WOUND HEALING ON ARTIFICIAL EXTRACELLULAR MATRIX PROTEINS

Thesis by

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To my family and friends

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Abstract

Collective cell migration is a key process in tissue repair, and in drawing parallels from complex multi-cellular events such as tumor morphogenesis and embryogenesis. Mechanisms of wound healing have been studied extensively *in vitro*. Extracellular matrix (ECM) is required to support cell migration and ensure rapid coverage of the wound area. The main challenge in designing biomaterials for tissue repair is to provide cells with the appropriate biological and mechanical cues. Hence, understanding key cell-ECM interactions during wound healing is necessary for effective biomaterial design.

Genetic engineering provides a convenient avenue to customize materials for any given application. The artificial protein-based biomaterials discussed in this work were derived from fibronectin and elastin. These proteins have a modular design, and have

material properties that can be fine-tuned according to specific applications. The artificial extracellular matrix (aECM) proteins prepared by previous members of our laboratory have been shown to promote attachment of endothelial cells. In this work, we studied extensively epithelial and fibroblast wound healing behavior on these aECM biomaterials.

Crosslinked aECM protein films of varying RGD densities have been prepared by mixing aECM proteins with the RGD cell binding domain with aECM proteins containing the scrambled RDG sequence. Corneal epithelial wound healing was observed on aECM films with 100% RGD but not on aECM films with 2.5% RGD. Surprisingly, we found a five fold difference between the wound closure rates between these surfaces, but individual cell speeds did not increase significantly. We proposed that the five fold increase in wound closure rate was determined by the rate of crossing the boundary between the wound area and the area underneath the cell sheet. Both simulation and experimental data verified that the rate of boundary-crossing was sufficient to account for five-fold difference in wound closure rates between 100% RGD and 2.5% RGD surfaces.

Full-length fibronectin domains have also been incorporated to improve the overall cell binding properties of the aECM proteins. The aECM proteins containing full-length fibronectin domains were shown to facilitate rapid spreading of Rat-1 fibroblasts. The aECM protein containing both fibronectin domains 9 and 10 exhibited an increased binding affinity to the $\alpha_5\beta_1$ integrin. More importantly, these aECM proteins also promoted rapid wound closure, which was comparable to that on fibronectin. We showed that aECM proteins containing full-length fibronectin domains also promoted higher

phosphorylated levels of focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK), consistent with the faster cell migration and proliferation observed.

To try to understand how cells select wound healing mechanisms, wound healing of Madin-Darby Canine Kidney (MDCK) epithelial cells were examined *in vitro*. On surfaces containing the aECM protein bearing the fibronectin domain 10, characteristic healing patterns were observed in MDCK wound healing. These patterns are defined by the formation of leader cells at regular intervals of actomyosin purse strings. The spacing between consecutive leader cell groups was also found to be independent of the wound diameter. This spacing however, was found to decrease with increasing myosin II inhibition. These observations could be explained using a simple force transmission mechanical model. Consistent with the model predictions, we demonstrated that wounds with a zigzag geometry biased the selection of the wound healing mechanism along the wound edge. These zigzag wounds also healed nearly eight fold faster than wounds with straight edges.

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