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Comparative antibacterial properties of zinc oxide microstructures and silver nanoparticles grown on PVDF-HFP films against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

The rise of antibiotic-resistant bacteria, particularly *Escherichia coli* and *Staphylococcus aureus*, poses a critical threat to public health and food safety, as these pathogens are increasingly detected in processed foods. This study evaluates the antibacterial potential of inorganic particle/polymer composites by incorporating silver (Ag) nanoparticles and zinc oxide (ZnO) microstructures into poly (vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) films with micropillar surfaces. PVDF-HFP@Ag and PVDF-HFP@ZnO films were fabricated using solvent casting and hydrothermal growth methods, respectively, and their microstructures were characterized by FESEM, EDX, and AFM analyses. Antibacterial activity was tested against *E. coli* (ATCC 11303) and *S. aureus* (ATCC 25923) using a Live/Dead™ BacLight™ staining protocol and confocal laser scanning microscopy (CLSM). PVDF-HFP@Ag films reduced *S. aureus* viability to 0.6% ± 1.1% and *E. coli* to 41% ± 3.7%, while PVDF-HFP@ZnO films showed enhanced efficacy against *E. coli* (1.36% ± 0.98%) and suppressed *S. aureus* (3.8% ± 6%). These effects are attributed to synergistic mechanisms, including membrane disruption, ROS generation, and metal ion toxicity. The findings demonstrate the potential of these composites as scalable, non-antibiotic antimicrobial materials for food-contact surfaces and active packaging, offering a promising strategy to mitigate antimicrobial resistance and foodborne pathogens.

Keywords: S. Aureus, E. Coli, Antibacterial, Antimicrobial resistance, Polymer, Foodborne pathogens, Inorganic materials, Food packaging.

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1. Introduction

Antimicrobial resistance (AMR) has become a critical global public health issue, contributing to an estimated 4.95 million deaths annually [1]. Leading pathogens such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* are at the forefront of this crisis [1]. Their ability to develop antibiotic resistance transforms them into dangerous foodborne pathogens, significantly increasing the risk of human infections through contaminated food. Foodborne diseases contribute to an estimated 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually in the United States [2]. Among these pathogens, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) are of particular concern as they account for the highest mortality associated with antimicrobial resistance (AMR) [1]. Moreover, their remarkable resilience to advanced sterilization techniques, including conventional thermal treatments and various non-thermal interventions, further exacerbates the threat to public health and food safety [3]. The detection of antimicrobial-resistant strains of *S. aureus* and *E. coli* in packaged foods, especially ready-to-eat products, underscores their survival against conventional antimicrobial and food processing methods [4, 5].

To address this challenge, alternative strategies are urgently needed to combat antimicrobial-resistant strains of *S. aureus* and *E. coli*. Inorganic materials such as silver (Ag), zinc (Zn), and copper (Cu) have emerged as promising candidates due to their potent antibacterial properties [6]. When incorporated into polymer matrices, the microstructures of these materials can form effective antibacterial films [7]. Furthermore, introducing microstructures onto the polymer surface can enhance surface area and potentially improve antibacterial efficacy [8]. This study investigates and compares the antibacterial properties of silver and zinc microstructures embedded in PVDF-HFP films, examines their mechanisms of action, and evaluates their potential applications in the food industry to mitigate the risk of foodborne pathogens.

2. Method of Testing

2.1. Material Preparation

Four film types were prepared: (1) PVDF-HFP film without micropillars as a control sample, (2) Plain PVDF-HFP with micropillars, (3) PVDF-HFP with silver nanoparticles (PVDF-HFP@Ag film), and (4) PVDF-HFP with ZnO microstructures grown on the film's micropillars (PVDF-HFP@ZnO film).

To prepare PVDF-HFP films, a solution of dimethylformamide (DMF) and acetone (70:30 wt.%) was prepared by mixing 3.44 g of DMF with 1.48 g of acetone. Next, 1.08 g of poly (vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) was dissolved at 80°C under continuous stirring until fully dissolved. A commercial template with micropillars will be prepared for the study. The cooled solution was cast onto a commercial micropillar template and allowed to dry for 48 hours, forming the control PVDF-HFP films with micropillars.

