Polym. Bull. (2010) 65:411–423
DOI 10.1007/s00289-010-0266-1

ORIGINAL PAPER

Biological evaluation of poly-L-lactic acid composite containing bioactive glass

Zhihua Zhou · Jianan Zhou · Qingfeng Yi · Lihua Liu · Yanmin Zhao · Huidong Nie · Xiaoping Liu · Jianpeng Zou · Lianglong Chen

Received: 28 November 2009 / Revised: 25 February 2010 / Accepted: 7 March 2010 /
Published online: 14 March 2010

Abstract Biodegradable and biocompatible materials are the basis for medical application. As an initial step for developing bone internal fixation materials, the biological evaluation of poly-L-lactic acid/bioactive glass (PLLA/BG) composite in vitro and in vivo, including the hemolysis test, pyrogen test, acute systemic toxicity test, genetic toxicity test, anaphylaxis test, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test, direct cell culture and in vivo implant experiment, was performed. The results indicated that PLLA/BG composite showed no acute systemic toxicity, genetic toxicity, anaphylaxis reaction, and pyrogen reaction, and the hemolysis ratio was 0.39%. MTT assay indicated that no cytotoxic effect was observed for the PLLA/BG composite, and in addition, a significant increase in cellular activity compared to the negative control group was found. Excellent adhesion between fibroblast and PLLA/BG material was observed, the fibroblasts cultured on the PLLA/BG composite substrates revealed a higher proliferation and differentiation rate than those on the pure PLLA substrates. In vivo implant experiment showed that the PLLA/BG composite could maintain the mechanical properties during the course of fracture therapy, and the malleolar

Z. Zhou (✉) · J. Zhou · Q. Yi · L. Liu · Y. Zhao · H. Nie · X. Liu
Key Laboratory of Theoretical Chemistry and Molecular Simulation of Ministry of Education, School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan, People's Republic of China
e-mail: zhou7381@126.com

Z. Zhou · J. Zou
State Key Laboratory for Powder Metallurgy, Central South University, Changsha, People's Republic of China

L. Chen
Department of Orthopedics, The Second Xiangya Hospital, Central South University, Changsha, People's Republic of China

fracture of rabbits was healed in 8 weeks on the whole. Therefore, PLLA/BG composites have a promising biological response as a potential implant material.

Keywords Poly-L-lactic acid · Bioactive glass · Composite · Biological evaluation · Cytotoxicity · Internal fixation

Introduction

Poly-L-lactic acid (PLLA) and its copolymer have been very attractive to biomedical materials due to their controllable biodegradability and the safety of their degraded products [1, 2]. However, these polymers may elicit inflammatory response in the host tissue because of the release of acidic degradation products and lack bioactivity [3, 4]. On the other hand, these synthetic resorbable polymers are more easily fabricated into complex structures yet are too weak to meet the demands of orthopedic surgery [5, 6].

Bioactive glasses can bond to living bone directly, which is due to the formation of biologically active carbonate containing hydroxyapatite on their surfaces when implanted in bony defects [7]. The benefit of the direct bonding of bioactive glasses to bone is the tight fixation of the implant with bony tissues. However, there is limitation on clinical application because of their inappropriate mechanical properties, such as high Young's modulus, low toughness, and brittle character [8]. The composite of bioactive glass with PLLA has been developed to achieve a mechanical performance comparable to that of natural bone as well as bone-bonding ability [9, 10]. The PLLA/BG composite is of greater initial hardness and rigidity, and the speed of early degradation is decreased, which is helpful to the early cicatrisation of bone fracture, so the synostosis of the composite is enhanced. The composite has good mechanical properties; moreover, in clinic, the secondary operation is not needed owing to its degradability and it does not release any metallic ions that may cause series of adverse reaction [11, 12].

However, other conditions should be met for a material to be considered suitable for biomedical use. For successful functioning biomaterials, the biocompatibility and the absence of cytotoxicity of the materials are the essential requirement. Being considered as "the ability of a material to perform with an appropriate host response in a specific application" [13, 14], the biocompatibility of biomaterial is closely related to cell behaviors in contact with them, such as cell adhesion, morphology, proliferation, and differentiation [15–17].

