100a Understanding Endothelialization: Rate Limitations in Chemotaxis,

William H. Velander and Cicely Washington

Physiological wound healing is a process that consists of a highly orchestrated sequence of events stimulated by various growth factors. The biological half-lives of growth factors are short so in order to apply them therapeutically they must be delivered in an efficient manner. Immobilization of growth factors at the target site may potentially enhance their effect. A 43.3-kDa collagen-binding fusion protein (CBDR136K) consisting of both R136K, FGF-1 with the Arginine at the 136 site changed to Lysine via site directed mutagenesis, and a collagen binding domain derived from Clostridium histolyticum collagenase was produced. The changes to FGF-1 do not alter its mitogenic nor chemotactic response to HUVEc or NIH 3T3 cells. In the presence of thrombin R136K and CBDR136K maintain their chemotactic properties while that of FGF-1 is reduced by a half. Surface plasmon resonance biosensing was used to quantify binding interactions to a soluble synthetic collagen peptide analog. The binding affinity constant to a KW(POG)8 peptide with FGF-1, R136K, CBD, CBDR136K were 5.1 x 10–6 M, 3.4 x 10–6 M, 0.89 x 10–8 M, and 1.1 x 10–8 M respectively. Because the CBDR136K retains its mitogenic potential, it may be feasible to use it or similar chimeric proteins to immobilize growth factors at the site of damaged tissue and increase efficiency of endothelialization of wound sites or acellular, collagenous surfaces.