

Hydroxyapatite (n-HA)/Unsaturated Poly(ester-amide) Nanocomposites for Bone Fixation Material

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ABSTRACT: A new type of unsaturated poly(ester-amide) viz maleic anhydride-phthalic anhydride-ethylene glycol-neopentylene glycol-glycin copolymer was prepared by melt polycondensation. The copolymer was characterized by FT-IR, gel permeation chromatography, and thermal gravimetric analysis. The molecular structure of crosslinked unsaturated poly(ester-amide) was determined by wide-angle X-ray diffraction. Hydroxyapatite (n-HA) was used to boost up the new unsaturated poly(ester-amide), the flexural properties of n-HA/unsaturated poly(ester-amide) nanocomposites with different n-HA content were measured. Studies of degradation behavior were carried out in simulated body fluid at pH 7.4 and 37°C, the flexural strength changes and cumula-

tive mass loss of n-HA/unsaturated poly(ester-amide) nanocomposites were measured at different degradation times. The n-HA/unsaturated poly(ester-amide) nanocomposites was hydrolyzed in 1M NaOH standard solution at room temperature to study the mass loss with different n-HA contents. All the preliminary results suggested that n-HA/unsaturated poly(ester-amide) nanocomposites might be potentially used as a new type of bone fixation material. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 121: 2591–2596, 2011

Key words: unsaturated poly(ester-amide); hydroxyapatite (n-HA); nanocomposites; flexural properties; degradable properties

INTRODUCTION

Compared with metallic implants, biodegradable polymers as bone fixation materials can eliminate the second surgery of removal, avoid problems caused by stress shielding and can simultaneously deliver anti-infectious drugs and/or bone growth-enhancing factors. To overcome problems associated with bulk degradation of the widely used polyester bone fixation materials (such as poly(L-Lactic acid) (PLLA), poly(glycolic acid) (PGA) and their copolymers),^{1–5} crosslinking polyanhydrides have been developed in the past few years. In contrast with polyesters, polyanhydrides undergo the surface-eroding degradable mechanism, which effectively maintains the mechanical integrity by preserving the molecular weight of the polymer and exhibits a gradual loss in size. This permits bone to grow little by little.⁶ Besides, crosslinked polyanhydrides can be easily fabricated into the de-

sirable shapes which demonstrate a great potential in orthopedic applications and might be a better alternative than polyesters as the internal fixation materials. However, the mechanical strength, especially the flexural strength of these reported materials is still much lower than the mechanical strength of cortical bones. The flexural strength needs to be further increased to meet the need of load-bearing bones during rehabilitation.

Many methods have been used to increase the mechanical properties of biodegradable polymers, such as composite with fibers or particles. Among these methods, a relatively common way^{7–10} is to combine inorganic particles, particularly hydroxyapatite (n-HA) into the biodegradable matrix and form composites. As a primary constituent of human hard tissues, n-HA has excellent biocompatibility and non-toxicity. Thus, n-HA has received extensive attention as a bioceramic and has been used as prosthetic devices and implanted materials.

In this article, a new type of unsaturated poly(ester-amide), namely “maleic anhydride-phthalic anhydride-ethylene glycol-neopentylene glycol-glycin” copolymer was prepared by melt polycondensation. The characterizations have been investigated before/after crosslinked using vinyl acetate as crosslinker. Instead of traditional composite methods, *in situ* polymerization has been used to prepare

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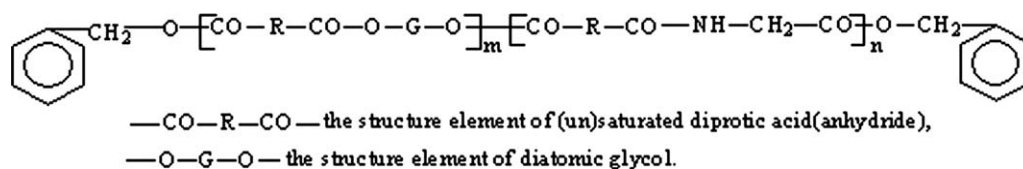


Figure 1 The structure formula of synthesized unsaturated poly(ester-amide).

nanocomposites of the unsaturated poly(ester-amide) and n-HA. The flexural and degradable properties of the nanocomposites with different n-HA contents have been investigated.

