Radiolytic Synthesis and Characterization of Selenium Nanoparticles: Comparative Biosafety Evaluation with Selenite and Ionizing Radiation

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Research Article

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Abstract

Selenium nanoparticles (SeNPs) were synthesized by irradiating a solution containing sodium selenite (Se^{+4}) as the precursor in YPG liquid medium with gamma-rays. Spherical particles were formed after reactions of sodium selenite with hydrated electrons (e_{aq}^-) and hydrogen radicals ($\text{H}^\bullet$) produced following water radiolysis. No hazardous reducing agents were employed. The obtained nanoparticles were morphologically characterized, and their physicochemical and structural parameters were analyzed. SeNPs characterization showed all selenium in the Se^0 state. We incubated *Saccharomyces cerevisiae* cells with the SeNPs for 24 h and then challenged the cells with ionizing radiation. After radiation exposure, cells were assessed for cell viability, lipid peroxidation, protein carbonylation, free radical generation, and total sulfhydryl content. The synthesized SeNPs were considered safe and less toxic at the concentration employed than the same selenite concentration. Except for the protein carbonylation results, there were no other significant modifications in viability or the oxidative stress parameters in SeNP-treated cells. It was concluded that 1 mM of the synthesized SeNPs does not trigger oxidative stress. Furthermore, we verified that SeNPs attenuate the reactive oxygen species generation after *in vitro* ionizing radiation exposure. These observations open up tremendous possibilities for radiosensitizer development.

Highlights

- Synthesis of selenium nanoparticles with gamma irradiation and sodium selenite
- Generation of spherical, amorphous, and zero-valent selenium nanoparticles
- *In vitro* nanoparticle treatment reduces reactive oxygen species after irradiation
- Selenium nanoparticles are safe, nontoxic, not provoking oxidative stress *in vitro*
- Selenium nanoparticles as possible platform for radiosensitizers development

Introduction

The use of nanoparticles and nanomaterials has increased vertiginously due to their applicability in different domains, including agriculture, business, food, clothing, cosmetics, and medicine. Concerning medical uses, nanoparticles are employed as therapeutic or diagnostic agents for various pathologies. This field is also known as "nanomedicine" and has been associated with significant benefits (Foulkes et al., 2020).

In terms of safety, efficacy, physicochemical properties, drug distribution and kinetic properties, nanomedicines present certain advantages over conventional therapies (Choi and Han, 2018). Different selenium-containing compounds are among the arsenal of conventional medicine drugs with clinical uses. For example, P-phenylenebis (methylene) selenocyanate and triphenylselenonium chloride have chemopreventive properties, Ebselen is an antibacterial and anti-inflammatory agent, (Nogueira et al., 2004) and selenazofurin and 7-methyl-8-selenoguanosine exhibit antiviral activity (De Clercq, 2016). In

addition to these characteristics, selenium compounds have been reported to elicit antioxidant, antimutagenic, anticarcinogenic, antifungal, antiparasitic, and neuroprotective effects (Bodnar et al., 2012).

Despite the plethora of beneficial properties, selenium can also produce harmful effects (Rayman, 2020). These effects are usually related to the selenium-containing compound's chemical formula and concentration. Moreover, organic compounds are typically less toxic than inorganic ones, (Barceloux, 1999; Rohn et al., 2018) and elemental selenium (Se^0) is the least toxic (Barceloux, 1999).

Due to the advantages of nanomedicines, the low toxicity and beneficial properties of nanostructured selenium, as reviewed by (Khurana et al., 2019) we sought to synthesize selenium nanoparticles. We also assessed whether these SeNPs induce oxidative stress since many nanoparticles such as metallics, metal oxide, carbon nanostructures, fullerenes and others lead to free radical generation, a significant limitation of this therapeutic approach (Fu et al., 2014).

It is well known that oxidative stress is caused by an imbalance between the generation and elimination of free radicals and reactive oxygen species (ROS). The failure to reestablish this equilibrium can damage biological macromolecules (i.e., nucleic acids, proteins and lipids) (Katerji et al., 2019). Herein, we performed toxicity tests and characterized the synthesized nanoparticles. We evaluated oxidative stress biomarkers *in vitro* by monitoring lipid peroxidation, protein carbonylation, free radical generation, total sulfhydryl (SH) content, and cell viability. All experiments were conducted using *Saccharomyces cerevisiae* cells. Besides toxicity, the potential of SeNPs to display sensitizer or protector properties when challenged with ionizing radiation was also verified. The selected yeast cells as an experimental model because nowadays, there is a pursuit for toxicological models that diminish the use of animals, which are associated with increased ethical constraints and high economic costs.

