A vascular fabrication method based on sacrificial material and spraying process

Jian Qi, Jia Li, Shuxian Zheng and Jie Liu
Tianjin key laboratory of equipment design and manufacturing technology, school of mechanical engineering, Tianjin University, Tianjin, 300354, China

*Corresponding author e-mail: sxzheng@tju.edu.cn, qijian78@126.com, jli@tju.edu.cn, ljlqq@tju.edu.cn

Abstract. In tissue engineering, the lack of vascular structures often leads to massive cell death inside the scaffold, it is necessary to construct vascular structures in scaffolds. This paper presented a method for fabricating blood vessels based on sacrificial materials and spraying process. Sucrose was used as the sacrificial material to prepare the vascular core, the SA/PVA gel formulated from sodium alginate (SA) and polyvinyl alcohol (PVA) was used as vascular material. The optimal proportion of SA/PVA gel material was determined by orthogonal experiments: SA: 4 wt%, PVA: 5 wt%, CaCl$_2$: 4 wt%, and its elastic modulus and porosity were 993.19 kPa and 89.23%. The spraying process utilized high-pressure air flow to coat the SA/PVA gel on the vascular core, and then it was cross-linked in CaCl$_2$ solution, and the hollow vascular structure can be obtained by removing the vascular core in an aqueous solution. This method allows rapid, low-cost preparation of vascular structures.

1. Introduction

Tissue engineering, as an interdisciplinary field, is aimed at constructing artificial tissues and organs to repair or reconstruct the damaged tissues or maintain the function of organs [1]. However, there are some problems in the current research, such as oxygen and nutrition cannot go deep into the scaffold, resulting in a large number of cell deaths within it, which greatly limits the clinical application of tissue engineering [2]. Therefore, to ensure the cells in the scaffold can acquire enough oxygen and nutrients, it is necessary to construct a vascular structure in the interior [3].

Vascular manufacturing is a method worth studying, which can be classified as direct forming and indirect forming. Direct forming, including weaving [4] and 3D printing methods [2], refers to fabricate the blood vessels directly; indirect processing uses metal [5] or sacrificial materials [6] to make a vascular core, then the vascular material is deposited on the vascular core surface using electrospinning or 3D printing method to create a coating that forms a hollow vascular structure. These methods have achieved good results in the preparation of blood vessels, but expensive or special equipments are needed. In this paper, we proposed a low-cost and rapid method of manufacturing blood vessels based on sacrificial materials and spraying processes, which can quickly fabricate complex vascular structures.
2. Materials and experiments
In this study, sodium alginate (SA) and polyvinyl alcohol (PVA) were used to formulate the SA/PVA gel, PVA can increase viscosity of hydrogel, and calcium chloride (CC) solution was used as a cross-linking agent. The elastic modulus of the hydrogel determines the strength of the vessels, and porosity of vessels affects the exchange efficiency of nutrients and oxygen. In order to make the artificial blood vessel have the ideal strength and nutrient exchange efficiency, the orthogonal experiment was used to find the optimal material proportion of elastic modulus and porosity.

The components used in the gel preparation were SA, PVA, and CC. After pre-experiments, the concentration range of each material was determined: SA was 2-4wt%, PVA was 5-7wt%, and CC was 2-4wt%. The SA, PVA, and CC concentrations were used as the influencing factors for orthogonal experiments, and each factor was divided into three levels, then the L9 (4^3) orthogonal experiment table was used for experiment design. The elastic modulus and porosity of the gels with different experimental proportions were measured, the results were shown in Table 1.

| Exp.No. | SA (wt%) | PVA (wt%) | CC (wt%) | Elastic Modulus (E/kPa) | Porosity (P/%) |
|--------|----------|-----------|----------|------------------------|--------------|
| 1      | 2        | 5         | 2        | 333.95                 | 89.20       |
| 2      | 3        | 5         | 3        | 607.56                 | 89.61       |
| 3      | 4        | 5         | 4        | 993.19                 | 89.23       |
| 4      | 2        | 6         | 3        | 499.53                 | 86.72       |
| 5      | 3        | 6         | 4        | 756.67                 | 86.52       |
| 6      | 4        | 6         | 2        | 740.67                 | 86.58       |
| 7      | 2        | 7         | 4        | 783.73                 | 83.68       |
| 8      | 3        | 7         | 2        | 706.84                 | 83.39       |
| 9      | 4        | 7         | 3        | 1158.41                | 83.79       |

After the range analysis of the experimental results, we found that the order of factors affecting the elastic modulus of the SA/PVA hydrogel were SA>CC>PVA, and the order of factors affecting the porosity were PVA> SA>CC. To ensure the strength of blood vessels and the exchange efficiency of nutrient and oxygen, the large elastic modulus and higher porosity were selected as the optimum gel material ratio, the optimal material proportion is SA: 4wt%, PVA: 5wt%, and CC: 4wt%, which is the 3rd group of experiments in Table 1, and the elastic modulus and porosity are 993.19 kPa and 89.23%.

3. Blood vessel fabricating method
Due to the small scale and complicated structure of blood vessels, the vascular manufacturing process has always been a challenge, and current vascular preparation methods place high demands on equipment. In this paper, we proposed a low-cost and rapid method of manufacturing blood vessels based on sacrificial materials and spraying processes. This method used sucrose as sacrificial material to make a vascular core, SA and PVA were used to prepare the SA/PVA gel, which was attached to the surface of vascular core by spraying process, then the vascular core was removed to obtain a vascular structure.

To illustrate the blood vessel fabrication process, two vascular models were designed based on reference [7], the spatial dimension of vascular model was 60mm×60mm×3mm, the wall thickness was 0.5mm, and inner diameter of blood vessel was 1mm, outer diameter of blood vessel was 2mm, as shown in Fig.1(a).