EXPERIMENTAL

Materials

Phthalic anhydride, maleic anhydride, glycin were purchased from Tianjing Central Pharmaceutical Corp. (Tianjing, China). Ethylene glycol, benzyl alcohol, neopentylene glycol, and vinyl acetate were purchased from Zibo Guangtong Chemical Co. (Zibo, China). n-HA were produced by our study team.

Unsaturated poly(ester-amide) synthesis

In a typical reaction used in this article, 0.024 mol (3.59 g) phthalic anhydride, 0.046 mol (3.49 g) ethylene glycol, and 0.049 mol (5.09 g) neopentylene glycol were added to a flask with two side arms electrodynamic stirring and oil water separation and polymerized at 160°C for 1 h. It was then cooled to 120°C. 0.076 mol (7.42 g) maleic anhydride and 0.0125 mol (0.937 g) glycin were added. The mixture was kept at the temperature for 1 h. Then the reaction temperature was raised to 180°C for 1 h and polymerized at 195°C for 4 h under a flow of argon. After it was cooled to 120°C, 2.05 g benzyl alcohol was added and reacted for 1 h, the temperature was again raised to 180°C for 1 h after reacting at 160°C for 1 h. When the temperature dropped to 100°C, the ropy liquid products were moved to a P₂O₅ desiccator before usage. The structure formula of synthesized unsaturated poly(ester-amide) is shown in Figure 1.

Preparation of n-HA

n-HA was prepared via the method reported by Suzuki. In short, with vigorous stirring, 25 mL 0.05 mol/L Ca(NO₃)₂ solution (pH11) was dropped into 25 mL 0.03 mol/L(NH₄)₃PO₄ (pH11) via a peristaltic pump under the effect of 5.0 wt % dispersant A-6114 (polymethylmethacrylate amid, *Mw* = 10,000). After that, the reaction mixture was poured into an autoclave and aged for 20 h at 150°C. White n-HA pow-

ders were recovered by filter and washed with deioned water for several times and dried at 40°C.

Preparation of n-HA/unsaturated poly(ester-amide) composites

As a typical reaction, 8.2 g synthesized unsaturated poly(ester-amide) containing 10 wt % n-HA was dissolved into a definite store of acetone via long-time ultrasonic wave treatment and strength electrodynamic stirring. And n-HA had been dispensed into the unsaturated poly(ester-amide) equably and the acetone had already been volatilized. 1.8 g vinyl acetate containing 0.06 g benzoyl peroxide and 0.005 g *N, N*-Dimethylaniline were added to the even intermixture of unsaturated poly(ester-amide)/n-HA. 0.005 g *N, N*-Dimethylaniline was dropped into the intermixture after being mixed equably, then placed to a mould for some time until the intermixture was crosslinked completely at room temperature, the crosslinked samples were autoclaved for 1 h under 1 MPa pressure at 195°C, then was heated at 195°C for 20 h. The rectangle samples (5 mm × 5 mm × 45 mm) were stored in a P₂O₅ desiccator until they were used.

Unsaturated poly(ester-amide) characterization methods

IR spectra were obtained with a Bruker EQUINOX55FT-IR spectrometer. The synthesized unsaturated poly(ester-amide) was on NaCl plates directly for recording IR Spectra. Molecular weights of the unsaturated poly(ester-amide) before being crosslinked were determined on a water gel permeation chromatography system consisting of a water 600 pump, Water 410 Differential Refractive Index detector. Samples were eluted in dichloromethane through Varian Micropak G4000 and G3000 column installed in series at a flow rate of 1.0 mL min⁻¹. Molecular weight was determined relative to the narrow dispersed polystyrene standards with a molecular weight range from 1000 to 200,000. Thermal analyses were performed on Perkin-Elmer system consisting of DSC 7 and thermal gravimetric analysis (TGA) 7 analyzers with TAC7/DX instrument controller. Data were processed by UNIX thermal analysis system software on a DEC computer station. For TGA, an average sample weight of 10 mg was

heated at $10^{\circ}\text{C min}^{-1}$ under a flow of argon. The decomposition temperature was detected by TGA. Wide angle X-ray diffraction of crosslinked unsaturated poly(ester-amide) was recorded on a RIGAKU D/Max-38 X-ray diffractometer by nickel filtered CuK source.

