

Preparation and Characterization of Poly (ϵ -lysine)-chitosan Cross-linked by Glutataldehyde

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Abstract. A novel composite material: Poly-(ϵ -lysine)-chitosan cross-linked by glutataldehyde (GTA) was prepared in this study. The composite polymer was investigated through FTIR spectroscopy, X-ray diffraction to confirm the structure. Then porous structure was fabricated by freeze-drying the material, the results of the scanning electron microscopy (SEM) and compress property measurement indicated that the composite material is a promising candidate material for scaffold in Tissue Engineering.

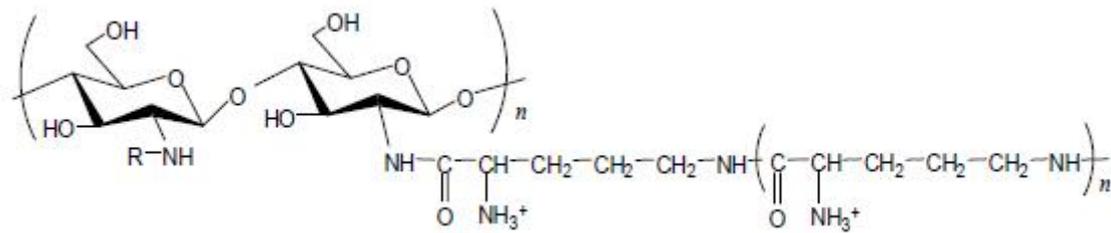
1. Introduction

Poly- ϵ -lysine (ϵ -PL) is an unusual cationic, naturally occurring homopolyamide made of L-lysine, having amide linkage between ϵ -amino and α -carboxyl groups[1-2]. It is water soluble, biodegradable, edible, and has the function of supporting attachment and growth of cell, and nontoxic toward humans and the environment[3-4], thus, it has been taken notice in recent years for a broad range of applications such as, biodegradable fibers, drug carriers, anticancer agent enhancer and food preservatives. It is a good candidate for biomaterials. However, the dominant limitations of ϵ -PL in applications as biomaterials are its relatively poor mechanical properties.

Chitosan is a partially deacetylated found in crustaceans and insects, which is structurally similar to glycosaminoglycan's[5]. It's good potential as a biomaterial derives from its cationic and high-charge density properties, and the free amino groups of chitosan can be chemically derivatized, in mild reaction conditions, to promote biological activities and modify mechanical properties[6]. Chitosan has also been shown to have wound healing properties, is nontoxic, and has minimal foreign body response with accelerated angiogenesis [7-8]. For improving the mechanical or biological properties of chitosan over a broad range, blending with other polymers is widely investigated[9-10].

In this work, we have prepared a novel bio-composite, namely ϵ -PL/chitosan cross-linked by glutataldehyde[11] [GTA(ϵ -PL/chitosan)], in order to combine the advantages of ϵ -PL and chitosan and overcome the limitations in mechanical properties. The structure of ϵ -PL/chitosan was shown in Scheme 1.





Scheme 1. Structure of ϵ -PL/chitosan

2. Experimental

2.1. Material

Chitosan was purchased from Acros Organics, ϵ -PL was supplied by Tianjin University of Science and Technology, GTA was purchased from Tianjin Standard Science Technique co.ltd. All other reagents were local products of analytical grade.

2.2. Preparation of ϵ -PL/chitosan cross-linked by GTA composite material

Amount of predetermined chitosan powder (1 g) was added into 100 ml acetic acid solution (1%). After dissolved thoroughly, ϵ -PL powder (1 g) was transferred into the solution aforementioned and then stirred mildly for 2 h at 50 °C. The resultant was sublimated via vacuum extraction filtering method. Glutataldehyde solution (5 ml, wt 20%) was added into the filtrate for cross-linking ultimately.