Various methodologies have been employed for synthesizing nanoparticles. Herein, we chose to utilize ionizing radiation in the form of gamma-rays from a ^{60}Co source. In this situation, the radiolytic reactions in aqueous solutions dominate. When a radiation beam crosses a system containing sodium selenite dissolved in water, it can promote the radiolysis of the water molecules, producing large amounts of reducing agents, including hydrated electrons (e_{aq}^-) and hydrogen radicals ($\text{H}^\bullet$) (Le Caër, 2011) and oxidizing species like hydroxyl radicals ($\text{OH}^\bullet$). It has been reported that e_{aq}^- and $\text{H}^\bullet$ display a strong reducing capacity with standard reduction potentials (E^0) of -2.88 V and -2.31 V, respectively. These E^0 values are sufficient for reducing Se^{+4} to Se^0 (El-Batal et al., 2020) On the other hand, the E^0 of $\text{OH}^\bullet$ is 1.9 V, producing more elevated selenium oxidation states (Armstrong et al., 2015).

Using γ -ray irradiation for nanoparticle synthesis has been gaining attention and offers some advantages over other synthetic routes. For example, it utilizes low energy, requires minimal or no oxidant or other noxious chemical addition, and has a relatively uncomplicated synthesis design (Flores-Rojas et al., 2020). In the protocol employed in this study, the SeNP synthesis was carried out without the addition of stabilizing and/or scavenging substances, consistent with "Green" approaches. After synthesis, we

characterized the obtained nanoparticles in terms of morphological, physicochemical and structural properties using UV–visible (UV–Vis) spectroscopy, X-ray diffraction (XRD), dynamic light scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and X-ray Photoelectron Spectroscopy (XPS).

Materials And Methods

Synthesis of SeNPs by gamma radiation

Pentahydrate 99% sodium selenite (Sigma Aldrich, St. Louis, MO) was dissolved in distilled water and autoclaved. This solution was used as the precursor for the SeNPs synthesis. Selenium concentration was calculated based on 2.19 g/L of Na_2SeO_3 containing 1 g/L of selenium. Typically, 1 mM of selenium was added to 100 mL of YPG medium (1% yeast extract, 2% peptone 2% and 2% glucose) and divided equally into two 50 mL polypropylene centrifuge tubes for irradiation.

The irradiation procedure was carried out in the Gamma Irradiation Laboratory at the Nuclear Technology Development Center, using a ^{60}Co source. At the time of the experiments, samples were placed 50 cm from the source and delivered 25 kGy doses at 532.96 Gy/h.

Following irradiation, the solution was centrifuged (30 min $\times$ 2000 $\times$ *g*), and the supernatant was discarded. The resultant pellet was washed three times with distilled water (30 min $\times$ 2000 $\times$ *g*), collected, dried at room temperature for three days, and weighed using an analytical balance.

Characterization Of The Senps

The Zeta potential, hydrodynamic size and polydispersity index of the isolated SeNPs were determined by Dynamic Light Scattering (DLS) on a Malvern 3000 Zetasizer NanoZS instrument. Data from at least three independent experiments conducted in triplicate were used to calculate the average values. We evaluated the absorption properties by placing the samples in a Perkin Elmer 2300 Multilabel Reader and recording absorbance from 230–800 nm. The data was collected using the Enspire software. The morphology and size of the synthesized nanostructures were characterized using Scanning Electron Microscope (SEM) (SIGMA VP, Carl Zeiss Microscopy, equipped with an Energy-Dispersive X-ray Spectroscopy (EDS) – XFlash 410-M (Bruker Nano GmbH controlled by ESPRIT software and Transmission Electron Microscope (TEM) (Tecnai G2-20 – SuperTwin FEI, at 200 kV. The TEM samples were diluted with autoclaved water, then dispersed by ultrasound (USC – 1800 A, 40 kHz frequency, 132 W) for 30 minutes, and subsequently deposited onto copper grids covered with *Holey Carbon*.

For crystallinity analysis of samples, X-ray diffraction (XRD) was carried out with a Rigaku DMAX diffractometer, and the scanning was done in the 2θ . The oxidation state of selenium was verified by X-ray Photoelectron Spectroscopy (XPS) in a SPECS PHOIBOS 150 MCD model, equipped with a monochromatic Al K α X-ray of 1486.6 eV. C 1s peak, at 284.6 eV, from adventitious carbon, was used as

energy reference, and the high-resolution spectra were acquired with a step of 0.1 eV using pass energy of 20 eV. Following the assessment of SeNPs characterization, this work aimed to verify the nanoparticles' influence on the cells.

Cell Culture And Treatment

Yeast strain and culture conditions

Wild-type *Saccharomyces cerevisiae* strain BY4741 (*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) was obtained from the European *Saccharomyces cerevisiae* Archive for Functional Analysis (EUROSCARF), Frankfurt University, Germany. The cells were pre-incubated in YPG medium (1% yeast extract, 2% peptone and 2% glucose). When a solid medium was needed, 2% agar was added to the medium. Cells (10^8 cells/mL) were then transferred to a broth, herein referred to as Yeast Nitrogen Base (YNB) media, containing 0.67% YNB; 2% glucose; 0.52% sodium citrate; 0.70% citric acid; histidine, uracil and methionine (20 mg/L each) and leucine (60 mg/L).

