CHARACTERIZATION OF CELL BEHAVIOR ON PATTERNED PHOTODEGRADABLE HYDROGELS
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ABSTRACT
This paper reports a characterization of cell behavior on photodegradable hydrogels, which were composed of an activated-ester-type photocleavable crosslinker (NHS-PC-4armPEG) and biocompatible polymers containing amino moieties, such as amino-terminated tetra-arm poly(ethylene glycol) (amino-4armPEG) and gelatin. We demonstrated that cell patterning on the photodegradable hydrogels can be precisely controlled with microscale by micropatterned light irradiation.

KEYWORDS: photocleavable crosslinker, photodegradable hydrogel, micropatterning, cell culture

INTRODUCTION
A number of research groups have reported micropatterning of hydrogels for tissue engineering using biocompatible hydrogels such as polyethylene glycol [1]. Also, cell behavior (i.e., cell adhesion, cell elongation, and cell proliferation) on the hydrogels is important to fabricate biomimetic tissue contract [2]. Recently, photodegradable hydrogels have attracted significant attention because they are suitable for the creation of 3D microstructures for biomaterials and tissue-engineering research of their tunable 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 amino-4armPEG [4]. In this study, we characterized cell behavior on photodegradable hydrogels.

EXPERIMENTAL

Figure 1. Schematic diagram of synthesizing photodegradable hydrogels prepared with photocleavable NHS-PC-4armPEG and either amino-4armPEG or gelatin.
\(N\)-hydroxysuccinimide (NHS)–terminated photocleavable tetra-arm PEG (NHS-PC-4armPEG; Mw = 12,062) crosslinker was synthesized (Figure 1). A prepolymer solution containing either 10 mM amino-4armPEG (Mw = 9,617) or 5% w/v gelatin was prepared in a 1:1 mixture of PBS and 0.3 M HEPES buffer (pH 7). A solution of the synthesized NHS-PC-4armPEG crosslinker (10 mM, 12.1% w/v) was prepared in 10 mM phthalate acid buffer with 140 mM NaCl. The prepolymer and crosslinker solutions were mixed at a 1:1 v/v ratio. Immediately after the two components were mixed, the mixture was transferred to an amino-coated glass slide. The mixtures were then incubated at 37 °C for 30 min to form photodegradable hydrogels. A photomask was placed on the cover slip on the photodegradable hydrogel. Subsequently, the hydrogel was exposed to light from the ultra violet light source through the photomask. To develop the photodegraded region in the hydrogel, the sample was immersed in PBS solution at 37 °C for 24 h. For evaluation of the cell growth, a droplet of cell suspension was pipetted on the hydrogels, and then cells were cultured in a 5% \(CO_2\), 37 °C incubator.

RESULTS AND DISCUSSION

To characterize the cell behavior on the photodegradable hydrogels, HUVECs were cultured on the photodegradable hydrogels prepared with either 4armPEG or gelatin as a main polymer. The prepolymer and crosslinker solutions were mixed in a 1:1 (v/v) ratio and a droplet of the cell suspension was pipetted onto the photodegradable hydrogels. After 1 and 3 days of culture, phase contrast images were captured by the inverted microscope. As expected, few cells adhered onto hydrogel surfaces prepared with amino-4armPEG, because PEG is inert to cell adhesion (Figure 2a). The differences in cell behavior on the hydrogels prepared with 1.25% gelatin stemmed from the concentration of the NHS-PC-4armPEG (Figure 2b). A similar phenomenon was observed for the hydrogel prepared with 2.5% gelatin: higher cell growth was observed on the hydrogel with 1.0% NHS-PC-4armPEG than on that with 5.0% NHS-PC-4armPEG (Figure 3a). These results suggest that the free amino acid residues in the gelatin related to cell binding are probably important for cell adhesion and proliferation, because free amino acid residues remain intact at lower crosslinker concentrations. Also, to demonstrate the cell patterning, HUVECs on the photodegradable hydrogels prepared with 2.5% gelatin and 1.0% NHS-PC-4armPEG were irradiated with light through photomasks. The resulting cell patterns reflected hydrogel degradation (Figure 3b).
To evaluate the effects of light irradiation and hydrogel degradation on the cell viability, a live/dead assay was performed. After light irradiation of the elongated cells, the cells were stained by Calcein and EthD-1. The cell viability in the exposed regions was similar to that in the unexposed regions (Figure 3b), demonstrating that light irradiation and hydrogel degradation did not alter cell viability during this process.

CONCLUSION

In this work, we demonstrated that cell patterning on the photodegradable hydrogels can be precisely controlled with microscale by micropatterned light irradiation. The resulting patterned cell on photodegradable hydrogels would be useful to fabricate 3D tissue structures in the near future.

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REFERENCES


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