

Investigation of Antifungal and Antibacterial Effects of Fabric Padded with Highly Stable Selenium Nanoparticles

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ABSTRACT: In this article, highly stable selenium nanoparticles (SeNPs) were padded onto fabric to obtain, for the first time, antifungal and antibacterial fabric. SeNPs are prepared from a simple food-grade redox system by using polysaccharide–protein complexes (PSPs) isolated from the mushroom sclerotia of *Pleurotus tuber-regium* (tiger milk mushroom) as a modifier or stabilizer. The novel PSP–SeNPs are highly stable, size-controllable, and water-dispersible. Different amounts of PSP–SeNPs were applied onto fabric by using the pad–dry–cure method. It was found that the fabric treated with PSP–SeNPs can inhibit more than 99.7% of *Trichophyton rubrum* growth over a testing period of 7 days. The inhibition of *Staphylococcus* is effective in the first 12 h. The fabric treated with PSP–SeNPs is a promising material that can potentially be used inside shoes as insoles or shoe material to reduce the possibility of tinea pedis infection usually caused by the *T. rubrum* fungus. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 40728.

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INTRODUCTION

Selenium (Se) is an essential trace element for human health, which is also an antioxidant that can remove free radicals *in vitro*. It improves the activity of seleno-enzymes and glutathione peroxidase, which can prevent free radicals from damaging cells and tissues *in vivo*.^{1–3} The intervention of Se is also advantageous for reducing the risk of incidences in all forms of cancers, especially liver, prostate, colorectal, and lung cancers.^{4,5} Se compounds have been used in nutrition and medicine for a long time, such as topical antifungal medications that contain 2.5% Se sulfide have been recommended for treating tinea versicolor, which is caused by the *Malassezia globosa* fungus.⁶ Compared with Se compounds, the elemental Se in a nano size [i.e., Se nanoparticles (SeNPs)] has become the new research target, because it is found to possess excellent bioavailability, low toxicity, and contribute to a wide spectrum of health-promoting as well as disease prevention and treatment activities.⁷ However, SeNPs are very unstable and easily aggregate when there are no other surfactants or stabilizers. Different approaches have been suggested to prevent the aggregation of SeNPs, such as those by Mondal et al.⁸ and Chen et al.⁹ who prepared Se nanorods and/or nanospheres, respectively, via simple solution routes with the assistance of L-cysteine. Some researchers have prepared SeNPs

by using a biosynthesized method which is simple and eco-friendly.¹⁰ Zhang et al.¹¹ obtained SeNPs via a selenious acid solution with ascorbic acid in the presence of polysaccharides, such as chitosan, konjac glucomannan, acacia gum, and carboxymethyl cellulose. Although the anticancer functions of SeNPs have been widely studied, their antifungal or antibacterial properties or any textile materials treated with SeNPs have not been reported.

Tinea pedis, also known as athlete's foot, is a form of ringworm associated with highly contagious yeast–fungi colonies including *Trichophyton rubrum*. This disease affects 15% of the population in the world and causes patients to greatly suffer due to the uncomfortable itching and burning sensations on the feet accompanied by malodor.^{12,13} Pharmacological treatment of tinea pedis usually involves the use of topical antifungal agents, such as terbinafine, clotrimazole, miconazole, fluconazole, or ciclopirox creams.¹³ However, research data have shown that this kind of treatment fails to cure about one-third of patients with tinea pedis. Most of the relapses are caused by the fungi in shoes or exposure to certain environments.^{14–17} In light of this, it is necessary to develop products with antifungal properties especially for the treatment of tinea pedis. There have been many studies on the antifungal and antibacterial properties of

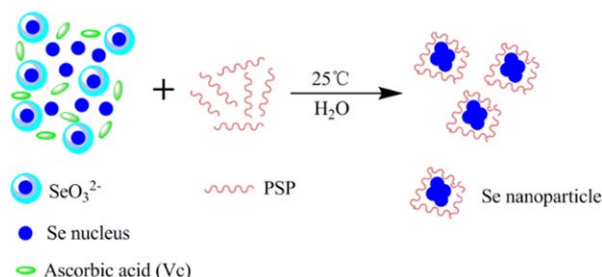


Figure 1. Schematic illustration of PSP–SeNP preparation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fabrics or fibers coated with golden, silver, chitosan, or other types of nanoparticles for common use.^{18–24} However, the antimicrobial activity of these materials has been mainly investigated for their antibacterial properties and less so for antifungal activity,^{22–24} and many of these products may not be suitable for patients with tinea pedis because of their negative effects on the body tissues.¹⁸

