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Microfabrication of biocompatible hydrogels by proton beam writing

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ABSTRACT

Functionalization of biocompatible materials is expected to be widely applied in biomedical engineering and regenerative medicine fields. Hydrogel has been expected as a biocompatible scaffold which support to keep an organ shape during cell multiplying in regenerative medicine. Therefore, it is important to understanding a surface microstructure (minute shape, depth of flute) and a chemical characteristic of the hydrogel affecting the cell culture. Here, we investigate the microfabrication of biocompatible polymeric materials, such as the water-soluble polysaccharide derivatives hydroxypropyl cellulose and carboxymethyl cellulose, by use of proton beam writing (PBW). These polymeric materials were dissolved thoroughly in pure water using a planetary centrifugal mixer, and a sample sheet (1 mm thick) was formed on polyethylene terephthalate (PET) film. Crosslinking to form hydrogels was induced using a 3.0 MeV focused proton beam from the single-ended accelerator at Takasaki Ion Accelerators for Advanced Radiation Application. The aqueous samples were horizontally irradiated with the proton beam through the PET cover film, and then rinsed with deionized water. Microstructured hydrogels were obtained on the PET film using the PBW technique without toxic crosslinking reagents. Cell adhesion and proliferation on the microfabricated biocompatible hydrogels were investigated. Microfabrication of HPC and CMC by the use of PBW is expected to produce new biocompatible materials that can be applied in biological and medical applications.

1. Introduction

In recent years, natural polymers and their derivatives, such as cellulose, chitin, chitosan, hydroxypropyl cellulose (HPC), sodium carboxymethylcellulose (CMC), collagen and gelatin, have been widely used in numerous biomedical applications. For example, they have been used as artificial kidney membranes, coatings for drugs, blood coagulants, additives in pharmaceutical products, supports for immobilized enzymes, stationary phases for chromatographic optical resolution and in tissue engineering [1–4]. HPC, a non-toxic, water soluble, transparent and biodegradable material that can exhibit liquid crystallinity has already been applied as a binder, thickener, lubricating agent and emulsion stabilizer in the pharmaceutical and food industries. Moreover, HPC has been used as an additive for copolymerization in scaffolds for tissue engineering, resulting in improvements to the biocompatibility, hydrophilicity and mechanical properties. CMC, also non-toxic, water soluble, biodegradable and disposable, has protective colloid properties and is used as a thickener, food additive, water-retaining regent, and anti-adhesive material [5]. CMC-based microgels modified with amines as crosslinkers have been applied to encapsulate cells for further fabrication of scaffolds in tissue engineering [2]. However, the biocompatibility of chemically treated HPC and CMC decreases with increasing amounts of artificial copolymer or crosslinker. Ionizing radiation methods, such as γ-rays and electron beam irradiation, are widely used as convenient tools for the crosslinking modification of natural polymers, without requiring any toxic chemical reagents [6–11]. These modified natural polymers have been used as polymer gel dosimeters, water absorbents, wound dressings and so on [12–14].

Recently, biocompatible materials are expected to be applied widely in stem cell technology and biomedical engineering fields. Hydrogel has been expected as a biocompatible scaffold which support to keep an organ shape during cell multiplying in regenerative medicine [15]. Therefore, it is important to understanding a

Abbreviations: PBW, proton beam writing; PET, polyethylene terephthalate; HPC, hydroxypropyl cellulose; CMC, sodium carboxymethylcellulose; TARRI, Takasaki Advanced Radiation Research Institute; QST, National Institutes for Quantum and Radiological Science and Technology.

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surface microstructure (minute shape, depth of flute) and a chemical characteristic of the hydrogel affecting the cell culture.

On the other hand, proton beam writing (PBW) technique has a capability to fabricate next-generation micro-devices and microbio devices [16,17]. PBW has gained recognition as a unique technique for 3D microfabrication of resist materials by direct writing using a focused beam of MeV protons. The depth of proton penetration into the sample is controlled by the beam energy. Microfabrication of artificial polymers, such as poly(methyl methacrylate) [18], poly(dimethylsiloxane) [19], negative photoresist [20], and Teflon [21], by PBW has already been reported. In the case of natural polymers, however, only agar gel on a Petri dish has been microfabricated by PBW for patterned cell growth [22]. The agar is also one of polysaccharide and acts as positive resist in the PBW. Since HPC and CMC have been crosslinked by electron beam irradiation as described above, these polysaccharides have a potential to act as a negative resist in the PBW technology which can freely draw a pattern.

In this study, we fabricate HPC and CMC hydrogels, without the use of highly toxic chemicals, using the ionizing radiation of proton beam. Radiation-induced crosslinking of HPC and CMC is investigated by exploring the chemical yields and swelling. HeLa cell adhesion to the microfabricated HPC and CMC hydrogels is investigated following PBW, to validate their applicability for use in microfluidic devices and as cell scaffolding materials.

