IC\textsubscript{50} Evaluation of Platinum Nanocatalysts for Cancer Treatment in Fibroblast, HeLa, and DU-145 Cell Lines

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**ABSTRACT:** Cancer is a major public health problem being one of the main causes of morbidity and mortality today. Recent advances in catalytic nanomedicine have offered new cancer therapies based on the administration of nanoparticles (NPs) of platinum (Pt) dispersed in catalytic mesoporous nanomaterials (titania, TiO\textsubscript{2}) with highly selective cytotoxic properties and no adverse effects. A half maximal inhibitory concentration (IC\textsubscript{50}) study was carried out in cancerous cell lines (HeLa, DU-145, and fibroblasts) to evaluate the cytotoxic effect of different nanomaterials [Pt/TiO\textsubscript{2}, TiO\textsubscript{2}, and Pt(acac)\textsubscript{2}] synthesized by the sol–gel method at concentrations 0–1000 μg/mL. The assays showed that IC\textsubscript{50} values for Pt in functionalized TiO\textsubscript{2} (NPt) in HeLa (53.74 ± 2.95 μg/mL) and DU-145 (75.07 ± 5.48 μg/mL) were lower than those of pure TiO\textsubscript{2} (74.29 ± 8.95 and 82.02 ± 6.03 μg/mL, respectively). Pt(acac)\textsubscript{2} exhibited no cytotoxicity. Normal cells (fibroblasts) treated with NPt exhibited no significant growth inhibition, suggesting the high selectivity of the compound for cancerous cells only. TiO\textsubscript{2} and NPt were identified as antineoplastic compounds in vitro. Pt(acac)\textsubscript{2} is not recommendable because of the low cytotoxicity observed.

**INTRODUCTION**

Cancer represents one of the main causes of morbidity and mortality worldwide, being the second leading cause of death globally.\textsuperscript{1} In 2012, 14 million new cases were reported with 8.2 million deaths;\textsuperscript{2} this number increased to an estimate of 9.6 million deaths in 2018.\textsuperscript{3} In Mexico, each day, 204 people die of carcinomas, with breast (13.3%) and cervical cancers (10.4%) in women\textsuperscript{4} and prostate (15.0%) and lung cancers (12.0%) in men being the most common cancers.\textsuperscript{5} Because of the high incidence and mortality of cervical and prostate cancers, special attention was given to them in this study.

Traditional treatments for cervical cancer include hysterectomy, radiotherapy, and chemotherapy;\textsuperscript{6–8} the latter is based on cisplatin administration in doses of 40 mg/m\textsuperscript{2} with radiotherapy at maximum doses of 50 Gy.\textsuperscript{9} In the same way, modern treatments for prostate cancer include androgenic blockage and radiotherapy, as well as chemotherapy in cases where hormonal management fails.\textsuperscript{10} Although systemic therapies for metastatic diseases have been tested, a significant progress is still needed in the area of nonandrogen ablative approaches.\textsuperscript{11}

Despite being the main chemotherapeutic utilized, cisplatin exhibits severe dose-dependent adverse effects, including neurotoxicity, nephrotoxicity, ototoxicity, gastrointestinal symptoms, fever, hypotension, altered sleep–wake cycle, myelosuppression, as well as alterations in the liver, skin, and respiratory apparatus.\textsuperscript{12–17} Among the recent approaches for cancer treatment, nanostructured chemotherapeutics have exhibited disruptive alternatives for drug delivery, diagnosis, and imaging because of their uniquely appealing features that greatly differentiate them from regular small molecules: <100 nm particle size, high surface area, selectivity, mesoporous nanostructure, point defects, and catalytic activity.\textsuperscript{18} Platinum nanoparticles (NPs) are well-known for their cytotoxicity on both cancer and normal cell lines,\textsuperscript{23–31} including cervical\textsuperscript{32,33} and prostate cancer cell lines.\textsuperscript{35,36} Their mechanisms of actions are based on increasing the levels of reactive oxygen species (ROS), malondialdehyde, nitric oxides, and carbonylated proteins, which leads to the loss of mitochondrial integrity.\textsuperscript{35} However, the highly toxic profile of the compounds and the lack of selectivity for cancer cells lead to substantial dose-limiting acute and chronic toxicities.\textsuperscript{37} Regarding the latter, in past works, we have reported the design and characterization of an inorganic nanocatalyst (a nanoparticulated catalyst with organic functionalization agents that mimic cellular conditions) based on a nanostructured functionalized titania matrix with catalytic properties; the titania NPs were impregnated with platinum at low concentrations (1%). The nanocatalyst exhibited antineoplastic
effects by directly catalyzing the breakage of carbon—carbon and carbon—nitrogen bonds present in the DNA molecule instead of generating secondary compounds that would carry out the cytotoxic outcome.38–42 The nanocatalysts adhere to the surface of cancerous cells through a hypothesized ligand—receptor interaction facilitated by the functionalizing agents in the compound, making it selective to cancer cells without interacting with normal cells.40,43 In the same way, the low concentrations of Pt used decrease the general toxicity related with the platinum NPs. The NPs synthesized were tested in a pediatric ependymoma, exhibiting high selectivity and efficiency in terms of tumor elimination, with no adverse effects detected.44 Because of its mechanism of action based on catalysis, the nanocatalyst is believed to be effective in different types of cancer.

