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ORIGINAL RESEARCH

Antibacterial biocomposites: efficacy of MWCNT-coated Hanji cellulose paper against *E. coli*

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Abstract Cellulose paper has been studied for its application as an antibacterial filter, due to its robust porous structure, minimal ecological footprint, biocompatibility, and inherent antibacterial properties. Moreover, its properties can be further enhanced via surface functionalization with metallic nanoparticles (NPs) such as silver, zinc, etc. However, the synthesis of metallic NPs is challenging, costly, and environmentally harmful. On the other hand, carbon nanotubes (CNTs) are highly suitable as an additive to cellulose paper due to their high electrical conductivity, excellent mechanical strength, ease of fabrication, and antibacterial properties. In this study, we coated multi-walled carbon nanotubes (MWCNTs) on Hanji, a traditional Korean paper, using a simple dipping method and investigated its antibacterial activity against *Escherichia coli (E. coli)*. The MWCNT-coated Hanji exhibited an inhibition efficiency of ~93% against *E. coli* cells. Moreover, the MWCNT coating resulted in improved mechanical strength, enhanced electrical conductivity, and increased hydrophobicity of the Hanji. Furthermore, it was observed that MWCNTs exhibited exceptionally stable adhesion to the Hanji surface. Our finding shows that MWCNT-coated Hanji could be utilized as an antibacterial material that is used as masks, air pollution filters, and wallpapers in hospitals and residential complexes, and an efficient platform for antiviral studies.

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Introduction

There has been a growing urgency for the replacement of synthetic materials given their significant ecological footprint (Rinaudo 2007; Rostami et al. 2019). Cellulose, an organically derived biopolymer, has been increasingly investigated over the past few decades as a desirable alternative due to its biodegradability, biocompatibility, and renewability. In addition, it also possesses great mechanical properties, a low mass density, and inexpensive production costs (van de Ven and Sheikhi 2016). Furthermore, cellulose is very resistant to acids, extreme temperatures, and proteolytic enzymes (Pandey et al. 2012; Peng et al. 2011). Among many cellulose papers available, Korean traditional cellulose paper, Hanji, is an especially intriguing alternative to synthetic polymer, as one of the most stable and durable papers with a life span exceeding 1000 years (Choi et al. 2012). It is made of bast fibers of paper mulberry trees and comprises long cellulose fibers and pores, showing a 7000 ~ 9000 range of high degree of polymerization (Jeong et al. 2014). In the current industry, it is mainly used as wrappers or packaging material. It has low inherent antibacterial activity, but its antimicrobial performance can be improved by surface treatment (Jung et al. 2016). As such, Korean paper has already been used for antibacterial activity in combination with different types of metallic NPs (zinc, titanium, gold, silver, etc.) (Du et al. 2013; Kamal et al. 2022; Yang et al. 2023; Anwar et al. 2021; Maslana et al. 2021; Onyszko et al. 2020). However, synthesizing metallic NPs is challenging, costly, and environmentally harmful (Exbrayat et al. 2015; Jamkhande et al. 2019). Nowadays, carbon-based nanomaterials are attracting attention in combating microbial contamination. Especially, carbon nanotubes (CNTs) show antibacterial activity (Deokar et al. 2013) due to their ability to rupture the bacterial cell walls. Therefore, a composite of CNTs and cellulose paper would result in a highly efficient product that has the ability to deal with antimicrobial resistance.

Recently, the increasing concern over air pollution has led to a growing demand for filters. The development of filters has become crucial, not only for their primary function of filtering out particulate matter, fine dust, and volatile organic compounds from the atmosphere but also for their antimicrobial ability against bioaerosols such as bacteria. Poor air quality has been linked to around four million premature deaths annually (Asefa and Mergia 2022). Globally, air pollution contributes to 1 out of 10 deaths and caused approximately 5.5 million deaths in 2013 (Gonzalez-Martin et al. 2021). Additionally, poor air quality can reduce employee productivity by 10–15% in workplaces (Cincinelli et al. 2016). On the other hand, over the past 60 years, we have been losing the battle against bacteria as they are continuously developing resistance to antibiotics. Consequently, these disease-causing agents are now becoming antibiotic-resistant. The European Center for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMEA) estimated annual losses of over €1.5 billion due to antimicrobial resistance (Serwecińska 2020). In 2013, the USA reported over 23,000 deaths caused by resistant infections (Dhingra et al. 2020). The European Commission also reported approximately 33,000 deaths per year due to antimicrobial resistance (AMR). A World Health Organization (WHO) report confirmed a global shortage of effective antibiotics due to AMR problems (Aljeldah 2022). Therefore, it is essential to find efficient and cost-effective substitutes for conventional antibiotics.

