Composite Biomaterials Active against Opportunistic Pathogens

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Statement of Purpose: The present study focuses on the preparation and characterization of nanoparticulate materials based on chitosan and hop constituents. Chitosan, an important biomaterial due to its biodegradability, biocompatibility and wide availability, has been reported as an antibacterial and antifungal agent (Qin C. Carbohydr Polym. 2006; 63: 367-374). In recent years, chitosan nanoparticles have been reported as drug delivery systems, as insulin or gene carriers. Their antibacterial activity may be due to their overall positive charge and high surface potential, clogging of protein channels, penetration into cells, binding of DNA and interfering with transcription, binding metabolic components (Leonida MD. Int.J. Nano & Biomat. 2011;3:316-334). Hop (*Humulus lupulus*) components have been studied and they found multiple applications. Lupulone is known for its strong antibacterial effect against Gram-positive species and xanthohumol, with a similar but more modest effect, is the most potent phytoestrogen known, and a cancer chemopreventive agent. In spite of their outstanding properties the big drawback of the hop extracts is their extremely low bioavailability. To address this problem, in the present study, nanocomposites were prepared from chitosan and lupulone (NCL) and xanthohumol (NCX), respectively. Different synthetic conditions were investigated. The antibacterial effect of the composite biomaterials was assayed against several opportunistic microbial species. The biologic activities of the nanoparticles were compared to those of chitosan-based nanoparticles enhanced with antibacterial metallic species, prepared using the same synthetic procedure. Potential application of the biomaterials synthesized in wound healing and skin aging was also investigated.

Methods: Chitosan-based nanoparticles were prepared from chitosan of different molecular weights (Sigma) using sodium tripolyphosphate (TPP) as a cross-linker, in different ratios, with and without ultrasonication, respectively. The first set of biomaterials contained lupulone/xanthohumol as additives, respectively, and the second set of nanocomposites contained Cu/Ag, respectively. The composite materials were characterized chemically (by colloidal titration for the ratio of residual amino groups, by titration with thiocyanate for silver content, by electrogravimetric analysis for copper content), by UV spectroscopy (for lupulone/xanthohumol content), by IR spectroscopy (to evaluate crosslinking), scanning electron microscopy, and antimicrobial assays against *Staphiloccocus epidermis* (Gram-positive), *Pseudomonas aeruginosa* and *Pseudomonas putida* (both Gram-negative). The minimum inhibitory concentrations (MIC) were determined and used to assess the stability in time of the composites.

Results: SEM micrographs showed no significant morphological differences between the nanoparticles prepared from different molecular weight chitosans. IR spectra showed, in all cases, modifications consistent with chitosan nanoparticles (NC) formation (Qi L. Carbohydr. Res.2004;339:2693-2700). Chemical analysis determined the amount of additive entrapped in each type of chitosan-based composite. The antibacterial assays showed similar activity of the nanoparticles against Gram-positive and Gram-negative bacteria . The nanoparticulate composites exhibited higher activities compared to the starting chitosan/plant component/NC/NC+L/NC+X, respectively. While the synergistic effects were expected in the case of *S. epidermis,* since both hops components are active against Gram-positive bacteria, those obtained against the Gram –negative species were unexpected, since lupulone and xanthohumol are inactive against Gram-negative species. Important antibacterial effects were found for the metal-enhanced nanocomposites, higher for the silver-added ones against pseudomonads while the copper-added ones were more active against *S. epidermis*. There was not a clear dependence on ultrasonication during the ionic gelation procedure of these effects. Starting chitosan and NC maintained their antimicrobial activities over an eight months period. The particles containing hop extracts maintained half of their activity over the same period. This still makes them attractive since hop extracts are known for their low stability. The potential as wound healing agents was assayed as inhibitory activity on matrix metalloproteinase (MMP-1). While copper and silver inhibited MMP-1, chitosan, lupulone and xanthohumol, respectively, did not. NC acted as an inhibitor itself. Lower ratio chitosan:TPP increased inhibitory effect for NCL. Ultrasonication during ionic gelation resulted in lower inhibitory effect. This may be the result of an unfavorable interaction at the active site due to modification in size during ultrasonication.

Conclusions: The chitosan-based nanocomposites synthesized were active against Gram-positive and Gram-negative microbial species. Synergistic effects were observed in all cases. They are remarkable especially in the case of plant-added particles since hop components are not active against Gram-negative species. The biological activity of the composite materials was stable over eight months. Since chitosan is a biocompatible material, the nanocomposites presented herein show potential for applications as delivery systems for hop components (known for their bitter taste and low bioavailability). The biological effect, enhanced at the nanolevel, suggests more than just electrostatic interactions, possible interactions with intracellular components, hence enhanced bioavailability.