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RESEARCH ARTICLE

SYNTHESIS OF POLYCAPROLACTONE (PCL) NANOSPHERES CONTAINING POLYPHENOLS-RICH EXTRACT AND EVALUATION OF PCL TOXICITY IN HUMAN CELLS

Catia Santos Branco[1,3]., Kelly Todescatto[1]., Francine Girardello[1]., Silvia Guterres[2]., Valeria Weiss-Angeli[3] and Mirian Salvador[1,3]*

[1]Institute of Biotechnology, University of Caxias do Sul, Caxias do Sul, RS 95070 560, Brazil
[2]Department of Pharmaceutical Sciences, Federal University of Rio Grande do Sul, Porto Alegre RS 90610 000, Brazil
[3]Department of Life Sciences, University of Caxias do Sul, Caxias do Sul, RS 95070 560, Brazil

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ABSTRACT

Nanoparticles have been studied for drug delivery with enormous potential to reduce toxicity or side effects of drugs. Although these systems are usually reported as safe, there are few studies testing the possible cytotoxic effects of nanoparticles. For this study, we developed nanospheres (NS) using poly (ε-caprolactone) and evaluated its effects in the tumor (HEp-2) and non-tumor (MRC5) cells. We also assessed the effects of a polyphenols-rich extract (PE) and its association to the formulation of the NS. To address the mechanisms of action of NS, cell viability, oxidative stress parameters and morphological changes were evaluated in the tumor and non-tumor cells. We observed that the pH of NS in presence or absence of PE was stable between a range of 6.0; diameter was around 200 nm and both samples exhibited narrow distribution (polydispersity index up to 0.19) with zeta potential from -9.6 to -10.6 mV. Low association rate for NS *plus* PE (efficiency of 19.05 %) was found; therefore, further studies and formulations are needed to improve PE encapsulation. NS reduced viability with different levels of toxicity (IC_{50} values460 ± 25 µg.mL^{-1}for HEp-2 and 573 ± 51 µg.mL^{-1} for MRC5 cells; p=0.008,*t*-test).This reduction was associated with alterations in redox metabolism. Changes in symmetry and cell adherence at highest doses were observed in both cells. Results suggest that, depending on the applied concentrations and the cell type, NS may interfere with the cellular response.

INTRODUCTION

Nanomedicine is among one of the most promising fields, combining the pharmaceutical and biomedical sciences with nanotechnology. In the last decades, the number of different applications using nanoparticle materials for drug delivery or as drugs themselves has increased [1–3]. Nanocarriers are systems developed at the nanoscale (<1µm) and includes several different forms already approved by Food and Drug Administration (FDA) such as microcapsules and nanospheres [4–6]. The nanospheres are systems able to incorporate both hydrophilic and lipophilic substances, thus increasing the bioavailability of poorly soluble substances [7]. Among the aliphatic polyesters commonly used as drug carriers, the poly(ε-caprolactone) (PCL) has stood out due to their diverse applications[2]. Some of the main advantages of using PCL are a slow degradation rate, the absence of acidity during degradation and its high permeability to small drug molecules[8,9], showing broad pharmacological applicability.

The increasing number of diseases worldwide highlights the importance of the development of new pharmacological alternatives, and the polymeric systems for drug delivery have gained attention. Diseases like cancer have driven studies seeking to develop new nanotechnology-based therapeutics[10,11]. Nowadays, more than 50 % of the drugs used in cancer treatment is based on natural products[12] which contain phytochemicals, potent modulators of cellular signalling pathways [13-16]. Taking into account the low bioavailability of these compounds, their instability, and target specificity, the nanotechnology represents an innovative and promising approach. Our research group has been studying a polyphenols-rich extract (PE) derived from natural source, the *Araucaria angustifolia* (for review, see [17]), which possess an important and selective antitumor activity[18,19]. Despite its powerful bioactivity, the extract is labile. Therefore, a promising candidate for the generation of nanoparticles. The encapsulating is a valuable way to preserve its chemical characteristics and hence the stability. In fact, there are studies

reporting the use of the nanocarriers to incorporate chemically labile substances, such as lipoic acid[20], quercetin[21,22], and catechin [22]; however, there are few data in the literature showing employment of nanotechnology for association of plant extracts, a complex moisture of substances. Moreover, little is known about the possible toxicity of nanosystems *per se*. In view of this, the aim of this study was to prepare a formulation of nanospheres (NS) using poly (ε-caprolactone) and to evaluate its mechanisms of action in the tumor (HEp-2) and non-tumor (MRC5) human cells. Parallel, the association of the polyphenols-rich extract (PE) to the nanospheres was also investigated.

