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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.

The morphology of the samples was observed using field emission scanning electron microscopy (FE-SEM, SUPRA 55VP, Carl Zeiss, Germany). The freeze-dried samples were cut into 1 cm x 1 cm sized pieces and were placed on the conductive adhesive tape on aluminum stubs. The sample surfaces were coated with platinum using a sputter coater (SCD 005, BAL-TEC GmbH, Germany) before imaging. The imaging was performed in the Inlens mode at an acceleration voltage of 2 kV.

The permeability of pure BC and BC composite membrane was estimated with 1 mg/mL 200 Da, 6 kDa, 20 kDa PEG and 200 kDa PEO solutions using a home built permeation equipment under the mild shaking at 37 °C. The permeability of antibody was conducted using mouse IgG and the all release concentration was measured with a liquid chromatography (Waters 410, Waters, USA). The donor and receiver volumes were 0.5 ml and 10 ml, respectively and the exposed area for permeation was 2.0 cm^2. All data are the means ± standard deviations from three specimens.

2.5. Cell viability and proliferation

Cellular viability and proliferation were assayed using methylthiazolyldiphenyl tetrazolium bromide (MTT, Sigma Aldrich). BC and BC composite membrane were cut into the well size of 96 well plate and placed in the well. The empty well of the tissue culture plate was used as a control substrate. And then, the 1×10^4 cells were seeded on the specimen and incubated for 7 days with complete DMEM medium containing 7.5% heat-inactivated fetal bovine serum (FBS), 7.5% horse serum, 2 mM HEPES, and 44 mM sodium bicarbonate. Cultures were maintained at 37 °C in a 95% humidified incubator with 5% CO_2. After the incubation of cells for 1, 3 and 7days, the medium was removed from the well plates, which were washed with PBS. 20 μl of MTT (5 mg/mL in PBS) and 100 μl of medium were loaded to the well plates and incubated further for 2 min at 37 °C under 5% CO_2. The produced formazan crystals were dissolved in 100 μl of DMSO and the absorbance of the suspension was measured at 570 nm using a microplate reader (Powerwave XS2, Bio-tek, USA).

2.6. Cell adhesion and morphology

Cell adhesion to the composite membrane surface and the change of cell morphology were examined by FE-SEM. PC12 cells were incubated in the BC composite membrane kit for 7 days at 37 °C, 5% CO_2. The medium containing nerve growth factor-7S from mouse submaxillary glands (NGF, Sigma-Aldrich, USA) was changed every two days and washed with PBS after the incubation. The cells were fixed with 2% glutardaldehyde solution in water by incubating for 30 min followed by washing with DI water and ethanol subsequently. The samples were then sputter-coated with a platinum layer as described above.

2.7. Measurement of dopamine released through the BC composite membrane

PC12 cells (1.2×10^5 cell/ml) were cultured in BC composite membrane kit using complete DMEM medium containing 7.5% heat-inactivated fetal bovine serum (FBS), 7.5% horse serum, 2 mM HEPES, and 44 mM sodium bicarbonate. After the cell incubation for 5 days, the medium was replaced with NGF treated one. The fresh NGF treated medium was replaced every two days. The amount of dopamine released through the composite membrane was harvested at 1st, 3rd and 7th day of the incubation and quantified by Dopamine ELISA Kit (KA3838, Abnova, Taiwan). The donor and receiver volumes were 0.5 ml and 10 ml, respectively and the exposed area for permeation was 2.0 cm^2.

3. Results and discussion

In normal, macroencapsulation is defined as an encapsulation system of implant cells based on permeable membranes sealed to a mechanically supporting frame. The scheme of cell macroencapsulation kit was illustrated in Fig. 1. The kit was fabricated by BC composite membrane (BC/TOBC/collagen/alginate) and conventional dialysis cassette. The cells were grown on the membranes before the assembly of the kit in macroencapsulation. The two BC composite membranes were placed on top of each other with the cell layers facing each other. In the macroencapsulation system, the semipermeable composite membrane should allow the selective diffusion of small molecules such as oxygen, nutrients, and therapeutic proteins, whereas to large molecules like host immune molecules or cells should be blocked to support the survival of the cells loaded in the kit (Fig. 1A and C). In addition, the membrane should allow the release of bioactive molecules produced by the cells to the outside of the kit through it to be used for biomedical applications.

