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Visible light-induced crosslinkable gelatin

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ABSTRACT

A novel visible light-crosslinkable porcine gelatin was prepared for gelation and micropatterning. The preparation employed a photo-oxidation-induced crosslinking mechanism. First, furfuryl groups were incorporated into the gelatin. Second, the modified gelatin was mixed in water with Rose Bengal, which is a visible light sensitizer. Irradiation by visible light solidified the aqueous solution. In addition, when the solution was cast on a plate, dried and photo-irradiated in the presence of a photomask a micropattern was formed that matched the micropattern on the photomask. The gelatin-immobilized regions enhanced cell adhesion. It was also confirmed that the gelatin incorporating furfuryl and Rose Bengal have no significant toxicity. The photo-crosslinkable gelatin was employed as a direct pulp capping material in the dental field. Considering these results, this system could be useful as a new type of visible light-induced crosslinkable biosealant.

1. Introduction

Photo-induced crosslinking or polymerization is a fast and convenient way to produce gels or high molecular weight polymers. Many types of materials based on photo-triggered reactions have been developed for industry. Photo-induction is also useful for rapid curing, and it has been extensively employed for hard tissues in dental medicine [1]. Matsuda's group developed a photo-induced polymerizable system, including poly(ethylene glycol) diacrylate, derivatized gelatin and photo-induced radical initiators [2]. Similarly, the Hubbell group's visible light-induced photopolymerizable glue, based on hydrolytically degradable poly(ethylene glycol) diacrylate derivatives and a visible light-induced photoradical generator (eosin Y), is now commercially available [3]. Other types of polymerizable biomacromolecule derivatives have also been reported [4–16]. However, these systems usually utilize synthetic polymerizable monomers as their major component. For medical utilization it is desirable to have biological molecule-based sealants.

Some types of ultraviolet-crosslinkable biomacromolecules have also been reported [17–21]. Recently, visible light-crosslinkable gelatin [21] and fibrinogen [22] have been reported. In the present investigation we developed a new type of visible light-induced crosslinkable biosealant. Tajima et al. found that a furan-containing polymer formed a gel in the presence of fullerene by photo-oxidation polycondensation [23,24]. Here, instead of fullerene, which is insoluble in water, Rose Bengal, a food dye that conjugates with furan groups in water, was employed for formation of the gelatin gel. After visible light irradiation by a photosource for dental use, the mixture was transformed from solution to gel by a photo-oxidation crosslinking (POC) mechanism, as illustrated in Fig. 1. Rose Bengal photosensitizes the oxygen molecules to generate singlet oxygen, and the resulting singlet oxygen reacts with furan derivatives to afford crosslinking through the formation of furan endoperoxide.

2. Materials and methods

2.1. Synthesis of furan-conjugated gelatin

Porcine skin gelatin (G2500) was purchased from Sigma (St Louis, MO), and 0.25 g was dissolved in 25 ml of water at

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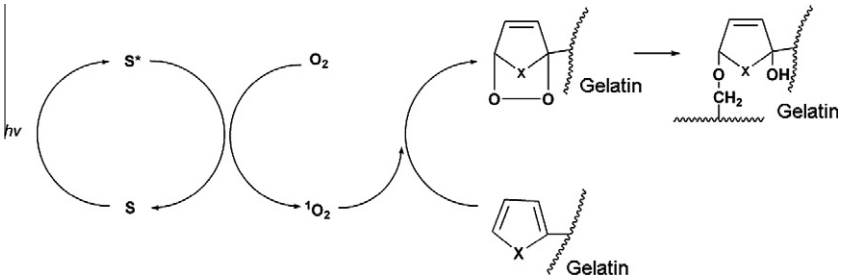

Fig. 1. Schematic drawing of the photo-oxidation crosslinking (POC) mechanism.

40 °C. An aqueous solution of NaOH was used to adjust the solution pH to 9.0. Furfuryl isocyanate (100 μl) in dimethylsulfoxide (2.5 ml) was added dropwise to the solution. After addition the solution was allowed to stand for 20 h at room temperature, after which it was neutralized with dilute HCl. Finally, the solution was dialyzed using a dialysis membrane for 2 days at 40 °C. The furfurylated gelatin is referred to as F-gelatin.