It is equally important to consider the changes in the local environment created by a composite which includes a highly reactive phase before in vivo testing of the material, as these changes may have considerable effects on the behavior of the cells [18, 19]. Cytocompatibility is a basic requirement for an implant material. The simplicity, sensitivity, and reliability of in vitro cell culture make it a useful initial screening method for biomaterials. Furthermore, it is possible to test any toxic effect on human cells, thereby assessing the same cellular response as in vivo [20–22].

In this study, biological evaluation of PLLA/BG composite was investigated including cell cytotoxicity, acute systemic toxicity, genetic toxicity, hemolysis,

pyrogen tests, and direct cell culture. The in vivo implantation of PLLA/BG screw for fixing and healing progress of rabbit condyle fracture was also investigated.

Experimental

Preparation of PLLA/BG composite and leaching liquor

The raw materials used in this study were PLLA with a weight average molecular mass of 8.75×10^5 Da and bioactive glass with a composition of 35CaO, 60SiO_2, 5P_2O_5 (mol.%). The PLLA/BG (90/10 in weight) composite was obtained by cold pressing and hot pressing methods at 185 °C according to the previous literature [12]. PLLA/BG composites were placed in tissue culture flasks with RPMI-1640 medium containing 10% fetal calf serum (FCS) at a ratio of 0.1 cm^2 mL^{-1} medium and incubated in a cell incubator for 48 h (100% relative humidity and 5% CO_2).

Fresh anti-coagulated rabbit blood

0.5 mL of 20 g L^{-1} potassium oxalate solution was added into each 10-mL aliquot of fresh rabbit blood for anticoagulation. Then each 8 mL of anti-coagulated blood was attenuated with 10-mL physiological saline.

Hemolysis measurement

4 g PLLA/BG composite was immersed into 10-mL physiological saline at 37 °C for 30 min. Then 0.2-mL prepared cony blood was added into the saline for 60 min of incubation at 37 °C. Two comparison groups were settled for comparison. Negative control group included the saline and blood but no material, while the positive control group included distilled water instead at saline of negative control group. After a centrifuge at 600 rpm, the supernatant liquid was taken for optical density (OD) examination at 545 nm. Three samples were carried out in each group. Hemolytic rate was calculated with the following formula:

$$\begin{aligned}\text{Hemolytic rate} = &\left(\text{OD}_{\text{material}} - \text{OD}_{\text{negative control}}\right)/ \\ &\left(\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}}\right) \times 100\%.\end{aligned} \quad (1)$$

Pyrogen measurement

Totally five healthy adult New Zealand rabbits with body mass 1.5–3.0 kg of either gender (female rabbit was not pregnant) were selected, which were screened by pre-measurenlent of body temperature. Qualified NaCl injection in pre-test was selected as extraction medium for the preparation of PLLA/BG leaching liquor. The rabbits selected for the experiment should be left in the same environment 1 or 2 days before the study. The changes of the room temperature during one experiment should not exceed 5 °C. Rabbits should be fixed on fixer to prevent agitation when the normal body temperature was measured. The first measurement started after 30 min Anal

thermometer was inserted into the anus of the rabbits at a depth of 6 cm for at least 2 min. Totally, 10 mL kg^{-1} of PLLA/BG leaching liquor (38 °C) was injected slowly into auricle marginal vein after 15 min if the rabbit was accorded with the requirement. The body temperature of the rabbit was measured every 1 h for a total of three times. The difference between the highest body temperature among the three measurements and normal temperature would be evaluated based on the criteria.

Acute systemic toxicity test

A total of 12 rabbits were weighed and randomly divided equally into experimental group, negative control group, and positive control group. Four rabbits in each group were injected abdominally with standard leaching liquor (50 mL kg^{-1}), saline and formaldehyde (volume fraction 0.05), respectively. The general state, toxicity appearance, and animal death number of each group were recorded 4, 24, 48, and 72 h after injection; each animal was also weighed 24, 48, and 72 h after injection. We used the toxicity grading system suggested by the Assembles.

Genetic toxicity test

Eighteen mice were taken into genetic toxicity test in three groups, with six animals in each. All mice were abdominally injected with leaching liquor (50 mg kg^{-1}), saline, and cyclophosphamide (50 mg kg^{-1}), respectively. All mice in genetic toxicity test were injected twice, the second injection was carried out 24 h after first injection, and mice were killed 6 h later to obtain femur bone marrow smears, which were fixed with methanol and stained with Giemsa. We counted 10,000 polychromatic erythrocytes (PEC) for each mouse. The number of PEC with micronucleus was recorded and interclass statistics analysis, expressed as %, and made by Poisson distribution.

