

Article

The Antibacterial Properties and Safety of a Nanoparticle-Coated Parquet Floor

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Abstract: Floor antibacterial technology prevents the human body from cross-infection with bacterial diseases. The most commonly used approach to endow daily-used floors with antibacterial properties is to apply a thin film of antibacterial agents on the parquet floor surface. In the present study, five commercial antibacterial nanoparticles were first dispersed in melamine resin solution, and then applied on a floor. Afterwards, the antibacterial properties of the nanoparticle-coated floor were investigated, in which *Escherichia coli* was used as the target bacteria. The impact of the nanoparticle dispersing agents on the ultimate antibacterial properties of the floor were also investigated. The results showed that silver nanoparticle-loaded hydroxyl zirconium sodium phosphate (Ag-HZDP) was most suitable as the antibacterial agent of a melamine coating for parquet flooring. With the help of sodium hexametaphosphate, the antibacterial agent was able to disperse well in the melamine resin solution and was also able to disperse well on the floor surface. When the loading amount of Ag-HZDP was 1 wt % or higher, the prepared antibacterial floor was able to kill almost all the bacteria cultivated on its surface. Moreover, the prepared antibacterial floor had a lower toxicity compared with a pristine cedar substrate. The present study provides an effective way to provide daily-used parquet floors with excellent antibacterial properties.

Keywords: parquet floor; antibacterial properties; toxicity; silver nanoparticle; dispersing agent

1. Introduction

Nowadays, people are more inclined to design their home with natural style when decorating their houses. Thus, solid wood floors like oak, bamboo, and white ash are popular to most people when they choose their ground decoration materials [1–3]. Traditionally used solid wood floors use high-quality woods as the raw materials, which have the merits of low toxicity, high comfort, and delectable appearance [4–6]. However, pure wood floors consume abundant forest resources. Since most high-quality trees, such as black alder and oak, have relatively long growth cycles, the production of pure wood flooring may lead to excessive deforestation [7].

Comparatively, parquet flooring, which is also called a tongue-and-groove floor, is composed of wood laminate surface ply, wood stripe center ply, and veneer ground ply, or veneer surface ply and plywood substrate [8]. A parquet floor uses large amounts of fast-growing woods as its raw materials, resulting in a reduction of the high-quality wood consumption [9,10]. Moreover, the tongue-and-groove structure, along with the multilayer structure of parquet flooring, can avoid anisotropy of the wood's inner structure, leading to longtime use stability [11,12]. Besides, a parquet floor still keeps the merits of natural grain and a comfortable feeling, making it a promising substitute for pure wood floors [13].

It is generally known that wood is mainly composed of carbohydrates with a multi-void structure [14,15]. Many studies have shown that wood species have antimicrobial capability [16–18]. However, when the wood is used as a ground decoration material, its surface antimicrobial capability

is more likely to be inhibited due to surface coating before practical service and surface contamination after long-time use [19,20]. Meanwhile, as ground decoration materials, which are mainly used in public and home flooring, the spread of bacteria on the floor surface is inevitable, bringing about serious threats to human health [21]. Thus, it is necessary to conduct antibacterial treatment for the floor. The most commonly-used approach to endow polymers with antibacterial properties is to incorporate antibacterial ingredients into the polymers [22–24]. For wood decoration products, it is easy to apply antibacterial agents, especially antibacterial nanoparticles, on the surface of a parquet floor [25,26]. During the production process of a parquet floor, it is usual to use melamine-impregnated paper to veneer the floor surface [27]. Based on this fact, it is feasible to fabricate a parquet floor with antibacterial coatings through dispersing antibacterial nanoparticles in the melamine resin solution first, and veneering the antibacterial agent-loaded, melamine-impregnated paper on the parquet floor afterwards [28]. Since the dispersed state of the nanoparticles affects the final antibacterial properties remarkably, it is also of significance to choose a suitable dispersing agent for the antibacterial nanoparticles in melamine solution [29]. To the best of our knowledge, although the concept of an antibacterial floor has been already proposed, the detailed study of antibacterial nanoparticle-coated flooring is still lacking.