MATERIALS AND METHODS

Chemicals

Poly (ε-caprolactone) (PCL) (Mw = 14,000 g mol^{-1}) and sorbitan monoesterate (Span 60®) were supplied by Sigma-Aldrich ® (St. Louis, MO, USA). Polysorbate 80 was obtained from Henrifarma® (São Paulo, Brazil). Acetone was acquired from Nuclear® (Diadema, Brazil). The chemicals used in cell culture were Complete Dulbecco's Modified Eagle Medium (DMEM), FetalBovine Serum (FBS), trypsin–EDTA and penicillin–streptomycin, all were purchased from Gibco BRL (Grand Island, NY, USA). Other reagents and solvents were obtained from Sigma (St. Louis, MO, USA). All chemicals were of analytical grade.

Preparation of NS

NS were prepared by interfacial deposition of preformed polymer [23]. The organic phase composed of PCL, sorbitan monosterate and acetone was prepared at 40 °C. Separately, polysorbate 80 was dissolved in water (aqueous phase). The organic solution was injected into the aqueous solution, and maintained for 10 min under magnetic stirring, at room temperature. After complete homogenization, the acetone was eliminated and the suspension was concentrated under reduced pressure at 40 °C (Rotavapor Buchi, model R210). For preparation of NS containing the polyphenols-rich extract (PE) from *Araucaria angustifolia* sterile seeds (bracts), two different approaches were employed: a) it was used an aqueous liquid extract using 5 g of bracts in 100 mL of distilled water (under reflux at 100 °C) for 15 min; and b) it was used a lyophilized powder obtained from PE as described above, under vacuum conditions at -54 ± 5 °C (Lyophilizer LIOBRAS model L-101); both were added to the aqueous phase (polysorbate 80 and water) containing, respectively, 1.43 $mg.mL^{-1}$and 1.27 $mg.mL^{-1}$ of catechin equivalents. PE is rich in phenolic compounds including catechin, epicatechin, rutin, quercetin, apigenin, 4'-methoxytectorigenin, 3-glucoside-dihydroquercetin and the biflavonoid amentoflavone 4',4''',7,7''-tetramethyl ether, as described previously by our group, which catechin is the major compound present in PE [18,24,25].

Chemical characterization of NS suspensions

The physicochemical characterization of the suspensions was performed through the determination of the pH, particle size, analysis of polydispersity index (PDI) and zeta potential (ZP).The pH values of the suspensions were measured without previous dilution using a calibrated potentiometer (TECNOPON model Mpa-210). Quantifications of particle diameters and PDI were performed by dynamic light scattering using a Zetasizer ZS (Malvern, UK). Measurements of ZP were carried out by electrophoretic mobility technique using a Zetasizer ZS (Malvern, UK).Morphology and structure of NS were analyzed by scanning electron microscopy (SEM) using a Shimadzu SSX 550, equipped with a CCD camera, after the suspensions were previously dried by lyophilisation (Lyophilizer LIOBRAS model L-101) and. In order to quantify levels of polyphenols in the NS formulation containing PE, an aliquot of the suspension was combined with methanol, and submitted to ultrasonic extraction to promote the dissolution of the nanospheres. The solution was then filtered in Millipore equipment (pore size, 0.22 μm; Millipore Corp.) and analyzed by High-Performance Liquid Chromatography (HPLC). The chromatographic analyzes were carried out using HPLC model HP 1100 UV/VIS (Palo Alto, CA) by Zorbax SB C18 column (250 x 4 mm) with a flow rate of 0.5 ml min^{-1}. The encapsulation efficiency of the PE to the NS was evaluated by centrifugation at 12,000 rpm, 4 °C for 15 min using a microcentrifuge tube coupled with filter (Amicon® Ultra 0,5 10.000 NMWL; Millipore Corp.). The aqueous phase was analyzed by HPLC as previously described.

Assays with human cells

To study the effects of NS in human cells, the laryngeal tumor cells (HEp-2) and lung fibroblast cells (MRC5) were used, both acquired from American Type Culture Collection (ATCC bank; VA, USA). Cells were cultured in DMEM medium, supplemented with 10% FBS, and 1% penicillin–streptomycin (10,000 UmL^{-1}), and were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37°C until experimentation.

Analysis of cytotoxicity of NS – MTT assay

The possible cytotoxicity of the NS in human cells was evaluated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- 2H-tetrazolium bromide] assay [26]. This assay reflects the activity of mitochondrial dehydrogenases by viable cells in convert MTT salt. The HEp-2 and MRC5 cells were seeded (1×10^4cells mL^{-1}), and incubated at 37 °C in 5%CO_2 for 24 h to attach. After this time, the medium was removed and replaced by fresh complete medium containing 0; 0.03; 0.3; 3; 30; 300 and 750 $\mu g.mL^{-1}$ of PCL nanospheres for 24 h. MTT (1mg mL^{-1}) was then added to the wells and incubated for 3 h. Subsequently, MTT solution was removed and the resulting formazan violet product was dissolved in dimethylsulfoxide (DMSO), stirred for 15 min, and the absorbance was measured using a microplate reader (Victor-X3, multilabel counter, Perkin Elmer, Finland) at 517 nm. Cell viability was expressed as percentage (%) of the control from five independent experiments. The IC_{50}, *i.e.* the inhibitory concentration needed to reduce 50% of the cell proliferation was also quantified.

