PEEK composite suspension, the negative control of physiological saline and the positive control of the fresh ox bone suspension. Both the abdominal cavity and the muscle around rats' backbone were injected into the suspensions with doses of 1 mL and 0.2 mL respectively under gnotobasis, and the rats were examined in 7 days.

Hemolytic Test. Totally, 10 mL blood sample of New Zealand rabbit was added with 0.5 mL potassium oxalate solution (0.1 mol/L) for anticoagulation, then this anticoagulated blood sample was diluted with physiological saline at a ratio of 1 : 1, and the as-prepared blood sample was used for the following tests as the anticoagulated blood sample.

Totally, 0.5 g of 5.6 vol %HA/PEEK composite powders and 0.5 g of fresh ox bone powders were added into 10 mL of physiological saline, respectively, to make their suspensions, and the samples were preserved at 37°C for 30 min in test tubes. The suspensions were charged with 0.2 mL of the anticoagulated blood sample and shaken slowly. After the samples were kept at 37°C for 60 min, the supernatant of the samples was obtained by centrifugation at 4000 r/min for 5 min. 0.2 mL of the anticoagulated blood sample was also added to 10 mL physiological saline as negative control, and to 10 mL distilled water as positive control. The absorbance of the samples was examined at 545 nm by a spectrophotometer. The hemolytic ratio (HR) of the samples was evaluated from the equation:

$$\mathrm{HR}(\%) = [(D_t - D_{nc})/(D_{pc} - D_{nc})] \times 100$$

where D_t is the absorbance obtained from the samples, D_{nc} and D_{pc} were the absorbances of negative control and positive control, respectively.

Sensitization Test. Totally, 5.6 vol % HA/PEEK composite powders and fresh ox bone powders were kept in physiological saline with a concentration of 0.1 g/mL at 37°C for 72 h, respectively, and then the leached liquors were taken for test. Thirty SD rats were divided into three groups for the sensitization test of the leached liquors from the 5.6 vol %HA/PEEK material, the negative control of physiological saline, and the positive control of the fresh ox bone. The rats were injected into 0.1 mL liquors and examined for skin reaction after 24, 48, and 72 h, respectively.

Pyrogen Test. Nine New Zealand rabbits with weights between 3.0 and 3.2 kg were anesthetized by 846 combined narcotics with a dose of 0.4 mL/kg. The rectal temperature of the rabbits was measured three times, and the average value was used as the normal body temperature. Two suspensions of 5.6 vol % HA/PEEK composite powder and fresh ox bone powder in physiological saline with a concentration of 0.1 g/mL were intravenously injected into the rabbits with a dose of 5 mL per rabbit, respectively. Their body temperatures were measured at 24, 48, and 72 h, respectively, after injection, and compared with their normal body temperatures.

Intradermal Test. Five grams of 5.6 vol % HA/PEEK composite powders and fresh ox bone powders were dispersed in 10 mL of physiological saline, respectively. After high pressure-high temperature (HPHT) sterilization, the supernatants were obtained by centrifugation. The New Zealand rabbits with a weight of 22.21 ± 0.92 kg) were shorn at the two sides of rachis where the samples were injected by a dose of 0.5 mL. The skin reaction was observed at 24, 48, and 72 h, respectively, after injection with physiological saline as negative control.

Toxicity Assay Test of the Composite Materials on Tissues and Cells. The lab-synthesized PEEK powder and 5.6 vol % HA/PEEK composite powder were suspended in saline with a concentration of 0.9 g/100 mL and injected into the abdominal cavity of the rats, respectively. After one week, the tissues surrounding the powders were obtained through operation and

