Evaluation of Chitosan-Coated Liposome Potentials as Nano-Carriers for Laryngeal Cancer Treatment

Christian R. Moya-Garcia, M.Phil.[1], Nicole Li-Jessen, Ph.D.[1,2,3], , Maryam Tabrizian, Ph.D.[1,4]

[1]Department of Biological and Biomedical Engineering, [2]School of Communication Sciences and Disorders, [3]Otolaryngology – Head and Neck Surgery, [4]Faculty of Dentistry, McGill University, Montreal, Canada

Statement of Purpose: Local delivery of lipophilic neoplastic agents in the laryngeal mucosae is challenging due to potential dislodge to surrounding healthy tissues and limited chemotherapy drug solubility in blood stream. Liposomes are spherical nano-carriers that have been frequently used as a vehicle for lipophilic drug delivery (Bozzuto G. Int J Nanomedicine. 2015;10:975–99). Liposome nano-carriers are composed of phospholipids and cholesterol (Bozzuto G. Int J Nanomedicine. 2015;10:975–99) and have a similar amphipathic bi-layer membrane as that of cells. Since chitosan has a mucoadhesive and positive in nature (Mizrahy S. Chem Soc Rev. 2012;41(7):2623–40), this characteristic is particularly appropriate to evade dislodge of the chitosan-coated liposomes (chitosomes) to healthy tissues as the lung. The primary goal of this study was to compare the coating of chitosan on liposomes and evaluate the cytotoxicity of chitosan-coated liposomes on laryngeal cancer and stromal cells.

Methods: To prepare the chitosan solution, 1.8g of chitosan and 3mL of 0.06M HCL were placed in 300mL of MilliQ-water. The solution was stirred in a fume hood until the chitosan was completely dissolved. The solution was filtered with a 0.45µm and 0.22µm filters. Ethanol injection methodology was performed to fabricate the liposomes. Liposomes composed of DSPC, DSPE-PEG2000 and cholesterol were coated with chitosan to create positively charged liposomes.

First, standard fabrication of the uncoated liposomes was performed. After analyzing the size of the liposomes via nanoparticle tracking analysis, analysis of the liposome charge (zeta potential) was carried out using the dynamic light scattering. Then, 110µL of chitosan were added to 1.5mL of the liposomal solution. The liposomal solution was then sonicated in a water-bath for 20 min. The mixtures were dialyzed using 12-14kDa membranes at room temperature for 3 days against the 0.5% Tween 80 (pH 7.4) to separate free chitosan from the liposomes. The chitosome formulation was then stored at 4ºC until use or at 37ºC for stability studies. FTIR spectra was acquired in transmission mode with OPUS software version 7.2 at a spectral resolution of 4 cm^{-1} within a 4000–600 cm^{-1} range and averaging 128 scans per sample using a Bruker Tensor 27 spectrophotometer equipped with an Attenuated Total Reflection module, a Mid Infra-Red source, and a Mercury Cadmium Telluride detector cooled with liquid nitrogen.

Immortalized vocal fold fibroblasts (VFFs) and laryngeal cancer cells (LSCCs) were culture on 8-chamber slides for LIVE/DEAD staining and MTT analysis. Both cell types were exposed to liposomes coated and uncoated with chitosan after 1 and 3 days of culture.

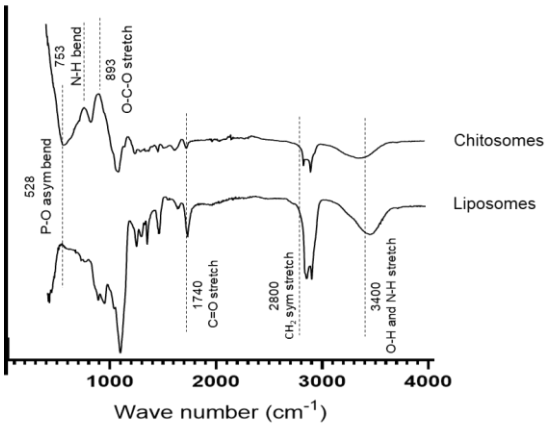

Figure 1. FTIR spectra of chitosan-coated and uncoated liposomes.

Results: Uncoated liposomes showed a negatively charged surface of -26.04 ± 2.03 mV and size of 99.4±1.5nm (polydispersity index < 0.3). Charge positively increased reaching 24.8±2.6mV on the chitosomes ranging in size of 120±2.2nm (polydispersity index < 0.3). This stabilization may be attributable to the chitosan coating on the liposome surface and colloidal stability.

Results from FTIR spectroscopy analysis showed that for the uncoated liposomes, the peak at 1740cm^{-1} represented the C=O stretching of the ester bond. This bond linked to the head group with the fatty acid tail of the phospholipids. In addition, the CH_2 symmetric stretch peak was depicted at 2800 cm^{-1} and the O-H and N-H stretching was shown at 3400cm^{-1}. The peak at 528cm^{-1} represents the P-O asymmetrical bending of the phospholipids (Alinaghi A,. Int J Pharm. 2014;459(1–2):30–9.). For the chitosan coating on the liposomes, the peaks were found at 753cm^{-1} representing the N-H bending and at 893cm^{-1} related to the glycosidic C-O-C stretching (Rumengan IFM. Int J Fish Aquat Sci. 2014;3(1):12–8) (**Figure 1**).

Results from the cell cytotoxicity study showed over 90% viability in all experimental and controls groups throughout the 3 days of chitosome and liposome exposure.

Conclusions: FTIR spectroscopy and zeta potential analyses confirmed the presence of chitosan coating on liposomes. Immortalized VFFs and LSCCs exhibited a high viability status upon exposure with liposomes and chitosomes. To further evaluate the potentials of chitosome-coated liposomes for controlled, effective delivery of lipophilic cancer therapeutics, ongoing work includes drug loading and release kinetics analyses and drug sensitivity studies of chitosomes.