In this study, we synthesized multi-walled carbon nanotubes (MWCNTs) coated Hanji paper using a simple dipping method and investigated their antibacterial activity against *Escherichia coli* (*E. coli)*. We coated MWCNTs onto Hanji paper solely through the dipping method without acid modification of the MWCNTs, and it is more cost-effective and easily synthesized than SWCNTs. Furthermore, the MWCNT-coated Hanji paper showed improved mechanical strength, enhanced electrical conductivity, increased hydrophobicity, and exceptionally stable adhesion to the Hanji surface. We suggest that MWCNT-coated Hanji paper could be used as a mask, air pollution filter, and wallpaper in hospitals and residential complexes, reducing the risk of infections.

Experimental details

Materials

Pristine MWCNTs (Jenotube 6A; diameter ~6 nm, wall number 3~5 layers) were purchased from JEIO, South Korea. N-Methyl-2-pyrrolidone (NMP) anhydrous of 99.5% grade, was purchased from Sigma-Aldrich. The Gram-negative bacterium *E. coli* was obtained from the Korean Collection for Type Cultures (KCTC No. 2791, https://kctc.kribb.re.kr/, Jeongeup-si, Jeollabuk-do, Korea).

MWCNT dispersion

To ensure homogeneous coating of MWCNTs on Hanji, aggregation of the MWCNTs must be prevented by overcoming van der Waals attraction. Given that the dispersion of MWCNTs in NMP has been effective for more than 7 days (Sabri et al. 2020), NMP was used to disperse our MWCNTs. So, 8.6 mg of MWCNTs were mixed in 40 mL of NMP solvent (0.0215 wt %) in a 50 mL plastic conical tube (SPL Life Sciences Co. Ltd.). The conical tube was then put in a 200 mL plastic beaker surrounded by ice cubes. Next, sonication (Ultrasonic homogenizer, KUS-650, 100 W, 6 HORN, 25 °C, ON; 2 s, OFF; 3 s) was performed for 3 h for MWCNT dispersion in NMP. The ice was constantly replenished to maintain a constant temperature.

Characterization

Optical microscopy (Nikon ECLIPSE LV150N) and scanning electron microscopy (SEM, Nova NanoSEM) were performed to analyze the surface morphological changes of normal and MWCNT-coated Hanji. SEM was performed in secondary electron mode with an acceleration voltage of 10 kV. Raman Spectroscopy (XperRam 200) was used to characterize MWCNTs. X-ray photoelectron spectroscopy-XPS (K-alpha, Thermo Scientific) was conducted using monochromatic Al Kα radiation (1486.6 eV) to investigate the surface chemistry.

Leaching assessment of MWCNT

To assess whether MWCNTs coated on paper are leaching into the water, we immersed MWCNT-coated paper in water and collected samples over time. We obtained UV-Vis spectra of the samples contained in quartz cuvettes using a double-beam UV-visible spectrophotometer (V-670, Jasco) in the 240–600 nm range. We confirmed the quantity of MWCNTs in the water by comparing the absorption values around 260 nm, which is known as the absorption peak of CNTs (Njuguna et al. 2015).

Zone of inhibition in *E. coli* culture

The *E. coli* was cultured in nutrient broth (MBcell, KisanBio Co., Seoul, Korea) at 37 °C with shaking at 180 rpm for 16 h. The pre-cultured *E. coli* cells were suspended in a phosphate-buffered saline (PBS) solution (pH 7.2) to obtain a cell concentration of $9.3 \pm 4.0 \times 10^5$ colony-forming units per milliliter (CFU/mL). Aliquots (100 μL) of the suspension were spread on 90 mm nutrient agar plates (MBcell, KisanBio Co.). Subsequently, 3×3 cm of the normal Hanji and MWCNT-coated Hanji were transferred onto the plates and put into contact with the surface of the agar plates for 60 min. Each paper was then removed from the plates, and the paper was analyzed by SEM. The plates were statistically incubated at 37 °C for 16 h. After a given incubation, the plates were photographed. The experiments were performed in triplicate.

