Polymeric nanoparticles as carrier system: the investigation of citotoxicity and genotoxicity

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INTRODUCTION AND OBJECTIVE

The development of nanotechnology has resulted in a growing public debate on the toxicity and environmental impact of direct and indirect exposures to nanoparticles. The nanoparticles toxicity, including human health and environmental implications, is still considered not completed elucidated and relatively unexplored (Nel *et al.,* 2006). Concerning to human health, studies have demonstrated that nanoparticles have toxic effects at the cellular, subcellular and biomolecular levels, such as genes and proteins (Chi *et al*., 2009).

The objective of this study was evaluate the cito and genotoxicity of polymeric nanoparticles (nanospheres and nanocapsules) used as carrier systems for bioactive compounds.

MATERIAL AND METHODS

Reparation of nanoparticles The preparation of the nanoparticles was made the emulsion of oil in water and evaporating the solvent and the other was the method of nanoprecipitation. According to the method of emulsification, 400 mg of polymer PCL and oil were dissolved in chloroform and pre-emulsified with acetone. The emulsification was performed ultrasonically for 1 minute at 100W. The pre-emulsion was added to an aqueous solution of PVA as a surfactant and was sonicated for 8 minutes to form an emulsion. Then the organic solvent was removed and the emulsion was concentrated to a final polymer concentration of 1mg/mL (Zhou *et al*., 2010). In order to produce nanocapsules (NC), the oil was used in organic phase and nanopsheres (NS) were produced with absence of oil (triglycerides of capric and caprylic acids).

Size and polydispersion The light scattering technique was used to determine the average size and size distribution of nanoparticles (polydispersity). This evaluation was performed by diluting the suspension of nanoparticles with deionized water (1:100 v:v), using a particle analyzer Zetasizer Nano ZS 90 (Malvern, UK) at a fixed angle of 90 ° and at 25 ° C. The size distribution is given by polydispersion index. Each result was expressed as mean of three measurements (Melo *et al.,* 2011).

Zeta potential The zeta-potential reflects the surface charge of the nanoparticles. This parameter can be influenced by the composition of particles and the dispersing medium. Nanoparticles with values of approximately ± 30 mV are more stable in suspension (Melo *et al.,* 2011). The zeta potential value as mV was determined by an analyzer zeta potential, Zetasizer Nano ZS 90 (Malvern, UK). The analyzes were performed by diluting the suspension of nanoparticles in deionized water (1:100 v:v) and the results were expressed as mean of three measurements.

Toxicity analyses

CELL VIABILITY The cytotoxicity was available utilizing 3T3 cells that were exposed for 1h to nanoparticles. The cells viability was determined by Tali (Image Based Citometry). For the analyses of results the following indexes were calculated: relative viability index (IR_viab.), relative death index (IR_death) and relative apoptosis index (IR_apop.) were calculated.

CYTOGENETIC ANALYSIS The cells were incubated with treatment for 72 hours at 37°C with 5% CO_2. The colchicine was added to each culture 4 hours before the end of the incubation period. After 72 hours, the cells were subjected to progressive hypotonization followed by fixation. The mitotic index (MI) was calculated by dividing the number of cells in division by the total cells counted and the relative mitotic index (RMI) was determined by the treatment mitotic index divided by negative mitotic index (Lima *et al.,* 2010).

ALLIUM CEPA ASSAY When the roots reached a length of 2 cm, the seedlings were placed in contact with treatment. Ultrapure water was used as a negative control. After 24 hours of treatment, the roots were fixed, after that they were subjected to acid hydrolysis and finally to Schiff-base reaction. The results of the *Allium cepa* tests were used to calculate the relative chromosomal aberration score (RCAS=Treatment chromosomal aberration index/Negative treatment chromosomal aberration index) (Parida *et al.*, 2011).

COMET ASSAY The lymphocytes were submitted to treatment with the nanoparticles for a period of one hour. Negative control employed phosphate buffered saline (PBS). The comet assay was performed as described by Azqueta *et al.* (2011).

RESULTS AND DISCUSSION

Characterization of the formulations After the nanoparticles preparations we have investigate the mean diameter, polydispersity and zeta potential of the particles (Table 1).

Table 1 - Values of mean diameter (hydrodynamic diameter. nm), polydispersity, zeta potential (mV) and pH of the suspensions of polymer nanoparticles (ne-ee and nc-ee).

Parameter	PCL-NS	PCL-NC
Mean diameter (nm)	445.5	452.9
Polydispersity	0.068	0.080
Zeta potential (mV)	-5.2	-4.7

PCL-NE = PCL nanospheres and PCL-NC = PCL nanocapsules by emulsification/evaporation.

The results presented in Table 1 showed that there were no differences between PCL-NC and PCL-NS nanoparticles parameter and that the suspensions of polymeric nanoparticles have a diameter and polydispersity index compatible with colloidal suspensions (Melo *et al.,* 2011). In this way, in order to investigate the cito and genotoxic effect of this particles the only difference observed between the formulations is the presence of the triglycerides of capric and caprylic acids.

Cytotoxicity The cytotoxicity analysis using Tali method shows no difference in cell death after treatment with polymeric nanoparticles (Figure 1).

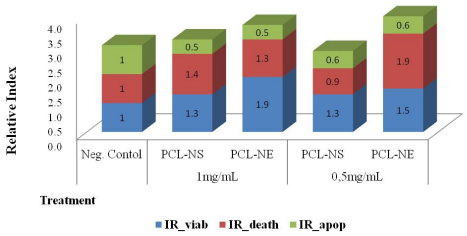

Figure 1 – Effects of different PCL nanoparticles on the IR_viab., IR_death, IR_apop. analyses using 3T3 cells exposed to the PCL nanoparticles at the polymer concentrations of 0.5 and 1mg/mL.

Genotoxicity After the comet analysis using lymphocyte cells exposure to nanoparticles, the results showed that the PCL-NS and PLC-NC (1mg/mL) induced significant DNA breaks in the cells in relation to negative control (Figure 2) indicating some genotoxic effect of these nanoparticles.

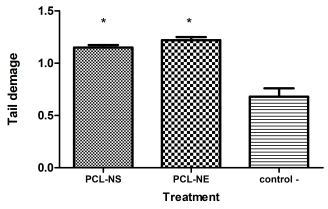

Figure 2 - Results of tail damage obtained from lymphocyte cells after treatments with the PCL nanoparticles at a polymer concentration of 1mg/mL.

Another investigation was done by the *Allium cepa* assay. The results of the *Allium cepa* were used to determine the relative chromosomal aberration score (RCAS) for the cells exposed to polymeric nanoparticles (Figure 3).

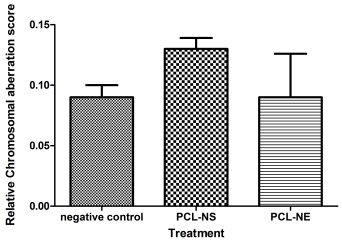

Figure 3 – Effects of different types of PCL nanoparticles on the relative chromosomal aberration (RCAS).

The results showed that there was no significant difference in the RCAS, indicating that the PCL nanoparticles did not present genotoxicity to this cell type.

CONCLUSIONS

We conclude the polymeric nanoparticles present no significant cytotoxicity (in fibroblast cells) and do not produce significant chromosomal aberration (in *Allium cepa* cells*)*, but in comet assay (lymphocyte cells) the results showed a significant genotoxic effect for both nanoparticles. In this way, further studies will be needed in order to determine the genotoxicity mechanism of action by these polymeric nanoparticles.

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