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The effect of BC/XG/CeO_2NPs nanocomposite on proliferation of NIH/3T3 cell line

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Abstract

Bacterial cellulose (BC) and Xanthan gum (XG) are used as renewable polymers in various fields, such as food and biomedical industries due to their unique physical properties. Today, the improvement of BC using nanoparticles (NPs) to create a new matrix has been attracted many researchers. In this study, BC was produced using *Komagataeibacter xylinus* (BPR 2001). Also, cerium oxide (CeO_2) NPs were synthesized using a green method. The FTIR and XRD analyses were performed for characterization the BC and CeO_2 NPs. Then, BC/XG/CeO_2 nanocomposite was prepared by casting method. The results confirmed the formation of both of BC and CeO_2 NPs. Therefore, the cytotoxicity effect of BC/XG/CeO_2 nanocomposite against NIH/3T3 cell line was evaluated using MTT method. The results showed that this nanocomposite had no cytotoxicity and can be used as a safe and biocompatible matrix for various biotechnological applications.

Key words: Bacterial Cellulose, Xanthan Gum, CeO_2 NPs, NIH/3T3 cell line

1. Introduction

Bacterial cellulose (BC) is a natural biopolymer which can be produced specifically by *Acetobacter xylinum*. BC has unique features including natural purity, biodegradability, biocompatibility, and non-cytotoxicity (1). Compared to conventional dressings, BC has an improved ability to repair chronic injuries and burns, mainly due to its moist and hydrophilic character, which provides an ideal environment for cell adhesion and pain control (2, 3). Although BC wound healing properties have been presented, it lacks intrinsic antimicrobial features (4). Therefore, several antimicrobial agents are introduced to be added into BC matrix to facilitate its application in biomedicine. Polysaccharide-

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based hydrogel materials are useful compound for biomedical purposes as they resemble biological systems. Xanthan gum (XG) is a branched and high molecular density polysaccharide with acidic characteristics, regularly produced by *Xanthomonas campestris*. This biopolymer has been used in the food and cosmetics industrials. (5). Cerium Oxide (CeO_2) nanoparticles (NPs) have been used in several applications due to their unique surface chemistry, high stability, and biocompatibility. It is mostly used in the fabrication of sensors, cells, catalysis, therapeutics agents, drug delivery careers, and anti-parasitic ointments (6). Furthermore, the applicability of CeO_2 in biomedical fields has been studied by several researchers (7). A recent study revealed that nano cerium, as a dual-functional therapeutic nanocarrier platform, could address pilocarpine to the inner components of the eye for glaucoma therapy (8). Furthermore, nanoceria held anti-apoptotic effects on macrophages, cardiomyocytes, and pancreatic islet cells and decreased signs of autoimmune encephalomyelitis (9-11). Chen et al. (12) indicated that ceria NPs has anti-apoptotic effects and preserves the endothelial cells. Hence, CeO_2 NPs can enter the body and affect the physical health when it is used in biomedical applications.

In this study, the bacterial cellulose/ xanthan gum/ CeO_2 nanocomposite were prepared and its cell cytotoxicity using MTT method against NIH/3T3 cell line was evaluated.

2. Materials and Methods

1-2- Materials

Fetal bovine serum (FBS), trypsin, Dulbecco's modified Eagle's medium (DMEM), and trypsin-EDTA were purchased from Gibco, and 3-[4,5-dimethylthiazole-2- yl]-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Aldrich. Xanthan gum (fufeng, China), bactopeptone (Quelab, Canada), disodium phosphate, cerium(IV) sulfate, citric acid, and glucose were purchased from Merck.

2-2- Bacterial Cellulose Production

Komagataeibacter xylinus (BPR 2001) was cultivated on modified medium, that was made of 0.5% (w/v) glucose, 1.5% (w/v) Fructose, 0.4 (%v/v) Corn steep liquor (CSL), 0.27% (w/v) disodium phosphate, and 0.115% (w/v) citric acid (13). The flask was incubated statically at 30 °C for seven days. The bacterial cellulose pellicles were settled into 0.1 M NaOH for 90 min at room temperature to remove the culture liquid's cells and elements, and further washed with distilled water. The purified cellulose pellicles were stored in distilled water at 4 °C to prevent drying (14). The BC was further characterized by FTIR analysis was used to confirm bacterial cellulose production.