Anaphylaxis test

A total of 20 white guinea pig of either gender aged 1–3 months with a body mass 300–500 g were randomly divide into sample group ($n = 10$) and control group ($n = 10$) according to random number table. Saline was used as extraction medium for the extraction of PLLA/BG leaching liquor for intradermal injection and local application. 5 g L^{-1} of formaldehyde solution was used as positive control, and saline was set as negative control. The experiment was performed through intradermal injection and local skin patch test, and the provocation test was carried out after 14 days of local skin patch test. The reaction of the provocation site was observed at 24, 48, and 72 h after the removal of the application.

MTT test

Cell culture studies were carried out with L929 mouse fibroblast cell line. MTT assay was optimized for the cell lines used in the experiments. Briefly, three groups of cells were seeded in 96-well plates with cell seeding densities of 1×10^5 in a

humidified incubator containing 5% CO_2 at 37 °C for 24 h. Then, cells were incubated for 24 h in PLLA/BG leaching liquor, negative control medium, and positive control medium. Positive controls contained media of 0.64% phenol and negative controls contained only RPMI-1640 medium containing 10% FCS. For the purposes of the experiments at the end of the incubation time (7 days), cells were incubated for 4 h with 10 μmol L^{-1} of MTT. Washing with PBS was followed by the addition of DMSO (150 μL), gently shaking for 10 min so that complete dissolution was achieved. The OD was measured at 492 nm. The values reflect the viable cell population in each well.

Cell morphology on PLLA and PLLA/BG composite surface

Cell attachment and cell morphology on PLLA and PLLA/BG films were studied. PLLA and PLLA/BG composite films were obtained by a solvent evaporation technique. Cover-slides coated with PLLA/BG were placed into 24-well plates (Costar) and washed three times with PBS. 3-mL medium was added to the wells to prevent the cover slide from floating during cell seeding. Into each well, 1.0×10^5 cells (in 1 mL of medium) were then placed, and the plates were incubated at 37 °C and 5% CO_2 for 7 days. The cover-slides were washed three times with PBS and fixed with 3% glutaraldehyde in PBS at room temperature for 30 min. After thorough washing, the cells were dyed with one drop of Giemsa stain (SIGMA) for 30 min, washed with distilled water, and dried in air. Cell attachment and cell morphology were observed under in scanning electron microscope (SEM, KYKY-2800) after being sputter-coated with gold.

In vivo implant

Twelve rabbits were randomly selected and randomly divided into two groups (the experimental group and the control group). After feed for 7 days separately, the rabbits were anesthetized with 1 mL kg^{-1} ketamine and 1 mL kg^{-1} proazamine, then fixed onto the operation table for routine skin preparation and sterilization. The skin and periosteum of femoral condyles of left anterior limb were cut off curvedly and turned over to expose the condyles of femur. The condyle was cut off from outer top to inner underside by bistoury, and a condyle fracture was formed. For the experimental group, the sample screw of PLLA/BG was implanted into the drilled bone cavities (Φ 2.5 mm × 4 mm) among condyles of femur sites of the rabbits (the rod could be s lightly higher than the surface of bone), then the samples were covered by periosteum and skin, the wounds were washed by normal saline, and the periosteum and skin were sutured delaminately. For the control group, nothing was implanted and the animals were taken as controls. After the surgery, the rabbits could move freely without external fixation. Penicillin (4.0×10^5) IU and gentamicin (8.0×10^4) IU were intramuscularly injected for continuous 3 days to avoid infection. After operation six rabbits were killed, respectively, after 1, 4, and 8 weeks implantation. The position of the implants in relation to the bone defect was examined radiographically. Bone healing progress was evaluated with further X-ray films taken in anteroposterior view.

Statistical analysis

All the quantitative results were obtained from triplicate samples. Data were expressed as a mean ± standard deviations. Statistical analysis was carried out using the unpaired Student's t test. A value of $p < 0.05$ was considered to be statistically significant.

