International Journal of Biological Macromolecules 121 (2019) 233–238

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

A collagen film with micro-rough surface can promote the corneal epithelization process for corneal repair

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ARTICLE INFO

Article history:
Received 16 August 2018
Received in revised form 27 August 2018
Accepted 7 October 2018
Available online 09 October 2018

Keywords:
Collagen
Corneal
Micro-rough surface
Keratoplasty
Epithelial repair

ABSTRACT

Corneal disease is a major cause of blindness and keratoplasty is an effective treatment method. A freeze-dried collagen film (FD-Col) with micro-rough surface structure for corneal epithelial repair was reported in our previous studies. In this research, we conducted a more comprehensive study on the FD-Col film. Besides with excellent mechanical property and optical transparency, the FD-Col film also has good penetrating ability in nutrient solutions. The permeability coefficient of the FD-Col in NaCl and tryptophan solution is $(2.58 \pm 0.47) \times 10^{-6}$ cm^2/s and $(2.67 \pm 0.13) \times 10^{-7}$ cm^2/s, respectively. In addition, the morphology change of the FD-Col film before and after water absorption is relatively stable suggesting that this film can be fabricated with various dimensions easily. Moreover, corneal lamellar keratoplasty shows that the FD-Col film can be sutured in rabbit's ocular surface and the re-epithelization process in vivo is complete in about 12 days, and the transparency is restored quickly in the first month. Corneal rejection reaction, neovascularization and keratoconus are not observed within 2 months. This FD-Col film, which can be prepared in large quantities and at low cost, should have potential application in corneal repair in the future.

1. Introduction

Corneal epithelial lesion is a major cause of blindness and keratoplasty is considered as an effective method for visual rehabilitation of patients with corneal blindness [1,2]. However, the availability of high quality allogeneic corneal tissues fails to meet the demand in many countries and regions [3–5]. To solve this problem, various efforts have been made to reconstruct corneal tissue substitutes by using natural biological materials [6–9]. Collagen, as the main component of corneal tissue and some connective tissues, has been extensively studied as cornea tissue engineering scaffold materials [10–14]. Although a number of collagen materials for corneal repair have shown good biocompatibility [15–19], but the reepithelization rate on the corneal substitutes in vivo still needs to be further improved.

A freeze-dried collagen film (FD-Col) with micro-rough surface structure was fabricated in our previous studies [20]. Compared with the air-dried collagen film (AD-Col), this freeze-dried collagen film (FD-Col) with micro-rough surface structure shows good physical and chemical properties. Besides, the adhesion and proliferation rate of human corneal epithelial cells on the micro-rough surface of FD-Col film is higher than that on the smooth surface of AD-Col film. In this paper, the penetration capacity of nutrients and the morphology change of the collagen films were measured. Furthermore, the comprehensive repair effect of FD-Col film in vivo was evaluated by rabbit corneal transplantation experiments.

2. Material and methods

2.1. Materials

Type I collagen (HM Biotech Ltd., Guangzhou, China) was extracted from bovine tendon. 1 Ethyl 3 (3 dimethyl aminopropyl) carbodiimide (EDC) and *N* hydroxysuccinimide (NHS) were purchases from GL Biochem Ltd. (Shanghai, PR China). Phosphate buffered saline (PBS) was prepared from the tablet form (Calbiochem Corp, Germany). Deionized water was obtained from a water purification system (Millipore S.A.S., France). New Zealand white rabbits of either gender (12 week old and 2.5–3 kg) were used as animal transplant recipients.

2.2. Preparation of films

The preparation of collagen film was reported previously [20]. In brief, collagen was dissolved in 0.01 M HCl (7.5 mg/mL). Then, EDC and NHS were dissolved to the collagen solution and thoroughly mixed with a mass ratio of EDC:NHS:Col = 0.5:0.5:6 at 4 °C. Crosslinking reaction was carried out by stirring the mixture for 4 h.

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https://doi.org/10.1016/j.ijbiomac.2018.10.026
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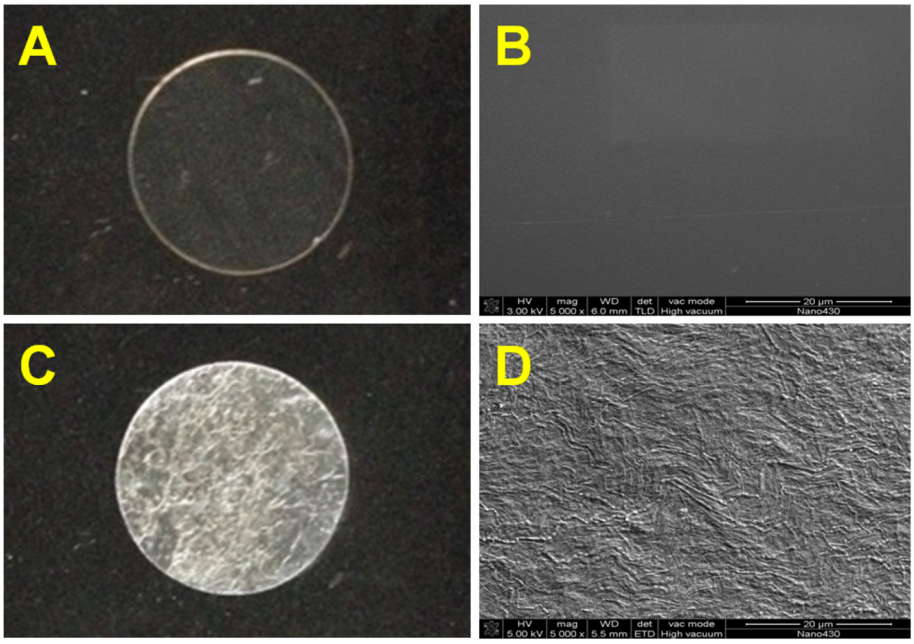