3-2- Preparation of CeO_2 NPs

CeO_2 NPs was prepared using bacterial cell lysate supernatant (CLS of luminescent *Vibrio sp. VLC* (15). Briefly, the bacteria was cultured in SWC medium and centrifuged to get cell precipitate. Then, the cell pellets were resuspended in distilled water to release the enzyme for use in biogenic synthesis of CeO_2 NPs. Cerium (IV) sulfate (10 mM) was added to the CLS of *Vibrio sp. VLC* and heated for 90 min at 100 °C. The pale yellow precipitate was formed which it shows CeO_2 NPs was prepared . The precipitate was centrifuged and washed several times with deionized water and finally dried in a vacuum oven at 60 °C for 16 h. The powder was analysed by XRD for detection of CeO_2 NPs in sample.

4-2- Preparation of BC/XG/CeO_2 nanocomposite

The wet BC pellicle was blended in domestic blender. Then, the obtained BC (5 %wt) was mixed with 0.5% XG solution for 30 min at 80 °C. After that, CeO_2 NPs was added to the mixure and stirred for 15 min. Finally, it was casted to petri dish and dried by air oven at 45 °C for 24 h. Two nanocomposites were prepared: BC/XG without NPs as control and the BC/XG/CeO_2 as a test sample.

5-2- NIH/3T3 cell culture and treatment

NIH/3T3 (mouse fibroblast) cells were cultured in DMEM containing 10% FBS, 1% penicillin, and streptomycin. The cells were incubated at 37 °C in humid condition with 5% CO_2. Different sizes of BC/XG and BC/XG/CeO_2 were used to evaluate biocompatibility of these nanocomposites. For this purpose, nanocomposites were prepared in 3 sizes (1×1, 2×2, and 4×4 cm) and after sterilization they were maintained in DMEM for 24 h at room temperature to extract the released nanoparticles, and after that, the entire medium without nanocomposites was used for cell viability assessment using MTT assay.

6-2- Cell Viability

To assess the number of viable cells, the MTT assay was performed after 24 and 48 h of cell seeding in 96 well plates. The cultured samples were washed in PBS and incubated with MTT solution in DMEM at 37 °C for four hours. After removing the medium, 150 μL DMSO was added to dissolve the formazan crystals by incubation at 37 °C for 30 min. The optical density of the supernatant solution was then read at 540 nm using a microplate reader. The wells without nanocomposite were regarded as the baseline.

3. Results and Discussions

1-3- BC and CeO_2 NPs synthesis and characterization

The FTIR spectra analysis showed the presence of functional groups such as (-OH), (C-H), (β-1,4-glycosidic), (CH_2CH_2), and (C-O) related to BC structure (Figure1, Table 1).

Table 1-The assignment and wavenumber of BC (16)

Assignment	Wavenumber (cm^{-1})
O-H bending	400–700
β-Glucosidic linkages between the glucose units	~896
O symmetric stretching of primary-C alcohol	1040
C antisymmetric bridge-O-C stretching	1168
C-H deformation	1340
CH_2 bending or O-H in plane bending	1400
H-O-H bending of absorbed water	1650
CH_2	2700
CH stretching of CH_2 and CH_3 groups	2900
H-bond	3246
OH stretching	3500

The X-ray diffraction pattern of CeO_2 NPs (JCPDS no. 03-065-5923) biosynthesized using *Vibrio sp. VLC* was obtained using MATCH! Software (Figure 2). Results showed that CeO_2 NPs are successfully synthesized from *Vibrio sp. VLC* cell lysate supernatant.