Pleurotus tuber-regium (Fr.) Sing. also known as “tiger milk mushroom” in China, is an edible and medicinal mushroom mainly found in the tropical and subtropical regions, such as China, Australia, and Nigeria.²⁵ Previous *in vitro* experiments have shown that dietary fiber or nonstarch polysaccharides extracted from the sclerotia of this mushroom have antiproliferative activity toward tumor cell lines and do not inhibit the growth of a normal kidney cell line (Vero) which prove its innocuousness.^{26–29} Highly stable SeNPs have been successfully prepared by using mushroom polysaccharide–protein complexes (PSPs) isolated from the sclerotia of *P. tuber-regium*.³⁰ In this article, we would like to further study the antifungal and antibacterial properties of PSP–SeNPs, which have never been reported before.

In this study, SeNPs are prepared under a simple food-grade redox system by using PSPs isolated from the sclerotia of *P. tuber-regium* as a modifier or stabilizer. The novel PSP–SeNPs are highly stable, size-controllable, and water-dispersible. Different amounts of PSP–SeNPs are applied onto fabric by using the pad–dry–cure method, and the antifungal and antibacterial properties of the fabric are investigated. It was found that fabric treated with PSP–SeNPs can inhibit more than 99.7% of *T. rubrum* growth in a testing period of 7 days. The fabric treated with PSP–SeNPs is a promising potential material that can be used inside shoes to prevent tinea pedis infection.

MATERIALS AND METHODS

Materials

Sodium selenite (Na_2SeO_3) was purchased from Sigma (St. Louis, MO). Vitamin C was purchased from the Guangzhou Chemical Reagent Factory. PSPs were isolated from the sclerotia of *P. tuber-regium* as previously described.³¹ The water used in all the experiments was ultrapure because of the use of a double deionized water purification system from Millipore. *T. rubrum* was from American Type Culture Collection No. 40051.

Spacer fabric is a three-dimensional knitted fabric that consists of two separate knitted substrates that are joined together or

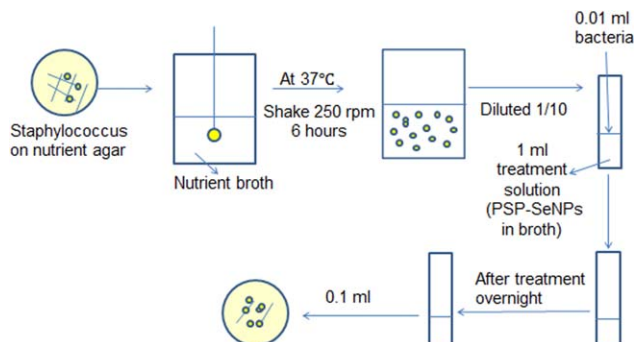


Figure 2. Study of antibacterial properties of PSP–SeNPs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

kept apart by spacer yarns.^{32,33} In this study, 100% polyester spacer fabric was chosen as insole material because of its high breathability, thus creating a moisture-free environment, which in turn, reduces the chances of skin maceration.

Preparation and Characterization of PSP–SeNPs

The aqueous mushroom PSP solution (0.25%) was mixed with a freshly prepared ascorbic acid solution (100 mM) under magnetic stirring prior to the drop-wise addition of aqueous Na_2SeO_3 solution (25 mM). After reconstituting the final volume to 25 mL with double deionized water, the mixture was further stirred for 12 h under room temperature followed by dialysis (molecular weight cutoff = 8000) against double deionized water until its Se content could not be detected by inductively coupled plasma–atomic emission spectrometry. The resulting dialyzate was centrifuged (4000 rpm; 20 min) and freeze-dried to obtain the PSP–SeNPs. Figure 1 is an illustration of the PSP–SeNP preparation.

