Macrophage response to Chitosan/Poly-(γ-Glutamic acid) nanoparticles carrying an anti-inflammatory drug

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Statement of Purpose: The natural body response to biomaterials implantation involves an inflammatory reaction [1]. The traditional concept of inflammation being detrimental has been gradually changing to the fact that controlling inflammation is required for physiological tissue repair/regeneration [2, 3]. Therefore, inert materials are being substituted by modified biomaterials to control inflammation, aiming to target specific immune cell populations, or modulate both cell activation or secretion of pro-/anti-inflammatory cytokines [4]. Among different immune cells, the macrophages play a key role in the inflammatory response to biomaterials [4]. Macrophage plasticity depends also on the type of stimulus. Therefore, these cells constitute an appealing cell target in the design of more advanced biomaterials. Here, the preparation of Chitosan (Ch)/Poly-(γ-glutamic acid) (γ-PGA) nanoparticles (NPs) as vehicle for a non-steroid anti-inflammatory drug (NSAIDs), Dicloefenac (Df), is described and the response of primary human macrophages to this system is evaluated.

Methods: Sodium Df, widely used in the musculoskeletal field, was incorporated in Ch/γ-PGA NPs. The effect of molar ratio of NPs components molar ratio and their order of addition was evaluated in NPs size and polidispersion. Df release kinetics was analyzed at pH 7.4. The NPs cytotoxicity, internalization and drug bioactivity were analyzed in vitro in cultures of primary monocyte-derived macrophages isolated from human peripheral blood and previously activated with Lipopolysaccharide (LPS). The production of pro-/anti-inflammatory cytokines was quantified.

Results: Df was incorporated in Ch/γ-PGA NPs at controlled pH (5.0) (max. 0.05 mg/ml). The components molar ratio and order of addition revealed to be critical to obtain NPs (315±50 nm with 0.36±0.06 polydispersion index). Df is released at physiological pH (~80% in 2h). This drug-delivery system was proved to be non toxic to macrophages, being rapidly internalized (95%). Importantly, efficacy of Df-NPs was confirmed by their ability of inhibit/revert PGE2 production of activated macrophages. Ch/γ-PGA NPs also reduce IL-6 production, without significant alterations in IL-10 and IL-23/23 levels. The effect of these particles on TNF-α□□□□centration was not so evident, being donor dependent.

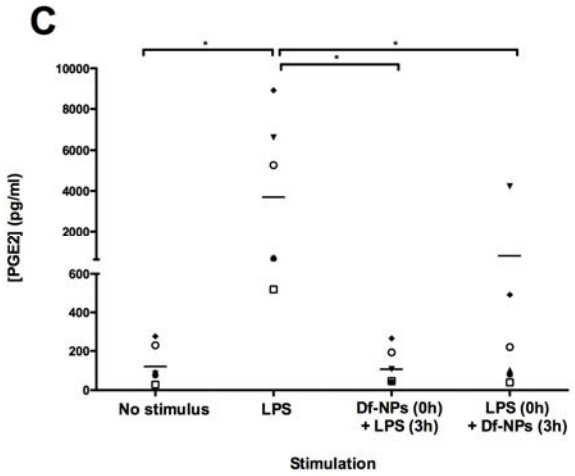

Fig. 1: [PGE2] of macrophages cultured: 1) without stimulus; 2) with LPS (10 □g/ml); 3) with Ch/Df/PGA NPs added 3h before LPS; and 4) with Ch/Df/PGA NPs added 3h after LPS stimulus. [PGE2] was determined 48h after stimulation by ELISA. Results of 6 independent donors are presented; the horizontal bar represents the mean [PGE2] for each condition. Statistical significance was considered for at least $p<0.05$ (*).

Conclusions: Ch/γ-PGA NPs can be used as Df carriers, being the molar proportion of the components and their order of addition critical to obtain particles with nano-size and an elevate Df content. At controlled doses, Ch/Df/γ-PGA NPs are not toxic for human macrophages, being rapidly phagocyted and slightly activating these cells. Overall, Df-NPs are an efficient anti-inflammatory drug delivery system, decreasing PGE2 concentration in LPS-activated macrophages, which confer to these NPs attractive properties to locally control macrophage response, and consequently promote inflammation resolution.

References: [1] Anderson J. Annu Rev Mater Res. 2001;31:81–110; [2] Mountziaris P, Tissue Engineering: Part B. 2011;17(6):393-402. [3] Medzhitov R. Nature. 2008;454:428-35. [4] Franz S, Biomaterials. 2011;32:6692-709.

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