Nanostructured biomedical selenium at the biological interface (Review)
Victoria le Ching Tan, Angelica Hinchman, Richard Williams, Phong A. Tran, and Kate Fox

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Nanostructured biomedical selenium at the biological interface (Review)

This paper critically reviews the current evidence of research in biomedical applications of selenium nanoparticles (SeNPs) and their effects at cellular and tissue levels. In recent years, interest in SeNPs as a natural trace element nanomaterial for nanomedicine has resulted in a number of studies evaluating their bioactivities, such as anticancer, antimicrobial, and antioxidant properties. Significant data have been generated to demonstrate the effectiveness of SeNPs alone or in combination with other reagents. Their activities are demonstrated through in vitro and in vivo experimentation; yet, the levels of efficacy need to be improved, particularly when compared with those of pharmaceutical drugs (such as antibiotics and cytotoxic chemotherapeutic drugs). However, promising evidence suggests decreased toxicity when using SeNPs, and more importantly their ability to perform as an interfacing biomaterial with cells and tissues. SeNPs have demonstrated unique antibacterial properties: they inhibit bacterial adhesion, growth, and/or quorum sensing and as a result prevent biofilm formation on medical devices, to name a few. Therefore, as with other nanomaterials, SeNPs warrant further study as part of the biomaterial-based therapeutic toolkit as an alternative to traditional pharmaceutical agents. This paper will provide a succinct review of recent studies on SeNPs to critically assess the findings in the light of effectiveness, particularly highlighting the roles of the cellular interface. Finally, an outlook of the potential of SeNPs will be presented to highlight the need for more intensive studies of material stability, mechanistic understanding at subcellular levels, and investigations into their combinational and/or synergistic effects with other bioactive reagents including pharmaceutical drugs. © 2018 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1116/1.5042693

I. INTRODUCTION

Selenium nanoparticles (SeNPs) are promising candidates for development in biomedical applications. Selenium is an essential microelement in mammals and is present in 25 selenoenzymes. It functions as a cofactor for glutathione peroxidases and thioredoxin reductases, with remarkable growth promoting activities including pharmaceutical drugs (such as antibiotics and cytotoxic chemotherapeutic drugs). However, promising evidence suggests decreased toxicity when using SeNPs, and more importantly their ability to perform as an interfacing biomaterial with cells and tissues. SeNPs have demonstrated unique antibacterial properties: they inhibit bacterial adhesion, growth, and/or quorum sensing and as a result prevent biofilm formation on medical devices, to name a few. Therefore, as with other nanomaterials, SeNPs warrant further study as part of the biomaterial-based therapeutic toolkit as an alternative to traditional pharmaceutical agents. This paper will provide a succinct review of recent studies on SeNPs to critically assess the findings in the light of effectiveness, particularly highlighting the roles of the cellular interface. Finally, an outlook of the potential of SeNPs will be presented to highlight the need for more intensive studies of material stability, mechanistic understanding at subcellular levels, and investigations into their combinational and/or synergistic effects with other bioactive reagents including pharmaceutical drugs. © 2018 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1116/1.5042693

Phong A. Tran,1,6,7,d),e) and Kate Fox8,9,d),e)  

1Faculty of Medicine, The University of Queensland, Brisbane, Queensland 4072, Australia  
2Ochsner Clinical School, School of Medicine, The University of Queensland, Brisbane, Queensland 4072, Australia  
3School of Engineering, RMIT University, Carlton, Victoria 3000, Australia  
4Queensland University of Technology, 2 George Street, Brisbane, Queensland 4000, Australia  
5Institute of Health and Biomedical Innovation (IHBI), Queensland University of Technology, Brisbane, Queensland 4059, Australia  
6Interface Science and Materials Engineering Group, School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology, Brisbane, Queensland 4059, Australia  
7Center for Additive Manufacturing, RMIT University, Carlton, Victoria 3000, Australia  
8School of Engineering, RMIT University, Carlton, Victoria 3000, Australia  
9Faculty of Medicine, The University of Queensland, Brisbane, Queensland 4072, Australia

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This paper critically reviews the current evidence of research in biomedical applications of selenium nanoparticles (SeNPs) and their effects at cellular and tissue levels. In recent years, interest in SeNPs as a natural trace element nanomaterial for nanomedicine has resulted in a number of studies evaluating their bioactivities, such as anticancer, antimicrobial, and antioxidant properties. Significant data have been generated to demonstrate the effectiveness of SeNPs alone or in combination with other reagents. Their activities are demonstrated through in vitro and in vivo experimentation; yet, the levels of efficacy need to be improved, particularly when compared with those of pharmaceutical drugs (such as antibiotics and cytotoxic chemotherapeutic drugs). However, promising evidence suggests decreased toxicity when using SeNPs, and more importantly their ability to perform as an interfacing biomaterial with cells and tissues. SeNPs have demonstrated unique antibacterial properties: they inhibit bacterial adhesion, growth, and/or quorum sensing and as a result prevent biofilm formation on medical devices, to name a few. Therefore, as with other nanomaterials, SeNPs warrant further study as part of the biomaterial-based therapeutic toolkit as an alternative to traditional pharmaceutical agents. This paper will provide a succinct review of recent studies on SeNPs to critically assess the findings in the light of effectiveness, particularly highlighting the roles of the cellular interface. Finally, an outlook of the potential of SeNPs will be presented to highlight the need for more intensive studies of material stability, mechanistic understanding at subcellular levels, and investigations into their combinational and/or synergistic effects with other bioactive reagents including pharmaceutical drugs. © 2018 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1116/1.5042693

I. INTRODUCTION

Selenium nanoparticles (SeNPs) are promising candidates for development in biomedical applications. Selenium is an essential microelement in mammals and is present in 25 selenoenzymes. It functions as a cofactor for glutathione peroxidases and thioredoxin reductases, with remarkable growth promoting properties.1,2 The incorporation of selenium into nanoparticles has emerged as a promising approach to harness both the therapeutic benefits of Se and the unique properties of nanoparticles.3–5 Significant research efforts have seen SeNPs synthesized via physical techniques (UV radiation and laser ablation), chemical methods (catalytic reductions, acid decomposition, and precipitation), and biogenesis (localization within a microorganism).6 There are several advantages of nanoparticles as shown in Fig. 1. Nanoparticles can be targeted to exploit the enlarged and leaky blood vessels in tumors, which have pore sizes of 100–800 nm, as compared to those of healthy tissues which are much smaller at 2–6 nm.10 These nanoparticles with diameters between 10 and 100 nm thereby show enhanced permeation and retention, allowing them to penetrate tissues containing tumors and selectively kill cancerous cells while avoiding damage to the surrounding healthy tissue and cells. Their high surface area to volume ratio allows for a greater density of active sites for interactions (such as surface decoration/functionization with other molecules) compared to conventional micron-sized particles.11

Selenium is a naturally occurring element that is essential for the operation of multiple biological processes. The reductive
and oxidative effects of biologically synthesized selenoproteins are utilized in many systems within the body, including endocrine, reproductive, cardiovascular, and immune processes. The thyroid contains the highest concentration of selenium within the human body. The majority of the selenium consumed is stored here through sequestration via selenoprotein P (SePP), which transports selenium from the plasma and extracellular fluid and retains it in the thyroid. Within the thyroid, the selenoprotein, glutathione peroxidase, plays a vital role as a cofactor for iodothyronine deiodinase. This enzyme is responsible for the conversion of thyroxine (T4) into activated thyroid hormone, tri-iodothyronine (T3), which assists with the regulation of metabolic rate, bone maintenance, brain development, muscle control, mood, heart, and digestive function.

After the thyroid, the testes are the next most targeted organ for selenium sequestration by SePP. The testes are dependent on selenium for the formation and development of spermatocytes, by first protecting the developing sperm cells from oxidative stress and then polymerizing glutathione peroxidase into a structural protein that is later incorporated into the midpiece mitochondrial sheath thereby enabling sperm motility. The antioxidant effects of selenoprotein may also manage the production of eicosanoids and protect against hypertension associated with pre-eclampsia via regulation of vascular tone in pregnant women. Glutathione peroxidase’s effect on vascular tone also protects against cardiovascular diseases, such as atherosclerosis where it downregulates platelet aggregation inside the blood vessel by the breakdown of hydroperoxides. If uninhibited, the buildup of hydroperoxide promotes the production of thromboxane and thereby enhance vasoconstriction and platelet aggregation, increasing the risk of thrombus formation. In addition, the antioxidative effects of selenoprotein prevent trapping of phospholipids and cholesteryl esters of lipoproteins within the artery wall, thus impeding further inflammatory response within the vessels.

