Cross-linking and polymer immobilization of decellularized blood vessel for bioscaffold application

Kwangwoo Nam[1)], Ayako Murakoshi[1)], Tsuyoshi Kimura[1)], and Toshiya Fujisato[2)], and Akio Kishida[1,2)].
[1)] Division of Biofunctional Molecules, Institute of Biomaterials and Bioengineering,
Tokyo Medical and Dental University, Tokyo, Japan
[2)] Department of Regenerative Medicine and Tissue Engineering,
National Cardiovasucular Center Research Institute, Osaka, Japan
TEL: 03-5280-8029 FAX: 03-5280-8005 E-mail: bloodnam.fm@tmd.ac.jp.

Statement of Purpose: Constructing an engineered tissue for cell culture has been a major interest in bioscaffold field. It is very important to construct a matrix that is physically and biologically suitable for cell culturing. Synthetic and bioscaffold is widely used for the cell culture, but, synthetic scaffold causes problems such as too rigid property, complicated structure, and inflammatory reaction. Bioscaffold shows toxicity, and shrinkage when culturing the cell, and it is mechanically weak. Furthermore, calcification is also reported [1]. In this study, we report on physical and biological affect of cross-linking of bioscaffold which was obtained from ultra high pressure method [2]. And based on the cross-linking result, we immobilized an anticoagulant polymer [2-methacryloyloxyethyl phosphorylcholine (MPC) polymer], which is known for its good hemocompatibility [3], to obtain a bioscaffold having advantageous property of synthetic- and bioscaffold.

Methods: Decellularized blood vessel tissue was obtained by ultra high-pressure method [2] was cleansed using ethanol. Then glutaraldehyde, and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC)/ *N*-hydroxysuccinimide (NHS) was added to the aqueous solution (pH7.4) containing decellularized tissue for the cross-link. Each cross-linked bioscaffold was named GVat and EVat. In order to prepare a bioscaffold with suppressed protein interaction, MPC polymer was cross-linked with tissue using EDC/NHS as cross-linker (MiVat) at 4°C for 24 hours. And to obtain a bioscaffold with higher amount of MPC polymer, we reimmobilized the MPC polymer on MiVat (MdVat). With these bioscaffold, we investigated the surface structure and cross-linking structure of the bioscaffold. Furthermore, physical property and biological property of the bioscaffold was characterized.

Results/Discussion: The cross-linking of the tissue was corfirmed by detecting amount of free amine groups. Free amine groups decreased as the tissue was cross-linked. Tissue that was cross-linked with polymer showed much lower free amine group contents, indicating that the cross-linking of the polymer was successful. The cross-linking is increasing the shrinkage temperature, implying that the strong network is formed inside the engineered tissue. Figure 1 shows the picture of decellularized tissue, EVat, GVat, MiVat, and MdVat that are kept in the oven at 80°C for 24 hours. It shows that cross-linked bioscaffold shows that almost no shrinkage had occurred. Morphology of the tissue observed with SEM showed that after cross-linking, the pores of the tissue are becoming smaller and forms much ordered layered-structure.

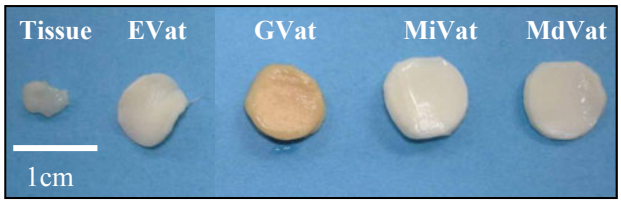
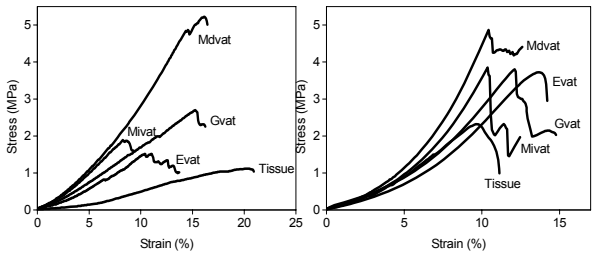


Figure 1. Picture of tissue samples after 24 at 80°C.



(a) (b)

Figure 2. Stress-strain curve of respective bioscaffold. (a) longitudinal and (b) circumferential.

Mechanical property test of the bioscaffold showed that the cross-linking is increasing the elongation modulus (Figure 2). For decellularized tissue, since there is no force that can hold the microfibrils together, it performs relatively low elongational modulus. In the case of cross-linked and polymer immobilized tissues, the strong network is holding the microfibrils together.

The strong network prohibits the degradability caused by the collagenase. The strong network is protecting the bioscaffold from degradation. After 2 weeks in the collagenase aqueous solutions (100IU/mL, at 37°C), approximately 50% of the polymer-immobilized bioscaffold remained, while decellularized tissue, EVat, and GVat degraded completely

The cell (L-929) that was seed on the modified bioscaffold proliferated without any problem. The toxicity by the cross-linker or the immobilized polymer was not shown. The contraction of the bioscaffold by the cell did not occur. It is thought that the stronger network inside the bioscaffold is holding the microfibrils tightly.

Conclusions: Polymer-modified bioscaffold showed physical stability and toughness. It was non-toxic and no sign of calcification was seen. The cells proliferated without any problem.

References: [1] Wissink MJB et al., Biomaterials 2001; 22:151-163. [2] Fujisato T. et al., *in* Cardiovascular Regeneration therapies using Tissue Engineering approaches. Springer, 2005;pp.83-94. [3] Ishihara K. Sci Technol Adv Mater 2000;1:131-138.