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Synthesis and Application of Biodegradable Crosslinking Agent

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Abstract: A novel kind of amphiphilic and biodegradable films were developed. The film mainly is composed of three components: a novel biodegradable crosslinking agent, which is used to adjust the degradable rate of the film; a hydrophilic monomer N-vinyl pyrrolidone (NVP), which is used to improve the material's hydrophilicity, and ultimately improve the interaction between material and cells; a macromonomer 2-hydroxyethyl methacrylate-terminated polylactide acid (HEMA-PLA), which is used to control the film's mechanical strength. These films hopefully will have practical applications in tissue engineering.

Keywords: crosslinking agent; NVP; HEMA-PLA; film; amphiphilic; biodegradable

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Tissue engineering is a new approach to reconstruction and/or regeneration of lost or damaged tissue and organ. One of the key technologies in tissue engineering is the preparation of scaffold material for cell culture and tissue repair. At present, a variety of biomaterials, including natural and synthetic ones, have been widely studied as scaffold materials for tissue engineering. Among these materials, polylactic acid (PLA) has become one of the most widely used biomaterials due to its biocompatibility, biodegradability, bioresorption and sufficient mechanical properties^[1-4]. But they have poor hydrophilicity and lack the ability to interact biospecifically with cells^[2]. Under physiological conditions, uncontrolled strong interactions exist between the surface of the material and the proteins. Furthermore, degradable rate and dynam attenuation to match tissue regeneration are also problems^[4].

To overcome the shortcomings of PLA based scaffolds, in this study, we synthesized a series of novel biodegradable crosslinking agents, and then prepared films using this crosslinking agent with hydrophilic monomer N-vinyl pyrrolidone and another macromonomer.

1 Experimental Part

1.1 Materials

Commercially available *D, L*-lactide (*D, L*-LA) was obtained from Beijing Conan Polymer R&D Center. The received product was recrystallization twice from ethyl acetate and then filtrated to eliminate the impurities. After filtration, the product was dehydrated at 40 °C under vacuum for 24 hours before used. Diethylene glycol (DEG) was purchased from Guangzhou Chemical Reagent Factory. It was distilled and collected under decompression (< 1.33 kPa) at 106 °C for purification. Stannous oc-

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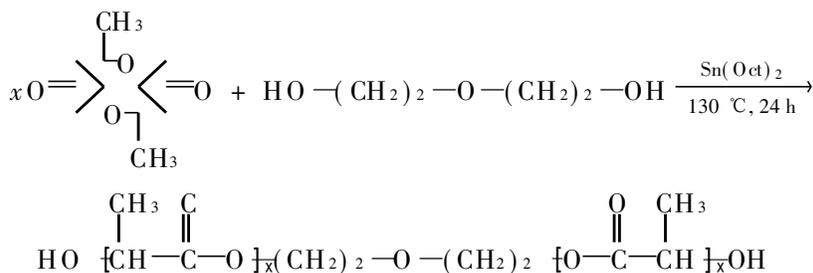
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toate ($\text{Sn}(\text{Oct})_2$) was provided by Shanghai Shuangxiang Auxiliary Agent Factory. It was also distilled and collected under decompression ($< 1.33 \text{ kPa}$) at $220\sim 240 \text{ }^\circ\text{C}$. The collected product was dissolved in *n*-hexane. 2-hydroxyethyl methacrylate (HEMA) was kindly provided by Fluka and was purified by vacuum distillation just before use. Triethylamine was obtained from Tianjin Bodi Chemicals Co. Ltd. . Acryloyl chloride and *N*-vinyl pyrrolidone (NVP) were both provided from Acros Organics. Azobisisobutyronitrile (AIBN) was purchased from Shanghai Sihewei Chemicals Co. Ltd. . All other chemicals were of analytical purity and were used as received.