Thus, in the present study, five commercial antibacterial nanoparticles are introduced, dispersed in melamine resin solution, and applied on a parquet floor surface. A multilayer cedar parquet floor was chosen as the floor sample, since cedar is an abundant planted fast-growing wood in Asia, Europe, and America [30]. The antibacterial properties of the nanoparticle-coated floor were investigated, in which *Escherichia coli* was used as the target bacteria. The impact of the dispersing agents on the dispersive state of the nanoparticles in the melamine solution [31] and the ultimate antibacterial properties of the floor were investigated in detail. Toxicity tests for the prepared antibacterial floor were also conducted.

2. Materials and Methods

2.1. Materials

A nine-layer cedar parquet floor substrate was used, in which the face veneer was Chinese fir, named *Cunninghamia Lanceolata*, with a density of 0.4 g/cm^3 , and the undercoated wood-based panels were plywood with 8-layer poplar veneers with backside anti-deformation grooves ($500 \times 60 \times 25 \text{ mm}^3$, water content: 10–12 wt %), which were supplied by Zhejiang Fudeli Wood Co., Ltd. (Shaoxing, China). The ultraviolet curing acrylic resin solution (white color, solid content >95%, viscosity: 1000–1200 mPa s) and the melamine resin solution (transparent appearance, solid content: 52.3 wt %, viscosity: 45 mPa s, pH value: 9) were supplied by Jiangsu Daya Wood Co., Ltd. (Danyang, China). Five commercial nanoparticle antibacterial agents, including silver nanoparticle-loaded hydroxyl zirconium sodium phosphate (Ag-HZDP), silver nanoparticle-loaded zeolite (Ag-Z), far infrared ceramic nanoparticles (FICN), Titanium oxide nanoparticles (Nano-TiO₂), and zinc oxide nanoparticles (Nano-ZnO), were all purchased from Nanjing Lisheng Chemical Company (Nanjing, China). Five dispersing agents, including polyacrylamide (PAA), sodium hexametaphosphate (Na₆P₆O₁₈), sodium carboxymethylcellulose (Na-CMC), sodium dodecylbenzene sulfonate (SDBS), and sodium polyacrylate (PAAS), were also purchased from Nanjing Lisheng Chemical Company. *Escherichia coli* (ATCC 11229) was purchased from China General Microbiological Culture Collection Center (Beijing, China).

2.2. Selection of Antibacterial Agent

In order to find the effect of the antibacterial agent on the properties of the melamine resin coating, five antibacterial agents were first added into the melamine resin solution, in which the weight content of the antibacterial agent in the melamine resin solution was 1 wt %. The prepared antibacterial agent and melamine resin solutions were then applied onto the surface of the cedar floor substrate. The optimized antibacterial agent was selected through investigating the color difference of the coated

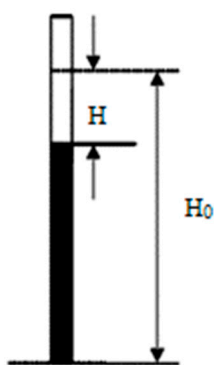
floor substrate after 7 days until the color of the floor did not change. The floor surface was first divided into three approximately even areas, named as the top, middle, and bottom parts, based on their position. The color difference of the floor was calculated using a WSC-S color difference meter (Shanghai Precision Technology Co., Ltd., Shanghai, China), based on ISO 105-J03:2009 [32], in which 3 spots were randomly selected from the pre-divided areas of the coated floor surface.

2.3. Selection of Dispersing Agent

Fifteen mL of melamine resin solution, 1.5 g of selected antibacterial agent, and 0.15 g of dispersing agents were added into a 20 mL glass tube. The mixture was mechanically shaken for ~2 h. Afterwards, the glass tube was put into a test tube rack and left for 24 h. The optimized dispersing agent was selected through investigating the stability of the antibacterial agents' dispersion in water, and calculating the subsidence value (H) of each antibacterial agent's dispersion. Specifically, H was calculated as:

$$H = H_0 - H_{\text{sub}} \quad (1)$$

where H_0 is the initial height of the antibacterial agent dispersion (mm), and H_{sub} is the final height of the antibacterial agent dispersion after 24 h of subsidence. The calculation of H was schematically illustrated in Scheme 1.