Antibacterial performance of MWCNT-coated Hanji paper against *E. coli*

One hundred microliters ($9.3 \pm 4.0 \times 10^3$ CFU/mL) of the *E. coli* cells were transferred onto the pristine Hanji and MWCNT-coated Hanji papers (1×1 cm) and kept the contact at 37 °C for 60 min. At the same time, 100 μL of the non-contact *E. coli* cells was prepared as a negative control. After the given contact duration, 900 μL of PBS solution was added to each paper and gently agitated to homogenize the solution. Aliquots (100 μL) of the solution were spread on the nutrient agar plates and the plates were statistically incubated at 37 °C for 24 h. The colonies grown on the plates were counted and the inhibition efficiency (%) of each paper was determined by comparing the CFU numbers of each sample (CFU_S) with a negative control (CFU_{NC}) using the following Eq. (1).

$$Antibacterial\ efficiency(AE\ ,\%) = (1 - \frac{CFU_S}{CFU_{NC}}) \times 100 \quad (1)$$

All the experiments were conducted in triplicate. These preparation, treatment, and measurement procedures were employed in the reusability experiment.

Reusability of MWCNT-coated Hanji paper as antibacterial surface

The reusability of the normal Hanji and MWCNT-coated Hanji was evaluated by measuring inhibition efficiency against the bacterium *E. coli*. The viable cell numbers of *E. coli* were measured upon exposure to either normal or MWCNT-coated Hanji and the inhibition efficiency of each paper was calculated as described above (AE^{1st}). After the experiment, each paper was air-dried under ambient conditions for 24 h and re-used for the antibacterial experiment. The paper was used three times and the inhibition efficiency of each experiment was determined as described earlier ($AE^{Repeated\ use}$). The relative antibacterial performance was calculated using the following Eq. (2).

$$Relative\ antibacterial\ performance(\%) = (\frac{AE^{Repeated\ use}}{AE^{1st}}) \times 100 \quad (2)$$

All experiments were carried out in triplicate.

Results and discussion

Fabrication of MWCNT-coated Hanji

As illustrated in Fig. 1a, MWCNTs were coated to fabricate the antibacterial biocomposite Hanji, denoted as MWCNT-coated Hanji. First, the MWCNTs dispersion solution was subjected to 15 min of bath sonication. A 100 mm diameter glass dish was cleaned using deionized (DI) water, isopropyl alcohol (IPA), and acetone to remove any contaminants. Subsequently, it was dried in an oven at 60 °C for 4 h to remove residual moisture. After completely drying and cooling the glass dish, 30 mL of the dispersed MWCNT solution was carefully poured into the dish using a pipette, fully submerging the Hanji (5 cm by 5 cm) in the solution (a process referred to as dip coating). After being immersed in the MWCNT solution for 40 s, the paper was transferred to an oven and left to dry for 2 h. During the drying process, the paper was flipped every hour to ensure proper drying from both surfaces. This step was repeated five times to enhance the coverage of MWCNTs on the surface of the Hanji (see details regarding the mass difference between Hanji before and after the MWCNT coating in Fig. S1).

To initially verify the presence of MWCNTs on the Hanji, we performed a comparative surface analysis of normal Hanji and MWCNT-coated Hanji via optical microscopy. Figure 1b shows the optical images of the normal Hanji. In the case of the normal Hanji, numerous cellulose fibers are easily observed, which are intertwined and bonded together. These long, slender, and tubular structures contribute to the rough texture of the Hanji surface. Optical images also enabled the observation of the porosity of the Hanji paper, including the presence of pores, voids, and interstitial spaces between fibers. Due to the porosity, Hanji exhibits high moisture absorption capability, which is consistent with the characteristics of typical cellulose papers (Fig. S2). Contrary to the white color of normal Hanji, MWCNT-coated Hanji exhibits a dark color as depicted in Fig. 1c. We observed that even after the MWCNT coating, the roughness, irregularities, and porosity resulting from the uneven arrangement of cellulose networks remain clearly visible, indicating the preservation of Hanji's distinctive porous structure. Additionally, the hydrophobicity was maintained in MWCNT-coated Hanji, in contrast to the hydrophilic nature of normal Hanji (Fig. S2). Since low moisture absorbance is a crucial and advantageous feature for antibacterial materials in minimizing microbial activity, coating Hanji with MWCNTs signifies the potential to create a more hygienic antimicrobial composite material by inhibiting the growth of bacteria, thus rendering it suitable for various antibacterial applications.