Selenoproteins also contribute immunomodulation via the upregulation of inflammatory mediators. Selenium has been associated with the increased density of interleukin-2 (IL-2) receptors on the surface of activated lymphocytes and natural killer (NK) cells allowing for increased binding and
upregulation of the JAK1/JAK3 intracellular pathway.\textsuperscript{12,14,15,18} IL-2 is an important cytokine that stimulates differentiation of Th1 cells and NK cells.\textsuperscript{12} Th1 cells then activate macrophages and cytotoxic T cells via the release of IFN-gamma. Cytotoxic T cells then go on to destroy viral infected and neoplastic cells by inducing apoptosis.\textsuperscript{18} This association may explain selenium’s inverse relationship to cancer incidence and mortality and chronic viral infections such as human immunodeficiency virus and hepatitis B and C.\textsuperscript{16} Selenium, in the form of glutathione peroxidase, is also protective against oxidative damage from ribonucleic acid (RNA)-viral genome that may lead to mutation or cellular damage.\textsuperscript{16}

II. SeNP INTERFACE: TARGETED APPLICATIONS

A. Role of selenium in antimicrobial applications

The discovery of the antibiotic penicillin, in 1928, by Alexander Fleming, has revolutionized medicine, drastically reducing infections and mortality. Since then, medicine has been equipped with an arsenal of various antimicrobials (antibiotics, antivirals, antifungals, and antiparasitics, to name a few) and it has become unfathomable to practice medicine without them. However, despite an early warning from Alexander Fleming regarding antibiotic resistance (penicillin-R \emph{Staphylococcus} identified in 1940), ongoing indiscriminate use of antimicrobials within the medical, agricultural, and food industries has led us into the global emergence of “superbugs”: multimicrobial resistant microorganisms which were becoming more widespread and harder to eliminate and treat using our very last line of potent antibiotics.\textsuperscript{19} In view of multiresistant antimicrobials, there has been a new urgency in seeking novel and alternative antimicrobial agents. Nanoparticles as potential broad-spectrum antimicrobials (including silver, gold, zinc, and iron nanoparticles) have been investigated extensively, as documented by a review by Yah and Simate.\textsuperscript{20} SeNPs have great potential as a broad-spectrum antimicrobial agent, with studies showing promising results against bacteria,\textsuperscript{6,21–29} viruses, fungi, and parasites.

The biomedical application of SeNP’s antibacterial properties has focused on the prevention of bacterial colonization on implanted medical devices. Notably, \textit{in situ} incorporation of SeNPs into common polymers used in medical devices [e.g., polyvinyl chloride (PVC), polyurethane, and silicone] was evaluated by Tran and Webster.\textsuperscript{30} Here, they demonstrated that the amount of antibacterial activity was directly correlated to Se-coating densities, which in turn were different in different polymers. The study also showed that SeNP coatings on the polymers significantly inhibited \emph{S. aureus} growth, compared to their respective uncoated polymers. A key finding highlighting the promise of this approach, they found that SeNP-coated PVC had greater antibacterial capacity compared to a commercially available Ag-coated PVC.\textsuperscript{30}

Biofilm formation by bacteria on medical device surfaces is an issue as they are hard to treat. SeNPs have been shown to inhibit biofilm formation.\textsuperscript{9,23,31–40} Wang and Webster\textsuperscript{38} had biosynthesized SeNPs using \emph{Bacillus licheniformis} JS2 which inhibited \emph{S. aureus} adherence, microcolony, and biofilm formation on different surfaces (polystyrene, glass, and catheter). Their study specifically alluded to the inhibition of bacterial adherence to the surfaces. These results have potential value in application as antibacterial coating on prostheses against \emph{S. aureus} and other medical devices against nosocomial infections.\textsuperscript{37}

In 2015, Shakibaie \textit{et al.}\textsuperscript{36} evaluated the antibiotic effects of biogenic elemental SeNPs against common biofilm-forming bacteria \emph{S. aureus}, \emph{Proteus mirabilis}, and \emph{Pseudomonas aeruginosa} which were clinically isolated from hospitalized-patient specimens in Iran. The elemental SeNPs were biosynthesized using \emph{Bacillus} sp. MSh-1, purified by the liquid–liquid extraction method, and characterized to be spherical in shape, with sizes ranging from 80 to 220 nm. A biofilm formation assay was first incubated with \emph{S. aureus}, \emph{P. mirabilis}, and \emph{P. aeruginosa} and then incubated with SeNPs and selenium dioxide (SeO\textsubscript{2}) at concentrations ranging from 0 to 16 \textmu g/mL. The results were expressed in biofilm formation (% of control); SeNPs were found to have reduced biofilm formation in \emph{S. aureus}, \emph{P. mirabilis}, and \emph{P. aeruginosa} samples (42%, 53%, and 34%, respectively), compared to controls, and SeO\textsubscript{2} reduced biofilm formation to 68%, 51%, and 55%, respectively. It was interesting to note that there was no significant difference (p \textless 0.05) in the reduction of biofilm formation between SeNPs and SeO\textsubscript{2}. However, the authors noted that SeNPs held an advantage over SeO\textsubscript{2} due to lower toxicity to human cells.\textsuperscript{3–5}

Nanoparticles as potential broad-spectrum antimicrobials (including silver, gold, zinc, and iron nanoparticles) have been investigated extensively.\textsuperscript{20} SeNPs have reported antimicrobial properties,\textsuperscript{6,21,28} with SeNPs having well documented antibacterial effects against common hospital pathogens, particularly \emph{S. aureus} and \emph{P. aeruginosa} (Fig. 2).\textsuperscript{9,23,26,28,31,36–38,41–44} The mechanism of SeNP’s antibacterial ability has been hypothesized to be due to oxidative stress\textsuperscript{45} and that SeNPs have a dual action, whereby they both disrupt the bacterial cellular and cytoplasmic membrane and cause deoxyribose nucleic acid (DNA) (Ref. 28). Tran and Webster\textsuperscript{39} demonstrated that elemental SeNPs strongly inhibited the growth of \emph{Staphylococcus aureus} by up to 60 times (compared to no treatment). While this preliminary finding can be considered low in microbiological terms, SeNPs have the capacity to be optimized further for more significant outcomes. One method for achieving this is to couple it with another antimicrobial element such as silver. In one example, Mittal \textit{et al.}\textsuperscript{46} synthesized bimetallic nanoparticles comprising both silver and selenium (Ag-SeNPs). Ag-SeNPs (30–35 nm) were surface-coated with bioactive flavonoids and phenolics, Quercetin (QC) and Gallic acid, to form Quercetin-Gallic Acid Ag-SeNPs (Qu-Gallic Acid@Ag-SeNPs). Measuring the zones of inhibition (mm) against Gram-negative \emph{Escherichia coli} (\textit{E. coli}) and Gram-positive \emph{Bacillus subtilis} (\textit{B. subtilis}) colonies on agar plates, they demonstrated that compared to the
antibacterial agent chloramphenicol (50 μg/ml) zone of inhibition (E. coli 24 mm, B. subtilis 31 mm), Qu-Gallic Acid@Ag-SeNPs (50 μg/ml) had comparable antibacterial activity for both strains of bacteria (E. coli 18 mm, B. subtilis 19 mm) and superior to both silver nitrate and sodium selenite (25 μg/ml each) (E. coli 11 mm, B. subtilis 12 mm) (Table I). The likely mechanisms for this are via the nanoparticles binding to bacterial cell surface, releasing the silver to destroy the bacteria. It may also be due to the interaction of silver nanoparticles with bacterial membrane proteins and DNA, and affinity for sulfur and phosphorus compounds, resulting in damage of these compounds.46 As a result, it was clear that the role of SeNPs could potentially serve as an adjuvant therapy alongside conventional antibacterial agents such as chloramphenicol.

Table I. Chloramphenicol (50 μg/ml) exhibited the largest zone of inhibition (E. coli 24 mm, B. subtilis 31 mm), while Qu-Gallic Acid@Ag-SeNPs (50 μg/ml) had comparable antibacterial activity both strains of bacteria (E. coli 18 mm, B. subtilis 19 mm), followed by silver nitrate and sodium selenite only (E. coli 11 mm, B. subtilis 12 mm). There was no antibacterial activity in Quercetin, Gallic acid only (E. coli 0 mm, B. subtilis 0 mm). Adapted with permission from Mittal et al., J. Colloid Interface Sci. 431, 194 (2014). Copyright 2014, Elsevier (Ref. 46).

