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Research Paper

Nanocellulose based asymmetric composite membrane for the multiple functions in cell encapsulation

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ABSTRACT

We describe the nanocomposite membrane for cell encapsulation using nanocelluose hydrogels. One of the surfaces of bacterial cellulose (BC) pellicles was coated with collagen to enhance cell adhesion and the opposite side of the BC pellicles was coated with alginate to protect transplanted cells from immune rejection by the reduced pore size of the composite membrane. The morphology of nanocomposite membrane was observed by scanning electron microscopy and the permeability of the membrane was estimated by the release test using different molecular weights of polymer solution. The nanocomposite membrane was permeable to small molecules but impermeable to large molecules such as IgG antibodies inferring the potential use in cell implantation. In addition, the BC-based nanocomposite membrane showed a superior mechanical property due to the incorporation of compared with alginate membranes. The cells attached efficiently to the surface of BC composite membranes with a high level of cell viability as well as bioactivity. Cells grown on the BC composite membrane kit released dopamine freely to the medium through the membrane, which showed that the BC composite membrane would be a promising cell encapsulation material in implantation.

1. Introduction

Cell encapsulation technology for curing diseases such as diabetes, neurological and sensory diseases (Zanin et al., 2012) and cell based drug delivery systems (Orive et al., 2009; Wilson & Chaikof, 2008) has been advanced in the past few years. Encapsulated cells are able to receive nutrients and secreted therapeutic proteins while receiving protection from host immune systems by semipermeable membrane (Sarker et al., 2014). Hydrogels have been investigated to encapsulate the cells since hydrogels have a property of semipermeable membrane and can suppress the immune reaction physically. In addition, it is a positive feature that they can provide three-dimensional environment to cells in culture (Beck, Angus, Madsen, Britt, Vernon & Nguyen, 2007). An alginate based hydrogel is one of the most used biomaterials (Ghidoni et al., 2008; Orive et al., 2015; Ruvinov & Cohen, 2016) and has excellent biocompatibility and ionic gelation characteristic with divalent cations in mild condition (Boontheekul, Kong & Mooney, 2005). Despite these advantages of alginate hydrogels, alginate could not enhance cell adhesion and cell proliferation because of the absence of cell adhesion molecules (Sarker et al., 2015). Furthermore, the mechanical property of alginate hydrogel is not strong enough to protect the encapsulated cells (An et al., 2015; Kang et al., 2014; Park, Lee & Hyun, 2015). Therefore a number of studies have been carried out to improve the alginate gel properties to be suitable for cell encapsulation (An et al., 2015; Kang et al., 2014; Park et al., 2015; Sarker et al., 2014).

Encapsulation of cells in micro-sized hydrogel has been proposed for improving of mass transport such as nutrient and secreted proteins. (Jacobs-Tulleneers-Thevissen et al., 2013; Ma et al., 2013; Wang et al., 1997; Weir, 2013). However, it is inconvenient to control the locations of the micro-gels without supporting matrix and to replace the cells when the transplant failures or medical complications occur. In addition the micro-gels happen to clump over time in the body. (An et al., 2015; Vaithilingam & Tuch, 2011; Jacobs-Tulleneers-Thevissen et al., 2013; Ma et al., 2013; Weir, 2013). For these reasons, macroscopic encapsulation devices have been studied recently as an alternative form for cell encapsulation (An et al., 2015; Dufrane, Goebbels & Gianello, 2010; Lathuiliere,

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Cosson, Lutolf, Schneider & Aebischer, 2014; Nyitray et al., 2015; Onoe et al., 2013).

In this work, we developed a nanocellulose based composite membrane for the macroscopic cell encapsulation kits. Bacterial cellulose (BC) is a cellulosic hydrogel synthesized by *Gluconacetobacter xylinus*, constituted of β-D-glucoses linked by 1–4 glycosidic bonds. BC consists of the nanofibers having diameter of several tens of nanometers and these nanofibers are connected forming a 3D networked structure. Its unique 3D-networked structure makes BC promising as a supporting frame material for the functional nano-biocomposites. (Czaja, Young, Kawecki & Brown, 2007; Park, Chang, Jeong & Hyun, 2013; Shah, Ul-Islam, Khattak & Park, 2013; Ul-Islam, Khan, Ullah & Park, 2015) This 3D networked nanofibric structure can provide superior mechanical property to a composite membrane compared with an alginate membrane. Furthermore, to promote cell adhesion and viability of the composite membrane, collagen, the most well-known natural polymer as a component of extracellular matrix (ECM) for enhancing the cell adhesion was introduced.

PC12 cells are susceptive to substances including growth factors, neurotransmitters and hormones, and secret a biomolecule such as dopamine. As a model secretory cell, PC12 cells were chosen to investigate the bioactivity of cells grown on the BC composite membranes and the differentiation of PC12 cells upon nerve growth factor stimulation and neurite growth were described. Another important feature of the cell encapsulation is the selective permeation of molecules through the membrane. The smaller size of molecules need to be permeable for the survival of encapsulated cells, meanwhile, the larger size of immune molecules impermeable for the protection of the cells. As a feasible cell encapsulation system, a heterogeneous BC-based composite membrane conjugated with collagen and alginate was designed and its characteristic properties were investigated. It provided the cell adhesive and immune protective layers with a superior mechanical property compared with alginate-based encapsulation.