To prepare the PVDF-HFP@Ag film, 0.1 g of silver nitrate ($AgNO_3$) was dissolved in 11 g of DMF and heated to 80°C until the solution turned brown. Then, 3.44 g of PVDF-HFP and 4.71 g of acetone were added (70:30 DMF-to-acetone ratio) and stirred at 100°C until fully dissolved. After cooling, the solution was cast onto the micropillar template to form PVDF-HFP@Ag films.

For PVDF-HFP@ZnO film, a ZnO seed solution was prepared by dissolving 1.0975 g of zinc acetate dihydrate (0.1 M) in 50 mL of isopropanol at 85°C, followed by the addition of triethylamine the resulting transparent solution aged for 3 hours at room temperature. Equimolar solutions of zinc nitrate hexahydrate and hexamethylenetetramine (0.025 M) were stirred for 24 hours to grow ZnO nanorods. PVDF-HFP films with micropillars were coated with a ZnO seed solution via an 8-minute immersion, rinsed with ethanol, heat-treated at 120°C for 1 hour, and dried for 24 hours. The ZnO nanorods were grown by submerging the seeded films in the growth solution, sealing them in glass vials, and heating them at 95°C for 8 hours. The PVDF-HFP@ZnO films were washed with deionized water and air-dried.

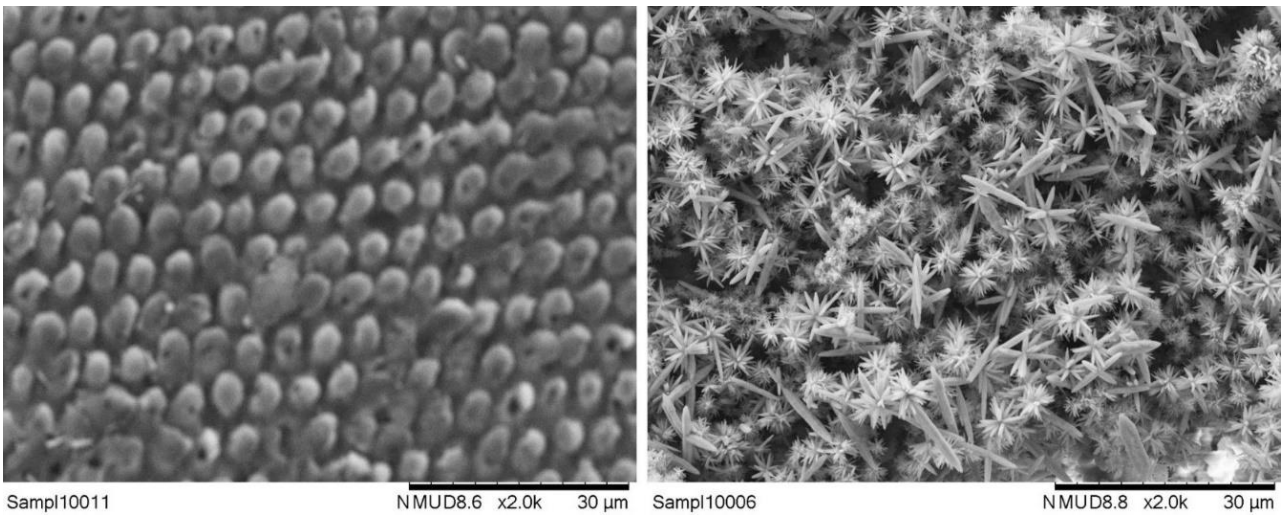

Figure 1,2.
Surface structure of PVDF-HFP@Ag and PVDF-HFP@ZnO film under Scanning Electron Microscope at the scale of 30 µm

2.2. Experimental Method

The antibacterial activity of the films was tested against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 11303). Bacteria were grown in Lysogeny broth (LB) for 18 hours at 37°C and diluted to OD600 ≈ 0.001. Film samples (0.5 × 0.5 cm) were immersed in 300 µL bacterial suspensions, incubated for 18 hours at 37°C, and rinsed with PBS. Bacterial viability on the films was analyzed using confocal laser scanning microscopy (CLSM) after staining with Live/Dead™ BacLight™ dye. Surface images were analyzed using Zen Black and ImageJ software, and the green-to-red pixel ratio was calculated to determine bacterial viability. Three random images were selected for each sample to assess antibacterial activity effectively.

