J Mater Sci: Mater Med (2011) 22:2555–2563
DOI 10.1007/s10856-011-4439-8

Bone substitute biomedical material of multi-(amino acid) copolymer: in vitro degradation and biocompatibility

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Received: 21 April 2011 / Accepted: 25 August 2011 / Published online: 6 September 2011

Abstract Degradable polymers with good mechanical strength as bone repair biomaterials have been paid more attention in biomedical application. In this study, a multi-(amino acid) copolymer consisting of 6-aminocaproic acid and five natural amino acids was prepared by a reaction of acid-catalyzed condensation. The results revealed that the copolymer could be slowly degradable in Tris-HCl solution, and lost its initial weight of 31.9 wt% after immersion for 12 weeks, and the changes of pH value of Tris-HCl solution were in range from 6.9 to 7.4 during soaking. The compressive strength of the copolymer decreased from 107 to 68 MPa after immersion for 12 weeks. The proliferation and differentiation of MG-63 cells on the copolymer significantly increased with time, and the cells with normal phenotype extended and spread well on the copolymer surfaces. When the copolymer was implanted in muscle and bone defects of femoral cortex of dogs for 12 weeks, the histological evaluation confirmed that the copolymer exhibited excellent biocompatibility and more effective osteogenesis in vivo. When implanted into cortical bone defects of dogs, the copolymer could be combined directly with the natural bone without fibrous capsule tissue between implants and host bone. The results indicated that the multi-(amino acid) copolymer with sufficient strength, good biocompatibility and osteoconductivity had clinical potential for load-bearing bone repair or substitution.

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1 Introduction

Degradable polymers are a versatile class of functional materials and have been extensively investigated as biomaterials for medical applications, which have shown great advantages when they are used as temporary substitutes, for example, bone defect fillers, bone fracture fixation, sutures, scaffolds for tissue engineering as well as controllable-released drug carrier [1–6]. Generally, biomedical polymer implants can be degradable in vivo and hence allow the host tissue growth till complete healing, while eliminate the need for the implant removal and can greatly reduce the pain for patients caused by the second operation. Ideally, the basic requirements for biodegradable polymers are biocompatible, and can be degradable at a controllable rate in accord with tissue growth. Meanwhile, the implants should not cause any excessive or chronic inflammatory response in vivo. Furthermore, they should provide sufficient mechanical properties and decompose into non-toxic products [7–10].

Presently, degradable polymers for medical applications consist of synthetic polymers (such as polyvinyl alcohol, linearity aliphatic series polyester etc.) and natural polymers (such as collagen, chitin, and cellulose etc.). However, some synthetic polymers, for example polyesters, show poor mechanical strength in clinical applications [11, 12]. Other synthetic polymers of aliphatic series polyesters (such as PLA, PGA and PLGA) present good mechanical strength but sometimes show a collapse degradation manner after implanted in vivo, which leads to the incompatibility of

mechanical properties between implants and host tissue [1, 13, 14]. Additionally, because these polymers give rise to acid degradation by-products, stimulation to tissue and even inflammation were reported in some literatures. Natural polymers (such as collagen) present poor mechanical strength and immune rejection as well as difficulties on sterilization, and thus are limited in clinical applications [15, 16]. Hence, it is imperative to explore new degradable polymers with good biocompatibility and sufficient strength.

Previous studies reported the synthesis of dual-(amino acid) copolymer [17, 18]. More recently, we have developed a novel degradable multi-(amino acid) copolymer and evaluated its mechanical performances [19]. The copolymer was composed of six amino acids, 6-aminocaproic acid, glycine, L-alanine, L-phenylalanine, L-proline and L-lysine. It was found that the content of 6-aminocaproic acid in the copolymer had significant effects on the mechanical strength and intrinsic viscosity of the copolymer. Based on the previous results, the in vitro degradation properties in Tris-HCl solution and the biocompatibility of both in vitro and vivo of the copolymer were evaluated in this study.

