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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 09, Issue, 12, pp.9079-9082, December, 2018

RESEARCH ARTICLE

DEVELOPMENT OF TiO_2-PEG-PTX NANOPARTICLE BASED DRUG SYSTEMS AND INVESTIGATION OF ANTICANCER ACTIVITY ON SH-SY5Y CELLS

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ARTICLE INFO

Article History:
Received 22nd September, 2018
Received in revised form
09th October, 2018
Accepted 02nd November, 2018
Published online 20th December, 2018

Key words:
Neuroblastoma, SH-SY5Y,
TiO_2-PEG-PTX, PTX, MTT.

ABSTRACT

The use of nanoparticles in biomedical and bioengineering fields has revolutionized cancer treatment. In this study, we aimed to investigate the anticancer activity of the nanotechnologically produced TiO_2-PEG-PTX drug on SH-SY5Y neuroblastoma cell lines. Our study, TiO_2 nanoparticles used were synthesized, coated with PEG, and PEG-TiO_2 nanostructure system was loaded with PTX.UV analyse of suspensions prepared at different concentrations of TiO_2, PEG-TiO_2, PTX, and PEG-TiO_2-PTX nanostructured system were performed. The synthesized drugs were performed to the SH-SY5Y neuroblastoma cell line and anticancer activity of these drugs were determined by using MTT method. The SH-SY5Y cells were treated with different concentrations of TiO_2 (5-100 µg/ml) for 24, 48 and 72 hours. The effects of these drugs on the SH-SY5Y cells were compared with the control group and IC50 values were determined for 24, 48 and 72 hours. In this study, it was shown that the effect of TiO_2-PEG-PTX nanocarrier system on SH-SY5Y cells was inhibitory to growth in cancer cells when compared with control group and PTX.

Citation: ***Ayca Tas, NeseKeklikciogluCakmak and Yavuz Silig*,2018.** "Development of TiO_2-PEG-PTXnanoparticle based drug systems and investigation of anticancer activity on SH-SY5 cells", *Asian Journal of Science and Technology*, 09, (12), 9079-9082.

INTRODUCTION

Neuroblastoma is the common pediatric neoplasm of the sympathetic nervous system. It also attracts attention with its heterogeneous clinical presentation and variable response to treatment (Matthay *et al*., 2016). Recently, although there has been significant progress in the treatment and prognosis of pediatric malignancies, high mortality rates still remain in the patients (Davenport *et al*., 2012). Neuroblastoma patients can only be treated with surgical or adjuvant chemotherapy (Strother, 2012; Baker *et al*., 2010). However, neuroblastoma, chemotherapeutic agents used in the treatment of drug resistance can occur (Louis *et al*., 2015). Traditional chemotherapy and radiation therapy do not have a satisfactory curative effect due to their high toxicity and the risk of secondary malignancy (Berthold *et al*., 2005; Matthay *et al*., 1999 Patterson *et al*., 2011). In the treatment of cancer, alkylating agents, antimetabolites, biological agents, etc. It is used. However, one of the major problems associated with the use of these molecules is the side effects caused by the difficulties in the distinction between cancerous and normal cells (Saloustros *et al*., 2008).

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Therefore, one of the new strategies for cancer treatment is the use of nanomaterials (Rasmussen *et al*., 2010). Recently, with the development of nanotechnology, there has been a significant improvement in the application of nanoparticles (NPs) for drug delivery systems, antibacterial agents, cosmetics, sunscreens and electronic materials (Kisin *et al*., 2007; Robertson *et al*., 2010). The introduction of NPs in the fields of biomedical and bioengineering has revolutionized the methods of cancer treatment (Liang *et al*., 2009). TiO_2NPs are a dynamic potential treatment agent in cancer treatment due to their excellent biocompatibility and unique photocatalytic properties (Bertrand *et al*., 2012; Paunesku *et al*., 2008; Chen *et al*., 2011). Also, TiO_2NPs have attracted much attention in the transport of chemotherapeutic agents (Oberdörster *et al*., 1992). However, recent research has shown that rats exposed to TiO_2 develop inflammation, lung injury, and lung tumors (Baggs *et al*., 1997; Mishra *et al*., 2008). This toxicity can be caused by the fact that these NPs can easily pass through the cell membrane and easily disrupt the biological systems by their effects (Wang *et al*., 2009).Therefore, in order to reduce the toxic side effects of the NPs, the surface is coated with non-toxic polyethylene glycol (PEG) and the surface is coated and biocompatible (Matsumura *et al*., 2009; Mahbubul *et al*., 2017). The aim of this study was generated TiO_2-PEG-PTX