The following groups were tested, all of which contained cells in YNB medium: Control (absence of treatment); SeNPs (supplementation with 1mM SeNPs); Selenite (supplementation with 1mM sodium selenite); 400 Gy (exposition to gamma radiation, with no other treatment); SeNPs + 400 Gy (supplementation with 1 mM SeNPs and exposition to gamma radiation), and Selenite + 400 Gy (supplementation with 1 mM sodium selenite and exposition to gamma radiation). The cell number used for all groups was 10^8 cells/mL. After 24 h under agitation on the orbital shaker (150 rpm), cells were collected by centrifugation ($2000 \times g$ for 5 min). The pellets obtained were washed with cold autoclaved water ($2000 \times g$ for 5 min) and then either exposed to or not exposed to 400 Gy of gamma radiation, as described in each figure. Cell viability was determined after 24 h of incubation when cell samples were collected, diluted in PBS to a final concentration of 10^4 cells/mL, and 100 μ L from each were spread onto plates containing solid YPG medium. After 48 h of incubation at 30°C, the plates were photographed, and the Colony Forming Units (CFUs) were counted.

Extract Preparation

After the 24-hour SeNP treatment, the cells were exposed to 400 Gy of gamma radiation and resuspended in a lysis solution (50 mM Sodium Phosphate buffer, pH 7.0; 1 mM EDTA; 1 mM PMSF) containing glass beads. The cell suspension was vortexed using eight intense 1-minute cycles with a 1 min rest on ice between each cycle (Costa-Moreira et al., 2016). The crude extract was centrifuged ($2000 \times g$ for 5 min), and the supernatant was collected and used for posterior analyses without further purification. Samples that were not submitted to gamma irradiation (400 Gy) were maintained at room temperature. The oxidative stress parameters of both the irradiated and non-irradiated samples were determined at the

same time. The total protein concentration of the supernatant samples was quantified using the Bradford assay, employing bovine serum albumin (BSA) for generating the standard curve.

Oxidative Parameters

Lipid peroxidation was evaluated using the Thiobarbituric Acid Reactive Species (TBARS) method, and malondialdehyde (MDA) was used to construct the standard curve. The final results are presented as pmol MDA/mg protein.

The total content of SH residues was determined by measuring Ellmann's reagent (DTNB) absorbance. Absorption was converted to concentration using the extinction coefficient $13,600 \text{ M}^{-1}\text{cm}^{-1}$. The results are expressed as $\mu\text{moles/mg protein/mL}$, as described previously (Costa-Moreira et al., 2016).

Protein carbonylation analysis was carried out using dinitrophenylhydrazine (DNPH), as described in (Lushchak and Gospodaryov, 2005). The molar extinction coefficient of $22,000 \text{ M}^{-1}\text{cm}^{-1}$ was used to calculate the concentration, and the results are presented as nanomoles/mg protein/mL (Lushchak and Gospodaryov, 2005). The spectrophotometric measurements described above were performed in 2300 EnSpire Multilabel Plate Reader (PerkinElmer) operating in spectrophotometric mode at the indicated wavelength as referred to in the cited methodologies.

Quantification of reactive oxygen species (ROS) was performed by incubating the samples with the fluorescent probe 2,7-dichlorofluorescein diacetate (0.05 mM DCFH-DA in PBS) in the dark, for 120 min at 30°C (Rhee et al., 2010). Fluorescence was recorded with a 2300 EnSpire Multilabel Plate Reader (PerkinElmer) operating in fluorometer mode using the following acquisition parameters, $\lambda_{\text{excitation}} = 485 \text{ nm}$ and $\lambda_{\text{emission}} = 500 \text{ nm}$. Each sample contained 2×10^8 cells/mL.

Statistical analysis

Results are expressed as the average of at least three independent experiments and presented as the mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was used to detect significant differences. The level of statistical significance between groups was set at $p < 0.05$.

Results

Physicochemical characterization

The irradiation of a sodium selenite solution in YPG medium was carried out in a panoramic irradiator using ^{60}Co as the gamma-ray source. As shown in the inset of Fig. 1a, following the gamma irradiation procedure, the characteristically brownish YPG medium changed to a reddish color, which is indicative of nanoscale material production. We then recorded UV-Visible absorption spectroscopy to evaluate the optical properties of the synthesized material. These analyses detected a strong absorbance

peak centered around 350 nm (Fig. 1a), further confirming the presence of nanoparticles. The elemental composition analysis, carried out by EDS, revealed that the SeNPs were mainly composed of selenium (Fig. 1c). It is worth mentioning that the X-ray diffractogram of the synthesized SeNPs did not contain the typical peaks of crystalline structure, suggesting a disordered, amorphous structure (Fig. 1b).

