Optimized Properties of Collagen Vitrigel Membranes for Ocular Repair and Regeneration Applications

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Statement of Purpose: The frequency of ocular injuries on the battlefield has been steadily increasing during recent conflicts. Such injuries are difficult to treat and solutions requiring donor tissue are not ideal and are often not readily available. Collagen vitrigels (CVs) have been developed for corneal reconstruction[1]. Important physical properties of CV, such as customizable dimensions and composition, and ease of manipulation make CV a feasible candidate for corneal tissue engineering, for both battlefield and clinical applications. However, improvements in properties such as transparency and strength are highly desirable for this application. In this study, by systematically varying vitrification temperature, relative humidity and time, the CV synthesis conditions were optimized to yield the best combination of high transparency and high mechanical strength.

Methods: CV preparation consists of three main stages: gelation, vitrification, and rehydration[2]. (1) Equal volumes of culture medium (Fetal Bovine Serum, 20 mM N-2-hydroxyethylpiperadine-N'-2-ethansulfonic (HEPES) acid buffer in Dulbecco's Modified Eagle Medium (DMEM) and 0.5% acid collagen solution are uniformly mixed. Gelation is initiated via incubation at 37 ˚C. (2) During vitrification, time, temperature, and humidity are controlled. (3) Following vitrification, the material is rehydrated with Phosphate Buffered Saline. Design of Experiments (DOE) was utilized to systematically vary the synthesis parameters (vitrification temperature (5, 10 and 40 °C), relative humidity (RH) (20, 40 and 60 %), and vitrification time (0.5, 1, 2 and 5 weeks)) and to understand the relationship with the resulting properties. The CV properties that arise from varying vitrification conditions will be compared to the original Takezawa et al. recipe which was developed for non-ocular applications. The characterization includes measurements of transmittance, ultimate tensile strength, denaturing temperatures and morphology.

Results: Varying the synthesis parameters facilitates control of the transparency and mechanical strength of the vitrigel by tailoring the underlying material structure (Figure 1). These structural changes include an increase in the collagen fiber diameter and density as temperature and time increase. CVs with transparency up to 85%, tensile strength up to 12 MPa, and denaturing temperatures that exceed the eye/body temperature have been synthesized at 40˚C, 40% RH for one week.

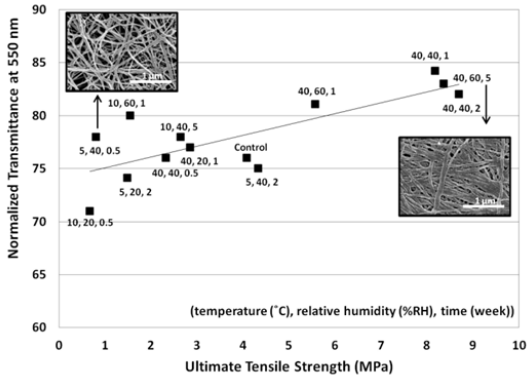

Figure 1: Normalized transmittance percentage as a function of ultimate tensile strength for different vitrification conditions.

Conclusions: The roles of the vitrification temperature, time and relative humidity in CV synthesis were investigated using a systematic DOE approach[3]. Altering the synthesis parameters facilitated control and custom tailoring of the structure and therefore the transmittance, mechanical properties and thermal stability of the biomaterial. The DOE analysis showed that the most influential parameters for the transmittance and tensile strength are time and the interaction between time and temperature. In addition, the systematic DOE and analysis performed in this work enabled improvements of 113% in tensile strength and 11% in transmittance, compared to the previously developed CVs, and a 3-week reduction in synthesis time, rendering the vitrigels a more practical solution.

References:

1. McIntosh Ambrose et al. J Biomed Mater Res B Appl Biomater. 2009;90B:818-31.
2. Takezawa T, et al. Cell Transplant. 2004;13:463-73.
3. X. Calderón-Colón et al. Biomaterials 2012; 33: 8286-8295.

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