Engineered Collagen-Mimetic Proteins for Wound Healing

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Statement of Purpose: Scarring is the result of a complex series of biological processes that occur during wound healing. Collagen I plays an important role in wound healing through signaling to cells via integrin interactions to promote migration and proliferation. We propose to employ a wound therapeutic based on Designer Collagens (DCs), a biomaterial platform developed in our laboratory that is established upon a collagen-mimetic protein from group A streptococcus (Scl2.28). DCs contain the GXY motifs that form collagen's triple-helix, which is required for integrin binding. However, unlike collagen, DCs serve as a biological "blank slate," lacking binding sites for cells and proteins. This property allows for modification of DCs with desired binding sequences. We previously inserted a collagen-based integrin α1β1 and α2β1 binding site into the template DC to form DC2. Here, we have incorporated both collagen- and bacterial-based extracellular matrix (ECM) protein binding sites in DC2 to promote its interaction with the ECM. We hypothesize that soluble DC2 with ECM binding sites will physically anchor in the wound bed after delivery to enhance its integrin-base healing effects.

Methods: Recombinant proteins: DC2 was expressed and purified as previously described. ECM-binding DC2 variants were generated with overlap extension polymerase chain reaction (PCR). Position 304 Gln → position 312 Pro were replaced with the collagen-based fibronectin binding site, GLAGQRGIVGLPGQRGER, to generate DC2-FN; SLNSLSGESGELEEPIESNEIDLTID-SDLRPKSSLQGAGSNSISYTDEIEEEDYDK from BBK32 to generate DC2-FN_{BBK}; IDFEESTHENSKHHA-DVVEYEEDTNPG from FNBPA4 to generate DC2-FN_{BPA}; or KYIKFKHDYNILEFNDGTFEYGARPQFNK-PA from Efb to generate DC2-Fg. All mutations were verified by sequencing, and proteins were expressed and purified as previously described. Circular Dichroism (CD): CD spectra of protein samples in 20 mM acetic acid were measured as previously described to assess the presence and thermal transitions of triple helices. Solid phase binding assays: Microtiter wells were coated with FN or Fg to assess DC protein adhesion to ECM proteins. After coating, wells were blocked, and then DC2, DC2-FN, DC2-FN_{BBK}, DC2-FN_{BPA}, DC2-Fg, or human collagen type I were added in serial dilution from 15 to 0 μM. After 1 hr of incubation, antibodies against DC2 or collagen were applied, followed by incubation with HRP-labelled secondary antibodies. SigmaFast OPD was utilized to detect bound ECM proteins via an absorbance reading at 450 nm. Integrin interactions with modified DC2 proteins: To assess retention of integrin binding in modified DC2 proteins, adhesion of (i) C2C12 cells, which do not natively express integrin α1 or α2 subunits, (ii) C2C12 cells modified to stably express human integrin α1 subunits (C2C12-α1), and (iii) C2C12 cells modified to stably express human integrin α2 subunits (C2C12-α2) was measured on DC-coated wells. Cells were seeded at 5,000 cells/cm^2 for 1 hr, washed, and lysed. Cell numbers in lysates from samples and from known standards were measured with the CytoTox 96® Non-Radioactive Cytotoxicity Assay.

Results: All ECM binding sites were successfully inserted, as confirmed by sequencing. All modified DC2 proteins retained a stable triple helix, as demonstrated by a positive peak at 220 nm on their CD spectra and by thermal transitions at ~35-38°C. Furthermore, integrin binding was maintained, as shown by C2C12-α1 and C2C12-α2 adhesion at or above levels shown on DC2, **Figure 1**. Increased adhesion to ECM-binding proteins is hypothesized to occur because of cellular production of FN and/or Fg in addition to integrin expression. This provides a second point of adhesion for cells and increases adhesion numbers. Solid phase binding assays demonstrated that the ECM binding proteins bind to either fibronectin (DC2-FN, DC2-FN_{BBK}, and DC2-FN_{BPA}) or fibrinogen (DC2-Fg) with apparent K_d's below 1 μM, **Table 1**.

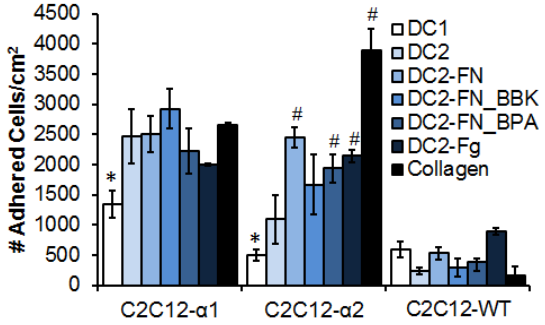

Figure 1. C2C12 cell adhesion to DC proteins.

Table 1. Apparent binding constants (K_D^{App}) of DC proteins to FN or Fg.

Sample	FN K_D^{App} (nM)	Fg K_D^{App} (nM)
DC2	n/a- non-saturated	n/a
DC2-FN	349	n/a
DC2-FN_{BBK}	278	n/a
DC2-FN_{BPA}	249	n/a
DC2-Fg	n/a	539

Conclusions: ECM binding sites were successfully inserted into DC2 to enable interactions with fibronectin or fibrinogen. This protein library harnesses both collagen-based healing cues and bacterial mechanisms of host invasion for use in wound healing. Future work includes assessment of the effects of ECM binding sites on human dermal fibroblast, human epidermal keratinocyte, and human umbilical vein endothelial cell adhesion and spreading as well as on healing rates and DC retention in a mouse excisional wound model.