Redox metabolism evaluation

Oxidative damage to lipids and proteins, along with enzymatic and non-enzymatic antioxidant defences were assessed in HEp-2 and MRC5 cells (1×10^7cells mL^{-1}) treated with 3; 30; 300 and 750 $\mu g.mL^{-1}$ of PCL nanospheres. The concentrations were chosen from MTT assay, considering the minimal doses able to reduce cell viability.Oxidative damage to lipids was monitored by formation of thiobarbituric acid reactive substances (TBARS) during an acid-heating reaction[27]. Results were expressed as nmol of TBARS per mg of protein. Oxidative damage to proteins was measured based on the reaction of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH)[28]. The results were expressed

as nmol of DNPH per mg of protein. Enzymatic antioxidant defences were assessed through Superoxide dismutase (SOD) and Catalase (CAT) activities [29,30]. SOD activity was determined spectrophotometrically and results were expressed as Unit of SOD (USOD) per mg of protein. One unit of SOD is defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50%. CAT activity was determined by hydrogen peroxide (H_2O_2) decomposition rate. The values were expressed as Unit of CAT (UCAT) per mg of protein. One unit of CAT is defined as the amount of enzyme that decomposes 1 mmol of H_2O_2 in 1 min at pH 7.4.Protein sulfhydryl content was quantified as non-enzymatic defence and it was determined by a reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)[31]. Results were expressed as mmol of DTNB per mg of protein. The protein concentration was measured by Lowry method, using bovine serum albumin as the standard [32]. All assays were performed in triplicate.

Cellular morphology analysis

Alterations in cellular morphology were analyzed in HEp-2 and MRC5 culture flasks after treatment with PCL nanospheres (3; 30; 300 and 750 $\mu g.mL^{-1}$) for 24 h. The changes in symmetry along with increase or decrease in cell number, growth and cell adherence, were analyzed. The images were obtained using an inverted microscope (Optiphase-403F, USA).

Statistical analysis

Results were expressed as the mean ± standard deviation (SD) from at least three independent experiments. Data were determined to be parametrical by using the Kolmogorov–Smirnoff test. Statistical significance was evaluated using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Relationships between the continuous variables were assessed using Pearson's correlation coefficient. Results were deemed significant if *p*-value was less than 0.05. The software SPSS 21.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analysis.

RESULTS AND DISCUSSION

NS characterization

The results about main characteristics of formulations of NS in the presence (NS_LYPE) or absence of lyophilized PE are shown in Table 1. Both evaluated formulations exhibited macroscopically homogeneous aspect. The NS formulation showed an opaque white color whereas the NS_LYPE had a slightly brownish appearance (very characteristic of the presence of proanthocyanidins on *A. angustifolia* extract).The formulation using NS plus liquid PE (NE_PE) was unsatisfactory; there was no interaction between the liquid extract and the raw materials used in the preparation of nanospheres, therefore, it was not possible to obtain and characterize it. More studies are needed to investigate the inability to associate the liquid extract to the system.

Table 1 Physicochemical parameters evaluated on NS formulations

Sample	pH value	Particle size (nm)	PDI	Zeta potential (mV)
NS	6.04 ± 0.20	189.6 ± 1.0	0.12 ± 0.01	-10.6 ± 1.0
NS_LYPE	5.94 ± 0.29	196.3 ± 1.0	0.19 ± 0.02	-9.6 ± 0.5

NS (nanospheres); NE_LYPE (nanospheres containing lyophylized polyphenols-rich extract); PDI (polydispersity index).

The pH found was around 6.0 for both the formulations and it was stable for a period of 30 days with the samples stored under refrigeration (4°C). According to the dynamic light scattering technique, the average particle size for NS spheres was around 190 nm, and for NS_LYPE was almost 200 nm. In addition, it was found low PDI values (up to 0.19), which is within the expected parameters for the system. As previously shown, the PDI values of 0.1 to 1 are considered suitable from the standpoint of uniformity in particle distribution [33]. Regarding zeta potential, we found values ranged from -9.6 to -10.6 mV in NS_LYPE and NS *per se*, respectively. Zeta potential represents the electrokinetic potential of nanoparticle surface and is a very important marker of interaction between nanosystems and biological membranes [34]. The negative values of ZP obtained in our study are in agreement with previous studies, which also found a negative superficial charge in nanospheres using PCL [35–37].