The biocompatible BC membrane in the centimeter scale can provide sufficient internal space for encapsulating large number of cells per kit. Prior to the collagen incorporation to the BC membrane, carboxylated TOBC nanofibers were introduced to the membrane surface by filtration process (Fig. 1B). First, well dispersed TOBC fibers were obtained by TEMPO-mediated oxidation of BC. After the oxidation, a BC hydrogel was disentangled into nanofibers due to the repulsive interaction of anionic charges from carboxylic acid at the fiber surface. The application of vacuum drove the TOBC nanofibers to be efficiently infiltrated through the surface pores of BC membrane. The inner side of the membrane in the kit was treated with collagen for enhancing the ability of cell attachment. Also, the alginate hydrogel was formed on the outer side of the membrane for the protection of encapsulated cells from the immune molecules or host cells (Fig. 1A).

The presence of carboxyl groups at the BC nanofiber surface was confirmed by conjugating FITC-PEG-NH_2 with the TOBC nanofiber surface followed by fluorescence microscopic observation (Fig. 2A). As shown in the figures, the BC/TOBC nanofibers conjugated with FITC-PEG-NH_2 emitted fluorescence signal strongly while it was not possible to observe the fluorescence with normal BC. The incorporation of TOBC nanofibers into the BC membrane was also confirmed with the strong asymmetric stretching band of carboxyl groups at 1602 cm^{-1} (Fig. 2B) (Isogai, Saito & Fukuzumi, 2011). The binding of TOBC at the BC surface was stable after the harsh ultrasonic cleaning confirming the strong binding between TOBC and BC. The amount of TOBC in the total weight of the BC/TOBC was about 23% in dry state. The binding of collagen to the BC/TOBC membrane was determined by the peaks at 1545 cm^{-1} and 1640 cm^{-1}, which were assigned for N—H bending vibration of amide II and carbonyl stretching vibration of the amide I, respectively as collagen was coated on the BC membrane (Fig. 2B) (Saska et al., 2012).

Fig. 3 shows SEM images of a BC composite membrane whose surfaces were modified with TOBC, collagen and alginate. A pure BC membrane had a networked structure of 50 nm thick fibers with a large volume of empty space (Figs. 3 A and 4 A). In the case of a BC/TOBC, the porous structure of a BC surface was densely filled because the TOBC nanofibers were incorporated in the available space of the porous BC during the process of filtration (Fig. 3B). The one side of BC composite membrane composed of BC/TOBC/collagen/alginate showed a smooth surface by the homogeneous coating of collagen (Fig. 3C). The other side of the BC composite membrane which was coated with the alginate was nonporous showing the possibility as a protecting layer against the penetration of immune molecules (Fig. 3D).