2.3. Fabrication of porous structure

Inject the composite material into a glass cylindrical container ($\Phi 20 \times 35$ mm) with a brass bottom (Figure 1), and immersed in liquid N₂, occurring the unidirectional growth of ice from the bottom to the upper face [12]. Then the container was set on a cooling stage at -50 °C equipped with a freeze-dryer. The subsequent freeze-drying produced a porous composite.

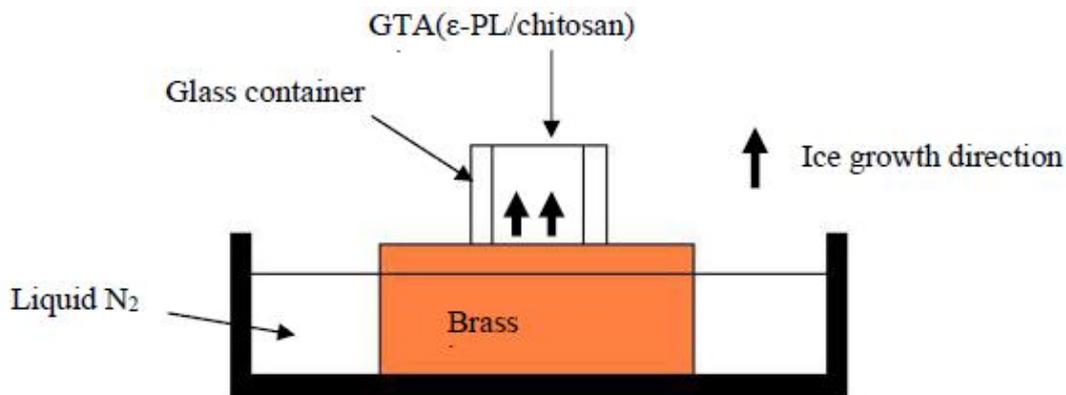


Figure 1. Schematic illustration of the freezing apparatus for unidirectional solidification

The container was composed of two parts: the bottom made of brass and the side of silicone. When the container was immersed in liquid N₂ (in this figure) or set on a cooling stage, the container face was only cooled to generate ice crystal growth along vertical direction.

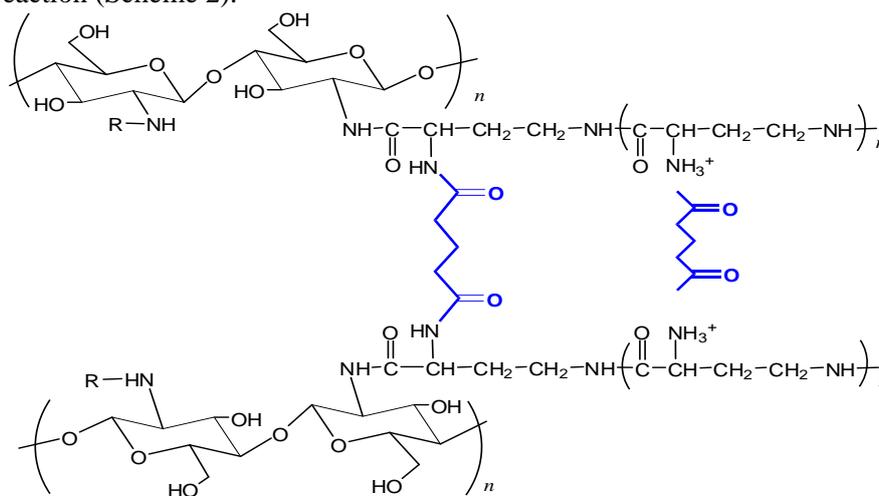
2.4. Characterization

The microstructure of the scaffold was observed under scanning electron microscopy (SEM). IR spectra were collected by a FTIR spectrophotometer (Bio-Rad FTS 6000) using an attenuated total reflection (ATR) method. The crystallinity of the polymer was determined by a Wide-angle X-ray diffraction (WAXD) apparatus (RIGAKU, D/MAX 2500) at room temperature.