Results and discussion

Hemolysis assay

There is absence of cracked erythrocyte and hemagglutination in the underlayer of the centrifuged blood. The results of OD value and hemolysis rate of PLLA/BG composite are listed in Table 1. The mean value of hemolytic rate is 0.39%. The hemolytic rate less than 5% can be considered to be satisfactory; therefore, hemolysis test showed that this material meets the standard requirement for biological materials.

Pyrogen assay

As shown in Table 2, the increments of body temperature of five rabbits were all below 6 °C with the total increment below 1.4 °C. Hence, this PLLA/BG composite accorded with the requirement of pyrogenic reaction test, which meant it passed this test.

Table 1 Optical density value and hemolytic rate of PLLA/BG sample

Sample	$OD_{PLLA/BG}$	$OD_{positive\ control}$	$OD_{negative\ control}$	Hemolysis rate (%)
1	0.005	0.580	0.006	0.17
2	0.005	0.623	0.003	0.30
3	0.012	0.543	0.008	0.70

Table 2 Results of pyrogenic reaction test

Rabbit	Body temperature before injection (°C)	Body temperature after injection (°C)			Increment of body temperature (°C)
		1	2	3	
1	38.40	38.55	38.50	38.65	0.17
2	38.50	38.75	38.70	38.70	0.32
3	38.55	38.70	38.65	38.75	0.15
4	38.50	38.75	38.70	38.65	0.20
5	38.45	38.65	38.60	38.70	0.20

Acute systemic toxicity assay

The symptom of toxicity was found in neither experimental group nor negative control group. After injection, the general state of animals maintained well, fed routinely, and no abdomen irritation, exhaustion, cyanosis, and death occurred, while six animals in positive control group showed slight and serious toxic reaction. Acute systemic toxicity test is a non-specific acute toxicity test to judge the acute toxicity effect of materials by intravenous or intraperitoneal injection with material leaching liquor [23]. In this study, all animals in leaching liquor group occurred no toxicity response, suggesting the safety of PLLA/BG composite.

Genetic toxicity assay

The polychromatic erythrocytes (PEC) with micronucleus are shown in Fig. 1. The polychromatic erythrocytes take on bice and the erythrocytes present nacarat. The PEC with micronucleus is single and round with slippery and soigne hem.

As shown in Table 3, the incidence of PEC micronuclei suggested no difference between three experimental and negative control groups ($p > 0.05$); however, there

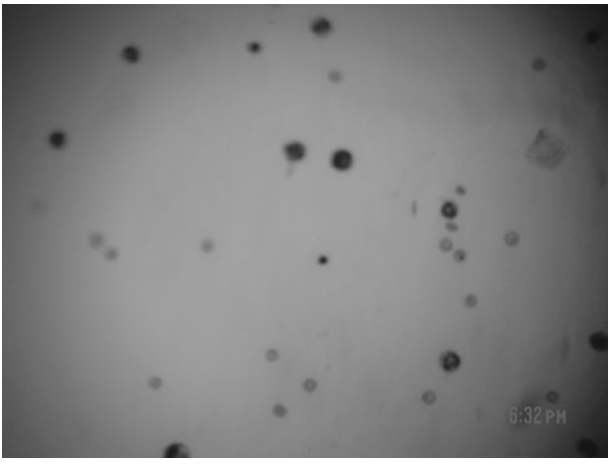

Fig. 1 Polychromatic erythrocyte

Table 3 Results of genetic toxicity test

Group	Animal number	PEC number	Number of PEC with micronucleus	Micronucleus formation/%	p Value
Leaching liquor	10	10000	19	1.9 ± 0.86	>0.05
1/3 Leaching liquor	10	10000	16	1.6 ± 0.78	>0.05
1/9 Leaching liquor	10	10000	22	2.2 ± 0.86	>0.05
Negative control	10	10000	14	1.4 ± 0.57	>0.05
Positive control	10	10000	458	45.8 ± 1.87	<0.05

was statistically significant difference between the positive and negative control groups ($p < 0.05$). Therefore, we initially concluded no teratogenic action or mutagenic action could be found in the material. Micronucleus test is a quick way to evaluate the chromosome damage and caryocinetic interference induced by material [24]. In this study, we could initially conclude the material with no teratogenic action or mutagenic action.