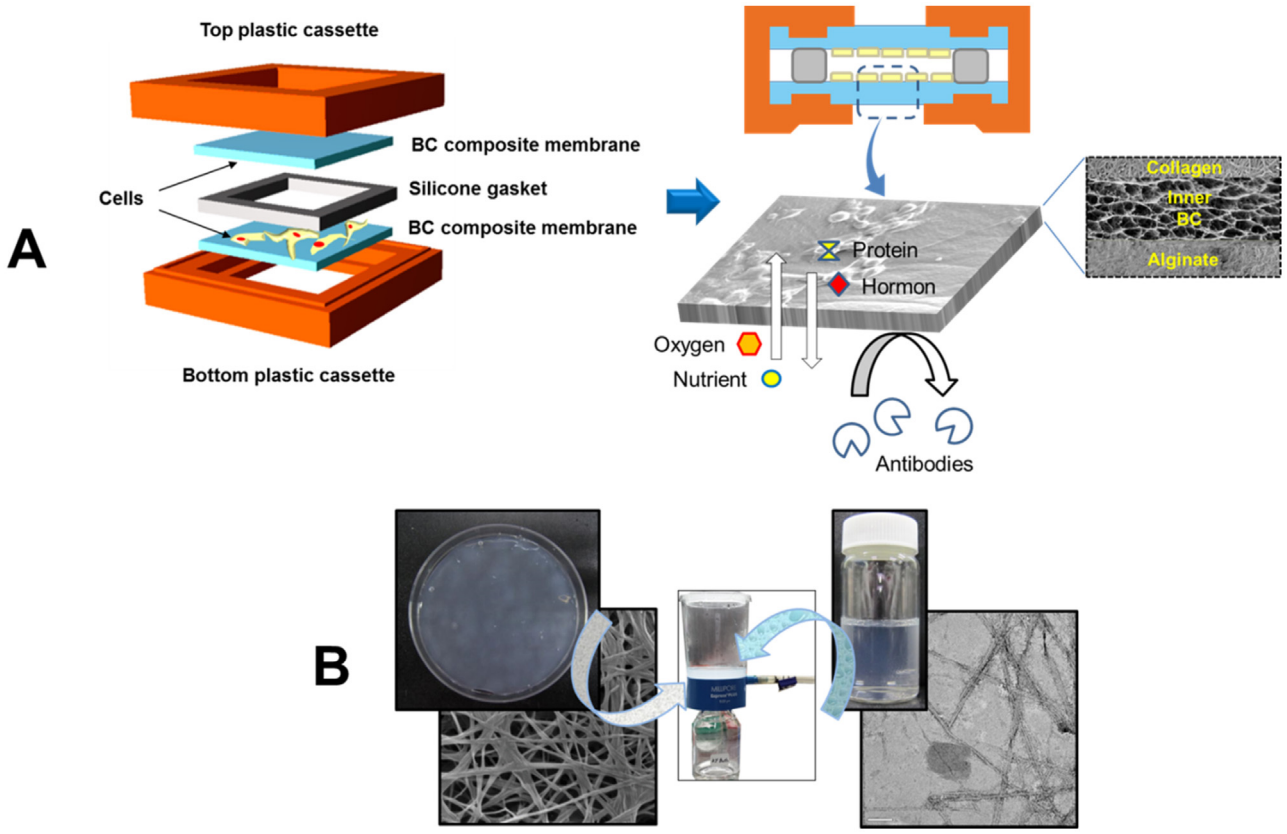

Fig. 1. Illustration of cell macroencapsulation. (A) Diagram of the macroencapsulation system for non-autologous cell implantation. The inner side of the composite membrane was treated with collagen for enhancing the ability of cell attachment. The alginate hydrogel was formed on the outer side of the membrane for the protection of encapsulated cells from the immune molecules or host cells. The composite membrane allows the selective diffusion of small molecules such as oxygen, nutrients, and therapeutic proteins to support the survival of the cells loaded in the kit. (B) Schematic of introducing TOBC nanofibers into BC membrane surface.