3. Results and discussion

3.1. FTIR Spectral Analysis

Figure 1 shows the FTIR spectra of ϵ -PL, ϵ -PL/chitosan and GTA (ϵ -PL/chitosan). The absorption peak at ~ 1071 cm^{-1} belongs to the characteristic band of chitosan. The absorption peaks located at ~ 1969 cm^{-1} were assigned to stretching vibration of amino cation ($-\text{NH}_3^+$) of ϵ -PL. The typical peaks of chitosan (at ~ 1071 cm^{-1}) appeared obviously in curve b and c, which suggested that the ϵ -PL/chitosan composite was prepared successfully. From the comparison of the three curves in Figure 2, the absorbance of characteristic peaks of amino cation (~ 1969 cm^{-1}) of ϵ -PL has decreased evidently. This demonstrated that $-\text{NH}_3^+$ had reacted to aldehyde groups of GTA according to the mechanism of crosslinking reaction (Scheme 2).



Scheme 2. Crosslinking reaction

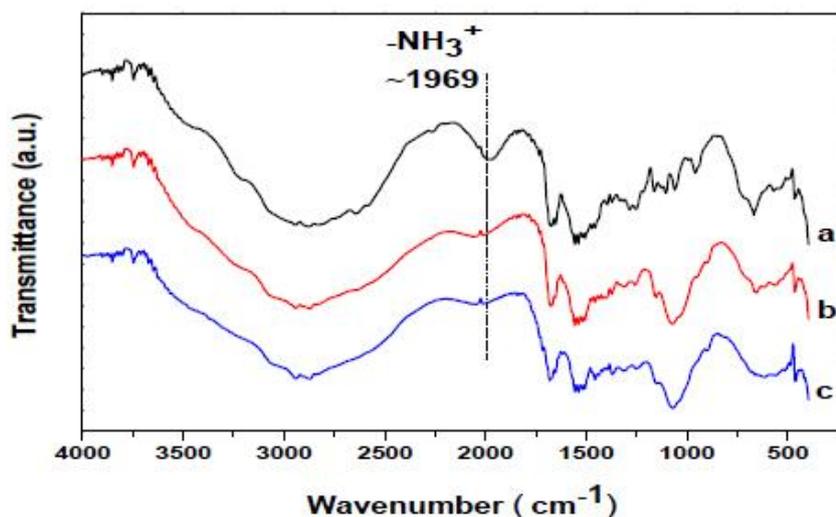


Figure 2. FTIR spectra of (a) ϵ -PL; (b) ϵ -PL/chitosan; (c) GTA (ϵ -PL/chitosan)

3.2. X-ray analysis

The X-ray diffraction patterns of ϵ -PL, ϵ -PL/chitosan and GTA (ϵ -PL/chitosan) are shown in Figure 3. The typical peaks of ϵ -PL were detected at 24 and became weak and broad with respect to the comparison of the profiles of curve a, b and c. It indicated that synthesis and crosslinking of ϵ -PL was effective as the crystallinity of ϵ -PL in curve b and c was decreased.

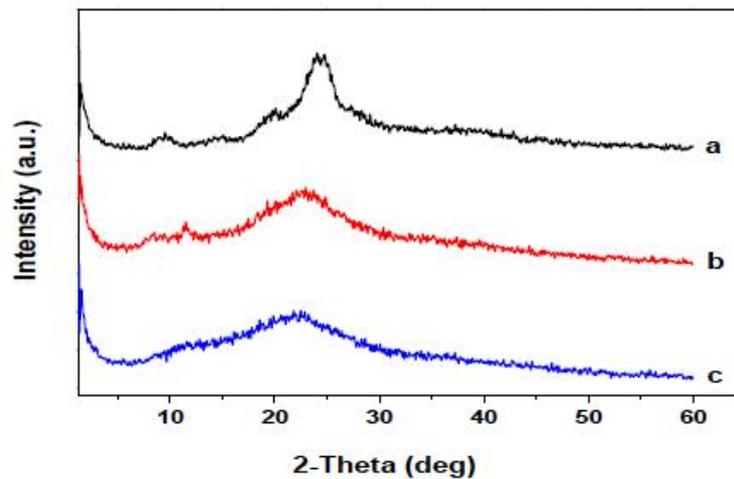


Figure 3. X-ray patterns of (a) ϵ -PL, (b) ϵ -PL/chitosan, (c) GTA (ϵ -PL/chitosan)

