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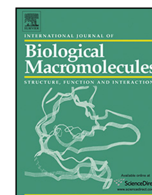

Fabrication of *Phaeodactylum tricornutum* extract-loaded gelatin nanofibrous mats exhibiting antimicrobial activity

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ABSTRACT

Microalgae have recently been recognized as a valuable resource for various applications. *Phaeodactylum tricornutum* is a diatom that lives in marine water and has an unusually high content of lipids. In this study, we added *P. tricornutum* into a gelatin dope solution to examine the effect of this diatom using electrospinning. The addition of *P. tricornutum* extracts increased the conductivity of the dope solution but had little effect on the viscosity. Due to the increased conductivity, the fiber diameter was reduced compared with the neat gelatin nanofiber. The loading of *P. tricornutum* extracts was confirmed using fluorescence microscopy, and the incorporation of lipids was detected through gas chromatography. The *P. tricornutum*-loaded nanofiber mat exhibited anti-microbial activity against *Escherichia coli* and multidrug-resistant *Staphylococcus aureus* (MRSA). The cell viability test showed that the *P. tricornutum*-loaded nanofiber has no cytotoxicity. We expect that this antimicrobial *P. tricornutum*-loaded gelatin nanofiber mat can be applied as a wound dressing.

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1. Introduction

The marine bio-environment encompasses a considerable part of the Earth's biodiversity, and novel chemical compounds from marine species are reported annually [1]. To date, more than 35,000 marine species have been discovered, and more than 18,500 chemical compounds have been identified [2,3]. Microalgae are unicellular microbial species found in marine water. In nature, microalgae are not only a primary producer in the marine ecosystem but also a significant producer of atmospheric oxygen through photosynthesis. In the industrial field, microalgae are proposed as infinite bioresources for various chemicals due to their biodiversity. High added-value compounds having antioxidative, anti-inflammatory, antimicrobial, antiviral and antitumoral properties have been identified, and some of these compounds are in the clinical trial state [3]. In addition, microalgae have received much attention as an excellent biomass for biorefinery. Components of microalgae can be converted into biofuels, bioplastics and platform chemicals through biochemical processes [4,5].

Phaeodactylum tricornutum, a diatom living in brackish and marine waters worldwide, can be easily cultured and is a candidate for the production of lipids and long-chain polyunsaturated fatty acids (PUFAs) for nutrition, pharmaceuticals, and cosmetics [6]. The water soluble extracts of *P. tricornutum* have exhibited anti-inflammatory, analgesic and free radical scavenging activities [7], but the primary interest on the *P. tricornutum* concerns the high PUFA content [8]. The anti-bacterial activities of *P. tricornutum* have been previously reported, and these activities are dependent on the morphotypes of *P. tricornutum*. The fusiform of *P. tricornutum* showed higher anti-bacterial activity than the oval morphotype due to the high PUFA content [9]. Moreover, the methanol extracts of *P. tricornutum* exhibited anti-bacterial activity against multidrug-resistant *Staphylococcus aureus* (MRSA) [10].

In the past, wound dressings were simply used to cover the wound to prevent infection. Currently, wound dressing requires additional properties to enhance the healing process of a wound. Functionalized biological and biochemical wound dressings have been developed for this purpose. These dressings provide a modified chemical environment at the wound site, thereby inducing more rapid healing and a longer period between dressing

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changes. Bioactive wound dressings are produced from a variety of biopolymers containing active ingredients, such as antimicrobials, antibiotics, growth factors, and vitamins [11]. However, antimicrobial activity is the primary requirement of any bioactive wound dressing.

Electrospinning is a simple and effective method used to fabricate ultra-fine fibers with nanometer scale range diameters. The ultra-fine fibers are collected on the metal collector in a form of flat nonwoven mats. Due to its ultra-fine diameter, the nanofibrous mat has high surface-to-volume ratios and can serve as adequate carriers for active ingredients. There have been several studies on the application of active ingredients containing electrospun nanofibers in wound dressing [11,12].

The ultimate goal of this study was to integrate the functionality of microalgae into the nanofibrous material as a wound dressing material. We selected gelatin as a carrier material and extracts of *P. tricornutum* as active ingredients. In this study, we identified the electrospinning condition of gelatin in the presence of a small amount of the *P. tricornutum* extracts. In addition, *in vitro* studies were performed to show the feasibility of the application of this material for wound dressing.

2. Materials and methods

2.1. Materials

Gelatin was purchased from Merck (Darmstadt, Germany). 2,2,2-Trifluoroethanol (TFE) and glutaraldehyde (GA) was purchased from Sigma Aldrich (Yongin, Korea). Bacto peptone, beef extract were purchased from Difco Lab (Detroit, USA). All chemicals were of analytical reagent grade and used without further purification.