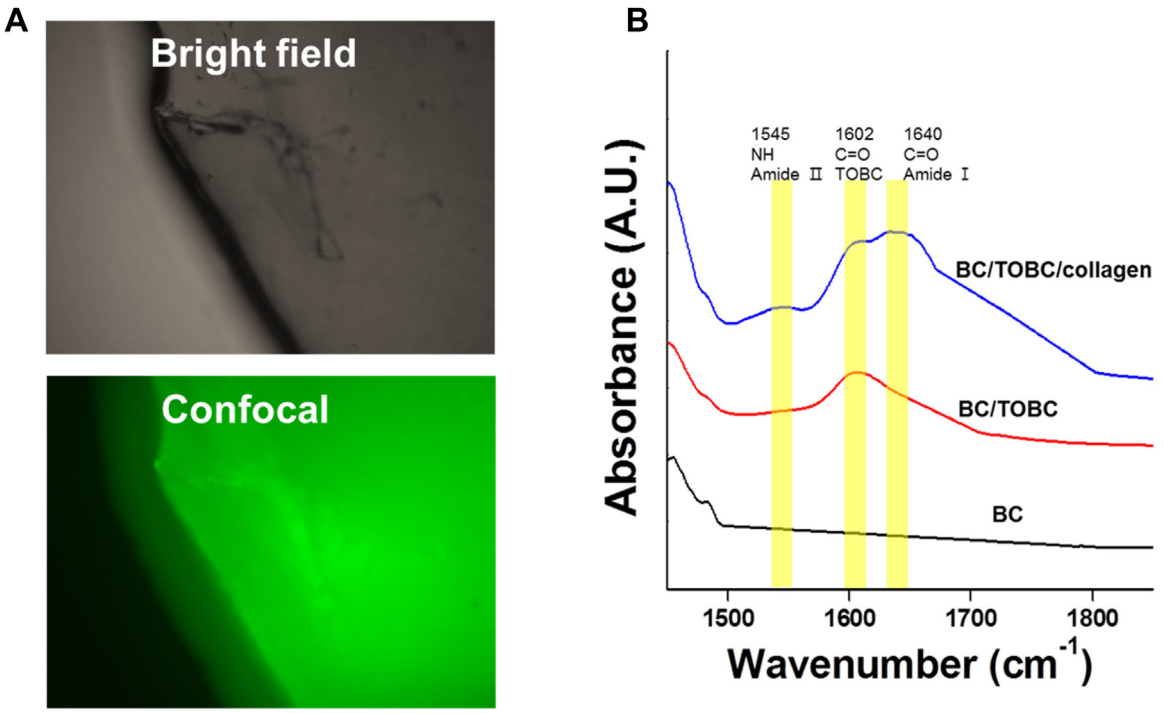

Fig. 2. Modification of BC membrane surfaces with TOBC and collagen. (A) Fluorescence image of BC/TOBC treated with FITC-PEG-NH_2. (B) FTIR spectra of BC, BC/TOBC and BC/TOBC/collagen.

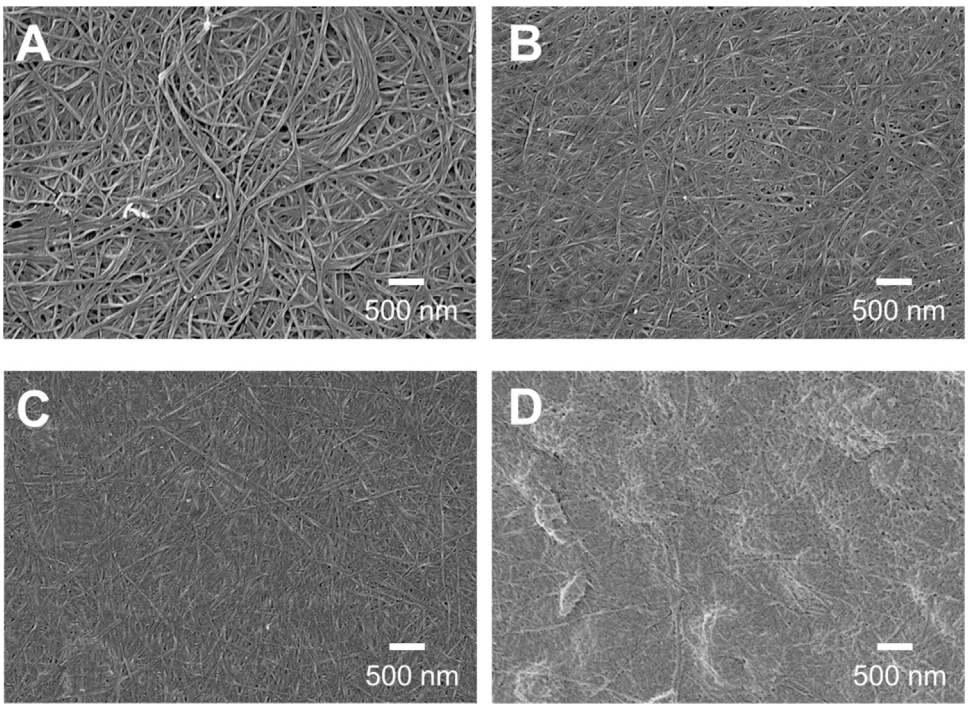

Fig. 3. Morphological FE-SEM images of (A) pure BC, (B) BC/TOBC, (C) collagen side and (D) alginate side of BC composite membrane (BC/TOBC/collagen/alginate).

