water, the actuation performance of the IPMC actuator cannot be recovered. The tip-displacement value at start point of the second test was just the same as that at end point of the first test, and continued to decrease until the actuation was stopped. However, the actuation performance was nearly completely recovered after performing enough cation exchange, and the mean values of tip displacement at each interval for the third test were almost the same as those for the first test. The amazing point for the 4th test is that only 1 h cation exchange can recover most of the actuation performance of the IPMC actuator. Fig. 3 shows the results of the durability test of two other IPMC actuators, which were conducted using lithium chloride solutions (1.5 and 3.0 N, respectively) at still higher frequency and otherwise the same conditions. It is interesting to notice that the IPMC actuated in the lithium chloride solution using a higher concentration showed a much better actuation performance.

The durability study performed here indicated that the number of hydrated ions in the polymer matrix, as well as the nature of the aqueous medium in which such IPMC works, was crucial to its durable actuation. This is important for the design of a new-generation of artificial muscles. Adopting appropriate techniques during the fabrication of IPMCs, which avoid the diffusion of mobile cations into their working medium, or which enable the lost cations to be supplied during actuation, such as by the incorporation of certain stimuli-responsive microcapsules that release ions in a controllable manner (Fig. 4), an artificial muscle with the ability of functional self-healing will be developed in the near future. If so, just as its natural counterpart, artificial muscle begins to perform metabolism.

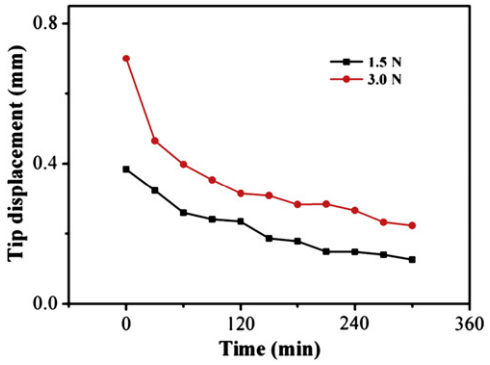

Fig. 3. Durability measurements conducted using lithium chloride solutions at 5.0 Hz.

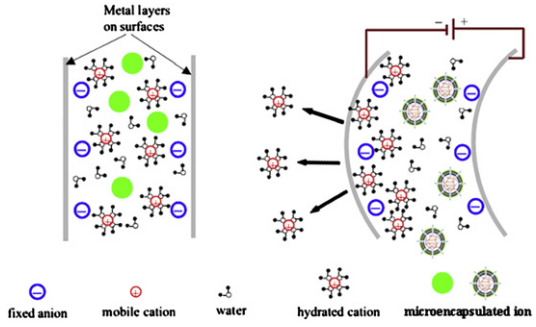

Fig. 4. Illustration of the actuation principle of self-healing IPMCs with embedded microencapsulated ions: before (left) and after (right) a voltage is applied.

Conclusion

In summary, the results of durability tests in this paper showed that the diffusion of mobile cations into the working aqueous medium during the actuation was the main reason for the performance deterioration of IPMCs. This should be assigned to the increased concentration gradient of such ions between the IPMC and the working medium such as deionized water, resulting from the higher ionic exchange capacity of the base polymer. The study presented here may be instructive to the design and fabrication of a new-generation of artificial muscles with unique functional properties such as self-healing.

Acknowledgments

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Genipin crosslinked gelatin nanofibers for tissue engineering

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Abstract summary

The objective of this study was to enhance the mechanical and thermo-mechanical properties of electrospun gelatin nanofibers, while maintaining their good bioactivity. In this work, genipin was used as crosslinker to achieve this goal. Electrospun gelatin nanofibers were crosslinked with genipin vapor at room temperature for 0 h, 6 h, 12 h, 24 h, 48 h and 72 h. The morphologies of the crosslinked nanofibrous mats were examined by scanning electron microscopy (SEM). The thermal and mechanical properties of the nanofibrous mats were examined. Bovine Serum Albumin (BSA) as a model drug was successfully incorporated into nanofibers. The release of BSA from fibrous mats crosslinked for different times was studied. The results indicated that the crosslinked nanofibrous mats have the potential of serving as tissue engineering scaffold or drug delivery vehicle.