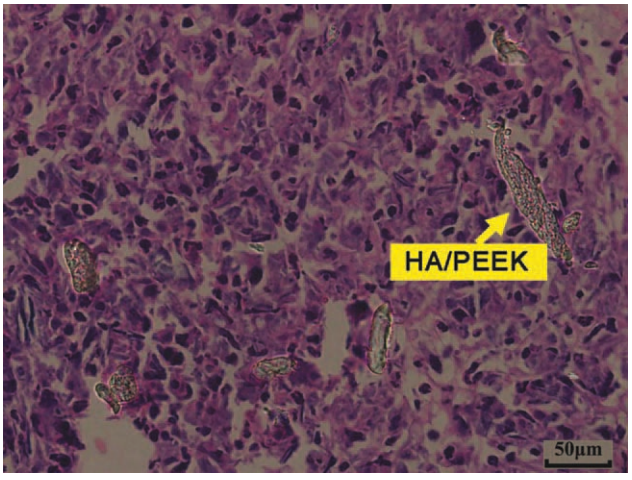

Figure 2. Histological micrograph of 5.6 vol % HA/PEEK powders and surrounding tissue. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

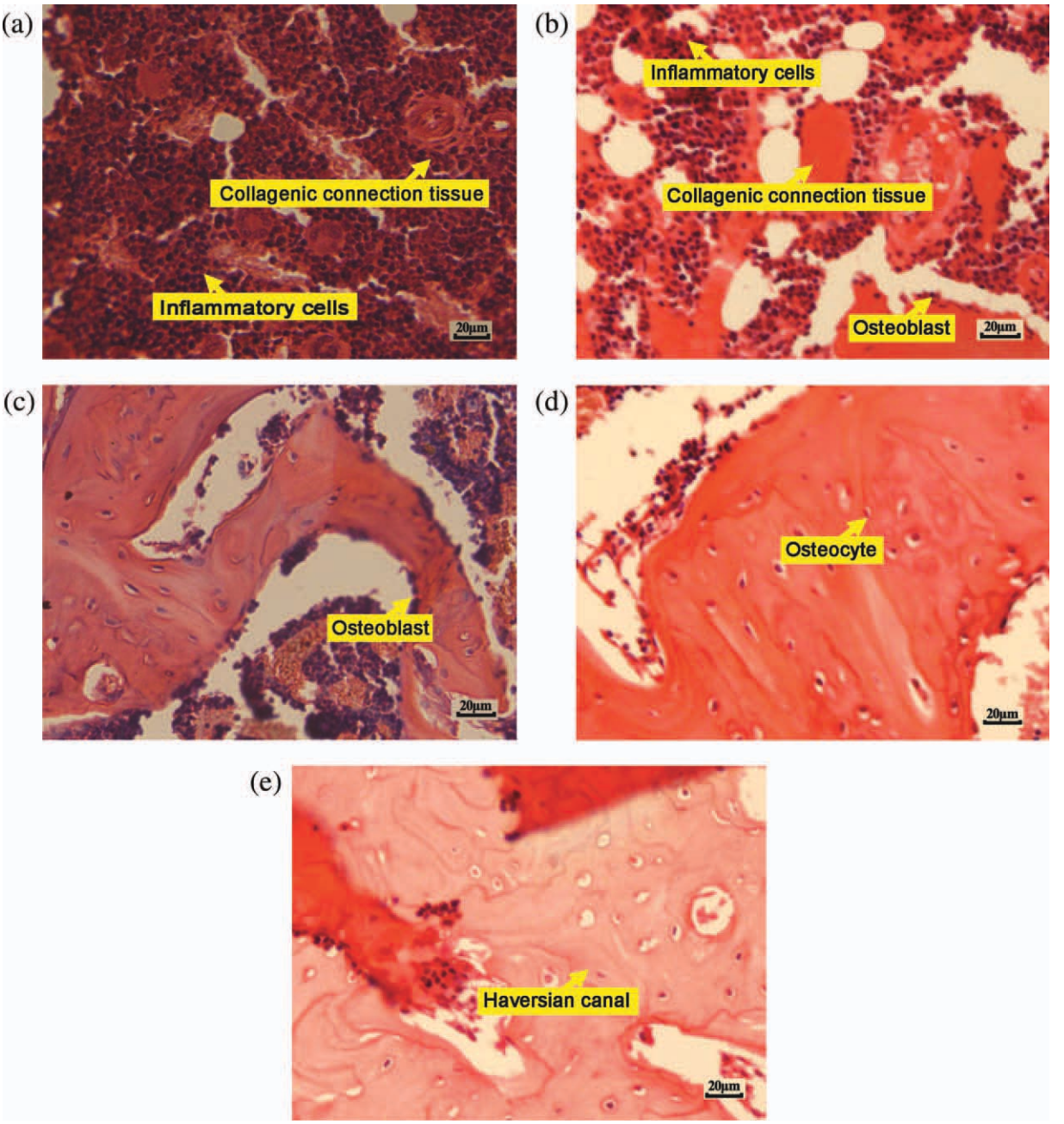

Figure 3. Histological micrographs of new bone tissue around the implants with various HA content for one month: (a) PEEK (b) 2.6 vol % HA/PEEK (c) 5.6 vol % HA/PEEK (d) 13.4 vol % HA/PEEK (e) 23.9 vol % HA/PEEK. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

stained with hematoxylin–eoxin (HE), followed by optical microscopy examination.

In Vivo Bioactivity of the Composite Materials. The implants with a cylindrical shape of a diameter of 3 mm and a height of 5 mm were sterilized by a steam autoclave at 120°C for 30 min. Under general anesthesia, the implants were inserted into the fracture part in the middle of the left femur of rat, and then the wound was fixed and sutured. The rats were