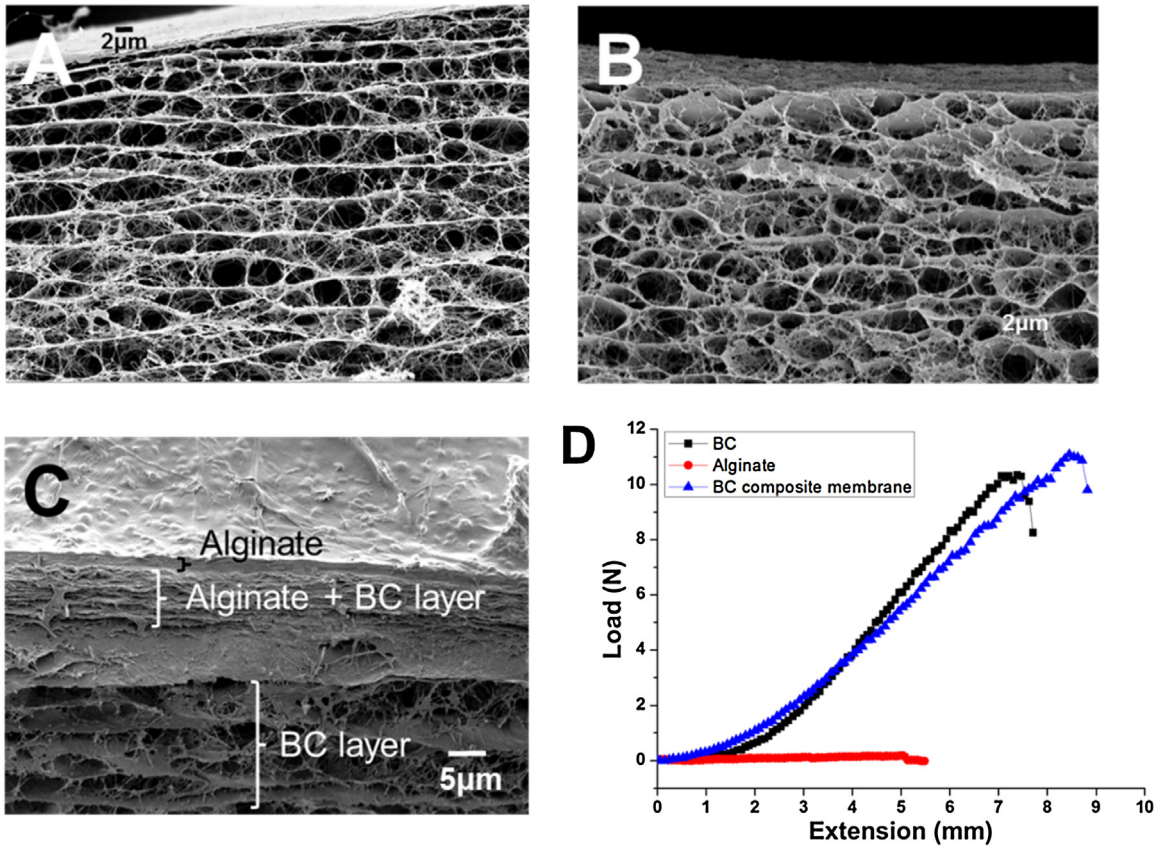

Fig. 4. Cross-sectional FE-SEM images of (A) pure BC, (B) BC/TOBC/collagen, and (C) BC composite membrane (BC/TOBC/collagen/alginate). (D) Tensile strength of BC composite membrane was superior compared with alginate hydrogel due to the 3D networked structure of BC.