Antimicrobial activity of Ag-SeNPs

| S. no. | Name                                      | Escherichia coli | Bacillus subtilis |
|-------|-------------------------------------------|-----------------|------------------|
| 1     | Ag-SeNPs                                  | 18 ± 0.5        | 19 ± 0.6         |
| 2     | Silver nitrate + sodium selenite          | 11 ± 0.2        | 12 ± 0.3         |
| 3     | Chloramphenicol                           | 24 ± 0.2        | 31 ± 0.3         |
| 4     | Quercetin dihydrate + gallic acid         | 0.0             | 0.0              |

(Fig. 2) Currently explored applications of SeNPs showing their diverse range of applications; adapted with permission from Wadhwani et al., Appl. Microbiol. Biotechnol. 100, 2555 (2016). Copyright 2016, Springer Berlin Heidelberg (Ref. 6).
membranes. The presence of Ach (used as a permeability-active unit in antibacterial agents) on Qu-Ach@SeNPs and Qu@SeNPs was found to facilitate the SeNP penetration of the bacterial cell membrane, resulting in increased antibacterial activity. The authors had also conducted a zone of inhibition test against MDR E. coli and S. aureus which also suggested that Qu-Ach@SeNPs had the highest antibacterial activity (2 cm), followed by Ach@SeNPs, and lastly Qu@SeNPs. However, it is important to note that the SeNP concentrations used in this experiment (25 mg/ml) were much higher compared to the SeNP concentrations (50 μg/ml) used in Mittal et al., this may have implications on SeNP-related toxicity in normal human cells.

Based on these studies, there are three hypothesized mechanisms behind the antibacterial property of the SeNPs: (i) interruption of bacteria cell membrane integrity by SeNPs; (ii) penetration of bacterial cells and hence disruption of DNA structures by SeNPs; and (iii) increased generation of bacterial intracellular reactive oxygen species (ROS) induced by SeNPs. To investigate this, Huang et al. quantified the destructive effect of SeNPs on MDR E. coli and S. aureus bacterial cell membranes. The results showed that samples incubated with Qu-Ach@SeNPs (25 μg/ml) and Ach@SeNPs (25 μg/ml) had significantly increased total cell membrane permeability and cytoplasmic membrane permeability (*p* < 0.01) (Table V), suggesting that the SeNPs had damaged the cell membrane. Qu@SeNPs did not result in a significant increase in bacterial cell permeability. Referring to the scanning electron microscope and transmission electron microscope of the SeNPs on bacterial morphology, it is clear that Qu-Ach@SeNPs (25 μg/ml) and Ach@SeNPs (25 μg/ml) induced bacterial cell lysis in MDR E. coli and S. aureus, with leakage of intracellular contents [Figs. 3(c), 3(d), 3(g), and 3(h)], compared to that of the untreated bacteria [Figs. 3(a) and 3(e)]. Qu@SeNPs had also caused significant changes to MDR S. aureus but had no effect on MDR E. coli [Figs. 3(b) and 3(f)].

One of the three mechanisms of SeNPs antibacterial ability hypothesized was SeNP-induced generation of ROS, which causes bacterial cell death via oxidative damage. To test this hypothesis, Huang et al. measured total ROS concentrations at 30 and 60 min after MDR E. coli and S. aureus and SeNPs (25 μg/ml) were incubated with 2',7'-dichlorofluorescein diacetate and hydroxyl radical (a type of ROS) concentrations with hydroxyphenyl fluorescein. The results showed that Qu-Ach@SeNPs (25 μg/ml) significantly increased the concentration of ROS in bacterial cells (*p* < 0.01) compared to the negative control (assay without Qu-Ach@SeNPs), whereas both Qu@SeNPs (25 μg/ml) and Ach@SeNPs (25 μg/ml) did not show any significant increase (Table V). The results from this study further demonstrate the compatibility and ability to conjugate a surface molecule such as Ach which binds to the corresponding receptor on bacterial cell membranes, further enhancing the efficacy of SeNPs’ antibacterial properties. Importantly, this may present a novel therapeutic agent against MDR bacteria with hopes to ameliorate the severity of widespread antimicrobial resistance.

These findings motivated further studies to determine whether the reported results could be linked to a possible antimicrobial resistance of MDR bacteria to SeNPs. To test this, Qu-Ach@SeNPs and Ach-SeNPs were incubated with MDR E. coli and S. aureus cultures. The MIC values of both SeNPs remaining unchanged after 30 passages, suggesting that there was no emergence of resistance to Qu-Ach@SeNPs and Ach-SeNPs. This is likely due to the existence of the possible multiple antibacterial mechanisms as detailed above.

Taking advantage of a possible synergy between SeNPs and other compounds and exploration of antibacterial activity, Prateeksha et al. investigated a new approach.
via quorum sensing (QS) interruption by honey polyphenol-SeNPs (HP@SeNPs). QS is a chemical process of the cell-to-cell communication system utilized by human pathogen *P. aeruginosa* for regulation of biofilm formation and virulence. The HP@SeNPs demonstrated enhanced anti-QS, antibiofilm, and antivirulence against *P. aeruginosa* in both *in vitro* and *in vivo* studies compared to HP and SeNPs alone. Using mice skin excision wound models on Swiss albino mice, the artificial skin wound was infected with *P. aeruginosa*. The results showed that SeNP@HP (4.5 μg/ml)-treated mice had significantly higher wound healing percentages compared to the control group [treated with distilled water (DW) only] at days 15 and 21 postinfection. In the mortality test, mice were infected with *P. aeruginosa* via intraperitoneal injections with treatments administered similarly and then observed for a duration for 55 h. The control group (DW only) had 100% mortality after 35 h, while SeNP@HP (4.5 μg/ml) treated mice had 100% survival rate after 55 h. In this study, mechanisms behind HP@SeNPs’ anti-QS property and antivirulence were determined, linking antivirulence activity of HP@SeNPs to molecular binding between HP and QS receptor LasR. This study shows that SeNPs has a promising role as a unique drug delivery system to prevent biofilm formation via QS interference.

### B. Role of selenium in wound healing

The reported antibacterial properties of SeNP have been further extended into the area of wound dressing materials. Biswas et al.\(^48\) compared the antibacterial activity of porous chitosan/polyvinyl alcohol (PVA) (CS) scaffolds decorated *in situ* with Se rods (CS-Se) against that of AgNPs (CS-Ag). The results demonstrated antibacterial activities of both CS-Se and CS-Ag against Gram-negative (*E. coli*), Gram-positive (*S. aureus*), and methicillin-resistant-*Staphylococcus aureus* (MRSA). The Ag and Se release was found to be dependent on the release medium used but also reported that the CS-Se scaffold was superior to the CS-Ag since it had a low toxicity toward mammalian cells (mouse embryo fibroblasts) compared to the evidence of significant cytotoxicity by CS-Ag. The authors also demonstrated clear bacterial membrane damage when cultured on Se-coated scaffolds.\(^48\)

In a complementary study, Ramya et al.\(^35\) investigated *in vivo* the effect of SeNPs on wound healing in Swiss albino mice.
The SeNPs were biosynthesized by *Streptomyces minutisceleroticus* M10A62. Reduction of Sodium selenite and were characterized to be spherical in shape, with sizes ranging from 100 to 250 nm. Swiss albino rats were given an artificial wound by shaving their dorsal sides using sterile blades and split into four different groups which received different treatments: untreated (control), standard antibiotic (Lyramycin), 5% SeNP concentration, and 10% SeNP concentration ointments. The results revealed that low-dose (5%, 5 mg of SeNPs) and high-dose (10%, 10 mg of SeNPs) SeNP ointment treatment healed artificial excisional wounds on the rats within 21 and 18 days, respectively, comparable to that of standard treatment with antibiotic (Lyramycin, 21 days) while the control group (without any treatment) took 30 days (Fig. 4).

C. Role of selenium in anticancer applications

Selenium has shown significant potential in the diagnosis and treatment of cancers. However, there is ongoing concern with the use of elemental selenium (e.g., sodium selenite) and naturally occurring selenium (e.g., selenocysteine and selenomethionine) in therapeutic cancer management due to selenium’s documented toxicity and bioavailability.49 As a result, studies have been conducted to demonstrate anticancer properties of nonmodified SeNPs against several cancer cell lines in a time- and dose-dependent manner.47,50–55 Highlighting selenium’s ability to significantly lower chemotherapy-induced toxicity, 56 SeNPs’ anticancer properties have explored extensively via *in vitro* studies.53,56–63 SeNPs have shown growth inhibition of prostate LNCaP cancer cells *in vitro* partially via caspase-mediated apoptosis.55 Furthermore, SeNPs reportedly suppress androgen receptor’s transcriptional activity via downregulation of its mRNA and protein expression. The *in vitro* results are further supported by *in vivo* studies, where nanorod elemental SeNPs orally administered to BALB/c mice bearing 4T1 breast cancer tumors were found to decrease tumor-related volume and hence resulting in a better prognosis (higher survival rate) for tumor-bearing mice.64

