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Featured Letter

Novel gellan gum incorporated TiO_2 nanotubes film for skin tissue engineering

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ABSTRACT

Novel gellan gum incorporated TiO_2 nanotubes (GG+TiO_2-NT) film was successfully fabricated using solvent casting method for skin tissue engineering. The physicochemical properties of the film was investigated by FTIR, XRD and SEM. FTIR studies show the existence of interactions between TiO_2 nanotubes and GG polymer matrix. XRD analysis revealed that the film was in amorphous state and the presence of TiO_2 nanotubes on the surface of film was proved by SEM images. Cell proliferation studies demonstrated that, no sign of toxicity and the number of cells were found to be increased, thus exhibiting an ideal characteristic in skin tissue engineering applications.

1. Introduction

Surface wound or skin loss is one of the oldest problems in the operative field. In the last 25 years, several skin substitutes and treatment strategies has been recommended for curing skin wounds. Skin tissue engineering is one of the most advanced technology for skin treatment to cover the defects on the skin caused by medical operation as well as other non-intention injuries like burns, soft tissue trauma, disfiguring scars, or tumor resection [1,2]. Nowadays, application of nanobiomaterials in skin tissue engineering is tremendously increasing as these biomaterials mimic the structure of extracellular matrices and provide a platform for cell attachment, differentiation and proliferation [3,4]. Owing to the great advantages of biocompatibility, biodegradability, good oxygen and water vapor permeability, gellan gum (GG) has been widely used for wound healing and tissue engineering applications [5]. Further modification on GG film was carried out in order to improve the cell proliferation. In this study, novel GG incorporated TiO_2 nanotubes (GG+TiO_2-NT) film was fabricated and studied for skin tissue engineering application.

2. Experimental

2.1. Materials and methods

Prior to film preparation, TiO_2 nanotubes was synthesized using similar method as reported previously [6]. For preparation of GG +TiO_2-NT (1 wt%) film, 0.01 g of synthesized TiO_2 nanotubes was dispersed into 100 mL of distilled water with stirring for 4 h and sonicated for 30 min using a bath-type ultrasonic cleaner (FS 140H, Ultrasonic Cleaner, Fisher Scientific, Pittburg, PA, USA). Then, 1 g of GG kelcogel type, 0.5 g of glycerol, and 5 mL of $CaCl_2$ (0.1 M) were added to the solution and continuously stirred for 2 h at 70 °C. The solution was poured into petri dish and allowed to dry in oven at 50 °C for 24 h. The films were peeled off from the petri dish and conditioned in a humidity chamber controlled at 25 °C and 50% RH for at least 48 h before the further test. For comparison, the pure GG film was prepared using similar method except the present of TiO_2 nanotubes.

2.2. Characterization

The morphology of TiO_2 nanotubes were examined using Tecnai Biotwin FEI transmission electron microscopy (TEM) coupled with energy dispersive x-ray spectroscopy (EDX) for elemental analysis and their distribution. The morphology of GG and GG+TiO_2-NT films was acquired using a JOEL JSM 6360 LA electron microscopy. Ultraviolet–visible (UV–Vis) transmission spectra of films were

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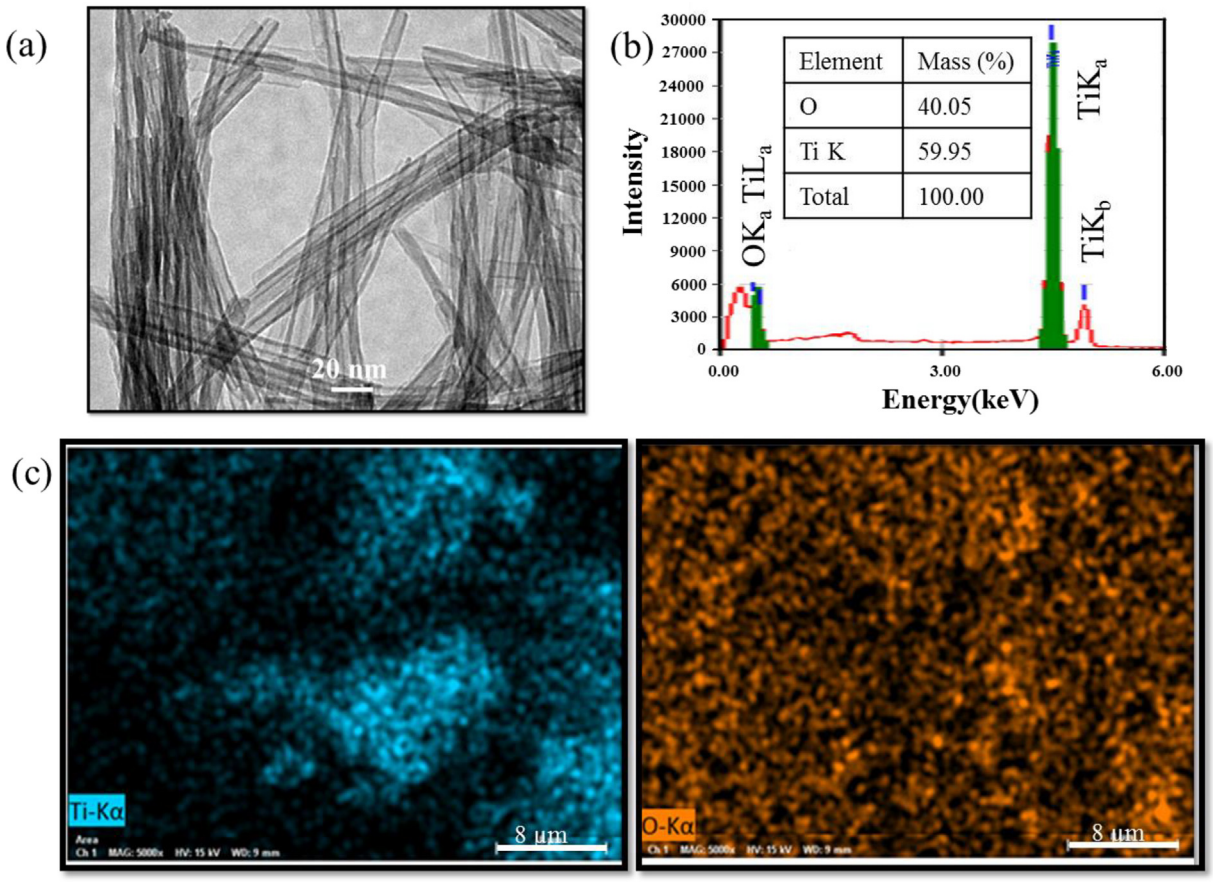

