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A novel use of cellulose based filter paper containing silver nanoparticles for its potential application as wound dressing agent

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ABSTRACT

The frequent use of antibiotics against microbial infections may lead to the emergence of antibiotic resistant microbial strains. To overcome these microbial strains, we need to fabricate alternative materials which can handle them. It is for this reason, we have fabricated cellulose (CE) based filter paper (FP) composite scaffolds comprising of adsorbed chitosan (CS) and sliver (Ag) nanoparticles (NPs). The AgNPs are incorporated in the CS layer of the composite scaffold. Prior to evaluate the efficacy of the scaffolds against gram positive and gram negative bacterial strains, the scaffolds were characterized for the presence of the Ag NPs with field emission scanning electron microscope (FE-SEM), fourier transform infrared (FTIR) spectroscopy and x-ray diffractometer (XRD). These techniques confirmed the presence of Ag NPs in the composite scaffold. The biocompatibility of the scaffolds was assessed by subjecting pristine FP, CS adsorbed FP (CS-FP) and Ag loaded CS-FP (Ag-CS-FP) composite scaffolds to *in vitro* studies. From the data obtained, it was observed that NIH3T3 fibroblastic cells adhered and proliferated onto all the scaffolds. Furthermore, the scaffolds exhibited good antibacterial activity against both strains of bacteria. It is, therefore, concluded that these scaffolds could find potential application in biomedical field, particularly as a wound dressing agent.

1. Introduction

Biopolymers, irrespective of their origin: which can be either natural or synthetic, are frequently used in numerous applications [1]. These polymers are often used as a supporting material in the materials development/fabrication mainly due to their excellent biocompatible and physio-chemical properties. However, despite their excellent biomcompatability, their role in biomedical field is somewhat restricted due to their inertness to microbes and non inhabition of bacterial growth (no antibacterial activity). These deficiencies are, however, overcome by researchers with the introduction of composite fabrication: where the syngernic effect of the components leads to the improvement of both the above mentioned deficiencies [2]. Biocomposites are fabricated by numerous techniques. Some of the techniques such blending, solution casting, salt leaching, and electrospinning are well established and widely documented in the literature, etc [2,3]. In these methods biocompatible biopolymers are loaded with materials, both polymers and NPs, that can inhibit antibacterial activity. The final material usually has dual properties, i.e., biocompatibility and antibacterial activity which make them of prime importance in biomedical field [3]. Living beings are mostly infected by various infections caused by microbes (viruses, bacteria, yeasts and mold) found in nature. With the emergence of the microorganism with antibiotic resistant strains, researchers are working day in and day out to introduce new cost effective methods to cope with these microbial strains [4,5]. Researchers have fabricated NPs of both metals and their oxide with antimicrobial efficacy. Among these NPs; Zinc oxide (ZnO), titanium dioxide (TiO_2), copper oxide(CuO), and Ag and its oxides are given top priority. These NPs are fabricated *via* different methods, which are mostly reported in the

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literature [6]. Compared to other NPs, Ag and its oxides have exhibited good antimicrobial activity and more specifically antibacterial activity against various bacterial strains. Thus, due to the aforementioned properties Ag NPs and their oxides are frequently used in fabrication of materials, which can be used to inhibit bacterial growth [4]. CE is a natural biopolymer produced by several microbial species.CE is an odourless and colorless polymer with good biocompatibility, nontoxicity, hydrophilicity and good mechanical strength. It has also exhibited tunable optical appearance. All these properties make CE an excellent candidate to be used in wide range of applications. CE has proved its pivotal role in biomedical field, particularly in drug delivery, tissue regeneration, and enzyme immobilization [7–9]. CE has the potential to be used in the fabrication of composites comprising of various biocompatible and antimicrobial materials. Pure CE is, however, mostly used as a template/supporting material due to its limited adsorption sites and good mechanical strength [10]. CS derived from chitin is a polysaccharide, which is usually coated on the CE to be used as an adsorbent [11]. The idea behind using CS as adsorbent on CE is the presence of hydroxyl (OH) and amine (NH_2) moieties in CS, which have the tendencies to bind with metal ions. The presence of OH and NH_2 moieties in CS enables the polymer to engange metal ions from aqueous solutions and bind them in the polymeric matrix *via* dipole- dipole and electrostatic interactions [12,13].

In this paper, we have produced an efficient and cost effective Ag-CS-FP microfibers composite scaffold, which can be used as a wound dressing agent. The Ag-CS-FP composite scaffold was fabricated by the adsorption of CS onto FP and reduction of adsorbed Ag^+ ions into the CS Layer with $NaBH_4$. After characterizating the scaffolds thoroughly using FE-SEM, XRD and FTIR, the scaffolds were subjected to *in vivo* cellular response (cell adhesion, cell proliferation) and antibacterial activity. The data revealed that Ag-CS-FP composite scaffold has the potential to be used in biomedical applications and more specifically as a wound dressing agent.

