Design of a sugar-responsive hydrogel for a sacrificial template to create vascularized tissue constructs

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Introduction: In the body, the transportation and delivery of necessary substances, such as nutrients and oxygen, to cells are facilitated by blood circulation. However, the vascularization design is not always considered for the present engineered tissues constructed from biomaterials and cells *in vitro*. Therefore, the biological functions of engineered tissue constructs will not be maintained due to the poor nutrients and oxygen supply. As one trial to tackle the issue, it is necessary to develop the methodology and technology for creating vascularized tissue constructs *in vitro*. In this study, a sugar-responsive hydrogel is designed as the sacrificial template that can be removed by the sugar-responsive water-solubilization without any cytotoxicity, to allow to create vessel-like structures in collagen gels.

Methods: Gelatin hydrogel rods with the property of sugar-responsive water-solubilization were prepared. Briefly, m-aminophenylboronic acid (APBA) of a sugar-responsive moiety was introduced into gelatin with a weight average molecular weight of 100,000 and an isoelectric point of 5.0 (Nitta Gelatin Inc., Japan) by using *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. An aqueous solution of the APBA-introduced gelatin was poured into a polypropylene dish and dried up at 4 ºC to obtain APBA-gelatin sheets. Then, the resulting sheet was cut into a rod with a diameter of 300 μm and a length of 1 cm, followed by labeling with fluorescein isothiocyanate (FITC). The FITC-labeled rod was embedded in gels of rat tail type I collagen at a neutral pH and 37 ºC, and the rod-embedding collagen gel was immersed into a culture media with sorbitol of a sugar to remove the rod from the collagen gel. After 60 min of the sorbitol addition, the water-solubilization of the FITC-labled rod in the collagen gel was evaluated both by confocal microscopic observation and fluorescence measurement.

To seed endothelial cells into the channel in the collagen gel, two different methods including post-seeding into the channel after the rod removal and pre-seeding onto the rod before being embedded into the collagen gel were investigated. As the post-seeding method, green fluorescent protein (GFP)-labeled mouse endothelial cells (MS-1) were seeded into the channel which was generated in the collagen gel by the rod removal. On the contrary, GFP-labled MS-1 were pre-seeded onto the surface of the rod and the resulting MS-1-attached rod was embedded into the collagen gel. Then, the rod was removed through the sorbitol addition to transfer the attached MS-1 to the channel surface.

Results: When placed in a culture media with sorbitol, the FITC-labeled rod was water-solubilized in the collagen gel to form a channel. However, in the absence of sorbitol, no water-solubilization of the rod was observed (Figure 1). It is likely that the sorbitol binding allows the boronic acid group to process negative charges, resulting in disrupted hydrophobic interaction between the APBA groups. This interaction disruption would lead to the water-solubilization of the rod.

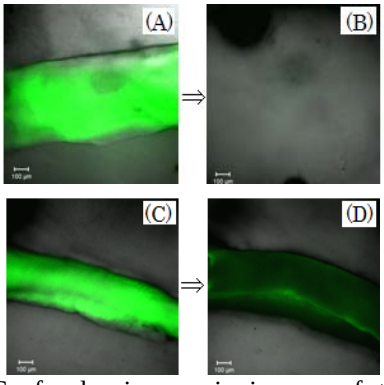

Figure 1. Confocal microscopic images of the FITC-labeled rod in collagen gels in the absence (A, C, D) and presence (B) of sorbitol. The images were obtained before (A, C) and after (B, D) changing media. The concentration of sorbitol is 10 mg/ml. Bars correspond to 100 μm.

After the sorbitol addition, the cell suspension of GFP-labeled MS-1 was injected into the resulting channel. As a result, GFP-labeled MS-1 were found in the restricted area that was formed by the removal of the rod (Figure 2A). Similar to the post-seeding method, GFP-labeled MS-1 were found on the channel surface after the removal of the MS-1-attached rod, probably due to transferring the attached MS-1 to the channel surface simultaneously with the rod removal (Figure 2B).

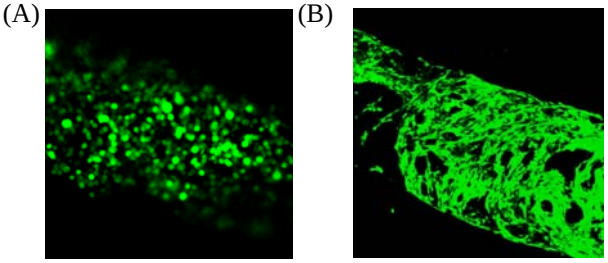

Figure 2. Confocal microscopic images of GFP-labeled MS-1 in the collagen gel. Post-seeding method (A) and pre-seeding method (B).

Conclusions: The present hydrogel system of sugar-responsive water-solubilization is a promising sacrificial template to create vascularized tissue constructs *in vitro*.

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