complex, whose cytotoxicity and antitumor efficiency were evaluated in human SH-SY5Y neuroblastoma cells.

MATERIALS AND METHODS

Synthesize of PEGylated TiO_2NPs and Drug loding on TiO_2-PEG: TiO_2NPs were produced by a sol-gel process (Mahbubul *et al*., 2017). Titanium iso-propoxide (TIP) was used as the starting precursor to synthesize TiO_2-NPs using the sol-gel method. PEG was used to increase the stability of the TiO_2 NPs and to coat the nanoparticles. 20 mL of TiO_2 NP (0.5 mg / ml) was added to the PEG solution and stirred for 24 hours. The TiO_2-PEG NPs were centrifuged at 12500 rpm for 30 minutes and were dispensed in 20 ml of ultrapure water. 1 ml of paclitaxel (PTX) (1 mg/ml) was added dropwise to the TiO_2-PEG NPs and stirred for 24 hours. The resulting TiO_2-PEG-PTX NPs were centrifuged at 12500 rpm for 30 minutes and stored at 4 °C. Furthermore, free PTX in the centrifugal supernatant was also collected to measure the loading efficiency of PTX onto TiO_2-PEG. The absorption of PTX at 250 nm was determined using a UV-visible spectrophotometer. The dose-absorption curve of PTX was calculated according to the different absorptions of PTX at 250 nm.

Characterization: The UV-visible absorption of TiO_2-PEG and TiO_2-PEG-PTX NPs was determined using a UV-visible spectrophotometer (UV-1280, Shimadzu, Japan).

Cell Culture: SH-SY5Y neuroblastoma cells were maintained in DMEM medium, containing 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (10 mg/L). Cells were grown in at 37°C, 5% CO2 and 95 % air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates.

Anti-cancer activity of TiO_2, PEG-TiO_2 and TiO_2-PEG-PTX on SH-SY5Y cells: Anti-cancer activity of the TiO_2-PEG-PTX, PEG-TiO_2, TiO_2, and PTX on SH-SY5Y cell lines was performed with the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to the Skehan's method. Briefly, cells were trypsinized and plated into 96-well plates (Corning, USA) in 0.1 ml of complete culture medium at a density of 1×10^5 cells per well and allowed to attach for 24 h. 1 µl of test substance at concentrations ranging between 5-100 µg/ml were added into each well containing the cells. Test substance was diluted with sterilized water into the desired concentrations from the stock. The plates were incubated at 37°C with an internal atmosphere of 5% CO2. After 24, 48 and 72 h incubation, with different concentrations of compounds, MTT (5 mg/ml dissolved in PBS) 10 µl/well was added directly to all the wells and incubated for 2 hours at 37°C. The supernatant was carefully removed from each well and 100 ml of DMSO was added to each well to dissolve the formazan crystals. After mixing with a mechanical plate mixer for 15min, the absorbance of plates was recorded at 570 nm on a microplate reader (Bio-Tek, USA). All drug doses were parallel tested in triplicate and were performed at least 3 times; control samples were run with 1% sterilized water.

