

# Nano-selenium and its nanomedicine applications: a critical review

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**Abstract:** Traditional supplements of selenium generally have a low degree of absorption and increased toxicity. Therefore, it is imperative to develop innovative systems as transporters of selenium compounds, which would raise the bioavailability of this element and allow its controlled release in the organism. Nanoscale selenium has attracted a great interest as a food additive especially in individuals with selenium deficiency, but also as a therapeutic agent without significant side effects in medicine. This review is focused on the incorporation of nanotechnological applications, in particular exploring the possibilities of a more effective way of administration, especially in selenium-deficient organisms. In addition, this review summarizes the survey of knowledge on selenium nanoparticles, their biological effects in the organism, advantages, absorption mechanisms, and nanotechnological applications for peroral administration.

**Keywords:** nanoparticles, biomedicine, drug delivery, oxidative stress, anticancer effect, antimicrobial activity, protective effect

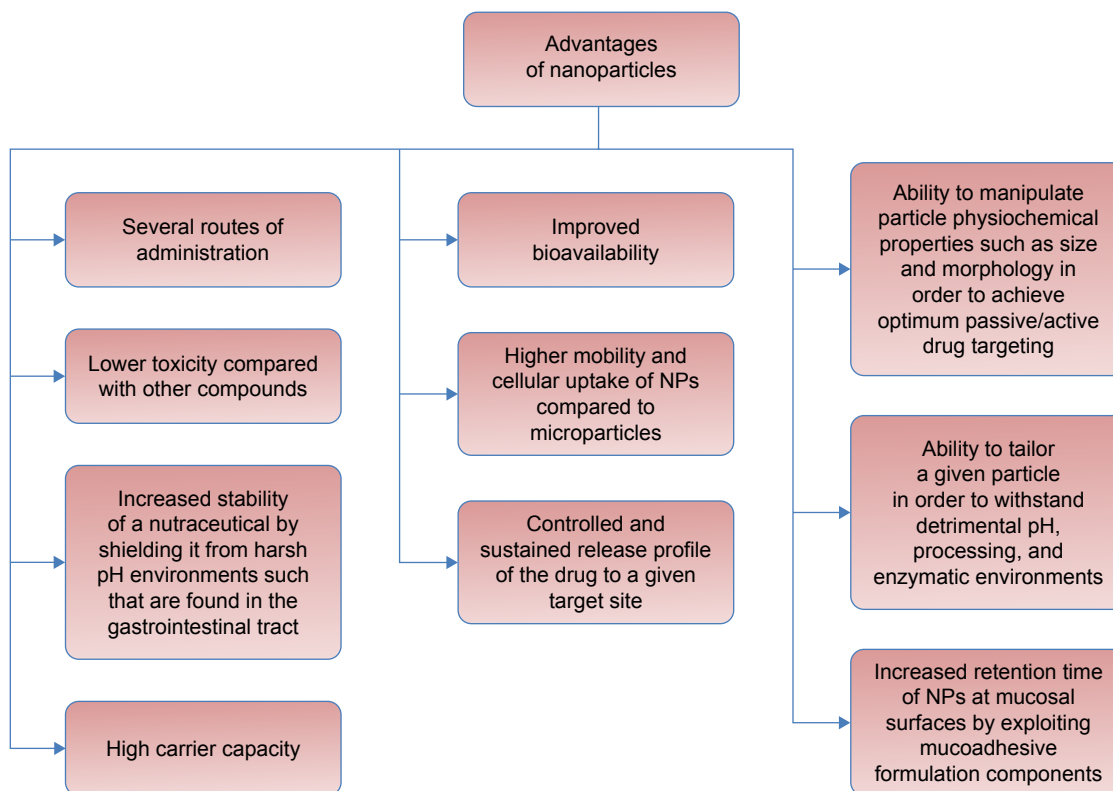
## Methodology of the review

To carry out this study, different databases such as Web of Science, PubMed, MEDLINE, and Google Scholar were employed. The findings of research studies (original articles, experimental studies) chosen from more than 1,000 viewed scientific publications were compared. The emphasis was put on original articles, while more than 100 review articles were not included. The search was based on phrases such as selenium nanoparticles and antioxidant activity/antimicrobial properties/antiviral effect/antibacterial activity/anticancer agent, selenium nanoparticles and synthesis, nano-selenium and bioavailability/toxicity/safety/drug delivery/orthopedic implants/protective effects/oxidative stress/metal intoxication/immunomodulation/reproduction/growth/gastrointestinal tract/hair, selenium nanoparticles and animals, advantages of nanoparticles, and effects of nano-selenium. The review includes research findings from the years 2000–2017.

## Nanotechnological modifications

Nanoparticle (NP) systems appear to be a promising alternative to peroral drug delivery<sup>1</sup> as well as nutritional supplements.<sup>2</sup> The effects of bioactive compound supplements – omega-3 and omega-6 fatty acids, probiotics, prebiotics, vitamins, and minerals – in nanoparticulate preparations have already been dealt by numerous studies.<sup>3–16</sup> Some applications of NPs in nutrition and medicine have already been approved for clinical use, and many others are at different stages of their development.<sup>17</sup>

The incentive to incorporate nanotechnology in nutrition has the following advantages: taste and smell, administration and solubility, protection against oxidation and



**Figure 1** Diagram showing the main advantages of nanoparticles (NPs).

**Note:** Data from various studies.<sup>2,22,28,201–204</sup>

enzymatic degradation, prolongation of residence time, and effective passage through the gastrointestinal tract enhancing the bioavailability of supplemented substances.<sup>18</sup> The use of most perorally administered supplements carries a number of drawbacks such as insufficient residence time, low permeability and solubility, and instability during the production process (temperature, presence of oxygen, light) or in the gastrointestinal tract environment (pH, enzymes, presence of other nutrients), which can reduce the activity and potential health benefits of supplements.<sup>19</sup> Thus, it is necessary to develop more appropriate products to ensure their physiological and therapeutic effect.<sup>20</sup>

The use of NPs is a promising alternative for the peroral application of supplements that could solve the mentioned shortcomings of traditional forms of supplements and preserve their health benefits.<sup>1</sup> The main advantages of NPs are shown in Figure 1.

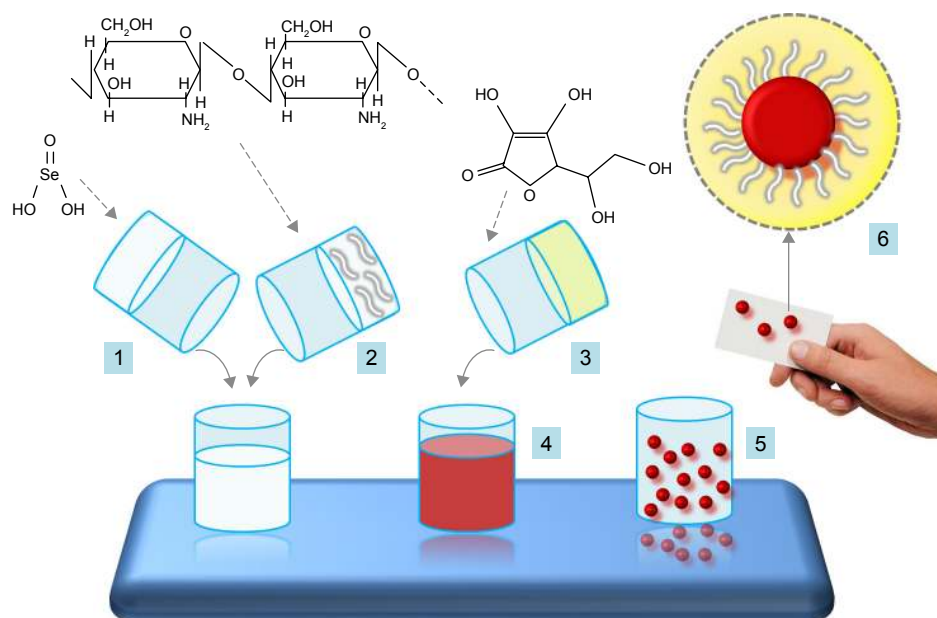
## Selenium NPs as a food additive

The nanoform of selenium attracts even more attention, thanks to its high bioavailability and lower toxicity than inorganic and organic forms,<sup>21–23</sup> where inorganic compounds are more toxic than organic ones.<sup>24</sup>

The biological properties of selenium nanoparticles (SeNPs) depend on their size: smaller particles have a greater activity.<sup>25</sup> Particle size affects the cellular intake of NPs; for example, in vitro absorption of 0.1  $\mu\text{m}$  particles was found to be 2.5 and 6 times higher compared to 1 and 10  $\mu\text{m}$  particles, respectively.<sup>26</sup> With respect to this fact, in the preparation of dietary supplements, an appropriate particle size and morphology, as well as encapsulation material, should be chosen.<sup>27</sup> A system based on the administration of food supplements encapsulated into NPs represents an important potential to improve the bioavailability of supplements with the possibility of modifying their properties, such as resistance to adverse pH, digestion, and enzymatic cleavage.<sup>28</sup>

The advantage of nano-selenium (Nano-Se) is the possibility of using selenium in zero oxidation state ( $\text{Se}^0$ ), which presents low toxicity and excellent bioavailability compared to other oxidation states ( $\text{Se}^{+IV}$ ,  $\text{Se}^{+VI}$ );<sup>22,25</sup> however, it is very unstable and easily transformed into an inactive form. Although, its stabilization can be achieved by encapsulation into suitable nano-vehicles, for example, chitosan (CS)<sup>15</sup> (Figure 2).