New drugs and compounds require to be tested for their toxicology profile before human application.45 In vitro cytotoxicity tests have proven to be an effective alternative for animal experiments in acute toxicity experiments as estimations are possible with high accuracy.46 The measurement of IC50 in NPs allows to identify the potential toxicity related with their persistent accumulation in organs,47 as concerns have been raised on the potential risk of using NPs in medical applications.48 The half maximal inhibitory concentration (IC50) is a measure of the dose of a drug that causes 50% inhibition in a tested population after a specified test duration; therefore, it is frequently used as a general indicator of a substance’s effectiveness.49 Regarding antineoplastic NPs, IC50 indicates the efficiency of the NPs to achieve growth inhibition in cancerous cells in terms of concentration needed, as well as their effect in normal cells.50

In this article, we evaluated the cytotoxic effect of a platinum-based inorganic nanocatalyst in cancerous cell lines of cervical (HeLa) and prostate (DU-145) cancers so as to obtain the IC50 of the compound, determining the concentration required to achieve 50% mortality. Furthermore, the selectivity of the NPs was tested by applying the nanocatalyst in normal cells (fibroblasts).

**RESULTS**

Electronic Microscopy Studies. The morphology and grain size of the samples TiO2 impregnated with platinum (NPt) and pure TiO2 were studied through scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Figure 1 shows SEM and TEM micrographs of NPt (a,b) and TiO2 (c,d). NPt NPs are observed to form amorphous and smooth conglomerates with sizes of the order of 500 nm; individual NPs are observed to exhibit sizes <100 nm in diameter (Figure 1a). The TEM analysis of the NPt NPs (Figure 1b) indicates particle sizes to be of the order of 10 nm, with clear crystalline formation (grain size ~20 nm). For the case of pure TiO2, the SEM micrograph (Figure 1c) shows the formation of conglomerates at ~200 nm in diameter. The conglomerates are spherical and smooth. The TEM study (Figure 1d) presents individual titania particles and confirms particle sizes to be of the order of 10 nm. The distribution of the atoms in the micrograph suggests a possible crystalline arrangement. In previous works, both NPt and pure TiO2 crystalline structures were identified as anatase (electron diffraction pattern shown in Figure 1b,d). High dispersion and small concentration of the platinum in the NPt sample do not affect the crystalline structure, as observed in Figure 1b,d.

**MTT Assay as the Cytotoxic Study in Cell Lines.** The cell lines were tested with NPt, the nonmetal matrix (TiO2) as a reference, and platinum acetylacetone [Pt(acac)2], which was the precursor utilized for the synthesis of NPt. For each cell line, IC50 was calculated. The effect of the NPs in the cancerous cells was evaluated at a microscopic and cellular level through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in triplicates (significant differences evaluated by ANOVA), which allowed to calculate the viability of cells by colorimetry. Viable cells synthesize formazan (deep purple); under the cytotoxic effect of the NPs, the number of viable cells diminishes, hence decreasing the color intensity.51 The mortality percentages were calculated by comparing the formazan synthesis in treated cells against the synthesis in untreated cells (0% mortality) for each NP concentration. The UV−vis study allowed to measure the NP concentration at which 50% of the cells died with each compound, and thus, IC50 was calculated from the cell mortality curves.