Statistical analysis: In our study, experiments were carried out in three replications and the mean ± standard error mean. Our results were analyzed using one-way variance analysis. Differences were considered significant at $p < 0.05$. IC_{50} values were determined by the statistical software program GraphPadPrism7 (Graph Pad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Synthesis and characterization of TiO_2-PEG and TiO_2-PEG-PTX: In this study, the TiO_2NPs were first synthesized, then the TiO_2 NPs were coated with PEG to increase their stability, and then PTX was added to the TiO_2-PEG NPs to form the TiO_2-PEG-PTX NPs. The reason for the addition of PEG to the surface of the nanomaterials may prevent rapid excretion of the renal and reticuloendothelial systems (RES) and greatly increase the half-life of the nanomaterials in the blood. Therefore, it increases the accumulation of nanomaterials in tumor tissue (Paunesku *et al*., 2008; Chen *et al*., 2011; Oberdörster *et al*., 1992). In addition, PEG-coated TiO_2 NPs can reduce the interaction between PTX and TiO_2, thus increasing drug release from NPs in tumor sites (Baggs *et al*., 1997; Mishra *et al*., 2008). The successful loading of PTX on TiO_2-PEG NPs was confirmed by UV-visible spectrum analysis. As shown in Fig.1 the characteristic peak of PTX occurred at about 250 nm. These results indicated that PTX is successfully loaded onto the TiO_2-PEG NPs.

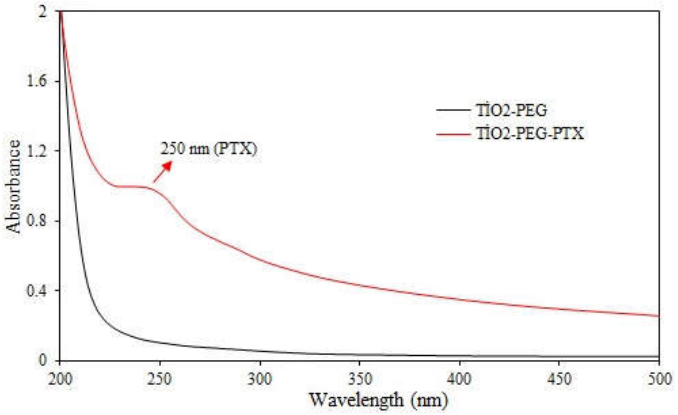

Figure 1. UV-Vis absorption spectra of TiO_2-PEG-PTX

Anti-cancer activity of TiO_2, TiO_2-PEG, TiO_2-PEG-PTX and PTX drugs on SH-SY5Y cells: To determine whether TiO_2-PEG-PTX, PEG-TiO_2, TiO_2, and PTX were intracellular anti-cancer activities, SH-SY5Y cells were exposed to certain concentrations of these drugs and their anti-cancer activities were determined by the MTT method (Figure 2). Figure 2 shows changes in cell inhibition for 24, 48 and 72 hours versus increasing concentrations of SH-SY5Y cell lines. x-axis shows cell types and varying time points, while the y-axis shows the inhibition rates of cancer cells relative to the control. Compared to the control group, TiO_2-PEG-PTX treated human SH-SY5Y neuroblastoma cells showed significantly decreased tumor survival rate after 24h, 48h and 72h of incubation. Compared to the PTX group, the TiO_2-PEG-PTX group had significantly reduced survival rate after 24h, 48h and 72 h of incubation. Cell survival rates in all groups after 24h, 48h and 72 h of incubation were significantly decreased than those in the control group. With elongated treatment time, the survival rate of tumor cells was significantly reduced. TiO_2-PEG-PTX, PEG-TiO_2, TiO_2, and PTX drugs on SH-SY5Y cells were the most active for 72 h of incubation. In addition, the most active TiO2-PEG-PTX and IC50 values for 24, 48 and 72 hours were 8,19 µg/ml, 7,27 µg/ml and 5,03 µg/ml respectively (Table 1). Also, TiO_2-PEG-PTX was found to be statistically significant compared to other drugs (p<0, 0001).