Anaphylaxis assay

Maximum dose was used. No erythema and edema were found in every provocation site at each time point in PLLA/BG group after removal of the application through the tests of injection induction, local skin patch test, and provocation. Therefore, the results of the PLLA/BG were evaluated as 0 level in skin reaction and thereby the composite passed skin anaphylaxis test.

Cell proliferation and viability

Cell cytotoxicity test is one of the important factors that affect the use of biomaterial composite for medical application. MTT test is a biochemical test widely used to assess cytotoxicity by measuring cell viability and proliferation in a qualitative way. This biochemical test is based in the reduction of MTT (which is water-soluble salt and has a yellow tonality) by the cell mitochondrial enzyme succinate dehydrogenase, yielding a purple-color salt insoluble in water [25]. The salt absorbs at a wavelength of 492 nm, and because only living cells have the capability to metabolize the MTT, it gives a measurement of the viable cells [26].

The results of toxicity of PLLA/BG by MTT assay is presented in Fig. 2. The percent of viable cells cultured in extracts of PLLA/BG is higher than that of

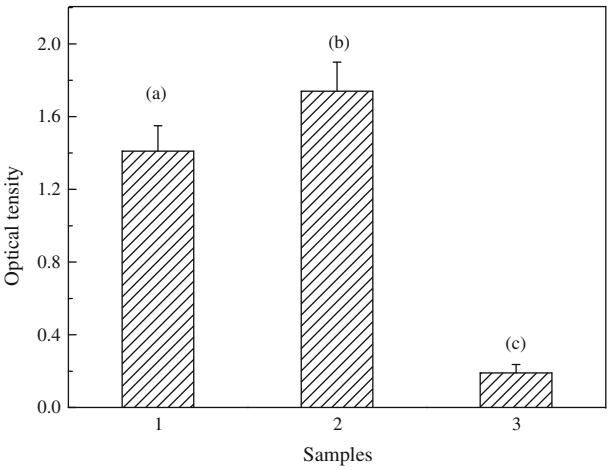

Fig. 2 MTT assay of the samples after 7-day culture: (*a*) negative control group; (*b*) PLLA/BG leaching liquor; (*c*) positive control group

negative control group ($p < 0.05$); however, positive control group shows lower percent of viability ($p < 0.05$). It indicates that no cytotoxic effect was observed for the PLLA/BG composite, and in addition, a significant increase in cellular activity was found, suggesting that the bioactive in the composite was able to stimulate cellular activity by creating a favorable microenvironment for cell proliferation and growth [27].

Cell attachment and morphology

Figure 3 shows representative SEM photographs of the cells attached on the surfaces of PLLA/BG and PLLA films after 7 days of incubation. Only rare cell was seen on the surface of the PLLA film (Fig. 3a); however, on the surface of PLLA/BG film, a large number of cells, mostly rounded in appearance, attached closely, and some fibroblast cells showed spread morphologies. The bulges of the main bodies of the fibroblast cells were still apparent, and the spreading cells were readily distinguishable from the substrate.

We chose to combine bioactive glass with degradable PLLA, because the ion dissolution products of the bioactive glass counteract the acidic degradation products of the polymer, which may otherwise lead to inflammatory responses [28]. Moreover, incorporation of bioactive glass within the PLLA matrix has been reported to play an important role in polymer surface wettability [29] and in determining the characteristics of polymer surfaces. Also, there is some evidence that bioactive glasses may increase collagen production by some cells [30]. As shown in Fig. 3, the presence of bioactive glass particles in PLLA may have positive biological effects. The PLLA/BG composites also demonstrated improved cell compatibility due to the good biocompatibility of the bioactive glass particles and the more uniform distribution of the glass particles on the film surface, and the loss of the bioactive glass particles in contact with the culture medium results in a coarse surface for the cell adhesion and proliferation. In addition, it is undoubted that incorporation of bioactive glass into PLLA introduced calcium ions in the surfaces of the PLLA/BG composite substrates. Therefore, the addition of bioactive glass into PLLA improved the interactions between cells and materials [31].