2.3. Microstructures

The surface morphology of the prepared PVDF-HFP@Ag and PVDF-HFP@ZnO samples was analyzed using ultra-high-resolution Schottky field emission scanning electron microscopy (FESEM, Hitachi SU7000) operated at an accelerating voltage of 10 kV (Figures 1 & 2). To enhance surface conductivity for SEM imaging, the samples were sputter-coated with a thin layer of gold at 18 mA for 60 seconds. Figure 3 displays the Energy Dispersive X-ray Spectroscopy (EDX) results, confirming the presence of Ag and ZnO elements in the modified films. These data validate the incorporation of antibacterial elements into the polymer, with strong Zn and Ag signals detected at peaks 2.1 keV and 3.0 keV. Complementary surface topographical analysis was conducted using atomic force microscopy (AFM) to visualize and characterize the samples.

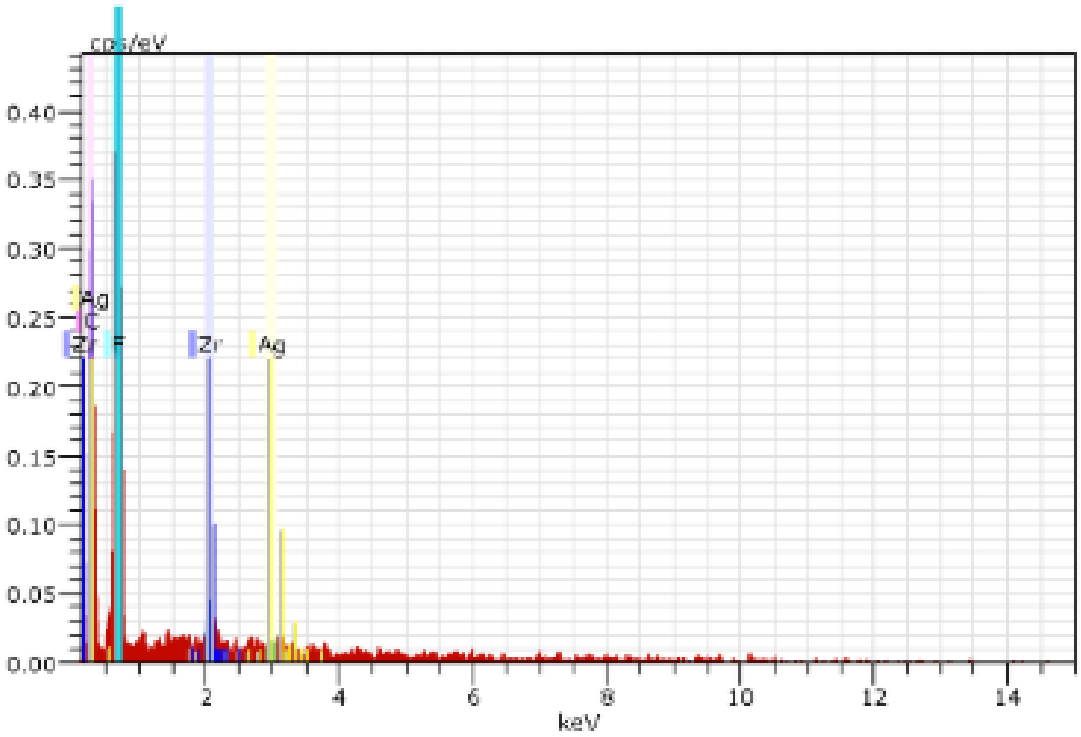

Figure 3.
EDX image of PVDF-HFP@Ag and PVDF-HFP@ZnO film at 30 µm.

