Enhancement of Cell Differentiation by Phospholipid Polymer Hydrogels with Tunable Physical Properties

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Statement of Purpose: A large number of lineage-committed progenitor cells are required for tissue engineering and cell-based therapies. Pluripotent stem cells have emerged as a vital source for generating transplantable cells for use in regenerative medicine due to their ability to differentiate into multiple cells types. The efficiency of differentiation into specified cell type is crucial topic in the usage of pluripotent stem cells. The differentiation process occurs during the cell turnover, and signal sensitivity is known to be associated with G1 phase of the cell cycle. We hypothesized that the enhancement of efficiency of lineage-restricted differentiation can be achieved using three-dimensional (3D) culture environments. Our purpose is to develop an ideal 3D cell culture environment that allows us to take control over cell turnover; converging the cells in to G1 phase, and synchronizing the restart of the cells turnover.

Methods: Poly(2-methacryloloxyethyl phosphoryl choline-*co*-*n*-butyl methacrylate-*co*-*p*- vinylphenylboronic acid) (PMBV) was synthesized[1]. The PMBV/poly(vinyl alcohol)(PVA) hydrogels were prepared by mixing of cell culture medium (DMEM with 10%FBS, 1% PC/SM) containing 5.0 wt% PMBV and 2.5 wt% PVA in 6/4 volume ratio to achieve PMBV/PVA hydrogel with storage modulus of 1.1 kPa. Medium with half the volume of the hydrogel was added to the PMBV/PVA hydrogel to swell. The storage modulus of the PMBV/PVA hydrogel was measured after the swelling. The non-oseteogenic mouse pluripotent cells, C3H10T1/2 were encapsulated in the PMBV/PVA hydrogel by suspending the cells in PMBV solution with the final density of 5.0 x 10^5 cells/mL. The proliferation and cell cycle of C3H10T1/2 were measured every 24 hours for 4 days: 1day before and 3 days after the swelling. The bone morphogenetic protein 2(BMP-2) was applied to the C3H10T1/2 cells encapsulated in the PMBV/PVA with storage modulus of 1.1 kPa for 1day. The hydrogel were swelled to lower its storage modulus to 0.78 kPa. The cells were cultured inside the hydrogel for 3days before the cells were recollected after dissociating the hydrogel with 0.3 M sorbitol solution. The differentiation efficiency was evaluated through gene expression of the osteoblast marker measured by PCR method.

Results: The storage modulus of the hydrogel was lowered by swelling and increasing its volume(Fig.1, left). The loss modulus stayed lower than the storage modulus, indicating the maintenance of the 3D network. The cells encapsulated in PMBV/PVA hydrogel were known to change its proliferation depending on the storage modulus of the hydrogel. The cells proliferate when the storage modulus of the hydrogel was between 0.5 kPa and 1.0 kPa, and it suppresses the proliferation when the storage modulus was over 1.0 kPa [1]. It was also true, when the storage modulus of the hydrogel were changed while the cells were encapsulated and cultured (Fig.2, right). More than 90% of the cells were converged to G1 phase in the first 24 hours. The differentiation efficiency of the encapsulated cells increased about 5.0-fold compared to the cells induced on the TCPS with same procedure. This is due to the proliferation and phase control of the cells by the PMBV/PVA hydrogel. The cells were converged to G1 phase when the differentiation signal was induced, and the proliferation was restarted at the same time.

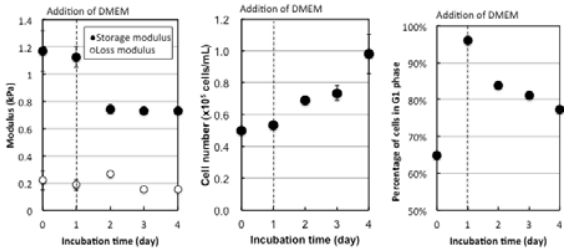

Figure 1. The storage modulus and the loss modulus of the PMBV/PVA hydrogel 1 day before and 3days after the swelling(left) the proliferation(middle) and the G1 phase ratio(right)of the cells encapsulated in the hydrogel during the modification of the storage modulus.

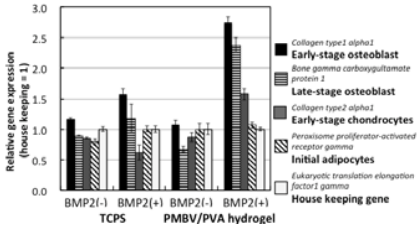

Figure 2. The gene expression of C3H10T1/2 cells, which differentiation signal BMP-2 was induced in hydrogel or on the TCPS.

Conclusions: More than 90% of the cells encapsulated inside the PMBV/PVA hydrogel converge in to G1 phase depending on hydrogel's storage modulus. The cells restart its proliferation as the storage modulus of the hydrogel was modulated. The differentiation is enhanced when this process is associated with differentiation signals. The PMBV/PVA hydrogel, which has controllable storage modulus, and ability to recollect encapsulated cells are suitable for preparation of homogeneous cell source for tissue engineering.

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References: [1] H. Oda, T. Konno and K. Ishihara. *Biomaterials*, **34**, 5891-5896 (2013)