ELASTICITY TUNABLE HYBRID HYDROGELS USING PHOTOCLEAVABLE CROSSLINKER

Fumiki Yanagawa¹, Takeomi Mizutani², Shinji Sugiura¹, Toshiyuki Takagi¹
Kimio Sumaru¹ and Toshiyuki Kanamori¹

¹Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Japan
²Faculty of Advanced Life Science, Hokkaido University, Japan

ABSTRACT

This paper reports a simple technique to synthesize elasticity tunable hybrid hydrogels with an activated-ester-type photocleavable crosslinker (NHS-PC-4armPEG) and non-photocleavable crosslinker (NHS-4armPEG). We demonstrated that synthesized hybrid hydrogels can control elasticity by light irradiation.

KEYWORDS: hybrid hydrogel, elasticity, photodegradation, photocleavable crosslinker

INTRODUCTION

The mechanical properties of the hydrogels are important for cell adhesion, migration, maintenance, and differentiation, which should be readily customized to match requirements for a particular application [1]. A host of research groups have reported elasticity patterning of hydrogels for tissue engineering using biocompatible hydrogels such as polyethylene glycol [2]. Recently, photodegradable hydrogels have attracted significant attention because of their tunable mechanical properties [3]. We also reported an activated-ester-type photocleavable crosslinker NHS-PC-4armPEG for preparing photodegradable hydrogels, which react with a biocompatible polymer containing amino moieties such as an amino-terminated tetra-arm poly(ethylene glycol) (amino-4armPEG) [4]. Recently, we applied this photocleavable crosslinker to preparation of elasticity tunable hybrid hydrogels [5]. In this paper, we report detailed structural analysis of the hybrid hydrogels analyzed by scanning electron microscopy (SEM).

Figure 1. Schematic diagram of synthesizing elasticity tunable hybrid hydrogels with photocleavable NHS-PC-4armPEG and non-photocleavable NHS-4armPEG using light irradiation [5].
EXPERIMENTAL

Figure 1 illustrates a schematic diagram for synthesizing the elasticity tunable hybrid hydrogels by crosslinking amino-terminated polymer using photocleavable NHS-PC-4armPEG and non-photocleavable NHS-4armPEG. A prepolymer solution including 10 mM amino-4armPEG (Mw = 9617) was prepared in a HEPES buffer. Also, a photocleavable crosslinker solution including 10 mM synthesized NHS-PC-4armPEG and a non-photocleavable crosslinker solution including 10 mM NHS-4armPEG were prepared in phthalate acid buffer. ‘crosslinker mixture solutions’ were prepared including 10 to 50 mol% of the photocleavable and non-photocleavable crosslinker solutions. The prepolymer solution and ‘crosslinker mixture solutions’ were then mixed at a 1:1 (v/v) ratio. Subsequently, the droplets of the mixture was placed onto a glass slide. The samples were then incubated for 24 h at 37 °C, allowing the crosslinking reaction to form hydrogel samples. After incubation, the samples were then exposed to UV light (365 nm, 30 mW/cm², 30 sec), allowing the photocleavage of o-nitrobenzyl moieties in the hydrogel (Figure 1). To erode the photocleaved polymer, the sample was immersed in MilliQ water for 24 h at 37 °C.

Atomic force microscopy (AFM) was employed to measure the elasticity of the hydrogels with micrometer-scale resolution, with a commercial microscope equipped with a piezo scanner and a silicon-nitride cantilever. The pyramidal tip located on the cantilever was indented on the hydrogel surface, and the force acting on the cantilever was recorded at room temperature (scan area = 90 μm × 90 μm) as a function of the displacement of the piezo scanner. Force curves were fitted to the Hertzian elastic contact model for a pyramidal indenter giving the local elasticity (Young’s modulus) of each point as a fitting parameter.

To evaluate the effect of light irradiation to hydrogels, the morphology images before and after light irradiation were scanned using a SEM. After the eroding, the samples were frozen in liquid nitrogen and then lyophilized using vacuum freeze dryer. The images of the microstructure in the hydrogels formed by light irradiation was scanned using SEM.

RESULTS AND DISCUSSION

The elasticity tunable hydrogels composed of amino-4armPEG with mixture of NHS-4armPEG and NHS-PC-4armPEG was synthesized. Using the crosslinker ≥ 70 % NHS-PC-4armPEG, the hydrogels were completely degraded when the hydrogels were exposed to light (365 nm, 30 mW/cm², 30 sec). AFM studies were performed to evaluate the elasticity of synthesized hydrogels. In the case more than 30 % of photocleavable NHS-PC-4armPEG, significant decrease in elasticity was observed after light irradiation. As expected, the higher concentration of NHS-PC-4armPEG led to the greater differences of elasticity between before and after irradiation. It was demonstrated that light irradiation alter the hydrogels elasticity, due to decreasing crosslink density by hydrogel degradation.

To evaluate the effect of light irradiation on the polymer network in the hydrogels, the SEM images of hydrogels before and after light irradiation were obtained (Figure 2). Using the crosslinker containing more than 70% (v/v) of photocleavable NHS-PC-4armPEG (less than 30% (v/v) of non-photocleavable NHS-4armPEG), the hydrogels in the exposed area were completely degraded. Interestingly, in the range of less than 50% (v/v) of photocleavable NHS-PC-4armPEG, the higher amount of photocleavable NHS-PC-4armPEG led to porous structure in hydrogels before and after light irradiation. This morphology differences were likely caused by the difference in the reactivity of the photocleavable NHS-PC-4armPEG with amino moieties of amino-4armPEG and that of the non-photocleavable NHS-4armPEG.

In the range of 30% and 50% (v/v) of photocleavable NHS-PC-4armPEG, the polymer network in the hydrogels exhibited more porous structure after light irradiation than that before light irradiation. In addition, the microstructure of the polymer network was homogeneous in the irradiated and non-irradiated area in all conditions. These results agree well the differences of elasticity, in which hydrogels was more soft in the hydrogels with high ratio of photocleavable NHS-PC-4armPEG.
CONCLUSION
In this paper, we introduced simple technique to synthesize elasticity tunable hybrid hydrogels with photocleavable NHS-PC-4armPEG and non-photocleavable NHS-4armPEG. This technique could provide novel platforms to precisely design elasticity of the hydrogels for mimicking soft and stiff microenvironment in tissue engineering field in the future.

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REFERENCES

CONTACT
*Fumiki Yanagawa, tel: +81-29-861-6286; fax: +81-29-861-6278, fumikiyanagawa@gmail.com