Exploring the potential of SeNPs one step further, there has been increasing attention in the field of surface-decorated/functionalized/modified-SeNPs whereby conjugation of SeNPs with functional ligands enhances the stabilization, cellular uptake, and bioactivity of SeNPs. It has been shown that positively charged SeNPs enhances anticancer efficacy and selective cellular uptake by cancer cells. This was demonstrated by Yu et al.62 with chitosan surface decoration of SeNPs. The development of conjugated-SeNP has also included various different compounds: ATP,55 *Spirulina* polysaccharides,56 mushroom polysaccharides,67 *Gracilaria lemaneiformis* polysaccharides,68 *Polyergus rhinocerus* polysaccharide–protein (PSP) complexes,69 *Undaria pinnatifida* polysaccharides,69 sialic acid,49 curcumin,72 amino acids —valine, aspartic acid, and lysine,73 ruthenium-thiol,74,75 polyethylene-glycol,58,63 11-mercapto-1-undecanol,76 transferrin,77 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox),78 and folate.79

Reviewing these studies is out of the scope of this paper; yet, one main goal of these SeNP conjugations is to provide targeting capability.80 In an *in vivo* study, Huang et al.77 looked into selective cellular uptake and apoptosis induction of SeNPs and their potential use as a cancer-targeted drug delivery system. The SeNPs were synthesized from sodium selenite (Na2SeO3) and conjugated with transferrin (Tf), chitosan (CS) and loaded with doxorubicin (DOX) to form Tf-CS-DOX-SeNPs (spherical, 130 nm). MCF-7 cells (breast adenocarcinoma) were injected into mice and tumors were grown to 50–75 mm3 before separated into three treatment groups where the mice were administered intravenously 2.5, 5.0, and 7.5 mg/kg of Tf-CS-DOX-SeNPs every 2 days. The results showed that mice administered with Tf-CS-DOX-SeNPs showed a decrease in tumor volume and weight, without a significant decrease in mice body weight, compared to mice injected with only phosphate buffered saline (PBS; Fig. 5). This suggested that the Tf-CS-DOX-SeNPs had inhibited the proliferation of MCF-7 cells in a dose-dependent manner. Although it suggested that the maintenance of body weight indicates minimal side effects.
effects of Tf-CS-DOX-SeNPs, 16 days may be a too short time duration to study the side effects of SeNPs without analysis of other parameters to determine mice well-being. Two mechanisms were noted to account for Tf-CS-DOX-SeNPs as a cancer-targeted drug delivery system: (i) Tf ligand targets cancer cells overexpressing Tf receptors, enhancing cellular uptake of DOX (as part of Tf-CS-DOX-SeNPs) through clathrin-mediated and caveolae/lipid raft-mediated endocytosis and (ii) Tf-CS-DOX-SeNPs induce intracellular ROS overproduction, activating p53 and MAPK pathways to promote cell apoptosis of cancer cells.77

SeNPs were also evaluated as a drug delivery system serving as a carrier of fluorouracil (5FU), a commonly used anticancer drug. SeNPs conjugated with 5FU (5FU-SeNPs) have been shown to have pronounced cancer cell selectively over normal cells evidenced by an enhanced cellular uptake in cancer cells by endocytosis via clathrin- and caveolae-mediated pathways, with a low cytotoxicity toward noncancerous cells. The efficacy of 5FU-SeNPs has been found across five cancer cell lines, A375, MCF07, HepG2, Colo201, and PC-3, where induced apoptotic cell death was described. The authors have postulated the possible development of topical 5FU-SeNPs therapeutic agents particularly for melanoma treatment.81

In 2013, Pi et al.79 investigated in vitro the effects of folic acid-conjugated SeNPs (FA-SeNPs) against MCF-7 breast cancer cells, with results collectively suggesting FA-SeNP’s anticancer potential and also its potential role in organelle-targeted drug carriers in cancer therapy. The FA-SeNPs were internalized via folate acid receptor (FAR)-mediated endocytosis and subsequently localized into the mitochondria, triggering mitochondria-dependent apoptosis of MCF-7 cells.79 Recently, further in vivo exploration of SeNPs as a low-toxicity cancer-targeted nanodrug delivery system to overcome multidrug resistance in cancer cells was assessed for drug-resistant hepatocellular carcinoma cells (R-HepG2).82

FA-SeNPs were loaded with fluorescent ruthenium polypyridyl which allows real-time direct imaging of cellular trafficking of FA-SeNP. FA surface modification of SeNP was shown to significantly increase cancer cell selectivity via cellular uptake of SeNPs by FAR which are overexpressed by cancer cells. FA-SeNP overcame multidrug resistance in R-HepG2 cells by inhibiting the expression of ABC family proteins. Cancer cell death was then induced via FA-SeNP triggered-ROS overproduction and p53 and MAPK pathway activation.82

D. Protective role of selenium

Selenium has been further found to have a chemoprotective role against chemotherapy-induced toxicity. Bhattacharjee et al.83 found that selenium had an effect on cyclophosphamide (CP)-induced hepatotoxicity and genotoxicity in Swiss albino mice. In the mice, SeNP reversed and/or ameliorated the effects of CP-induced toxicity: restoration of antioxidant enzyme activity, returning serum hepatic marker (alanine transaminase and aspartate transaminase) toward normal values, reduction of ROS and glutathione levels, reduction in chromosomal aberration in bone marrow, and DNA damage in both lymphocytes and bone marrow. In a follow-up study, Bhattacharjee et al.56 further demonstrated that SeNPs additionally have chemosensitizing and chemoprotective properties. After administration of SeNP adjutant therapy alongside CP in Ehrlich’s ascites carcinoma (EAC)-bearing Swiss albino mice, there was a significant reduction in viable tumor cell count, packed cell volume, and tumor volume while an increase in tumor-bearing hosts survivability, as well as attenuation of CP-induced hepatotoxicity and genotoxicity as per their previous study (Tables II–IV).

In a combined in vivo and in vitro study, Gao et al.54 successfully demonstrated SeNP as a chemotherapy preventative agent against chemotherapy-induced toxicity of irinotecan.

Table II. Effect of combination treatment of nano-Se and CP on hematological parameters in tumor-bearing mice. Vehicle control group, no Ehrlich’s ascites carcinoma (EAC) (VC), EAC-control group (E), only cyclophosphamide (CP)-treated control group (EC), only selenium nanoparticle (SeNP) concomitant-treated group (ED), only SeNP pretreatment group (PED), SeNP along with CP in the concomitant-treated group (ECD), pre-treatment with SeNP and CP group (PECD). Data were represented as mean ± SD (n = 6). Reproduced with permission from Bhattacharjee et al., Mol. Cell. Biochem. 424, 13 (2017). Copyright 2017, Springer US (Ref. 56).

| Groups   | Hemoglobin (g/dl) | RBC (x10^6) | WBC (x10^3) | Alanine transaminase (IU/ml) | Aspartate transaminase (IU/ml) |
|----------|------------------|-------------|-------------|-------------------------------|-------------------------------|
| VC       | 14.8 ± 0.52      | 10.08 ± 0.54 | 6.52 ± 0.46 | 23.72 ± 0.99                  | 51.8 ± 3.34                  |
| E        | 9.96 ± 0.57 a    | 7.08 ± 0.27 a | 14.32 ± 0.69 a | 30.4 ± 1.67 a                | 75.2 ± 3.63 a                |
| EC       | 6.92 ± 0.67 b,c  | 5.1 ± 0.23 b,c, e | 4.18 ± 0.5 b,c, e | 40 ± 1.41 b,c, e             | 118.8 ± 5.93 b,c, e          |
| ED       | 11.4 ± 1.06 b,c,e | 8.18 ± 0.48 b,c, e | 9.62 ± 0.42 b,c, e | 26 ± 1.4 b,c, e              | 56.4 ± 3.84 b,c, e           |
| PED      | 12.28 ± 0.48 b,c,e | 9.88 ± 0.52 b,c,d | 8.46 ± 0.46 b,c,d | 22.28 ± 1.63 b,c,d           | 52.72 ± 3.37 b,c,d            |
| ECD      | 11.72 ± 0.46 b,c,e | 7.92 ± 0.52 b,c,e | 5.6 ± 0.61 b,c,d,e | 32 ± 2.44 b,c,d,e            | 82 ± 2.44 b,c,d,e            |
| PECD     | 13.6 ± 0.4 b,c,d,e, f | 9.04 ± 0.47 b,c,d,e,f | 7.28 ± 0.42 b,c,d,e,f | 28.8 ± 2.28 b,c,d,e,f        | 59.6 ± 3.57 b,c,d,e,f         |

aSignificant (p < 0.05) as compared with VC.

bSignificant (p < 0.05) as compared with E.

cSignificant (p < 0.05) as compared with EC.

dSignificant (p < 0.05) as compared with ED.

eSignificant (p < 0.05) as compared with PED.

fSignificant (p < 0.05) as compared with ECD.
TABLE III. Effect of SeNP alone or in combination with cyclophosphamide (CP) on DNA damage in peripheral lymphocytes and tumor cells. Reproduced with permission from Bhattacharjee et al., Mol. Cell. Biochem. 424, 13 (2017), Copyright 2017, Springer US (Ref. 56).