The characterization of PSP–SeNPs was performed by transmission electron microscopy (TEM), scanning electron microscopy–energy dispersive X-ray analyzing system (SEM–EDX),

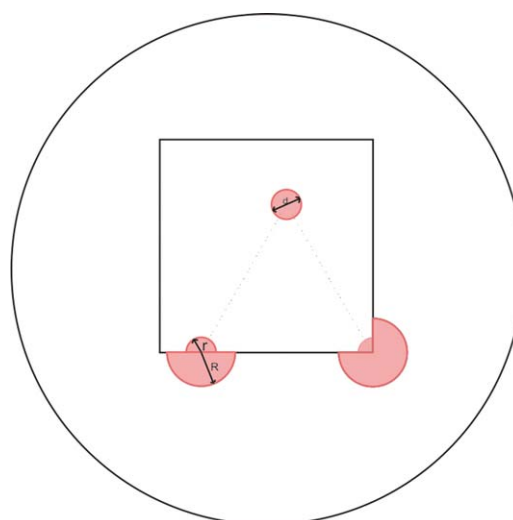


Figure 3. Schematic illustration of fabric strips placed on the agar and inoculation of *T. rubrum*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

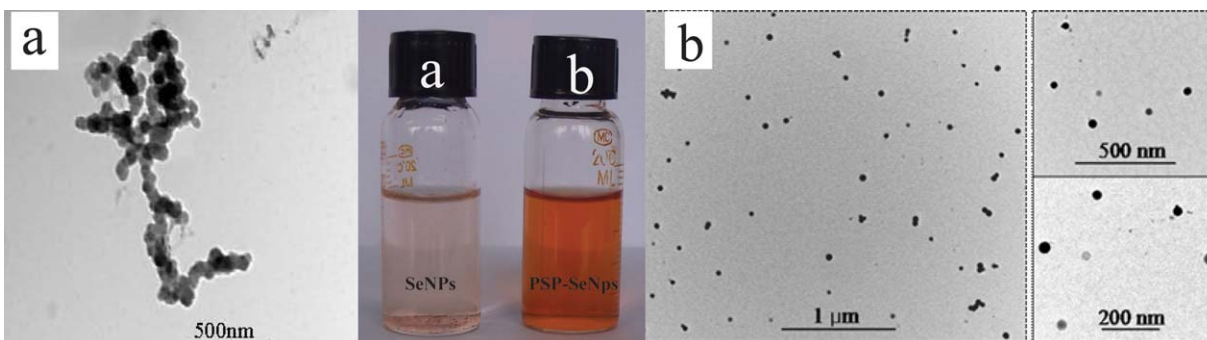


Figure 4. Transmission electron micrographs after 2 days of storage in refrigerator. (a) SeNPs alone; (b) PSP–SeNPs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

high-resolution TEM (HR-TEM), as well as Fourier transform infrared (FT-IR) spectroscopy as previously described.³⁰ In short, TEM samples were prepared by placing two drops of PSP–SeNP suspensions onto holey carbon film of copper grids, while the micrographs were obtained by TEM operated at an acceleration voltage of 80 kV. SEM–EDX analysis was performed to take SEM micrographs and analyze the elemental composition of the PSP–SeNPs. The HR-TEM images and the corresponding selected area (electron) diffraction patterns were obtained by HR-TEM performed at 200 kV. Besides that, the chemical surface binding of SeNPs to the PSPs was investigated using an FT-IR spectrometer in the range of 4000–500 cm^{-1} . The particle size of the PSP–SeNPs in water and broth mediums was studied by using a Zetasizer (Brookhaven Instruments Corp.) equipped with a 125-mW laser operated at $\lambda = 633 \text{ nm}$. Each measurement was carried out five times. The particle size was characterized by the Z-average size. The uniformity was characterized by the polydispersity index of the particle size data. A time-dependent study on the size distribution (i.e., stability) of the PSP–SeNPs was implemented before and after 6 months of storage in the refrigerator.

Preparation of Fabric Padded with PSP–SeNPs

A piece of 0.2 g spacer fabric was soaked in 5 mL of aqueous PSP–SeNP colloid solution (0.35 mM) and dipped twice, each time for 1 min at room temperature, followed by squeezing to obtain a wet pickup of 924%. Then, the padded fabric was dried and cured at 55°C for more than 2 h.³⁴ This pad–dry–cure process was repeated twice and thrice for the antifungal property study.

Morphology of SeNPs and Fabric Padded with PSP–SeNPs

In this experiment, 1 mL of PSP–SeNP colloid solution (0.35 mM) was centrifuged at approximately 8000–10,000 rpm for 10 min, and then washed with double deionized water two to three times to remove the PSPs. Then, the SeNP deposit was suspended with 10–30 μL of double deionized water. A high concentration of SeNP suspension was dropped onto a silicon wafer followed by drying overnight for observation. The control and padded fabrics were cut into 3 mm \times 3 mm slices and placed onto silicon wafers by using a conductive sticker. The morphology study was performed by field emission SEM (JSM-6335F).