Next, we used Dynamic Light Scattering (DLS) to determine the average size, Zeta potential and polydispersity index (PDI) of the SeNPs suspended in YNB medium (Table 1). The average size of the nanoparticles, considering the water layer, was estimated to be 150 ± 10 nm, the magnitude of Zeta potential was -16.2 ± 1.7 mV, and the PDI was 0.0132 ± 0.014 . These results indicate relative dispersity and some particle aggregation and are consistent with the previous interpretation of Fig. 1b.

Table 1 Zetasizer parameters	
Hydrodynamic size	154.9 ± 10.8 nm
PDI	0.132 ± 0.014
Zeta Potential	-16.2 ± 1.7 mV

Morpho-structural characterization

The morphological characterization of the SeNPs was performed using SEM (Fig. 2a, c) and TEM (Fig. 2b). The SEM image in Fig. 2a depicts nanoparticles with a predominantly round shape, with large clusters detected in several fields. The synthesized SeNPs were also imaged with SEM at a different magnification (Fig. 2c). The insert in Fig. 2c is in the same field as Fig. 2c but shows the presence of selenium (red), as determined by coupled EDS.

Next, we used TEM to verify that the radiolytically synthesized SeNPs are electron-dense. A representative TEM image of the selected area electron diffraction (SAED) (Fig. 2b, inset) of a randomly selected area also revealed an amorphous structure as in Fig. 1b. The image also shows SeNPs of various sizes close to one another. A closer examination of the size distribution found that the SeNPs range from 100 to 220 nm with an average size of 140 nm (Fig. 2d). The results presented in Fig. 2 demonstrate that the nanoparticles are primarily composed of selenium and support the previous size and aggregation state described in Fig. 1c and Table 1.

Selenium Oxidation State

To investigate the oxidation states of Se in the sodium selenite and SeNP samples, we acquired XPS high-resolution spectra of the Se 3d region. The Se 3d peaks were fit using a combination of Gaussian-Lorentzian functions of two components, Se 3d_{3/2} and Se 3d_{5/2}, and a Shirley background. A representative XPS spectrum of the selenite sample is shown in Fig. 3A. Two peaks exhibit binding

energies of 58.5 and 60.4 eV, typical 3d signals for Se^{+4} (Bai et al., 2020). Another peak at 64.1 eV is associated with sodium (Na) 2s, confirming the presence of sodium selenite.

In contrast, the 3d spectrum of the SeNPs is displayed in Fig. 3b and is typical of a metal sample. It should be pointed out that the peaks are narrower than in the sodium selenite sample. The binding energies associated with each peak correspond to 55.0 and 56.5 eV, typical 3d signals for Se^0 (Bai et al., 2020; Jain et al., 2015). Therefore, it can be inferred that the synthetically produced SeNPs are composed of metallic selenium.

In vitro viability and ROS generation

The *in vitro* analysis of nanoparticle toxicity is essential for further biological applications. In this sense, we evaluated the influence of the synthesized SeNPs on *S. cerevisiae* cell colony formation after 24 h of treatment with SeNPs, selenite, or control solutions. After 24 h, cells were submitted to 400 Gy radiation (or not). After that, aliquots of cells were transferred and spread onto solid YPG medium containing plates (without selenium) and incubated for 48 h at 30°C. Following this incubation period, the number of CFUs was recorded (Fig. 4a). The non-irradiated groups had more CFUs than the irradiated ones. We did not detect a significant difference in the number of CFUs between the groups treated with 1 mM SeNPs and a 400 Gy dose of irradiation. In contrast, the sodium selenite treatment attenuated the number CFUs (Fig. 4a). Moreover, treating the *S. cerevisiae* cells with both sodium selenite and ionizing radiation (400 Gy) inflicted substantial cellular damage, further reducing the number of colonies.

The reduced number of CFUs in the irradiated groups was not entirely unexpected since 400 Gy of radiation affects the yeast's ability to divide and form new colonies. Cells treated with SeNPs and then irradiated (group SeNPs + 400 Gy) had 49% more CFUs than the 400 Gy group (655 CFUs in the SeNPs + 400 Gy group versus 439 CFUs in the 400 Gy group). In this respect, the SeNPs protected the *S. cerevisiae* from the gamma-ray irradiation-induced damage.

As shown in Fig. 4b, in the cells exposed to ionizing radiation, ROS generation was not different from the control group. The SeNPs treated cells exhibited a significantly lower ROS production than the Control and 400 Gy groups. In the group treated with SeNPs followed by irradiation (SeNPs + 400 Gy), the ROS levels were higher than in the cells treated with SeNPs alone but significantly reduced compared to the control and 400 Gy groups. In contrast, the yeast cells treated with selenite or selenite followed by irradiation produced significantly higher ROS levels.

