SELF-VASCULAIZING THREE DIMENSIONAL COLLAGEN BY RECOMBINANT BACTERIOPHAGES

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ABSTRACT

Developing tissue inducing vascularization is of great important to the functional tissue design. We have genetically engineered recombinant bacteriophage displayed vascular endothelial cell growth factor (VEGF) on their coat proteins and combined it on the type I collagen scaffold, natural extracellular matrix. This functionalized tissue can lead angiogenesis and capillary morphogenesis itself without any VEGF supply. We showed that the functionalized collagen scaffold with VEGF conjugated recombinant bacteriophage was able to induce angiogenesis as well as capillary morphogenesis in three dimensions. There functionalized scaffold offers promising solution for therapies for graft surgery, such as acceleration of recovery after skin graft, or as intentional leading of vascularizing for preventing parenchyma necrosis.

KEYWORDS

collagen, bacteriophage, angiogenesis, vascular endothelial cell growth factor

INTRODUCTION

Bacteriophages are filamentous virus particles that can infect bacteria. Filamentous phages have been widely applied in nanotechnology, biomedicine, and tissue engineering.[1-3] Recently, the bacteriophage as a supramolecular template for a deliberate cell morphogenesis is receiving much attention, but these studies are just conducted with two dimensional system such as glass or MEMs patterned surface.[4] Because of these limitation, it is not sufficient to implement an in-vivo like situation.

This paper introduces a self-vascularizing tissue by vascular endothelial cell growth factor(VEGF) conjugated fd filamentous bacteriophage(fd-VEGF). Our group and nano-medicine team in KIST-Europe, Germany, developed a various type of fd-VEGF functionalized collagen block, inducing vascularizing itself. And to verify its capability, we applied a novel *in vitro* three dimensional microfluidic chip.[5] With these technologies, VEGF which is a soluble molecule is immobilized on a collagen fibrous structure by bacteriophage and the fd-VEGF functionalized collagen could generate angiogenesis itself.

EXPERIMENT

As illustrated figure 1, we developed two type of fd-VEGF functionalized collagen block(fd-VEGF collagen), bound and mixed type, inducing angiogenesis by VEGF on genetically modified bacteriophage. Also these are engineered to verify its capability by the novel *in vitro* three dimensional microfluidic chip.

First of all, to confirm an interaction between fd-VEGF and collagen, we conducted enzyme-linked immunosorbent assay(ELISA) in 96-well plates with fd filamentous phage(wild type) and fd-VEGF with various conditions, collagen coated surface for bound type and normal surface for mixed type. Each type of sample was incubated with different concentrations in each well. As a result, fd-VEGF successfully combined on collagen in both type. Secondly, we stained fd-VEGF to investigate combination morphology in collagen. We made each type of scaffold, normal, fd-VEGF mixed, and bound collagen. We put the antibody which could attach major coated protein p8 on the bacteriophage. As shown in figure 2, fd-VEGF is combined on the collagen fiber homogeneously, but two types, bound and mixed, expressed different combination morphology. While fd-VEGF showed a filamentous structure in bound case, fd-VEGF expressed dot like structure in mixed case.

To verify an angiogenic capability of the fd-VEGF collagen, we conducted the three dimensional microfluidic chip *in vitro* assay. Human micro-vascular endothelial cells(hMVECs) are seeded in the fd-VEGF collagen injected microfluidic chip. hMVECs express angiogenesis as shown in figure 3. hMVEC cultured on fd-VEGF mixed collagen sprouted more than hMVEC cultured on the fd-VEGF bound collagen, of course, the cells didn't express angiogenic morphology in normal collagen .

In conclusion, the fd-VEGF functionalized collagen blocks are successfully generated through the various combination of fd-VEGF and collagen and these angiogenic capabilities are confirmed. However, a combination mechanism and a different capability between bound and mixed type with quantified cell sprout should be explained. It has many applications for treatment for skin graft, bypass for clogging blood vessel, and so on.