Study of the Antibacterial Properties of PSP–SeNPs

To test the antibacterial property of the agent, 0.01 mL of *Staphylococcus aureus* broth culture (10^7 bacteria/mL) was sus-

ended in 1 mL of nutrient broth or PSP–SeNPs in a nutrient broth (0.7 or 0.35 mM) for various durations of time (0, 2, 12, and 72 h). Then, 0.1 mL of the *S. aureus* culture was grown on tryptone soya agar at 37°C to observe for any antibacterial ability. After incubation, the bacterial colonies in control plates and plates incorporated with PSP–SeNPs were visually compared (as shown in Figure 2).

Antifungal Property of PSP–SeNPs

The PSP–SeNPs (2 mL of 0.35 mM solution) were mixed with molten agar [Sabouraud's dextrose agar, (SDA)] and cooled to room temperature to solidify. The fungal species, *T. rubrum*, were inoculated onto PSP–SeNP agar at three locations arranged in a triangular position. The plates were incubated at 37°C. After incubation, fungal growth was visually assessed and compared with that in the control plates.

Study of the Antifungal Property of Fabric

The antifungal property of the fabric was tested in accordance with the following method (modified AATCC Test Method 30–2004).

Duplicate test specimens were cut into $(2.5 \pm 0.5) \text{ cm} \times (2.5 \pm 0.5) \text{ cm}$ strips from both treated and untreated samples. The culture medium was SDA. The fungal inoculum, *T. rubrum*, was grown on the SDA in three spots (2.5-cm triangle). The fabric strips were placed onto the agar surface, with one *T. rubrum* spot on one corner of the fabric, one spot on the

Table I. Time-Dependent Study on Size Distribution of PSP–SeNPs in Water or Broth Media Measured Before and After 6 Months of Storage in the Refrigerator

Sample	Catalogue	
	Particle size (nm)	PDI
Newly prepared PSP–SeNPs in water	131.0 \pm 2.6	0.167
PSP–SeNPs in water after 6 months of storage	147.7 \pm 0.5	0.206
Newly prepared PSP–SeNPs in broth	125.3 \pm 2.6	0.144
PSP–SeNPs in broth after 6 months of storage	132.8 \pm 2.6	0.204