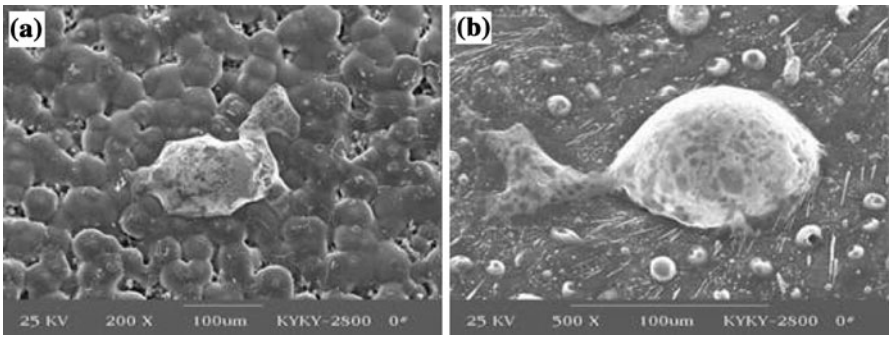

Fig. 3 SEM micrographs of cultured fibroblasts on the surfaces of materials after 7 days: (**a**) PLLA; (**b**) PLLA/BG

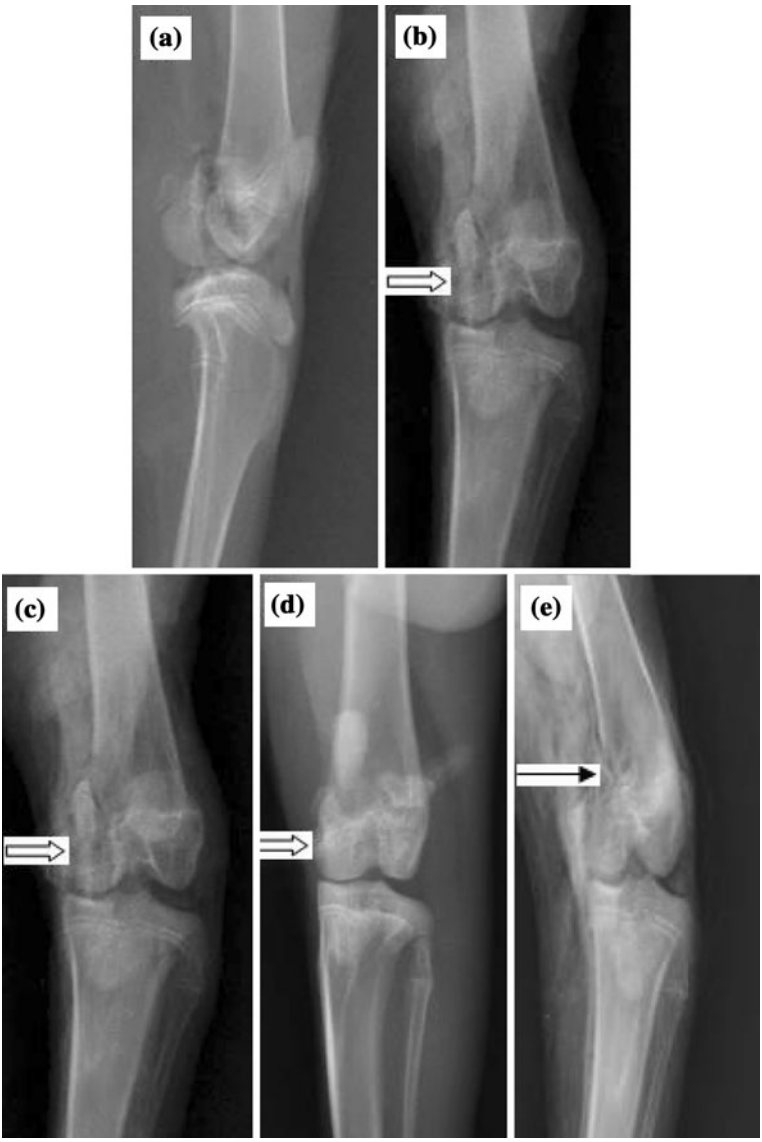

Fig. 4 X-ray photograph of rabbit fracture: (**a**) fracture model; (**b**) 1 week after operation; (**c**) 4 weeks after operation; (**d**) 8 weeks after operation (*white arrow* at the screw location); (**e**) 8 weeks after operation of the control group (*black arrow* at the catagmatic stria location)

Radiographic analysis after in vivo implantation

Animals appeared to recover quickly from the surgical procedure were observed to regain full movement within less than 3 weeks. The rabbits continued to exhibit no physical limitations, with normal behavior, and movement observed throughout the 8-week period.