2 Materials and methods

2.1 Preparation of multi-(amino acid) copolymer

6-aminocaproic acid, L-alanine, L-phenylalanine, glycine, L-proline, L-lysine were purchased from Hebei kairuijie amino co., Ltd., China. The multi-(amino acid) copolymer was synthesized by acid-catalyzed condensation reaction in three-necked flask with a continuously stirring. The reaction flask was charged with 6 g of L-alanine, 7 g of L-phenylalanine, 2 g of glycine, 108 g of 6-aminocaproic acid, 6 g of L-proline, 2 g of L-lysine and 50 ml water. 0.5 ml of phosphorus acid (1 mol/l) was used as the catalyst. The mixture was heated to 200°C and kept for a while until 50 ml of water was fully evaporated. Then the mixture was kept at 220°C for 2 h and at 230°C for 2 h subsequently. After cooled to room temperature, the copolymer was obtained, and then dipped in ethanol and water for 24 h, respectively. To avoid undesirable oxidation reactions, the reaction system was protected with a continuous flow of nitrogen gas. The scheme of synthesis of multi-(amino acid) copolymer (polymerization reaction) is shown in Fig. 1. Our previous results showed that the dosage of 6-aminocaproic acid affected the intrinsic viscosity and mechanical strength of the copolymer. When the content of 6-aminocaproic acid was 80% (mol), the intrinsic viscosity of the copolymer was 1.45 dl/g, and the polymer had good mechanical strength (compressive strength of about 107 MPa, bend strength of 78 MPa, and tensile strength of 91 MPa) [19].

2.2 In vitro degradation

2.2.1 *Weight loss and pH change of Tris-HCl solution*

The degradation of the multi-(amino acid) copolymer in Tris-HCl solution (pH = 7.4) was determined by measuring its weight loss ratio at 1, 2, 4, 8, 12 weeks. The initial dried weight (W_i) was obtained after the samples were dried at 80°C in vacuum oven for 4 h. The samples (∅ 8 × 12 mm) were immersed in Tris-HCl solution with 200 ml PE tubes at 37°C and a solid/liquid ratio of 1 g/200 ml. The tubes were capped and placed in a shaking water bath (37°C and 72 rpm) and the Tris-HCl solution was refreshed every week. After soaking, the specimens were removed from the liquid, and the weight (W_d) was obtained after rinsed with distilled water and dried in an oven for 4 h at 80°C. The weight loss ratio of the multi-(amino acid) copolymer in solution was calculated by the follow equation:

$$\textit{Weight loss ratio} = 100\%(W_i - W_d)/W_i$$

After soaking the multi-(amino acid) copolymer samples into Tris-HCl solution, the changes of pH value of the Tris-HCl solution was measured using an electrolyte-type pH meter (PS-25, Shanghai Leici inc., China) at 1, 3, 5, 7, 14, 28, 56 and 84 days under room temperature condition. Five samples were tested at each time point, and the results were expressed as Mean ± SD.

2.2.2 *Compressive strength and morphology changes of the copolymer*

The compressive strength of the multi-(amino acid) copolymer samples (∅ 8 × 12 mm) after soaking into Tris-HCl solution for 0, 2, 4, 8 and 12 weeks was determined with mechanical testing machine (REGER 30-50, Shenzhen Reger Co., Ltd., China). The cross-head speed was 5 mm/min, and the load was applied until the specimens were compressed to about 30% in height. Five replicates were carried out for each group, and the results were expressed as Mean ± SD. Furthermore, the surface morphology/microstructure of the multi-(amino acid) copolymer samples after immersion in Tris-HCl solution for 8 weeks was characterized by SEM.

2.3 In vitro cytocompatibility

2.3.1 *Cell morphology and proliferation*

The MG-63 cells were cultured on the polymer discs (∅ 6 × 2 mm). The cells were allowed to attach to the polymer substrates in a humidified atmosphere at 37°C and 5% CO_2, respectively. At each time point, the samples

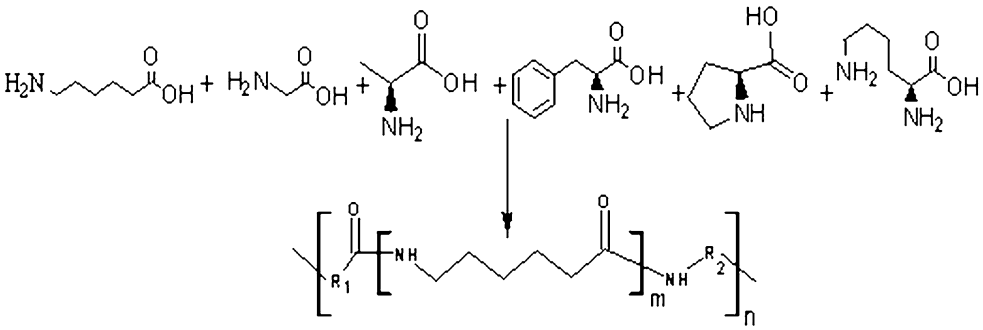

Fig. 1 Scheme of synthesis of multi-(amino acid) copolymer, R_1 and R_2 represent the natural amino acids

were removed and washed with phosphate-buffered saline (PBS) twice and fixed in 2.5% glutaraldehyde in 0.1 M sodium-PBS for 30 min. The fixed cells were washed with PBS three times, and then dehydrated in ascending concentrations of ethanol for 5 min. The specimens were prepared by first immersing in 50% alcohol-HMDS (hexamethyldisilazane) solution (v/v) for 10 min and then in pure HMDS for 10 min. Later, the samples were vacuum-dried at 37°C overnight, and the morphology of the cell on the polymer samples at 3 days was observed using SEM.