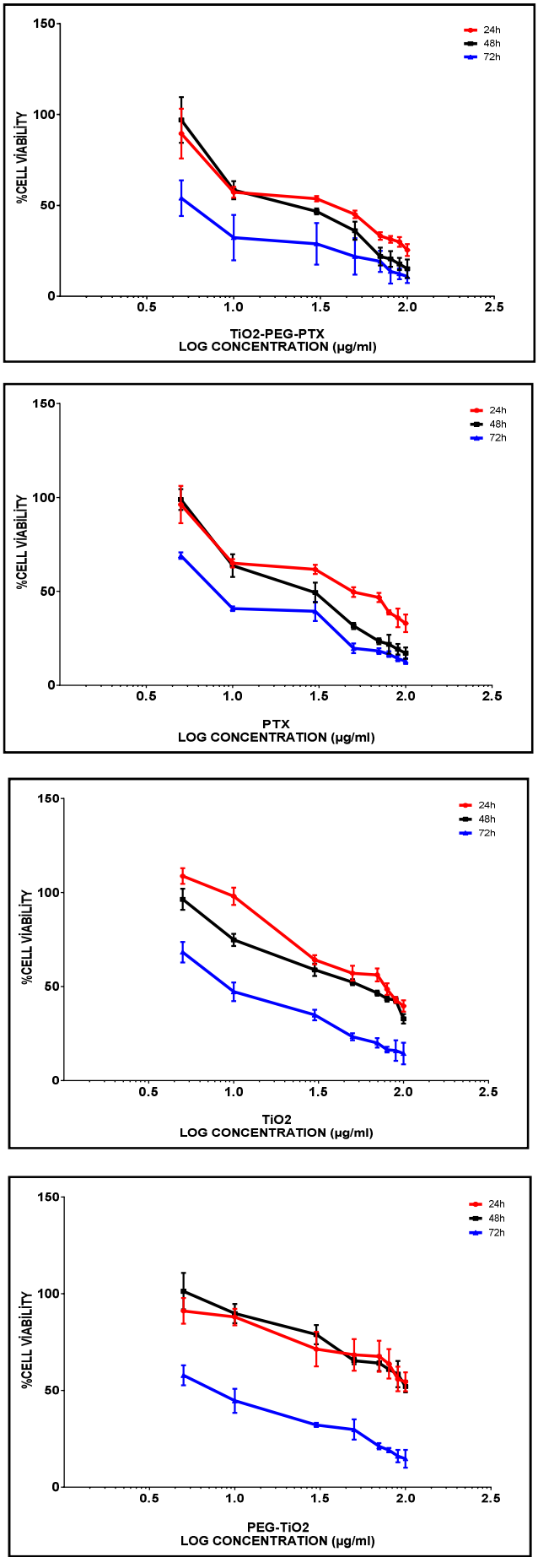

Figure 2. Anti-cancer activityofTiO_2-PEG-PTX, PEG- TiO_2, TiO_2, and PTX drugs on SH-SY5Y cell line

Table 1. Comparison of IC_{50} values between TiO_2-PEG-PTX, PEG-TiO_2, TiO_2, and PTX on SH-SY5Y after 24 h, 48 h and 72 h of incubation

	IC_{50} (µg/ml±SD*)		
Drugs	24h	48h	72h
TiO_2-PEG- PTX	8,19±0,38	7,27±0,21	5,03±0,15
TiO_2-PEG	65,29±013	49,40±0,21	31,00±0,25
TiO_2	21,78±0,19	14,55±0,22	11,35±0,26
PTX	10,17±0,28	9,95±0,34	8,60±0,23

*The mean standard deviation values of IC_{50} obtained from three independent experimental repetitions after 24 h, 48 h and 72 h incubation for the SH-SY5Y cell line.

In our study, we treated SH-SY5Y neuroblastoma cells with TiO_2-PEG-PTX, PEG-TiO_2, TiO_2 and PTX drugs for 24 and 48 hours approximately value of average IC_{50} with10µg /ml. Then we obtained images with a 10x magnification on the microscope (Figure 3). As shown in fig 3. it was noted that the TiO_2-PEG-PTX synthesized as nanotechnology compared to the control was more active on SH-SY5Y cells in 24 and 48h. PTX was similar to TiO_2-PEG-PTX, whereas PEG-TiO_2 showed the least effect. This reason of PEG-TiO_2 is less effective on cells may be because TiO_2 nanoparticle, which is toxic to the cells, is coated with PEG to form a non-toxic biocompatible molecule. Zhang *et al*. In another study, they found that PEGylated nanoparticles had less cytotoxic effects than uncoated ones (Zhang *et al*., 2011). Also, it increases the residence time in vivo by coating the nanoparticles with PEG, thereby reducing clearance through the reticuloendothelial system (RES) (Prencipe *et al*., 2009). This may lead to further circulation of nanoparticle-based synthesized drugs. Therefore, this situation supports our hypothesis.

