Design of Tetra-arm PEG-crosslinked Thermoresponsive Hydrogel for 3D Cell Culture

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We synthesized poly(N-isopropylacrylamide) gel containing cell-adhesive peptides, RGDS pendants, and crosslinked by a hydrophilic polymer. Since gelation occurs by simply mixing the polymers, cells can be simultaneously encapsulated inside the gel during gelation. This gel has roughly a 15% volume change between 25 and 37°C, and is transparent at both temperatures. Moreover, adhesion of encapsulated C2C12 cells to the gel could be observed. This thermoresponsive gel would be potentially useful as a cell culture material that can control cell fate by changing mechanical properties of the cell’s external environment.

Keywords Poly(N-isopropylacrylamide), hydrogel, thermoresponsive gel, 3D cell culture

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NIPAAm:NAPMAm = 98:2, $M_n$: 14000), $N$-hydroxysuccinimide-terminated tetra-branched polyethylene glycol (NHS-tetra-PEG) as a crosslinker, and RGDS peptide as a cell adhesive molecule in PBS (pH 7.4) were mixed and incubated for 1 h at 25°C for synthesizing the PEG-crosslinked PNIPAAm gel with RGDS pendants. The hydrogel is referred to as PEG-RGDS-PNIPAAm gel (Fig. 1). The temperature dependent swelling ratio and storage elastic modulus ($G'$) of the PEG-RGDS-PNIPAAm gel were measured by optical microscope observation (MZ16, Leica, Mannheim, Germany) and a rheometer (MCR 301, Anton Paar, Graz, Austria), respectively.

For the 3D cell culture, a solution containing stained C2C12 cells and the polymers were simply mixed and kept in a CO$_2$ incubator at 37°C to form a hydrogel and encapsulate the cells inside the gel simultaneously. The cell-laden hydrogel was observed under a fluorescence microscope (DFC 360FX, Leica) after incubation at 37°C for 24 h.

**Results and Discussion**

A cylindrical shaped gel was used to analyze the temperature dependency of the swelling ratio of the PEG-RGDS-PNIPAAm gel (Fig. 2(a)). Due to the thermoresponsibility of the PNIPAAm gel, the $d/d_0$ value decreased as temperature increased up to 40°C. The VPTT of the PEG-RGDS-PNIPAAm gel was less defined compared to a PNIPAAm gel prepared by using a conventional low molecular weight crosslinker. This is because our PEG-RGDS-PNIPAAm gel does not shrink easily due to bulkiness and hydrophilicity of PEG. Roughly 15 and 7% volume changes could be observed in temperature changes from 25 to 37°C and from 33 to 37°C, respectively. Moreover, the kinetics of the volume change of the gel from 25 to 37°C and from 37 to 25°C (Fig. 2(b)) showed that the volume changes were completed within 20 min. Thus, this gel can apply the volume change as a stimulus to encapsulated cells in under a half hour. Furthermore, transparency of the gel is specifically desired, as transparency allows the cells to be easily observed by microscopy. Usually, PNIPAAm gel becomes opaque in the shrunken state; however, since the PEG crosslinker is bulky and highly hydrophilic, the PEG-RGDS-PNIPAAm gel maintains enough transparency for cell observation even in the shrunken state (Fig. S1, Supporting Information). Storage elastic modulus ($G'$) of PEG-RGDS-PNIPAAm gel at 25, 33, and 37°C were 170, 190, and 210 kPa, respectively. The $G'$ values increased with increasing temperature. This result is attributed to the
thermoresponsive coil-globule transition of PNIPAAm chains. The aggregated network structure in the PNIPAAm gel above the VPTT showed more compact and rigid characteristics than the structure in the swollen state below the VPTT.

Relative viability of C2C12 cells exposed to NHS (Fig. 3(a)), roughly 10 mM of NHS, revealed that NHS produced during the PEG-RGDS-PNIPAAm gel and PEG-PNIPAAm gel after 24 h culture at 37°C. Some cells inside the PEG-RGDS-PNIPAAm gel became slightly deformed. Conversely, cells inside the PEG-PNIPAAm gel maintained a round shape. These results suggested that the RGDS sequence, which is a receptor for cell adhesion molecules, in the PEG-RGDS-PNIPAAm gel mediates cell adhesion by integrins on the cell surface. Because mechanical signals are delivered to the nucleus of a cell through integrins, the integrin-mediated cell adhesion is indispensable when applying mechanical signals to a cell. Therefore, the PEG-RGDS-PNIPAAm gel has the potential to regulate mechanical integrin-mediated signal transduction of a cell by thermoresponsive dynamic volume and elasticity changes.

Conclusion

In this study, we prepared PEG-crosslinked PNIPAAm gel as a smart 3D cell culture material, which changes its volume and storage elastic modulus according to temperature changes, by simply mixing two solutions in a nontoxic gelation process. Because it maintains transparency even in the shrunken state, encapsulated cells inside the gel could be observed by optical microscopy. By introducing RGDS into the network of the gel, adhesion of C2C12 cells could also be confirmed. This gel could be a promising tool that can apply reversible mechanical forces to cells in a spatio-temporal manner. Currently, we are investigating modifications to the molecular design of the gel to produce larger changes in mechanical properties by temperature stimuli. Additionally, biological studies using this material are currently under way.

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Supporting Information

Detailed experimental section and images of the PEG-RGDS-PNIPAAm gel can be found. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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