| Group  | Peripheral lymphocyte | Tumor cells |
|--------|-----------------------|-------------|
|        | Damaged cells showing comet (%) | Average tail length (μm) | Damaged cells showing comet (%) | Average tail length (μm) |
| VC     | 9.14 ± 0.47           | 7.88 ± 0.75 | —                         | —                      |
| E      | 44.46 ± 3.08*         | 64.1 ± 1.42 | 13.64 ± 2.44               | 8.19 ± 2.07            |
| EC     | 76.04 ± 2.39*         | 96.71 ± 1.25 | 56.29 ± 2.14*              | 32.15 ± 2.63*          |
| ED     | 19.37 ± 2.15*         | 29.42 ± 1.26 | 21.6 ± 1.89*               | 16.75 ± 2.98*          |
| PED    | 15.74 ± 1.04*         | 22.3 ± 2.24 | 35.99 ± 3.16              | 26.78 ± 2.67*          |
| ECD    | 33.41 ± 0.95*         | 58.19 ± 1.21 | 66.29 ± 2.65*              | 41.89 ± 2.08*          |
| PECED  | 23.75 ± 2.39*         | 37.91 ± 1.99 | 72.35 ± 4.35*              | 56.41 ± 6.1*            |

Data were represented as mean ± SD (n = 6).

*Significant (p < 0.05) as compared with VC.
*Significant (p < 0.05) as compared with E.
*Significant (p < 0.05) as compared with EC.
*Significant (p < 0.05) as compared with E.
*Significant (p < 0.05) as compared with PED.
*Significant (p < 0.05) as compared with ECD.

In a separate study, further supporting the promising role that SeNPs offer in the treatment of cancer, the dose-dependent effects of red-allotrope selenium nanoparticles (rSeNPs) on head and neck squamous cell carcinoma (HNSCC) and human dermal fibroblast (HDF) cells were evaluated. The results indicated that rSeNPs primarily induced HNSCC and were approximately four times as cytotoxic toward HNSCC than HDFs, with additional observations that rSeNP < 5 μg rSeNP/ml media actually resulted in cell proliferation.50 In another in vitro study, Rezvanfar et al.83 concluded that coadministration of SeNPs and cisplatin in mice had a protective effect against cisplatin-induced gonadotoxicity and had reduced spermatotoxicity, DNA damage, and chromatin abnormality. Cisplatin-induced nephrotoxicity was also ameliorated in an in vitro study done by Li et al.78 with the use of Trolox-surface-functionalized SeNP (Se@Trolox). It demonstrated dose-dependent antioxidant activity and prevented cisplatin-induced apoptosis in HK-2 cells via inhibition of ROS-mediated p53 phosphorylation and regulation of AKT and MAPK pathways.78

SeNPs are also being evaluated in the field of chemoradiotherapy and its potential role as a radiation sensitizer. External radiation therapy has been extensively utilized in cancer treatment, but there is inevitable damage to adjacent healthy cells by indiscriminate radiation. With an aim to reduce this adverse effect, chemo-radiotherapy with the use of a radioactive seed has been proposed to improve the precision and efficacy of radiation delivery. Bhattacharjee et al.83 investigated this concept via an in vitro and in vivo combination study, with the use of folic acid-conjugated SeNPs (FA@SeNPs) together with radioactive 125I seeds to realize enhanced anticancer efficacy. The combination of FA@SeNPs and 125I seeds showed better in vivo antitumor activity as well as lower systemic toxicity in MCF-7-bearing mice compared to individual treatment. In vitro, 125I seeds had a greater synergy with FA@SeNPs compared to x-ray.

TABLE IV. Effect of SeNP alone or in combination with cyclophosphamide (CP) on tumor growth, mean survival time, and % increase in lifespan in tumor-bearing mice. Reproduced with permission from Bhattacharjee et al., Mol. Cell. Biochem. 424, 13 (2017), Copyright 2017, Springer US (Ref. 56).

| Groups | Tumor volume (ml) | Packed cell volume (ml) | Tumor cell count (×10⁶) | Mean survivability (days) | Increase in life span (%) |
|--------|------------------|------------------------|------------------------|--------------------------|--------------------------|
| E      | 4.84 ± 0.47      | 2.32 ± 0.22            | 55.12 ± 3.42           | 22.1 ± 1.83               | —                        |
| EC     | 1.36 ± 0.16*     | 0.8 ± 0.24*            | 18.66 ± 2.24*          | 41.9 ± 2.26*              | 89.5                     |
| ED     | 2.96 ± 0.32*     | 1.84 ± 0.35*           | 34.88 ± 3.88*          | 27.55 ± 2.61*             | 24                       |
| PED    | 2.32 ± 0.3*      | 1.28 ± 0.22*           | 26.12 ± 1.67*          | 33.05 ± 2.19*             | 49.5                     |
| ECD    | 0.8 ± 0.14*      | 0.36 ± 0.15*           | 12.2 ± 1.14*           | 52.45 ± 2.47*             | 153.16                   |
| PECED  | 0.6 ± 0.15*      | 0.26 ± 0.08*           | 8.64 ± 1.55*           | 62.85 ± 3.46*             | 184.38                   |

Data were represented as mean ± SD (n = 6).

*Significant (p < 0.05) as compared with E.
*Significant (p < 0.05) as compared with EC.
*Significant (p < 0.05) as compared with ED.
*Significant (p < 0.05) as compared with PED.
*Significant (p < 0.05) as compared with ECD.
irradiation, evident from the increased ROS overproduction triggering apoptosis and G2/M arrest in MCF-7 cancer cells via DNA damage-mediated p53 and MAPK signaling pathways (Fig. 6). This was supported by earlier in vitro studies by Yu et al. The radiosensitizing properties of SeNPs were further reported by Yu et al. who used SeNPs in conjunction with irradiation on MCF-7 breast cancer cells in vitro, where it improved cancer cell sensitivity to toxic effects of irradiation and allowed the reduction of damage to surrounding normal tissue.

Karami et al. evaluated the radioprotective effects of SeNPs in irradiation-induced nephropathy in mice, as compared to sodium selenite. Mice were irradiated with gamma radiation (0, 2, and 8 Gy) for 14 days and were concurrently treated with either nothing (control), SeNPs, or sodium selenite intraperitoneally (0.1 mg/kg). Subjects were then assessed for the following parameters reflective of irradiation-induced nephropathy: serum creatinine, urea, cystatin C, and beta-2-microglobulin levels; renal antioxidant enzyme activity levels—superoxide dismutase, glutathione peroxidase, and catalase; and malondialdehyde levels. Renal tissue selenium content and histopathological features were also assessed (Fig. 7). Both SeNP (spherical, 20–50 nm) and sodium selenite administered intraperitoneally into the mice were found to normalize the aforementioned indicators for irradiation-induced nephropathy, though SeNP was more effective compared to sodium selenite.

E. Role of selenium in antifungal applications

SeNPs have been shown to possess antifungal activities in various applications, from tackling issues of treatment-resistant fungus, potential enrichment of probiotics, to potential antifungal and antibacterial fabric for prevention of tinea pedis (infection with Trichophyton rubrum) and S. aureus skin infections. Yip et al. evaluated the effects of biogenic PSP@SeNPs (synthesized using PSP complexes of tiger milk mushroom) applied onto fabric, discovering that they inhibited more than 99.7% of Trichophyton rubrum over 7 days and additionally demonstrating the effective inhibition of S. aureus for the initial 12 h. Shakibaie et al. also demonstrated in vitro the antifungal effects of biogenic elemental SeNPs (mediated via Bacillus sp. Msh-1) against Aspergillus fumigatus and Candida albicans (C. albicans) which had MICs of 100 and 70 μg/ml, respectively. This study proposed SeNPs as a novel antifungal agent in view of nystatin-resistant Candida.
sp., particularly in immunocompromised patients, and a more effective treatment than amphotericin B for invasive aspergillosis. However, contradictory results have been reported by Kazempour et al. into the postantibiotic/antifungal effects (PAEs), and sub-MIC effects of biogenic SeNPs (prepared via K. pneumoniae, 90–320 nm in size) have cast concerns over SeNPs’s therapeutic potential. PAEs describe the suppression of antimicrobial growth after short exposure of micro-organisms to antimicrobial agents and is a crucial pharmacodynamic parameter to establish dosage regimens. The study showed that pre-exposure to high concentrations of SeNPs (MIC, 2× MIC, and 4× MIC) did not produce any postinhibitory effects on Aspergillus niger (A. niger) and C. albicans and, subsequently, has actually stimulated the growth of A. niger at sub-MIC concentrations (0.01× MIC, 0.02× MIC, and 0.04× MIC). It was postulated that the mechanism is due to selenium’s physiological effect on particular enzymes in A. niger. The results may have direct implications on dermatitis caused by A. niger or C. albicans; the exposure to SeNPs may result in the regrowth of these pathogenic fungi on the skin. In addition, contrary to the MIC value of 100 μg/ml for Aspergillus fumigatus and 70 μg/ml for C. albicans reported by Shakibaie et al., this study reported a much higher MIC value of 250 μg/ml for Aspergillus niger and 2000 μg/ml for C. albicans.