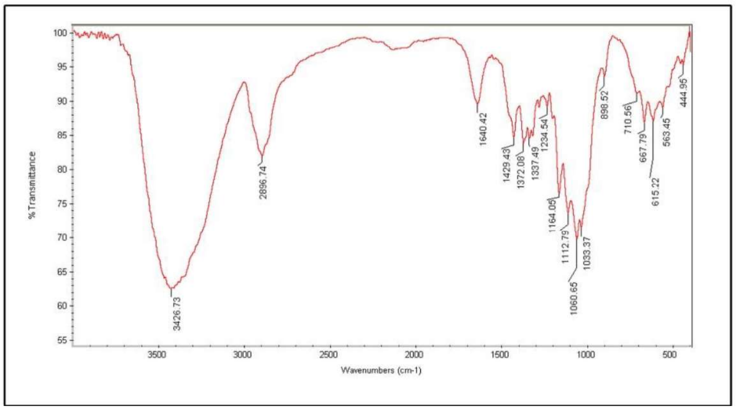

Figure 1 -The FTIR spectrum of BC

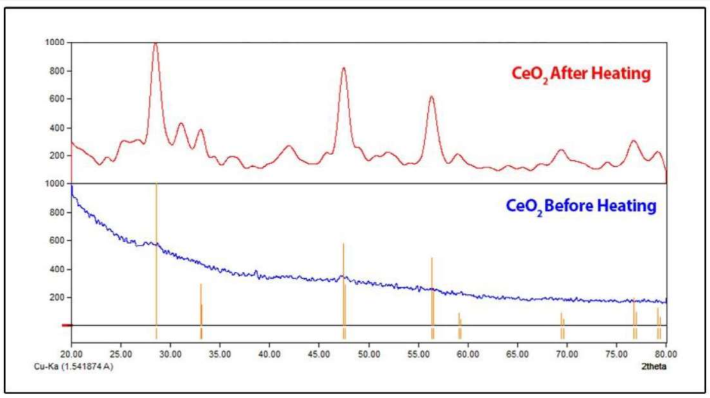

Figure 2- XRD pattern of CeO_2 NPs synthesized using *Vibrio sp. VLC*

2-3- Cell Viability

The MTT results assay showed that an addition of BC/XG/CeO_2 nanocomposites in different sizes (1×1, 2×2, and 4×4 cm) increases the growth of NIH/3T3 cells after 48 h incubation (Figure 3). Also, culture-treated cells containing nanocomposites had good biocompatibility with NIH/3T3 cell lines. However, with increasing the size of nanocomposite films, a significant difference was observed in the viability of NIH/3T3 cells exposed to them at 24 h. The viability of NIH/3T3 cells was further increased after 48 h possibly due to the synergistic effect of biopolymers (17). In conclusion, the results indicate that the newly constructed nanocomposite (BC/XG/CeO_2) forms a matrix for cell attachment and preservation of their morphology, which may affect the cell proliferation (18). As antimicrobial activity of CeO_2 NPs is well reported (19), its addition to BC-XG that resulted BC/XG/CeO_2 nanocomposite beside cell growth improvement, added antimicrobial properties to this bionanocomposite made it an appropriate structure for biomedical applications.

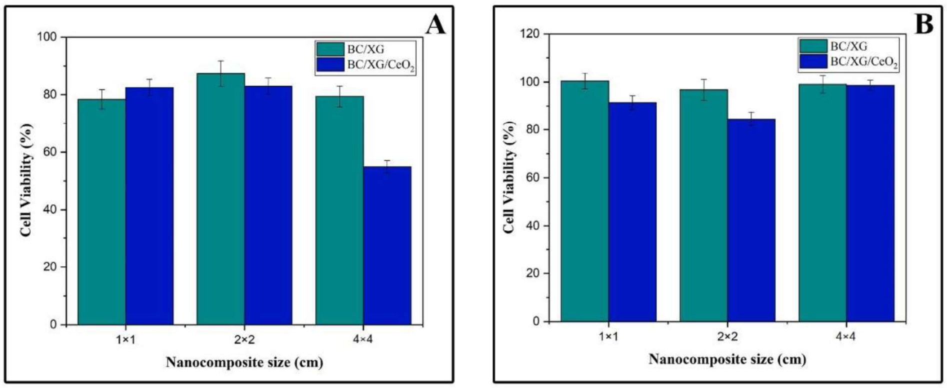

Figure 3- Results of MTT assay showing the cell viability of NIH/3T3 cells on all the synthesized nanocomposites. 24 h (A), 48 h (B)

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