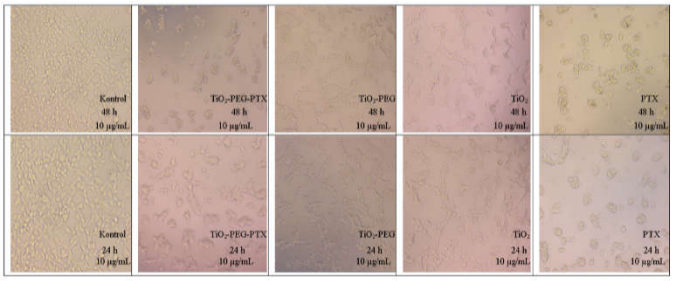

Figure 3. Morphological changes of SH-SY5Y cells after 24 and 48 hours of incubation with concentrations (10 µg/ml) of TiO_2-PEG-PTX, PEG-TiO_2, TiO_2, and PTX. the results presented are from that were carried out and photographed microscopically

Conclusion

In summary, we have developed modified PEGylated TiO_2 drug carriers (TiO_2-PEG-PTX) for targeting drug delivery and therapy. TiO_2-PEG-PTX complex effectively carries large amounts of PTX drug, elevates drug solubility in water, enhances PTX stability in aqueous solution, and improves biocompatibility of drugs. This study demonstrates the possibility of using TiO_2-PEG-PTX to inhibit the growth of SH-SY5Y and their anti-cancer activities for potential therapeutic treatments and offers a new method to develop molecule for cancer therapy. Therefore, based on the results of this study, further in vitro drug release and in vivo studies will be performed.

Acknowledgements

This study was carried out at Cumhuriyet University's Advanced Technology Application and Research Center (CUTAM).

REFERENCES

Baggs, R. B., Ferin, J. and Oberdörster, G. 1997. Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. *Veterinary pathology,* 34(6), 592-597.

Baker, D. L., Schmidt, M. L., Cohn, S. L., Maris, J. M., London, W. B., Buxton, A., ... and Shamberger, R. C. 2010. Outcome after reduced chemotherapy for intermediate-risk neuroblastoma. *New England Journal of Medicine,* 363(14), 1313-1323.

Berthold, F., Boos, J., Burdach, S., Erttmann, R., Henze, G., Hermann, J., ... and Simon, T. 2005. Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. The lancet oncology, 6(9), 649-658.

Bertrand, N., & Leroux, J. C. 2012. The journey of a drug-carrier in the body: an anatomo-physiological perspective. Journal of controlled release, 161(2), 152-163. S. Yamaguchi, *Photochem. Photobiol.,* 2010, 86, 964–971.

Chen, Y., Wan, Y., Wang, Y., Zhang, H. and Jiao, Z. 2011. Anticancer efficacy enhancement and attenuation of side effects of doxorubicin with titanium dioxide nanoparticles. International journal of nanomedicine, 6, 2321.

Davenport, K. P., Blanco, F. C., & Sandler, A. D. 2012. Pediatric malignancies: neuroblastoma, Wilm's tumor, hepatoblastoma, rhabdomyosarcoma, and sacroccygeal teratoma. *The Surgical clinics of North America,* 92(3), 745-67.

Kisin, E. R., Murray, A. R., Keane, M. J., Shi, X. C., Schwegler-Berry, D., Gorelik, O., ... and Shvedova, A. A. 2007. Single-walled carbon nanotubes: geno-and cytotoxic effects in lung fibroblast V79 cells. Journal of Toxicology and Environmental Health, Part A, 70(24), 2071-2079.

Liang, G., Pu, Y., Yin, L., Liu, R., Ye, B., Su, Y., & Li, Y. 2009. Influence of different sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress. *Journal of Toxicology and Environmental Health, Part A*, 72(11-12), 740-745.

