

6 Conclusion

6.1 Summary

A set of photoreactive artificial extracellular matrix (aECM) proteins was engineered to serve as novel biomaterials for the investigation of cell-material interactions. These proteins consist of a cell-binding domain from fibronectin to promote cell attachment and elastin-like repeats for elastomeric behavior. A phenylalanine residue introduced into the elastin backbone enables the incorporation of the photoreactive non-canonical amino acid, *para*-azidophenylalanine (pN_3Phe), into proteins when expressed in a bacterial host containing a mutant phenylalanyl-tRNA synthetase [1]. Photocrosslinking of proteins into films was achieved by irradiation at 365 nm, and the mechanical properties of the protein films could be tuned by altering the irradiation dosage or the incorporation level of pN_3Phe in the proteins.

Rat-1 fibroblast cells recognize specifically the RGD cell-binding domain within the protein when deposited on adsorbed and photocrosslinked protein films. Photolithographic techniques were used to generate protein patterns to serve as a template for cell adhesion, and the resulting cell patterns were stable for several hours. An initial examination of the transcriptomic profiles of Rat-1 fibroblasts deposited on aECM proteins compared to fibronectin was achieved using mRNA microarrays. Preliminary BONCAT (bio-orthogonal noncanonical amino acid tagging) results demonstrate that proteins involved in focal adhesion formation are newly synthesized in cells when seeded on fibronectin [2, 3].

A new method for the quantification and tracking of cell-mediated displacements of the extracellular matrix was achieved through the use of time-lapse laser scanning confocal microscopy and digital volume correlation [4]. It was observed that cells explore

their extracellular microenvironment by generating displacements in all three spatial dimensions. The measured displacements were found to be independent of the elastic modulus (range: 1–10 kPa) of the underlying material. However, the direction and magnitude of the displacement components fluctuated significantly during cell migration, illustrating the dynamic nature in which the cell investigates its surroundings. Analysis of the displacement profile of migrating cells also showed that cells simultaneously “push” and “pull” the underlying matrix during locomotion.

6.2 Future Directions

The modular nature of these aECM proteins offers a facile method for the construction of novel biomaterials with customized cell-binding domains for specific biomedical applications. Currently, other aECM variants have been investigated as artificial corneal onlays [5] and for promoting neurorestoration in the brain [6]. Future approaches may include directing stem cell differentiation by incorporating epitopes from growth factors or other extracellular matrix molecules as well as targeting skin regeneration by promoting keratinocyte proliferation. Microfabrication techniques such as microstamping and microfluidics can be employed to create patterns of various aECM proteins to promote the formation of organized heterotypic cell populations and hierarchical tissue-like structures.

The characterization of cellular response to aECM proteins as described in Chapter 4 can be extended to examine the effect of how modifying the cell binding domain density (mixtures of RGD and RDG) and mechanical properties of protein films alters mRNA transcript levels and protein expression. Future work may also investigate

transcription levels and newly synthesized proteins after longer incubation periods. This analysis can be expanded to include a variety of cell types in order to test whether differences between human and murine cell lines can be detected, an important consideration in translating these materials from animal studies into the clinic. BONCAT can also be used to investigate more complex biological processes such as host-pathogen interactions as well as stem cell differentiation.

Three-dimensional traction force microscopy can be used to correlate force generation with many cellular processes including malignant transformation and cell clustering. Another application of this technology would include monitoring the changes in force generation during leukocyte extravasation [7]. During this process, cells must adhere to a surface and then migrate into a three-dimensional interstitial space. This could be modeled *in vitro* by creating a gradient of soluble factors within a three-dimensional matrix composed of collagen or fibrin. Such an experiment would offer unprecedented insights into cellular behavior by enabling dynamic tracking of the pattern of cellular force generation from two dimensions into three dimensions. This method can also be used to quantify individual nascent and maturing focal adhesions during cell spreading.

6.3 References

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