The proliferation of MG-63 cells on the polymer samples with the size of (∅ 6 × 2 mm) was determined using 3-{4,5-dimethylthiazol-2yl}-2,5-diphenyl-2H-tetrazolium-bromide (MTT) assay. The medium was removed and 2 ml of MTT solution was added to each well. After incubation at 37°C for 4 h in a fully humidified atmosphere at 5% CO_2 in air, the medium was discarded and the precipitated formazan was dissolved in DMSO (200 ml/well), and optical density (OD) of the solution was evaluated using a Thermo VARIOSKAN FLASH at a wavelength of 570 nm. The analytical assays were performed 1, 3, 5, 7 days.

2.3.2 Alkaline phosphatase (ALP) activity

MG-63 cells were seeded on the multi-(amino acid) copolymer (∅ 6 × 2 mm) at 4×10^3 cells/sample, and tissue-cultured polystyrene (TCP) as a control. ALP activity of cells was measured at different time point. At 7 days of incubation, the culture medium in 24-well plates was aspirated. 200 μl, 1% Nonidet P-40 (NP-40) solution was added to each well at room temperature and incubated for 1 h. The cell lysate was obtained and centrifuged. 50 μl supernatant was added to 96-well plates, 50 μl, 2 mg/ml *p*-nitrophenylphosphate (Sangon, Shanghai, China) substrate solution composed of 0.1 mol/l glycine, 1 mmol/l $MgCl_2 \cdot 6H_2O$ was added and incubated for 30 min at 37°C. The reaction was quenched by addition 100 μl, 0.1 N NaOH, the absorbance of ALP was quantified at the wavelength of 405 nm using a microplate reader (SPECTRAmax 384, Molecular Devices, USA) to determine enzyme concentration.

2.4 In vivo biocompatibility

2.4.1 Implantation in muscle

Surgical operation was performed on nine skeletally mature dogs (male, 1–4 year old, 10–15 kg) under general anesthesia and sterile conditions. The experimental protocol was approved by the Animal Care and Experiment Committee of West China Hospital of Sichuan University, Chengdu, China Anesthesia was introduced by an intra-abdominal injection of sodium pentobarbital (30 mg/kg, body weight). For intramuscular implantation, the back was shaved and the skin was cleaned with iodine. Then a longitudinal incision was made and the paraspinal muscle exposed by blunt separation. Longitudinal muscle incisions were subsequently made by scalpel and muscle pouches were created by blunt separation. Four separate muscle pockets at least 2 cm apart were created in either side of the paraspinal muscle, and in each pocket one sample (∅ 5 × 8 mm) was implanted. The wound was closed in layers using silk sutures. As a control, poly ethylene (PE) was implanted according to the same method. Three groups of nine animals were sacrificed after the samples implanted into muscle for 4, 8, and 12 weeks. The implanted samples were harvested with surrounding muscle tissues and immediately fixed in 4% buffered formaldehyde solution (pH = 7.4) for 1 week. After that, the samples were dehydrated in a series of alcohol solutions and embedded in methylmethacrylate (MMA). Thin sections were made with a diamond saw, and then stained with methylene blue and basic fuchsin. Histological evaluation was carried out with a light microscopy.

2.4.2 Implantation in bone

For the implantation in bone, surgical operation was also performed on nine dogs (similar to implantation in muscle) under same conditions. Lateral side of either thigh was shaved and the skin was cleaned with iodine. Then a longitudinal incision was made and the thigh muscle was exposed by blunt separation to expose femoral bone. The periosteum on the femoral bone was subsequently detached and cut off. Four holes (∅ 5) were created by drill at a

distance of 1 cm and the materials (∅ 5 × 8 mm) were implanted in the holes. At the end, the wound was closed in layers using silk sutures. Following surgery, each dog received penicillin intramuscularly for three consecutive days to prevent infection. Calcein (2 mg/kg, body weight), Xylenol orange (50 mg/kg, body weight) and tetracycline-HCl (20 mg/kg, body weight) were intravenously injected 3, 6 and 9 weeks respectively after surgical operation to mark bone formation in different time periods.

The three groups of nine animals for were sacrificed after 4, 8 and 12 weeks of implantation. The implanted samples were harvested with surrounding tissues and immediately fixed in 4% buffered formaldehyde solution (pH = 7.4) for 1 week. After fixation, the samples were dehydrated in a series of alcohol solutions and embedded in MMA. Thin sections were made with a diamond saw, and stained with methylene blue and basic fuchsin. Histological evaluation was carried out with a light microscopy.

