Delivery of a collagen binding FGF-1 chimera from collagen hydrogels enhances smooth muscle cell proliferation

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Statement of Purpose: We have constructed a fusion protein consisting of R136K, which is a relatively thrombin-resistant mutant derivative of FGF-1, and a collagen binding domain (CBD). Previous research in our lab demonstrated that R136K-CBD had greater binding affinity to 2-D collagen surfaces compared with FGF-1, and had similar chemotactic and mitogenic effects on ECs in a 2-D culture system compared with FGF-1. We predicted that R136K-CBD would demonstrate greater retention in collagen scaffold compared to R136K and FGF-1. This would thereby prolong the bioavailable of this growth factor subsequently promoting both SMC proliferation and endothelialization. In the current study, we investigated the delivery of R136K-CBD with a type I collagen scaffold as the delivery vehicle to smooth muscle cells (SMCs) enhancing the proliferation for vascular tissue engineering.

Methods: R136K and R136K-CBD was constructed as described in our previous published protocols. The binding affinity of R136K-CBD to 3-D collagen scaffolds was investigated both in the presence and absence of cells and/or salts. R136K-CBD and SMCs were labeled using different fluorescent markers and 2-D and 3-D visualization of delivery of R136K-CBD into SMCs was accomplished by combined fluorescent and reflection confocal microscopy. The mitogenic effect of collagen-immobilized R136K-CBD on SMCs in 3-D collagen was studied by Cyquant assay at different time intervals.

Results: In the group devoid of salt and cells, no detectable release of R136K-CBD into overlying culture media was found, compared with burst-and-continuous release of R136K and FGF-1 over a 14-day period in all other groups (Fig. 1). The release rate of R136K-CBD was 1.7 and 1.6 fold less than R-136K and FGF-1 when media was supplemented with 2M salt (P<0.0001), and 2.6 and 2.5 fold less in cell-populated collagen hydrogels (Fig. 2) (P<0.0001) respectively. R136K-CBD showed essentially uniform binding to collagen and its distribution was dependent on that of the collagen scaffold. Internalization of R136K-CBD into SMCs was documented by confocal microscopy (Fig. 3). 3-D local delivery of collagen-immobilized R136K-CBD increased the proliferation of SMCs in the collagen matrix to significantly greater levels and for a significantly greater duration than R136K or FGF-1, with 2.0 and 2.1 fold more mitogenicity than R136K and FGF-1 respectively (P<0.0001) at day 7(Fig. 4).

Conclusions: In this paper, we demonstrated a novel growth factor delivery strategy employing a collagen binding fusion mutant protein-R136K-CBD in a 3-D collagen scaffold as a delivery vehicle and documented its 3-D binding characteristics, 3-D visualization, cellular internalization and mitogenic activity on SMCs. In the context of previous work from our lab which demonstrated positive effects of R136K-CBD on EC behavior, we believe the delivery system discussed in this paper should be a very promising tool for use in engineering clinically applicable blood vessels and other tissue constructs.

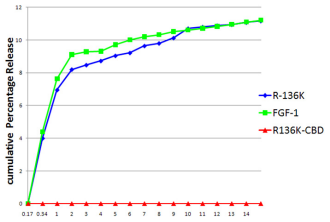

Figure 1

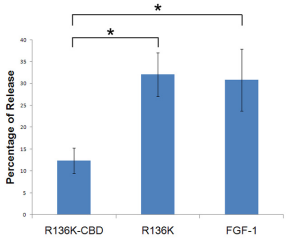

Figure2

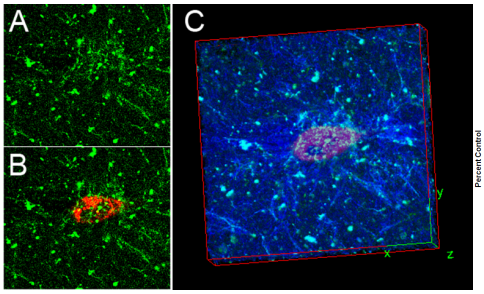

Figure 3

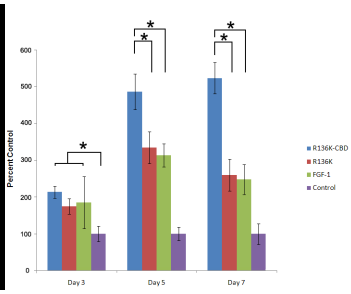

Figure 4

Fig. 1 The cumulative release curve of R136K-CBD, R136K and FGF-1. R136K-CBD showed undetectable release for up to14 days and is significantly less than release of R136K and FGF-1(P<0.0001).
Fig. 2 After 16-hour culture, R136K-CBD showed significantly lower percentage release in collagen hydrogels populated with SMCs compared with R136K and FGF-1(P<0.0001).
Fig. 3 A is the channel of Alexa fluor 488 (green) labeled R136K-CBD and B is the overlay of R136K-CBD and PKH26 (red) labeled SMC and C is the 3-D view of R136K-CBD internalizing into the SMC in collagen. Collagen fibers were visualized with reflection confocal microscopy (blue).
Fig. 4 Proliferation in response to R136K-CBD continued for the full 7 day period of times compared to the decrease in proliferation of R136K and FGF-1 stimulated groups at day 7.

References: Pang Y, HP Greisler, et al. The temporal and spatial dynamics of microscale collagen scaffold remodeling by smooth muscle cells. Biomaterials 2009;30(11):2023-31.

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