Degradation methods

In vitro, the degradation rate of rectangle samples ($5\text{ mm} \times 5\text{ mm} \times 45\text{ mm}$) in 50 mL 0.1M phosphate buffer solutions (pH 7.4) were performed in an incubator shaker (Model HQ45A, Factory of Scientific Instrument, Wuhan, CN). These samples were agitated at 60 rpm at a constant temperature of $37 \pm 1^{\circ}\text{C}$ in air bath. The phosphate buffer solutions were changed daily to maintain sink conditions throughout. The device was carefully frozen dried before its mechanical strength was measured. The degradation properties were measured by the weight loss of dry weight of the polymer sample. To mend its degradation speed, the rectangle samples ($5\text{ mm} \times 5\text{ mm} \times 45\text{ mm}$) were also performed in 1M NaOH standard solution at room temperature to hydrolyze. The cumulative mass loss was measured at different hydrolysis times. All measurements were carried out in triplicate by analyzing three separate devices. All the detected data were processed by Microcal Origin 6.1 software on PC computer.

Flexural properties

Flexural properties were measured on a Micro controls tension. The load speed was 1 mm min^{-1} and the sample size was $5 \times 5 \times 45\text{ mm}$

$$\sigma_b = \frac{3FL}{2BH^2} \quad (1)$$

$$E_b = \frac{FL^3}{4BH^3Y} \quad (2)$$

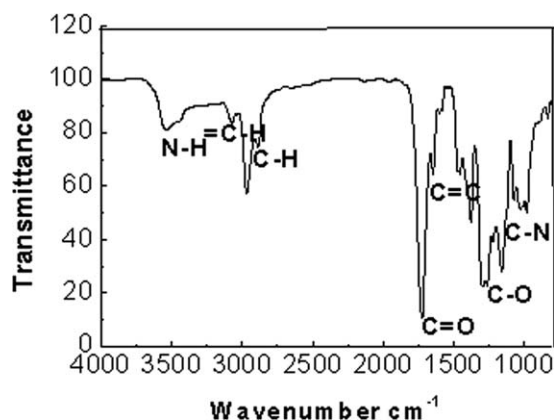


Figure 2 Infrared spectrum of the synthesized unsaturated poly(ester-amide).

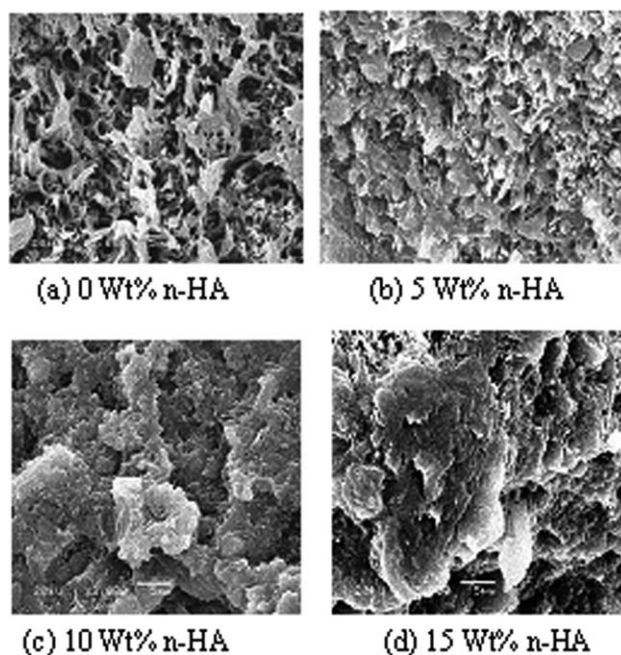


Figure 3 SEM of n-HA/unsaturated poly(ester-amide) nanocomposites with different n-HA content: (a) 0 wt % n-HA; (b) 5 wt % n-HA; (c) 10 wt % n-HA; and (d) 15 wt % n-HA.

wherein σ_b was flexural strength (MPa); E_b was flexural modulus, F was span; B and H were respectively breadth and ply and Y was camber.