Scheme 1. Illustration of subsidence value of the antibacterial nanoparticles in melamine resin solution.

2.4. Preparation of Antibacterial Cedar Parquet Floor

In 100 mL melamine solution, a certain amount of the selected dispersing agent and the antibacterial agent, in which the mass ratio between dispersing agent and antibacterial agent was 1/10, were put into the melamine resin solution. After 2 h of mechanical shaking, the antibacterial melamine resin solutions with antibacterial agent mass contents of 0.2, 0.4, 0.6, 0.8, and 1.0 wt % were obtained. At the same time, the cedar floor was brushed with an ultraviolet curing coat. The obtained antibacterial melamine resin solutions were then applied onto the surface of the coated cedar floor using a brush. The coated floor was then aged and dried under UV light at 110 °C for ~24 h, and the antibacterial cedar parquet floor was finally obtained. The thickness of the antibacterial coating was ~1 mm, which was measured by a micrometer. For comparison, the pure melamine-coated cedar parquet floor without the introduction of the antibacterial agent was also prepared.

2.5. Antibacterial Properties Analysis of the Cedar Parquet Floor

The antibacterial properties of the prepared cedar parquet floor were analyzed through cultivating the *Escherichia coli* on the floor surface and investigating the number variation after 24 h of cultivation. The analysis was mainly based on China enterprise standard of QB/T2591-2003 [33] (test for antimicrobial activity and efficacy). The detailed steps were as follows. The cedar parquet floor sample (50 mm × 50 mm) was first washed with 70% ethanol to kill the bacteria on its surface, and then fully dried naturally. At the same time, the *Escherichia coli* was cultivated in nutrient agar at 36 ± 1 °C for 16 h,

and was transferred into nutrient broth and cultivated at 36 ± 1 °C for 16 h. This cultivation broth was diluted 500 times with 1/500 nutrient broth ($\text{pH} = 7.0 \pm 0.2$). The *Escherichia coli* was then transferred and dispersed on a slope medium surface evenly, and cultured at 36 °C for 24 h. The bacteria was then moved to an agar culture medium and cultivated at 36 °C for 24 h. The transferring steps were repeated for ~10 days. Afterwards, a 0.4 mL solution of cultivated bacteria ($\sim 5.0 \times 10^5$ cfu/mL) was added onto the surface of the floor sample, and the floor surface was covered by a PE film (50 mm \times 50 mm). The floor sample was then incubated at a relative humidity of over 90% (37 °C) for 24 h. The floor sample was then completely washed with 30 mL of a 1% NaCl solution containing Tween 80 with a pH value of 7.0 ± 0.2 . The remained active bacteria was then counted. The final antibacterial ratio (AR) was calculated using the following equation:

$$\text{AR} = (B - C)/B \times 100\% \quad (2)$$

where AR is antibacterial ratio, B is the average remaining bacterial numbers of the pure melamine resin solution-coated cedar parquet floor, and C is the average remaining bacterial numbers of the antibacterial melamine resin solution-coated cedar parquet floor.

2.6. Free Formaldehyde Content (FFC) and Total Volatile Organic Compounds (TVOC) Tests of the Cedar Parquet Floor

FFC value (C_{FF}) was determined in accordance with Chinese National Standards (GB 18580-2001) [34] as follows. The cedar parquet floor sample (100 cm \times 100 cm) was first put into a testing chamber with an air exchange rate of 1.0 ± 0.05 h⁻¹ and a loading rate of 1.0 ± 0.02 m²/m³. The ambient temperature, relative humidity, and air velocity on the sample surface were set at 23 ± 0.5 °C, $50\% \pm 3\%$, and 0.1–0.3 m/s, respectively. The parquet floor sample was then put in the chamber for 5 days to release the formaldehyde. An appropriate amount of the air in the chamber was withdrawn at predetermined intervals and injected into an absorption bottle with distilled water. The content of the formaldehyde (C_f) in the air was quantified based on the absorbance value of 412 nm recorded by the ultraviolet spectrophotometer. The formaldehyde release equilibrium was reached when the C_f bias between two samples was lower than 5%. The CFF was then calculated as:

$$C_{\text{FF}} = C_f/V_{\text{air}} \quad (3)$$

where C_f is formaldehyde content in the sampling air (mg), V_{air} is the volume of the sampling air (m³).