For NS_LYPE samples, we also evaluated the total levels of catechin, a major compound of the extract (Figure 1), on the whole solution. Moreover, in order to assess the association rate (incorporation of PE to the polymer phase) the levels of catechin were also quantified inside the nanospheres. The results revealed the total catechin levels found in the whole solution were 0.42 mg mL^{-1}, greater than 80% of the total content added. The analysis of the association rate showed only 0.08 mg mL^{-1} of catechin into the spheres, indicating an association efficiency of 19.05 %. Previous studies have found different profiles on entrapment of bioactive compounds using plant aqueous extracts in nanosystems[38]. A study using an extract from *Phoenix dactylifera* reached an encapsulation efficiency of 70–78% in nanocapsules[39]. The anthocyanin-rich extract of *Hibiscus sabdariffa* was associated to liposomes reaching an association efficiency around 70% [40]. On the other hand, the association efficiency for an extract from Green tea (*Camellia sinensis*) in PCL nanoparticles was around 30%[41]. Some factors may influence the association rate, including the nature of the polymer and the physicochemical characteristics of the drug, and one of the most relevant is the difference of polarity between the drug and the polymer [42]. Alonso *et al*. (1991) improved the association efficiency to 55% by changing the molar ratio of drug and surfactant to increase the intrinsic drug solubility in nanoparticles[42]. In our work, we have a similar profile, which the PE is polar and the carrier is hydrophobic. Therefore, our data show us the employed polymerization conditions must be changed in order to reach high levels of incorporation using PCL spheres as a vehicle for a polyphenols-rich extract from *A. angustifolia*. Taking into account the few data in the scientific literature employing natural extracts on nanosystems, as well as the variability of surfactants, polymers and protocols, data found in this study can be useful to explore new delivery strategies using bioactive natural products.

Considering the low association rate we have obtained on NS_LYPE formulation, we decided to continue the biological experiments using only NS samples, since it is necessary to know the possible toxicity of these systems *per se*. By SEM analysis, the formulation exhibited structures with appearance spherical, heterogeneity of their sizes and empty inside, seemingly (Figure 2 A-B), being considered adequate for *in vitro* experimentation. According to Gaumet *et al*. [33], SEM is an appropriated technique to detect particle size from 50 nm to 100 µm. Regarding size, studies with PCL nanoparticles

already showed that different diameters, such as 363 nm [43] and 449.6 nm [36] can be found, depending on the employed measurement technique.

With a different level of cytotoxicity, the IC_{50} found in our study was 460 ± 25 µg.mL^{-1} for HEp-2 and 573 ± 51 µg.mL^{-1} for MRC5 cells being significant statistically (p=0.008; *t*-test),

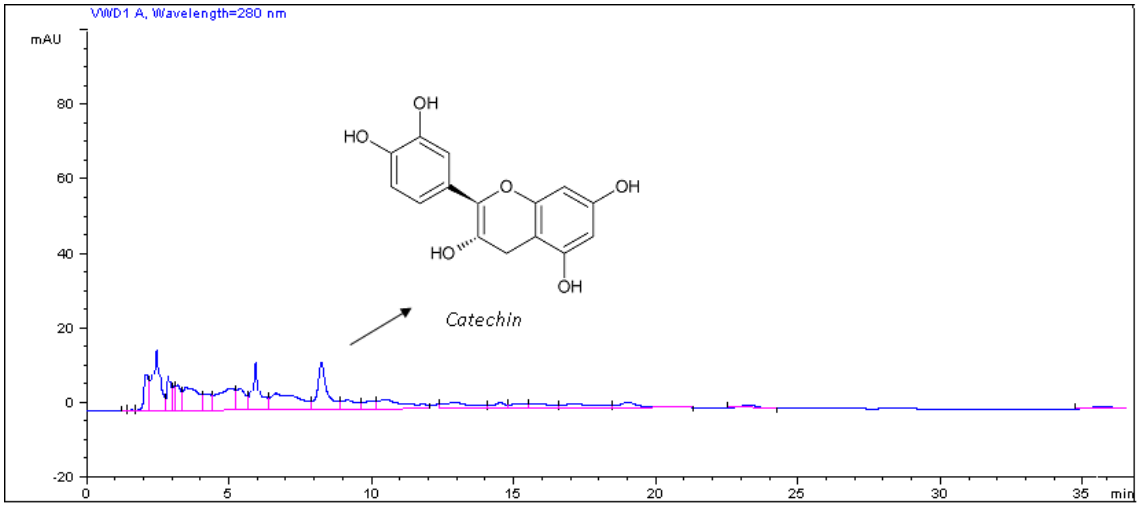


Figure 1 Chromatogram (HPLC) for tannins at 280 nm showing the presence of catechin in polyphenols-rich extract from A. angustifolia.

