Cell cycle unification based on tuning of cellular environments using cytocompatible and reversible forming phospholipid polymeric hydrogels

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Statement of Purpose: As the field of regenerative medicine and stem cell engineering started to play the important role in the new generation of bioengineering, the cells are starting to be treated as one of the materials to be controlled and optimized rather than those to be observed. Temporal and spatial encapsulation of the cells by the hydrogels are getting an attention as a novel way to handle cells in the three dimensional condition. Understanding the relationship between cell function and its environment is important to take passive control over the cell function through outer signals. Polymeric cellular environment is full of potentials for these needs since the environmental properties could be constructed. The 2-methacryloloxyethyl phosphorylcholine (MPC) polymers do not show adhesion of cells, which makes it possible to eliminate the chemical parameter of the polymeric environment by making polymer hydrogel with the MPC polymer. The moiety with boronic acid enables the hydrogels to dissolve under some condition, which made it easier to analyze the cells. The passive control of cell proliferation and cell cycle by physical property of the cellular environment were done in this study.

Methods: Poly(MPC-*co*-*n*-butyl methacrylate-*co*-*p*-vinylphenylboronic acid) (PMBV) was synthesized. The PMBV/poly(vinyl alcohol)(PVA) hydrogels were prepared by mixing of cell culture medium containing 5.0 wt% PMBV and 2.5 wt% PVA. The cross-link density of the PMBV/PVA hydrogels were controlled by changing the mixing ratio of the two polymer solutions and measured as its storage modulus. The non-oseteogenic mouse pluripotent cells, C3H10T1/2 were encapsulated in the PMBV/PVA medium (D-MEM with 10% FBS) hydrogel by suspending the cells in PMBV solution with the final density of 5.0 x 10^5 cells/mL. The number of cells were counted every 24 h of incubation, and the cell phase were determined by PI staining.

Results: The storage modulus of the PMBV/PVA hydrogels was controlled in the range between 0.3 kPa and 2.0 kPa. The cells proliferated and made spheroids only in the PMBV/PVA hydrogel with storage modulus between 0.5 kPa and 1.0 kPa. The further studies were done using PMBV/PVA hydrogel with storage modulus of 0.7 kPa (proliferated) and 1.1 kPa (non-proliferated)(Fig. 1). The 90% of the non-proliferated cells, which were encapsulated in 1.1 kPa PMBV/PVA hydrogel, were converged to G1 phase in 24 h of incubation (Fig. 2). Which is much higher than the cells encapsulated in 0.7 kPa PMBV/PVA hydrogel or the cells cultured on TCPS, which shows good relationship to the proliferation rate.

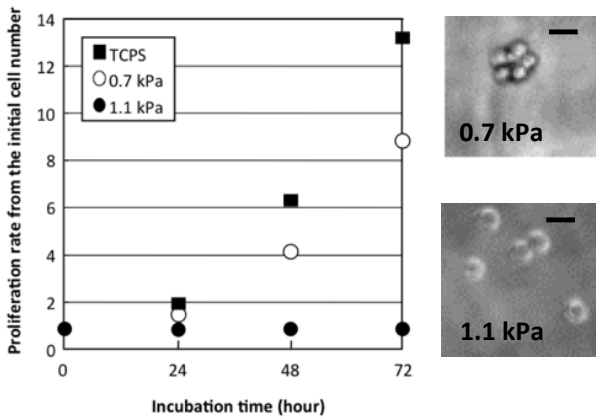

Figure 1. The proliferation rate of the cells during 72 h of incubation (left) and the phase contrast microscopic image of encapsulated cells after 72 h (right). The proliferation rate of the cells encapsulated in PMBV/PVA hydrogels with various storage modulus (0.7 kPa : ○, 1.1 kPa : ●) were suppressed compared to those cultured on TCPS (■). Scale bar : 25μm.

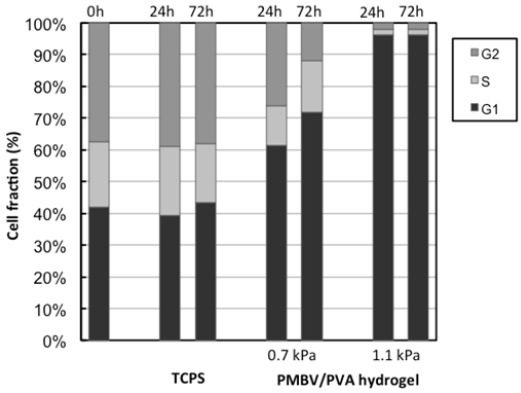

Figure 2. The cell cycle fraction of C3H10T1/2 cells in G1(black), S(light gray), G2(dark gray) phase after 24 h or 72 h incubation on TCPS or in PMBV/PVA hydrogels measured by flow cytometer. The initial fraction of cell phases are shown as 0 h.

Conclusions: The PMBV/PVA hydrogel provides a uniformed cells; especially high G1 phase ratio will lead to excellent differentiation efficiency. Moreover, PMBV/PVA hydrogel will realize the elucidation of relationships between environment's physical signals to cell functions, which will bring great insight to the bioengineering field.

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