The TVOC value (C_{TVOC}) was determined in accordance with (HJ571-2010) [35], and calculated using the following equation:

$$C_{\text{TVOC}} = xC_{\text{VOC}}/V_{\text{air}} \quad (4)$$

where x is the air exchange rate (m³/m² h), C_{VOC} is the amount of the VOC in the sampling air (mg), and V_{air} is the volume of the sampling air (m³). Specifically, CVOC was quantified by gas chromatograph equipment GC436 produced by Bruker Co., Ltd. (Billerica, MA, USA).

2.7. Cytotoxicity Tests of the Cedar Parquet Floor

The cytotoxicity effect of the cedar parquet floor was analyzed using the methyl thiazolyl tetrazolium (MTT) assay. Briefly, the floor sample was put into a 96-well plate with RPMI-1640 medium containing 10% fetal bovine serum (FBS), and the cells were allowed to grow in an incubator for 72 h at 37 °C. The cell culture fluid was then filtrated and kept at 4 °C as the extract liquid. Subsequently, liver cells were seeded and grown in 10^4 cells/well (200 μ L/well) in a 96-well plate for 24 h in a RPMI-1640 medium containing 10% FBS. After that, the media was removed and replaced with the prepared extract liquid for exposure. Then, the liquid was removed and the cells were incubated with 20 μ L of MTT (5 mg/mL in PBS) in fresh medium for 4 h at 37 °C. After the medium liquid was removed, the residual was dissolved in DMSO (150 μ L/well). The respective absorbance values (Abs.) were read

at 490, 570, and 630 nm using a microplate reader (Sunrise RC TS TC, Grödig, Austria) to determine the relative growth rate (RGR) value of the cells. The results were given as the mean of three independent experiments. The cytotoxicity grade was evaluated based on ISO 10993-5:1999 [36]. For comparison, the pristine cedar substrate was also assessed using the cytotoxicity test.

3. Results and Discussion

In order to analyze the effect of the antibacterial agent on the properties of the melamine resin solution coating, the selection of the optimized antibacterial agent was conducted first. Figure 1 shows the color difference of the melamine resin solution-coated floor with or without the introduction of the antibacterial agents. One could observe that compared with the floor coated with pure melamine resin solution, all the floor samples coated with melamine resin solution loaded with antibacterial agents showed a color difference. With the increase of the antibacterial agent loading amount, the color difference also increased. It could be seen that when the loading amount of the antibacterial agents reached 1 wt %, the color difference value of the floor coated with Ag-Z was as high as 20%. At the same time, the values of all the other four samples were lower than 15%, indicating Ag-HZDP, FICN, Nano-TiO₂, and Nano-ZnO did not affect the properties of the melamine resin solution much.

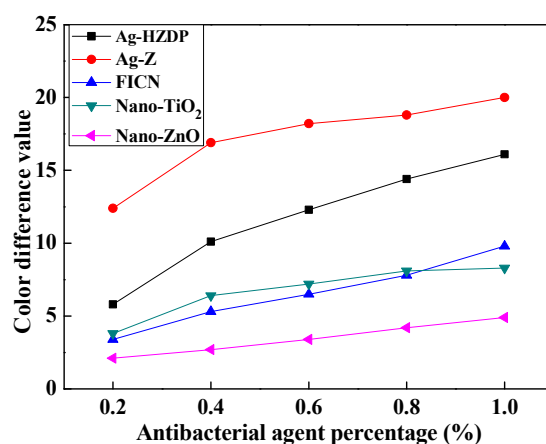


Figure 1. Effect of different quantities of antibacterial agents on the color of the coatings.

