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"Regeneration and Scar Tissue Formation: Developing Novel Approaches for Analyzing Macrophage-Mediated Regeneration"

The ultimate goal for optimal healing after traumatic injury or surgery is the complete regeneration of damaged tissue. While some animals such as zebrafish, salamanders, and certain species of rodents have the ability to successfully regenerate after complex tissue injury, most mammals have poor regenerative ability and instead form scar tissue. The African Spiny mouse (*Acomys cahirinus*) and common lab mouse (*Mus musculus)* are commonly used as a comparative mammalian model for studying the mechanisms of regeneration due to their effective regenerative and scar-forming abilities, respectively. Previous studies have found that macrophages play a key role in both guiding complex tissue regeneration in these highly regenerative animals and promoting scar tissue formation in humans and other mammals. In addition, regenerative macrophages have been found to secrete higher amounts of proteins such as PDGF-AA, LTF, and IL-1α compared to scar-forming macrophages, which is being further studied to determine the potential role of these proteins in the promotion of regeneration. PDGF-AA, in particular, has been shown to play a prominent role in promoting regenerative phenotypes in vitro.

The objective of this study was to further explore the factors that control regenerative ability through developing novel methodology for investigating macrophage-mediated regeneration. Polyhedrin Delivery Systems (PODS, Cell Guidance Systems), stable nanoscale (200 nm-5 µL) protein co-crystals built from the polyhedrin protein, were used as the primary method of focus. This sustained release growth factor technology encapsulates growth factors in a protein shell, protecting and preserving their function. In this study, RAW 264.7 cells, an immortalized macrophage-like cell line derived from Balb/c mice, were cultured and studied to determine their ability to phagocytize Human PDGF-AA PODS. Confocal microscopy, 3D Imaging, and live cell imaging were used to observe phagocytosis of PODS crystals into macrophages, and ELISA assays were utilized to measure the macrophage-mediated secretion of platelet-derived growth factor.

Live cell imaging revealed that most PDGF-AA PODS were phagocytized by RAW 264.7 cells over a 16-hour period. An analysis of macrophage behavior at different PODS concentrations showed that macrophages effectively phagocytize PODS cargo protein at 5 crystals per cell, as higher concentrations seemingly lead to partial cell death. In addition, the macrophages were found to secrete at biologically active levels (1 ng/mL) after incubation with PODS.

Overall, the preliminary results obtained from this study suggest that the PODS are a promising protein delivery system. Future research efforts will be aimed at applying the Polyhedrin Delivery System to mouse models to observe the difference in immortalized vs. primary cell responses to PODS. The PDGF-AA PODS-loaded macrophages will also be introduced into the scar-forming model to test the potential role of PDGF-AA on regeneration. The findings of these studies will contribute significantly to future efforts in developing a targeted approach to manipulating macrophages to control healing outcomes and advancing regenerative medicine.