Surface coating analysis of MWCNTs on Hanji

Next, we investigated and compared the surface of normal Hanji and MWCNT-coated Hanji. As shown in Fig. 2a, SEM analysis shows that normal Hanji exhibits a typical texture commonly observed in cellulose papers, characterized by a dense network of entwined cellulose fibers and macro-pores (Fig. 2a). MWCNT-coated Hanji (Fig. 2b) shows a similar texture to the porous normal Hanji in which cellulose fibers are interwoven throughout the whole structure (the left image

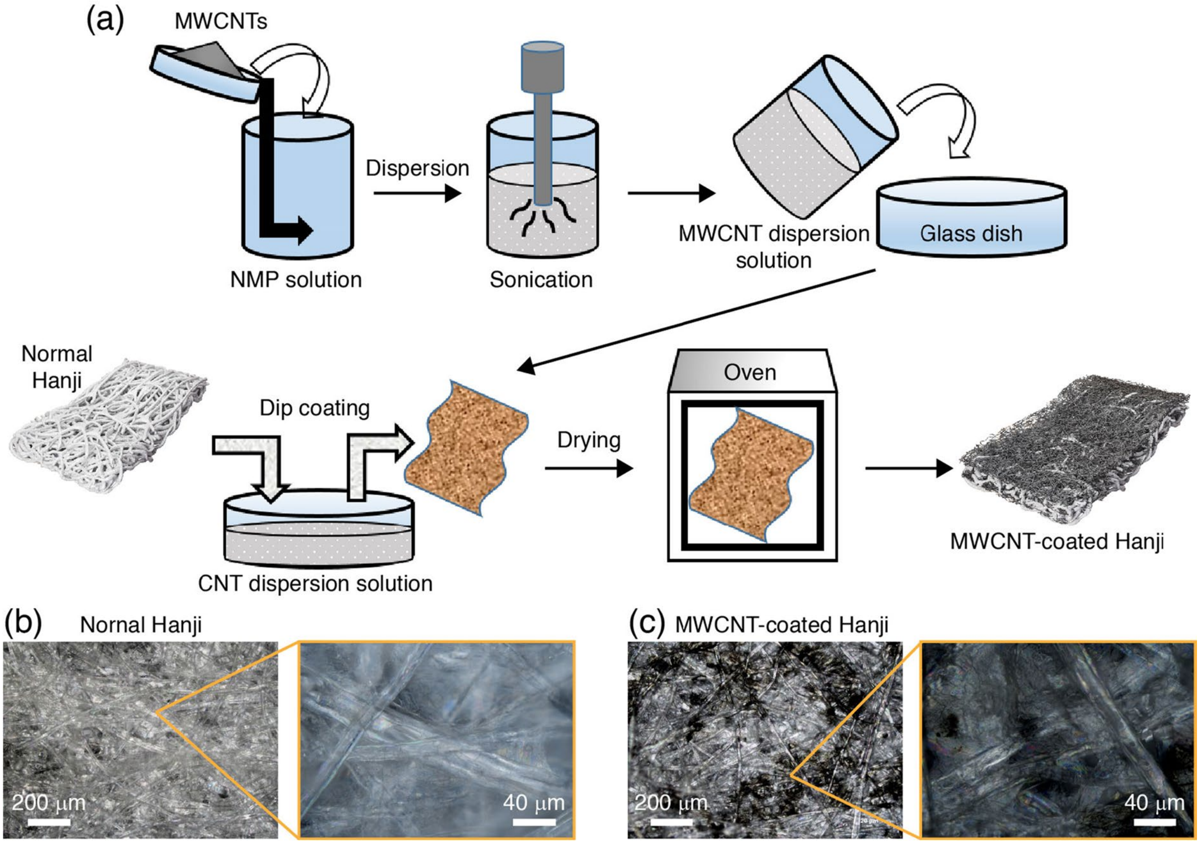

Fig. 1 Coating of MWCNTs on the surface of cellulose fibers in Hanji. **a** A schematic illustration describing the fabrication process of MWCNT-coated Hanji. Optical microscope images of **b** normal Hanji and **c** MWCNT-coated Hanji

in Fig. 2b). However, a closer observation of the cellulose fiber surface revealed that, unlike normal Hanji, the surface of cellulose fibers of Hanji is covered with MWCNTs (the right image in Fig. 2b). The results clearly reveal the successful coating of MWCNTs onto the surface of cellulose fibers of Hanji and the preservation of the interstitial voids between fibers. Through comprehensive SEM analysis, we validated the stochastic random dispersion of MWCNTs on Hanji surface (Fig. S3). Moreover, we confirmed the high adhesion stability of MWCNTs to the cellulose fibers comprising Hanji and the enhancement of the mechanical strength of Hanji after MWCNT coating (Fig. S4 and S5).

