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Short communication

Biochemical properties of *Hemigraphis alternata* incorporated chitosan hydrogel scaffold

M. Annapoorna, P.T. Sudheesh Kumar, Lakshmi R. Lakshman, Vinoth-Kumar Lakshmanan, Shantikumar V. Nair, R. Jayakumar*

Amrita Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham University, Kochi 682 041, India

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ABSTRACT

In this work, *Hemigraphis alternata* extract incorporated chitosan scaffold was synthesized and characterized for wound healing. The antibacterial activity of Hemigraphis incorporated chitosan scaffold (HIC) against *Escherichia coli* and *Staphylococcus aureus* was evaluated which showed a reduction in total colony forming units by 45-folds toward *E. coli* and 25-fold against *S. aureus* respectively. Cell viability studies using Human Dermal Fibroblast cells (HDF) showed 90% viability even at 48 h when compared to the chitosan control. The herbal scaffold made from chitosan was highly haemostatic and antibacterial. The obtained results were in support that the herbal scaffold can be effectively applied for infectious wounds.

1. Introduction

Chitosan is used as a haemostatic wound dressing due to its biodegradable and biocompatible nature. *N*-Acetyl-glucosamine is the degradation product of chitosan. It is an initiator of proliferation phase (Jayakumar, Prabaharan, Sudheesh Kumar, Nair, & Tamura, 2011) in wound healing. An infectious wound needs an active antimicrobial agent for its recovery which is not effectively met by chitosan alone. *Hemigraphis alternata* (*H. colorata*) is a well known folk medicine. The herb is a perennial, belonging to the family Apocyanacea. The foliage carries attractive violet color, growing well in the presence of sun light. The use of crude leaf extract for anti-inflammatory activity (Subramoniam, Evans, Rajasekharan, & Sreekandan Nair, 2001) has already been studied. The phytochemical screening of *Hemigraphis* leaves and their antibacterial activity has already been worked on (Anitha, Marimuthu, Antonisamy, & Jeeva, 2011). In the present work some of the properties of chitosan scaffold have been compared with the herbal scaffold in vitro.

2. Materials and methods

2.1. Materials

H. alternata was collected from Kakkanad, Kerala. Chitosan (molecular weight – 100 kDa and degree of deacetylation – 80%) was obtained from Koyo Chemical Co. Ltd., Japan. Bacterial samples (ATCC), *Escherichia coli*-25922 and *Staphylococcus aureus*-25923 were obtained from the microbiology department of Amrita Hospital.

2.2. Preparation of chitosan scaffold and HIC

Chitosan was dissolved in 1% acetic acid by constant stirring. The solution was neutralized by the addition of NaOH solution. To obtain hydrogel, the solution was centrifuged at 10,000 rpm for 10 min. The obtained hydrogel yield was 1250%. The pellet obtained was freeze dried to obtain the scaffold. Fresh crude extract was collected from *Hemigraphis* leaves. The extract was lyophilized and added to the hydrogel, stirred for uniform mixing and freeze dried to obtain HIC (Fig. 1).

2.3. Characterization

Surface morphology was done using SEM (JEOL, JFC-1600, Japan). Thermogravimetric analysis was done at a temperature

* Corresponding author. Tel.: +91 484 2801234; fax: +91 484 2802020.
E-mail addresses: rjayakumar@aims.amrita.edu, jayakumar77@yahoo.com (R. Jayakumar).

