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Synthesis and properties of regenerated cellulose-based hydrogels with high strength and transparency for potential use as an ocular bandage

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ABSTRACT

Cellulose is a biologically derived material with excellent wound-healing properties. The high strength of cellulose fibers and the ability to synthesize gels with high optical transparency make these materials suitable for ocular applications. In this study, cellulose materials derived from wood pulp, cotton, and bacterial sources were dissolved in lithium chloride/*N*,*N*-dimethylacetamide to form regenerated cellulose hydrogels. Material properties of the resulting hydrogels, including water content, optical transparency, and tensile and tear strengths, were evaluated. Synthesis parameters, including activation time, dissolution time, relative humidity, and cellulose concentration, were found to impact the material properties of the resulting hydrogels. Overnight activation time improves the optical transparency of the hydrogels from 77% to 97% at 550 nm, whereas controlling cellulose concentration improves their tear strength by as much as 200%. On the basis of the measured transmittance and strength values of the regenerated hydrogels prepared via the optimized synthesis parameters, Avicel PH 101, Sigma-Aldrich microcrystalline cellulose 435236, and bacterial cellulose types were prioritized for future biocompatibility testing and potential clinical investigation.

1. Introduction

Ocular injury is recognized to be highly prevalent in the battlefield environment and is associated with significant pain, visual disability, decreased personnel availability, and excessive cost [1–4]. Most battlefield injuries to the ocular surface are caused by particulate matter, such as sand or dirt that is generated by explosions, vehicles, or ordinance. Such injuries are very common and often cause severe pain and impaired vision that may last several days. Although the majority of injuries that affect only the ocular surface will eventually heal without causing permanent loss of vision, there is a significant risk of secondary infection and corneal scarring. In the civilian setting, when a corneal abrasion occurs, the patient is often treated with antibiotic ointment and an eye patch. However, if there is significant pain or the need to preserve the ability to work or drive, the patient can be seen emergently by an ophthalmologist or optometrist for the fitting of a soft contact lens over the injured eye. In this scenario, the contact lens acts like a bandage, protecting the cornea while reducing pain and permitting vision. Because such treatment is not feasible in a battlefield context, there is a significant need for a battlefield "ocular bandage" that could accomplish the same therapeutic goals.

Ideally, an ocular bandage should have material properties similar to those of contact lenses while also promoting wound healing and providing sustained delivery of pharmaceuticals. Additionally, the design and materials should be suitable for field application by medical corpsmen. Typical contact lenses are made of synthetic gels based on silicone or poly(2-hydroxyethyl methacrylate). These contact lenses have high optical transparency, 96–99% at 550 nm [5], tensile strengths ranging from 120 to 575 kPa, percent elongations ranging from 18 to 81%, and Young's moduli ranging from 364 kPa to 1.6 MPa [6]. Although these contact lens materials meet the requirements for their intended application, they do not offer many benefits for wound healing. As such, it is desirable to develop an ocular bandage from a natural source with similar material properties while also promoting wound healing.

Cellulose-based natural polymers offer several advantageous characteristics for biomedical applications, including hydrophilicity [7], nontoxicity [8], and low immunogenicity [9]. In addition, the matrix structure of the cellulose fibers provides a scaffold capable of supporting cell growth [9,10]. As the most abundant natural polymer [7], cellulose materials are also of interest from the perspective of "green" engineering and natural solutions, particularly for use within the human body. Challenges arise when designing cellulose materials because they are polymorphic polymers, i.e., their molecular weight, crystal structure, and percent crystallinity can vary greatly depending on the cellulose source and the way the cellulose was processed. All of these factors substantially

Abbreviations: BC, bacterial (microbial) cellulose; DMAc, *N*,*N*-dimethylacetamide; DSC, differential scanning calorimeter; LiCl, lithium chloride; MCC, microcrystalline cellulose; RH, relative humidity; SEM, scanning electron microscope; TGA, thermal gravimetric analysis; UTS, ultimate tensile strength; XRD, X-ray diffraction.

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impact the properties of the material. As such, it is necessary to determine which type of cellulose is the most suitable for preparing hydrogels for an ocular bandage application.

Bacterial (microbial) cellulose (BC) is chemically identical to cellulose found in plants (i.e., wood pulp and cotton linters) but has a unique three-dimensional fibrillar interconnected structure that imparts its nanoscale fibers with excellent mechanical properties; these properties include a Young's modulus of 138 GPa [11] and a tensile strength up to 2 GPa [12]. The BC fibers have also shown promise as optically transparent composite materials. Plant-based cellulose fiber diameters are approximately two orders of magnitude larger, resulting in lower mechanical strengths and less water-holding capability [13]; however, these materials still hold promise for ocular applications in hydrogel form. Saito et al. [14] have shown that pulp-based cellulose can be synthesized in a physically cross-linked hydrogel form that exhibits a tensile strength of 13.7 MPa and maximum transmittance of 88% at 550 nm; these values are in the range required for an ocular bandage.

