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Nanocellulose-alginate hydrogel for cell encapsulation

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ABSTRACT

TEMPO-oxidized bacterial cellulose (TOBC)-sodium alginate (SA) composites were prepared to improve the properties of hydrogel for cell encapsulation. TOBC fibers were obtained using a TEMPO/NaBr/NaClO system at pH 10 and room temperature. The fibrillated TOBCs mixed with SA were cross-linked in the presence of Ca^{2+} solution to form hydrogel composites. The compression strength and chemical stability of the TOBC/SA composites were increased compared with the SA hydrogel, which indicated that TOBC performed an important function in enhancing the structural, mechanical and chemical stability of the composites. Cells were successfully encapsulated in the TOBC/SA composites, and the viability of cells was investigated. TOBC/SA composites can be a potential candidate for cell encapsulation engineering.

1. Introduction

Cell encapsulation has garnered attention as a technology to provide immunoprotection for transplanted cells. The cells can be protected from the immune systems by a semipermeable membrane that allows nutrients and secreted proteins to permeate while isolating the cells from hostile immune reactions. Therefore, the transplantation of encapsulated cells has been suggested as a promising cell-based treatment for a variety of diseases, such as diabetes, metabolic deficiencies, liver failure, cancer, and neurodegenerative and cardiovascular diseases (Orive et al., 2003; Rokstad, Lacik, de Vos, & Strand, 2014; Schmidt, Rowley, & Kong, 2008; Zhang et al., 2013).

Alginate is a biopolymer that forms a hydrogel in the presence of divalent cations, such as Ca^{2+} (Draget, Steinsvag, Onsoyen, & Smidsrod, 1998). Because alginate hydrogels have excellent biocompatibility, they have been preferentially used to protect transplanted cells from immune rejection and as a matrix to increase the cell viability in cell encapsulation (Bratlie, York, Invernale, Langer, & Anderson, 2012; Orive, Tam, Pedraz, & Halle, 2006; Tam et al., 2011; Rokstad et al., 2014). However, the mechanical and chemical stability of alginate is not sufficient to achieve long-term transplantation. Consequently, reinforcing materials need to be added to an alginate matrix to overcome this limitation (Chan et al., 2011; Cordoba, Deladino, & Martino, 2013; Santagapita, Mazzobre, & Buera, 2012).

Bacterial cellulose (BC) biosynthesized by *Gluconacetobacter xylinus* has a high aspect ratio of high crystalline nanofibers (Klemm, Heublein, Fink, & Bohn, 2005; Park, Cheng, Choi, Kim, & Hyun, 2013b; Watanabe, Tabuchi, Morinaga, & Yoshinaga, 1998). BC nanofibers show better mechanical properties and higher hydrophilicity than celluloses from other sources (Hu, Chen, Yang, Liu, & Wang, 2011; Park, Chang, Jeong, & Hyun, 2013a). In spite of its promising properties, BC nanofibers cannot be easily applied as a component of composites due to their inter-connected 3D structure based on a large number of hydrogen bonds. Recently, a 2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-oxidation process was developed to modify the surface of cellulose, and well dispersed cellulose fibrils could be effectively obtained using the electrostatic repulsion of fibers under mild and environmentally friendly conditions compared to other methods (Isogai, Saito, & Fukuzumi, 2011; Saito, 2010).

In this study, TEMPO-mediated oxidized bacterial cellulose (TOBC) was used to improve the mechanical and chemical stability of an alginate hydrogel. TOBC and alginate have a similar chemical structure, and they both participate in the Ca^{2+} crosslinking process. The carboxyl groups on the surface of TOBC provided the possibility of participating in the construction of an alginate-based composite and played important roles in the structural, mechanical and chemical stability. The viability of cells encapsulated in the TOBC-alginate composite was investigated for possible biomedical application of the composite in the future.

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2. Experimental

2.1. Biosynthesis and purification of BC

G. xylinus (KCCM 40216) was obtained from the Korean Culture Center of Microorganisms. The bacterium was cultured on mannitol medium containing 2.5% (w/w) mannitol, 0.5% (w/w) yeast extract and 0.3% (w/w) bacto-peptone. Bacteria were introduced into Petri dishes containing culture medium at 28 °C for 5 days. After incubation, the BC membrane biosynthesized on the surface of the liquid culture medium was harvested and purified with 1 wt% NaOH (SAMCHUN Chemical, Korea) followed by washing with distilled water. This step was repeated to remove the medium components and bacteria. The membrane was stored in distilled water prior to use.

2.2. Preparation of the TEMPO-oxidized BC

The BC was oxidized using a TEMPO (Sigma-Aldrich, USA)-mediated system. To obtain the TEMPO-oxidized BC, 20 g of hydrogel (wet weight) was cut into small pieces and suspended in 500 mL of distilled water containing 20 mg TEMPO and 0.5 g NaBr (Sigma-Aldrich, USA). Subsequently, 15 mL NaClO (Sigma-Aldrich, USA) solution was added to the BC suspension to initiate the oxidation. The system was maintained at pH10 with NaOH. The mixture was vigorously agitated using a magnetic stirrer for 2 days. The oxidation was quenched by adding ethanol (SAMCHUN Chemical, Korea) to the suspension at the end of the reaction. The products were washed with deionized water, collected by centrifugation three times, and then freeze-dried for further experiments.