Louis, C. U. and Shohet, J. M. 2015. Neuroblastoma: molecular pathogenesis and therapy. *Annual review of Medicine*, 66, 49-63.

Mahbubul, I. M., Elcioglu, E. B., Saidur, R., and Amalina, M. A. 2017. Optimization of ultrasonication period for better dispersion and stability of TiO2–water nanofluid. Ultrasonics sonochemistry, 37, 360-367.

Matsumura, S., Sato, S., Yudasaka, M., Tomida, A., Tsuruo, T., Iijima, S., and Shiba, K. 2009. Prevention of carbon nanohorn agglomeration using a conjugate composed of comb-shaped polyethylene glycol and a peptide aptamer. *Molecular pharmaceutics,* 6(2), 441-447.

Matthay, K. K., Villablanca, J. G., Seeger, R. C., Stram, D. O., Harris, R. E., Ramsay, N. K., ... & Gerbing, R. B. 1999. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. *New England Journal of Medicine, 341*(16), 1165-1173.

Matthay KK, Maris JM, Schleiermacher G, *et al*. 2016. Neuroblastoma. Excellent review of the biology and clinical approach to neuroblastoma. *Nat Rev Dis Primers.* 2:16078.

Mishra, V. K., Mishra, S. K., & Jha, A. 2008. Application of nanomaterials in mesenchymal stem cell engineering. *Dig J Nanomater Biostructures,* 3, 203-208.

Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S. C., & Finkelstein, J. 1992. Role of the alveolar macrophage in lung injury: studies with ultrafine particles. *Environmental health perspectives,* 97, 193.

Patterson, D. M., Gao, D., Trahan, D. N., Johnson, B. A., Ludwig, A., Barbieri, E., ... & Kim, E. S.2011. Effect of MDM2 and vascular endothelial growth factor inhibition on tumor angiogenesis and metastasis in neuroblastoma. Angiogenesis, 14(3), 255-266.

Paunesku, T., Ke, T., Dharmakumar, R., Mascheri, N., Wu, A., Lai, B., ... & Larson, A. 2008. Gadolinium-conjugated TiO 2-DNA oligonucleotide nanoconjugates show prolonged intracellular retention period and T1-weighted contrast enhancement in magnetic resonance images. Nanomedicine: Nanotechnology, Biology and Medicine, 4(3), 201-207.

Prencipe, G., Tabakman, S. M., Welsher, K., Liu, Z., Goodwin, A. P., Zhang, L., ... & Dai, H. 2009. PEG branched polymer for functionalization of nanomaterials with ultralong blood circulation. *Journal of the American Chemical Society*, 131(13), 4783-4787.

Rasmussen, J. W., Martinez, E., Louka, P., & Wingett, D. G. 2010. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert opinion on drug delivery, 7(9), 1063-1077.

Robertson, T. A., Sanchez, W. Y., & Roberts, M. S. 2010. Are commercially available nanoparticles safe when applied to the skin? *Journal of biomedical nanotechnology*, 6(5), 452-468.

Saloustros, E., Mavroudis, D., & Georgoulias, V. 2008. Paclitaxel and docetaxel in the treatment of breast cancer. Expert opinion on pharmacotherapy, 9(15), 2603-2616.

Strother, D. R., London, W. B., Schmidt, M. L., Brodeur, G. M., Shimada, H., Thorner, P., ... and Murray, K. 2012. Outcome after surgery alone or with restricted use of chemotherapy for patients with low-risk neuroblastoma: results of Children's Oncology Group study P9641. *Journal of Clinical Oncology,* 30(15), 1842.

Wang, W., Xiong, W., Wan, J., Sun, X., Xu, H., & Yang, X. 2009. The decrease of PAMAM dendrimer-induced cytotoxicity by PEGylation via attenuation of oxidative stress. Nanotechnology, 20(10), 105103.

Zhang, Y., Xu, Y., Li, Z., Chen, T., Lantz, S. M., Howard, P. C., ... & Biris, A. S. 2011. Mechanistic toxicity evaluation of uncoated and PEGylated single-walled carbon nanotubes in neuronal PC12 cells. ACS nano, 5(9), 7020-7033.

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