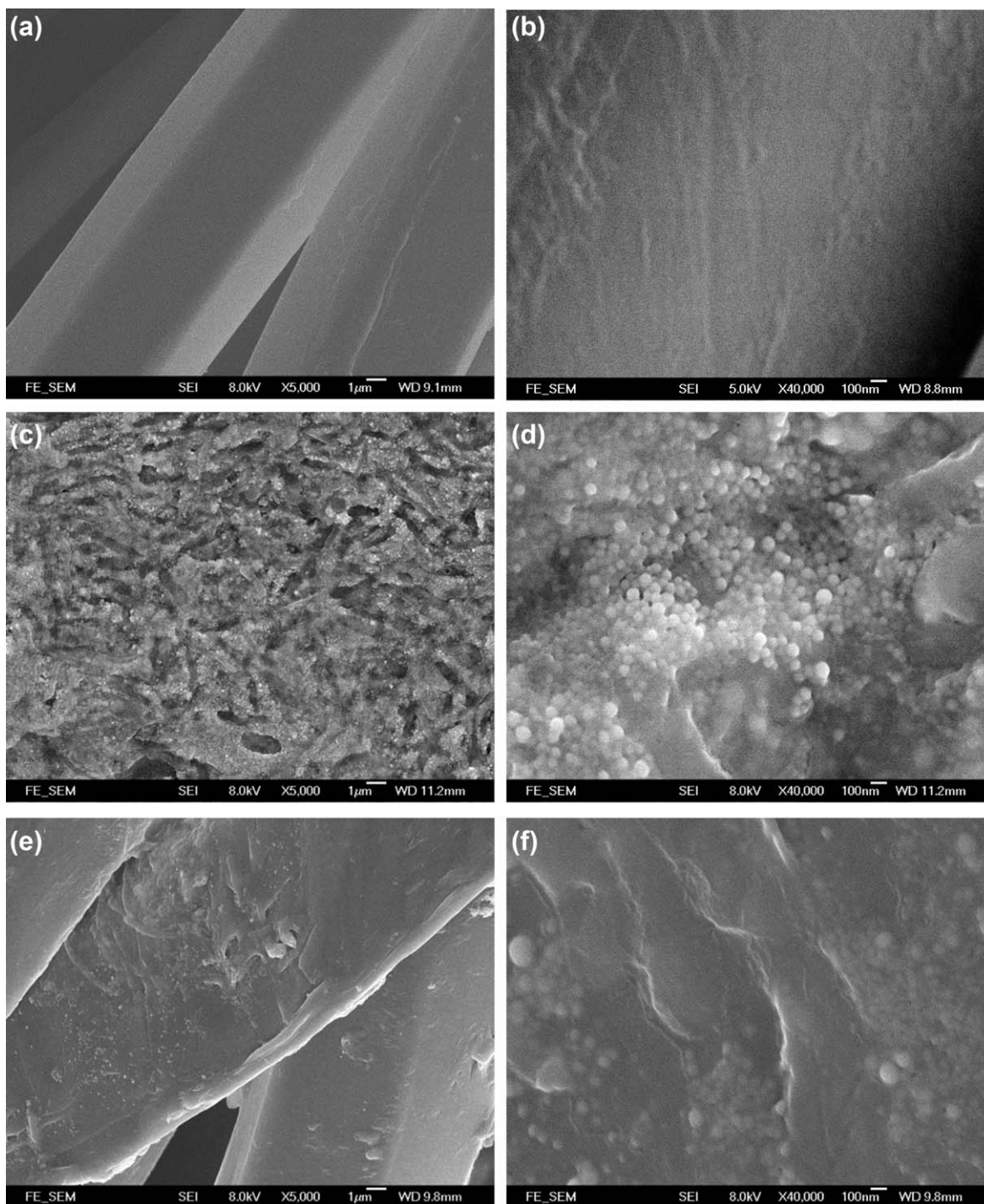


Figure 5. Morphology of SeNPs and fabric padded with PSP-SeNPs. (a) Control fabric surface $\times 5000$; (b) Control fabric surface $\times 40,000$; (c) SeNPs $\times 5000$; (d) SeNPs $\times 40,000$; (e) Padded fabric surface $\times 5000$; (f) Padded fabric surface $\times 40,000$.

edge of the fabric, and one spot covered by the fabric (Figure 3). A negative control fabric was used to ensure inoculum viability. All the specimens were incubated at a temperature of $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ($98.6^{\circ}\text{F} \pm 2^{\circ}\text{F}$) for 14 days.

At the end of the incubation period, the inhibition percentage was assessed for the extent of fungal growth on the surface area of the agar plate in the contact zone by eq. (1):

$$H = (R - r) / R \times 100\% \quad (1)$$

where H = the inhibition percentage, R = the distance of the fungi area in mm outside the fabric specimen, and r = the distance of the fungi area in mm covered by the fabric specimen.

An Olympus microscope ($\times 50$) was used during the measurement of R and r , and the values were averaged for three measurements.

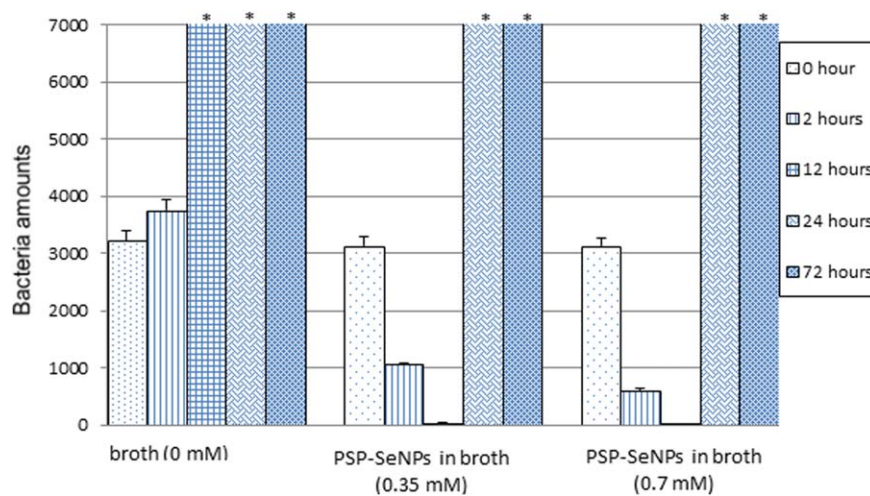


Figure 6. Antibacterial effect of PSP–SeNPs under two concentrations (0.35 and 0.7 mM) and various treatment durations. *Bacterial counts are over 30,000 (i.e., the inhibitory effect is very low). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