The X-ray photograph of rabbit fracture was shown in Fig. 4. It is shown that there was a clear catagmatic stria in the rabbit fracture model. For experimental groups, there was no distinct change after 1 week except that calluses were formed around the defects (seen in Fig. 4b). After 4 weeks of post-operation, the catagmatic stria became obscure; the catagmatic stria disappeared after 8 weeks and the fracture was healed (Fig. 4d). After 8-week implantation, the osteotomies showed the PLLA/BG screw had a tight combination with rabbit bone tissue, and there was fibrous tissue linkage between the two parts. There was no gross evidence of inflammation nor necrosis around the implant sites. The mechanical properties could maintain the concrescence of rabbit fracture. Besides, the composite has a resistance effect on X-ray, which is convenient for the clinical developing observation. However, roentgenographic examination of the control group showed a clear catagmatic stria and a small amount of peripheral callus at 4–8 weeks post-operation (Fig. 4e), this indicated that the osteotomy was not healed completely. The in vivo studies further demonstrated that 8 weeks post-transplantation bone repair in the experimental group was roentgenographically better than that of the control group.

Conclusions

In this study, the biological assessments, containing the hemolysis test, pyrogen test, acute systemic toxicity test, genetic toxicity test, anaphylaxis test, MTT test, direct cell culture, and in vivo implant experiment, of the PLLA/BG composite were studied. All the results indicated that PLLA/BG had good compatibility and had no cytotoxicity. The results showed that the PLLA/BG composites were more suitable for the cells adhesion than the pure PLLA due to their improved inorganic component. In addition, the fibroblasts on the PLLA/BG substrates proliferated and differentiated more quickly than those on the pure PLLA substrates. Therefore, it can be concluded that the addition of bioactive glass into PLLA stimulates the proliferation and differentiation of fibroblasts and the PLLA/BG composite is biocompatible and might be suitable for preparation of bone implant.

Acknowledgments This project was financially supported by the Foundation of State Key Laboratory for Powder Metallurgy, Central South University, P. R. China and the Doctoral Research Foundation of Hunan University of Science and Technology (No. E50858).

References

1. Middleton JC, Tipton AJ (2000) Synthetic biodegradable polymers as orthopedic devices. Biomaterials 21:2335–2346
2. Griffith LG (2000) Polymeric biomaterials. Acta Mater 48:263–277
3. Agrawal CM, Ray RB (2001) Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. Biomed Mater Res 55:141–150
4. He Z, Xiong L (2009) Preparation and in vitro degradation behavior of poly(L-lactide-co-glycolide) by direct-melt polycondensation. J Macromol Sci Phys 48:789–798

