

## A novel strategy for engineering vascularized grafts *in vitro*

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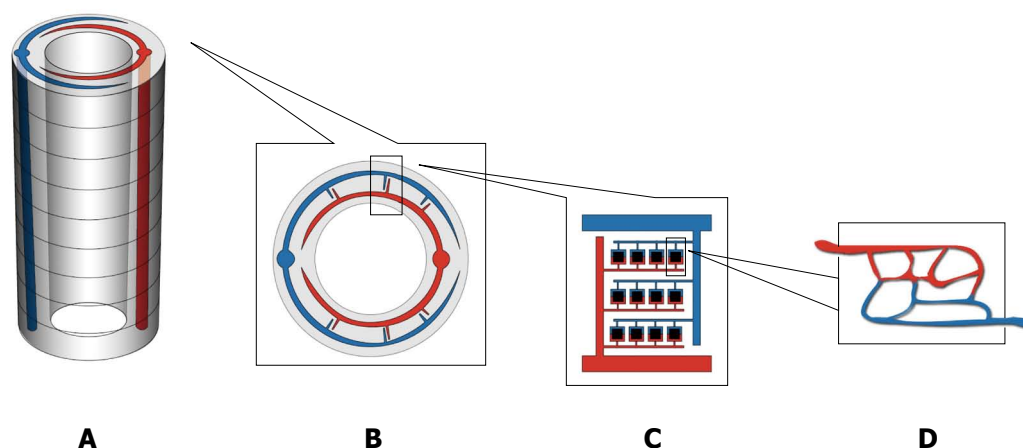
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### Abstract

Tissue engineering is an interdisciplinary field promising new therapeutic means for replacing lost or severely damaged tissues or organs. However, the fabrication of complex engineered tissues has been hampered due to the lack of vascularization to provide sufficient blood supply after implantation. In this article, we propose using rapid prototyping technology to prefabricate a scaffold with an inside hollowed vascular system including an arterial end, a venous end and capillary networks between them. The scaffold will be "printed" layer by layer. When printing every layer, a "low-melting point" material will be used to form a blood vessel network and a tissue-specific material will be used outside it. Hereafter the 'low-melting point' material will be evacuated by vaporization to ensure a hollowed vessel network. Then the inside hollowed capillary network can be endothelialized by using autologous endothelial cells in a cycling bioreactor while the outside material can be embedded with tissue-special cells. In the end, the new vascularized autologous grafts could be transferred to the defect site by using microsurgical techniques to connect the grafts with the host artery and vein. The strategy would facilitate construction of complex tissue engineering if the hypothesis proved to be practical.

### INTRODUCTION

Tissue engineering has tremendous potential for the treatment of a wide range of medical conditions. It represents a new concept that focuses on regeneration of neotissues from cells with the support of biomaterials and growth factors<sup>[1]</sup>. Many tissues have been engineered, including skin, bone, cartilage, tendon and periodontal tissues. But clinical use of engineered tissues and tissue substitutes is largely limited to thin or avascular tissues such as skin or cartilage because of the lack of transplant vascularization<sup>[2,3]</sup>. Fabrication of complex organs like heart or kidney still has a long way to go. Researchers have been working hard to overcome this limitation<sup>[4-6]</sup>. This research can be divided into four groups: scaffold design, angiogenic factor delivery, *in vivo* prevascularization and *in vitro* prevascularization<sup>[7]</sup>. Most of these strategies have their limitations: *in vivo* prevascularization requires two separate surgeries and *in vitro* prevascularization<sup>[8]</sup> is associated with a complex *in vitro* culture period that might not be easy to perform in a standard hospital situation. Moreover, at present there is no convincing evidence that any of these strategies will be sufficient to sustain tissue-engineered constructs that are larger than several millimeters after implantation<sup>[7]</sup>. So, it is a priority to find a new way of vascularization in tissue engineering.