2. Experimental section

2.1. Biosynthesis and purification of BC

Gluconacetobacter xylinus (KCCM 40216) was obtained from the Korean Culture Center of Microorganisms. The bacterium was cultured on mannitol medium composed of 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, 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 deionized(DI) water. This step was repeated to remove medium components and bacteria. The membrane was autoclaved at 120 °C for 20 min and stored in sterile water prior to use. The w/w ratio of BC to water was about 99%.

2.2. Preparation of the TEMPO-oxidized BC

The BC was oxidized by a 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO, Sigma-Aldrich, USA)-mediated system. To obtain TEMPO-oxidized BC, 20 g hydrogels (wetting weight) were cut into small pieces then suspended in 500 ml DI 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 start the oxidation in maintaining the system at pH10 with NaOH. The mixture was vigorously agitated using a magnetic stirrer for 2 days in room temperature. The oxidation was quenched by adding ethanol (SAMCHUN Chemical, Korea) to the suspension at the end of reaction. The products were collected by centrifugation at 15000 rpm for 15 min, dialyzed in a cellulose tube (Spectra/Por®, Spectrum Laboratories, Inc., USA, molecular weight cut off 12,000–14,000) against circulating DI water for 2 days at room temperature, and finally autoclaved at 120 °C for 20 min.

2.3. Fabrication of the cell encapsulating kit of the BC composite membranes

The composite membrane was fabricated by incorporating BC with TOBC, collagen (Sigma-Aldrich, USA) and alginate (Sigma-Aldrich, USA). The suspension of TOBC was sonicated for dispersion and vacuum filtered through the BC membrane (diameter of 8 cm) to form BC/TOBC membrane. Collagen dissolved in 0.1 M acetic acid solution was introduced to the surface of the BC/TOBC membrane. Covalent attachment of collagen to membrane was performed using 1-ethyl-3-(3dimethyl aminopropyl)carbodiimide (EDC, Sigma-Aldrich, USA) and *N*-hydroxysuccinimide (NHS, Sigma-Aldrich, USA). BC/TOBC membrane was immersed in a solution of 0.4 mg/mL EDC and 0.6 mg/mL NHS and gently shaken for 30 min. Activated BC/TOBC membrane was collected and dipped into 1 mg/mL collagen solution for forming covalent bonding with NH_2 groups of collagen and the carboxyl groups of TOBC. For alginate coating, autoclaved 5 ml of 3% alginate solution was placed on the opposite side of collagen-modified BC/TOBC membranes (BC/TOBC/collagen) at room temperature. After the incubation for 1 h, 3% $CaCl_2$ solution was poured carefully to form alginate gel on the BC/TOBC/collagen membrane. The final BC composite membrane (BC/TOBC/collagen/alginate) for cell macroencapsulation was washed with running DI water for 1 h and sterilized by UV. The frame of Slide-A-Lyzer™ gamma irradiated dialysis cassette (88250, Thermo Scientific) was used for the assembly of the BC composite membrane kit. The dimensions and the loading volume of the frame were 4.0 cm × 4.0 cm × 0.8 cm and 0.5 ml, respectively. The cellulose membranes of the commercial kit were replaced by 1.0 mm thickness of BC composite membrane and the kit was bonded tightly using ultrahigh-strength adhesive. The donor and receiver volumes were 0.5 ml and 10 ml, respectively and the exposed area for permeation was 2.0 cm^2. The cells were grown on the BC composite membranes before the assembly in cell macroencapsulation.

2.4. Characterizations

FITC-PEG-NH_2 (MW 3400, NANOCS, USA) as a substance exhibiting fluorescence was conjugated with BC/TOBC to confirm that TOBC was introduced on the BC membrane. Small pieces of BC or BC/TOBC membrane were activated using the EDC/NHS solution in the same way as mentioned above. Activated BC or BC/TOBC membrane was collected and immersed in the 1 mg/mL FITC-PEG-NH_2 solution for 3 h at room temperature with mild stirring. After the reaction was finished, samples were washed with DI water and observed by fluorescence microscopy (BX51, Olympus, Japan). The chemical structures of the samples were characterized by Fourier transform infrared spectroscopy (FT-IR spectroscopy, Nicolet iS5, Thermo Scientific, USA). The scan number was 32 times, a resolution of 8 cm^{-1} was used, and a wavenumber range of 4000–600 cm^{-1} was scanned.

The tensile strength of the samples was investigated using a universal testing machine (UTM, GB/LRX Plus, Lloyd, UK) fitted with a 500 N load cell at room temperature. The test specimens were rectangular, with 5 cm gauge length, 2 cm width, and 0.5 mm thickness in a wet state of the hydrogels.