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The preparation, characterization and evaluation of regenerated cellulose/collagen composite hydrogel films

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ABSTRACT

Porous structured regenerated cellulose films were oxidized by periodate oxidation to obtain 2,3-dialdehyde cellulose (DARC) films, which were then reacted with collagen to obtain DARC/Col composite films. The subsequent FT-IR spectra indicated that collagen was immobilized on the DARC matrix via the Schiff base reaction between $-NH_2$ in collagen and $-CHO$ in DARC backbone. Scanning electron microscopy revealed that DARC/Col exhibited a refined 3D network structure and its porosity and pore size decreased with increasing of collagen concentration. The composite films demonstrated a good equilibrium-swelling ratio, air permeability and water retention properties. The composite films also showed excellent mechanical properties, which was vital for practical application. Finally, the cytotoxicity of the composite film was evaluated using NIH3T3 mice fibroblast cells, the results revealed that DARC/Col composite films have good biocompatibility for use as scaffold material in tissue engineering.

1. Introduction

Recent advancements in tissue engineering have led to increased focus on the design and preparation of suitable polymer scaffolds capable of mimicking natural tissue (Palsson & Bhatia, 2004). In natural tissue, extracellular matrix (ECM) components, such as collagen, hyaluronic acid (HA) and fibronectin (FN), are important regulators for cellular growth, proliferation and differentiation in addition to key contributors in the tissue repair and regeneration processes (Adams & Watt, 1993; Clark, 1993). Polymeric scaffolds based on ECM components could replace corrupted or failing ECM in tissue to effectively promote rapid tissue healing and regeneration (Mostow et al., 2005; Vazquez et al., 2003). Collagen, as one of the major components of ECM, exhibits many attractive properties, including low immunogenicity, excellent biocompatibility and biodegradability, enhanced tissue regeneration, and promoted cellular adhesion, growth and proliferation (Lee, Singla, & Lee, 2001; Maeda et al., 1999; Seal, Otero, & Panitch, 2001). It is thus a strong candidate for use in tissue engineering scaffolds. However, when compared with ideal tissue scaffolds, collagen scaffold lacks mechanical properties and shows rapid degradation *in vivo* (Gleeson & O'Brien, 2011). In addressing these issues, extensive research has focused on mixtures of collagen with other biological materials for a composite with improved mechanical properties and stability. For examples, Salome Machado, Martins, and Plepis (2002) and Lu, Feng, Hu, et al. (2008) prepared collagen–chitosan and Collagen–fibroin composite scaffolds via blending and crosslinking; whereas Fischer et al. (2012) prepared collagen/hyaluronic acid porous network scaffold via electrospinning.

Cellulose is a large, linear-chain polymer with an abundance of hydroxyl groups, good biocompatibility and unique physicochemical properties, such as high crystallinity, high water-retention capacity, use of an ultrafine nano-fiber network, high tensile resistance and elasticity modulus. In addition, the fiber structure of cellulose hydrogel is similar to the collagenous fibers found in natural tissue. Cellulose hydrogel is thus considered a promising candidate for collagen-mimicking (Bäckdahl et al., 2008) and for application as a substrate in tissue engineering (Tan, Hong, & Shao, 2007). For tissue repair and regeneration, cellulose has high mechanical strength and good permeability for liquids, gases and electrolytes in a humid environment. It is effective in preventing the accumulation of bacteria and thus avoiding the infection of injured tissue (Gu, Xu, & Hou, 2005). However, cellulose is a polysaccharide and has lower bioactivity and chemical activity compared with proteins such as collagen that show effective cellular growth and proliferation as a result of cell surface receptors.

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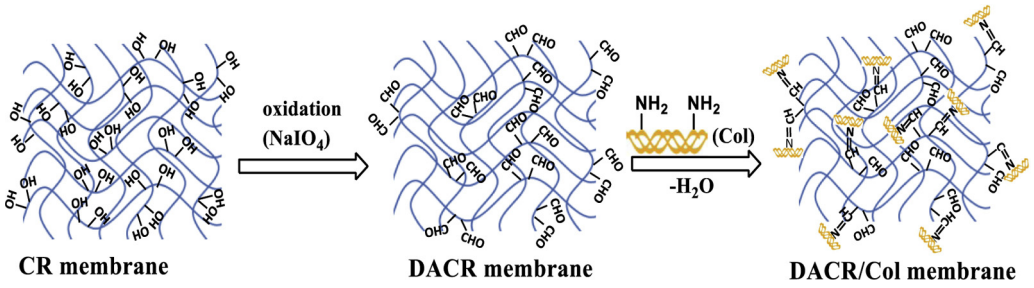

Fig. 1. The schematic diagram of the preparation of DARC/Col composite film.