5. Niiranen H, Pyhalto T, Rokkanen P (2004) In vitro and in vivo behavior of self-reinforced bioabsorbable polymer and self-reinforced bioabsorbable polymer/bioactive glass composites. Biomed Mater Res 69A:699–708
6. Boccaccini AR, Roether JA, Hench LL (2002) Composites approach to tissue engineering. Ceram Eng Sci Proc 23:805–816
7. Roether JA, Boccaccinib AR, Hench LL (2002) Development and in vitro characterisation of novel bioresorbable and bioactive composite materials based on polylactide foams and Bioglass® for tissue engineering applications. Biomaterials 23:3871–3878
8. Blaker JJ, Maquet V, Boccaccini AR, Jerome R, Bismarck A (2005) Wetting of bioactive glass surfaces by poly(alpha-hydroxyacid) melts: interaction between bioglass and biodegradable polymers. e-Polymers 23:1–13
9. Zhou Z, Chen L (2008) Morphology expression and proliferation of human osteoblasts on bioactive glass scaffolds. Mater Sci-Poland 26:505–515
10. Zhou ZH, Ruan JM, Zhou ZC, Zou JP, Chen LL (2008) Preparation and properties of bioactive composite based on bioactive glass and poly-L-lactide. Polym Plast Technol 47:451–457
11. Zhou ZH, Ruan JM, Zou JP, Zhou ZC (2007) Bioactivity of bioresorbable composite based on bioactive glass and poly-L-lactide. Trans Nonferrous Met Soc China 17:394–399
12. Zhou Z, Yi Q, Liu X, Liu L, Liu Q (2009) In vitro degradation behaviors of poly-L-lactide/bioactive glass composite materials in phosphate-buffered solution. Polym Bull 63:575–586
13. Groth T, Altankov G (1998) Cell-surface interactions and the tissue compatibility of biomedical materials. IOS Press, Washington, p 12
14. Lee CT, Lee YD (2006) Preparation of porous biodegradable poly(lactide-co-glycolide)/hyaluronic acid blend scaffolds: characterization, in vitro cells culture and degradation behaviors. J Mater Sci Mater Med 17:1411–1420
15. Huhtala A, Pohjonen T, Salminen L, Salminen A, Kaarniranta K, Uusitalo H (2007) In vitro biocompatibility of degradable biopolymers in cell line cultures from various ocular tissues: direct contact studies. J Biomed Mater Res A 83:407–413
16. He Z, Xiong L (2009) Assessment of cytocompatibility of bulk modified poly-L-lactic acid biomaterials. Polym Plast Technol 48:1020–1024
17. Fan X, Chen J, Ruan JM, Zhou ZC, Zou JP (2010) Preparation and properties of poly(lactide-co-glycolide)/bone meals composite materials. Polym Plast Technol 49:309–315
18. Fournier E, Passirani C, Montero-Menei CN, Benoit JP (2003) Biocompatibility of implantable synthetic polymeric drug carriers: focus on brain biocompatibility. Biomaterials 24:3311–3331
19. Barbanti SH, Santos AR Jr, Zavaglia CAC, Duek EAR (2004) Porous and dense poly(L-lactic acid) and poly(D,L-lactic acid-co-glycolic acid) scaffolds: in vitro degradation in culture medium and osteoblasts culture. J Mater Sci Mater Med 15:1315–1321
20. Bhang SH, Lim JS, Choi CY, Kwon YK, Him BS (2007) The behavior of neural stem cells on biodegradable synthetic polymers. J Biomater Sci Polym Ed 18:223–239
21. Karp JM, Shoichet MS, Davies JE (2003) Bone formation on two dimensional poly(D,L-lactide-co-glycolide) (PLGA) films and three-dimensional PLGA tissue engineering scaffolds in vitro. J Biomed Mater Res 64A:388–396
22. Johson HJ (1983) Biocompatibility test procedures for materials evaluation in vitro comparative test system sensitivity. J Biomed Mater Res 17:571–579
23. He Y, Yang SH, Li T (2008) Preparation and in vivo biocompatibility of a novel thermosensitive hydrogel. J Clin Rehab Tissue Eng Res 12:1157–1160
24. Pietrzak WS, Eppley BL (2007) In vitro characteristics of a bioabsorbable suspension screw and suture system for endoscopic brow lift surgery. J Craniofac Surg 18:429–436
25. Mosmann T (1983) Rapid colorimetric assay of cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
26. Hu X, Shen H, Yang F, Bei J, Wang S (2008) Preparation and cell affinity of microtubular orientation-structured PLGA (70/30) blood vessel scaffold. Biomaterials 29:3128–3136
27. Van Wachem PB, Beugelling T, Feijen J, Bantjes A, Detmers JP, Van Aken WG (1985) Interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities. Biomaterials 6:403–410
28. Heidemann W, Jeschkeit S, Ruffieux K, Fischer JH (2002) Degradation of poly(D,L)lactide implants with or without addition of calcium phosphates in vivo. Biomaterials 22:2371–2381

29. Yu D, Li Q, Mu X, Chang T, Xiong Z (2008) Bone regeneration of critical calvarial defect in goat model by PLGA/TCP/rhBMP-2 scaffolds prepared by low-temperature rapid-prototyping technology. Int J Oral Maxillofac Surg 37:929–934
30. Bosetti M, Zanardi L, Hench LL, Cannas M (2003) Type I collagen production by osteoblast-like cells cultured in contact with different bioactive glasses. J Biomed Mater Res 64A:189–195
31. Li H, Zhai W, Chang J (2008) In vitro biocompatibility assessment of PHBV/Wollastonite composites. J Mater Sci Mater Med 19:67–73