Preparation and Characterization of EDC-Modified Collagen Fiber Scaffold Materials

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Abstract: 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC) was selected as cross-linking agents to modify the collagen fiber scaffold materials (CFSM) and to get the EDC–CFSM. The physicochemical and biological degradation properties of CFSM and EDC–CFSM were investigated respectively. According to the analysis of FT-IR, the cross-linking reaction was carried out after the treatment of EDC. Through the cross-linking reaction, the denatured temperature and the degradation resistance of EDC–CFSM improved as the EDC concentration increased. When the concentration of EDC increased up to 14.0mM, the EDC–CFSM exhibited the most excellent properties with denatured temperature at 96.7 ℃, porosity at 80.91% and pore sizes about 70-180 μm.

Key words: collagen fiber; carbodiimide; cross-linking; scaffold material

1 Introduction

As the major structure protein in man, collagen has been extensively utilized as the scaffolding material for bioengineering due to its low antigenicity, biodegradability and biological compatible. Unfortunately, poor mechanical properties, rapid degradation rates of collagen scaffold often fail to meet the requirements of special applications. In that case, chemical modification was widely employed. Historically, glutaraldehyde (GA) [1] and dialdehyde starch (DAS) [2] have been used as a cross-linker, though they have been reported to exhibit toxicity during the degradation of materials. Conversely, 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC) [3, 4] is a zero-length cross-linker that has not been shown to cause any cyrotoxic reaction, extremely interesting collagen-based scaffolds. In this paper, CFSM were obtained as previously described[5] and cross-linked by EDC. In order to extending the application of collagen in medicine, the physicochemical and degradation properties of the scaffold were investigated.

2 Experimental procedures

2.1 Materials

Collagen fiber scaffold materials (CFSM) were prepared in the lab. 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide （NHS） were obtained from Medpep Co., Ltd. (China). 2-(N-morpholino) ethanesulfonic acid (MES) and lysozyme（Activity>20000U/mg）were purchased from Bioprimer Life Technology Co., Ltd. (China).

2.2 Cross-linking of CFSM

Collagen fiber scaffold materials (CFSM) were prepared as previously described[5]. Then, known quantity CFSM were immersed in a sterile buffer solution of 50mM 2-(N-morpholino) ethanesulfonic acid (MES) in 60% (V: V) ethanol-water with pH=5.5 for 30min, followed by adding a increasing concentrations of 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide（NHS）at a molar ratio of 1:1. The cross-linking reaction was carried out at ambient

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temperature for 6h. After that, the scaffolds were washed twice for 30min with 95% ethanol, 0.1M Na_2HPO_4, 1.0M NaCl solutions respectively and again with distilled water. Finally, the scaffolds were lyophilized.

2.3 Characterization

2.3.1 Differential scanning calorimetry (DSC)

Measures for the differential scanning calorimetry were performed with DSC-200PC thermal analyzæ DSC. A total of 2-5mg samples sealed in aluminum pans was scanned from 20 to 160℃ at a scanning rate of 10℃/min while sealed empty pan was used as reference.

2.3.2 FT-IR spectroscopy

The IR spectra of samples were run as KBr pellets on Nicolet 200SXV-IR in the frequency range 4000-450cm^{-1}.

2.3.3 Determination of porosity

EDC treated and untreated samples were immersed in 25mL ethanol at ambient temperature for 24h, followed by removing the redundant ethanol until the total volume back to 25mL and weighting the total mass. Then, the immersed samples were taken out while weighting the rest mass again. The porosity was calculated by following equation:

$$K(\%) = \frac{m_2 - m_3 - m_s}{m_1 - m_3} \times 100$$

Where m_1 is the weight of vessel containing 25mL ethanol, m_2 is the weight after removing the redundant ethanol, m_3 is the weight after removing the immersed sample, m_s is the weight of dry sample.

2.3.4 Scanning electron microscopy

The surface and cross section of the EDC-CFSM were investigated by a JSM-5900LV scanning electron microscope with accelerating voltage of 12KV.

2.3.5 Physical mechanical test

The elongation at break and tensile strength were measured by GT-AI-7000S Leather Tension Testing Machine at a speed of 5mm/min.

2.3.6 In vitro enzymatic degradation

EDC-CFSM were accurately weighed and immersed in PBS buffer solution (pH 7.7) with media containing 12mg/mL lysozyme, and incubated at 37℃ for up to 40 days. The weight of samples was assessed at 3, 6, 9, 12, 15, 18, 21, 24, 30 and 40 days.