2.5 Statistical analysis

Statistical analysis was conducted using one-way ANOVA with post hoc tests. The results were expressed as the mean ± standard deviation. A value of $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 In vitro degradability

3.1.1 Weight loss and pH change of Tris-HCl solution

Figure 2 shows weight loss ratio of the copolymer immersed in Tris-HCl solution over time. It was found that the copolymer had a fast weight loss rate in the initial 2 weeks, and then showed a slightly slow weight loss in the following time. 31.9 wt% of weight loss ratio could reach for the copolymer at 12 weeks. The results showed that the copolymer could be gradually degradable in the Tris-HCl solution. The changes of pH value of the Tris-HCl solution after soaking the copolymer for different time are shown in Fig. 3. It was noticed that the changes of pH value were in the range of 7.4–6.9 during soaking and only slight fluctuation occurred at 7, 14, 28, 56 and 84 days. A significantly decrease of pH was observed during the first 5 days, and no obvious changes were found after that time.

3.1.2 Compressive strength and morphology changes of the copolymer

The changes of compressive strength of the copolymer after soaking into Tris-HCl solution for different time

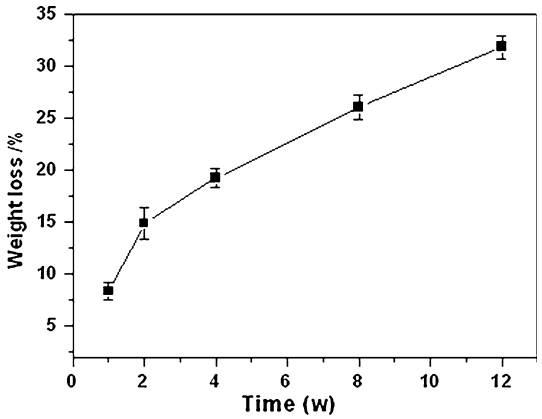

Fig. 2 Weight loss of the copolymer in Tris-HCl solution with time, each value is mean ± SD, $n = 5$

(2, 4, 8 and 12 weeks) are shown in Fig. 4. It was found that the compressive strength of the copolymer decreased with the immersion time. The initial compressive strength of the copolymer before immersion into Tris-HCl solution was 107 MPa. However, the compressive strength of the copolymer was 68 MPa after 12-week of soaking, decreased by 36.4%. Figure 5 shows the surface morphology of the copolymer after immersed into Tris-HCl solution for 8 weeks. Some deep cracks were found on the surface of the copolymer as shown in Fig. 5b. Clearly, the surface of the copolymer was eroded and formed many debris after soaking into Tris-HCl solution for 8 weeks.

3.2 In vitro cytocompatibility

3.2.1 Cell morphology

Figure 6 shows the scanning electron micrographs of MG-63 cells attached on the surfaces of the multi-(amino acid) copolymer at 3 days. It can be seen that the cells

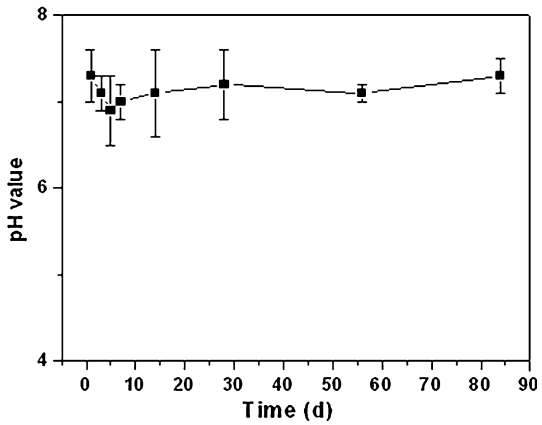

Fig. 3 Changes of pH value of the Tris-HCl solution after soaking the copolymer with time, each value is mean ± SD, $n = 5$

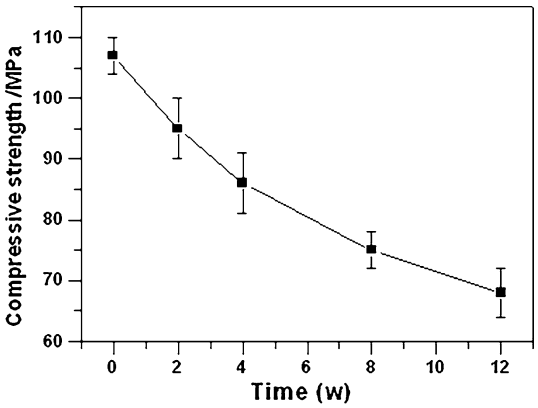

Fig. 4 Change of compressive strength of the copolymer after soaking in Tris-HCl solution with time, each value is mean ± SD, $n = 5$

spread and attach well onto the copolymer surfaces and formed a confluent layer with close attachment to the sample surfaces. The results indicated that the copolymer had good cytocompatibility, and showed no negative effects on cell morphology and viability.