Oxidative Parameter Markers

The irradiated group (400 Gy) displayed significantly higher lipid peroxidation levels than the control group, demonstrating that the 400 Gy dose caused oxidation of the yeast cell membranes. On the other hand, no significant changes in lipid peroxidation were detected in cells treated with 1 mM of the SeNPs

after the 24 h treatment (Fig. 5a) when compared to the control group. Similarly, *S. cerevisiae* cells maintained the same lipid peroxidation concentration when challenged with ionizing radiation after SeNPs treatment. In contrast, cells treated with sodium selenite or sodium selenite followed by irradiation produced high concentrations of lipid peroxidation that were significantly greater than all the other treatments (Control, 400 Gy, SeNPs and SeNPs + 400 Gy), thus demonstrating the toxic potential of these treatments (Fig. 5a).

Concerning protein carbonylation, exposing cells 400 Gy of radiation did not increase the presence of this irreversible post-translational modification (Fig. 5b). Interestingly, we observed a significant increase in this modification in cells treated with SeNPs for 24 h when compared to the control and 400 Gy groups. However, when the SeNPs were associated with gamma irradiation, protein carbonylation was significantly reduced compared to the SeNP group and not significantly different from the control and 400 Gy groups. It is unlikely that the increased protein carbonylation concentrations are not lethal since cell viability was unaffected by this treatment (Fig. 4a). Sodium selenite promoted significantly higher protein carbonylation concentrations compared to the other groups. Moreover, treating the cells with sodium selenite followed by gamma irradiation produced a further increase in protein carbonylation. It is plausible that the increased protein carbonylation associated with sodium selenite induced oxidative stress and could account for the observed reduction in CFUs capability.

Finally, we evaluated free sulfhydryl content to determine if there was a change in the amount of reduced glutathione after treatment with ionizing radiation, SeNPs, SeNPs + ionizing radiation, sodium selenite, or sodium selenite + ionizing radiation (Fig. 5c). When compared to control cells, gamma irradiation did not affect total sulfhydryl content. However, cells treated with SeNPs displayed significantly lower free SH residue content than the control and 400 Gy groups. This observation indicates that 1 mM of SeNPs has an oxidizing effect that significantly attenuates the GSH (reduced Glutathione) concentrations compared to the control and 400 Gy groups. Interestingly, the SeNPs + 400 Gy group had significantly higher free SH concentrations than cells treated with 1mM of SeNPs. However, the concentrations were significantly less than when exposing cells to 400 Gy of gamma irradiation alone. The sodium selenite and sodium selenite + 400 Gy groups contained low amounts of free SH residues compared to the other groups. Similar to protein carbonylation (Fig. 5b), the combination of sodium selenite and ionizing radiation (Selenite + 400 Gy group) further aggravated the reduced free SH concentrations (i.e., GSH levels).

Discussion

In the present study, we exposed a solution of 1mM of selenium in YPG broth to 25kGy of ^{60}Co Cobalt irradiation, a characteristic low LET radioactive source of ionizing radiation. It has been reported that the radiation dose is an important factor and may need to be adjusted depending on the precursor employed (Flores-Rojas et al., 2020). Indeed, the reduction of Se_2O_3 to Se^0 requires four electrons and a low radiation dose is not sufficient for this purpose. In previous experiments not shown here, decreasing the radiation dose from 25 kGy to 1 kGy generated larger-sized nanoparticles, an observation consistent with the scientific literature (Saion et al., 2013). Moreover, previous experiments from our group (data not

shown), and discussed by (Naghavi et al., 2010), showed that 2 and 5 mM sodium selenite produced NPs with a larger size than obtained with 1 mM of this compound. Considering the variables, we chose to perform all of the experiments using a 25 kGy dose of irradiation, 1 mM sodium selenite and placed the samples at the same distance (50 cm) from the ^{60}Co source.

The radiation beam applied to the samples randomly provoked water molecule radiolysis, consequently generating a great deal of oxidizing and reducing species. It is the oxidizing species, like e_{aq}^- and $H^\bullet$, that reduce selenite (Se^{+4}) to elemental selenium (Se^0) (Flores-Rojas et al., 2020). Notably, reducing species, such as $\text{OH}^\bullet$, can displace the reaction to a more positive oxidation state, even though the liquid medium contains glucose, peptone and yeast extract (Liu et al., 2014; Morelli et al., 2003), molecules that can perform different functions. For example, it has been shown that glucose scavenges $\text{OH}^\bullet$ (Morelli et al., 2003) and peptone and yeast extract are used as reducing and stabilizing agents, respectively (Liu et al., 2014; Zhu and Qian, 1996). It is worth mentioning that ^{60}Co gamma-ray irradiation produces highly pure nanoparticles. Moreover, this procedure only requires the substances in the YPG broth and water radiolysis products and does not require any hazardous substances. Consequently, no exogenous oxidants are incorporated into the synthesized nanoparticles. Thus, this methodology is based on the precepts of "green chemistry" (Anastas and Eghbali, 2010).