RESULTS AND DISCUSSION

Synthesized unsaturated poly(ester-amide) characterization

The IR spectrum of the synthesized unsaturated poly(ester-amide) in Figure 2 shows strong absorption peaks at $1710\text{--}1735\text{ cm}^{-1}$. These are characteristic absorptions of carbonyl stretching vibrations of ester groups, which have occurred in the IR spectra of all the products. The absorption bands at 3074 cm^{-1} are characteristic of $=\text{C}\text{--}\text{H}$ vibrations of unaliphatic alkyl; those at 1647 cm^{-1} are attributed to $\text{C}=\text{C}$ stretching vibrations; those at 2887 cm^{-1} are attributed to $\text{C}\text{--}\text{H}$ stretching vibrations; those at $1223\text{--}1159\text{ cm}^{-1}$ are attributed to $\text{C}\text{--}\text{O}$ stretching of ester group. The $\text{N}\text{--}\text{H}$ stretching vibrations peak and the $\text{C}\text{--}\text{N}$ stretching vibrations peak respectively appeared at about 3446 cm^{-1} and at about 980 cm^{-1} on Fourier transform infrared spectroscopy.

The weight-average molecular weights of all the prepared copolymers are over 2000. The value of decomposition temperature determined by TGA over 200°C for the copolymer suggests that the copolymers possess a desired thermal stability. X-ray powder-diffraction shows the copolymer is an amorphous material after heat treatment.

TABLE I
Flexural Properties of n-HA/Unsaturated Poly(ester-amide) Nanocomposites with Different n-HA Content

n-HA content (wt %)	0	2	5	7	10	15	20
Flexural strength (MPa)	136.1	141.4	148.3	153.7	158.5	153.0	147.9
Flexural modulus (GPa)	1.48	1.52	1.58	1.66	1.75	1.87	2.16

Flexural properties of n-HA/unsaturated poly(ester-amide) nanocomposites

n-HA is dispersed into the unsaturated polyester compound by ultrasonic treatment until n-HA is distributed at nanosized scale and initiation-accelerant agent is used to initiate crosslinking. Then, the nanocomposites of unsaturated poly(ester-amide) and n-HA have been formed. Figure 3 clearly demonstrates that as the content of n-HA increases, the pores are filled by n-HA step by step which results in the improvement of the mechanical strength of nanocomposites. But, with over 10 wt % n-HA content in nanocomposites, the n-HA can be reunited by itself. Figure 3(d) shows the n-HA has already been reunited seriously. When nanocomposites are stressed, the excessive reunited n-HA produce stress concentration which could debase the nanocomposites flexural strength [Table I].

As shown in Table I, the flexural strength and modulus of n-HA/unsaturated poly(ester-amide) nanocomposites are related to the content of n-HA in nanocomposites. The flexural strength and modulus can be improved gradually with the increase of n-HA content in the nanocomposite. The maximum flexural strength of n-HA/unsaturated poly(ester-amide) nanocomposites is increased about 19.11% of corresponding pure crosslinked unsaturated poly(ester-amide). However, with the content of n-HA over 10 wt %, the flexural strength is decreased. But the flexural modulus is always increased respectively.