subsequently sacrificed after 1, 2, and 3 months, respectively, and the implants together with surrounding tissues were obtained and pretreated in 4% formalin. The tissues were separated from the implant, fixed with paraffin, and then stained with hematoxylin–eoxin (HE) for subsequent histological analysis by means of optical microscopy. A part of implant samples with surrounding tissues, dehydrated in ethanol and acetone solutions, were also embedded in epoxy resin and cut with a diamond microtome (Leica UC6/FC6). The obtained samples were stained with toluidine blue for the observation of bone-implant binding by optical microscopy, which was further examined using SEM.

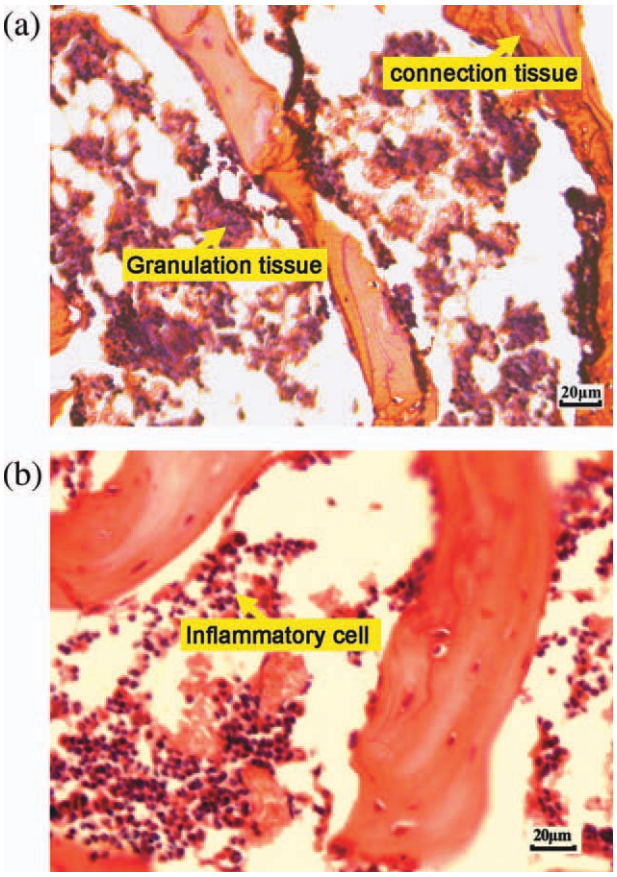

Figure 4. Histological micrographs of new bone tissue around pure PEEK implants: (a) for 2 months (b) for 3 months. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Biocompability of HA/PEEK Composites