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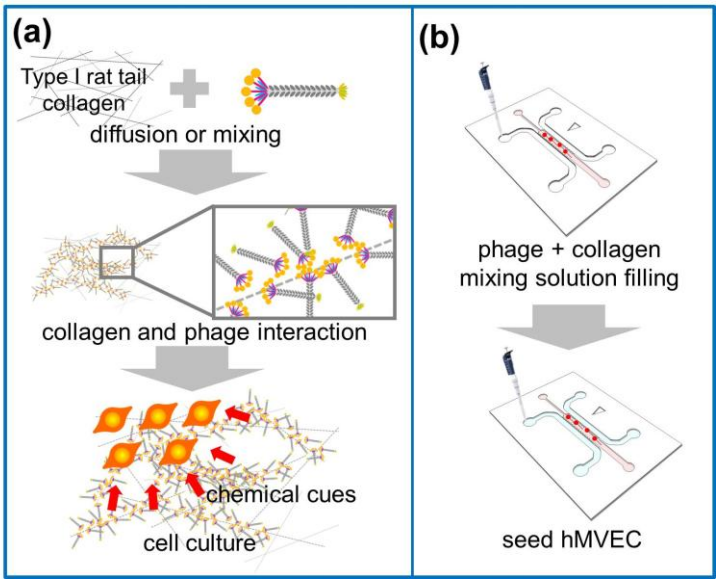

figure 1. (a) schematic concept of a combination between collagen and fd-VEGF and cell culture analysis, (b) three dimensional cell culture system

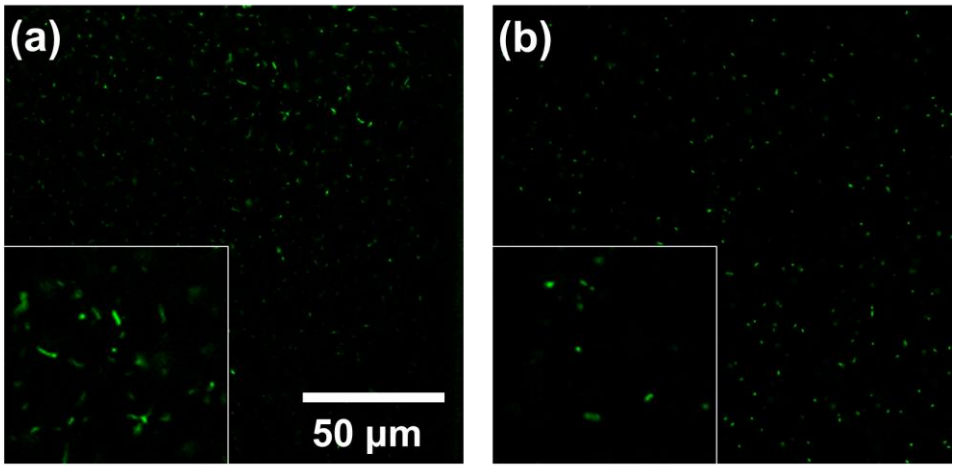

figure 2. fluorescence image of fd-VEGF bound(a) and mixed collagen(b).

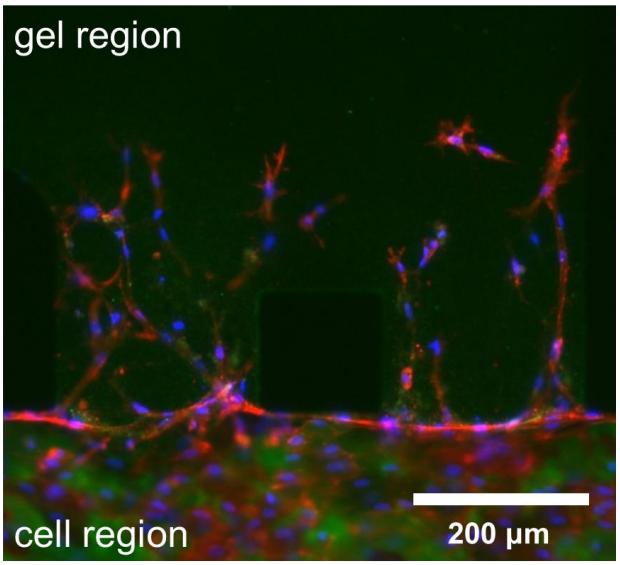

figure 3. angiogenesis of hMVECs on fd-VEGF bound collagen scaffold

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