Cytocompatible Cell Immobilization 3D Matrix by Reversible Phospholipid Polymer Hydrogel to Manipulate the Differentiation for Elaborating Stem Cell Niche

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Statement of Purpose: The purpose of this study is preparation of a cytocompatible polymer hydrogel to immobilize the functional cells in a 3D environment without any reduction of cell activity. The cytocompatible phospholipid polymer bearing phenylboronic acid moiety was synthesized to prepare a reversible polymer hydrogel using with polyols such as poly(vinyl alcohol) under the mild condition(1). Additionally, the inter-molecular interaction between phenylboronic acid and *cis*-diol can be changed after addition of sugar molecules. In this study, the phospholipid polymer hydrogel was applied as novel 3D environment, which can only immobilize the functional cells. This hydrogel can install the other cell manipulation techniques such as cell adhesive peptide, other scaffold and differentiation reagent delivery to guide the specific function including differentiation of the immobilized cells. The cytocompatible phospholipid polymer hydrogel system named as "Cell-Container" which can maintain the cell functions under the mild condition was powerful and useful platform hydrogel to create the future stem cell niche.

Methods: 2-Methacryloyloxyethyl phosphorylcholine (MPC) was synthesized by the method reported previously. The phospholipid polymer (PMBV) constituting from MPC, *n*-butyl methacrylate and *p*-vinylphenylboronic acid was synthesized by a conventional radical polymerization. The PMBV was purified by an ultrafiltration technique. The cell culture reagents were purchased from Invitrogen Co. Ltd. The PMBV was dissolved in cell culture medium at 5 wt%. The mouse embryonic stem (ES) cells (129/SV) were dispersed in the PMBV solution. The PMBV solution was gently mixed with equal amount of 2.5 wt% poly(vinyl alcohol)(PVA) solution. The PMBV hydrogel immobilizing ES cells was stored in an incubator. The immobilized ES cells were observed by a phase contrast microscopy. The PMBV hydrogel was dissociated by addition of DPBS containing D-fructose after 3 days culture. The recovered ES cells were plated under the conventional culture system. The undifferentiation characterization of recovered cells was performed after 3 days culture. The characterization of cells was estimated by alkaline phosphatase staining. In addition, the immobilized cells in the hydrogel accepted internalization of quantum dot (QD) nanoparticles decorated with cell penetrating peptide molecules(2). The confirmation of cell penetration was observed by confocal laser microscopy.

Results: The PMBV hydrogel was spontaneously formed after mixing of PMBV and PVA solution. The chemical structure of PMBV and crosslinking structure of hydrogel were shown in Fig. 1.

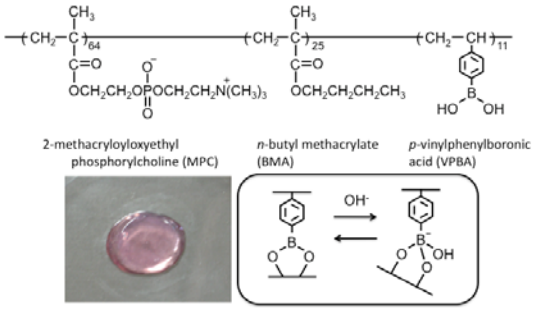

Figure 1. Chemical structure of phospholipid polymer containing phenylboronic acid moiety (PMBV), and crosslinking structure of hydrogel with PVA.

The crosslinking mechanism can be dissociated by changing reaction after addition of D-fructose. The fibroblast cells were immobilized in the hydrogel. The cells did not change their morphology during preservation period, and the cells were hardly proliferated. After dissociation of the hydrogel, the cells were collected with non-invasively, and the cells were directly replated on the conventional culture system. The cells were adhered, spread, and proliferated as usual. This result indicated that the immobilized cells were maintained their basically biological activity during preservation period. The PMBV hydrogel can maintain not only basic activity of the cells but also the specific bioactivity such as differentiation activity. In the case of ES cells, the ES cells were preserved for 3 days. After recover of the ES cells, the ES cells were grown as usual. The ES colonies were well-stained by alkaline phosphatase staining indicated the undifferentiation character of the ES cells.

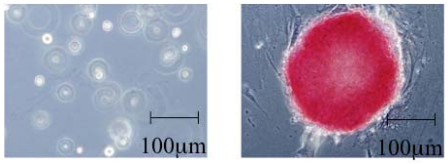

Figure 2. Phase contrast microscope images of ES cells immobilized in PMBV hydrogel for 3 days (left), and result of alkaline phosphatase staining of ES cell colony formed after dissociation of the hydrogel (right).

The confocal laser microscope observation was revealed that the QD nanoparticle conjugated with cell membrane penetrating peptide was well-penetrated into the cells in the hydrogel system (data not shown).

Conclusions: The cytocompatible polymer hydrogel with reversible property was prepared by using molecular interaction between two kinds of polymers. The hydrogel "Cell-Container" which can maintain the cell functions under the mild condition was powerful and useful platform hydrogel to create the future stem cell niche.

References:

(1) Konno T. et al., Biomaterials, (2007);28:1770-1777.
(2) Goto Y. et al., Biomacromolecules, (2008);9:3252-3257.