3.2.2 Cell proliferation

The MTT assay was used to evaluate the cytocompatibility of the multi-(amino acid) copolymer because OD values could provide an indicator for the cell proliferation on the biomaterials. It was found from Fig. 7 that the OD value for both the copolymer and control (TCP) increased significantly at 1 and 3 days, and then slowly increased at 5 and 7 days. No significant differences were found for both the copolymer and control through the cultured time. The results showed that the copolymer had no negative effects on MG-63 cells growth, and the cells proliferated on the copolymer similar to the control, suggesting this copolymer had good cytocompatibility.

3.2.3 ALP activity

The ALP activity was determined after the MG-63 cells cultured on the copolymer at 4 and 7 days, and the results are shown in Fig. 8. It was found that the ALP activity of MG-63 cells cultured on both the copolymer and control increased with time, and no significant difference was found at 4 and 7 days for both two samples. The results indicated that copolymer had good cytocompatibility, and the MG-63 cells could be differentiated on the copolymer.

3.3 In vivo biocompatibility

3.3.1 Implantation in muscle

The histological evaluation results of the multi-(amino acid) copolymer implanted into the muscle of dogs for 12 weeks are shown in Fig. 9, in which D, M represent copolymer and muscle, respectively. The results showed that the copolymer materials were closely connected with the muscle in intramuscular implantation, and the implants were surrounded by a thin layer of dense connective tissue (muscle). It could be suggested that the copolymer had little negative influences on the muscle, revealing good biocompatibility.

3.3.2 Implantation in bone

The histological evaluation results of multi-(amino acid) copolymer implanted into bone defects of dog femoral cortex for 12 weeks are shown in Fig. 10, in which D, B represent copolymer and bone tissue, respectively. It was found that the new bone tissue bonded tightly with the surfaces of the implant materials, the osteoid matrix was well distributed throughout the surface of the implants with some mineralization. The results indicated that the copolymer directly combined with the natural bone tissue

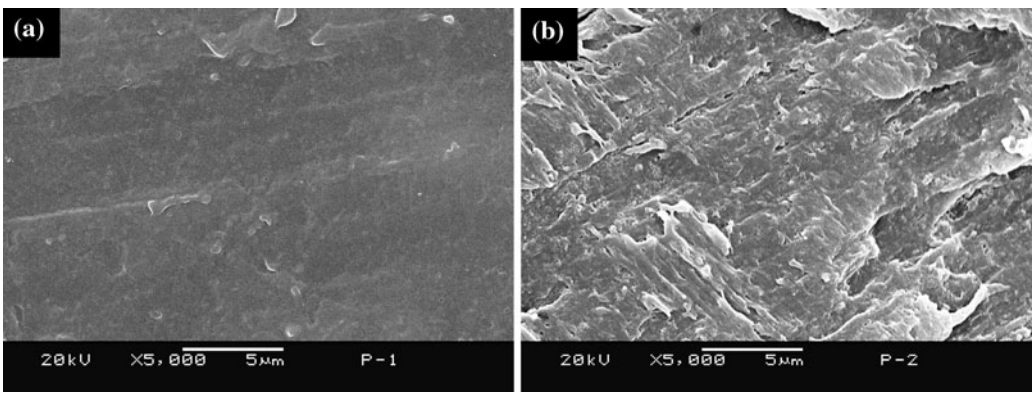

Fig. 5 Morphology of SEM images of the copolymer before (**a**) and after (**b**) soaking in Tris-HCl solution for 8 weeks

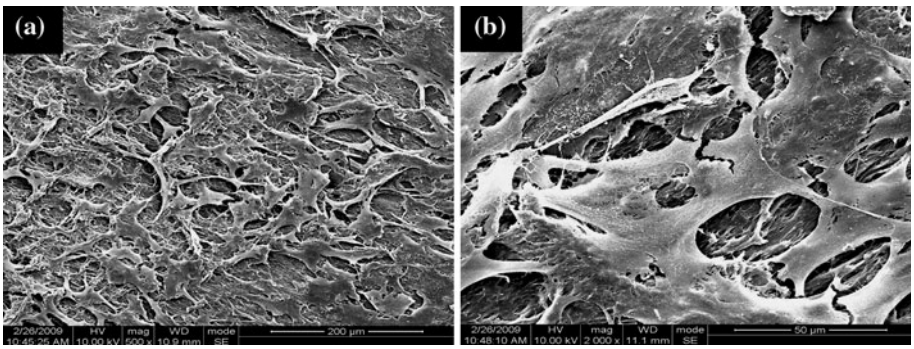

Fig. 6 SEM images of MG-63 cells cultured on the copolymer for 3 days under different magnifications