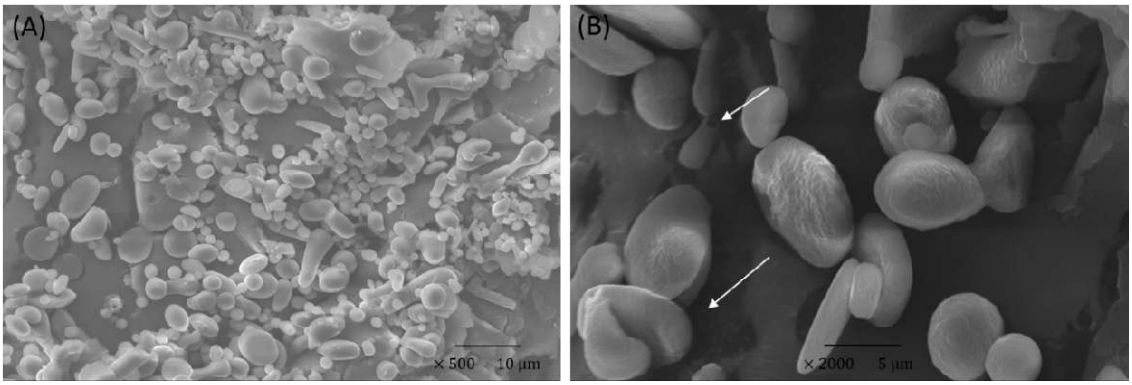


Figure 2 Representative SEM images of NS. (A) general aspect of the particles showing size heterogeneity; (B) image showing the formation of hollow structure (arrows). Samples were preparedas described in Section 2, and then photographed.

NS cytotoxicity in human cells

Although polymers generally employed in the preparation of nanosystems are regarded as nontoxic[44], few studies reporting the real effects of its administration on human cells were conducted so far. For that reason, we decide to investigate the possible effects of NS on metabolism in the tumor (HEp-2) and non-tumor (MRC5) cells, by assessing the cell viability (MTT assay). Both cell lines were chosen due to their epithelial characteristics in order to explore the differential cellular response in tumor and normal cells. Previously, cells were treated with increased concentrations of NS formulations (0.03; 0.3; 3; 30; 300 and 750 µg.mL^{-1}) for 24 h. Low doses of NS (0.03 and 0.3 µg.mL^{-1}) did not significantly reduce the viability of both cells. On the other hand, it was observed that NS treatments were able to significantly decrease the cell proliferation from the concentrations of 3 and 30 µg.mL^{-1} ($p<0.001$; Figure 3) for HEp-2 tumor and MRC5 non-tumor cells, respectively. The PCL toxicity was already discussed in few studies. In a recent study, it was showed time-dependent PCL cytotoxicity (from 2, 4 and 6 days) in all tested doses (25, 100 and 200 µg.mL^{-1}) in retinal pigment epithelium and retinal vascular endothelial cells [45]. Similar results were found in hepatocyte after an exposure to PCL at highest doses (300 and 1000 µg.mL^{-1}) at 72 h of treatment [43]. In opposition, no significant reduction in hepatocyte viability was found in 24 h of exposition to the PCL at concentrations up to 1 % (v/v) [2].

indicating that tumor cells are more sensitive to NS treatments than non-tumor cells. Indeed, tumor cells exhibit differential metabolism compared to normal cells, especially on energy generation and maintenance to cell growth and proliferation[46–51]. In order to understand the mechanisms behind the differential reduction in cell viability revealed by NS, the cytotoxic concentrations (3; 30; 300 and 750 µg.mL^{-1}; 24 h) were chosen to verify possible redox alterations on tumor and non-tumor cells.

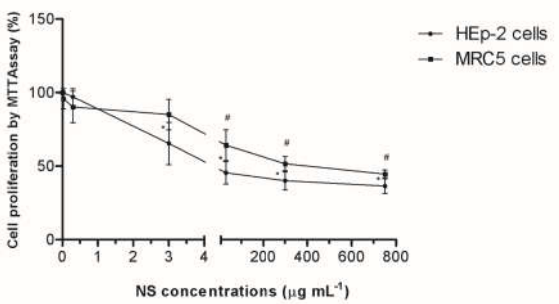


Figure 3 Viability of tumor (HEp-2) and non-tumor (MRC5) cells treated with increasing concentrations of nanospheres (NS) of polycaprolactone for 24 h by MTT assay. % viability = [(number of cells at time of observation/number of control cells) × 100]. Values are expressed as mean ± standard deviation (SD). The symbol # indicates a significant difference from the non-treated cells for MRC5 and * for HEp-2. Statistical significance according to analysis of variance (one-way ANOVA) and Tukey's post-hoc test ($p \leq 0.001$).

Oxidative stress and cell redox status

In order to study the cytotoxic mechanisms of the NS, we decide to evaluate oxidative stress levels, once reactive oxygen species (ROS) production has been identified as one of the earliest toxicity mechanisms for polymeric nanoparticles [43]. The oxidative stress evaluation was assessed through the membrane oxidative lipid damage (TBARS assay) and oxidative damage to proteins (Carbonyl assay). The treatment using cytotoxic concentrations (3; 30; 300 and 750 $\mu g.mL^{-1}$) of NS formulations was able to induce high levels of lipid peroxidation at doses of 300and 750 $\mu g.mL^{-1}$ in HEp-2 tumor cells when compared to control non-treated ($p<0.001$) and to MRC5 non-tumor cells ($p<0.05$; Figure 4 A).