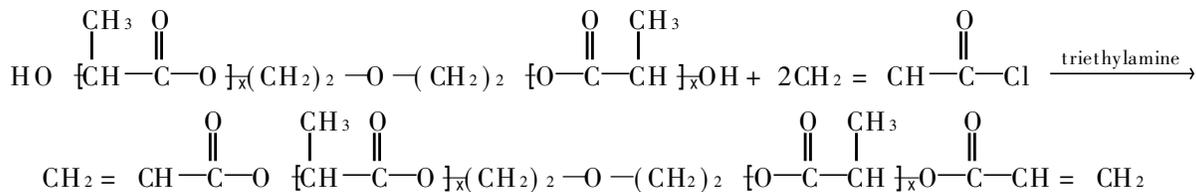
1.2 Methods

1.2.1 Synthesis of Biodegradable Intermediate Biodegradable PLA-DEG-PLA intermediate was synthesized by bulk ring-opening copolymerization of *D, L*-LA and DEG using $\text{Sn}(\text{Oct})_2$ as a catalyst. Synthesis of biodegradable intermediate:



Briefly, *D, L*-LA and DEG were enclosed in an ampoule ($x(\text{D, L-LA}) : x(\text{DEG}) = 10 : 1$). $\text{Sn}(\text{Oct})_2$ was added with the amount of 0.03% of total mass of *D, L*-LA and DEG. Then we vacuumized and sealed the ampoule, and put it in vacuum oven to react for 24 hours at $130 \text{ }^\circ\text{C}$. Collected product was dissolved in trichloromethane and then was precipitated by *n*-hexane. The sediment was dehydrated and stored in a desiccator. The final product was characterized by Fourier transform infrared (FTIR, EQUINOX 55, Bruker, Germany) and ^1H nuclear magnetic resonance (^1H NMR, UX-400, Bruker, Germany).

1.2.2 Synthesis of Biodegradable Crosslinking Agent PLA-DEG-PLA intermediate was dissolved by dichloromethane and then poured into a round-bottomed flask. Acryloyl chloride was added dropwise to the solution at $0 \text{ }^\circ\text{C}$ under nitrogen in the presence of triethylamine. The reaction mixture was stirred for 6 hours at $0 \text{ }^\circ\text{C}$ and additional 12 hours at room temperature. Synthesis of biodegradable crosslinking agent:



After filtration to remove the triethylamine hydrochloride sediment, ethyl ether was added to precipitate the formed crosslinking agent (CA). The precipitate was separated and dehydrated overnight in a vacuum oven at $45 \text{ }^\circ\text{C}$. The final product was characterized by FTIR.

1.2.3 Synthesis of HEMA-PLA Macromonomer The synthesis of methacrylate-terminated poly *D, L*-LA macromonomers (MCs) has been studied by several groups^[5-6]. In this paper, MC ($x(\text{HEMA}) : x(\text{D, L-LA}) = 1 : 80$) was prepared by bulk ring-opening polymerization of *D, L*-LA using HEMA and $\text{Sn}(\text{Oct})_2$ as the initiator system. The reaction was allowed to proceed at $130 \text{ }^\circ\text{C}$ for 12 hours. After the reaction, the resulting crude product was dissolved in tetrahydrofuran (THF) and then was precipitated in distilled water. The sediment was dehydrated under vacuum for several hours. The final product was characterized by FTIR.

1.2.4 Preparation of Amphiphilic, Biodegradable Films The procedure for the synthesis of polymer networks by crosslinking agent, hydrophilic monomer NVP and macromonomer HEMA-PLA is given here. Crosslinking agent was mixed with NVP and HEMA-PLA (different mass ratio). AIBN was dissolved in dichloromethane and then added to the mixture as initiator. After thorough mixing, the mixture was cast on a Teflon mold and to form a film at 70 °C under vacuum. Put the film in distilled water to remove the NVP homopolymers. The received film was characterized by contact angle meters (TANTEC CAM-PLUS, USA) and an universal mechanical testing machine (load cell: 1 kN, cross-head speed: 2 mm • min⁻¹, Shimadzu AG-I, Japan).

2 Results and Discussion

2.1 Synthesis of the Intermediate and the Biodegradable Crosslinking Agent

D, L-LA was ring-opening polymerized using DEG as the initiator to yield biodegradable PLA-DEG-PLA intermediate. This biodegradable copolymer contains a hydroxy group on each side, which can react with acryloyl chloride to yield functional biodegradable crosslinking agent with a double bond at each terminal. The resulting crosslinking agent can be used to prepare a novel biodegradable network with other alkene monomers when subjected to light or other initiators.