**Cytotoxic Effect in HeLa Cell Lines.** HeLa cells with no treatment (Figure 2a,e) showed a normal morphology (in spindle) and were confluent and adhered.52 HeLa cells under NPt treatment (Figure 2b,f) exhibited a drastic increase in cell mortality as early as 62.50 μg/mL with a reduction in formazan production (Figure 2f), achieving virtually no synthesis from 250.0 μg/mL. The morphology was also affected: the cells were visualized as amorphous, small, nonadhesive, and circular. For the triplicate test, there were no statistically significant differences between assays as determined by one-way ANOVA, F(2,24) = 0.33, p = 0.72. A similar effect was observed for pure TiO2 (Figure 2c,g), showing comparable mortality rates and effects in morphology, with no significant differences, F(2,24) = 0.03, p = 0.97. On the contrary, cells treated with Pt(acac)2 (Figure 2d,h) displayed no visible change in formazan production, which indicates no cytotoxic effect as the number of viable cells remained barely intact, F(2,24) = 0.40, p = 0.68. The ANOVA analysis between the three samples yielded significant variation, F(2,24) = 5.82, p = 0.01. A post hoc Tukey test showed that the Pt(acac)2 sample differed significantly at p < 0.05 from the other samples.

**Cytotoxic Effect in DU-145 Cell Lines.** The normal morphology of the prostate cancer DU-145 cells is observed in...
MTT assay probed the viability of the cells in terms of formazan synthesis (Figure 3e). DU-145 cells treated with NPt (Figure 3b,f) and TiO$_2$ (Figure 3c,g) presented a similar cytotoxic effect both on the morphology (amorphous and detached) (Figure 3b,c) and in the formazan concentration (Figure 3f,g), with no statistically significant differences between assays as determined by one-way ANOVA, $F(2,24) = 0.01$, $p = 0.99$ and $F(2,24) = 0.01$, $p = 0.99$, respectively. On the other hand, similar to the case of HeLa cells, Pt(acac)$_2$ administration (Figure 3d,h) caused no significant cell variation, neither in the morphology (Figure 3d) nor in formazan concentration (Figure 3h), no matter how much amount of the platinum compound is added, $F(2,24) = 0.09$, $p = 0.91$. The ANOVA analysis between the three samples yielded significant variation, $F(2,24) = 4.51$, $p = 0.02$. A post hoc Tukey test showed that the Pt(acac)$_2$ sample differed significantly at $p < 0.05$ from the other samples.

**Cytotoxic Effect in Fibroblast Cell Lines.** For the evaluation of the selectivity of the inorganic nanocatalyst, a viability test with NPt (highest cytotoxicity profile observed) was carried out in normal cells (fibroblasts) to evaluate possible cytotoxic effects in noncancerous cells. Having in consideration the poor cytotoxicity of the Pt(acac)$_2$ and the biocompatibility of sol–gel TiO$_2$ reported by Sidane et al.,$^{54}$ only the NPt NPs were tested for selectivity. The normal morphology of the cells was evaluated, while samples being confluent, adherent, and elongated (Figure 4a,c).$^{55}$ The triplicate MTT assay proved that there was no cytotoxic damage even at concentrations as high as 500 μg/mL of NPt (Figure 4b,d), maintaining both morphology and viability, $F(2,24) = 0.31$, $p = 0.73$.

**IC$_{50}$ Calculation.** Mortality curves (continuous) obtained from the MTT assays for each cell line are shown in Figure 5a,c,e. For the curve fitting, the four-parameter logistic (4PL)
A regression technique was applied, hence allowing to obtain the IC50 values for each bioassay. The fitted curves (dashed) are shown in Figure 5b,d,f. The IC50 values calculated are summarized in Table 1. For the assay in HeLa cells, NPt and pure TiO2 presented IC50 of 53.74 ± 2.95 and 74.29 ± 8.95 μg/mL (24 h), respectively. On the contrary, IC50 for Pt(acac)2 was as high as 800.70 ± 5.0% μg/mL (24 h). In the case of DU-145 cells, the IC50 obtained for NPt was 75.07 ± 5.48 μg/mL (24 h), while TiO2 exhibited an IC50 of 82.02 ± 6.03 μg/mL (24 h). By contrast, treatment with Pt(acac)2 displayed an IC50 of 1825.56 ± 5.0% μg/mL, which translates into no cytotoxic properties related to acetylacetonate. Finally, the IC50 obtained for NPt in the fibroblast cell line was of 159.62 ± 11.29 μg/mL (24 h), which represents a negligible toxicity, hence probing the biocompatibility of the compound in terms of the lack of cytotoxicity effect in normal cells.

