Abstract

Biomaterials play critical roles in tissue engineering, reconstructive surgery, and regenerative medicine. Constructing biomaterials through protein engineering offers a unique and powerful solution to the challenges posed by the creation of well-defined, multi-functional materials that must provide structural support, as well as guide cell and tissue behavior. This thesis describes the construction, characterization, and application of protein-based biomaterials composed of photoreactive artificial extracellular matrix (aECM) proteins. These proteins consist of mechanical and biological motifs derived from natural proteins: an elastin-like backbone containing a photocrosslinking site dictates the mechanical properties of the material, and an RGD binding domain from fibronectin mediates cell attachment. aECM proteins were assembled at the genetic level and expressed in a bacterial host. Manipulation of an aminoacyl-tRNA synthetase of the expression strain permitted the incorporation of the photoreactive noncanonical amino acid, *para*-azidophenylalanine (*p*N_3Phe), within the elastin subunit of the protein.

An evaluation of the mechanical and biological properties of photoreactive aECM proteins has been performed. Nanoindentation experiments using atomic force microscopy (AFM) demonstrated that the elastic moduli of photocrosslinked protein films lie within the range of native elastins and that the mechanical properties of films can be tuned by altering the incorporation of *p*N_3Phe in the protein or adjusting the irradiation dosage. Cell spreading experiments showed that cell attachment to protein films is sequence-specific to the presence of the RGD cell-binding domain. Patterning of protein films using standard photolithography methods allowed for the generation of cellular arrays.

A molecular characterization of cellular response to engineered aECM proteins has been initiated using mRNA microarrays and the proteomic technique, BONCAT (bio-orthogonal noncanonical amino acid tagging). These methods provide information regarding changes in the cellular expression of mRNA transcripts and proteins in response to the biochemical composition of protein-based materials.

Cell-mediated deformation of the extracellular matrix in all three spatial dimensions was tracked and quantified using a newly developed method that combines time-lapse laser scanning confocal imaging and digital volume correlation. Analysis of the displacement profiles of migrating cells illustrates that, in addition to in-plane (x,y) forces, cells also exert significant normal (z) forces. A new "push-pull" aspect of cell migration was detected in the normal displacement profiles of monitored cells suggesting that cells simultaneously "push" and "pull" the underlying matrix while exploring their microenvironment.