**Figure 1 Strategy for prefabrication a scaffold with hollow vascular network.** A: The scaffold is prefabricated in an layer by layer manner; B, C: The hierarchical vascular-like network made of 'low-melting point' material. The black squares represent the basic units; D: The basic unit of microcirculation-like net which is  $250\text{ }\mu\text{m} \times 250\text{ }\mu\text{m}$  in size. The diameter of the capillary-like line is  $10\text{ }\mu\text{m}$ .

Small-diameter tissue engineering arteries ( $< 6\text{ mm}$ ) have been successfully constructed *in vitro* but few studies have involved fabrication of capillary network *in vitro*. One reason is that a capillary is  $6\text{--}8\text{ }\mu\text{m}$  in diameter which is only  $1/10$  that of a single hair. On the other hand, a small tissue contains a great amount of capillaries. Development of advanced biomaterials and scaffold constructing technology give opportunity to accept the challenge. Rapid prototyping (RP) is a common name for several techniques which read in data from computer-aided design (CAD) drawings and manufacture automatically three-dimensional objects layer-by-layer according to the virtual design<sup>[9]</sup>. RP enables the control of the scaffold porosity, making it possible to fabricate applications with desired structural integrity. In this article, we present our hypothesis on the basis of the advanced material and RP technology, implying a new approach to prefabricate scaffolds with a hollowed vascular network and a new way of vascularization in tissue engineering.

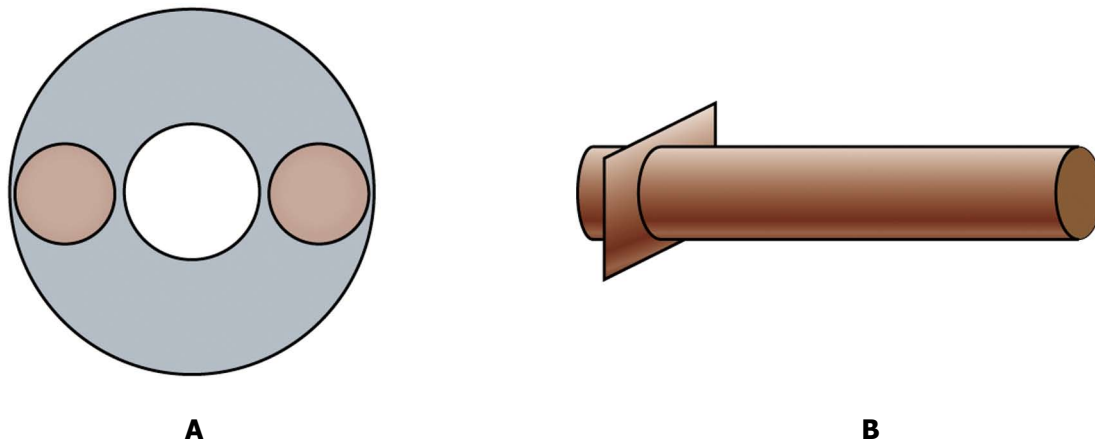
## HYPOTHESIS

To explore the possibility of fabrication of vascularized grafts, we hypothesize in this paper that, using computer-aided rapid prototyping technology and 'low-melting point' material, a scaffold with a hollowed vascular system of capillary networks with an arterial end and a venous end could be fabricated.

Firstly, two types of material will be used: the outside tissue-specific material and the inside 'low-melting point' material. The use of tissue-specific material depends on what kind of tissue is fabricated, for example, hydroxyapatite for bone engineering. The inside 'low-melting point' material will be shaped like a vascular network and will be eliminated in the end to form the inside hollowed vascular network. Secondly, rapid prototyping computer-aided 3D printing technology will be used to fabricate a particular 3D scaffold layer-by-layer<sup>[10]</sup>. Hundreds of layers of  $200\text{-}\mu\text{m}$  in