The reddish color, visually verified with the naked eye, results from the excitation of the surface plasmon resonance band that exhibits a sharp peak with maximum absorbance at 350 nm (Fig. 1a). The maximum absorbance wavelength is related to the particle size and shape, a distinctive feature of spherical nanoparticles (Begum et al., 2018). Herein, we observed a single peak at 350 nm, an observation consistent with previously reported results using different synthesis methodologies (Faramarzi et al., 2020; Wadhwani et al., 2017). Additionally, the color change from brown to red is a well-recognized feature of SeNPs that are about 100 nm in diameter (Lin and Wang, 2005). Subsequent particle size evaluations were used to confirm this relationship.

DLS analyses were used to assess the hydrodynamic size of the SeNPs dispersed in the YNB medium. This approach is considered more advantageous in toxicology studies since the YNB medium contains salts and organic molecules, resembling the intracellular environment and making the Zeta parameter determinations more realistic (Gollwitzer et al., 2016). These analyses revealed that our SeNPs were 150 ± 10 nm in diameter considering the water layer (Table 1) and are in accordance with the maximum absorbance peak at 350 nm (Fig. 1a). Concerning the Zeta potential, it was found to be -16.2 ± 1.7 mV. In general, negatively charged nanoparticles are less toxic than positively charged ones. This observation is because the lipid bilayer membranes of biological cells possess a net negative charge (Goodman et al., 2004). Lastly, the PDI was 0.0132 ± 0.014 , indicating relative dispersity and some particle aggregation.

As shown in Fig. 1c, the synthesized SeNPs were mainly composed of selenium, exhibiting characteristic peaks at 1.37 keV ($\text{SeL}\alpha$), 11.22 keV ($\text{SeK}\alpha$) and 12.49 keV ($\text{SeK}\beta$) (Zheng et al., 2014). We also detected the presence of some other element as carbon (C), chlorine (Cl), potassium (K), sulfur (S) and silicon (Si). These elements may have originated from YPG medium components, the tape used for depositing the

sample, or the microscope itself. It is worth pointing out that the peaks related to elemental oxygen were almost undetectable. It is plausible that this observation is due to the consumption of selenium oxides. Additionally, there were no prominent peaks in the SeNP diffractograms (Fig. 1b), a characteristic of crystalline structures with a clear geometric organization of the atoms. In other words, the absence of peaks (Fig. 1b) indicates an amorphous SeNPs structure composed of a disordered mixture of chains.

According to SEM images (Figs. 2a, c), the synthesized SeNPs ranged from 100 to 220 nm in diameter, with an average of 140 nm (Fig. 2d). Another study reported an average size of 70 nm for SeNPs synthesized using the same methodology (Zhu et al., 1996). This discrepancy could be because the authors used SeO_2 as the precursor, alcohol as the $\text{OH}^\bullet$ scavenger, and employed acidic and alkaline conditions (Zhu and Qian, 1996). In our study, we used 2% glucose as the $\text{OH}^\bullet$ scavenger (Morelli et al., 2003) as a potentially "green" reducing agent (Chen et al., 2016). It has also been shown that peptone, another YPG medium component, is a strong reducing agent with stabilizing capacity (Akçay and Avci, 2020; Kim et al., 2019).

The TEM image in Fig. 2b revealed agglomerated nanoparticles, further confirming the SEM results (Figs. 2a, c). It is worth mentioning that other studies have observed similar results (El-Batal et al., 2020; El-Sayed et al., 2020). The SAED technique (Fig. 2c, inset) showed that the SeNPs samples did not have illuminated spots or spots around the region, demonstrating the lack of a diffraction condition, which is characteristic of crystal formation. These results provided further evidence indicating that the synthesized SeNPs have an amorphous structure.

As shown in Fig. 3, we analyzed the sodium selenite and SeNPs samples by XPS to determine the oxidation state of the selenium present in these solutions. As previously discussed, the sodium selenite samples spectra present peaks with binding energies corresponding to Se^{+4} , whereas the SeNPs spectra revealed a typical 3d signal, with peaks at 55.0 and 56.5 eV, assigned to Se^0 , as previously reported (Bai et al., 2020; Jain et al., 2015). Based on these results, it was concluded that the synthesized SeNPs are primarily composed of Se^0 .

