Radiolytic synthesis and characterization of selenium nanoparticles: comparative biosafety evaluation with selenite and ionizing radiation

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Abstract
The goal of this work is to use a green chemistry route to synthesize selenium nanoparticles (SeNPs) that do not trigger oxidative stress, typical of metallic, oxide metallic and carbonaceous nanostructures, and supply the same beneficial effects as selenium nanostructures. SeNPs were synthesized using a radiolytic method involving irradiating a solution containing sodium selenite (Se4+) as the precursor in 1% Yeast extract, 2% Peptone, 2% Glucose (YPG) liquid medium with gamma-rays (60Co). The method did not employ any hazardous reducing agents. Saccharomyces cerevisiae cells were incubated with 1 mM SeNPs for 24 h and/or then challenged with 400 Gy of ionizing radiation were assessed for viability and biomarkers of oxidative stress: lipid peroxidation, protein carbonylation, free radical generation, and total sulfhydryl content. Spherical SeNPs with variable diameters (from 100 to 200 nm) were formed after reactions of sodium selenite with hydrated electrons (eaq−) and hydrogen radicals (H·). Subsequent structural characterizations indicated an amorphous structure composed of elemental selenium (Se0). Compared to 1 mM selenite, SeNPs were considered safe and less toxic to Saccharomyces cerevisiae cells as did not elicit significant modifications in cell viability or oxidative stress parameters except for increased protein carbonylation. Furthermore, SeNPs treatment afforded some protection against ionizing radiation exposure. SeNPs produced using green chemistry attenuated the reactive oxygen species generation after in vitro ionizing radiation exposure opens up tremendous possibilities for radiosensitizer development.

Graphical abstract

Keywords Biosafety · Oxidative stress · Radiolytic synthesis · Radiosensitizer · Saccharomyces cerevisiae · Selenium nanoparticles

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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. In the clinical setting, 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).

Nanomedicines present certain advantages over conventional therapies in terms of safety, efficacy, physicochemical properties, drug distribution, and kinetic properties (Choi and Han 2018). Different selenium-containing compounds are among the arsenal of conventional 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, anti-inflammatory, antiparasitic, and neuroprotective effects (Bodnar et al. 2012).

Despite many beneficial properties, selenium can also produce harmful effects (Rayman 2020). These results are usually related to the chemical formula and concentration of the selenium-containing compound. Moreover, organic selenium-containing compounds are typically less toxic than inorganic ones (Barceloux 1999; Rohn et al. 2018), with elemental selenium (Se0) being the least toxic (Barceloux 1999).

Additionally, many nanoparticles, such as metallics, metal oxide, carbon nanostructures, fullerenes, and others, generate free radicals, a significant limitation of this therapeutic approach (Fu et al. 2014). Thus, due to the advantages of nanomedicines (e.g., low toxicity) and beneficial properties of nanostructured selenium, as reviewed by (Khurana et al. 2019), there are extensive research and development efforts focused on synthesizing selenium nanoparticles (SeNPs) that do not induce oxidative stress.

It is well known that oxidative stress is caused by an imbalance between free radicals and reactive oxygen species (ROS) generation and elimination. The failure to reestablish this equilibrium can damage biological macromolecules (i.e., nucleic acids, proteins and lipids) (Katerji et al. 2019). In this sense, it is important to assess nanoparticle toxicity (e.g., cell viability) and monitor oxidative stress biomarkers [e.g., lipid peroxidation, protein carbonylation, free radical generation, total sulphydryl (SH) content] in vitro. Besides toxicity, the potential of SeNPs to display sensitizer or protector properties when challenged with ionizing radiation is also important to verify.

Various methodologies have been employed for synthesizing nanoparticles. Herein, ionizing radiation in the form of gamma-rays from a 60COberalt source was chosen. 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 (eaq−) and hydrogen radicals (H·) (Le Caër 2011) and oxidizing species like hydroxyl radicals (OH·). It has been reported that eaq− and H· display a strong reducing capacity with standard reduction potentials (E0) of −2.88 V and −2.31 V, respectively. These E0 values are sufficient for reducing Se4+ to Se0 (El-Batal et al. 2020). On the other hand, the E0 of OH· is 1.9 V, producing more elevated selenium oxidation states (Armstrong et al. 2015).

Using γ-ray irradiation for nanoparticle synthesis has gained 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 SeNPs synthesis was carried out without the addition of stabilizing and/or scavenging substances, consistent with "Green" approaches. After synthesis, the obtained nanoparticles were characterized 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). Furthermore, SeNP toxicity, sensitizer or protector properties and oxidative stress biomarkers were evaluated in Saccharomyces cerevisiae, a toxicological model that diminishes the need for laboratory animals, which are associated with increased ethical constraints and high economic costs.

