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Fungal chitosan based nanocomposites sponges—An alternative medicine for wound dressing

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ABSTRACT

The porous structured and cell proliferative biodegradable fungal chitosan (FCS) based composites with potential antibacterial property was prepared with *Aloe vera* extract (ALE) and the plant *Cuscuta reflexa* mediated biosynthesized silver nanoparticles (CUS–AgNPS) were developed for wound dressing applications by freeze drying method. Fungal chitosan was derived from *Cunninghamella elegans* a species belongs the family of Zygomycetes. The CUS–AgNPS were characterized by the UV–vis spectrum, XRD and SEM. CUS–AgNPS were loaded into the FCS-ALE sponges and were characterized by UV–vis spectrum, FT-IR and SEM. The nanocomposite sponges (FCS-ALE/CUS–AgNPS) showed prominent results against the different pathogenic bacteria and did not affect the cells were tested *in vitro* cell viability against human dermal fibroblast cell (HDF cells) which revealed significant cell viability. Based on these observations our composite formulation (FCS/ALE/CUS–AgNPS) could be suggested potential for wound dressing applications.

1. Introduction

Some of the biopolymers are the substituent's which could fulfill all the requirements of healing process in which they are recently occupying the place of wound dressing industries. Among the different biopolymers, chitosan have a great emphasis because of their different unique properties of polycationic, biocompatibility, biodegradability, muco-adhesiveness, antimicrobial and non-toxic [1–4] nature. Currently chitosan based nanocompsite sponges have got an ample attention in the field of wound dressing development [5–8].

Chitosan (CS) is a linear 1, 4-linked polymer composed of *N*-acetyl-D-Glucosamine residues, and deacetylated product of chitin which is the major source of crustaceans and fungi [9–11]. The chitosan isolated from crustaceans has the risk of affordability, seasonal supply, physio-chemical properties changes and toxic properties [11,12]. To overcome aforementioned problems, fungal chitosan (FCS) have been used in this study. Fungi, particularly the Zygomycetes group of fungi are rich source of chitosan [10,13], in which *Cunnighamella elegans* was chosen in the work for isolation of chitosan using simple and low cost isolation techniques.

Wound is defined as the physiological breakage in the skin architecture, trauma, fluid loss, leading to the infection and pain to the organisms [14]. Subsequently the wound healing is also a complex biological process such as angiogenesis, re-epithelialization and rebuild the skin strength [15]. Other than that wounded skin facilitating microbial growth, it delays the process of the healing. In general wound healing process is not easily accelerated without any antimicrobial agents, but many microbes have evolved against various commercial drugs making it resistant [16]. However, the biogenic silver nanoparticles (AgNps) overcome this problem since they have potential antimicrobial properties against multidrug resistant organism than the commercial drugs, depending on the dose depending manner against both gram positive and negative bacteria were recorded [17]. Hence, in this study biogenic silver nanoparticles were synthesized by using the medicinal plant *Cuscuta reflexa* (CUS–AgNPS).

Biologically active silver nanoparticles loaded chitosan nanocomposites had greatest consideration for its antimicrobial activity [2,18] and could be modified into various form like hydrogel [19], nanocomposite film [2,17,18], nanofibers [18], scaffolds and sponges [20].

However silver as metal exhibits toxicity even at a minimum level of concentration [21] and in this concern we have one hypothesis for reducing the toxicity of the cells from the silver nanoparticles and accelerating the cell proliferation without disturbing their properties of composites to aimed to taken well

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known natural and ancient wound healing agent *Aloe vera* extract were added to the nanocomposites. *Aloe Vera* is a succulent plant which comes under the family Liliaceae, which was used to cure the various skin diseases in the Indian traditional medicinal system. Still it has been used for treating wound because of their active major constituents such as vitamins, minerals, amino acids, glycoproteins, steroids, cholesterol, campestrol and β -sitosterol (anti–inflammatory), acemannan (macrophage activation on the skins) [22]. Aloe vera anti–inflammatory properties are higher than commercial wound healing drug [23,24] and are cost effective.

The aim of the present work was preparation of nanocomposites sponges using fungal chitosan isolated from the *Cunninghamella elegans*, *Aloe vera* extract and biosynthesized silver nanoparticles from medicinal plant *Cuscuta reflxa* for wound dressing application. The cost effective and promising antimicrobial metal nanoparticles loaded with the composites (FCS/ALE/CUS–AgNPS) were evaluated for its respective physiochemical and biological activities.

2. Materials and methods

2.1. Materials

Chitosan was isolated from the fungus *Cunninghamella elegans* obtained from the Culture Collection Centre, Centre for Advanced Studies in Botany, University of Madras, Chennai, Tamilnadu, India. The degree of deacetylation (Supplementary Fig. 1) was found to be 80.85% with molecular weight (Supplementary Fig. 2) ranging from 2 to 3 monomers. Silver nitrare ($AgNO_3$) and Muller Hinton agar (MHA) from Himedia, Acetic acid, other chemicals and solvents were procured from Merck and SD Fine chemicals.