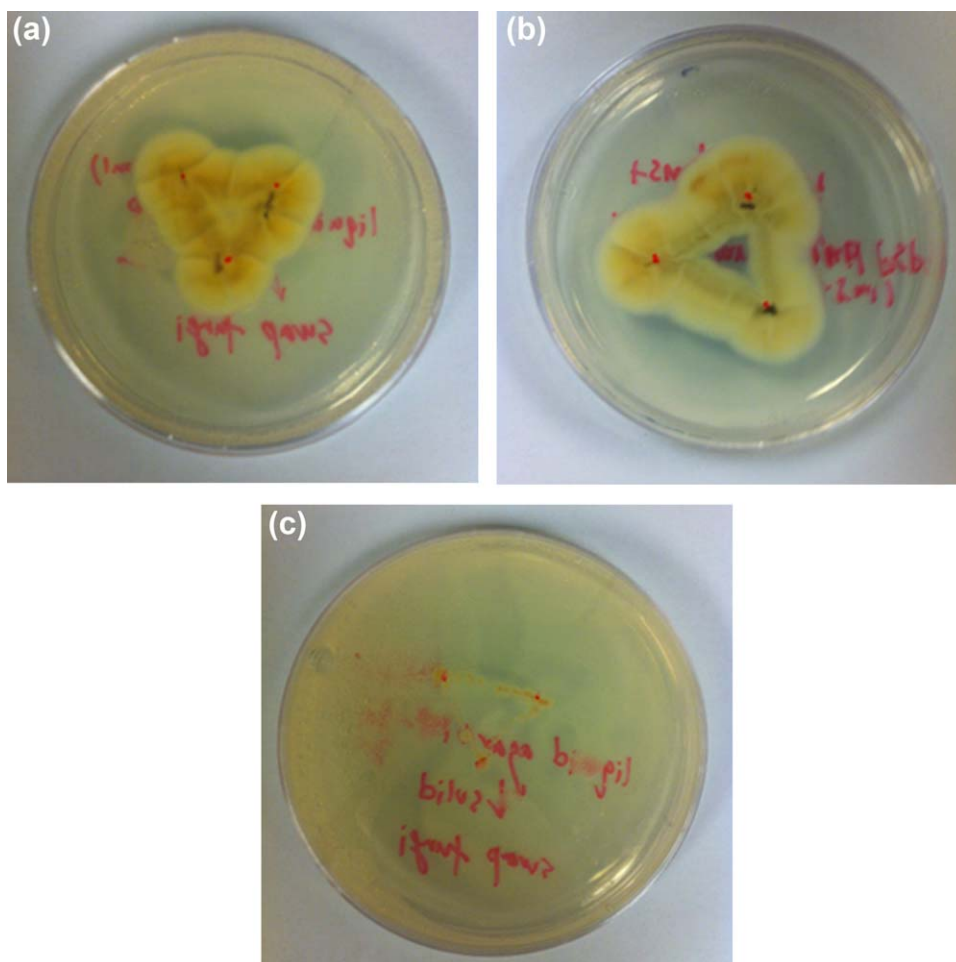


Figure 7. Growth of *T. rubrum* on agar. (a) Control. (b) Agar mixed with PSP solution. (c) Agar mixed with PSP–SeNP solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