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 Na2SeO3 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), divided equally into two 50 mL polypropylene centrifuge tubes and irradiated.
The irradiation procedure was carried out in the Gamma Irradiation Laboratory at the Nuclear Technology Development Center, using a 50Co Cobalt source. Samples were placed 50 cm from the source, and a 25 kGy dose at 532.96 Gy/h was delivered. The protocol was largely based on a previous report (Zhu and Qian 1996).

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

**Characterization of the SeNPs**

According to Jakubczak and Jastrzębska, many methodologies are commonly used to characterize nanomaterials (Jakubczak and Jastrzębska 2021). Herein, DLS, UV–Vis analysis, SEM, EDS, TEM, XRD, XPS were selected for SeNPs characterization.

Dynamic Light Scattering (DLS), performed on a Malvern 3000 Zetasizer NanoZS instrument, was used to measure the Zeta potential, hydrodynamic size and polydispersity index of the isolated SeNPs. Data from at least three independent experiments conducted in triplicate were used to calculate the average values. The absorption properties were evaluated by placing the samples in a Perkin Elmer 2300 Multilabel Reader and recording the absorbance from 230 to 800 nm. The data were 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.

The transmission electron microscope (TEM) experiments were conducted on a 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 min, and subsequently deposed onto copper grids covered with Holey Carbon.

X-ray diffraction (XRD) was carried out with a Rigaku DMAX diffractometer for crystallinity analysis of the samples, and scanning was done at 2θ.

The oxidation state of selenium was verified by X-ray Photoelectron Spectroscopy (XPS) in a SPECS PHOIBOS 150 MCD instrument, equipped with a monochromatic Al Kα X-ray of 1486.6 eV. The C 1s peak, at 284.6 eV, from adventitious carbon was used as the energy reference, and the high-resolution spectra were acquired with a step of 0.1 eV using a pass energy of 20 eV. Following the assessment of SeNPs characterization, this work aimed to verify the nanoparticles' influence on yeast cells.

**Cell culture and treatment**

**Yeast strain and culture conditions**

Wild-type *Saccharomyces cerevisiae* strain BY4741 (MATa; his3 Δ1; leu2Δ0; met15Δ0; ura3Δ0) was obtained from the European *Saccharomyces cerevisiae* Archive for Functional Analysis (EUROSCARF), Frankfurth 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. Cells (10⁸ 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). Culture conditions were based on a previous study (Pereira et al. 2018).

The following groups were tested, all of which contained cells in YNB medium: Control (absence of treatment); SeNPs (supplementation with 1 mM SeNPs); Selenite (supplementation with 1 mM 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 10⁸ cells/mL. After 24 h of growth in the orbital shaker (150 rpm), cells were collected by centrifugation (2000×g for 5 min). The pellets obtained were washed with cold autoclaved water (2000×g for 5 min) and then 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 10⁶ cells/mL, and 100 µL 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. The protocol was previously described (Porto et al. 2016).

**Extract preparation**

After the 24-h SeNPs 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-min cycles with a 1 min rest on ice between each cycle (Costa-Moreira et al. 2016). The crude extract was centrifuged (2000×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...
Results

Physicochemical characterization

A sodium selenite solution was irradiated in YPG medium in a panoramic irradiator using 60Co 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. UV–Visible absorption spectroscopy was used 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, DLS was used to determine the average size, Zeta potential and polydispersity index (PDI) of the SeNPs suspended in YNB medium (Table 1). Considering the water layer, the nanoparticles’ average size was estimated to be 150 ± 10 nm. Moreover, 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.

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, TEM was used 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

XPS high-resolution spectra of the Se 3d region were acquired to investigate the oxidation states of Se in the sodium selenite and SeNPs samples. The Se 3d peaks were fit using a combination of Gaussian–Lorentzian functions of two components, Se 3d3/2 and Se 3d5/2, 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 Se⁴⁺ (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 Se⁰ (Jain et al. 2015; Bai et al. 2020). 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, the influence of the synthesized SeNPs on S. cerevisiae cell colony formation after 24 h of treatment with SeNPs, selenite, or control solutions was evaluated. 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 incubation, the number of CFUs was recorded (Fig. 4a). The non-irradiated groups had more CFUs than the irradiated ones. There was no significant difference in
the number of CFUs between the groups treated with 1 mM SeNPs and a 400 Gy irradiation dose. In contrast, the sodium selenite treatment attenuated the number CFUs (Fig. 4a).