2.2. Fungal culture development for chitosan production

The fungus was maintained in Potato Dextrose Agar medium at 30 °C. Spore suspensions were prepared and adjusted to 10^7sporangioles/mL using a hemocytometer. The adjusted spore suspensions were inoculated into the PDB medium containing Potato extract 200 g, and dextrose 20 g per liter of water and incubated at 28 °C for 3–5 days in a static condition.

2.3. Fungal chitosan extraction

One gram of freeze-dried mycelia powder was grounded and soaked with 40 mL of one Molar sodium hydroxide (1 M NaOH) for12 h. The mixture was autoclaved at 121 °C for 15–20 min. Later, the mixtures were centrifuged at 8000 rpm for 20 min. The freeze dried pellet was washed twice with double distilled water, and subsequently washed with 95% of ethanol. The pellet (AIM – Alkali Insoluble Mass) fungal chitosan (200 mL) was dissolved in 0.5% acetic acid by continuous stirring at room temperature for 1 h. The obtained solution was precipitated by adding 1 M of NaOH with pH8.5 to 9.0. The retained fungal chitosan was freeze-dried with lyophilizer (FD–10 M Freeze Dryer, LARK) and was compared with practical grade chitosan (Sigma Aldrich) for further studies.

2.4. Preparation of Aloe vera gel extracts

Healthy and matured *Aloe vera* leaves were collected from natural environment and washed with distilled water. The rough portion of the leaves was peeled out, clear and smooth gelly portion were collected. Homogenized extracts were sonicated for 10 min and centrifuged at 10000 rpm for 15 min at 4 °C to remove the solid matters. It was then freeze dried and stored −20 °C for further use.

2.5. Green synthesis of silver nanoparticles by using Cuscuta reflexa

The *C. reflexa* was collected from south Tamilnadu, India. Healthy green plants were (10 g) were cleaned and added to distilled water at 60° C for 15 min. After cooling down, the extract was filtered twice using Whatman (No.1) filter paper. The filtered plant extracts were used for silver nanoparticle synthesis. The aqueous filtrates of plant C. *reflexa* (10%) was added to AgNO3 (10 mM) solution and the reaction flask was kept in the dark condition on magnetic stirrer. After 24 h of incubation, the plant filtrate (light green) added to the silver nitrate solution (colorless) was turned into a dark brown color, indicating the synthesis of silver nanoparticles. For further identification, the solution was evaluated under UV–vis spectrum at the range from 300 to 500 nm and also reading taken at different time interval for checking the stability of the biogenic silver nanoparticles.

2.6. FCS- ALE- CUS Ag NPs composites solution preparation

Chitosan (1%) was dissolved in 0.5% of acetic acid under stirring, then 0.2% of ALE was added drop wise into the completely dissolved FCS solution after that stirring was extended up to 2 h. Finally 0.5% of CUS Ag NPs was added to the FCS/ALE mixture under dark condition and continued stirring for another 6 h. The solution was pour into a round container (1 × 1 cm dia.), lyophilized and kept at −20 °C after the sample.

2.7. Characterization of FCS based composites sponges

2.7.1. UV–vis spectroscopy

The synthesized CUS–AgNPs and composite were analyzed using UV–vis spectroscope. CUS–AgNPs were recorded on different time intervals.

2.7.2. X- ray diffraction (XRD) analysis

Silver nanoparticles were characterized by X-ray diffraction analysis to confirm their crystallographic structures. The spectra were recorded in the scanning mode on a Bruker AXS D8 X-ray diffractometer employing CuKα radiation, in range of 2θ = 10°–80°.

2.7.3. Fourier transform infra red (FTIR) spectroscopy

Functional groups of fungal chitosan sponge was compared with the Sigma chitosan and analyzed by the potassium bromide pellet method using FT–IR spectrometer scanning in the range of 4000–400 cm^{-1}. Before being pelletized, the sponges were finely grounded and mixed with potassium bromide in water-free environment.

2.7.4. Scanning electron microscopy

The surface of the chitosan sponges and tetracycline cross-linked chitosan sponges were sputter coated with gold and observed by the field emission scanning electron microscope (Hitachi) with a precision of 15.0 keV.

2.7.5. Porosity of FCS based composites

Porosity of the control sponge and drug-loaded fungal chitosan sponge was calculated by liquid displacement method using ethanol [7] Vernier caliper was used to measure the dimensions of the sponges and the volume (V) was calculated. The initial weight (W_i) of sponges was recorded before they were immersed into known volume of ethanol in graduated cylinder for 24 h. After incubation of the sponges, the final weight (W_f) of the wet sponge was