Fig. 1. (a) TEM micrograph (b) EDX spectra and (c) EDX mapping of synthesized TiO_2 nanotubes.

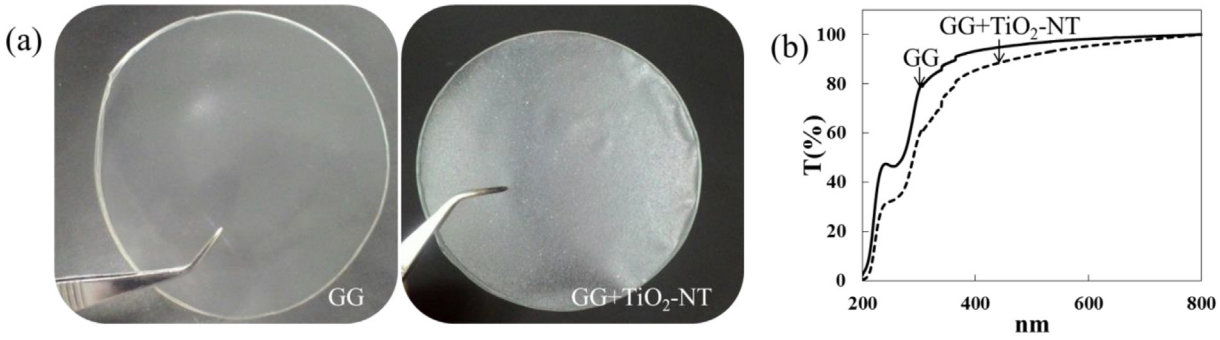

Fig. 2. (a) GG and GG+TiO_2-NT films (b) transmittance spectra of GG and GG+TiO_2-NT films.

scanned from 200 to 800 nm using Varian, Cary 50 spectrophotometer. Fourier Transform Infrared Spectroscopy (FTIR) spectra were recorded from 4000 to 600 cm^{-1} using a Perkin Elmer Spectrum 100 FT-IR spectrophotometer with a PIKE Miracle ATR accessory. XRD analysis were recorded at 2θ from 10° to 80° using Rigaku Miniflex (II) X-ray diffractometer. The mechanical properties of film samples was tested using an Instron Universal Testing machine (model 3366) with a load capacity ± 10 kN grips and cross-speed set at 10 mm min^{-1} according to ASTM standard method D882. Each sample was cut to 2.0 cm × 6.0 cm and five specimens for every sample were tested for reliable data.

2.3. Cell viability and proliferation testing

The performance of films for skin tissue engineering was tested using 3T3 mouse fibroblast cells. For 3T3 mouse fibroblast cells cultivation, the Dulbecco's Modified Eagle Medium (DMEM, ATCC, USA) supplemented with 10% (v/v) calf bovine serum (CBS, ATCC, USA) and 1% (v/v) antibiotic (penicillin/streptomycin, ATCC, USA) were used as the culture medium. The DMEM culture media without the presence of thin film samples were used as the negative control in this experiment. The viability of cells in contact with the thin film samples for 24 h, 48 h, and 72 h of incubation time was examined through a staining procedure of acridine orange/propidium iodide (AO/PI, Sigma Aldrich, USA) and observed by light microscope (Olympus TH4-200) equipped with fluorescence filter (Olympus U-RFL-T UV with blue light excitation). Meanwhile, the 3T3 mouse fibroblast cells' proliferations were quantified by using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Thermo Fisher Scientific, USA). The film samples and negative control were added with 50 µl of MTT assay solution and later incubated for 4 h. Then, the absorbance were recorded by