The acute toxicity tests show that all the rats in three groups did not die after 7 days, but the rats in the positive control group were observed to have such symptoms as mental and physical inactivity, instability in gait, appetite, and weight losses. The other two groups of rats injected with physiological saline and the composite suspension were observed to be normal, indicating that the composite suspension does not have acute toxicity. The results of hemolytic test are given in Table I, showing that the hemolytic ratio of HA/PEEK composites is below 5%, which meets the standards for clinical applications.[17,18] In sensitization test, there were no erythema and edema in the groups of rats injected with the extract from composites and physiological saline. However, the rats injected with the liquor from fresh ox bone were observed to be erythema with an area about 0.2 cm in diameter after 24 h, and the erythema disappeared in 72 h. The pyrogen tests indicate that the rabbits injected with the composite suspension have no obvious rising of body temperature (within 0.2°C), which also meet the standards of clinical applications (<0.4°C).[19,20] The intradermal test results exhibit that the rabbits in the positive control group inflame within an area of 1.2 cm in diameter, but the other two groups of rabbits injected with the physiological saline and the composite suspension are normal. The obtained tissues with 5.6 vol %HA/PEEK powder are shown in Figure 2, As seen, the tissues and cells are normal without pathological tissues, such as lymphocyte and macrophage infiltration, myocytolysis, and cytoclasis. All these results demonstrate that the lab-synthesized PEEK and HA/PEEK composites are biocompatible to rats without toxicity.

In Vivo Bioactivity of HA/PEEK Composites

After the implantation of the HA/PEEK composite samples, the incision part of the rats recovered normally without infection, suggesting that the HA/PEEK composites have no toxicity to tissues and cells. The bone tissues and cells in contact with the pure PEEK and HA/PEEK composite samples implanted in the rats for 1 month are shown in Figure 3. Generally, the bone repair in trauma can be characterized by the stages of hematoma formation, granulation tissue formation, fibrous callus formation, osseous callus formation and poroma recreation. For the pure PEEK samples, the granulation tissues with inflammatory cells are dominant with minor collagenic connection tissues [Figure 3(a)], indicating that the bone repair after one month is in the granulation tissue formation stage. For the composite samples with 2.6 and 5.6 vol % HA, there are a large quantity of collagenic connection tissues along with some osteoblasts are present besides a small quantity of granulation tissues [Figure 3(b,c)], implying that the bone repair after one month of implantation is

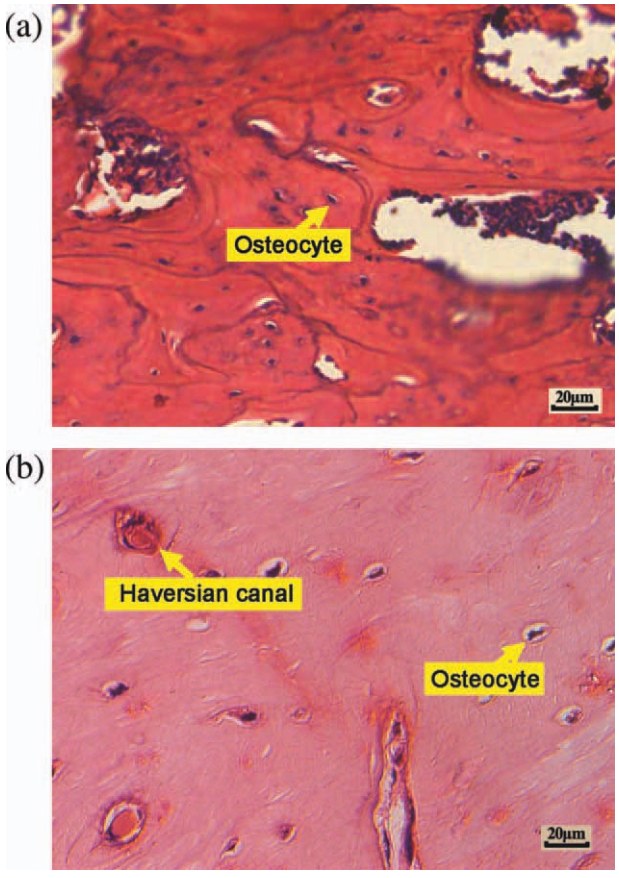

Figure 5. Histological micrographs of new bone tissue around 5.6 vol % HA-PEEKimplants: (a) for 2 months (b) for 3 months. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

