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A FACS STUDY TO SCREEN CYTOTOXICITY OF CHITOSAN AND CHITOSAN-BASED NANOSYSTEMS

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Despite chitosan (CS) has heretofore been regarded as a non toxic biomaterial, *in vitro* cytotoxicity can vary with both the type of cell line and CS's characteristics [1,2]. Cell manipulation (e.g. trypsinization) can contribute to increase cellular stress, which often hampers the correct interpretation of the cytotoxicity profiles. Even though the colorimetric MTT ([3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium]) assay has proved as an effective, simple and rapid *in vitro* strategy to analyze the cytotoxicity of different biomaterials, alternative techniques, such as fluorescence activated cell sorting (FACS) analysis, imply less manipulation of the cell culture and hence it allows an accurate quantitative study of the cell population. Indeed, FACS is a very powerful analytical tool to probe slight variations in cell's morphology and integrity induced by the type of treatment, dose, and exposure time.

In this work, a systematic FACS study was conducted to address the *in vitro* cytotoxicity of CS-based nanomaterials. A series of CS samples were used that varied in their degree of acetylation (DA~1, 9, 27, and 56 %) and *Mw*; here referred to as LDP (*Mw*~10 kDa) and HDP (*Mw*~100 kDa). The *in vitro* cytotoxicity induced by different CS-based systems, namely CS solutions, CS-based nanoparticles (NPs) and nanocapsules (NCs) was compared. To this end, two different human lymphoblastic-derived cell lines were utilized, namely K562 and THP1, which are able to grow in suspension (in complete RPMI medium) and hence require little manipulation. Equivalent doses of CS in all treatments were applied to cells (~1.0 x 10^4 cells/mL) during 24 or 48 h. Prior to FACS analysis (*FACScalibur*, BD Biosciences), the cells were washed and stained with propidium iodide (PI). The percentage of dead cells (i.e. PI positive), was detected in the red FL2 channel; while that of viable cells was calculated by difference. Data are represented here as cell viability relative to untreated cells. Lower cell viability was observed only for CS HDP DA 1% (Fig.1). Noticeably, a lower cytotoxicity was observed when the same CS was applied in the form of NPs or NCs (Fig. 2). Moreover, while a dose-response effect was evident for both the chitosan solution and the NPs, it was practically inexistent for comparable doses of NCs. Essentially similar results were observed for both cell lines and for exposure times of 24 or 48 h.

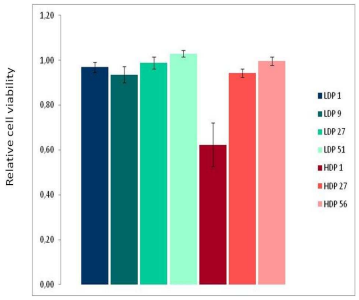

Fig. 1. Effect of different chitosans in solution on the cell viability (K562 cell line) (CS dose = 67 µg/well, 24 h treatment).

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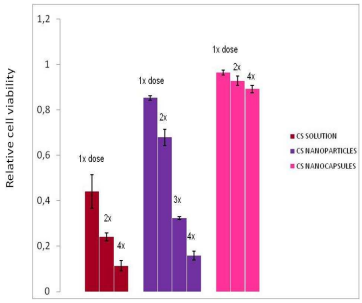

Fig. 2. Dose effect of different systems made of CS HDP DA 1% on the cell viability (K562 cell line) (1x CS dose = 67 µg/well, 24 h treatment).

The results of this study agree well with previous evidences reported in the literature of an enhanced cytotoxic effect promoted by chitosans with low DA [1]. The lower cytotoxicity observed for CS NPs, and especially so for NCs, highlights their great potential as cellular nanocarriers for bioactive molecules (e.g. genetic material) with enormous potential relevance in Nanomedicine**.**

ACKNOWLEDGEMENTS

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