Table 1. IC50 Calculated from the 4PL Fitted Curves for Each Compound in the Different Cell Lines Studied

| cell line | NPt      | TiO2     | Pt(acac)2 |
|-----------|----------|----------|-----------|
| HeLa      | 53.74 ± 2.95 | 74.29 ± 8.95 | 800.70 ± 5.0% |
| DU-145    | 75.07 ± 5.48 | 82.02 ± 6.03 | 1825.56 ± 5.0% |
| fibroblast| 159.62 ± 11.29 | nt^c      | nt^c      |

Numbers indicate the IC50 obtained for each compound in μg/mL after 24 h of incubation. Because of the large standard deviation obtained, the error range was established as 5.0% (instrument error). nt = not tested.

Figure 5. Cytotoxicity of the NPs (NPt, TiO2, and Pt(acac)2) in HeLa (a,b), DU-145 (c,d), and fibroblast (e,f) cell lines on 24 h incubation as determined by the MTT assay. For curve fitting, the concentrations were expressed in a logarithmic scale. The fitted curves are shown dashed.

- **DISCUSSION**

Platinum NPs are well-known for their cytotoxicity on both cancer and normal cell lines. However, the highly toxic profile of the compounds and the lack of selectivity for cancer cells lead to substantial dose-limiting acute and chronic toxicities. The typical synthesis methods (wet-chemical reduction, microemulsion, electrochemical processes, thermal decomposition, etc.) generate individual NPs with controlled particle sizes. For our inorganic nanocatalyst, we...
synthesized platinum NPs dispersed into an oxide matrix (TiO$_2$) with catalytic properties in a low concentration (1%) through the sol–gel method; the incorporation of noble metals in such mesoporous materials has been identified to enhance their catalytic features.$^{65}$ The NP structure observed in SEM and TEM micrographs of our nanocatalyst differed greatly from that of common platinum NPs, as platinum NPs were observed to be highly dispersed into the titania matrix without affecting the TiO$_2$ crystalline structure.

MTT assays for the three compounds indicate that both NPt and TiO$_2$ possess cytotoxic effects in cancerous cells, specifically in cervical and prostate cancerous lines, causing alterations at the cell level. Figure 5a–c shows the increase of mortality related to the increase in the concentration of the three compounds in the two cell lines. High mortality is observed at low concentrations for NPt and TiO$_2$, whereas mortality related to Pt(acac)$_2$ did not exceed 20% in both studies. On the other hand, NPt in fibroblast cell lines had no significant effect, showing a mortality not bigger than 5% (Figure 5e). The 4PL regressions were applied in the curves (Figure 5b,d,f) to obtain the concentration at which 50% of the cells died in the presence of the nanocatalysts.

NPt presented an IC$_{50}$ of 53.74 ± 2.95 μg/mL in HeLa cells and 75.07 ± 5.48 μg/mL in DU-145 cells both after 24 h, which correspond to a moderately cytotoxic compound. $^{66}$ TiO$_2$ showed higher IC$_{50}$ for the two cancer types: 74.29 ± 8.95 and 82.02 ± 6.03 μg/mL after 24 h, respectively. The lesser amount of NPt required against pure TiO$_2$ to achieve 50% of mortality demonstrates the higher antineoplastic efficiency of the first, in both cell lines. On the other hand, the high IC$_{50}$ observed for Pt(acac)$_2$ (800.70 and 1825.56 μg/mL, respectively) indicated that the compound was harmless as it exhibited no cytotoxicity against cancerous cells, that is, no antineoplastic properties. Despite proving to be effective to eliminate cancerous cells, the cytotoxic profiles of the compounds were considerably less effective than cisplatin, which exhibit IC$_{50}$ values of 28.77 μg/mL (24 h) for HeLa$^{67}$ and 57.81 μg/mL (24 h) for DU-145 cells. $^{68}$

The evaluation of NPt in fibroblasts yielded a high IC$_{50}$ (159.62 ± 11.29 μg/mL). The behavior observed for the nanocatalyst against cancerous and normal cells indicates that the NPs are harmless to this normal cell line, in contrast with its toxicity profile in cancerous cells. The aforementioned suggests the high selectivity of the NPt for malignant cells without affecting benign cells, in contrast with cisplatin and its high cytotoxicity against all types of cells. $^{69}$