3 Results and discussions

3.1 Infrared spectrometry

The IR spectra of EDC-CFSM and CFSM are presented in Fig. 1. As revealed in the Fig. 1, the representative absorption peaks in two samples are assigned to amide I (1660cm^{-1}), amide II (1556cm^{-1}), amide III (1453cm^{-1}) and symmetric stretching of carboxylate salts (1403cm^{-1}), and ester bond (1082cm^{-1}). Contrast with two samples, EDC-CFSM exhibits similar peaks with CFSM, but it is founded that after EDC treated, amide I, amide II, amide III were slight increased as well as the increase of ester bond (1082cm^{-1}), while the symmetric stretching of carboxylate salt (1403cm^{-1}) was decreased in compared to C-H bond (2961cm^{-1}), indicating that amidation reaction has occurred between carboxyl groups and available amino groups of lysine or hydrolysine residues.[6]

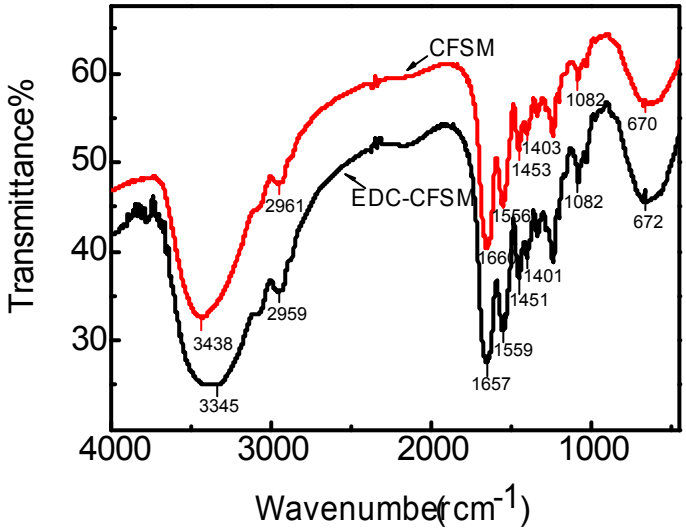

Fig. 1 FT-IR spectra of CFSM and EDC-CFSM

3.2 Thermal stability

The thermal denaturation temperatures of EDC-CFSM are presented in Fig. 2.

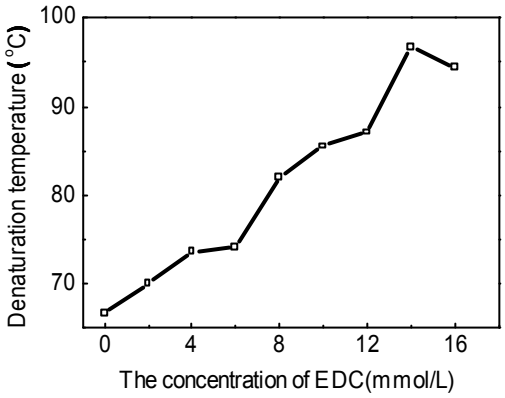

Fig. 2 Effect of EDC concentration on the denaturation temperature of EDC–CFSM

It is observed that the thermal denaturation temperatures significantly increase with increasing concentration of EDC, related to the cross-linking reaction caused by EDC. When concentration of EDC increases to 14mmol/L, the denaturation temperature reaches the peak at 96.7℃ which increase by 30.5℃ over the untreated sample.

3.3 Structural Morphology

It is reported that the comfortable porosity and pore size for scaffolds should be 80-97% and 50-100μm respectively, [7] which benefiting to the movement and migration of cells. [8] As the important parameters of three-dimensional porous scaffold materials, porosity and pore size were investigated (Fig. 3). It is observed that increasing EDC concentration up to 14mmol/L reduces the porosity of scaffold with minimum about 80.91%. Fig. 4 shows the surface and cross section of 14mmol/L EDC treated CFSM. It is found that EDC-CFSM of porous size is between 70 to 180μm, exhibiting clear 3-D structure,

homogeneous pore and suitable for cell regeneration.