thickness will be constructed layer by layer to form a special-shaped scaffold, for example a cylinder-shaped scaffold (Figure 1A). When constructing each layer, the outside tissue-specific material is deposited first and then the inside 'low-melting point' material is deposited to form a hierarchical vascular network under the control of a computer (Figure 1B, C and D). The basic unit (Figure 1D), like the physiological microcirculation system, is  $250\text{ }\mu\text{m}$  in length and  $250\text{ }\mu\text{m}$  in width and the diameter of a 'capillary' is  $10\text{ }\mu\text{m}$ . When the bio-material is printed layer by layer, the artery end and the vein end will connect with its counterpart of the adjacent layer (Figure 1A). In the end, a main artery and vein are formed throughout it automatically in the scaffold. In every layer the main artery or vein will send out its branches. Thirdly, the scaffold is put inside a vacuum and its temperature is increased to reach its vaporization point. Then the vascular-formed material (low-melting point material) is eliminated from the scaffold and a hollowed network is formed. Fourthly, targeted tissue cells are seeded into the outside tissue-specific material and cultured in a static manner while endothelium cells are seeded into the inside hollow surface in a rotative and dynamic way until the hollowed vascular network is endothelialized<sup>[11]</sup>.

Now we have a vascularized graft. But how can we transplant the graft into a host and connect its vessels with host axial blood vessels? We need two connectors. They are two tissue engineered blood vessels and will be used as bridges to connect the fabricated graft with host artery and vein by microsurgery (Figure 2). Tissue engineered blood vessels have been successfully fabricated and applied clinically<sup>[12]</sup> and so, using similar methods, we will fabricate the vessels. Specifically, the scaffold of the connection blood vessels are prefabricated with rigid material in one end like hydroxyapatite (HA) and soft material in the other end like polyglycolic acids (PGA). The rigid end will be plugged in to the artery or vein end of the fabricated scaffold and the soft end will be sutured with the host's vessels when the



**Figure 2** Fabrication of connective blood vessel graft to main scaffold and host vessels. A: The bottom of the vascularized graft with an arterial end and a venous end; B: The scaffold of connective blood vessel. The left part of it is made of rigid bio-material and can be plugged into the bottom of the vascularized graft. The right part of it is made of soft bio-material and can be sutured with the host vessels.

whole artificial graft is transplanted into a host.

## EVALUATION OF THE HYPOTHESIS

Some important issues should be considered to evaluate the feasibility of the vascularized graft. Although rapid Prototyping technology, such as 3D Printing<sup>[13]</sup>, offer great confidence to construct kinds of scaffolds prefabricated in the computer concisely and rapidly, fineness of the rapid prototyping machine should be considered. Is it possible to put a 10-um capillary-like deposition concisely in position? Advanced micro-nanoprocessing technologies may hold the promise to overcome the challenge<sup>[14]</sup>. Another consideration is the diameter of an endothelial cell which, as we know, is 10-50 um. Whether the endothelial cells would adhere to surfaces of the 10-um capillary-like tunnels or be obstructed before it is another problem. Endothelial cells have shown characteristics of preferring growth along micro-tunnels<sup>[15]</sup>. It is reasonable to speculate that endothelial cells could adhere to surface of the capillary-like tunnels in the above scaffold.

If the hypothesis is proved to be practical, the endothelialized graft could ensure the oxygen and nutrition supply once it is transplanted into a living host body and could improve the growth and differentiation of cells to form targeted tissues or organs. Considering the outside tissue-specific material and cells could be changed depending on our aim and the inside material and endothelialization could be used repeatedly, the method in this paper could be used as a model for complex tissue regeneration.

## CONCLUSION

Vascularization remains one of the main obstacles that need to be overcome before large tissue-engineered constructs can be applied in clinical applications. We propose a new strategy to fabricate a vascularized graft in tissue engineering. Based on advanced materials and rapid prototyping technology, a prefabricated scaffold with a hollowed

vascular-like network could be obtained. After endothelialization, the graft could be transplanted into a living host. The cells of the graft could proliferate and differentiate better and a complex and functional tissue or organ could be possibly constructed.

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