The rough AR of the coated floor was then investigated, with the results shown in Figure 2. In each melamine resin solution, the loading amount of the antibacterial agent was 1 wt %. It could be clearly seen from Figure 2 that compared with other antibacterial agents, antibacterial agents incorporated with silver nanoparticles, Ag-Z and Ag-HZDP, had the highest antibacterial efficiency; the ARs of these two agents could reach 90% or higher. It is generally known that silver ions can generate bacteria function disorder and ingredient destruction through contact reactions [37]. Even minute amounts of silver ions can firmly attach to the bacteria cytomembrane and coagulate the protein, resulting in destruction of synthetase activity [38]. Moreover, the silver ions can also destroy the transmission system and respiratory system of the bacteria, which results in excellent antibacterial properties for silver nanoparticle loaded antibacterial agents [39]. On the contrary, the semiconductor antibacterial agents, such as titanium oxide and zinc oxide nanoparticles, have a totally different antibacterial mechanism. Specifically, under the irradiation of incident light, especially ultra-violet light, the electrons (e) of the semiconductors delocalized from the conduction band (CB) to the valence band (VB). The holes (h) in the VB react with the H₂O molecules or O₂ molecules to generate highly oxidative radicals [40]. The radicals kill the bacteria through redox reactions. Since the antibacterial properties of these semiconductors require continuous light irradiation, and the electron/hole recombination rate is high, the antibacterial ability of these semiconductors is relatively low compared with silver nanoparticle loaded antibacterial agents [41]. Combined with the results from Figure 1, Ag-HZDP was selected as the optimized antibacterial agent and used for the following test.

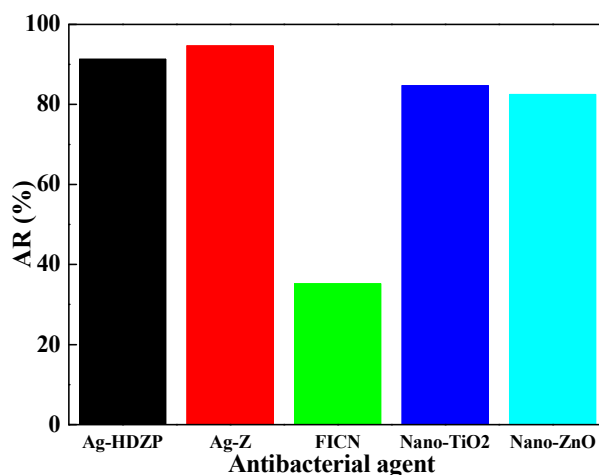


Figure 2. Antibacterial ratio of different antibacterial agents loaded with melamine resin coatings.

Since the dispersing status of the nanoparticles in the solution significantly affects the final antibacterial properties of the cedar parquet floor, the optimized dispersing agent was then selected. Table 1 shows the dispersion of Ag-HZDP in melamine resin solution using five alternative dispersing agents. It could be clearly seen from Table 1 that the Ag-HZDP was not well dispersed in melamine solution without the introduction of the dispersing agents, for which the subsidence value was ~2.5. When the dispersing agents were introduced into the system, the Ag-HZDP dispersed diversely in the melamine resin solution using different dispersing agents. One can clearly observe from Table 1 that the subsidence value of Ag-HZDP in both $\text{Na}_6\text{P}_6\text{O}_{18}$ and SDBS decreases, indicating a better dispersing stability of the Ag-HZDP in the melamine solution. Comparatively, the other three dispersing agents could not enhance the dispersing stability of the Ag-HZDP, which meant these three ones were not suitable as dispersing agents for the Ag-HZDP in melamine solutions. Combined with the final appearance and solution status shown in Table 1, it can be seen that with the assistance of the $\text{Na}_6\text{P}_6\text{O}_{18}$, Ag-HZDP was uniformly dispersed in the melamine resin solution without changing its color. Thus, the $\text{Na}_6\text{P}_6\text{O}_{18}$ was selected as the optimized dispersing agent and used for the following test.

