Dual Stimuli-responsive Sol-Gel Transition Polymers with Photodimerizable Groups for Regulating Cell Behavior

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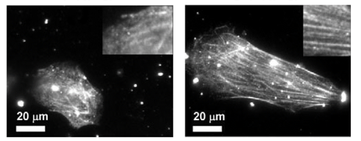
1. Introduction

Stimuli-responsive polymers that exhibit sol-gel phase transition in response to environmental changes such as temperature and pH have attracted considerable attention as injectable polymers and scaffolds for cell culture. Although numerous stimuli-responsive polymers that undergo a sol-gel transition have been reported, the literature contains few accounts of biomolecularly stimuli-responsive polymers that undergo a sol-gel transition in response to a specific biomolecule. We have designed a variety of stimuli-responsive gels that undergo the volume changes in response to a target molecule (Miyata, T. *et. al. PNAS* **2006**, *103*, 1190; Miyata, T. *et. al. Chem. Commun.* **2014**, *55*, 11101). In addition, biotin-conjugated four-armed poly(ethylene glycol) (PEG) that transformed from a sol to a gel state in response to avidin was synthesized as a molecularly stimuli-responsive sol-gel transition polymer (Miyata, T. *et. al. Polym. Chem.* **2017**, *8*, 6378). Our strategy for designing such molecularly stimuli-responsive polymers and gels uses biomolecular complexes as dynamic crosslinks. In this study, as dual stimuli-responsive sol-gel transition polymers for cell culture, we designed two kinds of PEG derivatives with functional groups for forming dynamic crosslinks, *i.e.* photo/molecule- and photo/temperature-responsive sol-gel transition polymers. Cells were cultured within the photo/molecule-responsive PEG derivatives with sol and gel states. Cell behavior was investigated on photo/temperature-responsive gels with different elastic modulus and hydrophilicities.

2. Background

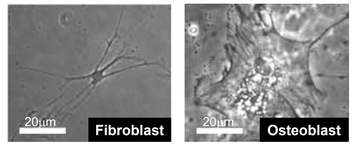
➢Effect of physical properties of scaffolds on cell behavior

Extension



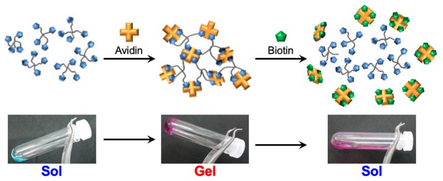
Engler, A. J., *J. Cell Sci.* **2008**, *121*, 3794.

Differentiation



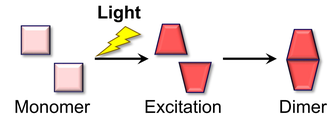
Engler, A. J., *Cell* **2006**, *126*, 678.

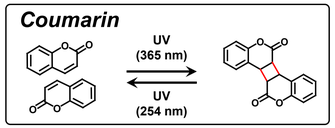
➢Sol-gel phase transition



Norioka, C.; Okita, K.; Mukada, M.; Kawamura, A.; Miyata, T. *Polym. Chem.* **2017**, *8*, 6378.