After the characterization analyses (Figs. 1, 2 and 3), we treated *Saccharomyces cerevisiae* cells with the synthesized SeNPs to evaluate their effects alone and with gamma radiation on viability and oxidative parameters. *Saccharomyces cerevisiae* cells were chosen because it is a eukaryotic experimental model that requires simple cultivation with fast reproduction. Due to a high gene homology, these yeast exhibit cellular responses similar to mammals. This experimental model is also devoid of selenoproteins, making it an ideal model for testing selenium compound toxicity (Herrero and Wellinger, 2015). We also decided to analyze the influence of SeNPs on oxidative stress since it has been shown that nanoparticles commonly induce this potentially harmful state in biological organisms (Manke et al., 2013). The selected *in vitro* oxidative stress parameters were based on the parameters previously proposed (Li et al., 2015).

In the present study, we first treated *S. cerevisiae* cells with the SeNPs for 24 h and then exposed the samples to ionizing radiation. The results were compared with sodium selenite, a stronger oxidative stress inductor (Porto et al., 2016; Salin et al., 2008). Concerning the ability to form new colonies (Fig. 4a), the SeNPs treatment was not toxic at 1 mM. Similar results using mammalian cells have also been reported. For example, Zhang et al. (2018) (Zhang et al., 2018) synthesized SeNPs using beta-lactoglobulin and ascorbic acid, treated normal (CCD-112) and cancerous (HCT-116) human colon cell lines with them, and found that the SeNPs were less toxic than selenite. In another study, Chen and colleagues (2018) treated cancer cells (human breast adenocarcinoma, MCF-7) with 1.1 mM of SeNPs and observed a minimal impact on cell viability (Chen et al., 2018). Furthermore, Gao and collaborators (2014) prepared SeNPs using BSA and glutathione and treated cancerous (HCT-8, human ileocecal adenocarcinoma cells) and normal (IEC6 rat intestinal epithelial cells) cells. The authors only observed a lower survival rate in cells treated with greater than 50 μ M of the SeNPs for 48 h (Gao et al., 2014). Nanoparticle toxicity has also been assessed *in vivo* (He et al., 2014). In that study, the authors treated mice with non-toxic (0.2 mg Se/kg) and higher concentrations (2 mg Se/kg) of SeNPs synthesized, using chitosan, ascorbic acid, and tripolyphosphate. It was concluded that neither SeNPs concentration was toxic to the animals (He et al., 2014).

The conflicting results involving the treatment of cells with SeNPs and selenite presented in this work are consistent with the literature and our previously published study (Porto et al., 2016). For example, we confirmed that sodium selenite is more toxic *S. cerevisiae* than SeNPs when applied at the same concentration (i.e., 1 mM). Treating the cells with SeNPs followed by ionizing irradiation (400 Gy) demonstrated that the SeNPs protect against the radiation (Fig. 4a). We chose to deliver a 400 Gy dose because it caused a reduction in the number of CFUs, but a significant CFUs number remained (compare the Control and 400 Gy groups in Fig. 4a). This detail is very important because completely inhibiting new colony formation was not the goal of this study. Notably, the radiation doses and nanoparticle concentrations used for treating mammalian and *S. cerevisiae* cells are different. For example, 400 Gy for yeast cells versus 4 Gy for mammalian ones, on average, has been reported. Despite both being eukaryotes with many ortholog genes, *S. cerevisiae* cells are more resistant to gamma-ray irradiation. It is well known that ionizing radiation directly reacts with cellular macromolecules, including nucleic acids, lipids, proteins and carbohydrates), or indirectly, through the radiolysis of water molecules and subsequent free radical generation. In both cases, cellular damage and cellular death can ensue (Reisz et al., 2014).

We first compared the biological activities of the SeNPs and selenite in terms of the cell proliferation capacity by quantifying CFUs. Additionally, we also challenged cells with ionizing radiation, a known inducer of oxidative stress, and monitored oxidative stress biomarkers, including lipid peroxidation, protein carbonylation, total SH content and ROS generation. As shown in Fig. 4b, we observed that the SeNPs treatment prevented ROS generation (SeNPs *versus* Control), even after gamma-ray irradiation (SeNPs *versus* SeNP 400 Gy). However, in the presence of SeNPs, ROS levels were still significantly lower than in the Control and 400 Gy groups. These results show that the nanoparticle treatment alleviates the effect of radiation. Therefore, SeNPs-mediated attenuation of ROS production was confirmed in this

study, an observation reported in another cell type (Rao et al., 2019). Furthermore, the results provide compelling evidence in favor of the radioprotective action of the synthesized SeNPs, as reviewed in a recent publication (Farhood et al., 2019).

