CHARACTERIZATION OF CROSSLINKED ARTIFICIAL PROTEIN FILMS

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Abstract

Genetically engineered artificial proteins are promising candidates for new biomaterials because their amino acid sequences can be precisely controlled. This work describes the characterization of crosslinked films of biomimetic artificial extracellular matrix (aECM) proteins with hybrid functions designed to meet materials needs in applications such as small diameter vascular grafts and corneal tissue implants. Elastin-derived polypeptides give the proteins flexibility, while RGD and CS5 peptide domains from fibronectin serve to adhere cells.

Techniques were sought to crosslink aECM proteins in ways that resulted in tunable mechanical properties. Hexamethylene diisocyanate was used to crosslink aECM proteins into uniform, transparent, highly-extensible hydrogel films with low water contents characteristic of native elastin. Their elastic moduli, 0.1 – 1.1 MPa, depended on crosslinker concentration and aECM protein length, and spanned the observed range of elastin fibers.

The suitability of biomaterials implants depends strongly on their susceptibility to proteolytic degradation *in vivo*. It was shown that small sequence changes in the elastin-like portion of aECM proteins were sufficient to decrease their rate of degradation by elastase sevenfold, illustrating a simple method to tune the protease sensitivity of designed proteins. The effects were seen in both soluble proteins and crosslinked films analyzed by measuring their decrease in elastic modulus during degradation.

An aECM protein was examined for its effectiveness as a corneal onlay, or permanent contact lens. The protein was crosslinked into transparent, elastic, water-rich lenses and was implanted into rabbit corneas. The onlays were stable and well-tolerated, and full re-epithelialization occurred within 4-7 days. Histological examination revealed normal regenerating epithelial cell morphology on the anterior surface, good interfaces between the onlay and surrounding tissue, and only minimal inflammation.

To create substrates for studying the coordinating effects of mechanical and biological signals on cell behavior, thin films were made from a photoreactive aECM protein containing the non-canonical amino acid *para*-azidophenylalanine. Atomic force microscopy (AFM) nanoindentation was used to calculate elastic modulus, and the technique was confirmed by bulk tensile measurements and finite element simulations. Film modulus could be tuned either by differential irradiation or variable incorporation of *para*-azidophenylalanine.

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