Keywords: Electrospinning, Nanofibers, Crosslinking, Release

Introduction

Electrospun nanofibrous mats as tissue engineering scaffolds have been widely reported [1]. Gelatin is a natural polymer derived from collagen. Because of its biodegradability and biocompatibility, and relatively low antigenicity, gelatin is commonly used for pharmaceutical and medical applications [2, 3]. Genipin is a naturally occurring biocompatible crosslinker, which can be obtained from its parent compound geniposide isolated from the Gardenia fruit extract [4]. Genipin has been widely used in Chinese herbal medicine and dark blue pigments, because of its spontaneous reaction with amino acids or proteins [5]. Gelatin nanofibers were crosslinked by saturated glutaraldehyde vapor to improve their water-resistant and thermo-mechanical performance [6].

In this study, gelatin nanofibrous mats were prepared by electrospinning. The morphologies of the nanofibrous mats were examined by scanning electron microscopy (SEM). The mechanical and thermal properties of crosslinked gelatin nanofibrous mats were studied and the protein release profiles from the electrospun nanofibrous mats were investigated.

Experimental methods

The electrospun gelatin nanofibrous mats were placed in a genipin vapor chamber at room temperature for 0, 6, 12, 24, 48, and 72 h. The thermal properties of the electrospun gelatin fibers crosslinked for different times were measured in a temperature range from 0 °C to 200 °C at a heating rate of 10 °C/min. The mechanical measurements were conducted by applying tensile tests on gelatin nanofibrous mats crosslinked by genipin on a materials testing machine at an elongation speed of 10 mm/min.

For the drug release studies, composite fibrous mats electrospun gelatin nanofibers crosslinked by genipin were soaked in PBS at 37 °C. BSA (at an optical wavelength of 280 nm) in the supernatant was determined by an UV–vis spectrophotometer.

Results and discussion

Fig. 1 shows the morphologies of nanofibrous mats after crosslinking for different times. Gelatin fibers were smooth and randomly oriented (Fig. 1a). The diameter of the fibers increased and eventually the fibers lost their original morphology with increase of crosslinking time (Fig. 1b–f). The fibers at junctions were fused together after long time crosslinking.

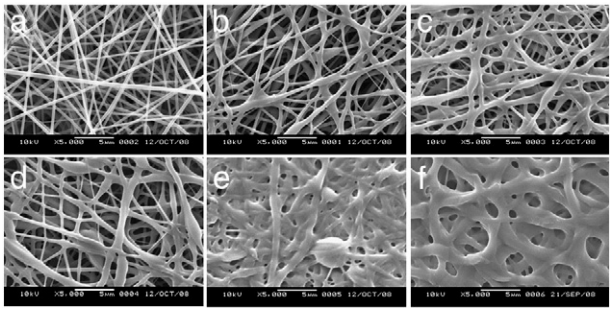

Fig. 1. SEM images of electrospun gelatin nanofibers after different crosslinking times. (a) 0 h, (b) 6 h, (c) 12 h, (d) 24 h, (e) 48 h, (f) 72 h.

Fig. 2 shows the DSC thermograms of electrospun gelatin nanofibers crosslinked with genipin. It is common that a gelatin fibrous mat contains 10–15% water. As shown in Fig. 2, the characteristic endothermic peaks have often been termed as denaturation temperature (Td), and the corresponding melting heat reflecting the 'crystallinity' is called denaturation enthalpy (ΔHd). The values of Td and ΔHd, obtained from the electrospun gelatin nanofibrous membranes before and after the crosslinking are summarized in Table 1. It is obvious that both Td and ΔHd of the electrospun gelatin fibers increased with increasing crosslinking time. DSC results indicated that the crosslinking treatment has appreciably enhanced the thermal stability of the electrospun gelatin fibers.

Table 1
DSC of electrospun gelatin nanofibers crosslinked for different times.

