VEGF-Fc delivery scaffold for promoting angiogenesis in biomaterials

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ABSTRACT SUMMARY
To enhance vascularization of implants in vivo, a VEGF-Fc fusion protein consisting of vascular endothelial growth factor (VEGF) fused to the immunoglobulin G (IgG) Fc domain was designed and delivered by PCL porous scaffold as an artificial extracellular matrix (ECM). VEGF-Fc was stably self-assembled into a monomolecular layer on the surface of the PCL scaffold due to the hydrophobicity of the Fc domain. The quantitative assay of VEGF-Fc immobilization by ELISA showed that the VEGF-Fc uniformly distributed throughout the PCL scaffold and the immobilization rate was above 96% that was significantly higher than that of commercial VEGF. Additionally, VEGF-Fc delivery significantly enhanced the adhesion and proliferation of human umbilical vein endothelial cells (HUVECs) and effectively up-regulated the expression of endothelial functional molecules to regulate the microvesse formation in the scaffold in vitro and in vivo. This study presents an alternative method for promoting angiogenesis in biomaterials, which may be a promising approach to vascularize of engineered tissues.

INTRODUCTION
Deficient vascularization, the preeminent problem in tissue engineering and regenerative medicine, results in apoptosis of the newly invaded cells due to insufficient oxygen and nutrient transportation. All vessels relay on the endothelium to regulate vascular permeability and normal vascular formation in vivo. Many studies have focused on the construction of endothelial cell-specific ECM by modifying scaffolds using bioactive molecules, such as vascular endothelial growth factor (VEGF), which is a key mediator of endothelial cell growth and vascularization. Meanwhile, recombinant growth factors (GFs) have been immobilized on scaffolds by combining them with either specially engineered peptides, such as collagen-binding domain (CBD), fibronectin domain (FND), arg-gly-asp tripeptide (RGD), or the immunoglobulin Fc (IgG-Fc) domain. The Fc domain, which homodimerizes due to the inter-chain disulfide bonds in the hinge regions, was selected as a candidate domain for a fusion protein mainly because it can improve the solubility and stability of its fusion partner.

In our study, we used recombinant genetic techniques to construct VEGF fused to the Fc domain and delivered the growth factor fusion protein as an endothelial specific ECM by PCL porous scaffold for promoting angiogenesis in biomaterial. The VEGF-Fc was biosynthesized in a eukaryotic expression system. The bioactivity of VEGF-Fc delivery scaffold was evaluated by assaying the adhesion, proliferation of HUVECs in vitro and microvessel formation in vivo.

EXPERIMENTAL METHODS
The VEGF-165 cDNA (GenBank Number: AB451451.1) was inserted into the BamHI and NotI recognition sites of pcDNA 3.1 vectors that contained cDNA encoding an Fc fragment. The VEGF-Fc was expressed using the FreeStyle MAX 293 Expression System (Invitrogen Corporation, USA) and purified with an rProtein A FF column (GE Healthcare Life Sciences, USA). The VEGF-Fc was analyzed by SDS-PAGE, Western blot and ELISA kit.

Porous PCL (Mw=80,000)(Sigma,USA) scaffolds were prepared by solvent casting and particulate leaching technique. VEGF-Fc or VEGF (40μl) at 500ng/ml was directly added to PCL scaffold which prepositioned in 48-well plate. After incubation at 4°C for 2 h, the plate and scaffold were washed with PBS three times. The amounts of bound VEGF-Fc or VEGF were quantified using VEGF ELISA kit (Human VEGF-A ELISA Kit, Neobioscience Technology Company, EHC108).

HUVECs (Cat. No. 8000, USA) were cultured at 37 °C and 5% CO2 in endothelial cell medium supplemented with 5% fetal bovine serum (FBS, Cat. No. 0025, ScienCell, USA), 1% endothelial cell growth supplement (Cat. No. 1052, ScienCell, USA) and 1% penicillin /streptomycin solution (Cat. No. 0503 ScienCell, USA). HUVECs between passages 2 and 6 were used in all experiments. The concentrations of glucose in culture medium were measured using glucose (Bioassay Systems, DIGL-100) assay kit.