3. Result

The antibacterial properties of PVDF-HFP without micropillars (Control Sample), plain PVDF-HFP with micropillars, PVDF-HFP@Ag, and PVDF-HFP@ZnO films were evaluated using the Live/Dead™ BacLight™ Bacterial Viability Kit under confocal laser scanning microscopy. Figure 4 presents the bacterial viability of the different film types, visually demonstrating a significant reduction in bacterial survival on the modified films. SYTO® 9 stained live cells (green fluorescence), while propidium iodide (PI) marked dead cells (red fluorescence).

Staphylococcus aureus showed high viability (87.4% ± 2.8%) on the plain PVDF-HFP film with micropillars, while the PVDF-HFP@Ag film significantly reduced viability to 0.6% ± 1.1% ($p < 0.00001$) (Figures 4 & Figure 5). The PVDF-HFP@ZnO film also lowered viability to 3.8% ± 6% ($p < 0.00001$). These results demonstrate the strong antibacterial activity of PVDF-HFP@Ag and PVDF-HFP@ZnO films against Gram-positive *S. aureus*.

For the Gram-negative *Escherichia coli*, the plain PVDF-HFP film with micropillars exhibited minimal antibacterial activity, with 90% ± 1.8% bacterial viability (Figure 4 & Figure 6). In contrast, PVDF-HFP@Ag reduced viability to 41% ± 3.7% ($p < 0.00001$), while PVDF-HFP@ZnO exhibited stronger antibacterial activity, lowering viability to just 1.36% ± 0.98% ($p < 0.01$). Although both films were effective against *S. aureus*, PVDF-HFP@ZnO demonstrated superior overall performance against *E. coli*.

