Immobilization of Vascular Endothelial Growth Factor onto Fibroblast-derived Matrix and the Effect on ECs Behaviors and Angiogenesis *in vivo*

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Statement of Purpose: Angiogenesis is typically involved in many physiological and pathological processes in the body. It is tightly regulated by multiple growth factors and surrounding extracellular matrix environment.[1,2] Vascular endothelial growth factor (VEGF) is one of the key cytokines in angiogenesis, which is an endothelial cell (EC)-specific mitogen and an angiogenic inducer as well as a mediator of vascular permeability.[3] Delivery of VEGF is a very important strategy to improve cell survival and new blood vessel formation for vascular tissue engineering.[4] In this study, we investigated that VEGF immobilized in the fibroblast-derived matrix (FDM), a decellularized extracellular matrix, could convey some signaling cues for advanced angiogenic response both *in vitro* and *in vivo*.

Methods: The FDM substrates were obtained by decellularization of *in vitro* cultured NIH3T3 fibroblast monolayer and then were activated using EDC/NHS chemistry in MES buffer for varying amounts of heparin conjugation. Different doses of VEGF were then loaded onto heparin-grafted FDM (hep-FDM) for 12 h at room temperature. Hep-FDM was encapsulated into alginate hydrogel (1wt%) for VEGF delivery in 3D environment. The immobilization efficiency and release profile were examined *in vitro* for up to 28 days. The effects of hep-FDM bound VEGF on ECs response were investigated in 2D and 3D environment *in vitro* as well as angiogenic response after the subcutaneous transplantation into ICR mice.

Results: The amounts of VEGF immobilization onto hep-FDM against the different initial doses of 100, 300, and 500 ng VEGF were 19.6±0.6, 39.2±3.2 and 62.1±8.6 ng, respectively. In addition, VEGF release profile from 2D hep-FDM was sustained for up to 4 weeks. Cell proliferation assay exhibited that hep-FDM bound VEGF poses significantly extended impact on EC proliferation in a dose-dependent manner compared with soluble VEGF added in culture media.

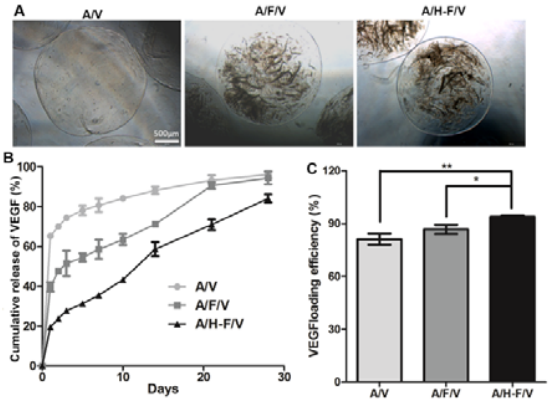

Figure 1. VEGF delivery using alginate capsules (ACs) (A), release profile (B), and loading efficiency (C). Statistical significance: ** (p<0.01), * (p<0.05).

Alginate capsules (ACs), prepared using alginate hydrogel with VEGF alone (A/V), with VEGF and FDM (A/F/V), and with VEGF and hep-FDM (A/H-F/V), were formed in uniform size and shape (Fig. 1A). Different VEGF release patterns were observed for 4 weeks with a significantly reduced initial burst release of A/H-F/V (Fig. 1B). In addition, VEGF loading efficiency of A/H-F/V was better compared with the other groups (Fig. 1C).

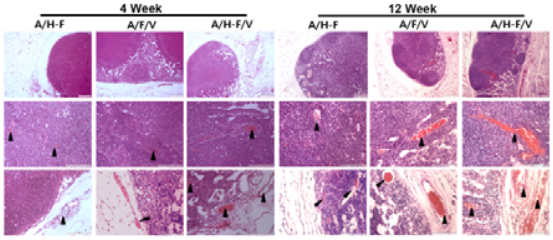

Figure 2. Histological analysis (H&E) of the retrieved ACs from the subcutaneous implantation.

Delivery of VEGF with hep-FDM in alginate hydrogel was successful in promoting new blood vessel invasion *in vivo*, compared to simple mix of VEGF with alginate or alginate with FDM (Fig. 2) at both 4 and 12 weeks. In addition, the quantitative analysis of blood vessel density and occupied vessel area for the implanted ACs exhibited that VEGF-tethered group (A/H-F/V) significantly improved neovascularization compared with A/V and A/F/V (data not shown).

Conclusions: The modification of FDM using heparin not only enhanced the loading efficiency of VEGF, but also improved the sustained release by reducing the initial burst releases in both 2D and 3D environments. Moreover, the bioactivity of VEGF immobilized in the hep-FDM resulted in better EC proliferation than soluble VEGF in medium. The present matrix-mediated VEGF delivery can be a valuable tool for vascular tissue engineering and therapeutic applications.

References

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