To further investigate the surfaces of both normal Hanji and MWCNT-coated Hanji, we performed XPS and Raman analyses. Figure 2c shows XPS survey spectra of both normal Hanji and MWCNT-coated Hanji. In the case of normal Hanji, typical O1s and C1s peaks commonly observed in cellulose fibers were detected (Kuzmenko et al. 2017; Johansson 2004), whereas MWCNT-coated Hanji showed an increase in the intensity of the C1s peak, indicating an increase in the amount of carbon-containing materials. *i.e.*, MWCNTs. The addition of MWCNTs, which are mainly composed of C−C bonds, resulted in a higher intensity of the C−C bonding peak in the high-resolution XPS data of MWCNT-coated Hanji (Fig. 2d). The C−C bonding peak of MWCNT-coated Hanji is higher than other oxygen-containing bonds (C−O and C=O, which are attributed to the presence of hydroxyl groups on the surface) commonly found in Hanji cellulose fibers (Kuzmenko et al. 2017). Raman analysis also clearly shows the presence of MWCNTs on Hanji cellulose fibers (Fig. 2e), providing strong evidence of the successful coating of MWCNTs on the Hanji surface.

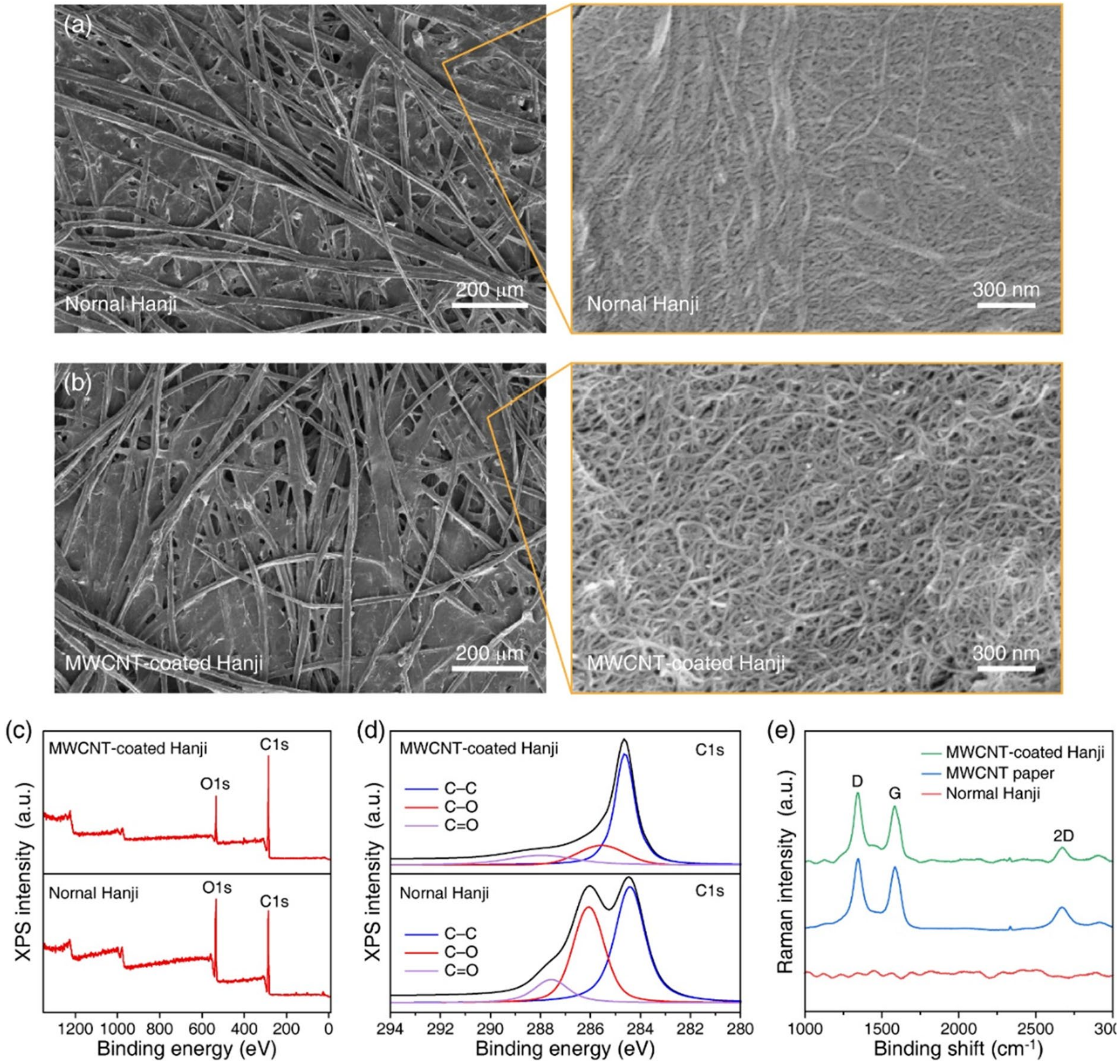