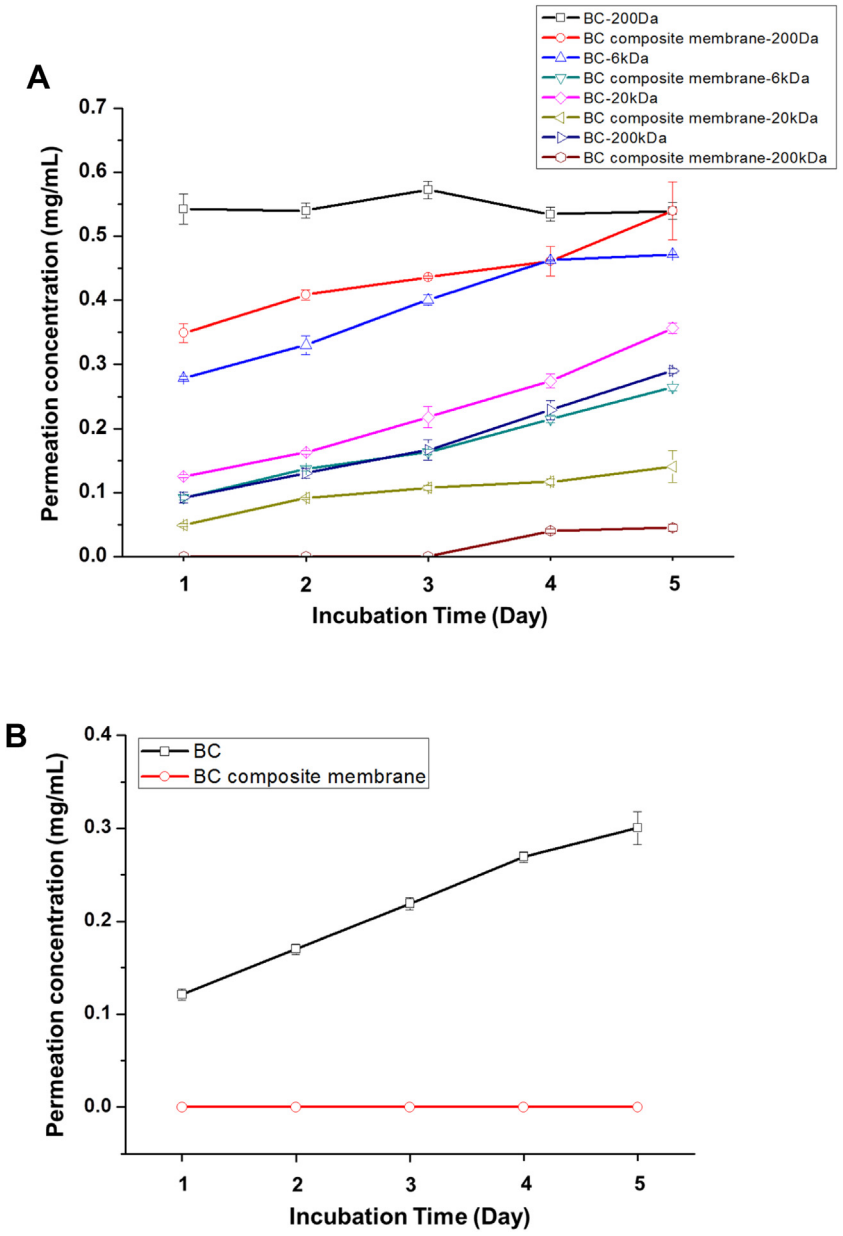

Fig. 5. Selective permeability of BC composite membranes. Cumulative concentration of model polymers (A) and IgG (B) released from BC and BC composite membrane as a function of time. Data are means ± standard deviations from three specimens. Standard deviation of red line in B could not be shown because the values were very small. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A BC membrane had a unique multilayered structure with spacing in microscale as shown in Fig. 4A. The incorporation of TOBC to the BC membrane and the subsequent conjugation of collagen to BC/TOBC membrane didn't deteriorate the spatial resolution of the inherent porous structure of BC membrane (Fig. 4B). Meanwhile, the alginate was deposited at the opposite side of the collagen modified BC membrane surface (BC/TOBC/collagen). The alginate penetrated a few of layers at the top of the BC membrane and created a dense layer about 20 μm thick which would function as a protection layer from the immune host cells or molecules (Fig. 4C).

In addition, the load-extension curves showed that the mechanical property of the BC composite membrane was greatly improved compared with alginate membrane since BC nanofibers cooperated well with alginate acting as a structural frame (Fig. 4D). The peeling or delamination of the alginate layer from the composite membrane was not observed probably due to the penetration of alginate into the BC layers as shown in Fig. 4C. The mechanical property of the membranes was important for the macroscopic cell encapsulation. In normal, an alginate membrane in hydrogel state could not be used as a macroscopic tool because it was too week to handle as a macro sized form. However, hydrogel-based macroscopic application could be available using BC composite membrane as its mechanical property was significantly enhanced enough to handle compared with alginate membrane (Fig. 4D).