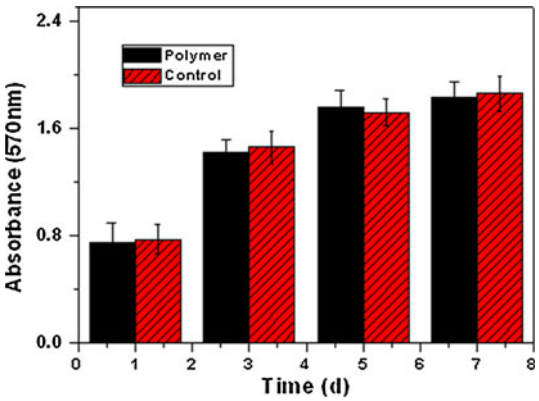

Fig. 7 MTT assay for proliferation of MG-63 cells cultured on the copolymer and control (TCP) at 1, 3, 5 and 7 days, each value is mean ± SD, $n = 5$

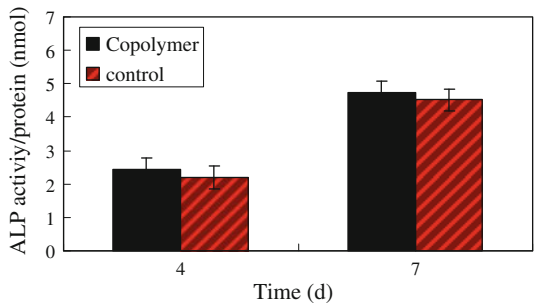

Fig. 8 ALP activity of MG-63 cells cultured on the copolymer and control (TCP) at 4 and 7 days, each value is mean ± SD, $n = 5$

without obviously fibrous connective tissue, showed excellent biocompatibility and osteoconductivity.

Figure 11 shows the fluorescent microscopic image of new bone formation surrounding the implanted materials at 3, 6 and 9 weeks. It was clearly found that the new bone tissue could form on the surfaces of the multi-(amino acid) copolymer implant at different time, and the new bone tissue surrounded the implant and formed tight combination with the materials. The results indicated that the multi-(amino acid) copolymer had good biocompatibility and osteoconductivity, showing faster and more effective osteogenesis.

4 Discussions

It is accepted that the biomaterials used for bone substitutes should be degradable and gradually replaced by newly formed bone tissue [1, 14, 20]. In this study, a novel bone substitute biomaterial of multi-(amino acid) copolymer was prepared by acid-catalyzed condensation reaction. The copolymer was composed of six amino acids, 6-aminocaproic acid, glycine, L-alanine, L-phenylalanine, L-proline and L-lysine. The 6-aminocaproic acid as main chain was the framework of the copolymer that was copolymerized with the other five natural amino acids, which contributed to degradability of the copolymer. Compared with the previous studies of the synthesis of dual-(amino acid) copolymer [17, 18], our results showed that the content of 6-aminocaproic acid in the copolymer had significant

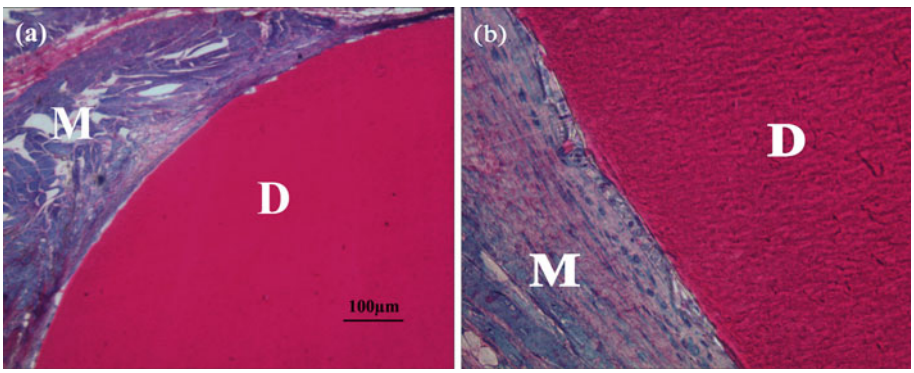

Fig. 9 Histological section of the copolymer implanted into the muscle of dogs for 12 weeks, *D* represent implant, *M* represent muscle; **a** ×4 and **b** ×20