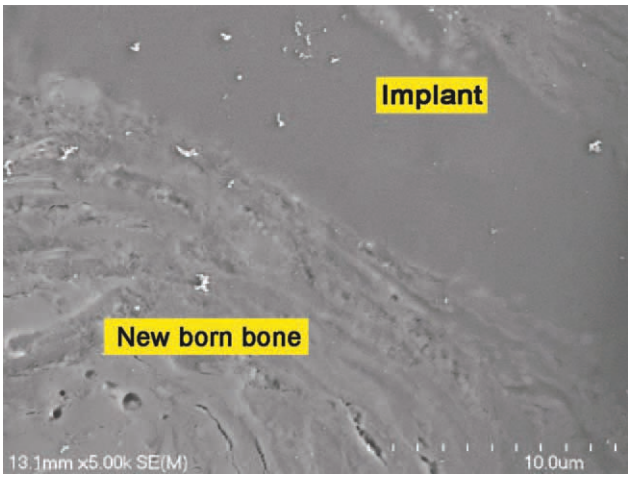

Figure 6. SEM micrograph of the interface between new born bone and implant containing. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in the stage of fibrous callus formation. The portion of connection tissues increases as the HA content increases from 2.6 vol % to 5.6 vol %. With further increasing HA content (13.4 vol % or over), the bone tissues with an orderly cell arrangement are present together with osteocytes and Haversian canals [Figure 3(d,e)], suggesting that the new bone tissues around the implant samples are maturer. These facts indicate that the HA possesses a good osteoconduction capability and can enhance bone regeneration because the HA in the composites is in contact with the body fluid and can provide a Ca^{2+} and PO_4^{3-} rich environment required for bone formation.[21,22]

Figures 4 and 5 show the tissues in contact with the implants of the pure PEEK and the 5.6 vol % HA/PEEK composite after 2 and 3 months of implantation, respectively. Obviously, the bone repair generally makes progress with implantation time. However, the process for the pure PEEK implant is slow and there are only a limited quantity of connective tissues along with inflammatory cells after 3 months (Figure 4). In contrast, the tissues in contact with the 5.6 vol %HA/PEEK composite sample are mature with a large quantity of connective tissues together with osteocytes and Haversian canals (Figure 5), suggesting that the composite materials possess an excellent bioactivity, which enhances the bone repair due to the introduction of bioactive HA.

The interfacial binding status between the 5.6 vol % HA/PEEK composite implant and the new bone after three months of implantation is shown in Figure 6. As seen, the new bone tissues are bound seamlessly at the implant surface without any fibrous tissues present between implants and bone tissues, indicating that the 5.6 vol % HA/PEEK composite material has a satisfactory bioactivity. Our previous study has shown that the 5.6 vol % HA/PEEK composite possesses excellent mechanical properties, and therefore the composite may be considered as a potential candidate for clinical test in the future since it achieves a balance between desirable bioactivity and acceptable mechanical properties.[23]

CONCLUSIONS

The biocompatibility and bioactivity of hydroxyapatite/polyetheretherketone composite materials containing different quantities of hydroxyapatite are investigated. The results demonstrate that the lab-synthesized materials possess a desirable biocompatibility without apparent toxicity to animals. The new bone tissues surrounding the composite implants grow faster with a higher HA content, and are more mature with a longer implantation period. The interface between new bones and 5.6 vol % HA/PEEK composite implants is seamless after three months of implantation, suggesting that the reliable biological fixation can be achieved. Accordingly, the *in situ* synthesized HA/PEEK composite materials are promising for hard tissue replacement since they possess a desirable bioactivity along with acceptable mechanical properties.

ACKNOWLEDGMENTS

This work was supported by the Technology Program of Nanshan district of Shenzhen in China.