Nanoscale selenium has a very wide range of biomedical applications. Its effect on the reduction of oxidative stress is



**Figure 2** Scheme of preparation of selenium nanoparticles (SeNPs).

**Notes:** For the synthesis of SeNPs, the selenious acid solution (aqueous solution of  $\text{SeO}_2$ ) (1) is mixed with an aqueous solution of a polysaccharide, for example, chitosan (2), and ascorbic acid solution (3), which gradually transforms the initially colorless solution into a red color solution (4). In the next step, elemental SeNPs are coated with chitosan, and the result is encapsulated nano-selenium form (5, in detail 6).<sup>50,213</sup>

very well known.<sup>12,29</sup> Gao et al<sup>30</sup> demonstrated the antioxidant properties of hollow spherical SeNPs reducing the risk of selenium toxicity. Besides its use as an antioxidant with reduced risk of toxicity, Nano-Se also possesses potential as a chemopreventive agent.<sup>22</sup> The results of numerous studies indicate that Nano-Se can be more helpful in cancer chemoprevention as a potential anticancer drug,<sup>9,31–34</sup> as well as an anticancer drug delivery carrier.<sup>32,35,36</sup> Many studies have shown the antimicrobial effect<sup>37–39</sup> and antifungal activity of Nano-Se.<sup>40</sup> In addition, its protective effects against metal intoxication were well documented.<sup>41–43</sup> Moreover, the immunostimulatory effect of nanoscale selenium was confirmed.<sup>8,44</sup> Last but not least, Nano-Se has beneficial effects on a number of physiological functions.<sup>13,45–47</sup>

Besides these unique abilities of Nano-Se, its antiprotozoal effect was described. Based on *in vitro* and *in vivo* studies, biogenic SeNPs can be considered as a novel therapeutic agent for the treatment of localized lesions typical of cutaneous leishmaniasis caused by *Leishmania major*.<sup>48</sup> The anti-leishmanial activities of SeNPs against *Leishmania infantum* were also described. SeNPs have more growth-inhibitory effect on promastigotes than selenium dioxide ( $\text{SeO}_2$ ), while the  $\text{IC}_{50}$  (half minimal [50%] inhibitory concentration) was determined to be 25 and 50  $\mu\text{g}\cdot\text{mL}^{-1}$ , respectively.<sup>49</sup>

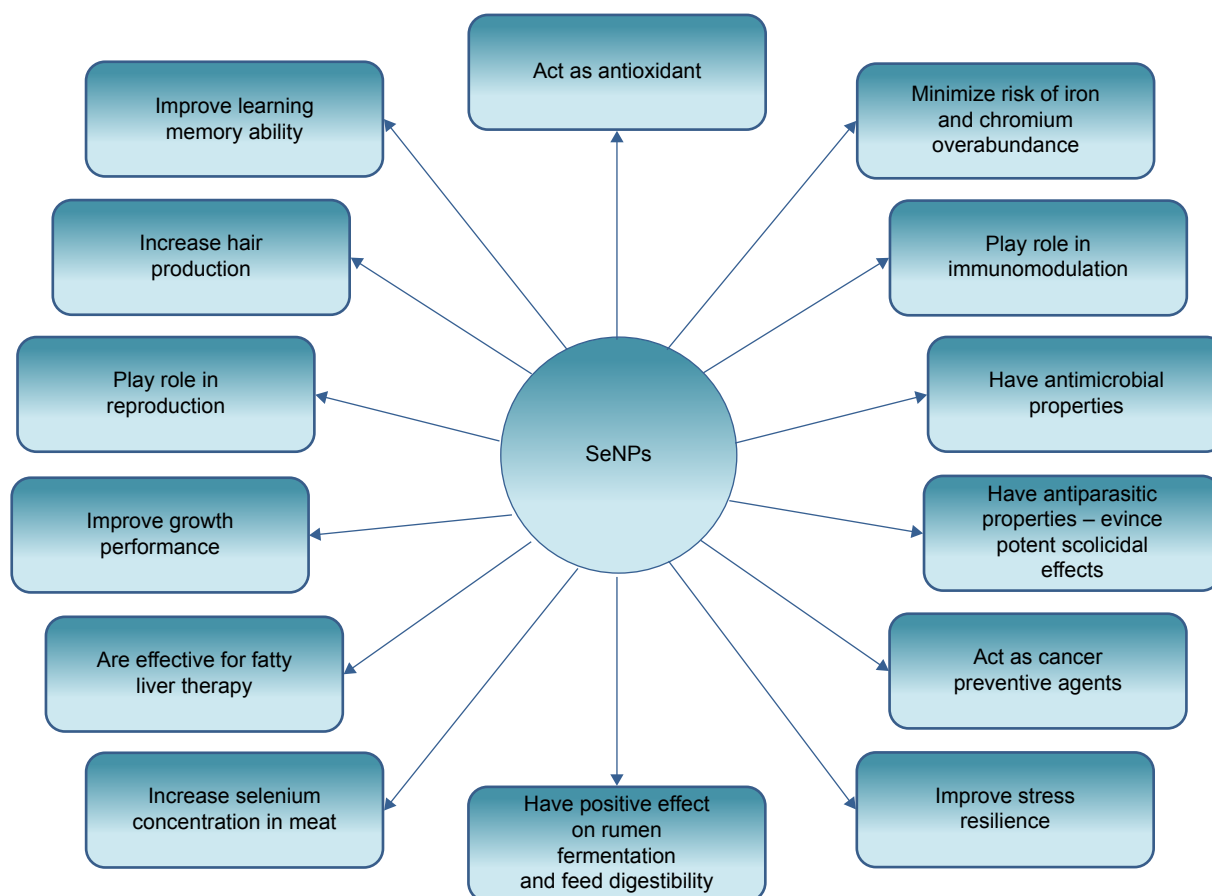
The main effects of the SeNPs found in various scientific studies are summarized in Figure 3.

## Synthesis of SeNPs

SeNPs can be synthesized chemically,<sup>50</sup> or using physical procedures,<sup>51</sup> or can even be obtained by biological way – using microorganisms or plant extracts, the so-called green synthesis.<sup>52,53</sup>

## Chemical synthesis

In terms of chemical synthesis, SeNPs are usually prepared by reduction of selenious acid solution by ascorbic acid in the presence of polysaccharides (Figure 2) such as CS, glucomannan, acacia gum, or carboxymethylcellulose.<sup>50</sup> CS is positively charged, biocompatible, non-immunogenic, nontoxic, pH sensitive, and biodegradable, and is therefore a suitable component for oral administration for a wide range of biomedical and nutritional applications.<sup>54–56</sup> It has been extensively examined in the pharmaceutical industry due to its potential in the development of medication delivery systems.<sup>57</sup> In the molecular structure of polysaccharides, reactive amino, hydroxyl, or carboxyl groups are present, which have a substantial effect on the formation, stabilization, and growth of SeNPs.<sup>50</sup> After the synthesis of NPs, their *ex situ* characterization is performed using different biophysical methods including electron microscopy. The obtained monodisperse spherical selenium particles are very stable in solution<sup>16</sup> and can be used as dietary additive.<sup>14</sup>



**Figure 3** Diagram showing the main effects of selenium nanoparticles (SeNPs).

**Note:** Data from various sources.<sup>7,10,12–14,21,29,43,46,47,52,155,176,180,205–212</sup>

The effects of selenium encapsulation into CS NPs were investigated by Zhang et al.<sup>2</sup> The study showed that encapsulation of selenium compounds into CS NPs is an effective way of delivering selenium to cells in which selenium retention increases, while reducing the risk of DNA damage. The development of NP–selenium compound systems may, in the case of low selenium level, significantly improve the bioavailability of selenium and facilitate the expression of selenoproteins.

Another example for chemical preparation of SeNPs is ionic liquid-induced synthesis with sodium selenosulfate as selenium precursor, in the presence of polyvinyl alcohol stabilizer, which can produce spherical SeNPs in the size range of 76–150 nm.<sup>58</sup>

### Physical synthesis

Among the physical techniques for the preparation of SeNPs, synthesis by pulsed laser ablation (PLA) or deposition was described in a study.<sup>51</sup> Generally, physical methods have distinct advantages over the chemical ones, since these often require a final calcination step, which makes them

unsuitable for certain applications. Furthermore, with sputtering and laser ablation, the stoichiometry of the material is maintained.<sup>59</sup> In PLA, the size of the nanoclusters can be controlled by laser parameters, such as fluence, wavelength, and pulse duration, as well as by ambient gas conditions, such as pressure and flow parameters.<sup>60</sup> However, physical techniques are not widely applied for producing SeNPs.

### Biological synthesis

Originally, SeNPs, like other NPs, were synthesized by various chemical methods. However, the high cost of production and the presence of toxic by-products led to the development of novel methods of NPs synthesis.<sup>61</sup> Biological organisms such as plants, fungi, or bacteria have the ability to convert some toxic metal ions to less toxic forms including metal precipitants or NPs.<sup>62–64</sup> Thanks to this advantage, researches focused on their use for synthesizing NPs with an ecofriendly approach.<sup>65–68</sup>

As for green synthesis, SeNPs were synthesized, for example, using the following plant extracts: aqueous extract of *Allium sativum*,<sup>69</sup> tea extract,<sup>70</sup> *Clausena dentata* plant leaf

extract,<sup>71</sup> *Undaria pinnatifida* polysaccharide solutions,<sup>31</sup> and *Terminalia arjuna* leaf extract.<sup>42</sup>

Biosynthesis of nanomaterials using plant extracts has more advantages than other biological methods because it is inexpensive and does not require any special conditions.<sup>52</sup>

NPs synthesis using bacteria is more effective than chemical synthesis, thanks to the following advantages: 1) high purity of selenium spheres (which are relatively regular and uniform, and their size depends on the bacterium), 2) cheaper and faster production process, and 3) better possibility to control the parameters.<sup>72</sup>

Microorganisms are capable of synthesizing metal NPs.<sup>53</sup> Different bacterial strains are able to reduce Se<sup>+IV</sup> (selenite) and (or) Se<sup>+VI</sup> (selenate) to less toxic Se<sup>0</sup> with the formation of SeNPs. The biogenic SeNPs have exhibited promising application perspectives in the field of medicine, biosensors, and environmental remediation.<sup>73</sup> Interestingly, two halotolerant *Bacillus megaterium* strains (BSB6 and BSB12), isolated from saline mangrove habitat without selenium contamination, were found to be capable of reducing Se<sup>+IV</sup> to elemental selenium, even in the presence of high salt concentrations.<sup>74</sup>

The synthesis of SeNPs by macro- or microorganisms, due to the diversity of reducing enzymes in organisms, involves morphological and shape changes of the particles. By changing the redox state, the reducing enzymes of microorganisms convert metal ions (Se<sup>+II</sup>) to SeNPs without charge (Se<sup>0</sup>). The biological activity of SeNPs includes their protective role against DNA oxidation.<sup>53</sup> It was found that certain anaerobic bacteria respire toxic selenium oxyanions and as a result cause extracellular accumulation of elemental selenium (Se<sup>0</sup>). The spectral properties differ considerably from those of amorphous Se<sup>0</sup> formed by chemical oxidation of hydrogen selenide (H<sub>2</sub>Se) and of black, vitreous Se<sup>0</sup> formed chemically by reduction of selenite with ascorbate. The microbial synthesis of Se<sup>0</sup> nanospheres results in unique, complex, with compact nanostructural arrangement of Se atoms. This probably reflects a wide diversity of enzymes involved in the dissimilatory reduction that are slightly different in various bacteria. Remarkably, these conditions cannot be achieved by current methods of chemical synthesis.<sup>66</sup>