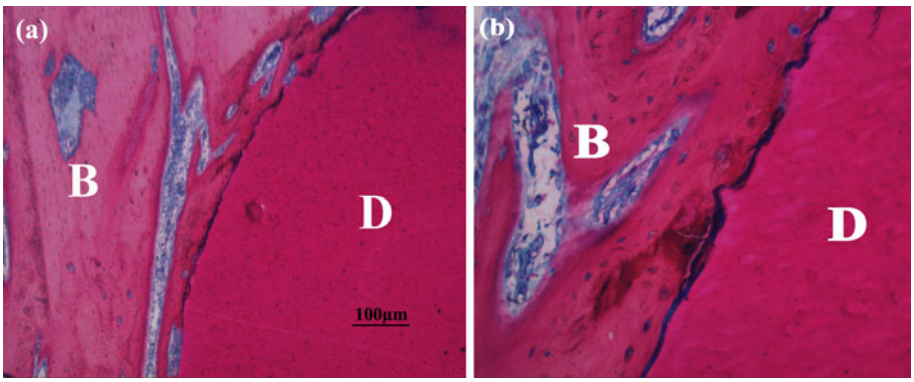

Fig. 10 Histological section of the copolymer implanted into bone defects of dog femoral cortex for 12 weeks. *D* represent implant, *B* represent bone; **a** ×4 and **b** ×20

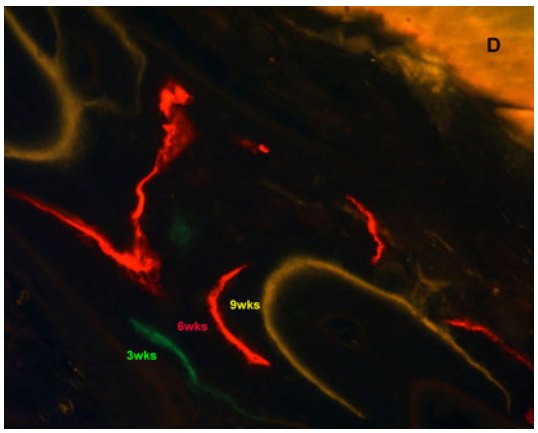

Fig. 11 Fluorescent microscopic image of bone formation surrounding the implant at 3, 6 and 9 weeks, *D* represents implant

effects on the mechanical strength and intrinsic viscosity of the copolymer.

Proper degradation in a physiological environment is one of the most important characteristics for a degradable biomaterial [14, 21]. In this study, the in vitro experiments demonstrated that the multi-(amino acid) copolymer lost 31.9 wt% of its initial weight after immersion into Tris-HCl solution for 12 weeks, which indicated the copolymer could be slowly degradable. In addition, the compressive strength of the copolymer decreased from 107 to 68 MPa after immersion in Tris-HCl solution for 12 weeks. The results showed the mechanical strength of the copolymer was not sharply decrease during soaking into Tris-HCl solution. Thus, compared with other bone repair biomaterials, such as PLA and PGA etc., the multi-(amino acid) copolymer had a slow degradation rate and could maintain good mechanical properties during soaking into solution. It was reported that the compressive strength of natural cortical bone was in the range of 50–150 MPa [22]. Natural polymers (such as collagen) present poor mechanical strength, thus are limited in clinical applications for load-bearing bone repair. Moreover, PLA, PGA and their copolymers biomaterials with good mechanical strength show a collapse degradation manner when implanted in vivo, which would cause the incompatibility in mechanical properties between implants and host bone tissue [1, 14, 21]. Therefore, the prepared multi-(amino acid) copolymer had sufficient mechanical strength for fundamental support during bone regeneration periodin this study.

One of the important properties of the degradable biomaterial is that the degradable products would cause the pH value changes in local environment when implanted in vivo. It has been reported that some biomaterials (such as PLA and its copolymer) would broke down acidic by-products and resulted in the decrease of pH value in the ambient solution, which was believed to induce the inflammatory reaction in vivo [14, 23]. In the present study, the results showed that the changes of the pH value of Tris-HCl solution were in the range of 6.9–7.4 during the copolymer soaking, which was quite closed to that of physiological fluid, and hence might not elicit the inflammation in vivo. It is suggested that the multi-(amino acid) copolymer would not elicit the inflammation when implanted in vivo, and might be better than PLA biomaterials for bone repair.