REFERENCES

1. Wang, M.; Bonfield W. *Biomaterials* **2001,** *22*, 1311.
2. Montazeri, A.; Naghdabadi, R. *J. Appl. Polym. Sci.* **2010,** *117*, 361.
3. Liu, Y.; Wang, M. *J. Appl. Polym. Sci.* **2007,** *106*, 2780.
4. Kurtz, S. M.; Devine, J. N. *Biomaterials* **2007,** *28*, 4849.
5. Pramanik, S.; Kar, K. K. *J. Appl. Polym. Sci.* **2012,** *123*, 1100.
6. Utzschneider, S.; Becker, F.; Grupp, T. M.; Sievers, B.; Paulus, A.; Gottschalk, O. *Acta Biomater.* **2010,** *6*, 4296.
7. Abu Bakar, M. S.; Cheang, P.; Khor, K. A. *Mater. Sci. Eng. A* **2003,** *345*, 55.
8. Abu Bakar, M. S.; Cheang, P.; Khor, K. A. *Compos. Sci. Technol.* **2003,** *63*, 421.
9. Wang, L.; Weng, L. Q.; Song, S. H.; Sun, Q. *J. Mater. Lett.* **2010,** *20*, 2201.
10. Tang, S. M.; Cheang, P.; Abu Bakar, M. S.; Khor, K. A.; Liao, K. *Int. J. Fatigue* **2004,** *26*, 49.
11. Wang, L.; Weng, L. Q.; Song, S. H.; Zhang, Z. Y.; Tian, S. L.; Ma, R. *Mater. Sci. Eng. A* **2011,** *528*, 3689.
12. Ma, R.; Weng, L.; Bao, X.; Ni, Z.; Song, S. H.; Cai, W. Q. *Mater. Lett. Mater. Lett.* **2012,** *71*, 117.
13. Abu Bakar, M. S.; Cheng, M. H. W.; Tang, S. M.; Yu, S. C.; Liao. K.; Tan, C. T. *Biomaterials* **2003,** *24*, 2245.
14. Yu, S. C.; Hariram, K. P.; Kumar, R., Cheang, P.; Aik, K. K. *Biomaterials* **2005,** *26*, 2343.
15. Tan, K. H.; Chua, C. K.; Leong, K. F.; Naing, M. W.; Cheah, C. M. *J. Eng. Med.* **2005,** *219*, 183.
16. Wong, K. L.; Wong, C. T.; Liu, W. C.; Pan, H. B.; Fong, M. K.; Lam, W. M.; Cheung, W. L.; Tang, W. M.; Chiu, K. Y.; Luk, K. D. K.; Lua, W. W. *Biomaterials* **2009,** *30*, 3810.
17. Znazen, R.; Kaabi, H.; Hmida, S.; Ben Abid, H.; Ben Tahar, S.; Zammit, I. *Transfus Clin. Biol.* **2006,** *13*, 341.

18. Sylvetsky, N.; Raveh, D.; Schlesinger, Y.; Rudensky, B.; Yinnon, A. M. *Am. J. Med.* **2002,** *112*, 622.
19. Hoffmann, S.; Peterbauer, A.; Schindler, S.; Fennrich, S. *J. Immunol. Methods* **2005,** *298*, 161.
20. Werner, L.; Tetz, M.; Mentak, K.; Aldred, M.; Zwisler, W. *J. Cataract Refr. Surg.* **2009,** *35*, 1273.
21. Hu, X. X.; Shen, H.; Shuai, K. G.; Zhang, E. W.; Bai, Y. J.; Cheng, Y. *Appl. Surf. Sci.* **2011,** *257*, 1813.
22. Laschke, M. W.; Strohe, A.; Menger, M. D.; Alini, M.; Eglin, D. *Acta Biomater* **2010,** *6*, 2020.
23. Torres, F. G.; Nazhat, S. N.; Sheikh Md Fadzullah, S. H.; Maquet, V.; Boccaccini, A. R. *Compos. Sci. Technol.* **2007,** *67*, 1139.