Figure 1 shows the FTIR spectra of the intermediate and the biodegradable crosslinking agent. The strong absorption band at 316 cm⁻¹ attributable to hydroxy groups in the spectrum of the intermediate (Figure 1, curve a) disappears in the spectrum of the crosslinking agent (Figure 1, curve b). And, a new absorption band appears at 1 631 cm⁻¹ in curve b, which corresponds to the terminal double bonds of the copolymer. Furthermore, both curve a and b have a strong absorption band at 1 756 cm⁻¹ which is attributed to the ester groups of the PLA chains. All the data indicate the acryloyl oxide groups were successfully grafted to the terminals of the PLA-DEG-PLA intermediate. ¹H NMR spectrum of the PLA-DEG-PLA intermediate also gives details about the intermediate structure (Figure 2). Briefly, ¹H NMR (recorded in CDCl₃) of materials a, b, c, d, e, f, g, there δ value is 1.4, 1.6, 3.6, 4.3, 4.4, 5.2 and 7.3.

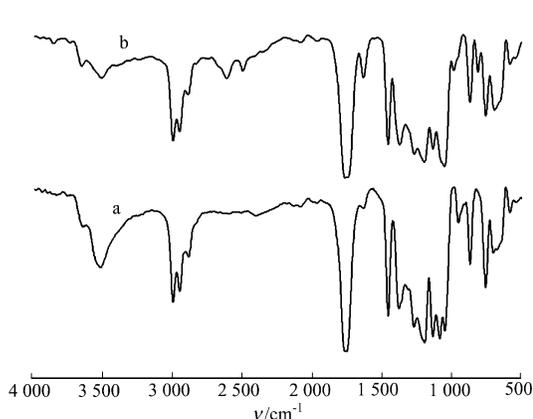


Fig. 1 FTIR spectra of biodegradable intermediate and crosslinking agent

图 1 生物降解中间体及交联剂的红外光谱图

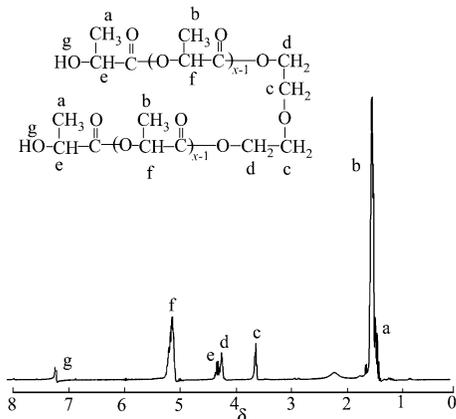


Fig. 2 ¹H NMR spectrum of the intermediate

图 2 中间体的核磁共振光谱图

2.2 Synthesis of HEMA-PLA Macromonomer

According to the mechanism of Sn(Oct)₂-initiated ring-opening polymerization of *D, L*-LA^[7], if we use HEMA as a co-initiator, which is biodegradable, hydrophilic, and has a terminal hydroxy group, we will undoubtedly prepare a HEMA-PLA macromonomer with a reactive terminal double bond.

Figure 3 shows the FTIR spectrum of the MC, which is similar to the FTIR spectrum of PLA. Two bands at 2996 cm^{-1} and 2948 cm^{-1} correspond to the stretching vibration of C-H, while the strong absorption band at 1756 cm^{-1} is attributed to the ester groups of the PLA chains. But the most obvious difference is that, in figure 3, a new C=C vibration bond appears at 1640 cm^{-1} which indicates the successful synthesis of HEMA-PLA.

The ^1H NMR spectrum of the MC is shown in Figure 4. ^1H NMR (recorded in CDCl_3) of macromer δ value is 1.6 (CH_3 , lactide), 1.9 (3H , CH_3 , methacrylate), 4.35 (4H , CH_2CH_2 , ethyl methacrylate), 4.38 (1H , CH , terminal lactide), 5.2 (CH , lactide), 5.6 and 6.1 (2H , CH_2 , methacrylate)^[5-6,8]. The molecular weight of the MC is calculated from the integrals of the characteristic ^1H NMR signals, which indicates that the experimental average molecular weight ($M_{n,\text{exp}}$) is rather close to the theoretical one ($M_{n,\text{t}}$). For example, when the feed molar ratio of HEMA to *D,L*-LA is 1:80, $M_{n,\text{t}}$ is 11650, while $M_{n,\text{exp}}$ is 12522.