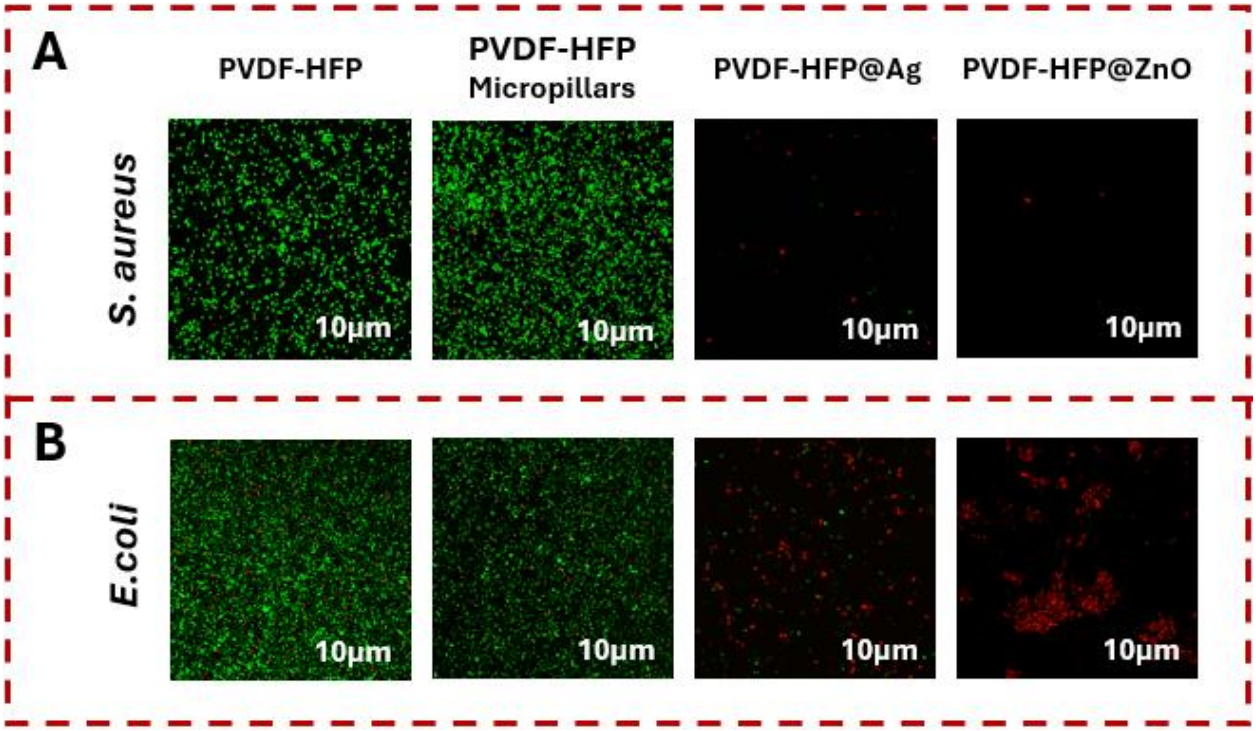

Figure 4.
Visibility of S. Aureus and E. Coli bacteria on PVDF-HFP film without micropillars (Control Sample), Plain PVDF-HFP with micropillars, PVDF-HFP@Ag and PVDF-HFP@ZnO samples.

The SEM images in Figure 1 confirm the presence of microstructures that enhance bacterial membrane disruption, supporting the antibacterial mechanisms of the films. Additionally, Figure 3 provides an elemental composition analysis of Ag and ZnO, reinforcing their contribution to antibacterial activity. The Live/Dead™ assay results in Figure 4 visually validate the significant reduction in bacterial viability, which aligns with the quantitative data in Figures 5 and 6. The comparative analysis indicates that both PVDF-HFP@Ag and PVDF-HFP@ZnO films effectively inhibit and eradicate Gram-positive *S. aureus*, with silver nanoparticles (AgNPs) demonstrating slightly superior antibacterial performance. However, the efficacy of the PVDF-HFP@Ag film against Gram-negative E. coli is lower and is outperformed by the PVDF-HFP@ZnO film.

4. Discussion of Results

The antibacterial performance of PVDF-HFP@Ag and PVDF-HFP@ZnO films reveals significant differences in their efficacy against *Staphylococcus aureus* (*S. aureus*, Gram-positive) and *Escherichia coli* (*E. coli*, Gram-negative) bacteria. The results highlight the superior antibacterial properties of the modified films compared to plain PVDF-HFP film with micropillars, which serves as a baseline control with no notable activity against either bacterial strain.

For *S. aureus*, the Gram-positive pathogen, plain PVDF-HFP film with micropillars exhibited a bacterial viability of 87.4% ± 2.8%, indicating almost no antibacterial activity (Figure 5). In sharp contrast, PVDF-HFP@Ag films performed exceptionally, reducing bacterial viability to 0.6% ± 1.1% ($p < 0.00001$) (Figure 5). This near-complete eradication of *S. aureus* can be attributed to the well-documented mechanisms of AgNPs, which include disruption of bacterial membranes, generation of reactive oxygen species (ROS), and the release of Ag^+ ions that interfere with critical cellular functions such as DNA replication and enzyme activity [9]. Similarly, PVDF-HFP@ZnO films significantly reduced bacterial viability, with

S. aureus viability recorded at 3.8% ± 6% ($p < 0.00001$) (Figure 5). ZnO's antibacterial efficacy arises from physical membrane damage caused by the sharp edges of its microstructures (Fig. 2), Zn^{2+} ion release, and ROS production [10, 11]. While both materials exhibit potent antibacterial activity against *S. aureus*, AgNPs demonstrated slightly superior performance compared to ZnO microstructures, likely due to their significantly higher cytotoxicity, which was reported to be up to ten times greater than ZnO [12].