Sample(time)	0 h	6 h	12 h	24 h	48 h	72 h
Td (°C)	80.38	83.67	84.13	84.77	88.50	88.62
ΔHd (J/g)	299.82	373.97	383.80	393.63	422.42	423.58

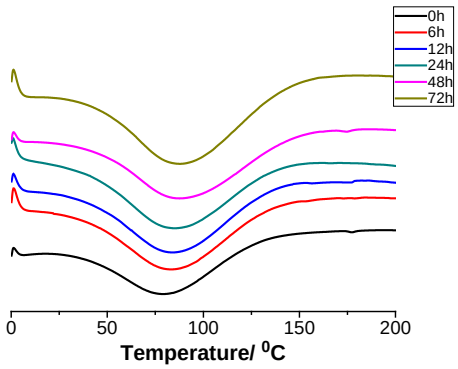

Fig. 2. DSC curves of electrospun nanofibers after different crosslinking times.

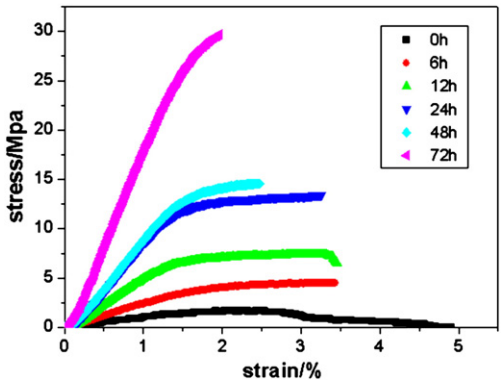

Fig. 3. Stress–strain curves of electrospun nanofibers with different crosslinking times.

For tissue engineering applications, mechanical properties of nanofibers are important. Typical tensile stress–strain curves of the electrospun gelatin fibrous membranes crosslinked for different times are plotted in Fig. 3. The results indicate that the mechanical performance of the gelatin fibrous mats was dramatically improved by crosslinking. After 72 h crosslinking, the breaking strength was enhanced to more than 16 times that of the non-crosslinked counterparts.

The release of BSA from gelatin scaffolds is shown in Fig. 4. There were initial burst releases from electrospun mats, and then the release rate decreased. It is clear that all the release curves have a similar shape. The initial burst release of fibrous mats without genipin treatment could reach 73%. However, with increasing crosslinking time, the initial burst release decreased. Only 47% of protein is

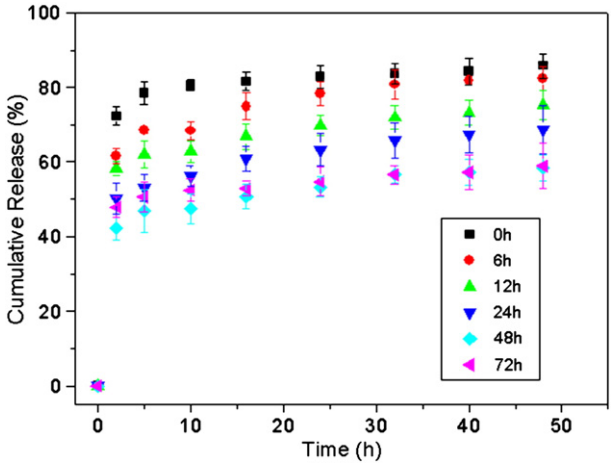

Fig. 4. Release profile of BSA from gelatin mats with different crosslinking times.

released from the fibrous mats after crosslinking for 72 h. The formed intra-molecular covalent bonds between BSA and gelatin and the bonds between the fiber junctions might be the reason for the reduced burst release. Formation of point-bonded structures favors the structural integrity of electrospun fibers and BSA, resulting in decreased release of BSA.

Conclusion

Gelatin nanofibers prepared by electrospinning with and without genipin crosslinking were systemically investigated. With increase of crosslinking time, thermal stability and mechanical properties are enhanced. Moreover, BSA as model drug was successfully incorporated into nanofibers. The release rate of BSA from fibrous mats decreases with increase of crosslinking time. These crosslinked nanofibrous mats may be interesting for tissue engineering or drug delivery applications.

Acknowledgments

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Novel pH-responsive polyphosphoester-based hydrogels with fast gelation

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Abstract summary

pH-Responsive polyphosphoester (PPE)-based hydrogels with rapid gelation were fabricated and evaluated for drug delivery application. Rheological studies indicated that the gelation occurs mainly due to the synergistic effect of ammonium persulfate (APS) and 2-(dimethylamino)ethyl methacrylate (DMAEMA). The hydrogels exhibited favorable pH-dependent swelling behavior and the swelling ratio changed regularly with the PPE content. The drugs loaded into the hydrogels during the gelation could be released continuously from the hydrogels.