Fig. 1. Surface morphology of the AD-Col and FD-Col film depicts that the two collagen films have different surface structure. Photographs of AD-Col (A) and FD-Col film (C), the diameter of the samples (A and C) is about 15 mm. SEM images of AD-Col (B) and FD-Col film (D), the freeze-drying procedure makes the FD-Col's surface produced some subtle convex areas.

After crosslinking, the collagen solution was dispensed into a specific mould and put into a constant temperature and humidity equipment to form a cornea shape film (AD-Col). The thickness of the dry collagen films is about 25 ± 5 μm. After that, the films were rinsed with deionized water and followed by an immersion in ultrapure water for water absorption. The wet collagen samples were frozen in an ultra-low temperature freezer and then placed into a freeze dryer for lyophilisation (FD-Col). The sample films were sterilized before use.

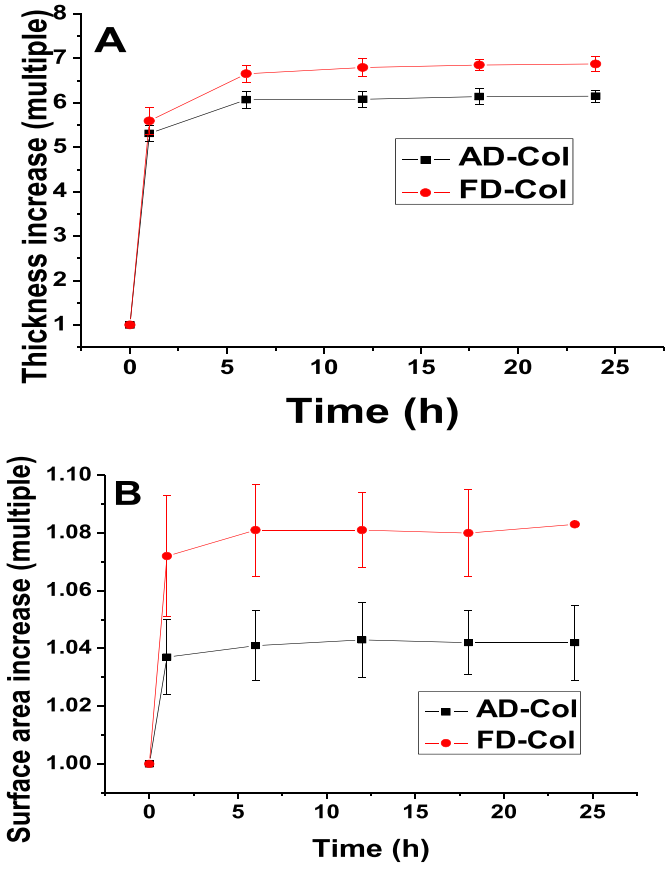

Fig. 2. Swelling properties of the AD-Col and FD-Col film: Variation of the dry and wet samples' thickness (A); Variation of the dry and wet samples' surface area (B). Values are expressed as the mean ± standard deviation (n = 10). The thickness and surface area of the films are controlled and repeatable.

2.3. Scanning electron microscopy observation

The surface morphology of the samples was examined using scanning electron microscopy (SEM). Prior to imaging, the dry collagen films were fixed horizontally by conductive tapes after which the specimens were coated with gold at about 2–5 nm thickness (Hitachi, Tokyo, Japan). Observations were performed with a ZEISS EVO 18, Oberkochen scanning electron microscope (Germany) at 10 kV.

2.4. Swelling characterization

Swelling properties of the films was measured by swelling them in PBS (pH = 7.4) at the normal physiological temperature of the cornea. Prior to the test, samples with known dimensions were immersed in PBS, and then the thickness and surface area of the hydrated samples were measured in each hour. The thickness and surface area of the samples were measured by micrometer caliper and ruler, respectively. Variations of thickness and surface area are calculated by the following equations:

$$\text{Thickness increase} = H_t/H_0; \quad (1)$$

$$\text{Surface area increase} = S_t/S_0; \quad (2)$$

Here, H_t and S_t are the thickness and surface area of the wet samples at target times, respectively. H_0 and S_0 are the initial thickness and surface area of the dry membranes, respectively. The values are expressed as the mean ± standard error (n = 10).

2.5. Evaluation of permeability

Nutrients for the survival of cornea cells are mainly depending on the aqueous humor and to a lesser extent from the limbal vasculature