Fig. 2 Surface analysis and comparison of normal Hanji and MWCNT-coated Hanji. SEM images of **a** normal Hanji and **b** MWCNT-coated Hanji. **c** XPS survey spectra and **d** high-resolution XPS C1s spectra of normal Hanji and MWCNT-coated Hanji. **e** Raman spectra of Normal Hanji, MWCNT paper, and MWCNT-coated Hanji

Verification of the antibacterial properties of MWCNT-coated Hanji

Antibacterial performance against *E. coli* of normal and MWCNT-coated Hanji was evaluated (Fig. 3a). Normal Hanji exhibited a weak inhibition efficiency against *E. coli* cells and the inhibition zone was negligible in the culture plate of *E. coli* (Fig. 3b, d). On the other hand, the growth of *E. coli* cells was inhibited upon contact with MWCNT-coated Hanji, with a clearly visible zone of inhibition (Fig. 3c). The MWCNT-coated Hanji showed an inhibition efficiency of 86.9±6.3% (Fig. 3d). The results indicate that the antibacterial performance of the Hanji was significantly improved by MWCNT coating. Moreover, we confirmed the reusability of MWCNT-coated Hanji for the antibacterial performance against *E. coli*. The relative antibacterial performance of MWCNT-coated Hanji paper decreased from 100±15.8 (1st use) to 79±13.3 (2nd use)