Different types of bacteria have been used for the biosynthesis of SeNPs, such as the species of phylum Proteobacteria (*Escherichia coli* ATCC 35218,<sup>75</sup> recombinant *E. coli*,<sup>76</sup> *Ralstonia eutropha*,<sup>77</sup> *Enterobacter cloacae* Z0206,<sup>78</sup> *Pseudomonas aeruginosa* ATCC 27853,<sup>79</sup> *Klebsiella pneumoniae*,<sup>80,81</sup> *Pantoea agglomerans*,<sup>25</sup> *Zooglea ramigera*,<sup>82</sup> *Rhodospseudomonas palustris* strain N,<sup>83</sup> *Shewanella* sp. HN-41,<sup>84</sup> *Azoarcus*

sp. CIB,<sup>85</sup> *Burkholderia fungorum*,<sup>86</sup> *Stenotrophomonas maltophilia*<sup>39</sup>), Firmicutes (*Lactobacillus casei*,<sup>72,81,87</sup> *Lactobacillus acidophilus* [LA-5],<sup>72</sup> *Lactobacillus helveticus* [LH-B02],<sup>72</sup> *Enterococcus faecalis*,<sup>53</sup> *Streptococcus thermophilus*,<sup>72</sup> *Staphylococcus carnosus*,<sup>88</sup> *Bacillus* sp. MSh-1,<sup>89</sup> *Bacillus subtilis*,<sup>65</sup> *Bacillus mycoides* SelTE01,<sup>38</sup> *Bacillus licheniformis* JS2<sup>90</sup>), Actinobacteria (*Streptomyces* sp. ES2-5,<sup>73</sup> *Bifidobacterium* BB-12<sup>72</sup>), and Cyanobacteria (*Arthrospira* [*Spirulina*] *platensis*<sup>91</sup>).

For in vivo synthesis of Nano-Se, unicellular eukaryotic organisms, such as the protozoa *Tetrahymena thermophila* SB210,<sup>92</sup> yeast *Saccharomyces cerevisiae*,<sup>93</sup> and a genetically modified *Pichia pastoris*,<sup>94</sup> or even multicellular organisms, such as a fungus from the phylum of Ascomycota (*Aspergillus terreus*<sup>95</sup>), were used.

## Problems with traditional forms of oral supplementation of selenium and potential benefits of SeNPs

The bioavailability of various chemical forms of selenium has been the subject of many studies. For instance, Mahan et al stated that the degree of selenium absorption is much lower in ruminants than in nonruminants; after oral supplementation, the selenium uptake in sheep was only 34%, whereas in pigs 85%.<sup>96</sup> In ruminants, the microbial digestion in the rumen and reticulum precedes its assimilation in the abomasum and microbial digestion in the small intestine.<sup>97,98</sup> Ruminal microorganisms partially transform selenium supplements into insoluble forms, such as elemental selenium and selenides, which are unavailable to animals, and thus, the absorption of selenium from the digestive tract is reduced.<sup>99</sup>

To avoid this negative phenomenon of reduced selenium absorption due to microbial digestion in ruminants and improve the availability of this microelement, polymer NP systems with in vivo potential have been proposed.

This technology was implemented using the Eudragit<sup>®</sup> RL and Eudragit<sup>®</sup> RS polymers.<sup>100</sup> These nonbiodegradable polymer NPs were already used in 2005 to encapsulate the lipophilic cyclic undecapeptide cyclosporin A (CyA) for oral administration in rabbits for the prevention of graft rejection and the treatment of autoimmune diseases. CyA was encapsulated by nanoprecipitation in mentioned NP polymers, and its relative bioavailability from both preparations ranged from 20% to 35%.<sup>101</sup> The design of selenium in the nanoform for oral administration to ruminants was proposed as late as 2010 by Romero-Pérez et al,<sup>100</sup> who monitored the in vitro effect of sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) encapsulated in polymeric NPs by nanoprecipitation and



emulsion-evaporation methods using two different solvents – ethanol and acetone. Nanoprecipitated NPs were spherical and had a greater particle size variability; on the contrary, the NPs produced by the emulsion-evaporation technique were both spherical and irregular in shape and homogeneous in size. The size of NPs ranged from 30 to 200 nm. Higher particle size, zeta potential, and polydispersity index were found for the NPs produced by the nanoprecipitation method using ethanol as the solvent of polymers. The release of selenium from NPs was monitored *in vitro* at different pH values and was higher in the strongly acidic environment (pH less than 4), which is a prerequisite for providing better accessibility of this element in the abomasum. At pH values lower than 4, selenium release increases by 62%<sup>100</sup> compared to pH 6. The pH in the rumen ranges from 5.5 to 6.5. Thus, the sheep rumen essentially does not absorb selenium.<sup>97</sup> On the contrary, the pH in the abomasum is less than 4; under this condition, the largest amount of selenium is released from the particles. NPs increase the bioavailability of this element because at pH 6 only a small amount of selenium is released (by rumen microorganisms) and the remaining, bigger part, is released then in the more acidic environment of the gastrointestinal tract, which guarantees its better availability.<sup>100</sup>

SeNPs evince, in addition to better selenium availability, much lower toxicity compared to other selenium compounds.<sup>22,23</sup> For example, in mice, SeNPs showed much lower toxicity measured by median lethal dose (50%, LD<sub>50</sub>), liver impairment, and short-term toxicity.<sup>23</sup>

## Mechanism of passage of NPs through intestinal mucosa

Oral administration of NPs is considered to be the most appropriate and cost-effective method of supplementation. However, absorption of NPs may also be made more difficult by the presence of absorption barriers in the digestive tract. It is necessary to overcome two barriers in the gastrointestinal tract for the absorption of NPs, which are mucus covering the intestinal mucosa and the intestinal mucosa.<sup>1</sup>

The intestinal epithelium is composed of a series of specialized cells, primarily enterocytes, goblet cells, and M cells. One of the main functions of enterocytes is to control the transition of macromolecules and allow the absorption of nutrients. The goblet cells secrete mucus consisting of a high-molecular-weight glycoprotein (mainly mucin) suspended in the electrolyte solution,<sup>102</sup> which covers with the adherent layer the mucous membrane of the intestine. Their primary function is to protect the intestinal mucosa from potential

pathogens or chemicals and maintain a different pH between the lumen and the mucosal surface of the intestine.<sup>1</sup>

NPs can theoretically pass through the intestinal epithelium in two ways: paracellular (between adjacent cells) or transcellular (through the cells) (Figure 4).<sup>102</sup> Under physiological conditions, the first way is restricted by a narrow region of intercellular spaces and by the tightness of the junctions between the epithelial cells (pore diameter is between 0.3 and 1.0 nm).<sup>103</sup> Transcellular transport of NPs takes place through a process called transcytosis, which starts with endocytosis in the apical membrane of the cells. Subsequently, the NPs are transported through the cells and released on the basolateral pole.<sup>104</sup>

Intestinal epithelial cells are capable of transferring NPs with mineral elements, although their capacity is limited. Transcellular transport begins with endocytosis (pinocytosis or macropinocytosis). It is an active process requiring energy for the internalization of NPs. Macropinocytosis is actin-dependent but is not regulated by receptors, and a large amount of fluid with particles smaller than 5 μm can be internalized through it.<sup>105</sup> The absorption of NPs is influenced by electric charge, surface hydrophobicity, and size.<sup>106</sup> The epithelial cell membrane of the gastrointestinal tract is composed of lipids, so that hydrophobic NPs have a higher absorption efficiency than hydrophilic particles. The absorption of NPs of 100 nm in the gastrointestinal tract is 15–250 times higher than that of larger-sized NPs.<sup>107</sup>

## Application of SeNPs through oral administration

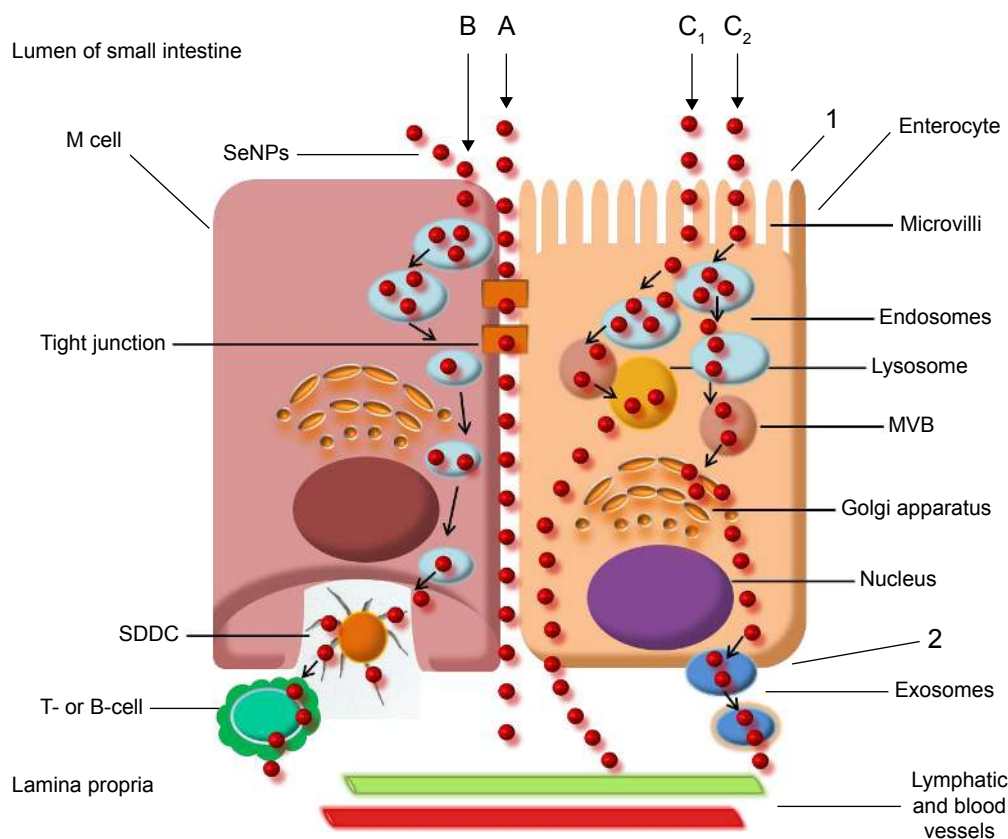
### Nano-Se as an antioxidant

Nano-Se possesses better antioxidant capability than other chemical forms of selenium while reducing the risk of selenium toxicity. Wang et al<sup>22</sup> demonstrated the antioxidant properties of SeNPs that evinced lower toxicity than selenomethionine (SeMet).