3.3. Morphology

The surface-section, cross-section and vertical-section morphologies of ϵ -PL/chitosan cross-linked by GTA are shown in Figure 4. Freeze drying resulted in uniformly size porous structure, the average pore size is nearly 25 μ m. The results indicate that the composite material is a good potential candidate for scaffold in Tissue Engineering.

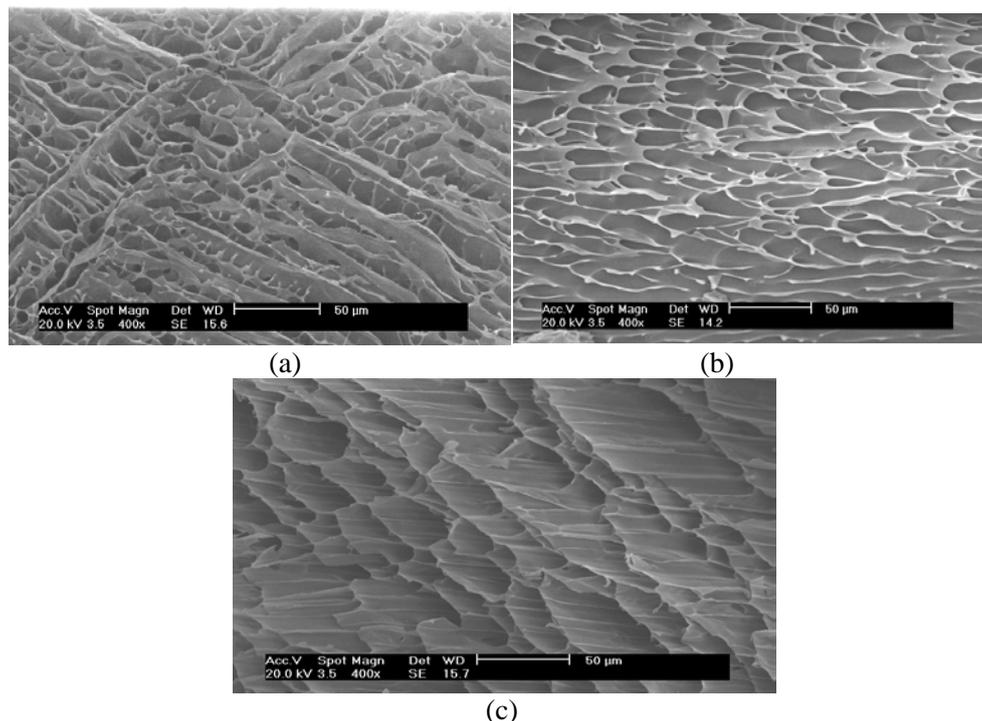


Figure 4. The SEM images of GTA (ϵ -PL/chitosan) $\times 400$. (a): surface-section; (b): vertical-section; (c): cross-section

4. Conclusion

Herein a new biomaterial ϵ -PL/chitosan cross-linked by glutataldehyde has been prepared successfully, and then fabrication the porous structure by freeze-drying. The FTIR analysis shows that the composite consisting ϵ -PL and chitosan is synthesized and able to be modified via crosslinking by GTA. The X-ray analysis revealed the crysallinity of the ϵ -PL/chitosan and GTA (ϵ -PL/chitosan) is

lower than that of pure ϵ -PL. The compress property treatment result and SEM porous structure suggested that it is a promising candidate material for scaffold in Tissue Engineering.

5. Acknowledgement

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6. References

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