To assess whether SeNPs can trigger oxidative stress, we monitored lipid peroxidation, protein carbonylation and total SH content towards the goal of estimating lipid oxidation, protein oxidation and total glutathione levels, respectively. The lipid peroxidation results are consistent with those obtained for cell viability following SeNPs treatment (Fig. 5a). In this case, we did not detect any alterations; thus, it is unlikely that the SeNPs disrupt the plasma membrane via lipid oxidation (Libardo et al., 2017). In fact, the SeNPs appear to protect cells, preventing radiation-induced lipid damage. Interestingly, male mice supplemented with SeNPs, or sodium selenite presented significantly lower MDA concentrations than the untreated control groups after exposure to 2 or 8 Gy of gamma radiation, (Karami et al., 2018) and these concentrations were even lower when SeNPs were administered. The authors concluded that SeNPs were more effective at increasing selenium levels in the tissues of mice and protecting against gamma-ray-induced nephropathy in these animals than sodium selenite. Indeed, selenium's protective effect during chemotherapy has been reported (Bhattacharjee et al., 2017). In that study, an Ehrlich tumor was implanted into mice and treated with cyclophosphamide. The animals treated with SeNPs had attenuated hepatic lipid peroxidation, highlighting the chemoprotective function of SeNPs. On the other hand, selenite produces the opposite response in the presence of ionizing radiation (Fig. 5b), an observation consistent with previously published studies (Porto et al., 2016; Zhang et al., 2001). It has also been reported that sodium selenite is seven times more toxic than nanoparticles in mice (Zhang et al., 2001).

Concerning protein carbonylation levels, the SeNPs appeared to protect the yeast proteins from this irreversible post-translational modification. Indeed, the protein carbonylation levels were similar when comparing the SeNPs + 400 Gy to those of the Control group (Fig. 5b). This result is in accordance with a previous study demonstrating the role of SeNPs in preventing protein glycation and carbonylation in three strains of mammalian cells (Yu et al., 2015). Moreover, El-Batal et al. described SeNPs-mediated protection against ionizing radiation in rats (El-Batal et al., 2012). In that study, the rats were administered SeNPs or lovastatin (Lov-Se) for fifteen days. At three-day intervals, the rats also received doses of gamma radiation, totaling 8 Gy. The results indicated that Lov-Se significantly improved protein carbonylation in irradiated rats. In this sense, the observed decrease in protein carbonylation in the SeNPs + 400 Gy group in the present study further demonstrates the protective role of these nanoparticles.

We measured the total free SH content to quantify GSH (Costa-Moreira et al., 2016; Porto et al., 2016) since a decrease in the free SH residue concentration corresponds to a diminution in GSH concentration, and consequently, attenuated antioxidant capacity. The oxidation of GSH maintains the redox balance when there are oxidants in the medium. In this sense, when GSH levels fall, there is a shift towards more oxidizing conditions, leading to oxidative stress. Therefore, SeNPs contribute to this balance by increasing the concentrations of GSH in cells exposed to ionizing radiation (Fig. 5c). This SeNP-mediated antioxidant effect was also observed in the study of Amin et al. when they showed augmented GSH

concentrations in rats that were administered acetoaminophen but did not receive an intraperitoneal injection of SeNPs (Amin et al., 2017). A similar conclusion was also reported in another study analyzing blood and liver samples from rats treated with SeNPs (Urbankova et al., 2018).

Concerning selenite's action, we were able to confirm this compound's toxic effects (Fig. 5c). These results are consistent with a previous study (Porto et al., 2016). In general, similar to other selenium-containing compounds, SeNPs display radioprotective actions – reviewed in (Farhood et al., 2019) – with low toxicities. Currently, Amifostine is the only radioprotector compound used in humans patients (King et al., 2020), despite its side effects and toxicity, the potential benefits reveal various avenues for future research.

Declarations

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Figures

Figure 1

Physicochemical characterization of the synthesized SeNP (a) UV-Vis spectrum. A photograph of the solution's irradiation mediated visual color change, from brownish (before) to reddish (after), is displayed in the inset, (b) Structure as determined by X-ray diffraction (XRD), (c) Elemental composition as determined by EDS

Figure 2

Morpho-structural characterization of the synthesized SeNP (a) SEM image. (b) TEM image (inset displays a SAED image). (c) SEM image (inset is the same image with EDS-detected selenium highlighted in red). (d) Size distribution as determined by SEM

Figure 3

XPS determination of selenium oxidation state (a) sodium selenite and (b) SeNP

Figure 4

Yeast colony forming unit capability and ROS generation (a) Cell viability, as evidenced by the number of Colony Forming Units (CFUs). (b) ROS production. In panels (a) and (b), p-values of < 0.05 are considered significant, where a is compared to the Control group, b is compared to 400 Gy group, c is compared to SeNP group; d is compared to SeNP + 400 Gy group, and e is compared to selenite group

Figure 5

Oxidative stress parameters (a) Lipid peroxidation, (b), protein carbonylation, and (c) total residues (SH). Comparisons with p-values of <0.05 were considered significant, where a is compared to the control group, b is compared to the 400 Gy group, c is compared to the SeNP group; d is compared to the SeNP + 400 Gy group, and e is compared to the selenite group

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