6 CONCLUSIONS AND FUTURE WORK

Protein-based biomaterials have gained considerable attention in recent years as potential candidates in various biomedical applications (*1*). Artificial proteins can be designed to present the appropriate biological and mechanical cues for directing cell behavior. For tissue repair, biomaterials are required to promote rapid cell migration and proliferation of cell sheets. In this thesis, we attempted to design artificial extracellular matrix (aECM) proteins for accelerating wound healing by incorporating relevant biological and mechanical functionalities.

We have demonstrated that human corneal epithelial cells attach and spread preferentially to aECM protein containing the RGD cell-binding domain. These surfaces also promote wound healing, in a fashion dependent on the RGD density. However, the rate of healing of epithelial cell monolayers was surprisingly not determined by the rate of cell migration. Instead, we found that the overall wound closure rates were dictated by the boundary-crossing rates; a result verified by simulation and experimental data.

In previous work from our laboratory (*2-3*) and others (*4*), cell responses on RGD surfaces were never identical to that of fibronectin. To improve the activity of aECM proteins, full length cell-binding domains were incorporated. We showed that Rat-1 fibroblasts attach and spread two fold faster on the aECM protein containing full-length fibronectin domains 9 and 10, compared to the short RGD variant. These proteins also supported rapid cell migration and proliferation, resulting in wound closure rates comparable to those observed on fibronectin. aECM proteins containing full-length fibronectin domains also promoted phosphorylation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK), accounting for the higher cell migration speeds and proliferation rates on these proteins.

Following this work, we used the aECM protein containing the fibronectin domain 10 to investigate how cells select the wound healing mechanism along the periphery of the wound. To create wounds with precise geometry, we used microfabrication to prepare micropatterned PDMS blocks to be used as barriers in the wound healing assay. MDCK wound healing on aECM surfaces exhibited unique patterns: leader cell groups are separated by constant regions of actomyosin purse string. The average spacing between consecutive leader cell groups was independent of wound size. However, this spacing was found to decrease with increasing myosin inhibition. These observations could be explained by a simple phenomenological model of force transmission. We also verified by experiments that the selection of wound mechanism along the wound edge could be controlled by wound geometry. These results were consistent with the model predictions.

Wound geometry could also be used to influence wound healing behavior of cell sheets. Using zigzag wounds, we created configurations where both lamellipodial crawling and actomyosin purse string act synergistically to accelerate the wound. In these zigzag-shaped wounds, cells located at the apex of zigzag wounds have a high propensity to develop into leader cells. At the same time, cells in concave regions are likely to undergo actomyosin contraction. Using a similar force transmission mechanism described in Chapter 4, we hypothesized that zigzag wounds with smaller angles at the apex have larger probability to develop into leader cells, and hence generate larger purse string contractions. Indeed, we observed especially strong purse string contraction in zigzag wounds with the smallest angle at the apex (i.e., $2\theta = 45°$) and these wounds healed nearly eight fold faster compared to wounds with straight edges.

Genetic engineering has demonstrated tremendous potential for creating protein-based materials for use in the body. Artificial proteins with novel mechanical and biological properties

could be fabricated to create complex 2D and 3D environments for directing cell sheet migration in tissue repair. For instance, the biological domains could also be expanded to include sequences from other types of ECM molecules or full length functional growth factors (*5*-*6*). Similarly, domains derived from other structural proteins such as silk (*7*) could be used to create materials with dramatically different mechanical properties. Since chemical and mechanical properties could also be controlled separately, artificial proteins provide a convenient system to decouple and study the effects of physical and chemical signals in collective migration. The wound healing studies could also be expanded to examine wound healing in 3D matrices, which are physiologically relevant to implantable biomaterial applications (*8*).

References

1. S. A. Maskarinec, D. A. Tirrell, *Curr. Opin. Biotechnol.* **16**, 422 (2005).
2. J. C. Liu, S. C. Heilshorn, D. A. Tirrell, *Biomacromolecules* **5**, 497 (2003).
3. J. C. Liu, D. A. Tirrell, *Biomacromolecules* **9**, 2984 (2008).
4. H. B. Streeter, D. A. Rees, *J. Cell Biol* **105**, 507 (1987).
5. J. A. Hubbell, *Swiss Med. Wkly* **136**, 387 (2006).
6. D. J. Geer, D. D. Swartz, S. T. Andreadis, *Am. J. Pathol.* **167**, 1575 (2005).
7. G. H. Altman et al., *Biomaterials* **24**, 401 (2003).
8. K. Y. Lee, D. J. Mooney, *Chem. Rev.* **101**, 1869 (2001).