Affinity-based Controlled Release Scaffolds for Modulation of Macrophage Phenotype in Chronic Diabetic Ulcers

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Statement of Purpose: Dysfunctional wound healing is a major complication of both type 1 and type 2 diabetes. Foot ulcerations, which occur in 15% of diabetic patients, lead to over 82,000 lower limb amputations annually in the United States (Dall TM. Health Aff. 2010; 29(2):297-303) with a direct cost of $5 billion per year (Weingarten MS. Wound Repair Regen. 2012; 20(6):911-7). Macrophages are the central cell of the inflammatory response and are recognized as primary regulators of wound healing, with their phenotype orchestrating events specific to the stage of repair. Macrophages exist on a spectrum of phenotypes ranging from pro-inflammatory, or "M1", to anti-inflammatory and pro-healing, or "M2." In early stages of normal wound healing, M1 macrophages secrete pro-inflammatory cytokines and clear the wound of debris. In later stages, macrophages switch to the M2 phenotype and promote ECM synthesis, matrix remodeling, and tissue repair. If the M1-to-M2 transition is disrupted and M1 macrophages dominate, the wound suffers from chronic inflammation and impaired healing, suggesting that macrophages hold promise as a theraputic target in design of next generation biomaterial and drug delivery technologies for wound healing. While abnormal macrophage activation in diabetic wounds has been thoroughly described in animal models of diabetes, it has not yet been assessed in human diabetic wounds. Therefore, in this work first we set out to assess expression profile of macrophage markers in human diabetic wounds. Next, utilizing the affinity interaction of the biotin-streptavidin complex we propose a novel affinity-based immunomodulatory scaffold to rectify the aberrant macrophage profile in nonhealing wounds by sustained and prolonged release of interlukin-4 (IL-4) that promotes macrophages to switch to a pro-healing M2 phenotype.

Methods: We selected a panel of genes that were highly indicative of macrophage phenotype using macrophages cultivated and polarized in vitro towards the M1 and M2 phenotypes as explained in Spiller, KL. Biomaterials. 2014; 35(15):4477-88. We then defined a combinatorial M1 over M2 score that converts gene expression data into a single score, resulting in higher scores for the M1 macrophages and lower scores for the M2 macrophages. In compliance with the study protocol reviewed and approved by Drexel University Institutional Review Board, we applied this score to debrided wound tissue obtained from human diabetic foot ulcers over the course of 4 weeks from the initial visit in order to describe differences in gene expression between healing and nonhealing diabetic wounds. Patients were followed for an additional 8 weeks and wounds that completely closed over the 12 week period were deemed "healing" while wounds that did not close during this time period were designated "nonhealing." These wounds were compared to healing acute wounds using data from a publicly available dataset of a longitudinal study of wound healing in acute burn wounds in humans (Greco, JA. Burns. 2010; 36(2):192-204). Utility of the biotin-streptavidin affinity interaction as a controlled release system was examined by attaching IL-4 (Peprotech) to collagen scaffolds (Davol) via biotin and streptavidin binding. Biotinylation of IL-4 and collagen scaffolds were achived using sulfo-NHS-LC-LC-biotin (Thermo Fisher Scientific) as per manufacturer's instructions. Biotinylated scaffolds were soaked in 0.5 ml of 172 mg/ml streptavidin (Thermo Fisher Scientific) for 1h, followed by washing 3 times in PBS. Scaffolds were then soaked in 375 ng biotinylated IL-4 in 0.5 ml of PBS for 1h. The release of IL-4 proteins from scaffolds was measured using ELISA (Peprotech).

Results: We showed that the combinatorial M1 over M2 score increased immediately after injury, and decreased back to baseline levels after 7 days of healing in acute wounds in agreement with studies of the temporal profile of numbers of M1 and M2 macrophages in animal models of healing wounds (Fig1a). Similarly, all of the diabetic ulcers that healed over the course of the study showed a decreasing M1 over M2 score. In stark contrast, all wounds that failed to heal showed increasing M1 over M2 scores over time corroborating reports that suggested an elevated inflammatory character in nonhealing chronic wounds as well as animal models that suggested a defective M1-to-M2 transition in diabetic wounds (Fig1b-c). Furthermore, we demonstrated that introducing affinity interactions between drug and scaffold allows for sustained drug release over extended period of time (Fig1d). This delivery method can be easily incorporated into wound dressings for controlled release of macrophage-stimulating cytokines without drastically altering the biofunctionality of the scaffolds as well as that of the proteins, which might occur with other controlled release systems.

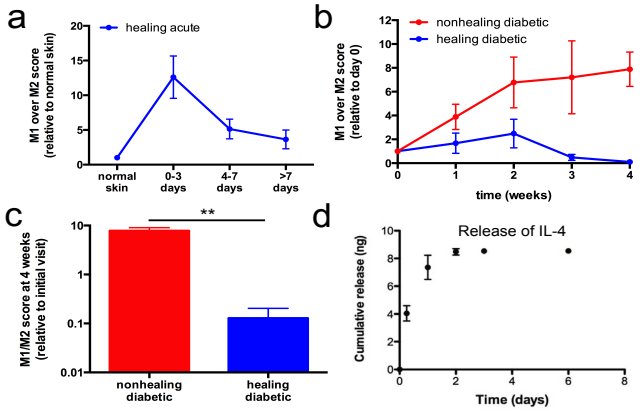

Figure1: Expression profile of macrophage markers in healing vs. nonhealing human diabetic wounds over time (a-c). Controlled release achived by utilizing affinity interaction between biotin and streptavidin (d).

Conclusions: A novel strategy to combine biomarkers derived from M1 or M2 macrophage gene expression signatures reveals striking differences between human healing and nonhealing diabetic foot ulcers. Affinity-based controlled release systems hold great potential in targeting the aberrant macrophage profile in nonhealing human diabetic wounds. Further work is required to investigate the utility of biotin-streptavidin binding in controlled release systems.