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# Preparation and characterization of a novel tobramycin-containing antibacterial collagen film for corneal tissue engineering

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ABSTRACT

Corneal disease is a major cause of blindness and keratoplasty is an effective treatment method. However, clinical treatment is limited due to a severe shortage of high-quality allogeneic corneal tissues and the bacterial infection after corneal transplantation. In this study, we develop a novel artificial and antibacterial collagen film (called Col-Tob) for corneal repair. In the Col-Tob film, the tobramycin, which is an aminoglycoside antibiotic to treat various types of bacterial infections, was cross-linked by 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide and N-hydroxysuccinimide onto the collagen. Physical properties, antibacterial property and biocompatibility of the films were characterized. The results indicate that the film is basically transparent and has appropriate mechanical properties. Cell experiments show that human corneal epithelial cells could adhere to and proliferate well on the film. Most importantly, the film exhibits excellent antibacterial effect in vitro. Lamellar keratoplasty shows that the Col-Tob film can be sutured in rabbit eyes and are epithelialized completely in15 ± 5 days, and their transparency is restored quickly in the first month. Corneal rejection reaction, neovascularization and keratoconus are not observed within 3 months. This film, which can be prepared in large quantities and at low cost, should have potential application in corneal repair.

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## 1. Introduction

Corneal disease is a major cause of blindness, and keratoplasty is considered to be an effective method for visual rehabilitation of patients with corneal blindness. However, its clinical application is limited due to a severe shortage of high-quality allogeneic corneal tissues [1]. Therefore, various efforts have been made to develop corneal tissue substitutes by using natural biological materials [2–6]. A tissue-engineered cornea should be biocompatible and transparent, and have appropriate mechanical properties. Collagen, the main load-bearing component in connective tissues, has been extensively studied as a scaffold material for tissue engineering corneas [7–11]. As reported, after implantation, the collagen scaffolds could replace the pathological corneal tissue in animals [12] or humans [13].

Although the collagen scaffold has many advantages for replacing pathological corneal tissue, its application is still limited by bacterial infection after keratoplasty [14–16]. Dropping antibiotics onto the wound in the first week is an efficient method to solve this problem, but it is difficult to fix the correct amount of the antibiotic, which could cause some of the drug to be wasted. In addition, dropping is also frequently troublesome. Drug carriers have been used for many years to encapsulate antibacterial agents for local administration. There are many such drug carriers, including calcium sulphate void filler and poly(lactic-co-glycolic acid) microspheres [17–21]. However, drug-loading in these physical delivery systems is always achieved by adsorption and encapsulation, which are difficult to control with regard to the drug-loading rate and drug release. As cornea is a thin, transparent and avascular tissue, these methods are not suitable for use with corneas because the presence of a physical carrier in the corneal repair materials may lead to a decrease in optical performance, mechanical properties or biocompatibility.

In this paper, we use the chemical cross-linking method to add tobramycin, a new kind of aminoglycoside antibiotic from *Micromonospora purpurea* that is effective against most species of both Gram-positive and Gram-negative aerobic bacteria [20], to collagen to prepare a novel tobramycin-containing antibacterial collagen film (Col-Tob) for corneal repair. The tobramycin was grafted onto the surface of a collagen film using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide

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(NHS) as cross-linking agents. The mechanical properties, light transmittance, antibacterial property and biological properties of the cross-linked film were characterized.

## 2. Materials and methods

### 2.1. Materials

Tobramycin (Tob) was purchased from Shandong Freda Biopharm Co., Ltd., China. Type I collagen (HM Biotech Ltd., Guangzhou, China) was extracted from bovine tendon. EDC and NHS were supplied by GL Biochem Ltd. (Shanghai, China). Phosphate-buffered saline (PBS) was prepared from tablet form (Calbiochem Corp, Germany). All cell-culture-related reagents were purchased from Sigma Chemical (St. Louis, MO, USA). Deionized water was obtained from a water purification system (Millipore S.A.S., France). New Zealand white rabbits of either gender (12 weeks old and 2.5–3 kg) were used as animal transplant recipients.

### 2.2. Preparation of films

Collagen (6.5 mg $ml^{-1}$) was dissolved in 0.01 mol $l^{-1}$ HCl solution and tobramycin was dissolved in deionized water with the concentration of 15 mg $ml^{-1}$. Then the collagen solution was dispensed into a specific mold and dried to form a cornea-shaped film. The collagen film was immersed in 15 ml of tobramycin solution, and EDC (the cross-linking agent) and NHS (the catalyst) were added to the solution to form a mixture with a mass ratio of EDC:NHS:Col-Tob = 1:1:6. The cross-linking was carried out by stirring the solution for several hours. After that, the Col-Tob film was rinsed three times with deionized water and dried again. The loading mass of tobramycin was determined by subtracting the mass of the residues in washing liquid. Cross-linked collagen film without tobramycin was also prepared as the control group. Scheme 1 displays the chemical reaction mechanism between Col and Tob.