Table 1. The subsidence condition and final appearance of Ag-HZDP in melamine resin solution using five dispersing agents.

| Dispersing Agent | Subsidence Value/Condition | Appearance |
|--------------------------------------|----------------------------|--|
| Nil | 2.5 mm | Normal color |
| PAA | Precipitates appear | Grey in color |
| $\text{Na}_6\text{P}_6\text{O}_{18}$ | 1 mm | Uniformly dispersed |
| Na-CMC | Na-CMC insoluble | Nanoparticle/Na-CMC separated |
| SDBS | 1.25 mm | Bubble appears, slight color change in solution |
| PAAS | Solution thickened | Viscosity increased, slight color change in solution |

Based on the above mentioned results, Ag-HZDP and $\text{Na}_6\text{P}_6\text{O}_{18}$ were used as the optimized antibacterial agent and dispersing agent, respectively, to prepare the final cedar parquet floor. Figure 3 shows the AR of the Ag-HZDP-melamine solution-coated floor with and without the $\text{Na}_6\text{P}_6\text{O}_{18}$. It can be seen that without the assistance of the $\text{Na}_6\text{P}_6\text{O}_{18}$, the AR of the floor reached ~90% only when the Ag-HZDP loading amount was higher than 1 wt %. Comparatively, for $\text{Na}_6\text{P}_6\text{O}_{18}$ assisted Ag-HZDP, the floor had an AR of higher than 90% when the loading amount of the Ag-HZDP was only 0.6 wt %. The results indicated that with the help of $\text{Na}_6\text{P}_6\text{O}_{18}$, the Ag-HZDP was better dispersed, both in the melamine resin solution and on the surface of the cedar parquet floor, resulting in better antibacterial effects.

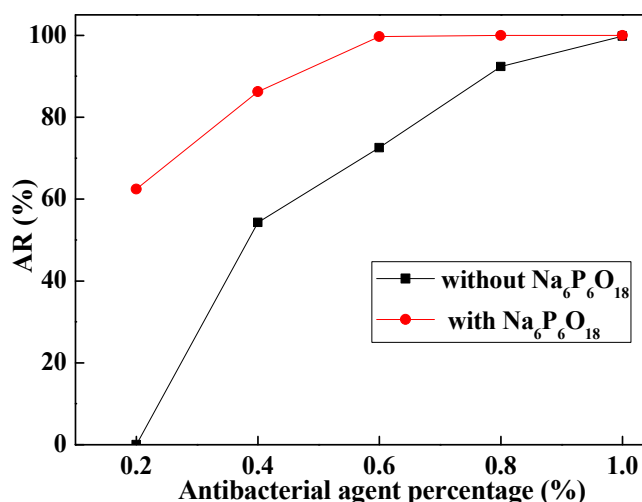


Figure 3. Antibacterial ratio of Ag-HZDP-melamine resin solution-coated cedar parquet floor, with and without $\text{Na}_6\text{P}_6\text{O}_{18}$.

Figure 4 shows the SEM images of the cedar parquet floor surface after cultivation of the *Escherichia coli*. It could be directly revealed from Figure 4a that the melamine-coated cedar parquet floor without antibacterial agent did not have any antibacterial ability. After 24 h of cultivation, numerous *Escherichia coli* colonies still grew on the floor surface. It could be also seen from Figure 4b that pure melamine resin did not have antibacterial ability either, resulting in abundant growth of the *Escherichia coli* on the surface of pure melamine resin-coated cedar parquet floor. Since the flooring is mainly used for public and home flooring, and the flooring cannot be used practically without coatings, the antibacterial treatment to the coatings is inevitable. After the antibacterial agent, Ag-HZDP, was added into the melamine resin solution, the antibacterial ability of the cedar parquet floor was dramatically enhanced. After 24 h of cultivation, nearly no *Escherichia coli* colonies still grew on the floor surface, due to the presence of the Ag-HZDP. From the magnified SEM image shown in Figure 4d, it can be seen that the morphology of some *Escherichia coli* had been changed. This indicated that the Ag-HZDP has promising antibacterial properties and is capable of being added into a melamine resin solution to enhance the antibacterial ability of cedar parquet flooring when combined with $\text{Na}_6\text{P}_6\text{O}_{18}$. Since one of the most common uses for floor products is in home flooring, where the floor is in contact with human skin continually, the toxicity of the floor is extremely significant. Thus, it is necessary to conduct conventional toxicity tests for a prepared antibacterial floor. The FFC and TVOC tests were conducted first. The C_{FF} and C_{TVOC} values of the floor were 0.084 mg/m^3 and $0.386 \text{ mg/m}^2\cdot\text{h}$, respectively, which were both lower than Chinese national standards and European Union standards [42,43].