Celluloses, particularly bacterial (sometimes referred to as microbial) and plant-based (e.g., wood pulp and cotton), have been widely studied in recent years because of their wound-healing properties, promise as tissue engineering scaffolds, and excellent mechanical and optical properties [7–9,15]. Biocompatibility and wound-healing enhancement are critically important considerations when selecting materials for an ocular bandage. Hydrogels composed of plant-based cellulose materials have been shown to be biocompatible in various in vivo applications [16–18]. BC has been shown to be both biocompatible and biodegradable (biosorbable) in vivo [19–22]. The most prevalent medical applications for BC have been in dressings for burn wounds or for chronic ulcers [20]. BC conforms to the external wounds, maintains a moist environment, reduces pain, accelerates re-epithelialization and the formation of granulation tissue, and does not stick to the burn or ulcer wound [20]. Additionally, BC is unique as a wound dressing material in that it can effectively absorb exudate and hydrate dry areas of a wound, in comparison with other dressings that have only a single function [23]. Adequate moisture on the ocular surface is also critical for conjunctival and corneal wound healing, suggesting that cellulose materials may hold promise for ocular bandage applications.

The objective of this work was to characterize the material properties of hydrogels synthesized from different cellulose sources (wood pulp, cotton, and bacterial). Based on requirements for high optical transparency and sufficient tensile and tear strengths, recommendations are made for the most appropriate cellulose source and synthesis method for preparation of a material suitable for use on the ocular surface. Biocompatibility testing will be the subject of future testing on down-selected materials.

2. Material and methods

2.1. Cellulose starting materials

Cellulose materials derived from wood pulp, cotton, and bacterial sources were investigated with the goal of comparing the structure and properties of resultant hydrogels. Table 1 lists the cellulose varieties under investigation along with each material's source information and approximate particle size (according to the manufacturer). The Avicel and Sigma-Aldrich microcrystalline cellulose (MCC) starting materials were commercially obtained, whereas the BC was grown according to the procedure described by Hestrin and Schramm [24]. Although the cellulose materials have the same molecular formula, they have different properties and characteristics based on their origin. Fig. 1 shows the scanning electron microscope (SEM) micrographs of three cellulose starting materials, which clearly range in size around the particle sizes provided by the manufacturers. Using the micrographs to compare the bulk differences in the as-received starting materials, the Avicel and MCC cellulose materials can be described as microscale particulates, whereas the BC is composed of nanoscale-diameter fibers.

2.2. Hydrogel synthesis

To prepare the hydrogels, 2–5 g of Avicel or MCC cellulose powder (denoted as 2, 3, 4, 5 wt/vol.% hereafter) was activated in 100 ml *N*, *N*-dimethylacetamide (DMAc) with stirring at 350 rpm for 24 h. Next, dissolution was initiated by adding 8 g of lithium chloride (LiCl) with continued stirring at 350 rpm and heating to 95 °C until the solution became clear. The resulting solution was poured onto glass plates or into silicone molds designed for various test geometries. These were then placed in a Tenney VersaTenn III Environmental Test Chamber at 35 °C and 73% relative humidity (RH) and left overnight to "gel." The gelled samples were gently washed in water until the excess LiCl/DMAc was removed and then stored in water before testing. Removal of the Li^+ and DMAc was verified by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Varian Vista-PRO) and Fourier transform infrared (FTIR) spectroscopy (PerkinElmer Spectrum 100), respectively. These synthesis conditions were arrived at after systematic study of the effect of each parameter, as is described in Section 3.1.

For BC, a 50 × 50 mm square was cut from the as-grown pellicle [24] and dried by pressing at 45 °C under 14 MPa of pressure for 5 min using a Carver Press (Hydraulic Unit Model #3912), followed by an overnight dry in a 90 °C convection oven. Five hundred milligrams of the resulting dry sheet was placed in 100 ml of 8% LiCl/DMAc solution and stirred at 500 rpm until the cellulose dissolved (up to 10 days). The resulting BC/LiCl/DMAc solution was then poured into silicone molds for gelation into test specimens, as described above for the plant-based cellulose varieties.

2.3. Thermal analysis: differential scanning calorimetry and thermal gravimetric analysis

To ensure that all materials would be stable for long durations at body temperature, the thermal stability of the hydrogels was studied using a Mettler-Toledo differential scanning calorimeter (DSC 1) under N_2 (99.99%) purge at 50 ml/min. Specimens with a mass of approximately 20 mg were used. Specimens were then hermetically sealed inside 40-μl aluminum pans to prevent moisture loss during the DSC scan. During the DSC scan, the samples were first cooled from room temperature to −20 °C, followed by ramping up to 200 °C at 20 °C/min. Denaturation peak temperature was analyzed by the STAR^e software.

Table 1
Source, natural origin, and dimensions of as-received cellulose powder or fiber starting materials.

	Avicel PH 101	Avicel PH 102	Avicel PH 105	Avicel PH 200	MCC 3	MCC 4	BC
Cellulose source	FMC biopolymer	FMC biopolymer	FMC biopolymer	FMC biopolymer	Sigma-Aldrich (310697)	Sigma-Aldrich (435236)	ATCC 23769 (*Gluconacetobacter hansenii*)
Origin	Wood pulp	Wood pulp	Wood pulp	Wood pulp	Cotton linters	Cotton linters	*Acetobacter xylinus*
Approximate particle/fiber size	50 μm	90 μm	20 μm	180 μm	20 μm	100 μm	20 nm × 50 nm × 100 μm fiber