Abstract (論 文 の 内 容 の 要 旨)

論文題目　　Cytocompatible polymer hydrogel matrices for functional control of encapsulated stem cells
（細胞適合性ポリマーハイドロゲルマトリックスに内包した幹細胞の機能制御）

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1. Introduction

Developments of advanced medicines based on cell engineering for applications in fields such as regeneration of tissues and organs and cell-based therapies have been expected to prosper in near future [1,2]. In such medicines, the cells are regarded as one of the materials to answer the needs. In this perspective, preparing cells with well-defined functional properties is important for use of cells in engineering bases [3].

The cells change its function in reaction to the surrounding environment in terms of both chemical signals and physical conditions. A lot has been known over this past few decades for the chemical signals. There are numerous chemicals to change cell proliferation or to induce differentiation. Physical conditions that changes the cellular state of being has also been demonstrated especially in two-dimensional culture, regarding its adhesion cites [4] and mechanical signals applied to the cells through the adhesion and substrate stiffness [5]. These ideas have been extended in a three-dimensional (3D) culture system as well, by creation of cell-interacting hydrogel as an artificial extra cellular matrix (ECM). The ECM consists of diverse parameters which interacts and influences with each other. It has been known that there are some features of the cells that could only be achieved through the 3D cell culture system for it is the environment the cells have *in vivo*.

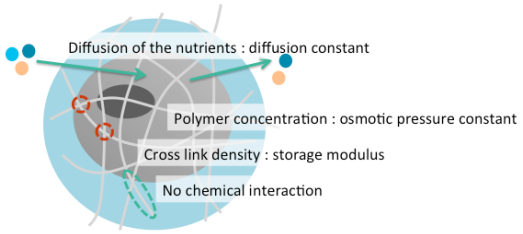

Fig.1 The illustrated image of the cytocompatible polymer matrices.

In this study, a new idea of the 3D matrix for cell culture is proposed [6]. The generation after mimicking the ECM will be a matrix beyond the ECM. By means of “beyond the ECM”, the

independency from of each parameters and the control of each parameters will be beyond the range of what it is in real ECM. This would not only provide deep understanding in the biological science, but also a new way to handle cells for application in a new medicine.

The matrix consists of two features. 1) A cytocompatible interface that does not have any direct interaction with the encapsulated cells. 2) A controlled and quantified physical properties (Fig.1). This is achieved through synthetic chemistry by creating a precise and describable structure. By using cytocompatible polymer, physical signals could be applied to the cells without changing the amount of chemical signals. The physical property of the matrix will be controlled through the mechanism of polymer network structure formation. Further more, the polymer network will be given a reversible binding formation for crosslinks to collect the encapsulated cells in times of needs.

2. Preparation of cytocompatible polymers for 3D polymer network

The water-soluble polymer with *p*-vinylphenylboronic acid (VPBA) unit, poly(2-methacryloloxyethyl phosphorylcholine (MPC)-*co*-*n*-butyl methacrylate (BMA)-*co*-VPBA) (PMBV, Fig. 2) was synthesized [6-8]. The monomer composition and molecular weight of the polymer were controlled through the monomer ratio in feed and the choice of the initiator. The binding constant of VPBA unit in PMBV with diol compounds was quantified as shown in Fig. 3. It was found that the polymer state inside the aqueous solution was important to enhance the binding ability of the VPBA unit. The polymer state was influenced by the BMA unit composition and polymer concentration. The best balance between the amount of BMA unit and the VPBA unit were achieved by PMBV622, which consists 22 mol% of BMA unit and 16 mol% of VPBA unit, respectively [7].