To evaluate the in vitro biocompatibility of the biomaterials, the cell culture experiments are useful approaches [24]. In this study, the MG-63 cells were used to test the cytocompatibility of the multi-(amino acid) copolymer. The results showed that the MG-63 cells could proliferate on the copolymer with time, as demonstrated by the MTT assay, suggesting positive cellular responses to this material. Thus, the copolymer was cytocompatible without negative effect on cellular viability, or proliferation. Furthermore, the ALP activity has been used as an early marker for functionality and differentiation of osteoblasts during in vitro experiments [24, 25]. The results confirmed that the ALP activity of the MG-63 cells cultured on the copolymer exhibited significantly higher levels of expression similar to the control of TCP at 4 and 7 days, indicating that the copolymer with good cytocompatibility. The biocompatibility of biomaterials is very closely related to the cell behaviors in contact with them and particularly to cell spread on their surface [25, 26]. The SEM results

showed that the cells spread well and formed a confluent layer with intimate attachment to the copolymer surfaces, while maintaining physical contact with each other. These results indicated that the copolymer with good cytocompatibility had no negative effects on cell morphology and viability.

The in vivo biocompatibility of the multi-(amino acid) copolymer was determined by implantation the materials into muscle and femoral cortex of dogs. The results showed that the copolymer had excellent biocompatibility with the surrounding tissue during the 12-week implantation. The results also indicated that neither the copolymer nor the degradation products had influences on bone metabolism: bone formation at the beginning and bone remodeling thereafter. Many studied reported that the degradable products of some polymers would cause the pH value changes in local environment (such as PLA, PGA and their copolymers) when implanted in vivo, these degradable biomaterials would broke down acidic by-products and resulted in the decrease of pH value in the ambient solution, which was believed to induce the inflammatory reaction in vivo [14, 23]. In this study, it was noticed that the pH value changes were ranged from 7.4 to 6.9 during soaking into solution for 84 days.

The morphology of the interface between the multi-(amino acid) copolymer implants and host bone tissue (cortical bone) after implantation for 12 weeks was observed by histological evaluation. The results showed that the multi-(amino acid) copolymer was directly connected with host bone tissues without obvious intervening connective layer, indicating that the copolymer had good biocompatibility. In addition, some new bone tissues were found to extend along the copolymer surface, which was known as bone-bonding. Bone-bonding could ensure that the implant integrated with natural bone through biochemical reaction at the interface between biomaterials and bone tissue, which was in favor of implant fixation in host bone [27]. If fibrous tissue surrounds the implants, the combination of the implants with bone is not of strong enough, which would result in loosening of the implant in bone, and ultimately, failure of the implant [14, 28]. The results showed that the multi-(amino acid) copolymer had excellent biocompatibility and osteoconductivity.

In this study, the degradable multi-(amino acid) copolymer with good mechanical strength as bone substitute had been prepared for biomedical application. The copolymer had slowly degradable properties, and did not cause obvious change of the pH value when soaked into degradable solution as compared with other degradable polymer such as PLA etc. The copolymer had good biocompatibility, which could promote the MG-63 cells proliferation and differentiation, and the cells maintained normal phenotype spread well on the copolymer surface. In vivo experiments, out results revealed that the copolymer exhibited excellent biocompatibility and more effective osteogenesis. Furthermore, the copolymer could be combined directly with the natural bone without fibrous capsule tissue between implants and host bone, showing good osteoconductivity, which was better than other polymer such as PLA, PGA and their copolymer [13, 14]. In conclusion, it could be suggested that the multi-(amino acid) copolymer with sufficient strength, good biocompatibility and osteoconductivity had clinical potential for load-bearing bone repair.

5 Conclusions

A biomaterial of multi-(amino acid) copolymer for bone substitute was synthesized by an acid-catalyzed condensation reaction. The copolymer could be slowly degradable in Tris-HCl solution, and the degradation by-products would cause no significant change of the pH value of soaking medium. The compressive strength of the copolymer decreased from 107 to 68 MPa after immersion for 12 weeks. The MG-63 cells could proliferate on the copolymer with time, and the ALP activity of the cells on the copolymer obviously increased at 4 and 7 days. The MG-63 cells with normal phenotype extended and spread well on the copolymer surfaces. Histological evaluations of copolymers implanted into both the muscle and osseous sites of dogs confirmed that the copolymers had excellent biocompatibility in vivo. When implanted in cortical bone of the dogs, the copolymer implants were directly connected with the host bone tissue without obvious intervening connective layer, exhibiting good osteoconductivity.

Acknowledgments The authors are grateful for the financial support from National Key Technology R&D Program in the 11th Five Year Plan of China (No. 2007BAE13B00), Nano special program of Science and Technology Development of Shanghai (No. 1052nm06600), and Key Medical Program of Science and Technology Development of Shanghai (No. 09411954900). They would also like to acknowledge Dr. Yuipin Yuan, for his assistance with animal experiments.

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