Keywords: Hydrogel, pH-responsive, Polyphosphoester, Drug delivery

Introduction

Hydrogels are three-dimensional polymeric networks absorbing a significant amount of water or biological fluids. Due to their tunable chemical and three-dimensional physical network structures, high water content and biocompatibility, hydrogels are of great interest for drug delivery, tissue engineering, and medical devices etc. [1, 2]. Among various hydrogels, injectable ones formed by *in situ* chemical polymerization or by the sol–gel phase transition have obtained a lot of attention [3]. The injectable systems are flowable aqueous solutions before administration, but once injected, rapidly gel under physiological conditions.

Polyphosphoesters (PPE), a class of biodegradable polymers with repeated phosphoester bonds in the backbone, has gained considerable attention in biomedical applications including delivery vehicles for drugs or gene, tissue engineering scaffolds because of their favorable biocompatibility, biodegradability, structure diversity and versatile functionality [4, 5]. In our earlier work, pH-sensitive anionic hydrogels based on PPE were synthesized and fully characterized [6]. In the present work, we report on a cationic and pH-responsive hydrogel consisting of PPE and poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA). We have unexpectedly found that the gelation can be completed at room temperature in a period from several to tens of minutes depending on the dosage of the components. The sol–gel transition and the properties of these hydrogels are systematically investigated. We think this report could provide a new route to construct hydrogels with fast gelation.

Experimental methods

The detailed synthesis procedure of the macrocrosslinker ($M_{n,\ GPC} = 6.5 \times 10^3$ g/mol) can be found in a previously reported paper [6]. The macrocrosslinker and DMAEMA was first dissolved in deionized water, APS was then added into the reaction vial and the mixture was gently stirred with the aid of a vortex mixer. Formulations reacted upon mixing, resulting in a transparent hydrogel (Fig. 1). The drug-loaded hydrogels were prepared using the same method except that the aqueous solution of doxorubicin hydrochloride with certain concentration was used to replace deionized water.

The gelation time was determined by the vial tilting method. Rheological tests were carried out with a RS 6000 rheometer (Thermo Haake) using a parallel plate (PP20H, 20 mm diameter, 0.5 mm gap) configuration at 25 °C in oscillatory mode. Release experiments were performed by placing the hydrogel (~1 mg drug) in 20 mL of PBS buffer (pH 7.4) in a shaking bath at 37 °C. Periodically, 5 mL of buffer was collected and the released drug was determined spectrophotometrically at a wavelength of 490 nm. Volume of the release medium in the vial was kept constant by adding 5 mL of fresh PBS after each sampling. All *in vitro* loading and release studies were performed in dark.

Results and discussion

As shown in Fig. 1, functional polyphosphoesters (HEMA-PEOP) were first prepared by ring-opening polymerization of cyclic phosphoester monomer in the presence of $Sn(Oct)_2$ and 2-hydroxyethyl methacrylate (HEMA). The macrocrosslinker (HEMA-PEOP-Ac) was then synthesized by the reaction of HEMA-PEOP with acrylic acid. After mixing of macrocrosslinker, ammonium persulfate (APS) and 2-(dimethylamino)ethyl methacrylate (DMAEMA), hydrogels with different crosslinking density could be obtained after standing for several minutes (crosslinking density: gel-1<gel-2<gel-3<gel-4).

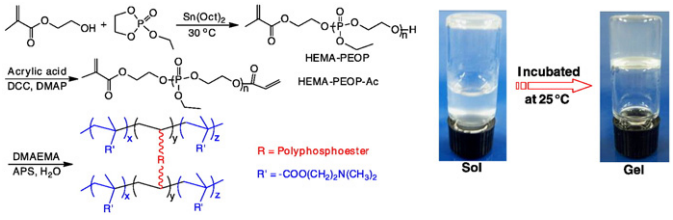

Fig. 1. Schematic illustration of the preparation of pH-responsive hydrogels (left), and digital photographs of the gelation process (right).