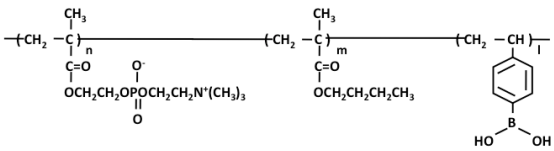

Fig. 2. The chemical structure of Poly(MPC-*co*-BMA-*co*-VPBA)(PMBV)

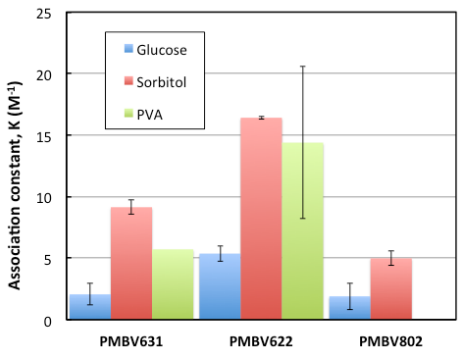

Fig.3 The association constant between PMBV and diol compounds. The number after PMBV indicates the monomer unit ratio of MPC, BMA, and VPBA respectively.

3. Preparation of property tunable 3D polymer matrices

Formation of PMBV/PVA matrix by mixing cytocompatible PMBV solution with PVA solution was confirmed. The storage modulus and osmotic modulus were analyzed as the physical property of the matrix. The storage modulus could be controlled through the VPBA unit density of the matrix (Fig. 4). The osmotic modulus was controlled through the addition of poly(MPC) (PMPC) to the matrix. The VPBA unit density and PMPC concentration could be controlled independently and thus, those two parameters were controlled separately. Finally, the protein diffusion inside the PMBV/PVA

matrix was analyzed and the diffusion constant of the bovine serum albumin was not influenced by its storage modulus. This is because there is no interaction between polymer and the protein, and the size of the mesh was large enough of the protein to diffuse in both storage modulus.

4. Cellular function control through 3D polymer matrices

Cells of two different lineage, murine mesenchymal stem cells (C3H10T1/2) and murine fibroblast (L929) were encapsulated in the PMBV/PVA matrix. The encapsulated cells were dispersed and kept its spherical shape inside the PMBV matrix. The viability of the cells was confirmed, and the cells could be collected from the matrix by dissociating the crosslinking by D-sorbitol. The proliferation of the cells was effected by the storage modulus of the PMBV/PVA matrix (Fig. 5). The proliferation of the cells was suppressed in the PMBV/PVA hydrogel. Yet at some storage modulus, the cells proliferated into a spheroid. The storage modulus of cell proliferation was different for each cell. C3H10T1/2 proliferated in the storage modulus of around 0.7 kPa, and L929 proliferated at 1.6 kPa. This result indicates that the cell proliferation was effected by the storage modulus, and not by the space the matrix creates.

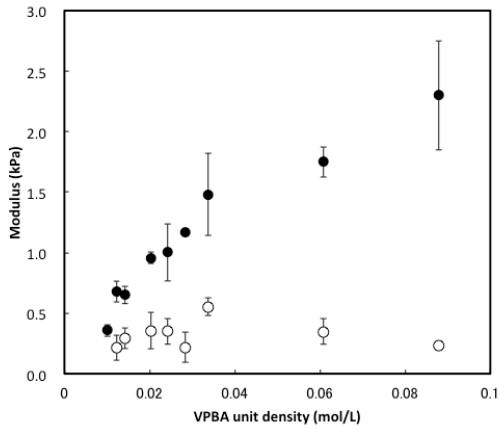

Fig.4 The relationship between the VPBA unit fraction and the storage modulus of the PMBV matrix.

Further analysis was done for function of C3H10T1/2 and the storage modulus of the PMBV/PVA matrix. When the storage modulus was more than 1.0 kPa, the proliferation was suppressed and the cell cycle was converged to G1/G0 phase. Not only the cell proliferation reacts to the initial storage modulus, the proliferation could be restarted by lowering the storage modulus by swelling the matrix. Fig. 6 shows the relationship between the storage modulus of the hydrogel and the proliferation rate of the cells. As the matrix was swelled at day 1 and the storage modulus was lowered, the cells started to proliferate and doubled its number by day 4. Through this process, the proliferation control through environment was achieved. Signal sensitivity of the cells was improved by the proliferation control, and it was demonstrated by differentiation induction of the encapsulated C3H10T1/2 (Fig. 7) [8].