Zhang et al<sup>108</sup> studied the effect of elemental Nano-Se on the activity of glutathione peroxidase (GPx) in the liver of weanling pigs (Duroc × Landrace × Yorkshire) in comparison to inorganic form of selenium. The animals had significantly higher activities of GPx at a concentration range of 0.50 and 1.0 mg Se·kg<sup>-1</sup> diet in the form of Nano-Se than Na<sub>2</sub>SeO<sub>3</sub>.

### Effect of SeNPs on reproductive performance

Oxidative stress affects the fertility potential of spermatozoa by lipid peroxidation which can lead to sperm dysfunction.<sup>109</sup> Selenium deficiency leads to the occurrence of abnormal mitochondria in goat spermatozoa, and Nano-Se supplementation



**Figure 4** Diagram of nanoparticle transport across the intestinal mucosa of the small intestine. Particles pass either paracellularly, that is, between adjacent cells, or by a transcellular pathway that has been more explored. The transcellular transition takes place via normal enterocytes or M cells. A, paracellular transport; B, transcellular transport: transcytosis through M cell; C<sub>1</sub>, transcytosis through enterocyte, first mechanism; C<sub>2</sub>, transcytosis through enterocyte, second mechanism; 1, apical membrane of enterocyte; 2, the basolateral membrane of the enterocyte. M cells form a row of vesicles before transfer from subepithelial dome dendritic cells (SDDCs) to T- or B-lymphocytes (B). Uptake of nanoparticles by microvilli of enterocytes is often followed by endosome formation, microvesicular bodies (MVBs) genesis, and their fusion with lysosomes, and then the particles are transported to lamina propria (C<sub>1</sub>). Another mechanism of enterocyte utilization for transepithelial transport involves uptake into endosomes, MVB formation, fusion with Golgi apparatus, and exosomal transfer to lamina propria (C<sub>2</sub>). This is followed by transport into the bloodstream and the lymphatic system.<sup>102,214</sup>

increases selenium concentration in testes, and testicular and semen GPx activity. In addition, it has a protective effect on membrane integrity and the tight arrayment of the midpiece of the mitochondria. To study the effects of elemental Nano-Se in the diet on the testes ultrastructure, ejaculate quality and GPx activity in goats were investigated. The animals were administered with Nano-Se in the declared selenium dose of 0.3 mg·kg<sup>-1</sup> of the feed dry matter (DM) for 12 weeks from weaning to sexual maturity. The results showed that the level of selenium in testes and the activity of GPx and ATPase in the ejaculate significantly increased in the group supplemented with nanoscale selenium compared to the control group. The quality of the ejaculate (volume, density, motility, and pH) was not affected by the addition of selenium, but the percentage of abnormal spermatozoa in the control group of goats was significantly higher compared to the SeNPs group. Using transmission electron microscopy, it was found that in selenium-deficient goats the sperm plasma

membrane was damaged, and there were abnormalities in the mitochondria midpiece of spermatozoa.<sup>13</sup>

### Use of Nano-Se for increasing hair follicle development and fetal growth

The study of Wu et al<sup>46</sup> demonstrated the importance of maternal administration of selenium at nano size for improving the hair follicle development and promoting the growth of fetus. This was attributed to the influencing antioxidant status in the fetal skin. An increase in the antioxidant defence results in a decrease of reactive oxygen species (ROS) generation, leading to upregulation of IGF-1 and its receptor (IGF-1R) which are crucial for the improvement of both properties.

In cashmere goats, SeNPs have a positive effect on the amount of wool. The administration of 0.5 mg·kg<sup>-1</sup> diet (containing 1,500 mg Se·kg<sup>-1</sup>) in the period from 30 days before conception to 110 days of gestation was manifested in their fetuses with significantly higher expression levels of

the *GPx*, *IGF-1*, and *IGF-1R* genes in skin. In addition, increased GPx and superoxide dismutase (SOD) activities, and IGF-1 and selenium concentrations in both skin and blood serum at 110 days were observed. Moreover, a significantly lower malondialdehyde (MDA) production in both skin and serum which affected the antioxidant status in skin of the fetuses, and significantly increased the number of their secondary hair follicles, was observed. The low level of ROS probably upregulates IGF-1 and IGF-1R, which favorably influences the development of hair follicles in the fetus. In addition, the weights of fetus and placenta were significantly higher in SeNPs-receiving group than those in control group.<sup>46</sup>

## Antiviral and antibacterial effects of SeNPs

SeNPs have attracted substantial attention due to their unique antimicrobial activity.<sup>53,110–114</sup> Selenium is an essential trace element regulated by cellular redox homeostasis<sup>115–117</sup> and is an integral component of selenoproteins controlling some crucial biological processes, such as ROS elimination and specific enzyme modulation.<sup>118,119</sup> Selenium deficiency can result in susceptibility to viral infections. The antiviral capability of SeNPs, together with other advantages such as low toxicity and excellent activity, has recently attracted increased attention. The mortality of the H1N1 influenza virus-infected selenium-deficient mice was 3 times higher compared to those receiving Na<sub>2</sub>SeO<sub>3</sub> at the dose of 0.5 mg Se·kg<sup>-1</sup>, and mice with low serum selenium concentrations showed a marked reduction in body weight (BW) and lower levels of TNF- $\alpha$  and IFN- $\gamma$ .<sup>120</sup> For improving the immune response in the body, the administration of SeNPs can also be an efficient realizable approach.

Li et al<sup>121</sup> synthesized oseltamivir (OTV) surface-modified SeNPs (Se@OTV) with superior antiviral properties and restriction on drug resistance. Although the clinical application of OTV itself as an effective antiviral agent is ordinarily limited by the appearance of drug-resistant viruses, OTV decoration of SeNPs evidently inhibited H1N1 influenza virus infection and showed less toxicity. Se@OTV interfered with the entry of H1N1 into host cells through inhibiting the activity of influenza virus glycoproteins – hemagglutinin and neuraminidase. Modified NPs were able to prevent H1N1 from infecting Madin-Darby canine kidney cells and block chromatin condensation and DNA fragmentation. In addition, they inhibited the generation of ROS as well as the activation of phosphorylation of cellular tumor antigen p53 and Akt. Thus, ROS play a pivotal role in the antiviral action. The presence of H1N1 virus increased the intracellular ROS

generation from 100% (control) to 380%, and OTV and SeNPs slightly inhibited their production to 270% and 210%, respectively, whereas Se@OTV markedly decreased the ROS generation (120%). It follows that Se@OTV are promising efficient antiviral pharmaceuticals against highly infectious respiratory disease caused by H1N1,<sup>121</sup> which belongs to influenza A-type viruses.<sup>122</sup>

SeNPs also show excellent antibacterial activity. With SeNPs biosynthesized by *R. eutropha*, 99% inhibition of growth of *P. aeruginosa*, *S. aureus*, *E. coli*, and *Streptococcus pyogenes* at the concentrations of 100, 100, 250, and 100  $\mu\text{g}\cdot\text{mL}^{-1}$ , respectively, was observed. Moreover, the concentration of 500  $\mu\text{g}\cdot\text{mL}^{-1}$  of SeNPs was found to inhibit the growth of pathogenic fungi *Aspergillus clavatus*. The antimicrobial efficacy of SeNPs can be comparable with commercially available antibiotic ampicillin.<sup>77</sup> SeNPs synthesized by another bacterial species *E. faecalis* can be used as an antistaphylococcal element to effectively prevent and treat *S. aureus* infections.<sup>53</sup> SeNPs with application of bioactive glass scaffolds (45S5Bioglass<sup>®</sup>) and poly(lactico-glycolic acid) (PLGA) (45S5Bioglass<sup>®</sup>/SeNPs and 45S5Bioglass<sup>®</sup>/PLGA/SeNPs) showed a considerable antibacterial activity against Gram-positive bacteria, *S. aureus* and *Staphylococcus epidermidis*.<sup>114</sup> In another study, Yang et al<sup>123</sup> introduced the antibacterial effect of Qe/CdSe/ZnS (quercetin/cadmium selenide/zinc sulfide) nanoparticles (QCZNP) against drug-resistant *E. coli* and *B. subtilis* in vitro. QCZNP showed markedly more effective antibacterial activities than Qe or CdSe NPs. The in vitro study of Wang and Webster<sup>37</sup> showed that the selenium coatings on polycarbonate medical devices significantly inhibited *S. aureus* growth to 27% compared with an uncoated polycarbonate surface after 72 hours.

The antimicrobial activity of SeNPs depends on the way they are synthesized. Piacenza et al<sup>38</sup> evaluated the antimicrobial efficacy of spherical biogenic selenium nanostructures embedded in organic material produced by *B. mycooides* SelTE01 in comparison with two different chemical SeNP classes produced using L-cysteine or ascorbic acid. The antimicrobial activity was tested on *P. aeruginosa* and *S. aureus* biofilms grown onto hydroxyapatite-coated clinical devices and surfaces. After 6 or 24 hours of Na<sub>2</sub>SeO<sub>3</sub> exposure, biogenic SeNPs evinced the same effective antibiofilm activity against both tested strains at 0.078 and 0.3125 mg·mL<sup>-1</sup>, respectively. On the contrary, chemically synthesized SeNPs at the highest tested concentration (2.5 mg·mL<sup>-1</sup>) showed only moderate antimicrobial activity. Cremonini et al<sup>39</sup> reported that SeNPs synthesized by



Gram-negative *S. maltophilia* and Gram-positive *B. mycoides* achieved much stronger antimicrobial effects compared with synthetically prepared SeNPs. Both biogenic types of NPs were active at low minimum inhibitory concentrations against a number of clinical isolates of *P. aeruginosa*. It was interesting that after an exposure of human dendritic cells and fibroblasts to all three SeNPs, the following phenomena were not observed: 1) loss of cell viability, 2) increased release of ROS, and 3) significant increase in the secretion of pro-inflammatory and immunostimulatory cytokines. Therefore, SeNPs appear to be reliable candidates for safe medical applications, either alone or in combination with traditional antibiotics, to inhibit the growth of *P. aeruginosa* clinical isolates.

