Recoverable Polymer Hydrogel Composed of Novel Phospholipid Polymer for 3D-Tissue Culture

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Statement of Purpose: To make 3-dimensional hydrogel matrix with water-soluble polymers will be provided new fixation method of target cell and tissues. Also, the hydrogel can entrapped bioactive proteins inside of the polymer network and released them continuously. First gelation of the polymer chains without strong reaction condition is necessary for this purpose to avoid the reduction of activity of the immobilized cells and entrapped bioactive proteins. In our previous study, we succeeded spontaneous gelation of water-soluble polymers from their aqueous solution by hydrogen bonding formation under room conditions. Also, that hydrogel could be reversible dissolved by small change in pH. However, the pH inside of the hydrogel formed was low, so, it could not apply for cell culture and tissue generation. In this study, we prepared new gelation system with water-soluble polymers, which can be done in physiological pH range. The polymers are poly(2-methacryloyloxyethyl phosphorylcholine(MPC) -co-*n*-butyl methacrylate(BMA) -co-*p*-styrene boronic acid(SBa)) (PMBBa) and poly(vinyl alcohol)(PVA). The PMBBa has biocompatible moiety(MPC)[1] and hydrophobic cell attaching moiety(BMA) and reactive moiety for diol compound(SBa). This water-soluble polymer could react selectively with PVA chains and made polymer network under mild conditions. Using this novel hydrogel system, we cultured model cell and discussed cell viability.

Methods: The water-soluble polymer, PMBBa was prepared using corresponding monomers by a conventional radical polymerization. NMR and FT-IR spectroscopies confirmed the chemical structure. The molar fractions of the MPC, BMA, and SBa moieties in the polymer were 0.6, 0.3, and 0.1, respectively. The structure is shown in Fig. 1. The PVA was commercially available

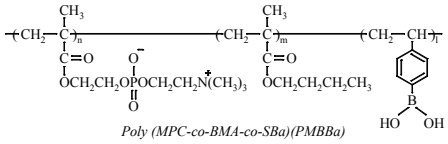
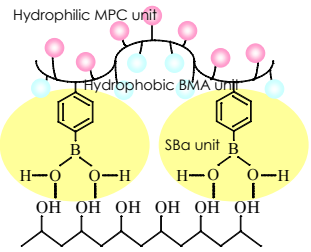


Fig. 1 Chemical structure of PMBBa

one. After preparing aqueous polymer solutions, two polymer solutions were mixed together with a given amount. Shaking gently for 5-10 sec, hydrogel was formed. In the case of cell culture, the polymer solutions were prepared with a cell culture medium. The hydrogel was prepared by gently shaking at room temperature. Then L-929 fibroblast cells in cell culture medium was poured on the hydrogel and kept at 37°C in 5% CO_2 incubator. We observed the shape and morphology of the L-929 cells with a phase-contrast microscope.

Results / Discussion: The boronic acid is well-known compound, which can react with hydroxyl groups selectively and reversibly and used as a protecting group of hydroxyl group in organic synthesis. If the boronic acid moiety is introduced in the polymer chain and the polymer is mixed with multivalent alcoholic compound, it is expected to make cross-linking reactions between these compounds[2]. So, we hypothesized that PVA is suitable polymer as that multivalent alcoholic compound to make polymer network to have a hydrogel. By considering biocompatibility and cell attaching ability, we designed a novel polymer, that is, PMBBa as a boronic acid-containing polymer. When the aqueous solution containing PMBBa and PVA was mixed, a hydrogel formed spontaneously under room temperature as shown in Fig. 2. The hydrogel was transparent and had sufficient mechanical strength as a cell culture scaffold. This polymer hydrogel can dissociate again by addition of other multivalent alcoholic compound such as glucose. The concentrations of the polymer were in the range between 0.63% and 5.0%. The L929 cells were applied on the hydrogel, and observed. After 3h the cells could not adhere and their shape was spherically. We took the cells and recultured on the normal tissue culture dish. The cells could adhere and spread on the culture dish after 1-day culture. On the other hand, in the hydrogel, cells still maintain their spherical shape. And this phenomena continuously observed for 3-days. That is, cells interacted very weakly with the hydrogel networks,



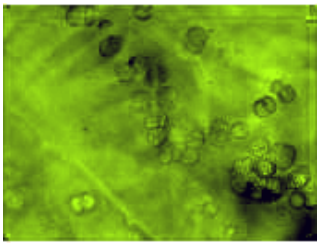


Fig. 3 L929 cells in the hydrogel after 3-days culture.

but maintained their original shape even after 3-days culture.

Conclusion: In this study, we obtained water-soluble novel phospholipids polymer, which can make spontaneously gelation with other multivalent polyol, PVA. The cell could permeate into the hydrogel and maintain original spherical shape. The cell detached from the hydrogel could spread on cell culture plate. No effect induced to the cells. That is, this hydrogel making system is effective for immobilizing cells. We will apply bioactive proteins to control cell function in the hydrogel.

Reference:

1) K.Ishihara, et al., J.Biomed.Mater.Res., 24, 1069 (1990).
2) S.Kitano, et al., J.Controlled Release, 19, 162 (1992).