Application of high-hydrostatic pressure technique for the preparation of PVA–heparin hybrid gel

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Statement of Purpose: Poly(vinyl alcohol) (PVA) is a one of the most actively studied polymer in the biomaterials field due to its excellent characteristics of biocompatibility and material properties that can be easily modified. However, PVA has many problems for long term implantation, such as complement activation and calcification. To overcome this problem, heparin is adopted to PVA for the hybridization to control release the heparin. However, the hybridization of the PVA and heparin is not easy for the possibility of the phase-separation during the hybridization. An effective method for the control release of heparin from the PVA is avoiding the phase separation between heparin and PVA at high heparin concentration is required.

We have found out that the PVA aqueous solution forms gel in high-hydrostatic pressure within 10 minutes due to the formation of hydrogen bond. [1)] Moreover, the incorporation of second component with PVA leads to the formation of intermolecular hydrogen bonding, which shows that high-hydrostatic pressure is an effective method for the hybridization of two different components. [2)] In this study, we are going to introduce a novel method for the preparation of PVA-heparin hybrid gel with high stability and good control release property.

Methods: Heparin (130IU/mg) and PVA (PVA-HC, polymerization degree: 1750, saponification degree: 99.6%) was mixed in a mold (10wt%) by autoclave before pressurizing the it in the cold isostatic pressurization machine at 980 MPa for 10 minutes. The hybridized gel (HHP gel) was taken out and preserved in saline buffer in 4°C. The structure and the distribution of the heparin within the HHP gel were confirmed with Scanning electron microscope (SEM). The swelling ratio, erosion of the gel, and the possible release of heparin from the gel in aqueous solution were evaluated. A PVA–heparin hybrid gel was also prepared by freeze–thawing (F/T) method using same mold with same PVA and heparin concentration for the comparative study.

Results: The antithrombogenenity of the heparin after autoclave and high pressure was executed prior to the hybridization. The activity of heparin was not be affected by the physical stimuli implying that the autoclaving or high pressure for the gelling would not affect the activity of heparin. Figure 1 shows the photographic image [(a) and (b)], SEM images of HHP gel and F/T gel [(c) and (d)], and the results of sulfur spectra within the gels [(e) and (f)]. The F/T gel and HHP gel were both white-opaque gel which possess elasticity. However, the time required for the gelling was much shorter for HHP gel (10 minutes). The distribution of sulfur, which represents the existence of heparin was concentrated outer part for F/T gel [Figure 1(e)]. On the other hand, the distribution of sulfur was relatively homogeneous for HHP gel [Figure 1(f)]. This is thought due to the fast gelling time. The swelling ratio of the HHP gel was approximately 3 times higher than that of F/T gel. The erosion during the swelling of the gel was lower for HHP, implying that the HHP gel is much highly stabilized for HHP gel. This is due to the difference in the gelling process where the F/T gel is formed by the crystalline lamellar structure during freeze-thaw process, and HHP gel is formed by the intermolecular hydrogen bond during high pressurization. The release of heparin was different for F/T gel and HHP gel. For F/T gel, approximately 80% of heparin was released within 24 hours. The release was almost completely within 3 days. On the other hand, HHP gel showed suppressed heparin release after initial burst at 1 hour (20 %). This is thought to due to the distribution of heparin within the PVA gel and the low erosion rate.

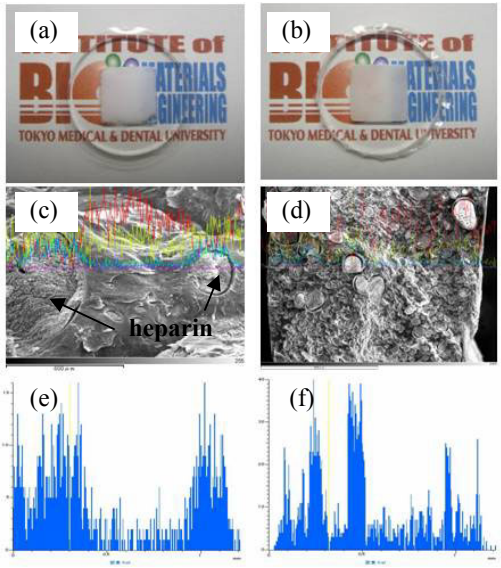

Figure 1. Photographic image [(a) and (b)], SEM images of F/T gel and HHP gel [(c) and (d)], and the results of sulfur spectra taken by EDX within the gels [(e) and (f)]. Left images are all F/T gels and right images are HHP gels.

Conclusions: By applying high pressure to the PVA-heparin aqueous solution, the hybridization of PVA and heparin with homogeneous distribution of heparin within the PVA matrix was achieved. The gelling process requires only 10 minutes. The release of heparin from HHP gel was suppressed. We believe that high-hydrostatic pressurization is an effective method for fabricating PVA hybrid material which can be applied for diverse biomaterials such as artificial blood vessel.

References 1) Kimura T.*et al.* Mater. Sci. Eng. C. 2004; 24:797–801. 2) Mutsuo S. *et al.* J. Polym. Sci. B: Polym. Phys. 2008;46:743–750.