Characterization of chitosan coated PLGA nanoparticles after formation of ocular protein corona in corneal route of ocular drug delivery

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Background: Data shows that out of 100 individuals in Europe, 40 will suffer from age related macular degeneration, an ocular condition that can cause sight loss as people age[1]. Treatments for this condition involve injections into the eye, which can have serious side effects. Topical drug delivery systems based on PLGA nanoparticles can be a potential treatment to replace ocular injections. Nanoparticles will go throught three routes within ocular tissues to reach the back of the eye and in each route they meet a series of ocular fluids that can from a layer of molecular moeties and proteins around them. This layer, called protein corona, can drastically change the properties of nanoparticles.

Methods: A simulated version of ocular fluids was made and optimized to be incubated with chitosan coated PLAG nanoparticles. After 15 minutes of incubation, nanoparticles were washed once by 15 minutes of high speed centrifugation, to separate protein corona-nanoparticle complexes from free protein, and redispersed by probe sonication at an amplitude of 60%, in water. Characteristics of nanoparticles with protein corona were investigated by Dynamic Light Scattering technology to measure particle size and surface charge, FTIR analysis and fluorescence spectrophotometry to confirm the formation of the protein corona.

Results: The size of the nanoparticles reached from 254.84 ± 19.53 nm to 399.44 ± 29.36 nm after incubation. The polydispersity index changed from 0.23 ±0.06 to 0.15 ± 0.04 and the zeta potential decreased from 25.18 ± 2.31 mV to 9.13 ± 1.23 mV. The coefficient of variance for all the data is below 10%. Presence of peaks at wavenumbers of 3359 cm^{-1}, 1587 cm^{-1} and 1387 cm^{-1} in comparison to the control samples in FTIR indicates the presence of protein in the sample having the protein corona. Additionally, Fluorescence intensity of the nanoparticle with protein corona was measured as 10.54 ± 0.54 RFU while chitosan coated PLGA nanoparticles had the intensity of 3.39 ± 0.34 RFU. Aggregation dynamics displayed that nanoparticles with protein corona coaggregated continuously and reached a size of 1425 nm within two hours while the control sample maintained their initial size for the same time.

Conclusions: A larger particle size is related to the formation of the protein corona layer which is the same case for the decrease in zeta potential. Positively charged chitosan on the surface of the nanoparticles could interact with the negatively charged. The increase in fluorescence intensity for nanoparticles with protein corona is due to the intrinsic fluorescence properties of tryptophan within the primary structure of proteins. The low zeta potential resulted from the formation of protein corona, triggered aggregation as there was not enough repelling force between nanoparticles to maintain the colloidal stability of the system. Changes in particle properties and the interactions between protein corona and the nanoparticle internalization mechanisms should be further investigated to check the capability of the formulation for reaching the back of the eye.

References: 1. Connolly *et al.* Prevalence of age-related macular degeneration associated genetic risk factors and 4-year progression data in the Irish population, *Br. J. Ophthalmol.*, 2018.