Platinum compounds, such as cisplatin, $^{70}$ are widely known for their antineoplastic properties regarding effects in DNA.$^{40,71,72}$ NPt is believed to exhibit cytotoxicity because of the platinum atoms distributed in the titania matrix, which display similar interaction with the DNA as reported by López $et$ $al.$ (2008)$^{73}$ in a Wistar rat model. Notwithstanding, the cytotoxicity profile observed for pure TiO$_2$ leads us to believe that the main DNA interaction is also related with the catalytic properties of the matrix as well; the platinum NPs not only act as the active compound but also as enhancers of the matrix.$^{65}$ Nanostructured titania (TiO$_2$) synthesized by the sol–gel method acquires Lewis acid properties (electron-pair acceptors), as metallic ions (particularly transition metal ions which have empty valence orbitals) act as Lewis acids.$^{40,74,75}$ When in contact with the membrane of cancerous cells, both NPt and TiO$_2$ adhere to the surface through a theorized ligand–receptor interaction carried out by the functionalizing agents present in both compounds.$^{40,76}$ Such interaction has been observed in a previous study to trigger endocytosis, hence allowing the uptake of the NPs.$^{44}$ Once inside the cell, the acid sites in both compounds destabilize DNA by electron heist, breaking the carbon–carbon and carbon–nitrogen bonds present in the nitrogenous bases of the structure.$^{81,77}$ In the same way, titania NPs have been observed to locate in the peri-region off the nucleus as aggregated particles, induce cell death by apoptosis, increase ROS, decrease reduced glutathione, and carry out the induction of oxidative stress-related genes such as heme oxygense-1, thioreredox reductase, glutathione-S-transferase, catalase, and a hypoxia-inducible gene. $^{78}$ Such interactions are directly related with the catalytic properties of the titania matrix.

In contrast with titania-based NPs, pure Pt(acac)$_2$ did not present cytotoxic effects related to Pt interaction with the DNA, which translates in no alteration of the cell functions. Generally, drugs are bounded with a component that interacts strongly with antigens or receptors that appear on the target cells. $^{79}$ A lack of ligand–receptor interactions that trigger cell uptake may be the main cause of absent cytotoxic effects for Pt(acac)$_2$. $^{75,79}$

Regarding the MTT assay in fibroblasts subjected to NPt, the high IC$_{50}$ obtained indicates no toxicity in the normal cell line and, therefore, biocompatibility in that cell line. A study carried out by Shoaebeddin $et$ $al.$, showed that fibroblasts (3T3 cell line, Swiss albino mouse embryo tissue) are more resistant to nanomaterial toxicity and titania cytotoxicity because of the presence of protective enzymes such as heme-oxygenase-1 and metallothioneines,$^{80}$ which regulate oxidative stress induced by NPs and facilitate detoxification.$^{81,82}$ This could be one of the possible factors that limit the NPs absorption in normal fibroblasts. Another possible explanation is the lack of ligand–receptor interactions in the plasmatic membrane that prevents the uptake of NPs.$^{77}$

### CONCLUSIONS

Cancer treatment drugs currently used, mainly cisplatin, cause adverse effects in patients, a situation that has stimulated the need for new treatments. NPs based on inorganic compounds, such as titania, have proven to exhibit anticancer properties without affecting the normal cells.

In this work, we evaluated the cytotoxic effect of three different NPs in cervical and prostate cancer cell lines and a normal cell line of fibroblasts. Stabilization of platinum in functionalized titania (NPt) and no-metal functionalized titania (TiO$_2$) probed to be cytotoxic for cancerous cells. Platinum in the TiO$_2$ matrix enhanced the cytotoxic properties of the matrix, probing the advantages of the sol–gel process as the synthesis method for NPs. In contrast, pure Pt(acac)$_2$ was determined to have low cytotoxicity, which indicates that the anticancer effect relies on the functionalized titania matrix.

Biocompatibility with a normal cell line was evaluated in normal fibroblast cells; probing NPt exhibited no cytotoxicity against this particular cell line, in contrast with what was observed for cancerous cells, hence suggesting high selectivity and biocompatibility. Further studies in other normal cell lines must be carried out to probe such properties.

These in vitro assays probed that NPs are an interesting alternative for cancer treatment, particularly in cervical and prostate cancer, which could substitute the use of cisplatin. In vivo studies must be carried out to evaluate possible adverse effects in these cancer lines. In the same way, future
cytotoxicity studies will be implemented to evaluate biocompatibility and selectivity in other normal cell lines.

**EXPERIMENTAL SECTION**

**NPt Synthesis.** The NPs were synthesized by the sol–gel method process previously reported by López et al., 2011 method patented under WO 2019/017723 A2. The NPs were synthesized 24 h before application in cell lines. Full characterization of the compounds is offered in previous works. With 1% Pt (NPt) were synthesized to determine the cytotoxic effect in cancerous cells and evaluate the properties related to the sol–gel process synthesis. NPs with no metal (TiO2) obtained by the same method reported above were tested to probe biocompatibility. Pure platinum(II) acetylacetonate (Pt(acac)2) was also evaluated to identify possible antineoplastic properties.