### Anticancer effects of SeNPs

SeNPs have also showed remarkable anticancer activity<sup>52,123–128</sup> and exhibit high potential in cancer chemotherapy and as drug carriers.<sup>129–131</sup> The anticancer effects of SeNPs are mediated through their ability to inhibit the growth of cancer cells through induction of cell cycle arrest at S phase.<sup>32</sup> The induction of the cell cycle arrest at the S phase is mediated by deregulation of the eIF3 protein complex.<sup>132</sup> A recent study revealed that cell membrane plays an important role in SeNPs-induced toxicity in cancer cells. SeNPs treatment changes the biomechanical properties of cancer cells, in particular remarkably decreases the adhesion force and Young's modulus.<sup>133</sup> Besides unique anticancer efficacy, SeNPs have been proved to present a better selectivity between normal and cancer cells than  $\text{Se}^{+IV}$  at similar concentrations.<sup>31</sup> SeNPs can be internalized selectively by cancer cells through endocytosis and induce cell apoptosis by triggering apoptotic signal transduction pathways.<sup>134–136</sup>

### Nano-Se as an anticancer drug

Nano-Se possesses higher anticancer efficacy than other selenium compounds.<sup>119</sup> A key mechanism for the chemopreventive effect is the induction of glutathione S-transferase (GST) by selenium.<sup>22</sup> The activity of GST in the case of Nano-Se administration increased much earlier and more markedly than in the case of SeMet and selenite.<sup>22,137</sup>

Yanhua et al<sup>127</sup> suggested that novel selenium-substituted hydroxyapatite NPs (SeHAN) could be a new promising anticancer agent to provide both survival advantage and lower toxicity in nude mice model of human hepatocellular carcinoma. The overall survival rate of nude mice in the control, pure hydroxyapatite, and SeHAN groups was 50.00%, 76.92%, and 100.00%, respectively. Blood biochemical

studies showed that SeHAN group had significantly lower toxicity on the liver and kidney functions.

In highly metastatic breast cancer mice model, a better prognosis could be achieved by oral administration of SeNP-enriched *Lactobacillus brevis*. Both components are immunostimulators, and enhanced immune response in cancer-affected mice. Moreover, lactic acid bacteria (LAB) can reduce selenium ions to elemental SeNPs and deposit them in intracellular spaces. When LAB in combination with SeNPs were administered to cancer-bearing mice, a significant increase in natural killer cell cytotoxicity and delayed-type hypersensitivity responses, as well as a high level of IFN- $\gamma$  and IL-17, compared to the control mice or mice that received *L. brevis* alone, was observed. In addition, an extended life span and a decline in the tumor metastasis to the liver were recorded in this group compared to the other two groups of mice.<sup>44</sup>

Chen et al<sup>31</sup> reported that SeNPs fabricated in *U. pinnatifida* polysaccharide solutions induced mitochondria-mediated apoptosis in A375 human melanoma cells. Treatment of this cancer cell line with Nano-Se resulted in a dose-dependent cell apoptosis manifested by DNA fragmentation and phosphatidylserine translocation.

Sonkusre et al<sup>34</sup> introduced biologically synthesized SeNPs by *B. licheniformis* JS2 and developed a method for extraction and purification of intracellular NPs. These neutral-charged, non-agglomerating SeNPs at a concentration as low as  $2 \mu\text{g Se}\cdot\text{mL}^{-1}$  were efficacious in inhibiting proliferation and inducing caspase-independent necrosis to human prostate adenocarcinoma cells (PC3) without causing any significant toxicity to human peripheral blood mononuclear cells.

Luo et al<sup>32</sup> examined the in vitro antiproliferative effects of SeNPs (Nano-Se<sup>0</sup>,  $10\text{--}40 \mu\text{mol}\cdot\text{L}^{-1}$ ) on HeLa (human cervical carcinoma) cells and MDA-MB-231 (human breast carcinoma) cells by optical microscopic inspection and MTT assay. Nano-Se<sup>0</sup> effectively inhibited the growth of cells of both the cancer cell lines in a dose-dependent manner. The morphology analysis with atomic force microscopy showed that the HeLa cells treated with  $10 \mu\text{mol}\cdot\text{L}^{-1}$  Nano-Se<sup>0</sup> were rough and shrunken with truncated lamellipodia at the terminal part of the cells. Flow cytometric analysis demonstrated that HeLa cells were arrested at S phase of the cell cycle after exposed to mentioned amount of Nano-Se<sup>0</sup> ( $10 \mu\text{mol}\cdot\text{L}^{-1}$ ).

It was found that anticancer activity of SeNPs correlates with the level of ER $\alpha$  in breast cancer cells. SeNP-induced cell death and expression of apoptotic markers (pp38,

Bax, and cytochrome c) were significantly higher in ER $\alpha$ -positive cells (MCF-7) but not in ER $\alpha$ -negative cells (MDA-MB-231).<sup>33</sup>

### Nano-Se as an anticancer drug delivery carrier

Besides their direct anticancer effects, SeNPs have been appointed as potential anticancer drug delivery carriers.<sup>32,35</sup> A key factor that usually contributes to nanomaterial-based drug cytotoxicity is cellular uptake.<sup>35</sup> The nanosize of these materials allows an efficient uptake by various cell types and selective drug accumulation at target sites.<sup>26</sup> Like other chemotherapeutics, the effective cytotoxicity of nanomaterial-based drugs usually requires a high level of accumulation within the cancer cells.<sup>138</sup> Nanomaterials tend to accumulate in cancer cells through a passive targeting process<sup>128</sup> and often serve as “nanocarriers” for chemotherapeutics.<sup>35,139–147</sup> Moreover, the usage of various surface decorators enhances the cellular uptake and anticancer efficacy of nanomaterials,<sup>35,128,135</sup> for example, Yang et al<sup>128</sup> developed a simple and solution-phase method for functionalization of SeNPs with *Spirulina* polysaccharides (SPS). In addition, they found that SPS surface decoration significantly enhanced the cellular uptake and cytotoxicity of SeNPs against several cancer cell lines. The action mechanism of SPS-SeNPs was grounded in inhibition of cancer cell growth through induction of apoptosis, as evidenced by an increase in sub-G1 cell population, DNA fragmentation, chromatin condensation, and phosphatidylserine translocation. In another study, Yang et al<sup>148</sup> designed and synthesized folic acid-conjugated SeNPs (FA@SeNPs) as cancer-targeting agents; the anticancer efficacy of FA@SeNPs was synergistically enhanced by radioactive <sup>125</sup>I seeds, and these NPs inhibited colony formation ability, which showed that functionalized SeNPs can be used as a radiation sensitizer for <sup>125</sup>I seeds for cancer therapy.

Chan et al<sup>129</sup> reported that, based on the Auger-electron effect and Compton effect of Se atoms, cancer-targeted SeNPs in combination with <sup>125</sup>I seeds achieve synergetic effects for inhibition of cancer cell growth and colony formation by inducing cell apoptosis and cell cycle arrest. In addition, these could be used as a safe and effective agent for next-generation cancer chemoradiotherapy in clinical applications. The action mechanism showed the activation of intracellular ROS overproduction to regulate p53-mediated DNA damage apoptotic signaling pathways and MAPKs phosphorylation as well as preventing the self-repair of cancer cells simultaneously.

SeNPs can be used as a carrier of 5-fluorouracil to achieve anticancer synergism, as introduced by Liu et al.<sup>35</sup> A panel of five human cancer cell lines (A375, MCF-7, HepG2,

Colo201, and PC3) evinced susceptibility to 5-fluorouracil surface-functionalized SeNPs (5-FU-SeNPs), with IC<sub>50</sub> values ranging from 6.2 to 14.4  $\mu$ M. Remarkably, despite this potency, the 5-FU-SeNPs possess great selectivity between cancer and normal cells. Induction of apoptosis in A375 human melanoma cells by 5-FU-SeNPs was evidenced by accumulation of sub-G1 cell population, DNA fragmentation, and nuclear condensation.

By comparing the effect of chemically prepared SeNPs on the behavior of cancer cells with that of other inorganic and organic selenospecies, similar alterations in terms of cell viability, proliferation, migration, and cell cycle arrest at the S-G2/M phase after exposure of hepatocarcinoma (HepG2) cells to SeNPs and selenocystine (SeCys2) were observed. In contrast, cells exposed to Se<sup>IV</sup> showed evident signs of toxicity such as strong induction of apoptosis and a significant population of cells in the sub-G1 phase compared to control cells. Se<sup>IV</sup>, SeMet, and seleno-methylselenocysteine (Se-MetSeCys) did not evince any significant differences in comparison to non-treated cells. While SeNPs only partially inhibited the Cdk1 expression, Se<sup>IV</sup> and SeCys2 reduced drastically its expression, which can be toxic for healthy cells. Cdk1 plays an essential role in cell cycle progression and mitosis entry, and its inhibition induces cell cycle arrest. These findings may pave the way for use of Cdk1-targeting SeNPs in mitotic cell death for cancer therapy.<sup>36</sup>

### Nano-Se as a promising orthopedic implant material and an agent reducing bone cancer cell functions

Currently used metallic orthopedic implants possess certain problems such as poor prolonged osseointegration, stress shielding,<sup>149</sup> and wear debris-associated bone cell death.<sup>150</sup> However, the greatest concern is corrosion as a result of continuous tissue exposure to metal. This limits orthopedic implant efficacy, especially in patients receiving implants due to bone cancer.<sup>151</sup> Unfortunately, current orthopedic materials are not designed to prevent the occurrence or reoccurrence of cancer.<sup>150</sup> Selenium, due to its chemopreventive and chemotherapeutic properties,<sup>150,152–154</sup> appears to be a promising anticancer biocompatible orthopedic implant material.<sup>151</sup> Moreover, SeNPs may be used to effectively prevent and treat *S. aureus*<sup>155</sup> and *S. epidermidis*<sup>114</sup> infections, which are one of the leading causes of implant failure.<sup>155</sup> In addition, selenium did not evince inhibitory effect on the osteoblastic cell growth.<sup>156</sup> Perla and Webster<sup>151</sup> reported increased osteoblast adhesion on particulate surfaces of the compacts made from selenium compared with nonparticulate wrought titanium sheets. Moreover, osteoblast density was further increased on the surfaces of the selenium compacts

with nanometer particles. Tran et al<sup>150</sup> fabricated titanium orthopedic material coated with a high-density selenium nanoclusters-doped surface. This novel biomaterial inhibited, compared to traditional untreated titanium, cancerous bone cell proliferation while promoting healthy bone cell functions (including adhesion, proliferation, alkaline phosphatase activity, and calcium deposition).