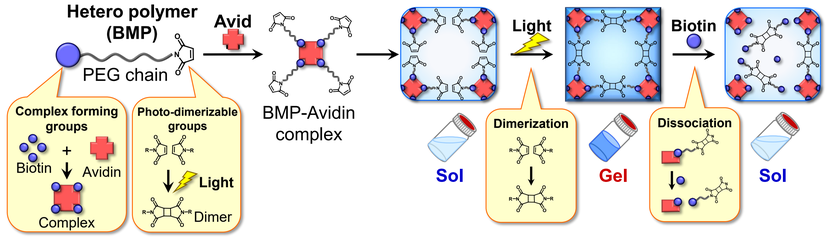
➢Photo-dimerization



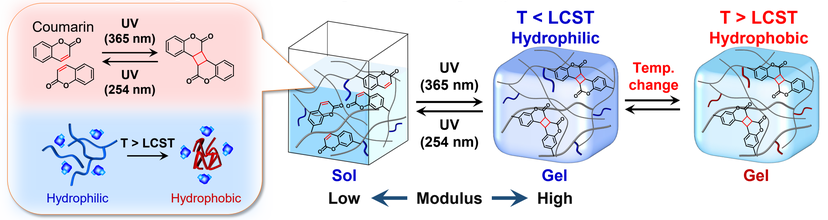


3. In this study

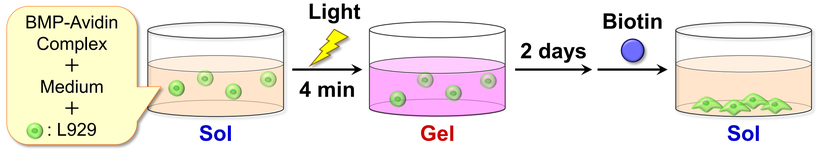
➢Photo/molecule-responsive polymers



➢Photo/temperature-responsive polymers



4. Cell culture in BMP-Avidin complex gel



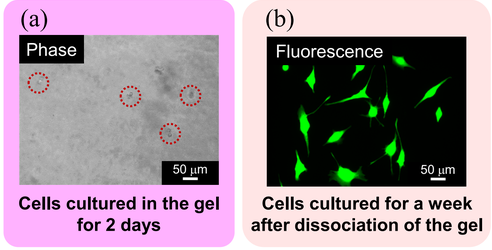


Fig. 1. (a) Phase contrast image of the L929 cells cultured within BMP-Avidin gel for 2 days. (b) Phase contrast and fluorescence image of the L929 cells cultured for a week after the gel changed to a sol state by the addition of free biotin. L929 cells were stained by calcein. (c) Number of L929 cells cultured without (●) and with (●) BMP-Avidin gel.

5. Properties of P(MAC-*co*-OEGMA)

➢Photo/Temperature-responsive behavior

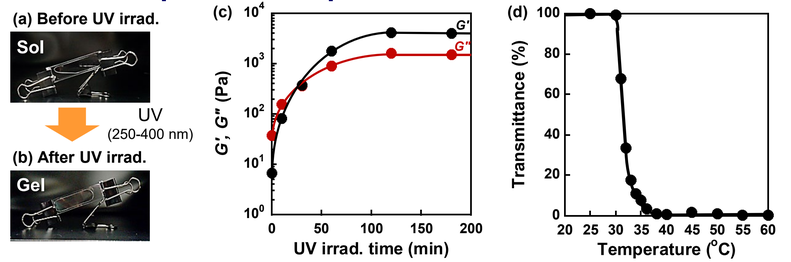


Fig. 2. Photographs of P(MAC_{20}-*co*-$OEGMA_{80}$) before (a) and after (b) UV (250-400 nm) irradiation for 1 h. The concentration of P(MAC_{20}-*co*-$OEGMA_{80}$) was 23 wt% in water. **(c)** Effect of the UV irradiation (300-400 nm) time on the storage elastic modulus (*G'*: ●) and loss elastic modulus (*G"*: ●) of the resulting P(MAC_{20}-*co*-$OEGMA_{80}$) gels. The polymer concentration of was 33 wt% in water. (d) Changes in transmittance (650 nm) of P(MAC_{20}-*co*-$OEGMA_{80}$) hydrogel formed by UV (300-400 nm) irradiation for 60 min as a function temperature.

6. Cell culture on P(MAC-*co*-OEGMA) gels

➢Cell culture on hydrogels with different elastic modulus

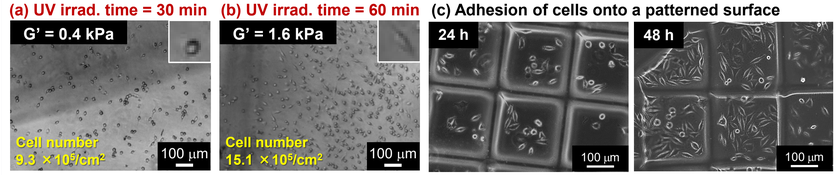


Fig. 3. Adhesion of L929 cells onto a surface of P(MAC_{20}-*co*-$OEGMA_{80}$) hydrogels formed by UV (300-400 nm) irradiation for 30 min (a) and 60 min (b). Cells were cultured on the hydrogel surfaces for 3 days and stained by calcein. (c) Adhesion of L929 cells onto a patterned surface of P(MAC_{20}-*co*-$OEGMA_{80}$) hydrogel exposed to UV (300-400 nm) for 120 min through a large square mesh (pitch = 250 mm, hole = 200 mm, and bar = 50 mm).

➢Cell culture on hydrogels at different temperatures

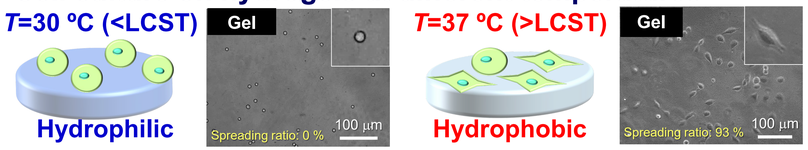


Fig. 4. Adhesion of L929 cells on a P(MAC_{20}-*co*-$OEGMA_{80}$) hydrogel, which was formed by UV (300-400 nm) irradiation for 180 min, at 30 and 37ºC. Cells were cultured on the hydrogel surfaces for 1 day.

7. Conclusion

- Cell behavior within the BMP-avidin complex hydrogel was quite different from that after the dissociation of the hydrogel by the addition of free biotin.
- Cell behavior on P(MAC-*co*-OEGMA) hydrogels was strongly influenced by their surface modulus and hydrophilicity.