2.2. Microalgae cultivation

P. tricornutum was cultivated in 30-L plastic cylinders at 20 °C, and air was continuously supplied at 5 L/min through airlift. Light was provided using 60-W fluorescent lamps at an intensity of 2500 lx. The algae were cultured in Conway medium [10] prepared from filter sterilized seawater, and the culture was continuously active during the 5 days after onset. The cells were flocculated with 200 ppm $Al_2(SO_4)_3$ (v/v) and recovered with centrifugation at 2000 rpm using high-speed refrigerated centrifugation (VS-24SMTi, Vision Scientific, Korea). The harvested biomass was lyophilized and stored at −70 °C.

2.3. Preparation and characterization of the dope solution

The dope solution for electrospinning was prepared by adding the lyophilized *P. tricornutum* powder to the gelatin solution. Typically, a pre-determined amount of gelatin was dissolved in TFE for 2 h, followed by adding *P. tricornutum* powder at a ratio of 0.5% or 1.0% (w/v) to the gelatin solution. After stirring the mixture for additional 2 h, the insoluble fractions of *P. tricornutum* were removed by filtering the solution with a non-woven filter before spinning. The conductivities of the dope solutions were measured using a conductivity meter (SC-170, Suntex, USA) and the viscosities were measured using a rotational viscometer (DV-E Viscometer, Brookfield, USA) in a temperature-controlled chamber (25 °C).

2.4. Electrospinning condition

For electrospinning, each dope solution was loaded in a syringe (10 mL) and placed on a syringe pump (KD Scientific, USA) to precisely control the flow rate. The syringe needle was connected to a power supply (Chungpa EMT high voltage supply, Korea). A rolling stainless drum was electrically grounded and used as a collector. The flow rate of dope solution was fixed to 1 mL/h. Electrospinning was performed at room temperature, with a relative humidity (RH) below 60%. The distance from the tip to the collector was maintained at 15 cm. The applied voltage was fixed at 15 kV.

2.5. Crosslinking of electrospun nanofiber mats

Crosslinking of electrospun nanofiber mats was performed according to a previous study [13]. Briefly, the gelatin nanofiber and *P. tricornutum*-loaded gelatin nanofiber mats were placed above a porous metal plate in a sealed chamber. GA aqueous solution was placed beneath the metal plate. The mats were crosslinked using GA vapor at room temperature (25 °C) for 48 h. After crosslinking, the samples were placed in a fume hood for 2 h, followed by post-treatment at 100 °C for 1 h to remove residual GA and partially enhance the crosslinking [14].

2.6. Characterization

The morphology of the electrospun *P. tricornutum*-loaded gelatin nanofiber mats was examined using field-emission scanning electronic microscopy (FE-SEM) (AURIGA, Carl Zeiss, Germany), and the obtained images were analyzed using image-analyzing software (Leopard, Korea). The fluorescence microscope images were examined using a photomicroscope (Axiophot, Carl Zeiss, Germany). Fatty acid methyl esters (FAME) were prepared to determine the fatty acid composition. Approximately, 100 mg of sample was placed in tubes with Teflon caps containing 340 μL of methylation mixture (MeOH:Benzene:DMP:H_2SO_4 = 39:20:5:2), and 200 μL of heptane were added to the sample and vigorously shaken. The tube was placed in a water bath at 80 °C for 2 h. After the heating step, the tube was cooled to room temperature. The upper phase, containing the FAME, was collected for the analysis. The composition of FAME was determined through gas-liquid chromatography (Agilent 7890A, Agilent Technologies Inc., USA), equipped with a DB-WAX Capillary GC Column (30 mm × 0.25 mm × 0.25 μm) with a split injector (1:20 ratio, 1 μL injected) and heated at 250 °C and a flame-ionization detector was operated at 280 °C.

2.7. Antimicrobial assay

The antibacterial activity of gelatin and *P. tricornutum*-loaded gelatin nanofiber mats against *Escherichia coli* (*E. coli*) ATCC 25922 and multidrug-resistant *Staphylococcus aureus* (MRSA) ATCC 33591 according to KSK 0693 was assessed. The nutrient agar was prepared by 5 g of bacto peptone, 3 g of beef extract in 1000 mL of distilled water and pH adjusted to 6.8 with 0.1 M NaOH. Gelatin and *P. tricornutum*-loaded gelatin nanofiber mats were inoculated with *E. coli* and MRSA. The concentrations of the cultures were adjusted to 1.5×10^5 colony-forming units (CFU) per mL, as verified using a spectrophotometer (λ = 660 nm). After that, additional inoculated control, gelatin and *P. tricornutum*-loaded gelatin nanofiber mats were incubated in the sealed chamber at 37 °C for 24 h. The bacteriostatic reduction rate was estimated using the standard equation

$$\text{Reduction}\,(\%) = \left(\frac{A-B}{A}\right) \times 100$$

where A and B represent the number of bacterial colonies in the control and experimental samples, respectively.

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