SeNPs precipitated on a common orthopedic tissue engineering material poly-L-lactic acid evinced, without simultaneous use of chemotherapeutics or pharmaceutical agents, selectively decreased long-term osteosarcoma cell density while promoting healthy, noncancerous, osteoblast functions (for instance, up to 2 times higher alkaline phosphatase activity on selenium-coated tissue culture plates compared to osteoblasts grown on typical tissue culture plates), which is an impetus for follow-up studies to replace tumorous bone tissue with healthy bone tissue.<sup>157</sup>

## Effect of SeNPs on oxidative stress parameters

The effect of Nano-Se on oxidative stress parameters was compared with the effect of organically bound selenium in a study. Nano-Se was found to have a comparable efficiency in increasing plasma GPx activity in mice as SeMet but exhibited much lower toxicity assessed on the basis of LD<sub>50</sub>, acute liver injury, and short-term toxicity. The results of the study showed that the nanoscale selenium can be administered as an antioxidant with a reduced risk of selenium toxicity.<sup>22</sup> Upregulation of selenoenzymes by elemental Nano-Se is also comparable to the effect of selenite and Se-MetSeCys, again with a significant reduction of acute toxicity.<sup>23,158,159</sup>

When comparing the effect of SeNPs (red selenium) (orally 1 mg·kg<sup>-1</sup> BW) and inorganic selenium (Na<sub>2</sub>SeO<sub>3</sub>) (orally 1 mg Se·kg<sup>-1</sup> BW) on antioxidant activities of neutrophils and hematological parameters in sheep for 30 days, it was found that on the 30th day thiobarbituric acid-reactive substance levels were significantly higher in both groups than in control animals, in contrary to the expected decrease in their levels. There were no significant differences between the packed cell volume and red blood cell count between the experimental groups and control group. The white blood cell count in the Nano-Se group showed a significant increase on the 20th and 30th days, and in the Na<sub>2</sub>SeO<sub>3</sub> group on day 20, compared to the control group. There was also a significant increase in neutrophil count and a significant decrease in lymphocyte count on day 10 in the Nano-Se group compared to the second experimental group and the control group, and on the 20th and 30th days in both the experimental groups compared to the control group.<sup>12</sup>

The effect of SeNPs on heat shock proteins (HSPs) and *HSP90* gene expression as additional oxidative stress parameters was investigated. Increased oxygen metabolism induces the formation of ROS.<sup>160</sup> Intensive training of trotter horses can lead to oxidative stress, the formation of ROS, and consequently lipid, protein, and DNA damage.<sup>161</sup> In addition to adaptive changes in protective enzymes (SOD, catalase [CAT],<sup>162</sup> GPx<sup>163</sup>), oxidative stress to cells is known to induce increased production of stress or HSPs.<sup>162</sup> Expression of HSPs is an adaptive mechanism against the disruption of cellular homeostasis<sup>164</sup> and integrity<sup>165</sup> during physical exercise.

A study of the effect of oral administration of SeNPs at 0.5 mg·kg<sup>-1</sup> concentration for 10 days on the expression of genes encoding HSP90 during intensive training in donkeys was performed.<sup>160</sup>

To assess the expression of the *HSP90* gene in gluteus medius muscle, the total RNA was amplified by semiquantitative reverse transcription polymerase chain reaction. The results showed that serum concentration of selenium increased and the expression of the *HSP90* gene decreased during rest after workload in the control group. As a response to intense exercise, in the experimental group with the 10-day administration of the SeNPs, both *HSP90* expression and serum selenium concentration significantly increased. The induction of HSP resulting from SeNPs administration protected the cells from otherwise lethal stress levels. The result may explain the positive effect of short-term oral supplementation of SeNPs to donkeys on cell stability under stress conditions such as intensive training.<sup>160</sup> Excessive dose of the antioxidants in combination with exercise may result in increased oxidative stress and impair exercise-induced adaptation, including direct induction of HSPs.<sup>166</sup> Kojouri et al<sup>160</sup> showed that oral supplementation of SeNPs in donkeys increased the expression of *HSP90* in the gluteal muscle after 24 hours of rest compared to the control group. Expression of *HSP90* induced by supplementation of SeNPs can alter the buffering capacity of the cells in response to stressful conditions and protects them from further damage. The epigenetic effect of SeNPs and mechanism of their action on gene expression remains misunderstood.

In addition, the effect of SeNPs on blood urea nitrogen (BUN) in donkeys was investigated. It is known that urea in high concentrations causes oxidative stress and DNA lesions in cells.<sup>167</sup> A study of the effect of oral administration of SeNPs on changes in BUN, creatinine, and total protein during intensive training in donkeys revealed that serum selenium concentration was significantly increased after SeNPs supplementation.<sup>29</sup> The creatinine concentration in the experimental and control groups was significantly increased

in 2 hours of rest after training and rapidly declined in 72 hours of rest after workout in the experimental group. A similar pattern was obtained with changes in BUN in the control group: its concentration was significantly increased in 2 hours of rest after training compared to the group which was dosed with SeNPs. These findings can explain the positive effects of SeNPs supplementation on serum changes in BUN levels and creatinine in response to intense training of donkeys. The positive effect of SeNPs could be related to the incorporation of selenium into proteins, such as seleno-cysteine (SeCys) and its preventive role in oxidative tissue damage.<sup>29</sup>

### Protective effects of Nano-Se SeNPs in prevention of cisplatin (CIS)-induced reproductive toxicity

CIS, an anticancer alkylating agent,<sup>168</sup> is widely used for cancer treatment.<sup>127,169–172</sup> Despite its abundant clinical use, CIS possesses many side effects. It is known that CIS induces DNA adducts<sup>173</sup> and forms DNA cross-links<sup>168</sup> which interfere with cellular metabolism, such as DNA replication and transcription, triggering cell death.

Rezvanfar et al<sup>168</sup> demonstrated that Nano-Se can be, due to its strong antioxidant potential, useful to prevent CIS-induced gonadotoxicity. Coadministration of SeNPs significantly improved the serum testosterone, sperm quality, and spermatogenesis and reduced CIS-induced free radical toxic stress and spermatogenic DNA damage in male rats.

### Protective effect of Nano-Se against polycyclic aromatic hydrocarbons

In mice exposed to oxidative stress induced by polycyclic aromatic hydrocarbon – 7,12-dimethylbenz(a)anthracene (DMBA), a known immunotoxin and carcinogen,<sup>174,175</sup> which were fed with lamb meat supplemented with selenium in the nanoform, the protective effect of Nano-Se against DMBA-induced immunotoxicity was observed. Compared to the control group of mice, also exposed to DMBA, survival of 2 times higher amount of leucocytes (of which 3 times higher amount of phagocytes) was found, and their recovery in the bone marrow was twice higher, and the regenerative capacity of granulopoiesis was 4 times higher. The study showed the functional dietary benefits of lamb meat enriched with selenium obtained by feeding lambs SeNPs.<sup>176</sup>

### Use of SeNPs for minimization of risk of iron overabundance

An in vivo study compared the effect of SeNPs and Na<sub>2</sub>SeO<sub>3</sub> (both were administered at a dose of 1 mg·kg<sup>-1</sup> BW for 30 days)

on iron homeostasis and expression of genes coding for transferrin and transferrin receptor in sheep.<sup>7</sup> The study showed that at the beginning of the administration selenium decreased the serum iron concentration and increased the total iron-binding capacity (TIBC). In addition, after 30 days, a reduction of the iron level in blood serum was observed. The group of animals dosed with SeNPs showed a significantly increased TIBC level after 20 days compared to the Na<sub>2</sub>SeO<sub>3</sub> and control groups, and significantly reduced iron serum level in comparison to the control group. At the beginning, the selenium administration stimulated the expression of the transferrin gene as well as the transferrin receptor gene, but at the end it suppressed their expression. This particular phenomenon was especially observed in animals that received Na<sub>2</sub>SeO<sub>3</sub>. This effect could be related to hypoferremia and cellular internalization of iron. Expression of both genes was significantly increased in the SeNPs group after 20 days compared to the Na<sub>2</sub>SeO<sub>3</sub> group.

### SeNPs in treatment of heavy metal intoxication

SeNPs were found to be a promising agent to check the chronic toxicity caused due to heavy metals exposure. Prasad and Selvaraj<sup>42</sup> studied the effect of SeNPs, synthesized using *T. arjuna* leaf extract, on human lymphocytes treated with arsenite (As<sup>+III</sup>). Studies on cell viability using MTT assay and DNA damage using comet assay revealed protective effect of SeNPs against As<sup>+III</sup>-induced cell death and DNA damage. This approach could be used in future for minimizing arsenic-induced ROS-mediated toxic hazard, especially in an area with arsenic-contaminated groundwater and prevalence of arsenicosis.

The protective ability of SeNPs was also found against hexavalent chromium-induced thyrotoxicity.<sup>43</sup> Toxic effect as a result of oxidative damage provoked by an intraperitoneal administration of single dose of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>; 60 µg·kg<sup>-1</sup> BW) to rats was manifested by significant decrease in free T<sub>3</sub> (triiodothyronine) and T<sub>4</sub> (thyroxine) and glutathione levels, and significant increases in CAT, SOD, and MDA in the chromium-treated group compared to the controls. SeNPs administration resulted in correcting the hormonal levels as well as oxidative stress biomarkers compared to the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-treated group.

Selenium also evinces protective effect against cadmium-induced nephrotoxicity.<sup>41</sup> Two-week oral administration of Na<sub>2</sub>SeO<sub>3</sub> to rats treated with repeated intraperitoneal injection of Cd at sublethal dose (1.50 mg Cd·kg<sup>-1</sup> BW for 14 days) reduced lipid peroxidation and restored GPx and SOD activities in the kidneys. Selenium supplementation facilitated



renal Cd accumulation in this group compared to Cd-exposed rats without selenium intake. Interestingly, X-ray diffraction analysis carried out on kidney fractions revealed CdSe and/or cadmium sulfide (CdS) NPs (about 62 nm in size). This indicates that Cd may induce the biosynthesis of red fluorescent CdSe and CdS NPs in the kidneys. The reduction of Cd-induced renal toxicity through selenium administration seems to lie in its ability to bind Cd in nanosized insoluble and fluorescent complexes. This implies that Cd complexation with Se or S at a nanoscale level could reduce oxidative stress induced by cadmium in the kidneys.