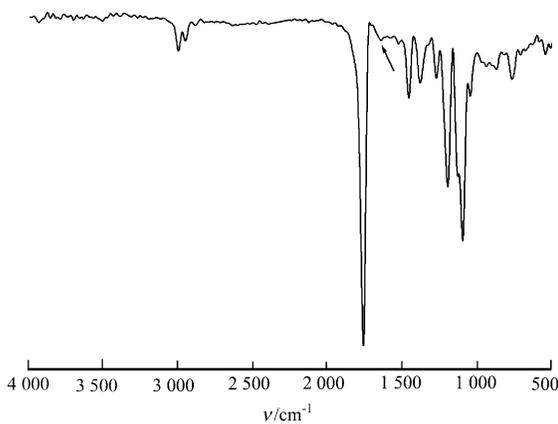


Fig. 3 FTIR spectrum of HEMA-PLA macromonomer

图3 HEMA-PLA 红外光谱图

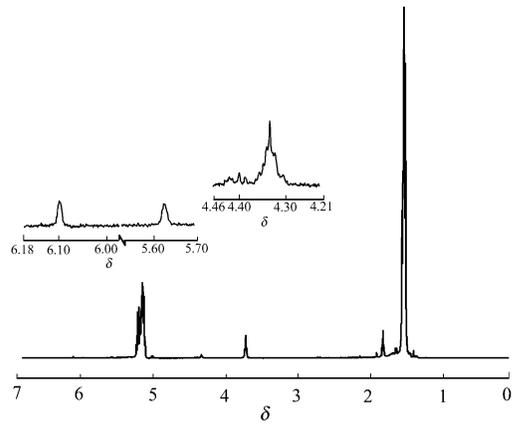


Fig. 4 ^1H NMR spectrum of HEMA-PLA macromonomer

图4 HEMA-PLA 核磁共振光谱图

2.3 Preparation of Amphiphilic, Biodegradable Films

To overcome the shortcomings of PLA scaffold, in our study, we synthesized a series of novel biodegradable crosslinking agents, and then prepared films using this crosslinking agent with hydrophilic NVP and another macromonomer HEMA-PLA to adjust the material's hydrophilicity and mechanical strength.

Data on tensile strength (F), tensile modulus (E) and contact angle (φ) are listed in Table 1. We can see from the table that when molar ratio of NVP/MC decreases, the film tensile strength and tensile modulus both increase,

and so does the contact angle. It is well known that the contact angle is related to the material hydrophilicity. The smaller the contact angle is, the better the material hydrophilicity. So the increasing contact angle data will lead to the decrease of the hydrophilicity of the crosslinking films at the same time. This is because when you increase the amount of MC, the relative amount of PLA chains also increases, which component is hydrophobic and has excellent strength property, while the relative amount of hydrophilic PVP chains decreases. Therefore, with the molar ratio of NVP/MC decreases, the film strength improves, while the film hydrophilicity falls.

Tab. 1 Properties of the biodegradable crosslinking films with different compositions

表1 不同组成生物降解交联膜的性能

Run	$x(\text{NVP}) : x(\text{CA}) : x(\text{MC})$	F/MPa	E/MPa	$\varphi/(\text{°})$
a	85: 10: 5	1.4	6.6	53.0
b	80: 10: 10	2.3	23.2	58.7
c	75: 10: 15	3.1	56.2	60.0
d	45: 10: 45	4.0	79.5	62.5

3 Conclusions

In this study, we developed a novel kind of amphiphilic and biodegradable films. First, we synthesized a series of novel biodegradable crosslinking agents, which have double bonds on the terminal. Then, we prepared films using this crosslinking agent with hydrophilic NVP and another macromonomer HEMA-PLA to adjust the material's hydrophilicity and mechanical strength. Finally, the influence of different feed molar ratios on the film mechanical strength and hydrophilicity were discussed. Results showed when the molar ratio of NVP/MC decreases, the film strength improves, while the film hydrophilicity falls. Further study will focus on the biodegradation and biocompatibility tests as well as its practical application in tissue engineering.

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新型生物降解交联剂的合成及应用

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摘要: 制备出新型两亲性可生物降解聚合物膜。膜由3种成分组成, 其一为新型的可生物降解交联剂, 用以调节膜的降解速率; 其二为亲水单体乙烯基吡咯烷酮, 用以提高材料的亲水性, 并最终提高材料与细胞的相互作用; 其三为羟乙基甲基丙烯酸酯封端聚乳酸, 用以调节膜的机械强度, 通过傅里叶红外光谱(FTIR)和核磁共振(NMR)分析, 结果表明, 成功合成可生物降解的交联剂及大分子单体HEMA-PLA, 并制备出可应用于组织工程的新型膜材料。

关键词: 交联剂; 乙烯基吡咯烷酮; 羟乙基甲基丙烯酸酯封端聚乳酸; 膜; 两亲性; 生物降解

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