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Protein-loaded chitosan nanoparticles modulate uptake and antigen presentation of hen egg-white lysozyme by murine peritoneal macrophages.

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Abstract – Chitosan binds to negatively charged tripolyphosphate (TPP) by an electrostatic interaction driven by its positively charged amino group. This interaction allows developing stable nanoparticles via ionotropic gelation, suitable as a targeted carrier and controlled release system for proteins, drugs or vaccines. We studied the effect of chitosan nanoparticles (CNp) on the uptake and antigen presentation of the model protein hen-egg white lysozyme (HEL) by Peyer's patches peritoneal macrophages isolated from mice.

Polymeric materials used for preparing nanoparticles for drug delivery must be biocompatible at least and biodegradable best. To this aim, many polymeric materials have been applied, including poly(lactic acid), poly(glycolic acid), polycaprolactone, polysaccharides (particularly chitosan), poly(acrylic acid) family, proteins or polypeptides (such as gelatin) [1, 2].

Chitosan biodegradability, immunological activity and bioadhesion, make it an excellent candidate for nanoparticle development. Chitosan microparticles and nanoparticles have been made by chemical cross-linking with glutaraldehyde, glyoxal, and ethylene glycol diglycidyl ether [3, 4]. Although these are very good cross-linkers, they are not preferred owing to their physiological toxicity. Chitosan can interact with negatively charged species such as tripolyphosphate (TPP) and sodium sulfate, due to its polycationic nature in acidic media. The interaction of chitosan with TPP leads to formation of biocompatible cross-linked chitosan nanoparticles (CNp), which can be efficiently employed in protein and drug delivery (Figure 1).

CNp were characterized according to its size, ζ-potential, surface morphology, protein loading and release properties. Results showed an increase on the size of the nanoparticles as the concentration of chitosan was increased, whereas the net positive charge decreases under the same conditions. Protein loading does not seem to affect significantly either particle size or surface charge of the nanoparticles. About 65% of the HEL loaded remained in the CNp after release studies for 4 h in phosphate buffer saline (PBS). After 4 hours of pre-incubation with a Tcell hybridoma line cocultured with murine peritoneal macrophages, only trace amounts of IL-2 were detected in the cocultures treated with HEL alone, whereas cocultures treated with HEL-CNp had already reached maximum IL-2 expression (Figure 2). Confocal microscopy studies showed that CNp had a higher uptake rate than the protein itself, after 30 min of incubation with peritoneal macrophages (Figure 3).

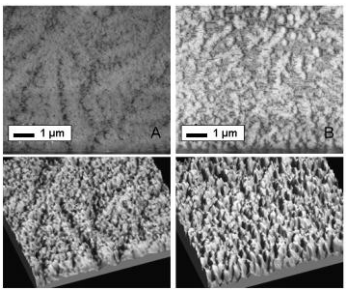

Figure 1. Atomic force (AFM) micrographs for unloaded chitosan nanoparticles (CNp) formulated with chitosan 0.10% w/v (A) and HEL-CNp loaded with HEL 1.0% w/v (B).

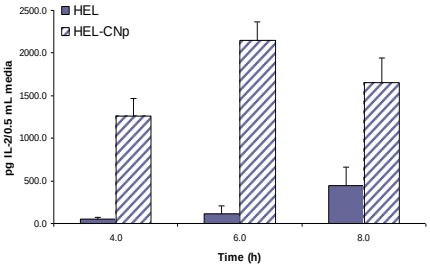

Figure 2. Effect of HEL-loaded CNp on interlukin-2 (IL-2) expression in 3A9 T cell hybridomas cocultured with mouse peritoneal macrophages.

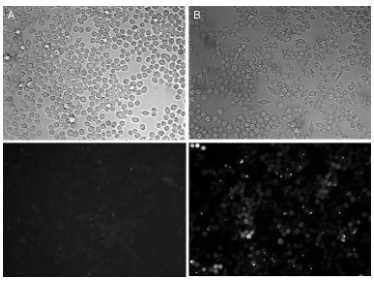

Figure 3. HEL was labeled with fluorescein and mouse peritoneal macrophages uptake of HEL alone (A) and HEL-loaded CNp (B) was determined at 30 minutes after adding the antigenic protein. Phase images on top (magnification of 20x) show the presence of macrophages.

References

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