

## Synthesis of Chitosan/Silver Nanocomposites: Antibacterial Properties and Tissue Regeneration for Thermal Burn Injury

**Authors :** B.L. España-Sánchez, E. Luna-Hernández, R.A. Mauricio-Sánchez, M.E. Cruz-Soto, F. Padilla-Vaca, R. Muñoz, L. Granados-López, L.R. Ovalle-Flores, J.L. Menchaca-Arredondo, G. Luna-Bárceñas

**Abstract :** Treatment of burn injured has been considered an important clinical problem due to the fluid control and the presence of microorganisms during the healing process. Conventional treatment includes antiseptic techniques, topical medication and surgical removal of damaged skin, to avoid bacterial growth. In order to accelerate this process, different alternatives for tissue regeneration have been explored, including artificial skin, polymers, hydrogels and hybrid materials. Some requirements consider a nonreactive organic polymer with high biocompatibility and skin adherence, avoiding bacterial infections. Chitin-derivative biopolymer such as chitosan (CS) has been used in skin regeneration following third-degree burns. The biological interest of CS is associated with the improvement of tissue cell stimulation, biocompatibility and antibacterial properties. In particular, antimicrobial properties of CS can be significantly increased when is blended with nanostructured materials. Silver-based nanocomposites have gained attention in medicine due to their high antibacterial properties against pathogens, related to their high surface area/volume ratio at nanomolar concentrations. Silver nanocomposites can be blended or synthesized with chitin-derivative biopolymers in order to obtain a biodegradable/antimicrobial hybrid with improved physic-mechanical properties. In this study, nanocomposites based on chitosan/silver nanoparticles (CS/nAg) were synthesized by the in situ chemical reduction method, improving their antibacterial properties against pathogenic bacteria and enhancing the healing process in thermal burn injuries produced in an animal model. CS/nAg was prepared in solution by the chemical reduction method, using  $\text{AgNO}_3$  as precursor. CS was dissolved in acetic acid and mixed with different molar concentrations of  $\text{AgNO}_3$ : 0.01, 0.025, 0.05 and 0.1 M. Solutions were stirred at  $95^\circ\text{C}$  during 20 hours, in order to promote the nAg formation. CS/nAg solutions were placed in Petri dishes and dried, to obtain films. Structural analyses confirm the synthesis of silver nanoparticles (nAg) by means of UV-Vis and TEM, with an average size of 7.5 nm and spherical morphology. FTIR analyses showed the complex formation by the interaction of hydroxyl and amine groups with metallic nanoparticles, and surface chemical analysis (XPS) shows low concentration of  $\text{Ag}^0/\text{Ag}^+$  species. Topography surface analyses by means of AFM shown that hydrated CS form a mesh with an average diameter of 10  $\mu\text{m}$ . Antibacterial activity against *S. aureus* and *P. aeruginosa* was improved in all evaluated conditions, such as nAg loading and interaction time. CS/nAg nanocomposites films did not show  $\text{Ag}^0/\text{Ag}^+$  release in saline buffer and rat serum after exposition during 7 days. Healing process was significantly enhanced by the presence of CS/nAg nanocomposites, inducing the production of myofibroblasts, collagen remodeling, blood vessels neof ormation and epidermis regeneration after 7 days of injury treatment, by means of histological and immunohistochemistry assays. The present work suggests that hydrated CS/nAg nanocomposites can be formed a mesh, improving the bacterial penetration and the contact with embedded nAg, producing complete growth inhibition after 1.5 hours. Furthermore, CS/nAg nanocomposites improve the cell tissue regeneration in thermal burn injuries induced in rats. Synthesis of antibacterial, non-toxic, and biocompatible nanocomposites can be an important issue in tissue engineering and health care applications.

**Keywords :** antibacterial, chitosan, healing process, nanocomposites, silver

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