In order to be used as an implantable cell encapsulating kit, the BC composite membrane should be permeable to small molecules such as oxygen (16 Da), glucose (180 Da) or medium size of growth factors ~30 kDa for the maintenance of cellular viability and function. Meanwhile, the BC composite membrane should not be permeable to host IgG antibodies (150 kDa) to protect the encapsu-

lated cells from the host immune system. In addition, it needs to be permeable to the therapeutic molecules such as dopamine 153 Da or insulin 6 kDa for the clinical application (Pedraz & Orive, 2010).

The permeability of BC composite membranes was determined by the release test using a model polymer of specific molecular weights. The cumulative permeation profiles of polymer solutions from the donor to a reservoir tube are shown in Fig. 5A. The donor tube-to-reservoir tube permeation results were dependent on the molecular weight of polymers. The pure BC membrane permeated 200 Da PEG completely out of the tube in a day. However, BC composite membrane permeated it about 70% after 1 day and completely after 5 days. For 6 kDa PEG, the pure BC membrane permeated about 94% after 5 days while the BC composite membrane (BC/TOBC/collagen/alginate) permeated about 53% after 5days. It was considered that the BC composite membrane was permeable to a low molecular weight of polymers.

For 20 kDa PEG, the cumulative permeation concentration of the pure BC membrane increased continuously up to about 71% after 5 days. The BC composite membrane also permeated about 28% of PEG after 5days, but the value was declined significantly compared with either 200 Da PEG or 6 kDa PEG. For 200 kDa PEO, the cumulative release concentration of the pure BC membrane was about 58% of PEG after 5 days. However, 200 kDa PEO was not permeated until 3 days and 8% of PEG was permeated after 4 days with the BC composite membrane. It indicated that the BC composite membrane (BC/TOBC/collagen/alginate) could remarkably reduce the permeation amount of large molecules compared with the BC membrane because of the nonporous alginate layer filling the pores of BC membrane. Permeability of antibody to the BC membranes was examined using mouse IgG (Fig. 5B). For a BC membrane, the amount of IgG permeation was increased continuously and approximately 60% was permeated after 5 days. On the other hand, a BC composite membrane did not permeate IgG at all even after 5days. PEO can permeate through the pores of BC composite membrane because it is a very flexible linear polymer. Meanwhile, proteins like IgG having a rigid structure are easily adsorbed at the pores of the membrane forming a founling cake layer. For the reason, 150 kDa IgG did not permeate through the membrane while the larger molecule of 200 kDa PEG did. It was inferred that the BC composite membrane was permeable to the low molecular weight of biological molecules that needed for the cell survival, but it enabled the protection of encapsulated cells from the immune molecules.

Prior to the PC12 cell encapsulation in the kit, the viability and proliferation of PC12 cells with the BC composite membrane were evaluated by MTT assays. As shown in Fig. 6, the relative cell number cultured on the normal BC membrane was lower than one on the tissue culture plate. To be used as a cell encapsulation material, the BC membrane needed to be modified for the improved cell attachment and proliferation. In general, the higher the cell adhesion capacity, especially good interactions between cells and underlying matrix, the faster the growth rate of the cells. The relative cell number cultured on the BC composite membrane which was coated with collagen showed a higher proliferation than cells cultured on the normal BC membrane as well as the tissue culture plate.

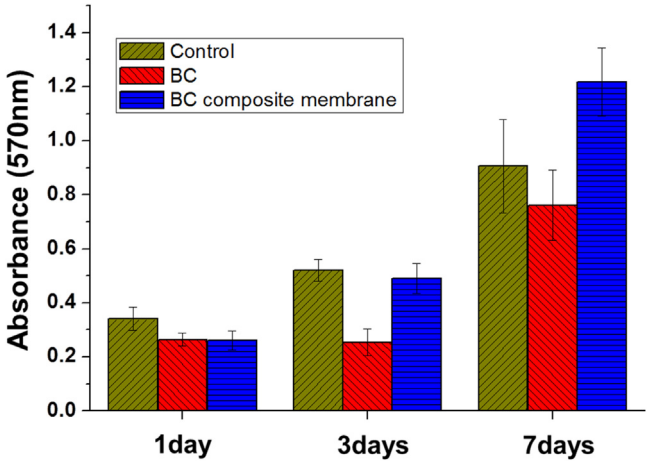

Fig. 6. Proliferation of cells by MTT assay on the TCP, BC and BC composite membrane. Empty 96 wells of tissue culture plates were used as a positive control. Data are means ± standard deviations from three specimens.