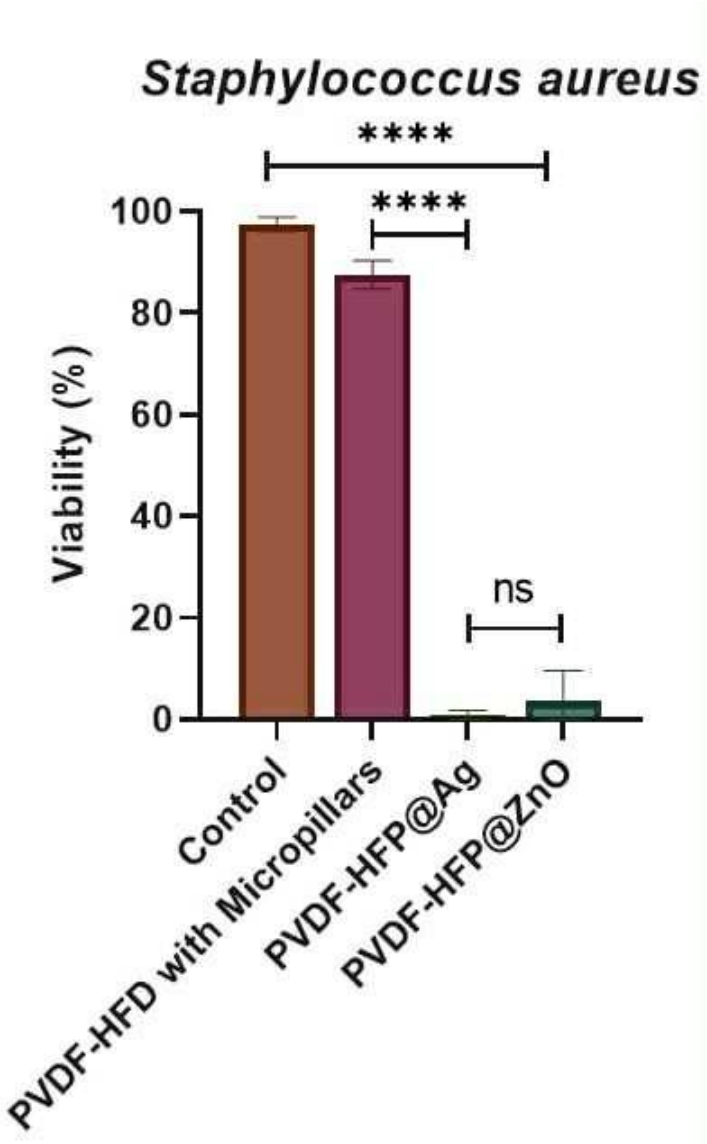

Figure 5.
Viability (%) of S. aureus bacteria on the surface of the PVDF-HFP without micropillars (Control Sample), Plain PVDF-HFP with micropillars, PVDF-HFP@Ag and PVDF-HFP@ZnO samples.

In contrast, the antibacterial efficacy against *E. coli*, a Gram-negative bacterium, demonstrated a different trend. Plain PVDF-HFP film with micropillars again showed no measurable antibacterial activity, with *E. coli* viability remaining at approximately 90% ± 1.8%, similar to the control (Figure 6). PVDF-HFP@Ag films significantly reduced bacterial viability to 41% ± 3.7% ($p < 0.00001$), demonstrating notable but incomplete inhibition (Figure 6). This moderate efficacy reflects the challenges posed by the outer lipopolysaccharide (LPS) layer of Gram-negative bacteria [13], which may provide an additional barrier to nanoparticle penetration and ion release. On the other hand, PVDF-HFP@ZnO films exhibited lower antibacterial activity against *E. coli*, with bacterial viability recorded at only 1.36% ± 0.98% ($p < 0.01$), which proved the exceptional antibacterial properties against Gram-negative bacteria of PVDF-HFP@ZnO film (Figure 6).