Therefore, the fixation of proteins or other active factors onto the surfaces of cellulose nano-fibrils could improve the biocompatibility and cytocompatibility of regenerated cellulose film, while preserving the mechanical properties of the scaffold materials (Lin et al., 2011). It is desirable to utilize a scaffold material that has the porosity necessary to support cell in growth and effective mass transport while also supplementing the mechanical properties of engineered tissue (Svenssona et al., 2005). Recently, many studies concerning cellulose/collagen composite materials were documented. They prepared cellulose/collagen composite film either by physical absorption method (Cai & Yang, 2011) or blending and crosslinking method (Li, Guo, & Lan, 2013; Steele, Huang, & Nguyen, 2013). However, physical absorption method can only immobilize tiny amount of collagen and lacks of stability; while blending and crosslinking method may destroy the triple helical conformation and degrade collagen chain, thus lose its bioactivity. Moreover, blending and crosslinking usually needs chemical crosslinking agents, such as glutaraldehyde, which are cytotoxic, and not suitable for *in vivo* application. In our current study, collagen was immobilized on cellulose film without conformation change and degradation, and no crosslinking agent was used. The effectiveness of periodate oxidized regenerated cellulose films for the stabilization of collagen was explored via the Schiff base reaction between —NH_2 in collagen and —CHO in DARC backbone (see Fig. 1). The composite material showed great potential for use as a tissue engineering scaffold due to its high strength in wet state, malleability in situ, good equilibrium-swelling ratio, air permeability and biocompatibility. To the best of our knowledge, this is the first report of self-crosslinked regenerated cellulose–collagen biocomposites as scaffolds for tissue regeneration. The effects of periodates oxidization on regenerated cellulose films were examined in relation to the amount of collagen fixation, swelling behavior, moisture-penetrability and water-retention abilities. The composite materials were also examined for their mechanical properties, microstructure and cell–material interactions to evaluate the potential of the matrix as a scaffold for tissue engineering application.

2. Experimental

2.1. Materials

Native cellulose (the cotton linter pulp) with a viscosity-average molecular weight (M_η) of 1.07×10^5 in cadoxen at 25 °C was supplied by Hubei Chemical Fiber Co. Ltd. (Xiangyang, China). Type I Collagen from bovine tendon with a molecular weight of 298 kDa was supplied by Wuxi Beidi Biological engineering Co. Ltd. (Wuxi, China). Other chemical reagents of analytical grade were supplied by the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used without further purification.

2.2. Preparation of regenerated cellulose (RC) hydrogel films

The freezing–thawing method was used for dissolving cellulose (Zhang, Ruan, & Gao, 2002). Briefly, native cellulose was dispersed into an aqueous lithium hydroxide/urea solution (4.6 wt%/15.0 wt%) and then placed in a freezer. After freezing, the solution was taken out and thawed at room temperature to obtain a transparent cellulose solution (5 wt%). The obtained cellulose solution was subjected to centrifugation at 7500 rpm and 15 °C for 10 min to eliminate the bubbles in the viscous solution. The bubble-free solution was then cast on a glass plate. The thickness of the solution was controlled at about 1 mm. It was then immersed into a coagulation bath containing 80 vol% ethanol where it coagulated and regenerated for 10 min. The RC hydrogel films were then washed and kept in deionized water until use.

2.3. Preparation of DARC and DARC/collagen hydrogel films

Cellulose hydrogel films were immersed into a 200 mL sodium periodate solution (3%, w/v, 0.14 mol/L) and kept at 37 °C for various time intervals under dark conditions. After the reaction, excess IO_4^- was removed using ethylene glycol and repeated rinsing with distilled water. The yielded wet DARC hydrogel films (oxidized for 2 h) were then reacted with collagen for 24 h at room temperature to prepare DARC/Col hydrogel films. After the reaction, the samples were washed multiple times with distilled water to remove excess collagen. DARC and DARC/Col hydrogel films were then obtained through a freeze drying process.

2.4. Dialdehyde content of DARC hydrogel films

The dialdehyde content of each sample was determined by the Schiff base reaction between aldehyde groups and hydroxylamine hydrochloride (Rosenau et al., 2001). The dialdehyde content (DC) of each sample was calculated through Eq. (1) by Meng, Feng, Liang, et al. (2005).

$$\mathrm{DC}(\%) = \frac{161(V_2 - V_1)}{C_{(\mathrm{HCl})} \times 10^{-3}} \times 100 \quad (1)$$

where V_1 is the amount of hydrochloric acid for titration in mL, V_2 is the amount of hydrochloric acid for control titration in mL, $C_{(\mathrm{HCl})}$ is the concentration of hydrochloric acid in mol/L, m is the weight of each sample, 161 is the average molecular weight when glucose units were translated into 50% dialdehyde.

2.5. Characterization of DARC and DARC/collagen hydrogel films

Fourier-transform infrared spectroscopies (FT-IR) of the samples were recorded with FT-IR spectroscopy (FT-IR 615, Japan). The samples were ground into powders, mixed with KBr and pressed to form a sample disk for FT-IR measurement.