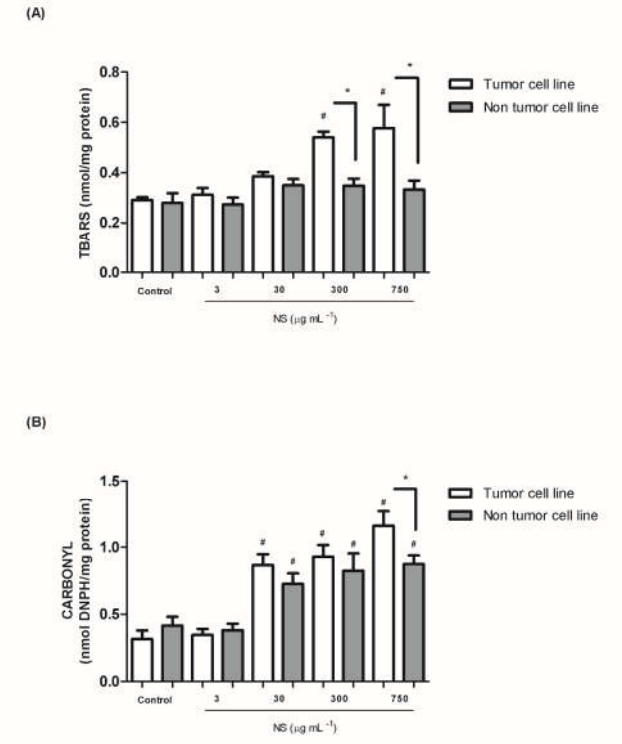


Figure 4 Oxidative damage to lipids (A) and proteins (B) in tumor (HEp-2) and non-tumor (MRC5) cells treated with different concentrations of nanospheres (NS)of polycaprolactone for 24 h. Values are expressed as mean ± standard deviation (SD). The symbol # indicates a significant difference from the controls, and * indicates difference between the cell lines by analysis of variance (one-way ANOVA) and Tukey's post-hoc test.

Additionally, the treatments were not able to induce lipid peroxidation in non-tumor cells. The doses at 30; 300 and 750 $\mu g.mL^{-1}$ induced a significative increase on carbonyl levels in both tumor and non-tumor cells in relation to the controls ($p<0.001$).The highest concentration (750 $\mu g.mL^{-1}$) was able to significantly increase carbonyl levels in HEp-2 ($p<0.05$; Figure 4 B) in relation to MRC5 cells. Oxidative damage to lipids and proteins observed is associated with the production of reactive oxygen species (ROS) along with alterations on redox defence systems. We assessed total thiol levels measured by sulfhydryl content as a non-enzymatic antioxidant marker and found this parameter significantly altered in all NS tested doses (3to 750 $\mu g.mL^{-1}$) in HEp-2 tumor cells ($p<0.001$; Figure 5 A) than compared to MRC5 non-tumor cells. Moreover, it was observed that HEp-2 cells present lower levels of sulfhydryl even on control non-treated. Although the higher dose has reduced sulfhydryl levels on non-tumor cells ($p<0.001$) when compared to their respective control, in general, these cells exhibit increased levels of this marker, indicating normal cells present enhanced ability to neutralize ROS. Besides sulfhydryl content, we also evaluated activities of superoxide dismutase (SOD) and catalase (CAT), the first line of defence against oxidative injury. The SOD enzyme catalyzes the dismutation of radical anion superoxide ($O_2^{\cdot-}$) producing H_2O_2, which can be eliminated by the action of CAT. The results showed HEp-2 tumor cells naturally present high levels of SOD on control non-treated ($p<0.001$; Figure 5 B) than compared to MRC5 non-tumor cells. In addition, at the highest PCL dose (750 $\mu g.mL^{-1}$), HEp-2 cells also exhibited increased levels of SOD in relation to MRC5 cells ($p<0.05$). We also observed that in tumor cells the treatments at all doses reduced the SOD activity when compared to their respective control ($p<0.001$). This data seems to indicate that NS treatments may induce the generation of $O_2^{\cdot-}$, the main ROS produced by mitochondria[52–54]. Besides SOD, we also quantified CAT activity in NS-treated cells, however, no significant differences were found in both HEp-2 and MRC5 cells (Figure 5 C).