The cytotoxicity of the prepared antibacterial floor was then analyzed using MTT method. Table 2 shows the RGR values of the floor. On the basis of the RGR values, it can be seen that the toxicity grade of the pristine cedar substrate was 3 according to United States Pharmacopeia (USP). When the antibacterial coating was applied on the cedar floor surface, all the RGR values of the prepared antibacterial floor were obviously enhanced compared with the value of the pristine cedar substrate. Moreover, the cytotoxicity grade of the prepared antibacterial floor was similar to that of the cedar floor with antibacterial-agent-free melamine coating. The results indicated the prepared antibacterial floor had a lower toxicity grade and was promising for daily use as a flooring product.

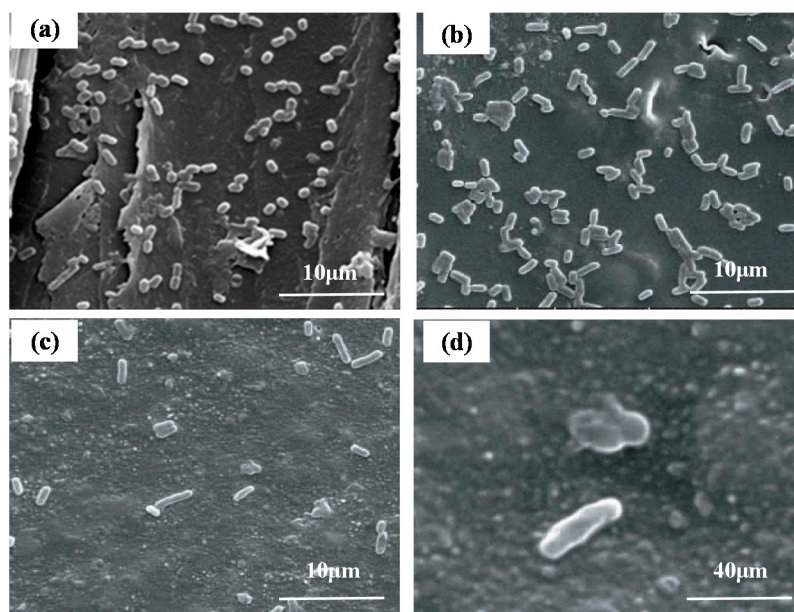


Figure 4. SEM image of cedar parquet floor (a) without coating, (b) with pure melamine resin solution, (c) with Ag-HZDP-loaded melamine resin solution, and (d) magnified cedar parquet floor with Ag-HZDP-loaded melamine resin solution.

Table 2. Cytotoxic effects of the prepared antibacterial floor.

| Sample | 490 nm | | | 570 nm | | | 630 nm | | |
|--|--------|-------|-------|--------|-------|-------|--------|-------|-------|
| | Abs. | RGR/% | Grade | Abs. | RGR/% | Grade | Abs. | RGR/% | Grade |
| Pristine cedar substrate without melamine coating | 0.1090 | 32.09 | 4 | 0.5287 | 25.82 | 4 | 0.4643 | 33.27 | 3 |
| Cedar floor with melamine coating (no antibacterial agent) | 0.2221 | 68.45 | 2 | 0.9921 | 60.43 | 2 | 0.4619 | 33.10 | 3 |
| Prepared antibacterial floor | 0.2052 | 63.25 | 2 | 0.8943 | 54.45 | 2 | 0.4618 | 32.89 | 3 |

4. Conclusions

In summary, a novel antibacterial cedar parquet floor was prepared in the present study. The optimized antibacterial agent and the dispersing agent were selected, and the antibacterial properties and the toxicity tests were conducted in detail. The results showed that Ag-HZDP and $\text{Na}_6\text{P}_6\text{O}_{18}$ were suitable as antibacterial and dispersing agents in a melamine coating for flooring. With the Ag-HZDP-loaded melamine resin solution applied onto the floor surface, the prepared antibacterial floor could inhibit and kill almost all the *Escherichia coli* bacteria cultivated on its surface. Moreover, the prepared antibacterial floor had a lower toxicity compared with pristine cedar substrate. The present study provides an effective way to fabricate daily-used parquet flooring with excellent antibacterial properties and lower toxicity.

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