# STRATEGIES TO OVERCOME MONONUCLEAR PHAGOCYTE SYSTEM PHAGOCYTOSIS OF POLYSACCHARIDE NANOPARTICLES

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## ABSTRACT

RAW 264.7 cell line was used to investigate the macrophage retention capacity of insulin-loaded nanoparticle formulations that have previously presented *in vivo* oral hypoglycaemic effects. The internalization of different nanoparticles (NP) containing insulin, labelled with fluorescein isothiocyanate (FITC), by macrophages was assessed by fluorescence microscopy and flow cytometry. Fluorescence microscopy images confirmed that chitosan-based nanoparticles were neglectfully internalized by RAW 264.7 cells, 20-fold less than fluorescent carboxylated latex nanoparticles used as positive control, and not attached in the cell surface.

## INTRODUCTION

The use of polymeric nanoparticles to deliver therapeutic proteins like insulin has been studied as an approach to treat diabetes, either orally or intravenously. Nanoparticulate delivery systems have the potential to improve protein stability against enzymatic degradation in the intestinal environment, increase the duration of the therapeutic effect and permit administration through non-parental routes.[1, 2] A major limitation facing the pharmacological activity of insulin-loaded polymeric nanoparticles is their rapid elimination from the systemic circulation by cells of the mononuclear phagocyte system (MPS). [3-5] A promising strategy is the use of multifunctional polymers, exhibiting permeation enhancing properties, like alginate and chitosan, which have also been described as biocompatible, biodegradable and mucoadhesive.[2] Dextran sulfate is also a biodegradable and biocompatible polymer used as an alternative to encapsulate insulin as it contributes for longer circulation times *in vivo*.[6]

## EXPERIMENTAL METHODS

Nanoparticles were prepared by alginate or dextran sulfate core, followed by chitosan polyelectrolyte complexation. The chitosan used was previously labelled with FITC [7,8] for cellular imaging in a fluorescence microscope and for fluorescence intensity determination in flow cytometry assays.

RAW 264.7 cells were seeded onto coverslips or 6 well culture plates and grown overnight in complete culture medium. Uptake experiments were initiated by adding nanoparticles solution followed by incubation at 37ºC. At pre-determined times samples were taken. To control the phagocytic capacity of the cells, carboxylated nanospheres (FluoSpheres®, 20nm diameter) were incubated for 1 hour with the cells.

## RESULTS

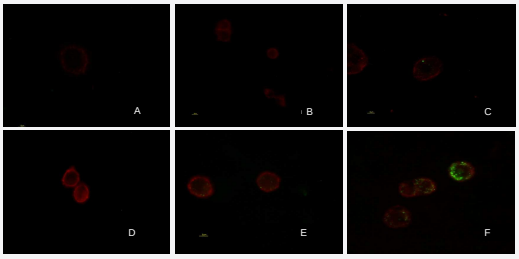


Figure 1. Fluorescence microscopy images of macrophage cells, incubated with Alginate/Chitosan nanoparticles for (A) 30 min, (B) 1h, (C) 2h, (D) 3h, (E) 4h and (F) incubated with carboxylated nanospheres for 1h. Cell membrane was stained red with Alexa Fluor WGA. Magnification 60x.

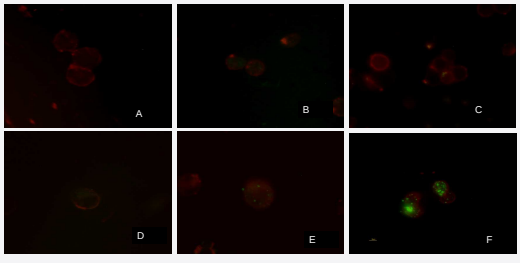


Figure 2. Fluorescence microscopy images of macrophage cells, incubated with Dextran sulfate/Chitosan nanoparticles for (A) 30 min, (B) 1h, (C) 2h, (D) 3h, (E) 4h and (F) incubated with carboxylated nanospheres for 1h. Cell membrane was stained red with Alexa Fluor WGA. Magnification 60x.

## RESULTS

**Chitosan nanoparticles are not externally adsorbed at the cell membrane, and seem to be neglectfully internalized by macrophage cells, while carboxylated nanospheres are phagocytosed as it is observed by the strong green fluorescence inside the cells.**

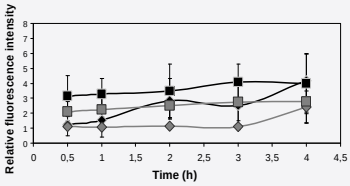


Figure 3. Relative fluorescence intensity of macrophage cells incubated with different formulations of NP at 37ºC and at 4ºC (♦ Alginate/Chitosan NP 37ºC, ■ Dextran sulfate/Chitosan NP 37ºC, ♦ Alginate/Chitosan NP 4ºC and ■ Dextran/Sulfate/Chitosan NP 4ºC). The results are expressed as the ratio between fluorescence of macrophages incubated with NP and the auto-fluorescence of macrophage cells.

## RESULTS

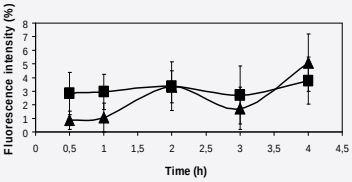


Figure 4. Percentage of fluorescence intensity of macrophage cells when incubated with different formulations of NP (▲ Alginate/Chitosan and ■ Dextran Sulfate/Chitosan NP). The results, expressed as the percentage of NP uptake relative to the nanospheres uptake (considered 100% of phagocytosis), are the mean of at least three experiments.

**After 4 hours of incubation, the uptake was 3.7% and 5.1% for alginate/chitosan and dextran sulphate/chitosan nanoparticles, respectively. The reduction on chitosan nanoparticles uptake was similar at 37ºC and at 4ºC.**

**To confirm the strength of the methodology, experiments with carboxylated nanospheres (FluoSpheres®) showed that at 4ºC the phagocytosis process was inhibited in 80%, showing that the lack of chitosan nanoparticles uptake were due to their properties to avoid macrophage uptake.**

## CONCLUSIONS:

**Results suggested that chitosan nanoparticles are not retained by macrophages *in vitro*, revealing an additional explanation to the prolonged insulin blood levels observed when administered *in vivo*.**

## REFERENCES:

1. Sarmento, B., *et al.*, J Nanosci Nanotechno, 2007. 7(8): 2833-2841.
2. Sarmento, B., *et al,* Pharm Res, 2007. 24(12): 2198-2206.
3. Zahr, A.S., *et al.*, Langmuir, 2006. 22(19): 8178-8185.
4. YaShu Y., *et al.*, J Controlled Release 2007. 123: 27-38
5. Champion, J.A., *et al.*,. Pharm Res, 2008. 25(8): 1815-1821.
6. Sarmento, B. *et al.*, Colloids Surf., B, 2006. 53: 200-209.
7. Hiraku Onishi, Y.M., Biomaterials, 1999. 20: 175-182.
8. Hasegawa, T.I., *et al*., Colloids and Surf., B, 2008. 63: 209-216