## Nano-Se as an immunostimulator

Chronic oxidative stress reduces the respiratory burst response of neutrophils.<sup>177</sup> Nano-Se, possessing an immunostimulating potential,<sup>44</sup> evinces, in comparison with Na<sub>2</sub>SeO<sub>3</sub>, a stronger and faster effect in supporting the antioxidant defense system, based on increased chemotactic activity and respiratory burst activities of neutrophils.<sup>8</sup> The effect of nanoscale selenium and Na<sub>2</sub>SeO<sub>3</sub> (both supplements were administered at 1 mg·kg<sup>-1</sup> BW, per os for 30 days) on neutrophils' characteristics in sheep was compared. To determine the chemotactic activity and respiratory burst activities of the neutrophils, the leading front assay and the nitroblue tetrazolium (NBT) test (it is based on the reduction of the oxidant substances inside the phagocytic cells by NBT revealed by color change) were carried out on heparinized blood samples. It was found that in the Nano-Se group and the Na<sub>2</sub>SeO<sub>3</sub> group, on the 10th, 20th, and 30th days, the neutrophil chemotactic activity increased significantly compared to its basal value. In contrast to the Nano-Se group, in animals supplemented with an inorganic form of selenium, chemotactic activity at day 30 compared to day 20 decreased significantly. In the SeNPs group, a significant increase was observed after 10, 20, and 30 days compared to the control group and at 10 days as compared to the Na<sub>2</sub>SeO<sub>3</sub> group (increase on day 10 was 24% in Nano-Se group and 5% in the group administered with Na<sub>2</sub>SeO<sub>3</sub>, compared to basal levels). In the Na<sub>2</sub>SeO<sub>3</sub> group, a significant increase in chemotactic activity was observed after 20 days compared to the control group. Respiratory burst activity value showed a significant increase in both groups from day 0 to day 30.

## Effect of Nano-Se on microbial fermentation, nutrients digestibility, and probiotics support

The nanoscale selenium has also shown positive effects on rumen fermentation and increased nutrient conversion.

A study of the effect of Nano-Se and selenium yeast (Se-yeast) supplements (both at a dose of 4 g·kg<sup>-1</sup> feed DM to meet the need for 4 mg Se) on food digestibility, ruminant fermentation, and urine purine derivatives in sheep<sup>14</sup> demonstrated a decrease in ruminal pH, ammonia nitrogen concentration, molar amount of propionate, and acetate/propionate ratio. In addition, an increase in total ruminal volatile fatty acids in the Nano-Se and Se-yeast groups was also observed. Selenium intake significantly improved the in situ degradation of ruminal amylase-treated neutral detergent fiber (aNDF) from *Leymus chinensis* and crude protein (CP) from soybean meal. The digestibility of DM, organic matter, CP, ethereal extract, aNDF, and acid detergent fiber throughout the digestive tract and urinary excretion of purine derivatives were also significantly affected by the addition of selenium. Ruminal fermentation was improved and feed conversion efficiency increased with Nano-Se compared to Se-yeast. Nano-Se can be considered as a preferentially available source of selenium for ruminants.<sup>14</sup>

Kheradmand et al<sup>40</sup> investigated the effect of Nano-Se on antifungal activity of probiotics on *Candida albicans*, usually a commensal organism in humans, which can become pathogenic especially in immunocompromised individuals. After exposure to SeNPs-enriched *Lactobacillus* spp. (*Lactobacillus plantarum* and *Lactobacillus johnsonii*), a greater decline in viability of *C. albicans* than after its exposure to non-Se-enriched LAB was observed. This indicates an increase in the antifungal activity of both bacterial strains by means of selenium. This phenomenon could potentially be used in anti-*Candida* probiotic formulations in future.

## Nano-Se in treatment of metabolic disorders

Recently, promising nanocarriers for oral delivery of antidiabetic supplement to potentiate its curative effect were developed. Yin et al<sup>45</sup> designed selenium-coated nanostructured lipid carriers (SeNLCs) for enhancing the oral bioavailability and strengthening the hypoglycemic action of berberine, an antidiabetic phytomedicine. Berberine-loaded SeNLCs administered orally to rats greatly enhanced the bioavailability of berberine 6.6 times and evinced significantly higher hypoglycemic effect than berberine solution as well as berberine-loaded nanostructured lipid carriers. Both properties were improved due to better sustained drug release and intestinal absorption of selenium nanocarriers.

Nano-Se has also been shown to be efficacious in the treatment of fatty liver disease (FLD). Hepatic steatosis (FLD) or fatty liver is a sign of a metabolic disorder affecting

50% of dairy cows immediately after calving, caused by ectopic fat deposition in the liver.<sup>178</sup> Fatty liver can cause an inflammatory response in the liver that can lead to permanent liver damage and even to death. Without proper treatment, the mortality may reach up to 25%.<sup>179</sup> Apart from changes in dietetic arrangement, the application of Nano-Se may be effective for the therapy of this disease.<sup>180</sup> Hegedüs et al<sup>47</sup> reported a study of the effect of Nano-Se on FLD therapy in male Wistar rats which showed a low level of inflammation and free radical release in sick animals compared to the control group. This was confirmed by transmethylation ability and histological analysis of samples.<sup>47</sup> Bioactive nanoscale selenium appears to be effective, but confirmation of its therapeutic effect requires further experiments.<sup>180</sup>

## Safety and toxicity concerns of orally delivered SeNPs for use as food additives and drug carriers

Despite the growing interest of scientists on SeNPs and reporting of their wide range of positive effects, there are some concerns about their toxicity and objections to their use in clinical practice.

Many studies have demonstrated that the administration of selenium can prevent cancer and reduce its incidence.<sup>35,181,182</sup> Moreover, another potential of selenium was conclusively showed in the fight against cancer – through combination with chemotherapeutic and hormonal agents.<sup>183,184</sup> Numerous investigations have shown that both tissue and cell distribution profiles of anticancer drugs could be improved by nanotechnology.<sup>35,185,186</sup> Nanosized anticancer drugs displayed increased antitumor efficiency and reduced serious side effects.<sup>187</sup> It was revealed that selenium can sensitize cancer cells to conventionally used anticancer drugs. For example, Hu et al<sup>183</sup> reported that selenium sensitized hormone-refractory prostate cancer cells to apoptosis induced by anticancer drug paclitaxel (Taxol) through enhancing multiple caspases. Li et al<sup>184</sup> showed that the combination of doxorubicin with selenium enhanced apoptosis in the MCF-7 human breast cancer cell line.

However, selenium evinces a narrow margin between beneficial and toxic effects. An effective dose of selenium as an anticancer agent approaches the toxicity limit, which substantially limits its clinical application. Nevertheless, the beneficial and toxic effects of selenium on health are greatly dependent not only on its concentration but also on its chemical form.<sup>35</sup> While some studies reported that exceeding the tolerable upper intake level of 400  $\mu\text{g}\cdot\text{day}^{-1}$  in humans can lead to selenosis,<sup>179,180</sup> Reid et al<sup>181,182,188</sup> observed no obvious

symptoms of selenosis in patients receiving 1,600  $\mu\text{g Se}\cdot\text{day}^{-1}$  in the form of selenized yeast with SeMet as the major selenium species. Even in those with an intake of 3,200  $\text{Se}\cdot\text{day}^{-1}$ , only some symptoms of selenium toxicity did occur.

Studies performed with Nano-Se revealed that elemental selenium at nanoscale is much less toxic in comparison with the organic selenium compounds, such as SeMet<sup>22</sup> and Se-MetSeCys,<sup>23</sup> and exhibited comparable efficacy to them in upregulating selenoenzymes and tissue selenium levels.

The *in vitro* free radical-scavenging efficiency of SeNPs was also found higher than organic and inorganic selenocompounds.<sup>22,159</sup> For instance, based on the data of  $\text{LD}_{50}$ , the *in vivo* toxicity of SeNPs was about 4–6 times lower than SeMet and Se-MetSeCys.<sup>22,23,159</sup> The higher toxicity of selenite, SeCys2, and  $\text{SeO}_2$  is associated with their ability to initiate the oxidation of the thiol groups of proteins,<sup>189</sup> which may lead to a change in the activity of essential enzymes containing the sulfhydryl group.<sup>190</sup> Selenite, compared to the same dose of Nano-Se, more markedly reduces hepatic GPx level and increases the production of MDA which is the product of lipid peroxidation as well as reducing the activity of antioxidant enzymes SOD and CAT in the liver, than the same dose of nanoscale selenium.<sup>159</sup> Wang et al investigated the effect of SeMet in mice which, compared to those with Nano-Se supplementation, had strongly elevated levels of liver enzymes such as alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase in the blood, over a long period of time, indicating acute severe liver injury.<sup>22</sup> Acute toxicity due to Nano-Se occurs at a much higher dose compared to organically bound selenium forms. The median lethal dose ( $\text{LD}_{50}$ ) is 92.1  $\text{mg Se}\cdot\text{kg}^{-1}$  for Nano-Se, 25.6  $\text{mg Se}\cdot\text{kg}^{-1}$  for SeMet,<sup>22</sup> and 14.6  $\text{mg Se}\cdot\text{kg}^{-1}$  for Se-MetSeCys.<sup>23</sup>

Studies showed that Nano-Se has a similar bioavailability in rats and much less acute toxicity in mice compared with selenite.<sup>191</sup> The dose of red elemental Nano-Se that causes acute toxicity was approximately seven times that of  $\text{Na}_2\text{SeO}_3$ : the  $\text{LD}_{50}$  was about 113 and 16  $\text{mg Se}\cdot\text{kg}^{-1}$  BW, respectively. The reaction ratio of red elemental Nano-Se with glutathione *in vitro* was one-tenth of that of  $\text{Na}_2\text{SeO}_3$ .<sup>192</sup>

Moreover, the design of SeNPs-loaded CS microspheres (SeNPs-M) can offer a new way for further development of SeNPs with a higher efficacy and better biosafety.<sup>115</sup> Based on acute toxicity test, SeNPs-M were much safer than selenite; the  $\text{LD}_{50}$  was around 18-fold of selenite. Furthermore, SeNPs-M possessed a strong antioxidant activity, as evidenced by a dramatic increase in selenium retention and also in the activities of GPx, SOD, and CAT. Oral administration of SeNPs-M can be considered as an effective way to supply selenium.