### F. Role of selenium in antiparasitic applications

There have been studies conducted which have investigated the antiparasitic property of SeNPs, specifically on those belonging to genus Leishmania. Biogenic SeNPs’ (biosynthesized by Bacillus sp. Msh-1) effects on both promastigote and amastigote forms of Leishmania major (L. major) were evaluated via in vitro and in vivo methods by Beheshti et al. (Fig. 8). In vitro study of the SeNPs showed a time-dependent reduction in promastigote proliferation via the presence of DNA fragmentation (indicator of apoptotic cell death) measured via gel electrophoresis, compared to the absence of DNA fragmentation in negative control. Similar time-dependent cytotoxicity results were demonstrated against intracellular amastigotes. Male BALB/c mice were infected with L. major subcutaneously and then treated with SeNPs injected intraperitoneally. Their results suggested that when the SeNPs were given prophylactically (5 or 10 mg kg⁻¹ day⁻¹, Groups 1 and 2) prior to infection by L. major, they could reduce the severity and progression/size of lesions (mm). When administered at the same doses (Groups 4 and 5) after the cutaneous lesions have developed, complete elimination of lesions was achieved after 14 days. Infected mice with no SeNP intervention served as control and had a high degree of disease progression. Studies were conducted without the use of established antileishmaniasis treatment.

Similar results of leishmanicidal activities of biogenic elemental SeNPs (mediated by Bacillus sp. MSh-1) were demonstrated in vitro against Leishmania tropica (L. tropica) and Leishmania infantum. Soflaci et al. had evaluated the antileishmanial property of SeNPs in both promastigotes and amastigotes of Leishmania infantum compared to SeO₂. The study identified a dose- and time-dependent antileishmanial property for both SeNPs and SeO₂ via in vitro studies, though SeNP demonstrated a greater effect than SeO₂. They have also...
shown that SeNPs reduced cytotoxic effects in SeNP treatment compared to SeO₂ (on uninfected BALB/m mice peritoneal macrophages) than SeO₂.95 Mahmoudvand et al.97 delved deeper into the issue of treatment-resistant leishmaniasis, evaluating the SeNPs’ (80–220 nm) effectiveness against meglumine antimoniate (MA/Glucantime)-resistant and MA-sensitive *L. tropica* in vitro. Similarly, these results showed dose-dependent inhibition of promastigote, reduced intramacrophage amastigotes viability in both strains, as well as significant effect with prophylaxis treatment. It is also noted that SeNPs displayed an enhanced antileishmanicidal activity when combined with MA, compared to SeNP or MA alone.

G. SeNPs in medical device coatings and delivery systems

Selenium-doped coatings, in addition to SeNPs, have valuable potential in medical devices,29,30,38 particularly for orthopedic implants.38,42,59,98–101 The ideal orthopedic implant material would have a combination of characteristics that not only has both *in vivo* antitumor and antimicrobial properties but also have osteogenic properties, the capability to enhance/ promote normal osteoblast proliferation for osteointegration.101 Tran et al.99 evaluated the potential utilization of SeNP as a surface-coating on titanium orthopedic implants. The titanium substrate was immersed in reduced selenite allowing for the nucleated elemental selenium to adhere to the composite surface in hemispherical clusters (~80 nm). Three different surface densities were constructed and evaluated for adhesion, proliferation, alkaline phosphatase (ALP) activity, and calcium deposition. Results from this study demonstrated inhibition of cancerous mouse osteosarcoma cells and increased healthy osteoblast proliferation.99 Other forms of selenium-related nanoparticles have also been widely studied. As an example, Wang et al.59 undertook an *in vitro* - and *in vivo*-combination study, in which elemental selenium was modified onto a bone mineral nanoparticle (70 nm) to form selenium-doped hydroxyapatite nanoparticles (Se-HANs). Findings from this study showed induction of osteosarcoma cell apoptosis in both *in vitro* and *in vivo* mice evaluation, whereby Se-HANs inhibit tumor growth via induction of tumor apoptosis while having reduced systemic toxicity.59

Hemalatha et al.98 took the opposite approach instead using SeNP as the substrate material. Instead, hydroxyapatite

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**Fig. 9.** (a) *In vivo* imaging of tumor-bearing mice after administration of Ru@L-SeNPs-siRNA at 0.5, 2, 4, 8, and 24 h. (b) *Ex vivo* fluorescence images of tissues including the heart, liver, spleen, lung, kidney, and tumor collected at 4 h postinjection of Ru@L-SeNPs-siRNA (chiral SeNPs modified with dinuclear Ru(II) complexes (Ru@L-SeNP). Control: No injection (only injected one time for fluorescence images 4 h before). (c) The images of dislodged tumors from the mice of five groups after last injection. (d) *Ex vivo* analysis of the histological characteristics of tumor and CD31 immunofluorescent staining. Reproduced with permission from Chen et al., Nanomedicine Nanotechnol. Biol. Med. 11, 1773 (2015). Copyright 2015, Elsevier (Ref.103).
was coated onto SeNP to form hydroxyapatite-SeNP (HASnp). The study looked at the potential use of SeNPs in orthopedic bone implants against human osteosarcoma cell line (SaOS-2). The analysis of results showed that in HASnp-treated cells, there was enhanced cell proliferation and increased ALP activity. ALP is an indicator of bone formation and holds a crucial function in bone mineralization. There was a concentration-dependent increase in SaOS-2 cell viability at HASnp concentrations of ≥50 μg/ml compared to control. In addition, HASnp-treated cells had ALP activity at HASnp ≥50 μg/ml on the 7th and 14th day compared to the control.98

SeNP has shown to serve as a multifunctional nanocarrier-based delivery system, delivering siRNA against specific oncogenic genes.102–107 In another in vivo- and in vitro-combination investigation, Chen et al.103 delved further into the functions of Ru-SeNP and demonstrated the crucial differential effect of chirality in siRNA delivery. Chiral SeNPs were modified with dinuclear Ru (II) complexes (Ru@L-SeNP/Ru@D-SeNP) and shown to be promising vectors for siRNA targeting tumor-MDR1 gene in cisplatin-resistant adenocarcinomic human alveolar basal epithelial cells (A549 cells). Ru@L-SeNP was found to have a much more remarkable stereoselective interaction to proteins and pDNA compared to Ru@D-SeNP. Nude mice that received cisplatin-resistant A549R xenografts were then treated with Ru@L-SeNPs-siRNA to investigate its biodistribution via fluorescent imaging and antitumor effects in vivo. Results showed Ru@L-SeNPs-siRNA-treated cisplatin-resistant A549R cells had enhanced cytotoxicity compared to Ru@L-SeNP and controls, high tumor-targeted fluorescence and decreased systemic toxicity (Fig. 9). The study also suggests that apoptosis induced by Ru@L-SeNP-siRNA involves the regulation of MAPK and PI3K/Akt signaling pathways.

III. FUTURE PERSPECTIVES AND CONSIDERATIONS

There is mounting evidence that selenium can provide positive outcomes when used as a medicinal nanomaterial. Selenium research continues to investigate and show both in vitro and in vivo that SeNPs may be used to prevent bacterial attachment and lifetime while at the same time providing a nontoxic cellular material. However, as Fig. 10 and Table V show while positive findings are routinely shown in the literature, many questions remain unknown. The main four are detailed below.