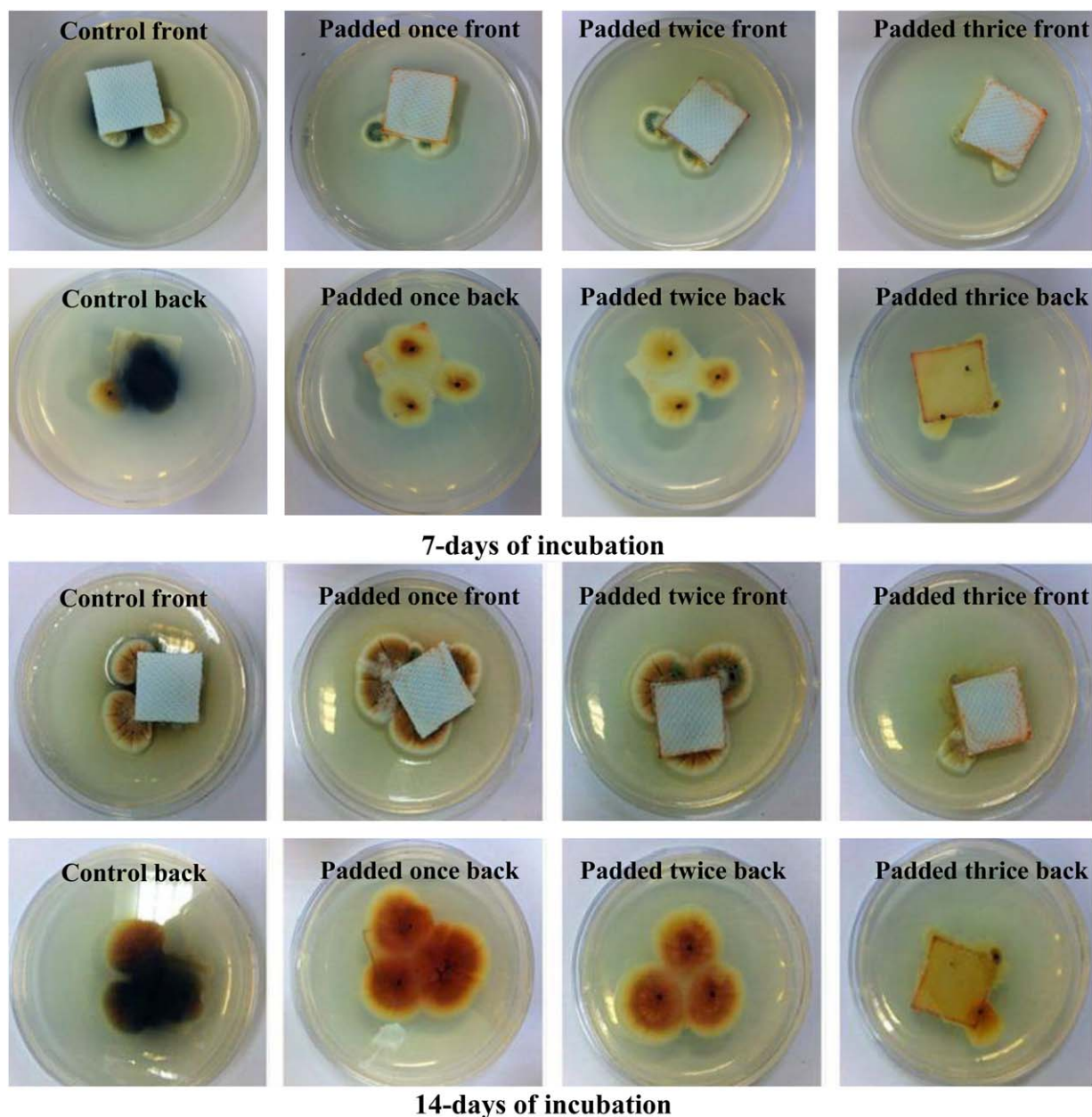


Figure 8. *T. rubrum* on control fabric and PSP–SeNP padded fabric at 7 and 14 days of incubation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Characterization of PSP–SeNPs

The TEM results showed that the SeNPs alone (622.7 ± 8.6 nm) easily aggregate and precipitate after just 2 days [Figure 4(a)]. However, by using PSP as the stabilizer (300 mg/L), the PSP–SeNPs formed into well-dispersed spherical particles in the aqueous solution [Figure 4(b)], with an average diameter of 131 ± 2.6 nm. The results obtained from the EDX and FT-IR analyses confirmed that the spherical SeNPs would likely be stabilized by the PSP molecules through the formation of Se–O

and Se–N bonds, thus leading to a good dispersion of SeNPs in the water.³⁰

A time-dependent study on the size distribution (i.e. stability) of PSP–SeNPs measured before and after 6 months of storage in the refrigerator is shown in Table I. It was found that the particle size of PSP–SeNPs in both water (from 131.0 ± 2.6 to 147.7 ± 0.5 nm) and broth (from 125.3 ± 2.6 to 132.8 ± 2.6 nm) media did not change much after 6 months of storage. This implies that the PSP–SeNPs colloid solution could maintain stability for more than 6 months.

Table II. Percentage Inhibition of Fungal Growth on Padded Fabric Treated with Different SeNP Concentrations After 7 Days of Incubation

Sample drug loading ($\mu\text{g/g}$)	Inhibition percentage after 7 days
Control (no padding)	12.5 ± 0.5
0.255 (padded once)	66.7 ± 2.6
0.510 (padded twice)	71.4 ± 1.9
0.765 (padded thrice)	99.7 ± 3.5

Morphology of SeNPs and Fabric Padded with PSP–SeNPs

The morphology of the SeNPs and fabric padded with PSP–SeNPs are shown in Figure 5. The average particle size of the SeNPs is about 122 nm. It can be clearly seen that the SeNPs are attached to the fibers after the padding process. The pad–dry–cure method is a simple and effective method for preparing the loading of the fabric with SeNPs. Our previous study found that PSPs isolated from the sclerotia of *P. tuber-regium* is a naturally good stabilizer for the preparation of SeNPs.³⁰

Antibacterial Property

Figure 6 shows the bacterial amount in the broth and PSP–SeNP/broth solution; the inhibition of *Staphylococcus* was effective in the first 12 h, which is consistent with a prior study,³⁵ but after 12 h, the effect of the inhibition was very low. The main reason may be because bacteria grow very fast and the dosage of the PSP–SeNP/broth solution was relatively low. It is thus impractical to use PSP–SeNPs as a long-term antibacterial agent in fabric.