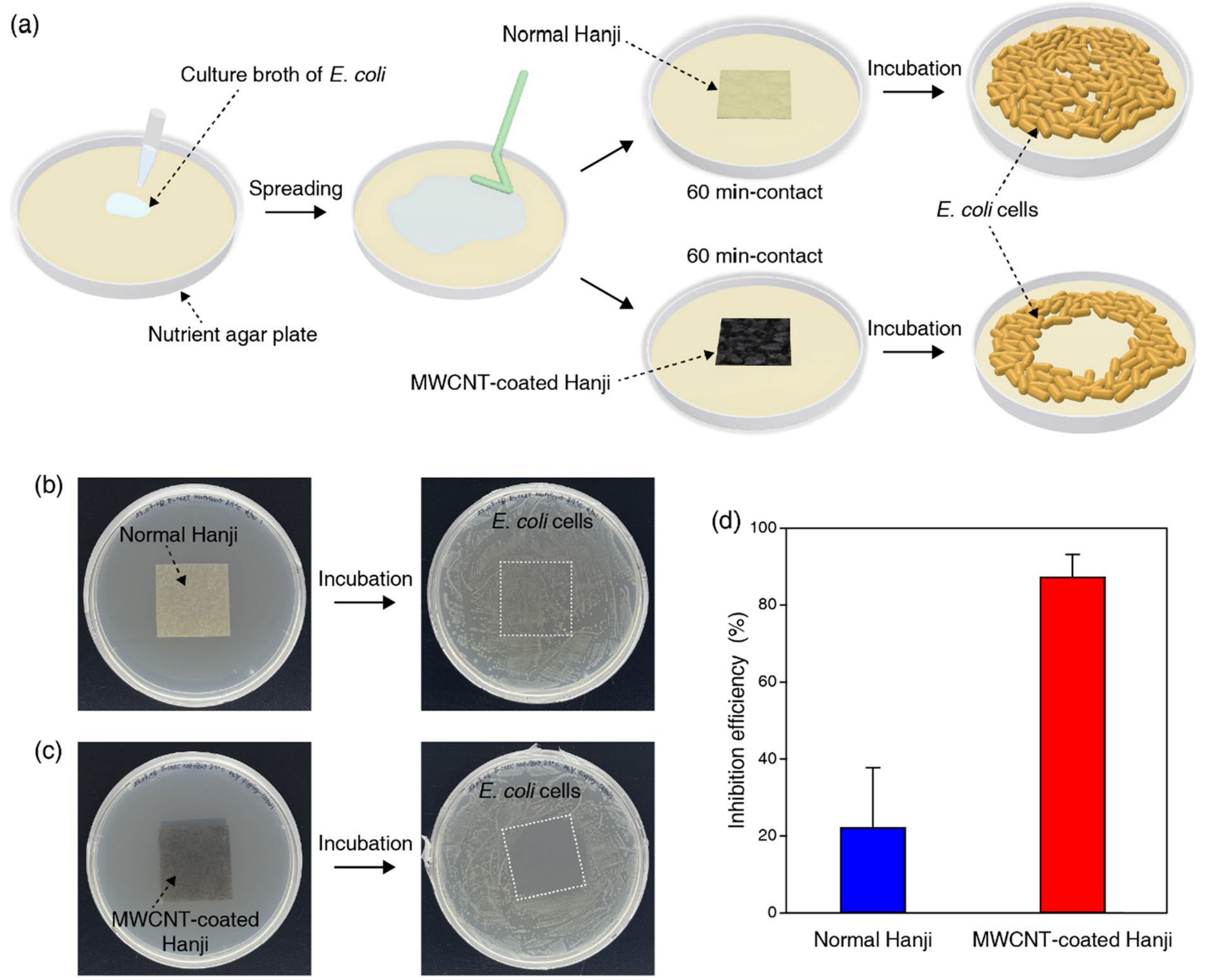

Fig. 3 Assessment of antibacterial activity of normal and MWCNT-coated Hanji. **a**–**c** Comparison of growth inhibition zones of *E. coli* cells upon contact with normal and MWCNT-coated Hanji. **d** Comparison of inhibition efficiency (%) of normal and MWCNT-coated Hanji paper *E. coli* cells

and 73.3 ± 13.1% (3rd use) upon repeated use. Despite a notable decrease in the inhibition efficiency of both Hanji papers upon repeated use, the MWCNT-coated Hanji nevertheless achieved greater antibacterial performance than normal Hanji.

The *E. coli* cells were observed on the surface of both normal and MWCNT-coated Hanji, displaying a typical rod shape (Fig. 4). The *E. coli* cells were mostly intact when they were in contact with normal Hanji (Fig. 4a, b). On the other hand, the *E. coli* cells were shown to be coated with MWCNTs upon contact with MWCNT-coated Hanji paper, and surface damage of *E. coli* was observed in SEM (Fig. 4c, d). While the process and mechanism by which *E. coli* cells are encapsulated by MWCNTs have yet to be fully elucidated, the results clearly show that MWCNTs on the Hanji play an important role in the antibacterial performance of the Hanji against *E. coli*.

Hydrophobicity has been found to inhibit the growth of bacteria and reduce bacterial adhesion and biofilm formation to the surface (Cheng et al. 2007; Doyle 2000; Freschauf et al. 2012). Hydrophobic surfaces reduce moisture uptake and repel water, preventing the creation of a stable water film required for bacterial adhesion, resulting in reducing the likelihood of colonization and subsequent growth (Hidouri et al. 2022; Manivasagam et al. 2022; Shen et al. 2020). Hydrophobic surfaces also hinder the transport and diffusion of nutrients required