Moreover, treating the *S. cerevisiae* cells with 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 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, ROS generation was not different in the cells exposed to ionizing radiation compared to 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) 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 SeNPs 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 further increased 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.

Finally, free sulfhydryl content was employed 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). Gamma irradiation did not affect total sulfhydryl content compared to control cells. 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 1 mM 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, a solution of 1 mM of selenium in YPG broth was exposed to 25 kGy of 60Co irradiation, a characteristically low LET radioactive source of ionizing radiation. Including the YPG medium was advantageous because the solution has a mild acid pH (5.6–6.0), meaning that a presence of H+ ions favors protonation of solvated electrons producing H+ and, at the same time, diminish OH− production (Siwek and Edgecock 2020). Notably, these reactions facilitate Se4+ reduction to Se0. 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). For example, the reduction of Se2O3 to Se0 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 to 1 kGy generated larger-sized nanoparticles, an observation consistent with another study (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. Therefore, all experiments were conducted with 1 mM sodium selenite and 25 kGy of irradiation 50 cm from the 60Co Cobalt source.

The radiation beam applied to the samples randomly provoked water molecule radiolysis, generating many oxidizing and reducing species. It is the oxidizing species, like eaq− and H·, that reduce selenite (Se4+) to elemental selenium (Se0) (Flores-Rojas et al. 2020). It is important to point out that reducing species, such as OH−, can displace the reaction to a more positive oxidation state, even though the
liquid medium contains glucose, peptone and yeast extract (Morelli et al. 2003; Liu et al. 2014), molecules that can perform different functions.

For example, it has been shown that glucose scavenges OH· (Morelli et al. 2003) and peptone and yeast extract are used as reducing and stabilizing agents, respectively (Zhu and Qian 1996; Liu et al. 2014). The 60Co gamma-ray irradiation method described herein produces highly pure nanoparticles, requiring only the YPG broth and water radiolysis products and no 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). A single peak at 350 nm was observed herein, consistent with previously reported results using different synthesis methodologies (Wadhwani et al. 2017; Faramarzi et al. 2020). Additionally, the color change from brown to red is a well-recognized feature of spherical nanoparticles (Begum et al. 2018). A single peak at 350 nm was observed herein, consistent with previously reported results using different synthesis methodologies (Wadhwani et al. 2017; Faramarzi et al. 2020). Additionally, the color change from brown to red is a well-recognized feature of spherical nanoparticles (Begum et al. 2018).

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, including the water layer (Table 1) and are in accordance with the maximum absorbance peak at 350 nm (Fig. 1a). Moreover, the Zeta potential was found to be −16.2 ± 1.7 mV. In general, negatively charged nanoparticles are less toxic than positively charged ones 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 particle aggregation.

As shown in Fig. 1c, the synthesized SeNPs were mainly composed of selenium, exhibiting characteristic peaks at 1.37 keV (SeLα), 5.55 keV (SeKα) and 12.49 keV (SeKβ) (Zheng et al. 2014). The presence of other elements such as carbon (C), chlorine (Cl), potassium (K), sulfur (S) and silicon (Si) was also detected. 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 indetectable. It is plausible that this observation is due to the consumption of selenium oxides. Additionally, there were no prominent peaks in the SeNPs 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 SeO2 as the precursor, alcohol as the OH· scavenger, and employed acidic and alkaline conditions (Zhu and Qian 1996). In our study, we used 2% glucose as the OH· 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 (Kim et al. 2019; Akçay and Avcı 2020).

The TEM image in Fig. 2b revealed agglomerated nanoparticles; thus, confirming the SEM results (Fig. 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 characteristic of crystal formation. These results provided further evidence indicating that the synthesized SeNPs have an amorphous structure.

As shown in Fig. 3, the sodium selenite and SeNPs samples were analyzed 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 Se4+, whereas the SeNPs spectra revealed a typical 3d signal, with peaks at 55.0 and 56.5 eV, assigned to Se0, as previously reported (Jain et al. 2015; Bai et al. 2020). Based on these results, it was concluded that the synthesized SeNPs are primarily composed of Se0.