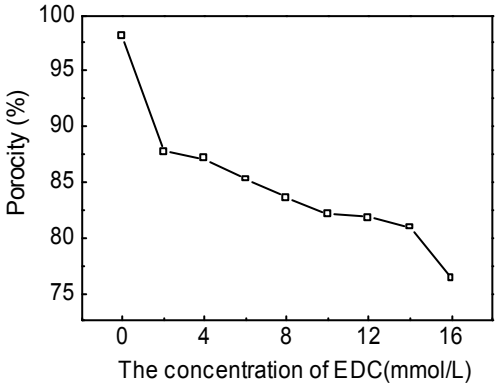

Fig. 3 Porosity of EDC-CFSM

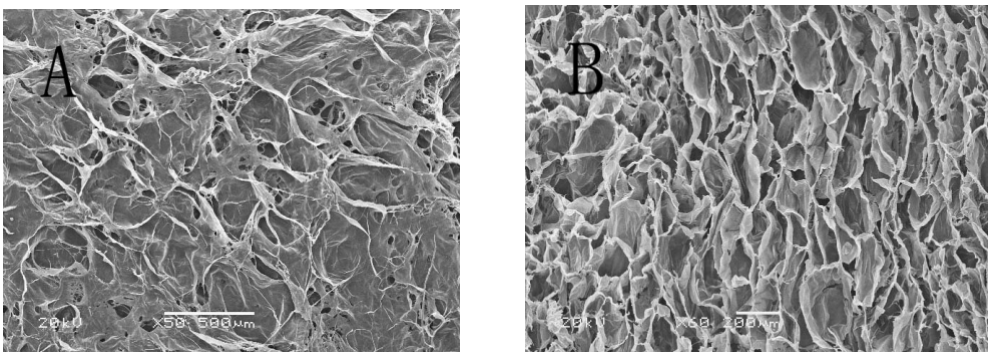

Fig. 4 SEM photograph of surface (A) and the cross section (B)

3.4 Mechanical Properties

The mechanical properties of EDC-CFSM including elongation at break and tensile strength are presented in Fig. 5. It is observed that the elongation at break of EDC-CFSM increases as the increasing of concentration of EDC, which exhibiting the same trend as tensile strength. When adding the concentration of EDC up to 14mmol/L, the elongation at break reaches 210.26% which increasing by 102.66%, while the tensile strength is enhanced to 3.014Mpa that increasing by 43.1% compared with CFSM. It is suggested that the mechanical properties of CFSM are improved after EDC treated, which attributed to the cross-linking reaction caused by EDC.

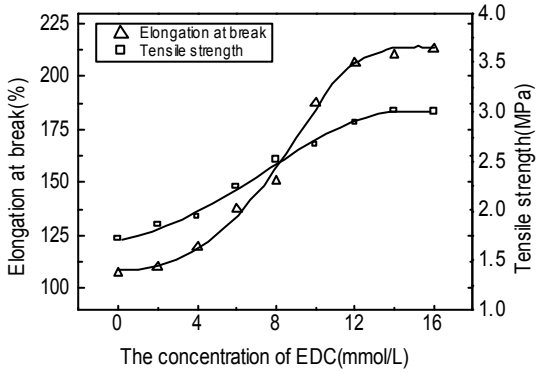

Fig. 5 The influence of concentration of EDC on mechanical properties of EDC-CFSM

3.5 In vitro biodegradability

Degradation of EDC- CFSM and CFSM were carried out in the medium of PBS buffer solution with lysozyme. It is revealed that the time of complete degradation of 8mmol/L treated EDC-CFSM is increased up to 40d in compared to CFSM of 21d (Fig. 6), indicating that the increase of concentration of EDC contributes directly to the improvement of bio-stability. Moreover, it is found that when the concentration of EDC reaches 14.0mmol/L, it takes 52d to degrade EDC-CFSM completely.

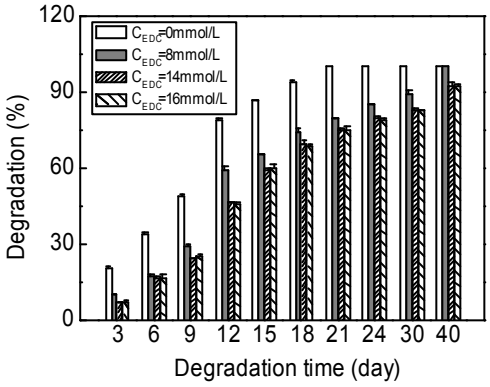

Fig. 6 The degradation of EDC-CFSM in lysozyme

4 Conclusions

EDC has been demonstrated to perform as an efficient cross-linker that improves both thermal stability and bio-stability of collagen scaffold. According to the analysis of IR, it is indicated that amidation reaction has occurred between carboxyl groups and available amino groups of lysine or hydrolysine residues. It is found that with the increase of concentration of EDC up to 16mmol/L, the denaturation temperature increased while their bio-stability is enhanced. An offer of EDC up to 14mmol/L has been estimated to be an optimum concentration that providing the scaffold with high denaturation temperature at 96.7℃, appropriate porosity at 80.91% and pore size between 70 to 180μm. Moreover, The EDC-CFSM, with clear 3-Dstructure, connected and homogeneous pores, is expected to be a novel collagen scaffold for tissue-engineering.

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