2. Experimental

2.1. Materials and methods

High molecular weight CS powder (deacetylation >75%), Sodium borohydride ($NaBH_4$, 99%), acetic acid (CH_3COOH), Silver Nitrate ($AgNO_3$) and CE based FP(Whatman) with 12.5 cm diameter and 0.3 mm thickness were purchased from Sigma-Aldrich. CE based FP is normally used for filtration purposes in scientific laboratories. Fibroblastic cells (NIH3T3) cell line was purchased from the Korean cell bank (Seoul, South Korea). Fetal bovine serum (FBS), phosphate-buffered saline (PBS) and penicillin G-streptomycin were purchased from Gibco, Tokyo, Japan. All products were of analytical grade and used without further purification. Double distilled water was used throughout this work.

2.2. Coating of CS onto FP

Cs solution was prepared in accordance with the method reported [1]. Briefly1% w/v concentration of CS solution was prepared using binary solvents (dilute acetic acid aqueous solution (10% v/v)) and kept at room temperature for stirring overnight. CS was then coated onto the FP by immersing the FP into the CS solution. After wiping the excess amount of CS solution, the CS coated FP (CS-FP) scaffold was dried and stored.

2.3. Ag NPs preparation in CS coated layer

The Ag NPs were fabricated *via in-situ* method inside the CS layer coated onto FP using the protocol reported for the reduction of Ag ions [1]. Briefly the CS-FP scaffold prepared in the above mentoined step was immersed in the aqueous solution of $AgNO_3$ for 2 h to load/adsorb Ag^+ ions into the CS layer of the CS-FP scaffold. The saturation of Ag^+ ions into CS-FP scaffold was determined as function of time. After the completion of the saturation step, the CS-FP scaffold loaded with Ag^+ ions was gently washed with double distilled water to remove the unadsorbed Ag^+ ions and re-immersed into 100 mL of 0.1 M $NaBH_4$ aqueous solution. The CS-FP scaffold was left immersed in the solution for another 2 h to cause the reduction of Ag^+ ions into Ag NPs. After the completion of this step, the Ag NPs loaded CS coated FP (Ag-CS-FP) was gently washed with double distilled water and preserved for characterization, cellular response and antibacterial activity. All the experimental septs are sketched in Scheme 1.

3. Characterization

The surface morphology of the pristine FP, CS-FP and Ag-CS-FP composite scaffolds was assessed by FE-SEM (400 Hitachi, Tokyo, Japan). The samples were fixed onto the holder using carbon tape and sputter-coated with platinum. The platinum-coated samples were then examined by FE-SEM under high vacuum. The x-ray diffraction (XRD, Rigaku D-MAX IIB, Tokyo, Japan) scans were recoreded at 2θ between 10 and 80° at 40 kV and 30 mA. Fourier transform infrared spectroscopy (FTIR, Galaxy 7020A; Mattson, Fremont, CA, USA) spectra were obtained in the region of 800–4000 cm^{-1}. Prior to analysis, the samples were mixed with KBr pellets and molded into a disc shape under hydraulic pressure.

4. Cellular response and antibacterial activity

4.1. Cell adhesion

For the evaluation of the interaction between fibroblastic cells (NIH3T3), pristine FP, CS-FP and Ag-CS-FP composite scaffolds, the scaffolds were subjected to cell culture studies [14]. Briefly, the scaffolds were shaped into round disc, fitted inside a 4-well culture dish and dipped into minimum essential medium (MEM) containing 10% FBS. After soaking the scaffolds, 1 mL of the NIH3T3 cell solution with (3×10^4 cells/mL) density was seeded on the surface of the scaffolds and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 1 and 3 days. After incubation, the supernatant was gently removed and the scaffolds were washed thrice with PBS, fixed with 2.5% glutaraldehyde solution for 10 min. On completion of the fixation step the scaffolds were dehydrated/dried with a critical point drier and sputter coated with gold. The surface morphology of the composite was observed by FE-SEM.

4.2. Cell proliferation

The proliferation of NIH3T3 fibroblastic cells on pristine FP, CS-FP and Ag-CS-FP composite scaffolds was evaluated using MTT assay [2]. Briefly, fibroblastic cells at a density of 3×10^4 cells/mL were cultured on FP, CS-FP and Ag-CS-FP composite scaffolds, which were fixed in a 4-well plate. The proliferation of NIH3T3 fibroblastic cells was observed for 1 and 3 days of incubation. MTT solution (50 μL, 5 mg/mL in PBS) was added to each well and incubated at 37 °C for 4h in a humidified atmosphere containing 5% CO_2. After removing the medium, the converted dye was dissolved in acidic isopropanol (0.04N HCl-isopropanol) and incubated for 30 min at 25 °C in the dark. From each sample, 100 μL medium was transferred to a 96-well plate and the converted dye was measured under ultraviolet light at a wavelength of 570 nm on a kinetic microplate reader (EL x 800, Bio-Tek Instruments, Inc., Highland Park, VT, USA).