

# AgNPs Improved Feather Keratin Based Bio-Membrane

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## Short Communication

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# Abstract

AgNPs (Silver Nanoparticles) is successfully prepared and then loaded into feather keratin (FK) based bio-membrane by electrospinning. The morphology, thermal stability, mechanical properties and bacteriostatic activity of the as-prepared AgNPs/FK/PVA bio-membrane are completely developed in this study. Microstructure show that the AgNPs has been dispersed in FK-based bio-membrane without agglomeration. The results suggest that the addition of AgNPs enhanced effectively the performances of FK-based bio-membrane, and the appropriate amount of AgNPs is 1%-2%. The combination of AgNPs and FK can not only ensure the uniform dispersion and antibacterial stability of AgNPs, but also give play to the biocompatibility effect of FK, which makes it natural, safe, stable and degradable, and broaden its application in the field of biomedicine.

## 1 Introduction

Feather keratin(FK)which has a wide range of sources is a kind of natural polymer material. FK has good biocompatibility and biodegradability, especially the arginine glycine aspartic acid (RGD) and leucine aspartic valine acid (LDV) in keratin molecules, which can promote the adhesion, migration and proliferation of animal cells on keratin membrane. And it can be used to prepare skin wound dressing and accelerate wound healing<sup>[1-3]</sup>. However, keratin has poor mechanical properties and weak antibacterial ability, which limits its application in biomedical materials. It needs to be modified to enhance the properties of keratin based materials to meet the complex requirements of clinical medical materials.

The modification of keratin based materials mainly focused on the blending<sup>[4-5]</sup>, cross-linking<sup>[6]</sup> and adding nanoparticles<sup>[7]</sup>. Nanoparticles have the advantages of large specific surface area, high unsaturation and outstanding nano size effect, which can significantly improve the mechanical properties, hydrophobic properties and thermal stability of biomacromolecule materials<sup>[8-9]</sup>. Silver nanoparticles (AgNPs) has many natural advantages in nanomaterials, such as high safety, good heat resistance, broad-spectrum antibacterial and non-drug resistance. It is widely used as an inorganic antibacterial additive in biomedical materials, which can not only improve the comprehensive performance of materials, but also endow materials with antibacterial properties. But, AgNPs are small in size, high in surface activity and easy to agglomerate, which leads to the loss of physical properties and functions of nanoparticles<sup>[10]</sup>. It is an effective method to maintain the properties of AgNPs by fixing and dispersing the AgNPs on the support.

Electrospinning is a good method to refine the solution with certain viscosity by static power and finally obtain nanofibers, which has the characteristics of fast, high efficiency, simplicity and low cost. However, it is difficult to spin pure keratin. It has been proved that the spinnability of keratin can be improved by mixing keratin with synthetic polymers, such as PVA<sup>[11]</sup>, PCL<sup>[12]</sup>, PLLA<sup>[13]</sup> and PEO<sup>[14]</sup>. The as-prepared keratin based nanofibers membrane has high porosity, specific surface area and connectivity, which can make AgNPs disperse evenly in the membrane and prevent the agglomeration and oxidation of AgNPs to lose antibacterial properties. AgNPs grafts polymer molecular chains from keratin based membrane

materials to form a polymer particle hybrid interpenetrating network structure by electrospinning<sup>[15]</sup>. The unique cross entanglement structure can further enhance the molecular chain entanglement of polymer, make the two phase affinity and stronger force and compatibility, thus effectively improve the mechanical properties of keratin based membrane<sup>[16]</sup>.

Many studies have shown that the combination of AgNPs and natural biological macromolecules can not only ensure the uniform dispersion of AgNPs, but also give play to the biocompatibility of keratin, which makes it natural, safe, stable and degradable, and broaden its application in the field of biomedicine<sup>[17]</sup>. Wang et al.<sup>[18]</sup> developed a novel nanofibrous mat of AgNPs/Keratin/PU for wound dressing, and studied its antibacterial properties. Antibacterial test results showed that AgNPs/Keratin/PU mats had inhibitory effect on the growth of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), and had stronger antibacterial effect on *S. aureus*. Dashdorj et al.<sup>[19]</sup> used electrospun technique to fabricate zein/Ag nanomats. Results showed that zein nanofibers loaded with AgNPs have good antibacterial activity which is important criteria for an effective wound dressing. Also, in vitro cell tests indicated that zein/Ag nanomats have good bioactivity, cell growth and proliferation as well as adhesion. Shen et al.<sup>[20]</sup> prepared a kind of AgNPs/Keratin/PA6 composite nanofiber membrane with enhanced filtration and antibacterial properties from goat wool. The results suggested that the AgNPs/Keratin/PA6 composite membrane had strong antibacterial activity and filtration efficiency against *S.aureus* and *E.coli*, the antibacterial activity achieved 99.62% and 99.10%, and the filtration efficiency (BFE) was up to 96.8% and 95.6%, respectively.

