## Preparation and characterization of succinyl chitosan and succinyl chitosan nanoparticle film: In vitro and in vivo evaluation of wound healing activity

N T Thu Thao<sup>1</sup>, H M S M Wijerathna<sup>2</sup>, R Saravana Kumar<sup>3</sup>, Dongrack Choi<sup>1</sup>, S H S Dananjaya<sup>4</sup>, A P Attanayake<sup>5</sup>

<sup>1</sup>Zerone Bio Inc., Dankook University, 3(rd) Floor, Sanhak Building, Dandae-ro 119, Dongnam-gu, Cheonan Si, Chungcheongnam-do 31116, Republic of Korea.

<sup>2</sup>Department of Aquaculture and Aquatic Resources Management, University College of Anuradhapura, Sri Lanka.

<sup>3</sup>Department of Physics, Government College of Arts and Science, Idappadi, Salem 637102, Tamil Nadu, India.

<sup>4</sup>Zerone Bio Inc., Dankook University, 3(rd) Floor, Sanhak Building, Dandae-ro 119, Dongnam-gu, Cheonan Si, Chungcheongnam-do 31116, Republic of Korea. Electronic address: sajith@zeronebio.com.

<sup>5</sup>Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka. Electronic address: anojaattanayake@med.ruh.ac.lk.

## Abstract

Development of novel wound dressing materials having the ability to prevent bacterial infections and capable of accelerating the tissue regeneration process is utmost important, since the wounds in patients can cause severe health issues. In the present work, we synthesized novel N-succinyl chitosan nanoparticles (N-SuC NPs) films and tested their antimicrobial, cytotoxicity, and in vitro and in vivo wound healing activity. N-SuC NPs were synthesized by ionic gelation method, and subsequently N-SuC NPs films were prepared by solution casting method using synthesized N-SuC NPs. The prepared N-SuC NPs films showed significant antimicrobial activity against Escherichia coli and Staphylococcus aureus with a minimum inhibitory concentration of 6 mg/mL and <8 mg/mL, respectively. The biocompatibility and the in vitro wound healing activity of N-SuC NPs films were analyzed using human dermal fibroblast (HDF) cells. In vivo cutaneous wound healing of the N-SuC NPs film was investigated using the Wister rat model, and the studies showed that the N-SuC NPs film significantly accelerated the wound healing process by inducing more blood vessels formation and tissue granulation. The experimental results showed that synthesized N-SuC NPs film had excellent antimicrobial, cytotoxicity and wound healing activity, indicating that it could be used in biomedical applications.

**Keywords:** Cytotoxicity; N-succinyl chitosan; N-succinyl chitosan film; Nanoparticles; Wistar rats; Wound healing.