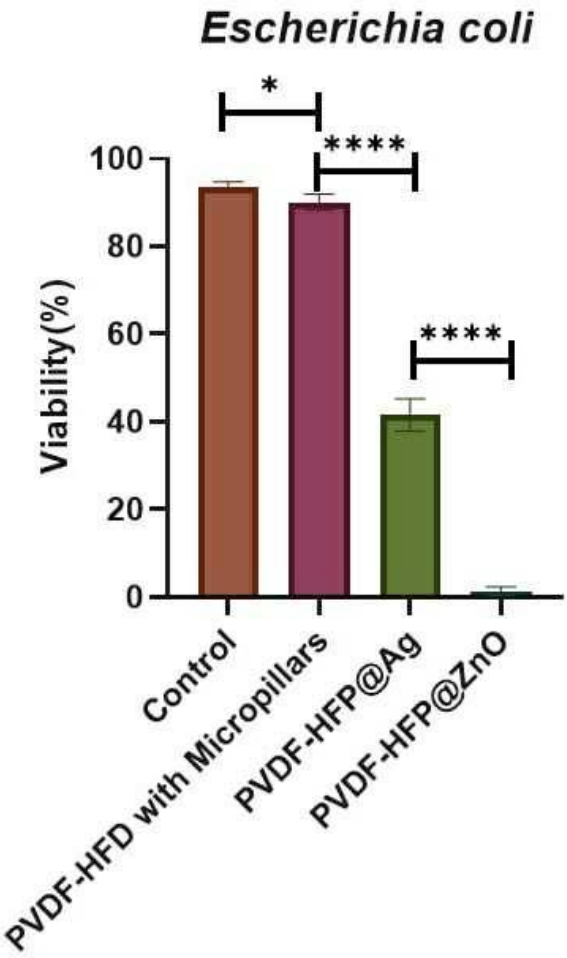

Figure 6.
Viability (%) of E. Coli bacteria on the surface of the PVDF-HFP without micropillars (Control Sample), Plain PVDF-HFP with micropillars, PVDF-HFP@Ag and PVDF-HFP@ZnO samples.

This discrepancy in antibacterial performance can be attributed to both the structural differences between Gram-positive and Gram-negative bacteria and the distinct antimicrobial mechanisms of silver (Ag) and zinc oxide (ZnO) microstructures. Silver nanoparticles (AgNPs) are renowned for their broad-spectrum antibacterial activity, which operates primarily through disruption of membrane integrity, generation of reactive oxygen species (ROS), and the release of Ag^{+} ions that interfere with DNA replication and protein synthesis [9, 14]. While these mechanisms are effective against many bacterial strains, their efficacy can be reduced against Gram-negative bacteria such as *E. coli* due to the presence of an outer membrane rich in lipopolysaccharides (LPS), which acts as a barrier, impeding nanoparticle penetration and ion diffusion [15, 16]. Additionally, Gram-negative bacteria can produce flagellin, a structural protein involved in motility, which has been reported to induce AgNP aggregation, further diminishing their antimicrobial effectiveness [17, 18]. This phenomenon does not occur in Gram-positive bacteria [18], providing a plausible explanation for the reduced activity of PVDF-HFP@Ag against *E. coli* relative to *S. aureus*. Moreover, the thicker peptidoglycan layer in the cell wall of Gram-positive bacteria offers more binding sites for AgNPs and Ag^{+} ions, enabling stronger interactions and deeper penetration compared to Gram-negative bacteria [19]. This results in progressive structural damage and eventual cell wall disruption, which may explain the high antibacterial efficacy of AgNPs against *S. aureus* but reduced effectiveness against *E. coli* [19].

In contrast, ZnO microstructures exhibit a broader and more potent antimicrobial spectrum, particularly against Gram-negative bacteria [20]. Their antibacterial action involves a combination of Zn^{2+} ion release and substantial ROS generation [10, 11]. Zn^{2+} ions interfere with essential enzymatic activities and disrupt bacterial membrane proteins, including those involved in flagellin synthesis and cellular integrity [21, 22]. This may explain the enhanced antibacterial performance of PVDF-HFP@ZnO against *E. coli* compared to *S. aureus*. Meanwhile, ROS, including hydroxyl radicals, hydrogen peroxide, and superoxide ions, inflict oxidative damage on bacterial membranes, lipids, proteins, and nucleic acids [19, 23-25]. Gram-negative bacteria, such as *E. coli*, possess a thinner peptidoglycan layer and comparatively weaker oxidative defense mechanisms than Gram-positive bacteria like *S. aureus* [24], making them particularly vulnerable to ROS-induced stress [26, 27]. This vulnerability is especially relevant to the antibacterial activity of ZnO, which is primarily attributed to ROS generation that disrupts active transport, amino acid metabolism, and enzymatic systems in bacteria [6, 12, 19]. In contrast, AgNPs primarily exert antibacterial effects through interactions with the bacterial outer membrane, ultimately leading to cell death [12, 28]. Furthermore, the unique morphology of ZnO microstructures, such as nanorods, offers a high surface area and

