Abstract
Chronic, non-healing wounds are a major complication of diabetes and are characterized by chronic inflammation and excessive protease activity. While once thought to function primarily as a pro-apoptotic serine protease, granzyme B (GzmB) can also accumulate in the extracellular matrix during chronic inflammation and cleave extracellular matrix (ECM) proteins that are essential for proper wound healing, including fibronectin. We hypothesized that GzmB contributes to the pathogenesis of impaired diabetic wound healing through excessive degradation of the ECM.

In the first part of the thesis, we demonstrated that the majority of GzmB was secreted by mast cells and localized in the wound edges and granulation tissues of diabetic mouse wounds at higher levels. Subsequently, we observed that GzmB induced detachment of mouse embryonic fibroblasts and also showed that co-incubation with a mouse GzmB inhibitor, serpina3n (SA3N), abrogated this effect. Finally, we administered SA3N to diabetic mouse wounds and found that wound closure including both reepithelialization and contraction were significantly increased in wounds treated with SA3N. Histological and immunohistochemical analyses of the SA3N-treated wounds revealed a more mature, proliferative granulation tissue phenotype as indicated by increased cells with proliferative activity, vascularization, contractile myofibroblasts, as well as collagen deposition in remodeling tissues. Skin homogenates from SA3N-treated wounds also exhibited greater levels of full-length intact fibronectin when compared to control wounds.

In summary, our findings suggested that GzmB contributes to the pathogenesis of diabetic wound healing through the proteolytic cleavage of fibronectin that are essential for normal wound closure, and that inhibition of GzmB can promote granulation tissue maturation and collagen deposition. These results offer preliminary evidence that a GzmB inhibitor may be a relevant therapeutic target in wound management therapy