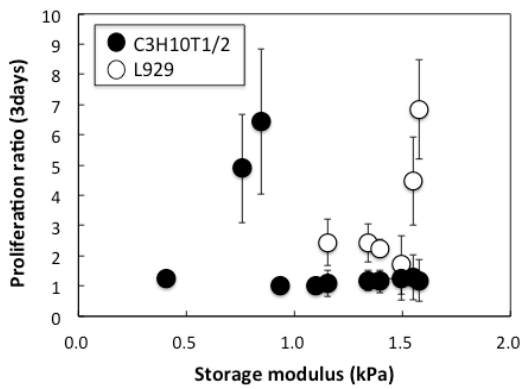

Fig.5 The relationship between the proliferation ratio of the encapsulated C3H10T1/2 and L929 and the storage modulus of the PMBV matrix

The expression of membrane protein, integrin and cadherin were quantified during the 3 days culture in the PMBV/PVA matrix. Both protein expressions increased, indicating the active output of the protein on the membrane. The hypothesis that chemical reaction inside the cells change in reaction to the pressure or tension that is caused by its surrounding environment, and the threshold of the pressure is dependent of the cell lines were introduced through this result.

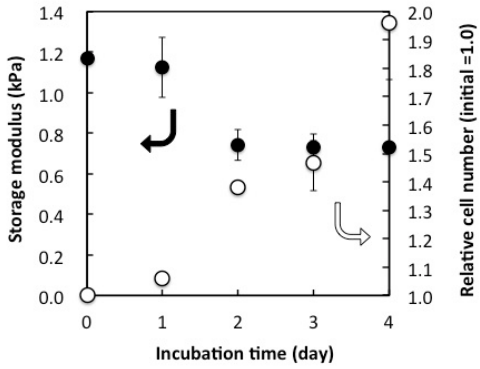

Fig. 6 The storage modulus of the PMBV/PVA matrix and the encapsulated cell number. The medium was added at day 1 to swell the hydrogel.

5. Conclusion

Cytocompatible polymer hydrogel matrices with tunable physical properties were prepared. The storage modulus and the osmotic modulus were actively controlled, while there was no chemical interaction between proteins or encapsulated cells. The encapsulated cell proliferation was controlled by the storage modulus of the PMBV/PVA matrix. When the cell proliferation was suppressed, the cells were converged into G1/G0 phase of the cell cycle. The sensitivity to differentiation signal had increased while both cell proliferation and cell cycle were controlled by the physical properties of the matrices. Encapsulation of stem cells in the matrix before signal induction will provide a new step for cell source production for regenerative medicine by enhancing the number and quality of the cells. Also well-defined physical properties of the matrices will contribute to the further understanding of the cellular reaction toward its surroundings. In conclusion, a new idea of cellular matrix is introduced and it is hoped that this matrix will bring new step to the field of cellular engineering.

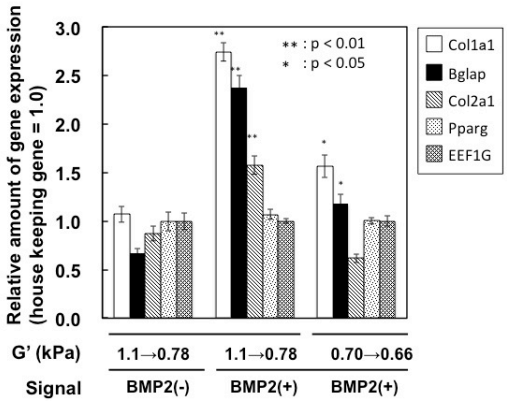

Fig. 7 The gene expression of C3H10T1/2 cells three days after addition of BMP-2. The expression of Col1a1, Bglap, Col2a1, Pparg are normalized to EEF1G, a house keeping gene.

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