A. Toxicity evaluation

Selenium is known to have dose-dependent toxicity. The nanomaterial form of selenium therefore presents a complex system which is expected to have toxicity dependent on both the dose and the size/shape of the SeNP. In vivo studies have shown that biogenic SeNPs have lower toxicity than sodium selenite108 and selenium dioxide.7,36 Nevertheless, even though SeNP toxicity is dose dependent, no biochemical changes occurred for oral administration of 2.5, 5, and 10 mg/kg of SeNPs. Rather, administration of only a relatively...
| SeNPs function | Assessment mechanism                                                                 | Outcome                                                                 | Ref. |
|----------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------|------|
| Antioxidant    | Antioxidant assays—azino-bis-ethyl benzthiazoline-sulfonic acid, diphenylpicrylhydrazyl, MTT | 50 μg/ml Qu-Gallic Acid@Ag-SeNPs                                        | 46   |
|                |                                                                                      | Antioxidant activity: 59%–62% compared to control                        |      |
|                |                                                                                      | IC50 values: 30–66 (μg/ml) compared to control                           |      |
| Antibacterial  | Agar well diffusion method; *E. coli*, *B. subtilis*                                  | Chloramphenicol (50 μg/ml): exhibited maximum zone of inhibition (E. coli 24 mm, B. subtilis 31 mm) | 46   |
|                |                                                                                      | Qu-Gallic Acid@Ag-SeNPs (50 μg/ml): had comparable outcomes (E. coli 18 mm, B. subtilis 19 mm) |      |
|                |                                                                                      | Silver nitrate (25 μg/ml) + sodium selenite (25 μg/ml): (E. coli 11 mm, B. subtilis 12 mm) |      |
|                |                                                                                      | Quercetin, Gallic acid: (E. coli 0 mm, B. subtilis 0 mm)                |      |
| Antibiofilm    | Zone of inhibition assay, bacterial viability assay, ROS assay, membrane permeability assay, β-galactosidase assay | ROS assay: Qu-Ach@SeNPs significant ↑ (p < 0.01) vs control for both E. coli and S. aureus | 30   |
|                |                                                                                      | Membrane permeability assay: Qu-Ach@SeNPs and Ach@SeNPs significant ↑ (p < 0.01) compared to control |      |
|                |                                                                                      | β-Galactosidase assay test, UV–vis spectroscopy: Qu-Ach@SeNPs and Ach@SeNPs (20 μg/ml) significant ↑ compared to control |      |
|                | 24 h Incubation of polyphenols of honey (HP)@SeNPs in *P. aeruginosa* using a crystal violet (CV) staining assay | Biofilm formation was reduced >90% by the treatment of HP@SeNPs (4.5 μg/ml) compared to SeNPs only (7.5 μg/ml) and HP only (0.6%) | 33   |
|                |                                                                                      | The greatest reduction in biofilm formation across all six bacterial strains was 3.2 μg of SeNPs, followed by 2.4 and 1.2 μg, compared to positive control (without SeNPs) |      |
| Anticancer     | Cell density assays: human osteoblasts cells (incubated for 4 h and 1 day) and mouse osteosarcoma cells (incubated for 4 h, 1 day, and 3 days) seeded on titanium substrates coated with SeNPs at different selenium nanocluster densities | After 4 h of incubation: Healthy osteoblast densities significantly increased on the high-nSe-Ti compared to control (uTi) (p < 0.05) and low-nSe-Ti (p < 0.05) | 99   |
|                |                                                                                      | After 1 day: No significant difference was found for the cancerous osteoblast densities on the four types of titanium substrates (i.e., uTi, low-nSe-Ti, medium-nSe-Ti, and high-nSe-Ti) |      |
|                |                                                                                      | After 3 days: cancerous osteoblast density on high-nSe-Ti was significantly reduced in comparison to all other substrates. Cancerous osteoblast densities on medium-nSe-Ti were also significantly lower than that on uTi |      |
|                | Dalton lymphoma (DL) cell lines; MTT assay                                           | Ag–SeNPs (50 μg/ml): 20% DL cell viability                               | 46   |
|                |                                                                                      | Ag–SeNPs (100 μg/ml): 10% DL cell viability                              |      |
| SeNPs function | Assessment mechanism | Outcome | Ref. |
|----------------|----------------------|---------|-----|
| Anticancer     | MCF-7 breast adenocarcinoma cells (MCF-7 cells) xenograft mice model | Tf-CS-DOX-SeNPs inhibited the proliferation of MCF07 cells in a dose-dependent manner, demonstrated by the decrease in tumor volume and tumor weight without a significant decrease in mice body weight (Fig. 5) | 77 |
| Cancer-targeted drug delivery system | Three treatment groups (SeNPs 2.5, 5.0, and 7.5 mg kg\(^{-1}\) day\(^{-1}\)) IV injections and control group (PBS only, without SeNPs). Tumor size was measured every 2 days and then excised after 16 days with their weight measured | Growth was stimulated in both A. niger and C. albicans at 2× and 4× MIC of SeNPs without toxicity. A. niger had the greater response to SeNPs | 89 |
| Antifungal     | Fungi: A. niger and C. albicans: incubated for 1 h with either 0×, 1×, 2×, or 4× MIC of SeNPs, previously determined by serial dilution. Growth kinetics of the fungi were evaluated in control and nontoxic SeNPs (0.01× MIC, 0.02× MIC, or 0.04× MIC) cultures | High concentrations of SeNPs elicited no postinhibitory effects on either fungi Truce amounts stimulated growth in a dose-dependent manner | 89 |
| Assessment of antibacterial resistance to SeNPs Chemoprotective | 30 Passages of SeNPs to MDR E. coli, MDR S. aureus cultures—MIC comparison | Qu-Ach@SeNPs and Ach@SeNPs: MIC of MDR E. coli, MDR S. aureus cultures remained constant after 30 passages compared to starting MIC | 28 |
| Chemoprotective | Cyclophosphamide administered (25 mg/kg body weight) and SeNPs (2 mg SeNPs/kg b.w.) to Swiss albino mice against Ehrlich’s ascites carcinoma (EAC) cells | Increased levels of serum hepatic marker, hepatic lipid peroxidation, DNA damage, and chromosomal aberration in CP-treated mice were significantly (\(p < 0.05\)) reversed by SeNPs. | 35 |
| Low cytotoxicity | HCT-8 tumor lines were treated with SeNPs (10 mmol/l), irinotecan (100 mmol/l, 5 h) and SeNPs \(\square\) Irinotecan, respectively | Antioxidant enzymes increased in tumor-bearing mice after CP treatment by SeNPs Tumor volume, packed cell volume, viable tumor cell count decreased by SeNPs when administered with CP | 51 |
| Radioprotective | Cytotoxic assay—human embryonic kidney (HEK 293T) cells—MTT and LDH release assays—cell viability (%) | In vivo experiment showed SeNPs at a dose of 4 mg kg\(^{-1}\) day\(^{-1}\) significantly alleviated adverse effects caused by irinotecan (60 mg/kg) treatment, while SeNPs alone did not induce any toxicity | 28 |
| Low cytotoxicity | Cells viabilities of SeNPs exceeded 80% at concentrations \(\geq 100 \mu\text{g/ml}\); the concentration of Qu-Ach@SeNPs exceeded the MIC of the SeNPs against E. coli and S. aureus by 20-fold | No significant effects on human embryonic kidney cells | 10 |
| Radioprotective | Three groups of 15 Mus musculus mice each, received doses of gamma radiation either 0, 2, or 8 Gy for 14 days, and were further subdivided and concurrently treated with either nothing (control), SeNPs, or sodium selenite intraperitoneally (0.1 mg/kg). 48 h after the final day of treatment animals were euthanized | 8 Gy-exposed mice with SeNPs or Se (0.1 mg/kg) significantly reduced (\(p < 0.05\)) radiotherapy-induced rise in renal function biomarkers (creatinine, urea, cystatin, B2M) compared to 8 Gy-exposed controls (without SeNPs or Se). SeNPs were more effective than sodium selenite | 28 |

8 Gy-exposed mice with SeNPs or Se (0.1 mg/kg) given had significantly reduced (\(p < 0.05\)) renal tissue pathology (glomerular sclerosis, focal glomerular necrosis, and tubular epithelium necrosis) compared to 8 Gy-exposed controls (without SeNPs or Se)
is clear that further toxicity evaluation is required in  
in vivo  

tation, routes of administration, and what the fate of the SeNP  
as how much the SeNP toxicity is attributed to the concentra-

There remain also important questions such as  

will become more complex when SeNPs are used in combi-

surface on the overall toxicity of SeNPs. The toxicity studies  
in vivo  


TABLE V. (Continued.)

| SeNPs function | Assessment mechanism | Outcome | Ref. |
|----------------|----------------------|---------|-----|
| Wound healing | Mice wound excision model and mice mortality test | Mice treated with HP@SeNPs (4.5 μg/ml) and HP only (0.6%) mice had significantly reduced a bacterial load of 4.3 cfu/ml (log_{10} p < 0.01) and 6.2 cfu/ml (log_{10} p < 0.05), respectively, when compared to control group (distilled water only) | 33 |
| Wound healing | 5 g ointment base blended with SeNPs (5 and 10 mg) applied on Swiss albino rats. Rats had dorsal sides shaved with sterile blade Four groups: untreated group (control), standard antibiotic (Lyramycin), 5% concentration SeNPs, 10% concentration SeNPs. Measurement of % wound contraction | The number of days taken for complete healing of artificial wound for control (untreated), antibiotic (Lyramycin), 10 mg SeNPs ointment, and 5 mg SeNPs ointment are 30, 21, 18, and 21 days, respectively | 35 |

high dose of 20 mg/kg resulted in mice having signs of toxicity (lower body weight, clinical and hematological parameters). However, contradictory results were reported  in vivo by Li et al. 108 when measuring selenium bioaccumulation and subsequent clearance in the Medaka fish liver, gills, muscle, and whole bodies; results showed that elemental SeNPs had higher toxicity than sodium selenite in selenium-sufficient fish when treated over 10 days of SeNPs and selenite (100 g Se/l). SeNPs were found to be more hyperaccumulated in the liver than selenite and had a fivefold increase in the lethal concentration required to kill 50% of the population (LC_{50}) compared to selenite. It was concluded that nanoparticle toxicity can be highly variable between species. It is clear that further toxicity evaluation is required in  in vitro and  in vivo to understand better the contribution of the SeNP surface on the overall toxicity of SeNPs. The toxicity studies will become more complex when SeNPs are used in combination or synthesized as nanoparticles conjugated with other bioactive agents. There remain also important questions such as how much the SeNP toxicity is attributed to the concentration, routes of administration, and what the fate of the SeNP is  in vivo.