**Electronic Microscopy Studies.** The grain’s size, morphology, and texture were characterized by SEM in a JEOL JSM-6010LV microscope. The NPs’ size was determined by TEM in a JEOL JEM-2100F.

**Cell Culture.** The cell lines for cervical cancer (HeLa [ATCC CCL-2]), prostate cancer (DU-145 [ATCC HTB-81]), and primary dermal fibroblasts ([ATCC PCS-201-010]) were cultured to generate a cell bank in order to place them in ELISA boxes. The cell lines were maintained with Dulbecco’s modified Eagle medium (DMEM) ([CAISSON DML 19–500 ML]) enriched with 4 mM L-glutamine and 12.5 mL of HEPES 25 Mm and a phosphate buffer (PBS1X). For the cell growth medium, we used 90 mL of DMEM added with 10% fetal bovine serum, filtrates, and 1% antibiotic (penicillin at 10,000 U/mL and streptomycin [CAISSON] at 10,000 μg/mL). Cell growth was performed at 37 °C in a 5% CO2 incubator.

**IC50 Determination.** The dissolution of NPs were prepared with DMEM growth medium (90 mL) and kept under refrigeration. The culture medium in the ELISA boxes was removed with a Pasteur pipette connected to a Büchner flask with a vacuum. The bottom of the boxes was not touched as cells had adhered to it. The wells were rinsed with 200 μL of PBS and extracted with the Pasteur pipette. The cells were exposed to the NPs [NPt, TiO2, Pt(acac)2] at concentrations of 7.81, 15.62, 31.25, 62.50, 125.00, 250.00, 500.00, and 1000.00 μg/mL with DMEM for 24 h at 37 °C in a 5% CO2 atmosphere. The NPs were solved in the growth medium because of their easy-suspension formation. The study was carried out by triplicates. The control only contained 200 μL of the growth medium.

**MTT Cell Viability Test.** The cytotoxic effect of the NPs in the cancerous and normal cell lines was evaluated through the MTT assay which is used to quantify the cell viability by colorimetry because of the amount of formazan produced by the living cells. The assay was conducted in triplicates. After the 24 h, the medium and the NPs were removed with the Pasteur pipette. 100 μL of growth medium with 10 μl of MTT dissolved in 5 mg/mL of PBS and 100 μL of DMSO was added on each well with the unwashed cells and incubated for formazan synthesis for 4 h at 37 °C in a 5% CO2 atmosphere. The supernatant was put in a new ELISA box, and a UV–vis study was carried out with a spectrophotometer adjusted to a wavelength of 595 nm (680 model, Bio-Rad brand) per each plate.

**Data Analysis.** The data obtained were processed in Excel 2013 and OriginPro 9.0. The mortality percentage was calculated comparing the formazan concentration in treated cells against the synthesis in the untreated ones (0% mortality) for each concentration of NPs. The mortality curves were plotted against the concentration in a logarithmic scale, and the IC50 was calculated through the four-parameter logistic (4PL) regression, a regression model often used to analyze bioassays as they often are linear across a specific range of concentration magnitudes. The equation that described the curves was of the form

\[
y = b + \frac{a - b}{1 + \left(\frac{x}{c}\right)^d}
\]

where \(y\) is the mortality percentage, and \(x\) is the concentration; the four parameters represent the (a) minimum value that can be obtained (0 dose), (b) the maximum value that can be obtained (infinite dose), (c) the point of inflection, and (d) the Hill’s slope of the curve, which is related to the steepness of the curve at point c. IC50 represents the concentration value (x) at which the mortality (y) achieves the point of inflection. The linear regression provided the four parameters, and IC50 was calculated by identifying the point of inflection in the curves.

To determine whether significant statistical differences were present both in the triplicate assays for each sample and between the samples, the data were analyzed by ANOVA with a 95% confidence interval (\(p < 0.05\)). Tukey’s range test was utilized as a post hoc when significant differences were identified by the ANOVA analysis.

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# ABBREVIATIONS
IC_{50}, half maximal inhibitory concentration; NPs, nanoparticles; Pt, platinum; NPs, platinum nanoparticles stabilized in functionalized titania; TiO_{2}, functionalized titania nanoparticles; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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