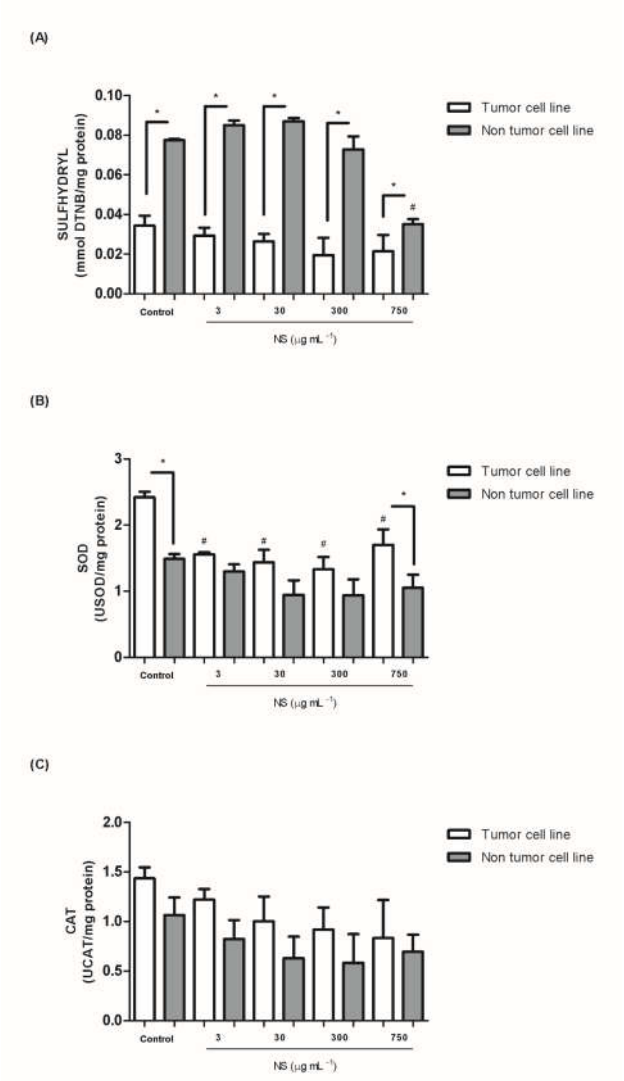


Figure 5 Evaluation of cellular redox metabolism in tumor (HEp-2) and non-tumor (MRC5) cells treated with different concentrations of nanospheres(NS) of polycaprolactone for 24 h. Total thiol content (A); superoxide dismutase (B) and catalase (C) activities. Values are expressed as mean ± standard deviation (SD). The symbol # indicates a significant difference from the controls, and * indicates difference between the cell lines by analysis of variance (one-way ANOVA) and Tukey's post-hoc test.

Pearson's correlation analysis between cell viability andredox status markers was performed and is shown in Table 2. In HEp-2 cells, viability was positively correlated with catalase activity (r = 0.646; p = 0.009); however, it was negatively correlated with oxidative damage to lipids (r = −0.894; p = 0.001) and proteins (r = −0.768; p = 0.001). On the other hand, in MRC5 cells, the viability was positively correlated with catalase activity (r = 0.545; p= 0.036) and with sulfhydryl content (r = 0.868; p=0.001). These data indicate that, at least in tumor cells, the reduction in viability induced by NS treatments is linked to oxidative damage. In addition, in normal cells, the maintenance of cell viability was associated with antioxidant defence systems, which suggest a more efficient cellular defence mechanism by non-tumor cells. In the present study, the alterations found in the MTT assay, which measures mitochondrial dehydrogenases activities, along with SOD activity levels seems to suggest NS may alter mitochondrial dynamic and function, which, depending on cell type, can modify the cell energetic balance.

Cellular morphology

Microscopic analysis of MRC5 non-tumor and HEp-2 tumor cells treated with different concentrations of NS (3; 30; 300 and 750 $\mu g.mL^{-1}$) is presented in Figure 6.

The images reveal NS treatments caused alterations on symmetry and cell adherence at highest doses in normal cells (Figure 6 G and I). In HEp-2 tumor cells, it was also observed for the same NS doses (Figure 6 H and J).