B. Biogenic versus synthetic SeNPs

A number of studies have reported different bioactivities of SeNPs when synthesized chemically or biologically. The latter is often produced by certain type of soil bacteria, fungi, or plants. A 2016 review by Wadhwani et al. 8 looked specifically into biogenic synthesized SeNPs, categorizing the various organisms utilized, location, size, and shape of SeNPs produced. Mechanistic understanding is yet missing in the difference in biogenic SeNPs and synthetic SeNPs bioactivities. With the same chemistry (i.e., elemental Se), these two types of SeNPs will need to be compared in terms of their size, shapes, and surface groups. The latter is expected to be of significant importance because surface groups strongly dictate the binding of the nanoparticles to cells and tissue and subsequently their effects at cellular levels. Synthetic SeNPs have well characterized surface functional groups which were designed by researchers; yet, this level of control is not guaranteed in the case of SeNPs being synthesized by bacteria or plants. Analytical techniques such as XPS, Raman spectroscopy, FTIR, together with ELISA would be important in understanding not only the composition but also the conformation of these functional groups on biogenic SeNPs.

C. Amorphous versus crystalline SeNPs

There is increasing evidence suggesting different bioactivities with SeNPs of different crystallinity. In their demonstration of SeNP’s antibacterial properties  in vitro against  \textit{P. aeruginosa} biofilm formation when coated onto poly(ether ether ketone) (PEEK) medical devices, Wang et al. 29 had also showed that the antibacterial effects were greater in rSeNP-coated PEEK than grey selenium nanorod-coated PEEK. Red selenium exists as amorphous powder, while grey selenium as crystalline hexagonal structure with red selenium having higher thermodynamic stability. The reasons behind the difference in antibacterial activities were hypothesized to be that red SeNP-coated PEEK had more nanoscale features and surface area, resulting in more interaction with the bacteria. We believe that the more systematic
investigations of bioactivity of amorphous versus crystalline SeNPs are needed to fully differentiate the effects from size, shapes, and oxidation states. When these parameters are controlled, it is expected that SeNPs of different crystallinity would have different dissolution kinetics, different in surface free energy that in turn could influence interactions at cellular and subcellular levels.

D. Combination of SeNPs and conventional anticancer or antimicrobial agents

Furthermore, several studies have looked into the combination of SeNPs with established and conventionally used chemotherapeutic drugs \(^{56,76,78,83,84,110}\) and antimicrobial agents. \(^{31}\) This strategy seeks to determine synergic effects and was seen by Bhattacharjee et al. \(^{56}\) where tumor-bearing mice showed that a combination of orally administered SeNP together with CP, a widely used chemotherapy drug, significantly increased the chemotherapeutic effect as well as reduced the CP-induced toxicity. This was supported by two earlier studies which showed first demonstrated in vivo the protective effects of SeNP against CP-induced toxicity in mice.\(^{83,110}\) It may be prudent to also note the mode of delivery of SeNPs in future in vivo experiments as it could impact bioavailability and pharmacodynamics; there has been oralavage administration in mice models\(^{65,84,110}\) while other studies utilized intraperitoneal injection of SeNP into mice.\(^{10,51}\) Looking at the combination use of SeNPs with antibiotics, Cihalova et al.\(^{31}\) investigated the effectiveness of antibacterial and antibiotic properties of SeNPs complexed with conventional antibiotics (ampicillin, oxacillin, and penicillin) to that of SeNPs alone and antibiotics alone. Their results showed that the individual components in SeNP–antibiotics complexes are able to act independently or synergistically. However, in a synergistic relationship, SeNP–antibiotics complexes had higher growth inhibition zone sizes and higher biofilm formation inhibition against both nonresistant \textit{S. aureus} \(^{18}\) and MRSA than SeNPs alone and antibiotics alone.

However, despite these promising results regarding combinations of SeNP and therapeutic drugs, further investigations are still required to fully characterize the mechanisms of activity, especially when synergistic effects were observed. As the drugs often have well characterized pharmacokinetics, bioavailability, and biodistribution, the same is not true for most of the SeNPs; this makes it even more challenging to understand how these two work together. Yet, given that selenium has been well studied as a trace element and from the biochemistry point of view, this foundation seems a good starting point when investigating SeNPs providing a better understanding of dissolution of SeNPs into soluble species in complex media.

In conclusion, there is an increasing volume of research into biomedical SeNPs with increasing evidence that suggests that SeNPs have a beneficial role in nanomedicine. SeNPs although promising still require a firm correlation of \textit{in vitro} performance and \textit{in vivo} efficacy as these materials often have different working mechanisms from the well-established pharmaceutical drugs. In particular, SeNPs appear to show promise in the inhibition of bacteria and cancer cells with increasing evidence of efficacy \textit{in vivo}. As with other nanomaterials such as silver, zinc, carbon nanotubes, cerium, and chitosan, SeNP research has been met with the same challenges in the mechanistic understanding, safety concerns, and applications.

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Victoria le Ching Tan grew up in Singapore, known as the “Little Red Dot” on the world map. She is almost a doctor, completing her final year in medicine at the University of Queensland. Prior to her medical studies she had completed a Bachelor of Science (Biomedical Science). She is working hard toward her ambition of becoming a surgeon and hopes to merge research and clinical practice and make a meaningful contribution to both fields. Victoria is also currently working on her muscle-ups in CrossFit.

Angelica Hinchman is a medical student at UQ (2017–present) with interest in orthopaedic surgery. She has a bachelor degree in chemistry from the University of Wisconsin Madison (2011–2014) and has assumed a number of biomedical research assistant roles both in the USA and in Australia.

Richard Williams is a senior lecturer and the theme leader in bioengineering at the RMIT School of Engineering.

Phong A. Tran (Ph.D., 2010, Brown University) is leading the Interface Science and Material Engineering group at Queensland University of Technology (Australia), focusing on material research for biomedical applications. His research has resulted in 50 refereed publications and more than 1000 citations. He has strong linkage with industrial and end-user partners, conducting translational research and developing technologies to address unmet biomedical needs. Two of the developed technologies have been taken up by medical device companies for production and commercialization. He is also passionate about teaching and is currently lecturing second year Physics and fourth year Biomaterials.

Kate Fox is a senior lecturer at RMIT in the School of Engineering. Graduating from Flinders University, South Australia, with a degree in Biomedical Engineering, she went on to undertake a Ph.D. in Applied Science at the Ian Wark Research Institute. After graduating, she became a patent attorney but very quickly worked out that she was better suited in academia (billable hours and pant-suits are not as fun as they sound). She does not want to discourage one from this career path, but it did involve a lot of writing and working alone. Two items that were not in Kate’s top list of fun tasks. In 2010, she moved across the border to Melbourne, where she joined the University of Melbourne to work with medical bionics implants. She has been involved in two of the biggest medical bionics projects in Australia, the Bionic Eye and the Stentrode device, a device capable of directly interfacing with the brain. At present, she is working at RMIT University researching the application of additive manufacturing for orthopedic implant applications, in particular, the use of new and exciting materials such as diamond. 3D printing is an exciting technology, but at present, the current material options do not really provide the advances in medicine compared to the traditional subtractive options. While we can now make patient-specific, one size fits one, implants, they remain expensive. By bringing in new technologies and in particular new materials designed to work at the implant–bone interface, we have the opportunity to really improve patient outcomes.

In her current role, Kate aims to inspire the next generation of engineers. Engineering is a very exciting career and it puts students at the interface of the newest technologies. When asked what is an engineer? The best definition she has is that “we exist to solve problems that are yet to exist.” Her advice to the next generation of women coming through

1. Be nice to your colleagues, as you get more senior so do they.
2. Network, network, network.
3. There is nothing wrong with being the linking person who brings together research groups but make sure sometimes you push yourself to the front.
4. Have fun and don’t worry about the small things. In the end they rarely matter.
5. As an engineer you have a rare opportunity to make a difference, use it!