2. Experimental

2.1. Sample preparation

Hydroxypropyl cellulose (HPC: NISSO HPC H type, Nippon Soda Co., Ltd., with an aqueous viscosity of ~1000–4000 mPa s in a 2% solution at 25 °C and a molar degree of substitution of 3) and sodium carboxymethyl cellulose (CMC: CMC DAICEL 1380, Daicel FineChem Ltd., with an aqueous viscosity of ~1000–2000 mPa s in a 1% solution at 25 °C and a degree of substitution of 1.36) were used without further purification. Ultra-pure water (with total organic carbon less than 4 ppb and resistivity of 18.2 MΩ cm) was supplied from Millipore Direct-Q UV5. HPC and CMC were dissolved in ultra-pure water using a planetary centrifugal mixer (ARE-310, Thinky Co., Japan). A 30% HPC aqueous solution and a 50% CMC aqueous solution were prepared in paste-like states, with densities of 1.10 ± 0.02 and 1.28 ± 0.03 g/cm^3, respectively. These solutions were placed between polyethylene terephthalate (PET) films with a 25 μm thickness and then pressed into a sheet of 1 mm thickness (150 × 150 mm^2) under a pressure of 15 MPa for 1 h.

2.2. Irradiation

The obtained HPC and CMC sheets were placed horizontally on a sample holder for irradiation with a 3.0 MeV proton microbeam formed at the light-ion microbeam system [23] connected to the single-ended accelerator at the TIARA (Takasaki Ion Accelerators for Advanced Radiation Application) facility in Takasaki Advanced Radiation Research Institute (TARRI), National Institutes for Quantum and Radiological Science and Technology (QST) [24]. The irradiation of the mircrobeam was applied onto the sample in the atmosphere. The beam was firstly focused onto the vacuum side surface of a beam exit window made of 7.5-μm-thick Kapton® foil, and the beam size measured from a secondary electron image of a cupper grid placed on the window was approximately 1 × 1 μm^2 in FWHM. On account of the 1-mm air gap for easy installation of the sample, the beam spot size on the sample was estimated at approximately 12 μm in FWHM by the SRIM-2013 code [25]. The penetration depths of the protons in the HPC and CMC aqueous solutions were approximately 97.5 and 87.5 μm, respectively, according to the PHITS code [26]. The beam current was typically 10 pA, as measured with a Faraday cup. The fluence (dose) was controlled by the beam current and exposure time, and were in the range from 1.4×10^{10} ions/cm^2 to 5.6×10^{10} ions/cm^2 (dose:25–100 kGy).

To compare the obtained gel by proton beam irradiation, the HPC and CMC sheets were also irradiated by use of the electron beam accelerator at the TARRI, QST. The acceleration energy and the beam current were 2 MeV and 2 mA, respectively. The sample sheets were uniformly irradiated by using a conveyor system. The penetration range of 2 MeV electron beam is approximately 1.2 g/cm^2. The radiation dose rate was 10 kGy/pass, which was evaluated with a cellulose triacetate film dosimeter FTR-125 from Fuji Film, Japan.

2.3. Estimating the gel fraction, swelling, elastic modulus

Formation yields of the chemically crosslinked HPC and CMC hydrogels obtained from the proton and electron beams irradiation were estimated from the gel fraction according to the equation:

$$\text{Gel fraction } (\%) = (W/W_0) \times 100 \quad (1)$$

where W_0 and W are the initial mass of the dried sample after irradiation and the final mass of insoluble product obtained after irradiation, washing with distilled water and drying, respectively. These values were measured using a precision balance (with a minimum sample weight of 0.01 mg, Sartorius ME215P). The swelling of the hydrogels in distilled water was estimated by dividing the mass of the dried hydrogel by the mass of swollen hydrogel. The errors in these measured values are estimated to be within the range 10% for proton beam experiments due to the small sample size. The elastic modulus of the hydrogel from the electron beam irradiation was estimated using a rheometer (creep meter: model II RE2-3305B, Yamaden Co., Ltd., Japan).

2.4. Cell cultures on the fabricated hydrogels

HeLa cells (RCB0007, BioResource Center, RIKEN, Wako, Japan) were cultured at 1 × 10^5/2 mL on the HPC and CMC hydrogels fabricated by the PBW in a culture medium [Minimum Essential Medium Eagle (Sigma-Aldrich) containing 10% fetal bovine serum (HyClone) and 1% penicillin-streptomycin-glutamine 100X (Thermo Fisher Scientific)] at 37 °C in a humidified atmosphere with 5% CO_2. Cell growth was observed with a microscope (Olympus IX70).

3. Results and discussion

3.1. Radiation induced crosslinking of HPC and CMC by proton and electron beam irradiation

The formation of HPC and CMC hydrogels has been reported in detail following electron beam irradiation at a dose of 10–100 kGy [6–8,13]. After careful washing with water, we obtained the insoluble products from both the proton and electron beam irradiated samples. The insoluble products were identified as hydrogels due to their soft structure following absorption of water, and hardness after drying. The mass of hydrogel obtained increased with increasing radiation dose. The gel fractions of the HPC and CMC hydrogels by the proton beam irradiation also increased with an increase in dose, and were similar to those by electron beam irradiation, as shown in Fig. 1. The swelling of the HPC and CMC hydrogels produced by PBW were also the same as that by electron beam, in

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