Mittal et al<sup>193</sup> synthesized bimetallic (Ag-Se) NPs which were used as anticancer agents for Dalton lymphoma cells. The Ag-Se NPs evinced strong anticancer activity at a lower concentration. In vitro viability of cells of the cancer line was reduced to 20% at 50  $\mu\text{g}\cdot\text{mL}^{-1}$  Ag-SeNPs.

Nano-Se is also less toxic than high-selenium protein. A study of the subchronic toxicity of these selenium forms performed in Sprague-Dawley rats<sup>191</sup> (in both genders) fed with diets containing individual compounds at different concentrations (0, 2, 3, 4, and 5  $\text{mg}\cdot\text{kg}^{-1}$  Se) for 13 weeks showed significant abnormal changes in BW, hematology, clinical chemistry, relative organ weights, and histopathology parameters at the doses of 4 and 5  $\text{mg}\cdot\text{kg}^{-1}$  Se. The toxicity was more pronounced in the selenite and high-selenium protein groups than in the Nano-Se group, at the dose of 3  $\text{mg}\cdot\text{kg}^{-1}$  Se. Significant growth inhibition and degeneration of hepatic cells were found in the selenite and high-selenium protein groups. On the other hand, no changes attributable to administration of Nano-Se at the dose of 3  $\text{mg}\cdot\text{kg}^{-1}$  Se were found. No-observed-adverse-effect level (NOAEL; the highest experimental point that is without adverse effect) of Nano-Se in male and female rats was considered to be 3  $\text{mg}\cdot\text{kg}^{-1}$  (equivalent to 0.22 and 0.33  $\text{mg}\cdot\text{kg}^{-1}$  BW $\cdot\text{day}^{-1}$  for males and females, respectively). On the contrary, the NOAELs of selenite and high-selenium protein in both genders were considered to be 2  $\text{mg}\cdot\text{kg}^{-1}$  (equivalent to 0.14 and 0.20  $\text{mg}\cdot\text{kg}^{-1}$  BW $\cdot\text{day}^{-1}$  for males and females, respectively).<sup>191</sup>

Therefore, it can be assumed that SeNPs have a much wider margin between beneficial and toxic effects than other selenocompounds and could serve as a suitable potential chemopreventive agent with reduced risk of toxicity.<sup>35</sup>

Higher  $\text{IC}_{50}$  of the SeNPs ( $41.5 \pm 0.9 \mu\text{g}\cdot\text{mL}^{-1}$ ) compared to  $\text{SeO}_2$  ( $6.7 \pm 0.8 \mu\text{g}\cdot\text{mL}^{-1}$ ) confirmed the lower cytotoxicity of the biogenic SeNPs on MCF-7 cell line.<sup>89</sup>

Regarding testing toxicity in mammalian models, Benko et al<sup>194</sup> compared the toxicity of different selenium species in mice which were administered for 14 days at concentrations of 0.5, 5, and 50  $\text{mg}\cdot\text{kg}^{-1}$  food, corresponding to an estimated 4, 40, and 400  $\text{mg}\cdot\text{kg}^{-1}$  BW $\cdot\text{day}^{-1}$ , respectively. A study on 14-day murine subacute toxicity showed that toxicity was more pronounced when an inorganic selenium ( $\text{Na}_2\text{SeO}_4$ ,  $\text{NaHSeO}_3$ ) was applied than after subacute application of Sel-Plex (a natural source of organic selenium consisting of predominantly selenoaminoacids [SeCys, SeMet] produced by *S. cerevisiae*), Nano-Se (synthesized by yogurt strains *L. acidophilus*, *S. thermophilus*, and *L. casei*), or LactoMicroSe (selenium-enriched yogurt powder – *L. acidophilus*, *S. thermophilus*, and *L. casei*). The toxicity of selenium species decreased in the following

order: selenate > selenite > Nano-Se > Sel-Plex > Lacto-MicroSe. He et al<sup>195</sup> reported a study on toxicity of Nano-Se in rats showing that supranutritional levels of SeNPs (at doses of 0.2, 0.4, 0.8, 2.0, 4.0, or 8.0  $\text{mg}\cdot\text{kg}^{-1}$  BW administered orally each day for 14 consecutive days) had no obvious toxic effects, and could be considered as potential candidates for cancer chemoprevention, although doses greater than 2.0  $\text{mg}\cdot\text{kg}^{-1}$  BW induced chronic toxicity.

Shakibaie et al<sup>196</sup> investigated the acute and subacute toxicity of the biogenic SeNPs synthesized by *Bacillus* sp. MSh-1 and  $\text{SeO}_2$  in mice. The biogenic SeNPs were much less (26-fold) toxic than the  $\text{SeO}_2$ . The toxicological evaluation showed that the  $\text{LD}_{50}$  values of  $\text{SeO}_2$  and SeNPs were 7.3 and 198.1  $\text{mg}\cdot\text{kg}^{-1}$ , respectively. No biochemical changes were observed from the administration of 2.5, 5, and 10  $\text{mg}\cdot\text{kg}^{-1}$  SeNPs, but a dose of 20  $\text{mg}\cdot\text{kg}^{-1}$  was accompanied with signs of toxicity including lower BW and changes in clinical chemistry and hematological parameters.

On the other side, studies on the toxicity of Nano-Se to aquatic organisms with relatively contradictory results appeared. Li et al<sup>137</sup> found that a 10-day exposure to SeNPs at a dosage of 100  $\mu\text{g}\cdot\text{L}^{-1}$  compared to the same amount of  $\text{Na}_2\text{SeO}_3$  in Medaka (*Oryzias latipes*) fish showed a greater toxicity due to hyperaccumulation. Gallego-Gallegos et al<sup>197</sup> evaluated the toxicity of SeNPs using 10-day waterborne and dietary exposures to larvae of *Chironomus dilutus*, a common benthic midge frequently used as a test organism for assessing the toxicity of sedimentary substances. They reported that even the lowest  $\text{Se}^0$  and SeNPs concentrations tested (2.81  $\mu\text{g}\cdot\text{L}^{-1}$  and 8.89  $\mu\text{g}\cdot\text{g}^{-1}$  dry weight, respectively) resulted in selenium bioaccumulation especially as SeMet. Inhibition of larval growth at higher concentrations due to both dietary and waterborne exposure was also observed.

However, both previous toxicological studies used chemically prepared NPs. Newer research works showed that the toxicity of Nano-Se also depends on the method of preparation; for instance, biogenic Nano-Se in comparison with that chemogenic is less toxic. Mal et al<sup>198</sup> investigated the toxicity of biogenic Nano-Se formed by anaerobic granular sludge biofilms on zebrafish (*Danio rerio*) embryos in comparison with selenite and chemogenic Nano-Se. The biogenic Nano-Se formed by granular sludge biofilms showed an  $\text{LC}_{50}$  (50% lethal concentration) value of 1.77  $\text{mg}\cdot\text{L}^{-1}$ , which was 3.2-fold less toxic to zebrafish embryos than selenite ( $\text{LC}_{50} = 0.55 \text{mg}\cdot\text{L}^{-1}$ ) and 10-fold less toxic than chemogenic, bovine serum albumin-stabilized Nano-Se ( $\text{LC}_{50} = 0.16 \text{mg}\cdot\text{L}^{-1}$ ). Interestingly, chemically synthesized Nano-Se particles of small (25–80 nm) and large (50–250 nm) size showed comparable toxicity on zebrafish embryos.

Moreover, a study of Khiralla and El-Deeb<sup>199</sup> also did not show any high toxicity of SeNPs biosynthesized by *B. licheniformis* assayed using larvae of *Artemia salina*, which have been suggested as a model organism in toxicity assessment of NPs.<sup>200</sup> No toxicity on *Artemia* larvae was demonstrated by SeNPs up to 100 µg·mL<sup>-1</sup>.

Furthermore, the method of extraction of biogenic NPs substantially affects the toxicity of SeNPs. Sonkusre et al<sup>34</sup> reported a novel method for the extraction and purification of intracellular SeNPs from the Gram-positive bacteria *B. licheniformis* JS2. These neutral-charged, non-agglomerating NPs at a very low concentration as low as 2 µg Se·mL<sup>-1</sup> were efficacious in inhibiting proliferation and excellent in inducing caspase-independent necrosis of human prostate adenocarcinoma cells (PC3) without causing any significant toxicity to human peripheral blood mononuclear cells.

## Conclusion

Selenium is an important essential element that interferes through selenoproteins in many physiological processes of the organism and affects the production and reproductive properties. By providing adequate supply of selenium in the diet, it is possible to effectively prevent health problems from its deficiency. Due to its high bioavailability, low toxicity and affordability, selenium in its nanoform appears to be the most appropriate for supplementation, especially in ruminants, in which traditionally used selenium compounds exhibit very low absorption in the digestive tract.

Future perspective is the possibility of global application of nanoscale selenium in nutrition as well as in clinical medicine. The development of new NP systems for the transport of selenium in the organism, with the possibility of modifying physicochemical properties of the particles, greater stability in the gastrointestinal tract, and allowing controlled release of selenium, offers a significant dietary and therapeutic potential. Although there are currently some concerns about the use of SeNPs for therapeutic purposes, many scientific studies suggested that many of these generally prevailing doubts have not been confirmed. However, it is still necessary to carry out further preclinical studies in animal models.

## Acknowledgments

This work was supported by the project for conceptual development of the research organization. The authors would like to thank The European Technology Platform for Nanomedicine for their cooperation.

## Author contributions

Bozena Hosnedlova performed the literature search, wrote the manuscript, and drew figures. Marta Kepinska, Sylvie Skalickova, Carlos Fernandez, Branislav Ruttkay-Nedecky, Qiuming Peng, Mojmir Baron, Magdalena Melcova, Radka Opatrilova, Jarmila Zidkova, Geir Bjørklund, and Jiri Sochor participated in writing and correction of the manuscript. Rene Kizek conceived the idea for this topic, proposed the concept, performed the literature search, and participated in writing and critical revision of the manuscript. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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