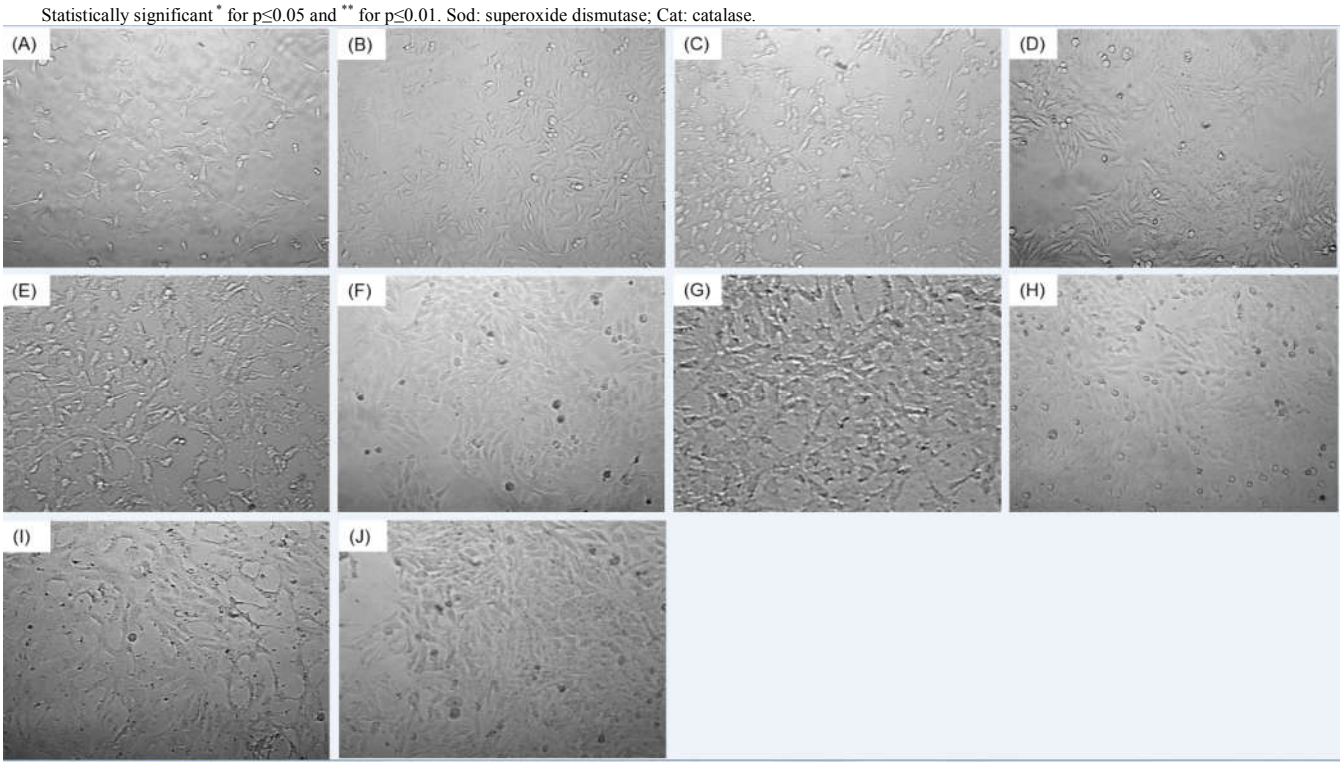
These data indicate that, at least on cellular morphology, both tumor and normal cells treated with NS showed the similar response. Taking into account the interaction between nanoparticle surface and cellular membranes, more studies are necessary to better understand this data.

Our results, taken together, show a possible link between NS toxicity and alterations on cellular redox metabolism of tumor and normal cells. Considering the few studies in the scientific literature about the toxicity of polymeric systems, these data can contribute to understanding the effects of PCL nanospheres in human cells.

Table 2 Pearson correlations between cell viability, lipid and protein oxidative damage, sulfhydryl content, and enzymatic antioxidant defenses in HEp-2 tumor (first line) and MRC5 normal (second line) cells treated with different concentrations of nanospheres of polycaprolactone (NS) for 24 h

	Viability	Lipid damage	Protein damage	Sulfhydryl	Sod activity	Cat activity
Viability	-	-0.894**	-0.768**	0.603	0.260	0.646**
	-	-0.440	-0.549	0.868**	0.482	0.545*
Lipid damage	-0.894**	-	0.811**	-0.772**	-0.537	-0.665**
	-0.440	-	0.757*	-0.370	-0.625	-0.386
Protein damage	-0.768**	0.811**	-	-0.661*	-0.461	-0.747**
	-0.549	0.757*	-	-0.454	-0.759**	-0.492
Sulfhydryl	0.603	-0.772**	-0.661*	-	-0.701*	0.370
	0.868**	-0.370	-0.454	-	0.139	-0.250
Sod activity	0.260	-0.537	-0.461	0.701*	-	0.471
	0.482	-0.625	-0.759**	0.139	-	0.682*
Cat activity	0.646**	-0.665**	-0.747**	0.370	0.471	-
	0.545*	-0.386	-0.492	-0.250	0.682*	-

Statistically significant * for p≤0.05 and ** for p≤0.01. Sod: superoxide dismutase; Cat: catalase.



CONCLUSIONS

The findings of our study demonstrated that the PCL spheres synthesized in the presence or absence of polyphenols-rich extract (PE) presented suitable physicochemical parameters, including size (mostly 200 nm), distribution (PDI lower than 0.19) and zeta potential (up to -10.6 mV). Nevertheless, a low association rate (around 19 % of incorporation) to the PE was found. The biological assays showed that the PCL spheres *per se* can induce cytotoxicity in concentrations higher than 3 and 30 $\mu g.mL^{-1}$ in tumor and normal cells, respectively. This effect was associated with oxidative damage to lipids and proteins along with alterations on antioxidant defence systems, mainly on sulfhydryl levels. Moreover, our findings showed that in contrast to non-tumor cells, tumor cells are more sensitive to the NS treatments. Although more studies are needed, these results suggest that nanoparticles made using PCL could be a candidate for antitumor drug delivery. Considering the cytotoxic effects of PE from *Araucaria angustifolia* in cancer cells, retries encapsulation of this extract will be carried out..

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