

Abstract

The phases of wound healing generally overlap and occur in a well-orchestrated manner, following the sequential steps of hemostasis, inflammation, proliferation, and remodeling. However, the presence of various pathophysiological conditions like diabetes mellitus (DM), venous insufficiency, thrombocytopenia, ischemia, blood dyscrasias, or pressure ulcers hinders the healing process due to systemic complications giving rise to chronic non-healing wounds. Despite being etiologically different, all chronic wounds share some basic characteristics or traits in common, like— enhanced pro-inflammatory cytokine levels, unregulated amounts of reactive oxygen species (ROS), proteases, and senescence cells, continued infection, and the presence of dysfunctional and deficient stem cells. Constant infiltration of neutrophils in the wound area increases the concentration of degenerative proteins called matrix metallo-proteinases (MMPs) that disturbs the equilibrium between these proteases and their tissue inhibitors (TIMMP). Unregulated MMP levels in chronic wounds degrade the deposited extracellular matrix (ECM), alter cytokine expression, and reduce proliferative factors that are quintessential for healing.

Among all the types of chronic wounds, diabetic wounds are the most difficult to heal as they lead to alteration of the immune system activation being associated with an autoimmune disease. In hyperglycemic wounds, the residual sugars present in the tissues and circulation react non-enzymatically with the amine residues of proteins, lipids, and nucleic acids in the oxidative environment to form complex structures called advanced glycation end products (AGEs). Taken together, uncontrolled hyperglycemia, oxidative stress, and upregulation of the AGEs make the diabetes wound microenvironment further complex to address.

To this end, this Thesis describes three different hydrogel-based approaches to regulate the chronic wound microenvironment to assist healing-

1. Sequential delivery of CHX and PDGF-BB from layer-by-layer hydrogel-based scaffold: Here, we designed a layer-by-layer scaffold (S_{L-B-L}) containing an antimicrobial agent and MMP-9 inhibitor, CHX, along with growth factor PDGF-BB to regulate healing of chronic wounds. We found that the initial burst release of CHX from the outermost layer of S_{L-B-L} controlled the protease-rich environment of the wound bed. Hence, the released PDGF-BB remained active and induced VEGF-A secretion over 21 days *in vivo*, overcoming two significant disadvantages associated with diabetic wound healing.
2. Inhibition of AGE/RAGE signaling to restore macrophage function to assist healing: We delivered RAGE inhibitor Ri loaded in hydrogel (Immuno-gel) containing antimicrobial agent CHX to downregulate AGE/RAGE signaling in wound bed. AGE/RGE upregulation hinders healing by preventing macrophage polarization from M1 to M2 macrophages in chronic wounds. Immuno-gel treatment further increased the M2 population in wound beds of diabetic rats and reduced the number of M1 macrophages making the overall microenvironment pro-healing.
3. Modulating chronic wound microenvironment via engineered M2Exo-conjugated hydrogel: Here, we conjugated M2-derived nanovesicles with a self-healing hydrogel (Exo-gel) and loaded it with both antimicrobial agent CHX and RAGE inhibitor Ri. Detailed studies indicated that Exo-gel induced faster healing in a diabetic rat wound model owing to the synergistic effect of all the components.