# Dextrin nanoparticles as a protein delivery system: The Interleukin-10 case study

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**INTRODUCTION:** Interleukin-10 (IL-10) is an anti-inflammatory cytokine, which active form is a non-covalent homodimer with two intramolecular disulphide bonds that are essential to its biological activity, which includes reduction of tumor necrosis factor α (TNF-α) synthesis and down-regulation of class II major histocompatibility complex (MCH-II) molecules on monocytes/macrophages [1]. Due to IL-10 potential applications in various medical fields, it is essential to develop systems that can effectively deliver the protein. A promising system is protein encapsulation by polymeric nanopartices (NPs), which minimize denaturation, and enables slow-release, while maintaining an effective concentration for the necessary period of time. In previous work, we have developed and characterized NPs obtained by self-assembling of hydrophobized dextrin [2] whose properties makes them promising for IL-10 delivery.

**METHODS:** The recombinant mutated (C149Y) murine IL-10 (rIL-10) was expressed, in form of inclusion bodies, in *E. coli* BL21 star. rIL-10 was recovered by a process of solubilization in 6M guanidine, renaturation and re-oxidation of disulphides. The dimeric form of rIL-10 was collected by gel filtration and further purified by ion-exchange chromatography. SDS-PAGE analysis was used to confirm molecular weight and purity. The amount of rIL-10 obtained was quantified by ELISA.

rIL-10 bioactivity was assayed by its ability to inhibit the production of TNF-α, quantified by ELISA, and the surface expression of MHC-II molecules, monitorized by FACS analysis, in lipopolysacharide (LPS) and IFN-γ activated bone marrow derived macrophages (BMDM).

To form the self-assembled NPs, lyophilized dextrin-VMA-$SC_{16}$ was resuspended in culture medium, at room temperature. NPs formation was confirmed by dynamic light scattering. The complex NPs/rIL-10 was formed by dissolving rIL-10 in culture medium and then by mixing lyophilized dextrin-VMA-$SC_{16}$. rIL-10 incorporation into NPs was verified quantifying the amount of rIL-10 free in solution by ELISA. The release of rIL-10 from the complex NPs/rIL-10 was assessed in a BMDM culture, by measuring free rIL-10 by ELISA. Bioactivity of rIL-10 released form the NPs/rIL-10 complex was assayed as described previously.

**RESULTS:** SDS-PAGE confirmed the expression and molecular weight (18 kDa) of the rIL-10 as well as their purity. Total recovery of dimeric rIL-10 was about 1-1.5 mg/L culture, quantified by ELISA. rIL-10 (in the range of 0.1 to 250 ng/mL) shown the ability to inhibit the TNF-α production and to reduce MHC-II expression on stimulated BMDM. rIL-10 incorporation by NPs was confirmed by quantifying, the amount of rIL-10 in free in solution, that was negligible. In the presence of 20% serum, rIL-10 is being released over time in a BMDM culture. After two hours of incubation with the NPs/rIL-10 complex, the rIL-10 reaches a maximum concentration; a stable value of about 35 ng/mL rIL-10 being estimated after twenty-four hours. The rIL-10 released from the NPs/rIL-10 complex was able to inhibit TNF-α production and MHC-II expression at the same level as the soluble rIL-10.

**DISCUSSION & CONCLUSIONS:**

IL-10 is a cytokine with a strong anti-inflammatory activity. A mutated form of murine IL-10 was successfully expressed in *E. coli*, recovered and purified from inclusion bodies. Its ability to reduce TNF-α synthesis and down-regulate MHCII molecules expression on activated BMDM was confirmed. Due to IL-10 potential applications in various medical fields, it is essential to develop systems that can effectively deliver the protein. For this purpose, dextrin NPs have been used and this work shows that dextrin NPs effectively incorporate IL-10 and enable the slow release of biologically active IL-10 over time. Altogether, these results demonstrate the suitability of dextrin NPs to be used as a system for the controlled release of IL-10.

**REFERENCES:**[1] R. de Waal Malefyt, et al (1991) *J Exp Med* **174**:1209-20. [2] G. Gonçalves, et al (2007) *Biomacromolecules* **8**:392-8.

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