All results were reported as the mean ± SD.

RESULTS AND DISCUSSION
Double enzymatic digestion of pcDNA3.1-VEGF-Fc showed that the eukaryotic expression vector (pcDNA3.1-VEGF-Fc) was constructed successfully (Fig. 1a). SDS-PAGE (Fig. 1b) showed that VEGF-Fc was efficiently purified by an rProtein A FF column; the purity of the VEGF-Fc measured using gray-scale analysis was nearly 85%. Western blot assays revealed that VEGF-Fc migrated as 46 kDa and 92 kDa bands under reducing and non-reducing condition, respectively (Fig. 1c), suggesting that VEGF-Fc formed homo-dimers in non-reduced condition. Additionally, Ogiwara reported that the Fc domain could induce the dimerization of other fusion proteins, even epidermal growth factor (EGF), which typically exists as a single polypeptide growth factor [3]. Therefore, it was considered that the Fc domain promoted the homo-dimerization of VEGF-Fc. It was also reported that the bioactivity of the VEGF-165 interaction with its receptors was enhanced by the dimerization of VEGF [4].

![Figure 1](https://example.com/fig1.png)

**Figure 1.** The construction and expression of VEGF-Fc. a Double enzymatic digestion of pcDNA3.1-VEGF-Fc. M: DNA marker (500-8000bp); 1: Plasmid vector pcDNA3.1-Fc (6112bp); 2: pcDNA3.1-VEGF-Fc digested by BamHI and NotI (573 bp, 6112 bp). b SDS-PAGE analysis of VEGF-Fc. 1: Purified VEGF-Fc. c Western blot analysis of VEGF-Fc. P: Human IgG kappa (Sigma, Cat. No. M15154) as a positive control; 1: Purified VEGF-Fc under reducing conditions; 2: Purified VEGF-Fc under non-reducing conditions.

The VEGF-Fc delivery PCL scaffold has open and interconnected pores with the size in 200-300μm, which was same as that without VEGF-Fc. The amount of VEGF-Fc immobilized on the scaffold increased in a dose-dependent manner and the VEGF-Fc uniformly distributed in the scaffolds showed by the VEGF ELISA assay. The immobilization rate of VEGF-Fc at different concentrations was above 96%, but that of commercial VEGF-165 on the scaffold was very low (below 20%), suggesting that VEGF-Fc was efficiently immobilized through the hydrophobic interaction between the Fc domain and the hydrophobic surface of the PCL scaffold.

In comparison with HUVECs cultured on non-coated, BSA-coated and IgG-coated scaffold, the adhesion and proliferation of HUVECs on the VEGF-Fc delivery scaffold significantly increased. Taguchi reported that VEGF-165 covalent bonding to polymer biomaterial surface induced the adhesion and enhanced the proliferation of HUVECs through the interaction between immobilized VEGF-165 and its receptors (Flt-1 and Flk-1/KDR) [5]. These results also suggested that the VEGF domain of VEGF-Fc was exposed on the outside of VEGF-Fc-immobilized surface.

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Glucose consumption rate of HUVECs in the VEGF-Fc delivery scaffolds during 1 week culture.

To assess the bioactivity of VEGF-Fc delivery scaffold in vivo, the scaffold was implanted subcutaneously into the interscapular region of rat for 1 month. The HE staining showed that the average vessel density in the VEGF-Fc delivery PCL scaffold was dramatically higher than that in the PCL scaffold with commercial VEGF (data not shown). It will be further investigated in the bioactivities and the molecular mechanism in vitro and in vivo.

**CONCLUSION**

VEGF-Fc was successfully immobilized on the PCL scaffold by the hydrophobic interaction of Fc domain and achieved handily delivery VEGF as an artificial ECM. VEGF-Fc delivery scaffold can mediate endothelial cell selective adhesion and enhance endothelial cell proliferation, which may be a promising approach to vascularize of engineered tissues.

**REFERENCES**


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