2.3. Preparation of SA/TOBC beads

The extrusion technique was used to prepare the alginate/TOBC beads. Briefly, 1 mL syringes (Korea Vaccine Co., Ltd., Korea) were filled with sodium alginate (Sigma-Aldrich, USA) solutions containing various amounts of TOBC. The solutions were extruded using syringe pump (US/KDS 100, KD Scientific, USA) and dropped into a calcium chloride (2%) bath aerated with N_2 for gelation. The total concentration of each sample was 20 mg/mL. Thus, 200 mg sodium alginate (SA), 180 mg sodium alginate and 20 mg TOBC (SA/TOBC10), or 160 mg sodium alginate and 40 mg of TOBC (SA/TOBC20) were dissolved in 10 mL of distilled water. To encapsulate the cells, 2 mL of a fibroblast cell (NIH3T3, obtained from KCLB) suspension was mixed with 2 mL of autoclaved (121 °C, 20 min) sodium alginate solution containing various amounts of TOBC. One milliliter of the cell suspension/polymer mixture was extruded through a syringe pump and dropped into a calcium chloride (2%) bath aerated with N_2 gas for gelation. The encapsulated cells were transferred to Petri dishes immediately after the gelation and stored in phosphate buffered saline (PBS, Gibco, USA) prior to use.

2.4. Characterization of SA/TOBC beads

The electron transmission images of SA/TOBC beads containing encapsulated cells were captured by energy-filtering transmission electron microscopy (EF-TEM, LIBRA 120, Carl Zeiss, Germany) at an acceleration voltage of 200 kV. The morphology of the samples was observed at an acceleration voltage of 2 kV using field emission scanning electron microscopy (FE-SEM, SUPRA 55VP, Carl Zeiss, Germany). The chemical structures of the samples were characterized by Fourier transform infrared spectroscopy (FT-IR spectroscopy, Nicolet iS5, Thermo Scientific, USA), and the crystal structure of the samples was determined using a high-resolution X-ray diffractometer (XRD, D8 DISCOVER, Bruker, Germany). The mechanical properties of SA/TOBC beads (compression test) were investigated using a Universal testing machine (UTM, GB/LRX Plus, Lloyd, UK) fitted with a 10 N load cell. For compression testing, 2 mm diameter spherical samples were prepared and compressed to 40% of their original thickness with a constant crosshead speed of 2 mm/min at room temperature. The chemical resistance properties and morphological change were observed by soaking the beads in 40 mM sodium citrate (Sigma-Aldrich, USA) solution for 1 h.

Model molecules with different molecular weights were used to evaluate the diffusion properties of SA/TOBC beads, i.e. dextran-conjugated FITC at 4 kDa and 150 kDa *MW* (Sigma-Aldrich, USA). SA/TOBC beads were incubated in 3 mg/mL of the model molecule solutions, and the permeability of the molecules was determined using confocal microscopy (LSM510, Carl Zeiss).

2.5. Proliferation and viability of encapsulated cells

To study the proliferation of cells encapsulated in the beads, the number of cells in each sample was counted as a function of the incubation day. The cells were encapsulated in beads of SA, SA/TOBC10, and SA/TOBC20 using 2 mL of fibroblast cell suspension (2.3×10^6 cells/mL) mixed with 2 mL polymer solutions according to the method mentioned above. After the gelation, the beads were washed with PBS and collected by centrifugation. After collecting the beads, they were re-dispersed in 20 mL of culture medium. One milliliter of cell-containing bead suspension was added to a Petri dish (SPL, 60 mm × 15 mm) with 1 mL of fresh culture medium. The culture medium consisted of Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), streptomycin (100 μg/mL), and HEPES (7.5 mM). The cells encapsulated in SA, SA/TOBC10, and SA/TOBC20 beads were cultured in a humidified 5% CO_2 incubator at 37 °C for 1, 3, and 5 days. After incubation, the encapsulated cells in Petri dishes were recovered using 80 mM sodium citrate and 2.5% trypsin (Gibco, USA), followed by centrifugation. The encapsulated cells from three Petri dishes were counted using a hemocytometer (Marienfeld, Germany). To investigate the viability of encapsulated cells, the cells were recovered from the beads using 80 mM sodium citrate and centrifugation. A live/dead viability/cytotoxicity kit was used to test the viability of mammalian cells (Invitrogen, USA). The indicated combined live/dead assay reagents were added to a Petri dish containing recovered cells, and the mixture was then incubated for 1 h at room temperature in the dark. Green (live cells) and red (dead cells) fluorescence images were collected separately using a fluorescence microscope (BX51, Olympus, Japan).

3. Results and discussion

BC was successfully selectively oxidized at the C6 carbon using TEMPO (Fig. 1A). The asymmetric stretching band at 1602 cm^{-1} of the FTIR spectra indicated chains modified by carboxyl groups. Moreover, the stretching vibration band of the C—H at 2896–2990 cm^{-1} and stretching vibration band of the —OH groups near 3345–3539 cm^{-1} were considerably reduced after the modification in TOBC (Dong, Snyder, Williams, & Andzelm, 2013; Fujisawa, Okita, Fukuzumi, Saito, & Isogai, 2011; Lin, Bruzzese, & Dufresne, 2012).

The effect of oxidation on the crystalline structure of BC was further investigated with XRD and TEM analysis. Because the TEMPO-mediated oxidation of BC fibers only occurred at the surface of cellulose fibers, the original morphology, such as the aspect ratio of fibers and the integrity of the crystal, were maintained. As shown in Fig. 1C, TOBC still distinctly presented the diffraction peaks of BC at 2theta angles near 14, 16, and 22 (Lin et al., 2012; Zhang et al., 2010). Fig. 1D–F shows the changes in the morphology