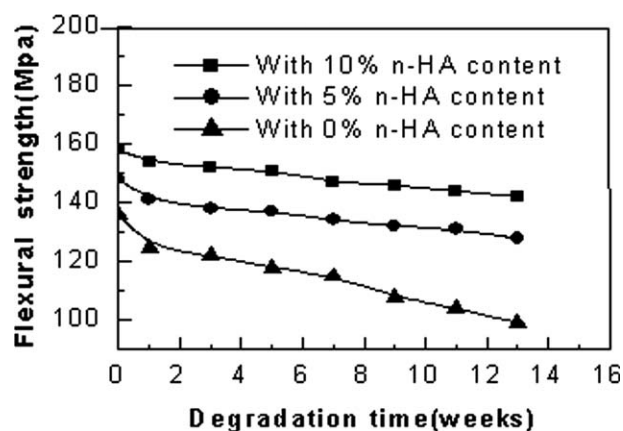


Figure 4 Flexural strength changes of n-HA/unsaturated poly(ester-amide) nanocomposites with different n-HA content at different degradation time in SBF at pH 7.4 and 37°C.

The reason why the maximum ratio of n-HA to unsaturated poly(ester-amide) is only 20 wt % is that unsaturated poly(ester-amide) is mostly an ester oligomer of seven monomers in a higher viscosity. Thus, the dispersion of n-HA particles by ultrasonic treatment becomes more difficult.

Degradable properties of n-HA/unsaturated poly(ester-amide) nanocomposites

Degradation properties of crosslinked unsaturated poly(ester-amide) and n-HA/unsaturated poly(ester-amide) nanocomposites are researched in simulated body fluid (SBF) at pH 7.4 and 37°C by using rectangle samples (5 mm × 5 mm × 45 mm). The flexural strength retention of the nanocomposites during 3 months degradation process is demonstrated in Figure 4. As shown in Figure 4, higher content of n-HA in nanocomposites has the less flexural strength loss. This clearly demonstrates that the addition of n-HA is crucial to increase the flexural strength retention of n-HA/unsaturated poly(ester-amide) nanocomposites during the degradation process.

As shown in Figure 5, at the same degradation time, the incorporation of n-HA in the unsaturated poly(ester-amide) can slower the mass loss rate, and as the content of n-HA in the nanocomposites become higher, the degradable rate of the nanocomposites will further decrease in the first 4 months of

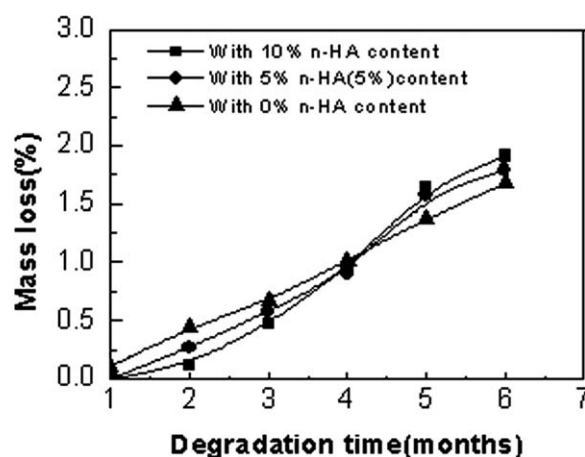


Figure 5 Mass loss rate of n-HA/unsaturated poly(ester-amide) nanocomposites with different n-HA content at different degradation time in SBF at pH 7.4 and 37°C.