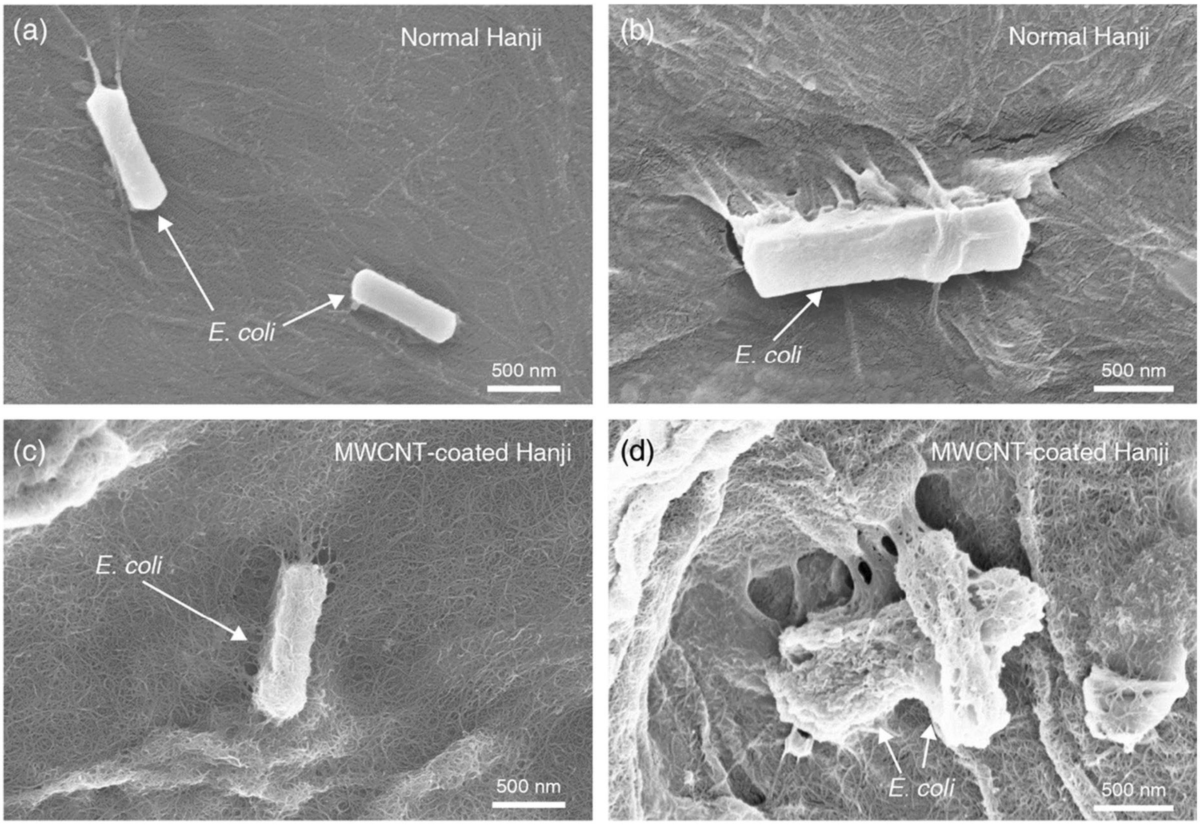

Fig. 4 SEM images of *E. coli* cells on the surface of **a**, **b** normal Hanji and **c**, **d** MWCNT-coated Hanji. The *E. coli* cells remain mostly intact on the surface of normal Hanji, but their typical cylindrical shape is destroyed on MWCNT-coated Hanji

for bacterial growth. This disturbance can lead to leakage of intracellular components, disruption of cellular processes, and even cell death (Wu and Shah 2014; Zheng et al. 2021). In this study, the MWCNT-coated Hanji exhibited a superior hydrophobicity to that of the normal Hanji (Fig. S2). We propose that this contributed to the higher inhibition efficiency of MWCNT-coated Hanji paper (i.e., 86.9 ± 6.3%) than that of the normal Hanji paper (26.1 ± 5.7%) (Fig. 3d). This is supported by our observation that a reduction in the inhibition efficiency of the MWCNT-coated Hanji paper occurred when treated with UV ozone (Fig. S6). Since UV ozone treatment generates oxygen radicals, primarily hydroxyl radicals (Bhandaru et al. 2020; Efimenko et al. 2005; Fan et al. 2019; Son et al. 2017), and decreases the hydrophobicity of the surface of MWCNT-coated Hanji, it results in the deterioration of the inhibition efficiency. Consequently, the hydrophobic surface properties of MWCNT-coated Hanji provide an unfavorable environment for bacterial survival, resulting in high inhibition efficiency. In addition, a decrease in electrical conductance, attributed to the chemical adsorption of oxygen radicals through UV ozone treatment, was clearly observed (Fig. S7).

Conclusions

Here we reported that MWCNTs-coated Hanji paper possesses significant antibacterial activity towards the Gram-negative bacterium “*E. coli*”. MWCNTs coating on Hanji cellulose paper was done by just dip and dry method and successfully confirmed by optical microscopy, Scanning electron microscopy, X-ray photoelectron spectroscopy and Raman spectroscopy. MWCNTs also showed strong adhesion stability. These results suggest that MWCNT-coated Hanji paper may present feasible alternative to conventional antibacterial agents such as silver, zinc, copper-based oxides. However, bacterial cell wall wrapping and rupturing phenomenon by MWCNTs deserves further investigation.

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Declarations

Competing interests The authors declare no competing interests

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