Rapid vascularization and anastomosis of a large vascularized construct of collagen/β-TCP scaffold fabricated by template-casting and electrochemical detachment technique

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Introduction: Major challenges of engineered tissue constructs are the formation of complete functional vasculature and rapid anastomosis with host vasculature, which limit the maximum size of engineered tissues. Most conventional approaches to engineer vascularization rely on the formation of channels first and then endothelialization of the inner surface of the microchannels. Most recently, a unique electrochemical detachment technique has been developed to rapidly engineer a capillary-like structure by separating an endothelial layer from a solid substrate while retaining a hollow lumen with structural integrity [1,2]. In this study, we engineered a large vascularized construct by incorporating a vascular-like structure into a collagen hydrogel and channeled β-calcium phosphate (β-TCP) macroporous scaffold, and examined its capability of vascularization and anastomosis. Briefly, we grew human umbilical vein endothelial cells (HUVECs) on gold-coated glass rods and detached them onto collagen using this technique. An artificial vascular-like structure was formed in the collagen hydrogel or collagen/β-TCP composite scaffold. Our hypothesis is that this engineered vascularized collagen/β-TCP composite scaffold can rapidly form blood vessels and facilitate anastomosis with host vascular *in vivo*.

Methods: Macroporous β-TCP scaffolds with a central macrochannel of 3mm in diameter were prepared by a template-casting method. The macroporous scaffolds were 5-6 mm in height, 8mm in diameter, and pore size of the scaffolds was approximately 350-500 μm. HUVECs constitutively expressing green fluorescent protein (GFP) were cultured onto gold-coated rods with 600 μm in diameter. After 7-day culture, the HUVECs became confluence on the rods and then were transferred into collagen hydrogels or collagen/β-TCP scaffolds in a customized chamber using electrochemical detachment technique. Then the specimens with HUVEC vascular-like structures were cultured under static and perfusion conditions. Immunofluorescent staining of human CD31 was used to study the network formation. The constructs were further subcutaneously implanted into nude mice with 7 weeks old. Hematoxylin and eosin (H &E) staining and immunohistochemistry staining were used to examine vascularization and anastomosis.

Results: I*n vitro* studies indicated that HUVECs on the vascular-like structures readily migrated into the collagens and collagen/TCP composite scaffolds, and covered a large area regardless of static or perfusion conditions (Figure 1 A and B). The migrating HUVECs appeared a radial pattern from the vascular-like structure (lumen). HUVECs can reach the periphery of β-TCP scaffolds in diameter of 8mm within 7 days. HUVECs formed many networks, and further developed into cords. H&E staining revealed many blood vessels formed in the collagen/β-TCP composite scaffold implants (Figure 1C). Numerous human CD31 positive lumens contained murine erythrocytes at 2 weeks after implantation, indicating anastomosis (Figure 1D).

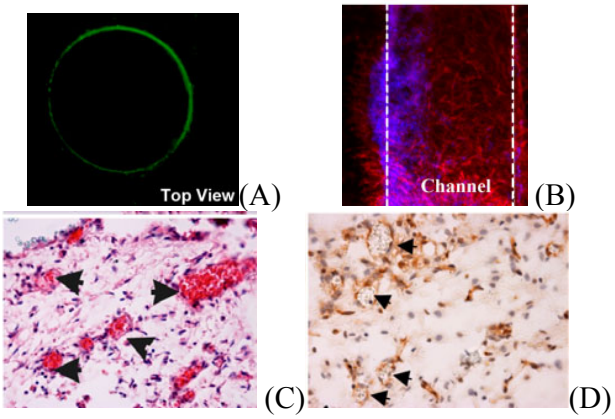

Figure 1 (A) Fluorescent image of the vascular-like structure, HUVEC lining microchannel in collagen *in vitro* (top view); (B) immunofluorescent image of human CD31 positive vascular-like structure *in vitro* (longitudinal view); (C) H&E staining showing blood vessel in the collagen/β-TCP composite scaffold in vivo; and (D) Immunohistochemisty staining of numerous human CD31 positive lumens containing murine erythrocytes.

Conclusions: In this study, we have successfully engineered a large vascularized construct by integrating the vascular-like structure, collagen and TCP scaffold. The large vascularized construct can rapidly form anastomosis and vascularization in vivo. Our next step is to engineer larger; multiple channeled constructs at a centimeter scale and test it in vivo.

References

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