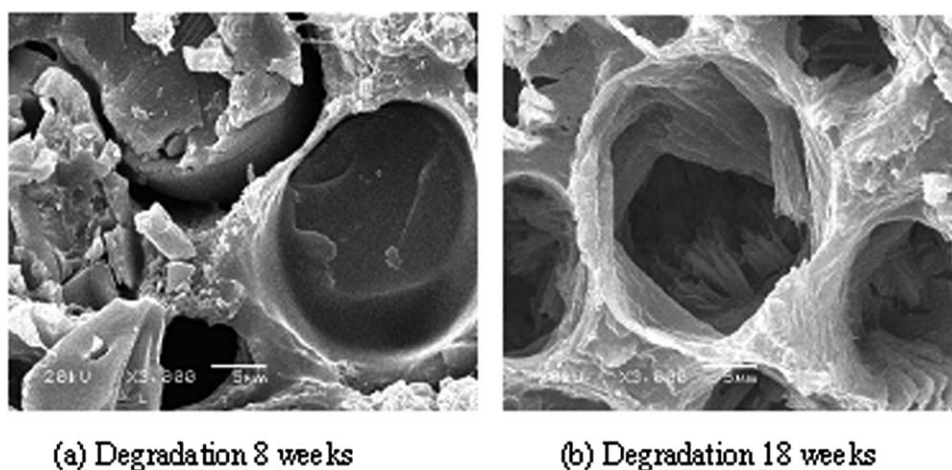


Figure 6 SEM of n-HA/unsaturated poly(ester-amide) nanocomposites with 10 wt % n-HA content after degradation different time: (a) Degradation 8 weeks; (b) Degradation 18 weeks.

degradation (Fig. 5). This phenomenon comes from n-HA partially neutralizing the degradable product acids and decreasing nanocomposites degradable rate, Figure 6(a) shows n-HA on the surface of nanocomposites has not been hydrolyzed completely in the first 4 months. In essence, the degradation of crosslinked unsaturated poly(ester-amide) is a process of the hydrolysis of ester bonds which can be accelerated by acids working as the catalyst in the first 4 months. However, after over 4 months, the hydrolysis products of crosslinked unsaturated poly(ester-amide) are just a large amount of organic acids which can act as the catalyst and accelerate the hydrolysis reaction of crosslinked unsaturated poly(ester-amide). This tendency will be enhanced with increase of the n-HA content in the nanocomposites after degradation for 4 months. For the n-HA on the surface of nanocomposites, it has already been hydrolyzed after degradation for 4 months and many pores have been left [Fig. 6(b)]. More pores appears when n-HA content is raised and more surface area of n-HA/unsaturated poly(ester-amide) nanocomposites can contact with the SBF solution. This can accelerate the mass loss of nanocomposites.

To accelerate the degradation, hydrolysis behavior of nanocomposites with different n-HA contents is determined by quantitative analysis of the mass loss in 1M NaOH standard solution at room temperature. The results are shown in Figure 7. As shown in Figure 7, in first 9 days, the nanocomposites with higher n-HA content have a lower mass loss rate. But, after being hydrolyzed for 10 days, as the content of n-HA in the nanocomposites becomes higher, the hydrolysis rate of the nanocomposites will be improved at the same hydrolysis time. This is because when higher n-HA content nanocomposites has been hydrolyzed, the hydrolyzed n-HA has left a number of pores. The mechanism is the same as

degradation for over 4 months, and many pores can be seen from Figure 6(b), which enables the nanocomposites to have a higher hydrolysis rate.

During the tested periods, the dimension of the devices is decreased step by step with the mass loss. The surface of the device is kept slick. It shows surface erosion property. After being hydrolyzed about 36 days, the dimension of the nanocomposites containing 10 wt % n-HA is decreased about half, and then gradually vanishes.

Preliminary biocompatibility

Compared with the clinically used biopolymer and absorbable gelatin sponge, the biocompatibility of the cross-linked unsaturated polyester has been evaluated in mice skin. Briefly, the tested device and the absorbable gelatin sponge (3 mm in diameter, 1 mm in thickness) are subcutaneously implanted in two

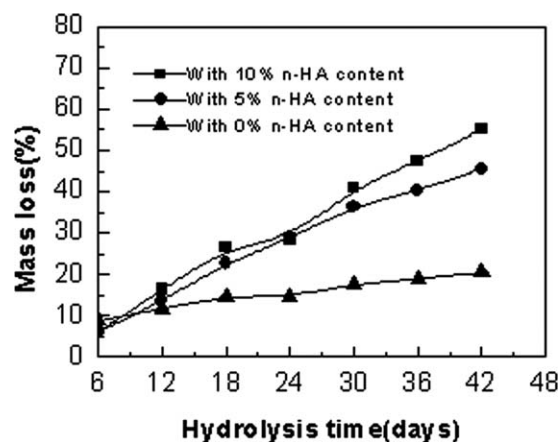


Figure 7 Hydrolysis rate of n-HA/unsaturated poly(ester-amide) nanocomposites with different n-HA content at different hydrolysis time in 1M NaOH standard solution at room temperature.