The blood vessel manufacturing process is mainly divided into three steps: (1) making the vascular cores with sacrificial material; (2) using the spray coating process to attach the SA/PVA gel to the surface of vascular core and cross-linked with CC solution; (3) removing the vascular core to form a hollow blood vessel. The diagram of fabrication was shown in Fig.1 (b).
3.1. Vascular core preparation
Since sucrose is soluble in water and has the characteristics of easy molding and bio-safety, then the sucrose was selected as the vascular core material in this study. Firstly, the vascular core was prepared by utilizing the high temperature plasticity of sucrose, then the sucrose was removed using its soluble properties, the preparation process is as follows:

(1) The sucrose was dissolved in deionized water (83wt%) and heated to 160°C, then stirred at room temperature for 20 minutes until it became a pale yellow transparent liquid. The sucrose solution was then lowered to 100°C and placed in a syringe with a 0.6 mm nozzle.

(2) The vascular model was projected onto a 2D plane and printed out. The sucrose solution was extruded from the syringe along the vascular branches, as the temperature decreases after extrusion, the sucrose solution solidified into the vascular core, as shown in Fig. 2(a).

(3) Adjust the local details of the vascular core. The excess sugar was removed using a heated blade, and then the vascular core was placed in a low temperature dry environment. The blood vessel core was shown in Fig. 2(b).

3.2. Spraying process
The blood vessel was prepared by spraying process. First, the SA/PVA gel was sprayed on the surface of the sucrose core, which was then cross-linked in CC solution. Second, the blood vessel with sucrose core was soaked in water for one hour to remove sucrose core to obtain SA/PVA gel blood vessel.

The equipment used in the spraying process was shown in Fig. 3(a). The SA/PVA gel was squeezed into the spray gun with a syringe, and the gel was then blown off by high-pressure gas to particles to coat the surface of the core; then it was cross-linked in CC solution and after removing the sucrose core structure in deionized water, a SA/PVA gel blood vessel wall was formed.

Figure 1. Vascular design models and manufacturing process. (a) vascular design models (b) Schematic diagram of blood vessel fabrication.

Figure 2. Blood vessel core and its manufacturing process. (a) manufacturing process of vascular core; (b) vascular core.

Figure 3. Equipment for spraying process.
The nozzle diameter of the spray gun was 1.3mm, the spray distance and width were 200mm and 120mm. The two ports of the spray gun were respectively connected to the air compressor and the syringe containing the gel. The proportion of SA/PVA gel material was (SA: 4 wt%, PVA: 7 wt%, CC: 4 wt%). Set the air compressor pressure to 0.29MPa, adjust the spraying distance to 100mm, and rotate the sucrose core while spraying so that the SA/PVA gel forms a uniform coat on the sucrose core. After that, the sprayed sucrose cores were cross-linked in CC solution for 30 minutes, and it was placed in deionized water to remove the cores, then the blood vessel was fabricated. The spraying process and result were shown in Fig.3 (b)(c).

![Figure 3. Schematic diagram of spraying process and spraying result (a)schematic diagram of spraying device; (b)spraying process; (c)spraying result.](image)

### 3.3. Manufacturing cases

According to the sacrificial materials plus spray coating method, the blood vessels in Fig.4 were fabricated in Fig.4 (a) and Fig.4 (d). Fig.4 (b) and Fig.4(c) were axial section and radial section of the blood vessel. It could be found that the topological structures of fabricated blood vessel were consistent with the design model, and the blood vessels had good internal connectivity. The inner diameter of the blood vessels were about 1 mm, the wall thickness were about 0.5mm, which were basically the same as the design sizes, as shown in Fig.4(c).

The blood vessel in Fig.4 (d) was infused with red ink to simulate blood flow, the results were shown in Fig.4 (e-h). The red ink can flow from the bottom of the blood vessel and flow out through the ends of the branches without occlusion and blockage. This showed that the blood vessels prepared by this method could meet the blood perfusion requirements and lay the foundation for the vascularization of tissue engineering *in vitro*.

![Figure 4. The fabricated blood vessels. (a) the fabricated blood vessel of case 1; (b) axial section of blood vessel of case 1; (c) radial section of blood vessel of case 1; (d) the fabricated blood vessel of case 2; (e-h) red ink perfusion schematic](image)
4. Discussion
In this study, blood vessels could be quickly and easily fabricated by using sacrificial materials plus spray coating method. However, the fabricate process was mostly depend on manual operation, and the uniformity of the blood vessel wall thickness has a certain difference from the design model, this method is suitable for preparing a blood vessel structure that does not require high precision. In addition, the perfusion experiments using red ink to verify the integrity of the blood vessels, yet no cell culture was performed on blood vessels. Using 3D printing methods to improve accuracy of vascular core and verify the biocompatibility of the material will be our future work.

5. Conclusion
In this paper, we proposed a vascular fabrication method based on sacrificial material and spraying process. The sodium alginate (SA) and polyvinyl alcohol (PVA) were used to prepare SA/PVA gel. The optimum proportion of the material was determined by orthogonal experiments: SA: 4 wt%, PVA: 5 wt%, CaCl₂: 4 wt%. The elastic modulus and porosity of the SA/PVA gel were 993.19 kPa and 89.23%. Blood vessels with 1 mm inner diameter and 0.4 mm wall thickness were fabricated using sacrificial material (sucrose) and spray coating method. It was found that the sizes and topological structures of fabricated blood vessel were consistent with the design model. After blood vessel perfusion experiments, we found the blood vessel structures were complete which have good internal connectivities. This sacrificial material plus spray method provides a rapid low cost way for blood vessel manufacture.

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