Gel permeation chromatography measurements were performed on samples dissolved in pure water using a TSK-GEL α-M column (Tosoh, Tokyo, Japan) at 0.6 ml min^{-1}. The eluted peaks were detected with a RI-2031 Plus detector (JASCO, Hachioji, Japan). The calibration was performed using a polyethylene glycol kit purchased from Polymer Laboratories (Varian Inc., Palo Alto, CA).

For the NMR measurements the sample was dissolved in D_2O. Measurements were performed using a JNM-AL400 spectrometer (JASCO).

2.2. Gelation by photo-irradiation

An aqueous solution of F-gelatin was prepared and mixed with Rose Bengal. The mixture was cast on a substrate and irradiated after drying with a halogen lamp (higher than 400 nm) or fluorescent lamp without or with a photomask, as shown in Fig. 2. Polyester disks (Thermanox™, NalgenNunc, NY) and 15 mm diameter glass disks (Matsunami, Osaka, Japan) coated with polyethylene glycol as previously reported [25] were used as substrates for micropatterning and cell culture, respectively.

2.3. Cell culture

A fusion cell of two mouse embryonic stem cells (EB3 and B6G-2) was cultured in Dulbecco's Modified Eagle Medium (high glucose, Wako Osaka, Japan) with 15% fetal bovine serum (Hyclone, Logan, UT), 1% glutamate (Sigma–Aldrich), penicillin–streptomycin stock (Sigma–Aldrich) at 10^{-2} dilution, 1% non-essential amino acids (Invitrogen Life Technologies, Carlsbad, CA), 0.1% 2-mercaproethanol (Invitrogen) and 0.1% Leukemia Inhibitory Factor (Wako). The cell suspension was added to a sample plate sterilized with 70% ethanol. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 in air for 2 days and then stained with Giemsa stain for observation by microscopy.

2.4. Cytotoxicity

COS-7 cells were cultured in Dulbecco's modified minimum essential medium (high glucose, no phenol red, GIBCO 21068) with 5% fetal bovine serum (Hyclone, Waltham, MA), and penicillin–streptomycin stock (Sigma–Aldrich). The modified gelatin was added to the culture medium. The cells (100 cells μl^{-1}) were incubated in a 96-well plate (50 μl $well^{-1}$) at 37 °C in an atmosphere of 5% CO_2 in air for 2 days. The cells were counted using a cell counting kit (Dojindo Molecular Technologies, Kumamoto, Japan).

2.5. Animal experiments

The use of photo-curable gelatin for direct pulp capping was investigated [26]. Cavities with an exposed pulp area were pre-

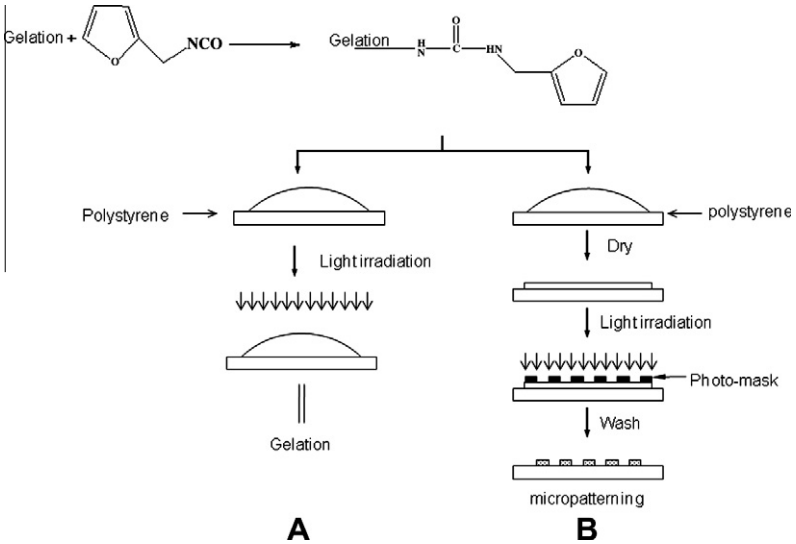

Fig. 2. Photo-crosslinking procedures: (A) photo-gelation; (B) photo-micropatterning.

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