### 2.3. X-ray photoelectron spectroscopy (XPS)

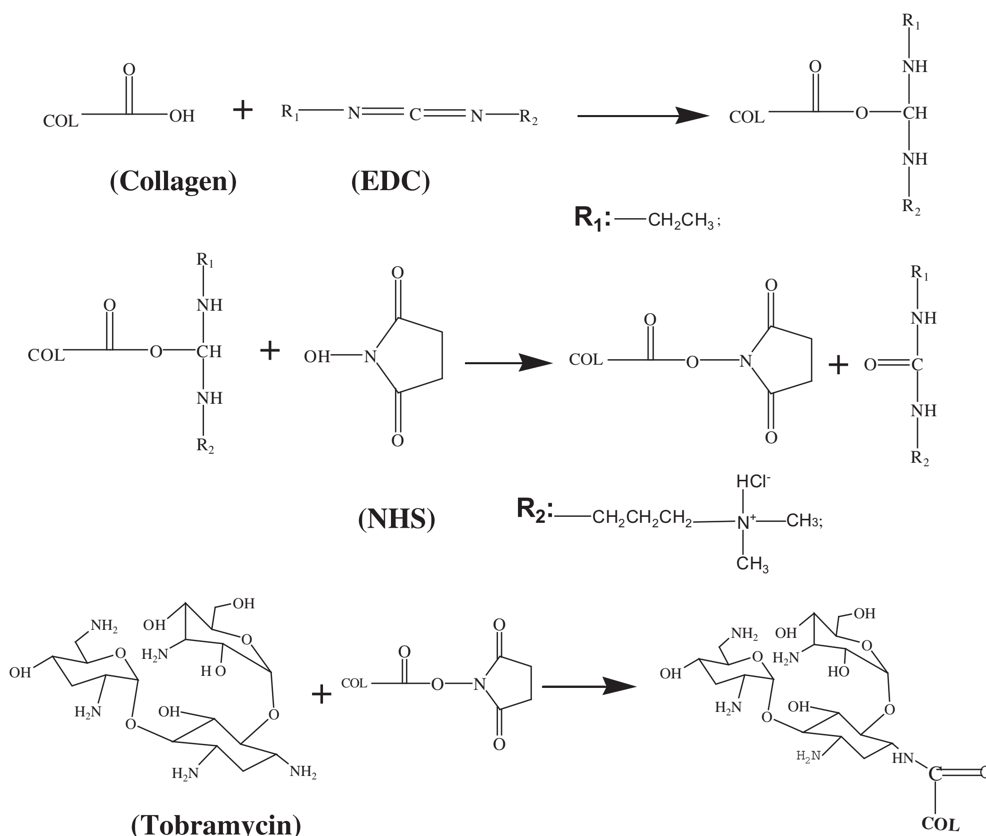
A Kratos Analytical (UK) model Axis Ultra X-ray spectrometer system was used with a single Al $K_\alpha$ X-ray source ($h\nu$ = 1486.6 eV, 150 W). The binding energy was calibrated by C1*s* of C–C as 284.6 eV. The wide scanning was operated with a pass energy of 160 eV at a scan rate of 1 eV per step over a range of 1105 eV, while high-resolution region scans were gathered with a pass energy of 40 eV and a step size of 0.1 eV. Elemental analysis and quantification spectra from the individual peaks were obtained with 40 eV pass energy. A Gaussian function was assumed for the curve-fitting process.

### 2.4. Fourier transform infrared (FTIR) spectroscopy

The infrared structure of the films was analysed using Fourier transform infrared-attenuated total reflectance (FTIR-ATR; Vector 33, Bruker, Germany). Before acquiring the FTIR spectrum of a sample, a background spectrum was collected. All the spectra were obtained from 3800 to 500 $cm^{-1}$.

### 2.5. Swelling test

Water absorption of Col and Col-Tob was measured by swelling them in PBS (pH 7.4) at 35 °C. After gently blotting the film surface with filter paper to remove the absorbed water, the wet weight of the samples was immediately weighed. Films with known




**Scheme 1.** The chemical reaction mechanism between collagen and tobramycin.

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