Representative SEM images of PC12 cells attached to the BC composite membrane were shown in Fig. 7A. Cells were attached to the surface stably. The network formation of long neurites at 7 days illustrated the bioactivity of cells in the encapsulated condition and it could be supported by the consistent result of dopamine release as shown in Fig. 7B. Encapsulated PC12 cells could survive and maintain their potential activity to differentiate into neurons on the BC composite membrane kit. After the PC12 cells were encapsulated in the kit, the amount of dopamine released from the kit was measured using ELISA. The dopamine concentration released through the membrane to the medium was greatly increased at 7days. It meant that a large number of cells differentiated into

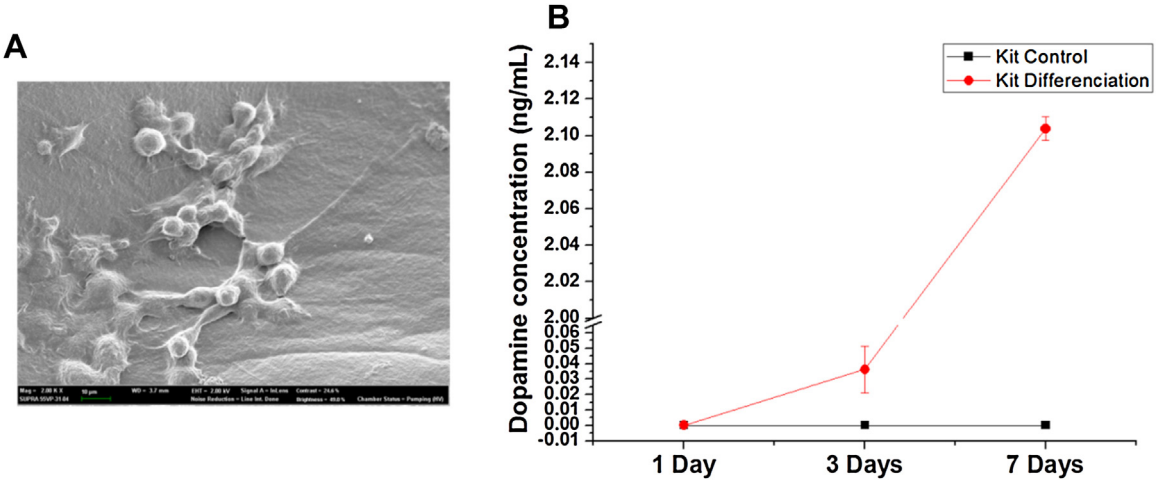

Fig. 7. PC12 cells cultured on the BC composite membranes. (A) FE-SEM image of PC12 cells cultured in the kit for 7days. (B) Amount of dopamine released from the kit. Data are means ± standard deviations from three specimens.

neurons after 7 days and the dopamine could penetrate the BC composite membrane.

4. Conclusion

The BC composite membrane (BC/TOBC/collagen/alginate) was fabricated by modifying the two sides of the BC membrane asymmetrically with different biomaterials. The one side of nanocellulose hydrogel was modified with TOBC and collagen subsequently for the improvement of cell adhesion and the other side was coated with alginate as a barrier to the penetration of immune molecules. The inner bulk layer of the BC composite membrane was highly porous whereas the alginate coated surface was non-porous. The BC composite membrane enabled the easy transfer of small sized molecules such as nutrients or oxygen but the large size of molecules having a similar size to immune molecules were not transferrable. The incorporation of BC with a thin alginate layer improved the cell viability as well as mechanical property due to the 3D networked and porous structure of BC compared with normal alginate. PC12 cells grown on the BC composite membranes released dopamine through the membrane to the medium with high cell viability and bioactivity.

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