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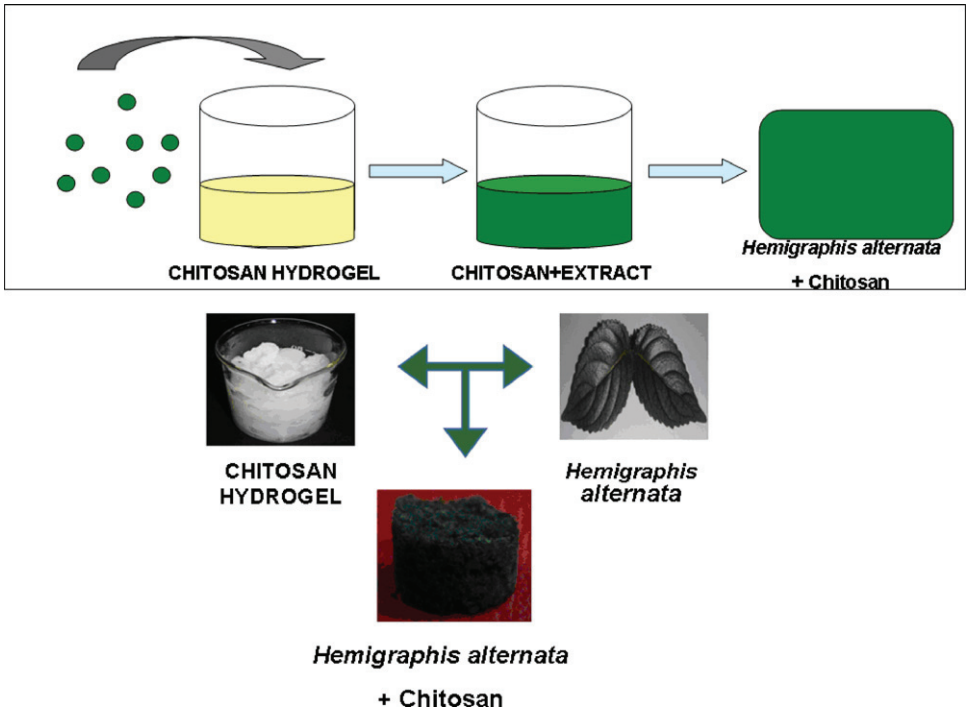

Fig. 1. Schematic representation of the preparation of *Hemigraphis alternata* incorporated chitosan scaffold.

range between 0 and 500 °C using TGA instrument (SII TG-DTA6200). Analysis using FTIR spectroscopy (Perkin Elmer Spectrum RX1) was done within a range of 400–4000 cm^{-1}.

2.4. Porosity, water uptake and swelling studies

Scaffolds of equal size were immersed in 95% ethanol for 24 h until saturation. The final weights of scaffolds were noted. Porosity can be calculated as follows

$$\text{Porosity} = \frac{\text{Wetwt} - \text{drywt}}{\text{Density} \times \text{volume}} \times 100$$

To determine the water uptake and swelling ratio, equally weighed scaffolds were immersed in water and PBS respectively followed by incubation at 37 °C for 14 days. The weights were noted on 1st, 7th and 14th days. The ratio can be calculated as

$$\text{DS/WU} = \frac{\text{Wtf} - \text{Wti}}{\text{Wti}}$$

where WU is water uptake, DS is degree of swelling, Wtf is the final weight and Wti is the initial weight.

2.5. In vitro biodegradation study

The scaffolds were equally weighed and immersed in PBS (pH 7.4) containing lysozyme followed by incubation at 37 °C. The final weights were noted after freeze drying the scaffolds. The percentage of biodegradation is calculated as

$$\% = \frac{\text{Wtf} - \text{Wti}}{\text{Wti}} \times 100$$

2.6. Platelet activation study and whole blood clotting assay

Plasma collected from fresh human (O+) blood samples were added to the scaffolds and incubated. The samples were fixed with gluteraldehyde, dried using ethanol and analyzed using SEM. The blood clotting property of HIC was compared with kaltostat and chitosan control. The samples for blood clotting studies were incubated with blood samples at 37 °C. Water was added slowly through the sides of the plate and free hemoglobin release at 540 nm was plotted.

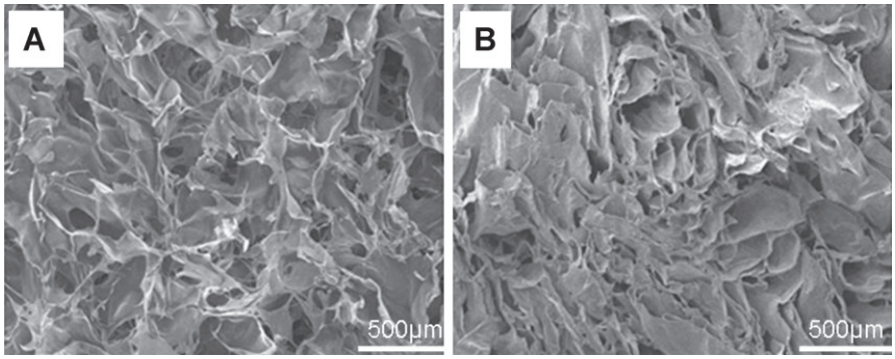

Fig. 2. (A) SEM image of chitosan control and (B) *Hemigraphis alternata* + chitosan.