After characterizing the SeNPs (Figs. 1, 2 and 3), S. cerevisiae cells were treated with the synthesized compounds and the effects, alone and with gamma radiation, on viability and oxidative parameters were assessed. S. cerevisiae cells were selected as the model system because these eukaryotic cells require simple cultivation and reproduce rapidly. Due to high gene homology, this yeast exhibits cellular responses similar to mammalians. This experimental model also lacks selenoproteins, making it ideal for testing selenium compound toxicity (Herrero and Wellinger 2015). The influence of SeNPs on oxidative stress was also determined 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 previously proposed parameters (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 inducer (Salin et al. 2008; Porto et al. 2016). 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) 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 et al. (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 μM 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 SeNP 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, when applied at the same concentration (i.e., 1 mM), sodium selenite was more toxic *S. cerevisiae* than SeNPs. Treating the cells with SeNPs followed by ionizing irradiation (400 Gy) demonstrated that the SeNPs protect against the radiation (Fig. 4a). It was decided to deliver a 400 Gy dose because it caused a reduction in the number of CFUs, but a significant number of CFUs also remained compared to the Control and 400 Gy groups (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, on average, 400 Gy for yeast cells versus 4 Gy for mammalian ones 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 situations, cellular damage and cellular death can ensue (Reisz et al. 2014).

Firstly, the biological activities of the SeNPs and selenite were compared in terms of the cell proliferation capacity by quantifying CFUs. Additionally, cells were challenged 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 were monitored. As shown in Fig. 4b, SeNPs treatment prevented ROS generation (SeNPs versus Control), even after gamma-ray irradiation (SeNPs versus Selenite). However, ROS levels were still significantly lower than in the Control and 400 Gy groups in the presence of SeNPs. 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 more recently (Farhood et al. 2019).

Lipid peroxidation, protein carbonylation and total SH content, for which elevated levels would indicate oxidative stress, were assessed by monitoring lipid oxidation, protein oxidation and total glutathione levels, respectively, following SeNPs exposure. The lipid peroxidation results are consistent with those obtained for cell viability following SeNPs treatment (Fig. 5a). In this sense, it is unlikely that SeNPs disrupt the plasma membrane via lipid oxidation since no alterations were detected (Libardo et al. 2017). On the contrary, SeNPs appear to protect cells by 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 in the SeNPs-treated group. The authors concluded that SeNPs were more effective at increasing tissue selenium levels in mice and protecting against gamma-ray-induced nephropathy in these animals than sodium selenite. Another study demonstrated selenium’s protective effect during chemotherapy by implanting an Ehrlich tumor into mice and treating them with cyclophosphamide. The animals treated with SeNPs displayed attenuated hepatic lipid peroxidation, highlighting the chemoprotective function of the compound (Bhattcharjee et al. 2017). On the other hand, selenite produces the opposite response in the presence of ionizing radiation (Fig. 5b); an observation consistent with previously published studies (Zhang et al. 2001; Porto et al. 2016). 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 mice to 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 administered SeNPs or lovastatin (Lov-Se) for fifteen days, and at three-day intervals, also received doses of gamma radiation, totaling 8 Gy (El-Batal et al. 2012). The results indicated that Lov-Se significantly improved protein carbonylation in irradiated rats. Therefore, the attenuated protein carbonylation in the SeNPs + 400 Gy group in the present study further demonstrates the protective role of these nanoparticles. Similar to the results presented in Fig. 4b, Izquierdo (2010) reported high levels of selenite-induced protein carbonylation, a likely consequence of the selenite-generated ROS (Izquierdo et al. 2010).

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 attenuation of 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 SeNPs-mediated antioxidant effect was also observed by Amin et al. when they showed augmented GSH concentrations in rats administered acetaminophen without an intraperitoneal SeNPs injection (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). Finally, selenite’s toxic effects were confirmed (Fig. 5c), consistent with a previous study (Porto et al. 2016).

In conclusion, this study showed that SeNPs with radioprotective properties and low toxicity, similar to other selenium-containing compounds as reviewed in (Farhood et al. 2019), can be produced using a simple methodology without hazardous chemical reagents. Despite its side effects and toxicity, it is important to highlight that Amifostine is currently the only radioprotector compound used in human patients (King et al. 2020). Notably, while radiotherapy plays a central role in cancer treatment, it damages tissues and cells that undergo rapid differentiation due to high cell division capacity (mitosis). Thus, there exists a possibility to counter this adverse effect with agents that can attenuate the injurious effects on normal cells in the surrounding cancerous tissue. The SeNPs synthesized and characterized herein reveal an alternative avenue for future research investigating their potential utility in protecting noncancerous cells during radiation therapy.

Authors’ contributions AGP conceived, designed and conducted experiments; LGLG and LMCM conducted experiments, analyzed and interpreted data; LSG analyzed and interpreted data and participated in manuscript redaction; PLG performed the XPS experiments and analyzed and interpreted data; MJN conceived, designed and wrote the manuscript. All authors have reviewed and approved the final version of the manuscript.

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Declarations

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