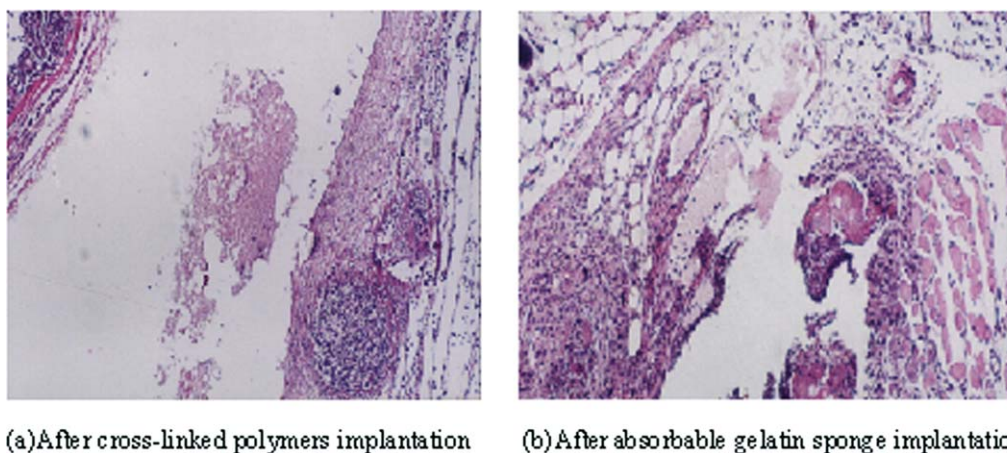


Figure 8 Hematoxylin-eosin stained photomicrograph (10 \times) of mice skin at day 30 after cross-linked polymers and absorbable gelatin sponge implantation [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com].

sides of the mice abdomen. The evaluation lasts for 1 month. At the predetermined time, mice are sacrificed and their skins are removed, and the microscopic histopathology is assessed by hematoxylin-eosin stained analysis. Macroscopically, results show that all the experimental mice survive healthily and actively to the date of their sacrifice. It indicates that sites are clean and the remainder devices are easily retrieved; no obvious damage to the locally implanted tissue is observed. Figure 8 shows the photomicrography of mice skin at day 30 after the implantation of cross-linked polymers and absorbable gelatin sponge, respectively. The tissue shows the characteristic of the convalescence of chronic inflammation tissue. The hyperplasias of fibrous tissues and capillary vessels have been observed, and many foam cells and multinuclear giant cells are surrounded. There is a clear verge between the local cell response region and the normal tissue region. Microscopically, histopathologic observations shows the responses of cells and tissue to the implanted polymer. There is no significant difference between the responses and those of the absorbable gelatin sponge. The results suggest that the cross-linked polymers are well tolerated by the skin tissue of mice. They may be used as materials for implantation.

CONCLUSION

The synthesized unsaturated poly(ester-amide) desired physicochemical properties have been successfully prepared by melt polycondensation with relatively high-molecular weights. All the prepared copolymers possess desired thermal stability. The n-HA/unsaturated poly(ester-amide) nanocomposites have been prepared through *in situ* photo-polymer-

ization and heat treatment to further increase the conversion of the monomers. The n-HA/unsaturated poly(ester-amide) nanocomposites are highly cross-linked nanocomposites. In contrast with unsaturated poly(ester-amide), the flexural properties of n-HA/unsaturated poly(ester-amide) nanocomposites have been increased. But the flexural strength of nanocomposites containing 10 wt % n-HA has a maximum value. Furthermore, the n-HA/unsaturated poly(ester-amide) nanocomposites have a lower mass loss and an increased retention of flexural strength than those of the corresponding unsaturated poly(ester-amide) during 4 months in the degradation process. Nanocomposites with higher n-HA content have the faster hydrolysis rate after being hydrolyzed over 10 days in 1M NaOH standard solution at room temperature. During the tested periods, it shows the surface erosion property.

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