improved contact with bacterial cell walls, enhancing both physical and chemical interactions [29, 30]. This synergy between mechanical disruption, ion toxicity, and oxidative stress likely accounts for the pronounced antibacterial efficacy of PVDF-HFP@ZnO, which reduced *E. coli* viability to 1.36% ± 0.98%, significantly outperforming PVDF-HFP@Ag (41% ± 3.7%).

These findings demonstrate the promising antibacterial potential of Ag and ZnO nanomaterials when incorporated into PVDF-HFP films. These results suggest that PVDF-HFP@Ag and PVDF-HFP@ZnO films hold significant potential for applications in antimicrobial surfaces, particularly in food packaging and food-contact materials [31, 32] where bacterial contamination is a major concern. Both silver (Ag) and zinc oxide (ZnO) present a transformative solution for the food industry to address the dual challenges of antimicrobial resistance and foodborne pathogens [21, 33]. As resistant strains of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) increasingly undermine food safety [34], conventional food packaging and handling methods are proving insufficient [3]. This study demonstrates the potent antibacterial activity of AgNPs and ZnO, achieved through mechanisms such as bacterial membrane disruption, reactive oxygen species (ROS) generation, and ion release [9-11]. These processes inhibit bacterial growth and survival [11, 35], making AgNPs and ZnO ideal candidates for integration into food-contact materials and active packaging systems. Incorporating these microstructures into polymer-based films, coatings, or edible packaging can significantly reduce bacterial contamination, extend the shelf life of food products, and minimize spoilage without compromising food quality. Furthermore, AgNPs and ZnO microstructures can be employed in disinfection solutions for food processing surfaces, equipment, and livestock environments, mitigating contamination risks across multiple stages of the food supply chain [36]. Their ability to target both *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) highlights their versatility and efficacy in combating foodborne pathogens and antimicrobial-resistant bacterial strains. By harnessing nanotechnology to develop sustainable and effective antibacterial materials, the food industry can enhance microbial control, reduce antibiotic reliance, and ensure safer, higher-quality food products for consumers.

5. Conclusion

This study demonstrates the significant antibacterial efficacy of PVDF-HFP films embedded with silver nanoparticles (AgNPs) and zinc oxide (ZnO) microstructures, offering a promising strategy to combat antimicrobial resistance and foodborne pathogens. Compared to plain PVDF-HFP films with micropillars, both PVDF-HFP@Ag and PVDF-HFP@ZnO films significantly reduced bacterial viability, confirming the effectiveness of microstructural integration. The findings highlight the adaptability of these microstructures for a wide range of applications, particularly in the food industry. Both AgNPs and ZnO microstructures can be incorporated into poly(vinylidene fluoride-co-hexafluoropropylene) films, coatings, and active packaging systems to reduce bacterial contamination, enhance food safety, and extend product shelf life. ZnO's superior performance against *E. coli* is attributed to the synergistic effects of mechanical disruption, elevated ROS generation, and effective Zn^{2+} ion release, while the higher cytotoxicity of AgNPs accounts for their stronger antibacterial effect against *S. aureus*. Incorporating these materials into food-contact surfaces and disinfection solutions for equipment and livestock environments presents a sustainable alternative to conventional antibiotics, addressing the urgent challenges of antimicrobial resistance and foodborne illnesses. Furthermore, exploring the synergistic combination of AgNPs and ZnO microstructure within a single polymer matrix may further enhance antibacterial performance, enabling more effective microbial control.

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