Mechanical property-tunable and cytocompatible phospholipid polymer hydrogels for cell encapsulation

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Statement of Purpose: As the fields of regenerative medicine and stem cell engineering started to play the important roll in the new generation of bioengineering, the cells are starting to be treated as one of the materials to be controled, rather than those to be observed. Temporal and spatially encapsulation of the cells by the hydrogels are getting the attention as a novel way to handle the cells in the three dimentional condition. It is important to be able to have control over the cell function through the environmental condition. The purpose of this research is to clarify the relationship between the physical property of the hydrogels and the cells. Making the hydrogels with polymer with biocompatible moiety made it possible for cells to be captured without damaging its functions. Also the cells are known not to adhere on the 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer surface, which makes it possible for the cells to stay in the three dimensional condition even though it is surrounded by the polymers. The moiety with boronic acid, (*p*-vinylphenylboronic acid, VPBA) enabled the hydrogels to dissolve under some condition, which made it easier to analyze the cells. Storage modulus is measured as one of the physical property, and the relationship between the multiplication rate of the cell was discussed in this study.

Methods: Three kinds of water-soluble polymer Poly (MPC-*co*-*n*-butyl methacrylate-*co*-VPBA)(PMBV, Figure 1) were synthesized by radical polymerization. Mixture of PMBV aq. solution and poly(vinyl alcohol)(PVA) aq. solution under the biological condition makes PMBV/PVA hydrogels. Storage modulus of the PMBV/PVA hydrogels with different number of cross-links was measured by rheometer. The number of cross-links was changed through changing not only the VPBA mole fraction of the polymer but also the PMBV ratio of the hydrogels. The combination between the boronic acid and diol of the PVA could be changed to other diols with stronger binding constant. Thus, PMBV/PVA hydrogels could dissolve in sugar solution. PMBV/PVA hydrogels was made with cell suspended medium (D-MEM with 10% FBS) with the density of 2.3×10^4 cells/mL, and the number of entrapped cells (non-osteogenic mouse pluripotent cells, C3H10T1/2) was counted by dissolving the gel after 4 days of incubation.

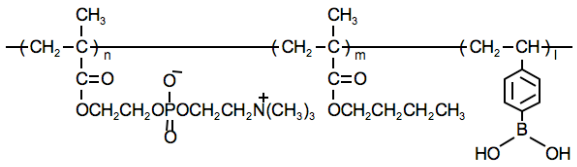

Figure 1. The chemical structure of PMBV

Results: PMBV was successfully synthesized with the VPBA mole fraction of 0.32, 0.16, and 0.12, determined by ^{1}H-NMR. The other properties such as molecular weight were almost the same for all three polymers. By changing the number of cross-links the storage modulus of the PMBV/PVA hydrogels varied from 108 N/m^2 to 720 N/m^2.

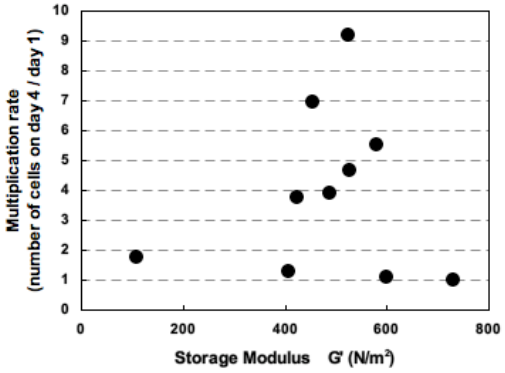

Figure 2. The relationships between the storage modulus of the hydrogels and the multiplication rate of the cells encapsulated in the hydrogels

All hydrogel maintaind cells more than four days after encapsulation. This is due to cytocompatibility of PMBV. Figure 2 is the combination of the results of the rheology mesurement and the cell number measurement. It shows that cells do not multiply when the storage modulus of the hydrogel entrapped is below 400 N/m^2 or above 600 N/m^2. When the hydrogel has storage modulus from 400 N/m^2 to 600 N/m^2, the number of cells do multiply. Also the encapsulated cells maintained the round shape during the four days of culture. One of the factors that change the storage modulus is the number of cross-links per volume, which also relates to the space provided for cell. It could be considered that when the storage modulus is more than 600 N/m^2, there will not be enough space for cell to proliferate.

Conclusions: The proliferation rates of the cells differ according to the storage modulus of the hydrogels the cells are encapsulated. When the cells are cultured three dimensionally in the hydrogels, the storage modulus of the hydrogels should be considered regarding to the proliferation function you wish the cell to have. For design the scaffold for cell engineering or regenerated medicine, mechanical properties of the scaffold may be one of the important factors to maintain cells actively.

References: K Ishihara, *et al. J Biomed Mater Res* 1998; **39**:323-330, T Konno, *et al. Biomaterials* 2007; **28**:1770-1777