Material Property Characterization of a Novel Hydrogel for Corneal Applications

Pravin Pattekari[1], Huiguang Zhu[4], Jean T. Jacob[5] and P. Sidney Sit[1,2,3]

[1]Molecular Science and Nanotechnology, [2]Biomedical Engineering, and [3]Institute for Micromanufacturing

Louisiana Tech University, Ruston, LA 71272

[4]Chemistry, Rice University, Houston, TX 77005

[5]Ophthalmology and Neuroscience, Louisiana State University Health Science Center, New Orleans, LA 70112

Introduction: Treatment for various corneal diseases such as Stevens-Johnson syndrome requires full thickness corneal replacement. However, there is limited success in the use of synthetic materials for corneal augmentation or replacement. By integrating novel chemistry and cell biology methods, a novel synthetic methacrylate-containing hydrogel scaffold material has been investigated for corneal applications. The hydrogel is based on poly(allyamine) (PAH)-glycidyl methacrylate (GMA), using poly(ethylene glycol) (PEG) as a crosslinker. Here, we present results on characterizing the material properties of the PAH/GMA hydrogel, in terms of pore size, morphology, optical clarity, surface wettability, swelling ratio, degradation potential, viscoelasticity and cellular biocompatibility.

Materials and Methods: All reagents were purchased from Sigma-Aldrich except stated otherwise. PAH, dissolved in water, was allowed to react with GMA at a ratio of 0.6 g PAH/ml of GMA. The PAH-GMA macromer, separated out using n-hexane, was then mixed with PEG diacrylate (PEGDA; 12.5% v/v) as a crosslinker and Irgacure 184 (8 mg/ml; Ciba Specialty Chemicals) as a photoinitiator. The mixture was irradiated with ultraviolet light at 254 nm for 3 min.

The material properties of the hydrogel were examined using various analytical techniques: 1. Contact angle goniometry operated at sessile drop method (OCA 15+, Dataphysics) was performed to measure surface wettability of the hydrogel; 2. Degradation potential based on the physical appearance of the hydrogel after soaking it for over one week in deionized water and phosphate buffer saline (PBS); 3. Gravimetric method, in which dry weight and wet weight of the hydrogel in deionized water, were recorded to determine the swelling ratio; 4. Scanning electron microscopy was used to study the morphology of the hydrogel; 5. Mercury intrusion porosimetry (MIP; AutoPore IV, Micromeritics) was used to determine the distribution of the pore size and specific surface area of the pore; 6. Viscoelastic measurements of the hydrogel were performed on a rheometer (C-VOR, Bohlin Instruments) equipped with parallel plate geometry of 20 mm in diameter. The rheological experiment was operated at amplitude sweep mode under controlled stress. A stress sweep was performed to assess the linear viscoelastic region (LVR). A controlled stress condition was carried out on the hydrogel between 0.1 Pa and 100 Pa. The viscoelastic properties of the hydrogel were expressed in complex modulus (G*), which is a function of the elastic modulus (G') and the viscous modulus (G") of the material; 7. Cellular biocompatibility was examined based on the support of the hydrogel to the growth of primary rabbit corneal epithelial cells.

Results and Discussion: The PAH/GMA hydrogel was highly transparent, a desirable property for corneal implant. It is revealed that a long UV exposure time would increase the opacity of the hydrogel. This is likely due to the increase in the number of crosslinks that compromises the clarity. An image was captured using a high speed video system with built-in charge-coupled device to determine the water contact angle. The measured contact angle was determined to be ~30°, suggesting that the hydrogel surface was primarily hydrophilic. The water droplet was absorbed in a less than a minute, which is likely due to the presence of hydrophilic domain (PEG) of the hydrogel, which makes it suitable for nutrient exchange to support cell proliferation in the hydrogel. The PAH/GMA hydrogel was stable in deionized water and PBS for over one week without any visible change of the hydrogel structure. The swelling ratio was determined to be about 500-600%. SEM images (Figure 1) did not show particular micrometer-sized pores. Results from MIP revealed that mean pore size of the hydrogel was 43 ± 17.54 nm (number of samples = 5). LVR showed elastic and viscous moduli were about 600 Pa and 80 Pa, respectively. The results indicate that the hydrogel is predominantly elastic in nature. When cultured on the hydrogel, primary epithelial cells from rabbit cornea showed increased adhesion and growth on intermediate level, but not on elevated and low level of PAH.

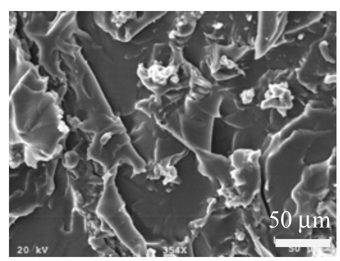

Figure 1. Scanning electron microscopic image of PAH-GMA hydrogel.

Conclusions: The results suggest that the concentrations of hydrogel components (PAH, GMA and PEG crosslinker) and UV exposure time can be varied to obtain variable material properties such that cellular responses can be optimized. The results suggest that the PAH/GMA hydrogel possesses materials properties (physiochemical, mechanical, optical, biological stability and cellular biocompatibility) that potentially are well-suited for corneal applications.

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