Antifungal Property

Figure 7 shows the growth of *T. rubrum* in the (a) control agar, (b) agar mixed with 1 mL of PSP solution (0.35 mM), and (c) agar mixed with 1 mL of PSP–SeNP colloid solution (0.35 mM). At the end of 14 days, it was clearly seen that the *T. rubrum* was inhibited in agar mixed with the PSP–SeNP solution. The results of our study revealed excellent antifungal activity of the PSP–SeNP-coated fabric against *T. rubrum*. The PSP–SeNP-coated fabric (drug loading at 0.765 $\mu\text{g/g}$) can inhibit 99.7% of the *T. rubrum*, and the *T. rubrum* fungi were killed rather than only inhibited, which was confirmed by the results after 14 days of incubation (Figure 8).

The inhibition percentages of the padded fabric after 7 days of incubation are listed in Table II. The padding process was repeated three times, and the drug loading for the thrice padded fabric was 0.765 $\mu\text{g/g}$. All of the incubations were duplicated, and the inhibition percentage was determined by using the average of the two.

The inhibition of *T. rubrum* was dose-dependent. When the fabric was drug loaded with SeNPs up to 0.765 $\mu\text{g/g}$, the fabric did not allow *T. rubrum* growth at all. Many other studies have proven that Se compounds can be used as antifungal agents. Cutsem et al.³⁶ studied the fungistatic and fungicidal activities of Se sulfide against *Pityrosporum*, a yeast thought to play a pathogenic role in seborrheic dermatitis and dandruff, and compared them with other ketoconazole and zinc pyrithione. The antifungal mechanism of Se remains unknown; however, there is evi-

dence which shows that inorganic Se generates free radicals when reacting with thiol groups in tissue proteins. Apart from inorganic Se, organic diselenides can also generate free radicals in the presence of thiols. Methyl-selenide formation also results in the formation of superoxide radicals.³⁷ Organoselenium compounds also exhibit a very high reduction of fungal colony growth at 200 mg/L *in vitro* testing against *Botrytis cinerea* (94%), *Fusarium culmorum* (100%), *Phytophthora cactorum* (97%), and *Rhizoctonia solani* (100%).³⁸ Our previous study found that PSPs isolated from the sclerotia of *P. tuber-regium* are a naturally good stabilizer for the preparation of SeNPs.³⁰

CONCLUSIONS

In this article, highly stable SeNPs have been used to develop antifungal fabric, especially designed for patients with tinea pedis and used as material for socks and insoles. SeNPs are prepared under a simple food-grade redox system by using PSPs isolated from the sclerotia of *P. tuber-regium* as a modifier or stabilizer. PSPs may modify the interface of nanoparticles, control the growth of nanoparticles, and stabilize nanoparticle solutions. The prepared PSP–SeNP solutions were very stable, which can be maintained for more than 6 months. They could be conveniently applied in the value-added industry for the development of antifungal fabrics.

PSP–SeNPs were coated onto fabric by using a simple pad–dry–cure method, and the coated fabric was found to have excellent antifungal ability. The antifungal ability of PSP–SeNP-coated fabric was dosage-dependent. The fabric had a 99.7% antifungal ability toward *T. rubrum* when the NanoSe loading was 0.765 $\mu\text{g/g}$. This study, for the first time, showed the excellent antifungal ability of PSP–SeNP-coated fabric against *T. rubrum*. The fabric has potential application in the treatment of tinea pedis as socks, insole, or shoe materials, and could be very effective.

The antibacterial properties of PSP–SeNPs were also studied. The inhibition of *Staphylococcus* was effective in the first 2 h, but after 12 h, the inhibition effect was very low. Meanwhile, the toxicity of SeNP-treated fabric on other normal skin cells has not been fully studied. More in-depth and long-term studies should be done to understand the mechanisms of the antifungal ability of SeNPs.

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