Modeling of Macrophage-Mediated Controlled Release System for the Treatment of Diabetic Wounds

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Statement of Purpose: Foot ulcerations occur in about 15% of diabetic patients, and lead to over 82,000 lower limb amputations each year in the United States (Dall TM. Health Aff. 2010; 29(2):297-303). Normal wound healing involves three stages: inflammation, proliferation, and remodeling. Diabetic wounds are known for being stalled in the inflammatory state. A treatment strategy that facilitates the transition of macrophages, the main regulatory cells of inflammation, from pro-inflammatory to pro-healing to pro-remodeling phenotypes, and at appropriate times, would restore the natural healing process. Exposure of resting macrophages to interleukin-4 (IL4) and interleukin-10 (IL10) induces a pro-healing (M2A) and pro-remodeling (M2C) phenotype, respectively (Spiller, K., manuscript under review). We have designed a hydrogel microsphere-based scaffold that exploits macrophage biology to cause sequential delivery of IL4 and IL10 in order to control the phenotypes of macrophages and their effects on healing. The hydrogel microspheres can be prepared from any hydrogel polymer with chemically conjugated avidin. A core of IL10 is formed by overnight immersion in a solution of IL10 conjugated to a ligand with specific affinity for avidin. Then, upon immersion in a solution of IL4 conjugated to a ligand with higher affinity for avidin, the IL10 is displaced by IL4 from the outside in, so that the thickness of the IL4 shell is dependent on the amount of time allowed for diffusion. IL10 molecules in the core of the microspheres, on the other hand, are trapped inside the inner core due to the high concentration of available binding sites provided by avidin and high affinity between the ligand and avidin molecules. Therefore, in order to allow release of IL10, it is conjugated to a ligand with affinity for avidin via a matrix metalloprotease (MMP)-sensitive peptide, so that IL10 molecules are released by the action of MMPs that diffuse into hydrogels. Because IL4 causes inhibition of MMP secretion by macrophages (Spiller, K. manuscript under review), the release of IL10 is delayed until such time as the IL4 depot is exhausted and the macrophages return to a resting phenotype with renewed ability to secrete MMPs. In this study, we describe the release profiles of IL4 and IL10 from this system using mechanistic modeling to determine the effects of various control parameters.

Methods: A mathematical model describing release of IL4 and IL10 from this system was developed under several assumptions. When IL4 is conjugated to small molecules with varying affinity for avidin, the following equation describes the interactions between IL4 and avidin:

$$[IL4 - avidin\ conjugate] \underset{K_r}{\overset{K_f}{\rightleftarrows}} [free\ IL4] + [immobilized\ avidin]$$

which yields $K_d = \frac{K_f}{K_r}$ as the dissociation constant of the IL4-avidin complex. Since IL4 is loaded primarily at the surface of the hydrogel spheres, only the forward reaction occurs, and release of IL4 is controlled by K_f and the loading of IL4: $\frac{\partial[IL4]}{\partial t} = -K_f[IL4 - avidin\ conjugate]$. Then, release of IL10 follows Michaelis-Menten kinetics. However, the concentration of MMP-sensitive linkages within the hydrogel is much higher than K_m (the substrate concentration needed to achieve a half-maximum enzyme reaction rate) and would saturate the enzyme (Patterson J. Biomaterials. 2010; 31:7836-7845), thus release of IL10 is governed by: $\frac{\partial[IL10]}{\partial t} = -K_{cat}[MMP]$

Results: Computer simulations demonstrate control over the release of IL4 by varying the affinity constants (K_d) of the peptides that link IL4 to avidin in the hydrogel (**Fig. 1A).** The rate of IL10 release, mediated by MMPs from the wound bed, can be controlled by modifying the sequence of the MMP-sensitive peptide that links IL10 to the hydrogel polymer in order to control K_{cat} (**Fig. 1B**). In addition, the duration of delay of IL-10 release can be controlled by varying the total amount of IL4 loaded, since the MMP-sensitive linkage retaining IL10 in its immobilized form cannot be cleaved until the supply of IL4 is exhausted and the M2A population subsides (**Fig. 1C**).

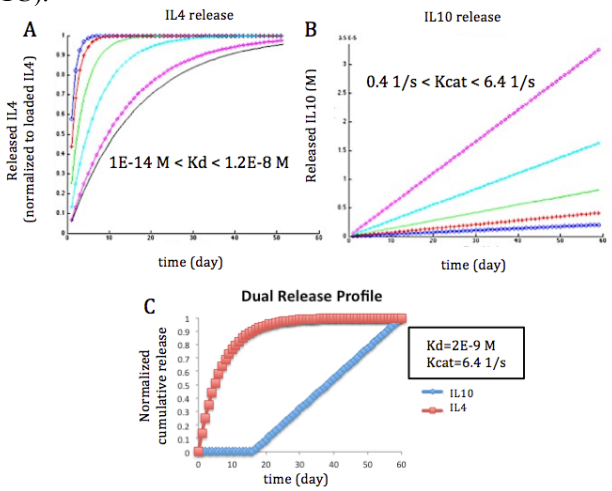

Figure 1: (A) Release of IL4 is controlled by varying Kd. (B) Release of IL10 is controlled by varying Kcat. (C) Sequential release of IL4 and IL10 can be achieved.

Conclusions: These results demonstrate that sequential release of IL4 and IL10 can be achieved by exploiting chronic wound biology via the actions of macrophages. Future studies will validate these results and evaluate the ability of the hydrogel scaffold to promote healing of chronic wounds.