In this study, AgNPs/FK/PVA composite bio-membranes were prepared by electrospinning with AgNPs as the functional agent and the biodegradable and biocompatible FK-based nanofibers membrane as the support, which solved the problem of agglomeration of AgNPs. The AgNPs/FK/PVA bio-membranes gave full play to the biocompatibility of FK and the high bacteriostatic efficiency of AgNPs, which provides a new idea for the preparation of novel medical materials with strong antibacterial and biological non-toxic.

## 2 Experimental

### 2.1 FK Extraction

The chicken feathers were collected from the waste market in Guangzhou city. The method of FK extraction was similar to that described in our previous work<sup>[21]</sup>. The feathers were put into peracetic acid solution (1:15, wt/wt) and stirred constantly at 60°C for 80 min. Then the as-prepared FK solution was dialyzed in distilled water at room temperature until the liquid in the dialysis bag(Oso-T8280, 12,000–14,000 Da; Union Carbide) is transparent and colorless. After dialysis, FK powder was obtained by filtering and drying.

### 2.2 AgNPs Preparation

The AgNPs was prepared by following process, as described elsewhere<sup>[22]</sup>. PVP and deionized water was added into a single flask and stirred at room temperature. Then tannic acid (2.4 mmol/L) and ammonia

water (0.5 mol/L) were mixed in the as-prepared PVP solution. Slowly added silver nitrate (120 mmol/L) to the single flask with an injection pump and stirred for 1h. The sample solution was concentrated at 60 °C for 4 h by rotating evaporator. A proper amount of acetone was added to destroy the gel structure of the system. The solid precipitate was collected by vacuum filtration and dried in a vacuum drying box for 24 h. The AgNPs powder was obtained and ground before using.

## 2.3 Electrospinning of AgNPs/FK/PVA nanofibers bio-membrane

0.5 wt% AgNPs solution was obtained by mixing a certain amount of as-prepared AgNPs powder and deionized water. Five parts of AgNPs/FK/PVA electrospinning solution were prepared and the mass amount of AgNPs in the above electrospinning solution was 0.6%, 1.2%, 1.8%, 2.4%, 3.0%, respectively. The AgNPs/FK/PVA nanofibers bio-membrane was electrospun and collected.

## 2.4 Testing and characterization

The microstructure of AgNPs/FK/PVA nanofibers membrane and the distribution of AgNPs in FK/PVA nanofibers membrane were observed by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) (EVO 18, Carl Zeiss, Germany), respectively. The average diameter of nanofibers were measured by the image analysis software (Image-Pro Plus) and 100 nanofibers selected randomly from each sample. The morphology of self-made AgNPs and AgNPs/FK/PVA nanofibers were observed by transmission electron microscopy (TEM)(JEM-2010HR, JEOL, Tokyo, Japan) at 120 kV accelerating voltage and 5s receiving time. The structures of AgNPs and AgNPs/FK/PVA nanofibers membrane were confirmed by the FTIR (spectrum 100, Perkin-Elmer, USA), carried out in a range of wavenumber from 4000 – 500  $\text{cm}^{-1}$ . X-ray diffraction analysis was performed by X-ray diffractometer (XRD, Empyrean, Holland) with Cu Ka ( $\lambda = 1.540 \text{ \AA}$ ) radiation at a step of 0.02°. Thermal stability of AgNPs/FK/PVA nanofibers membrane was assessed by thermogravimetric analysis (TGA, Instruments TG209F1, Netzsch, German) and differential scanning calorimeter (DSC, Mettler Toledo, Switzerland), respectively. The tensile properties of AgNPs/FK/PVA nanofibers membranewere determined by using a universal testing machine (CMT6503, Shenzhen MTS Test Machine Company Ltd., China)<sup>[23-24]</sup>. Staphylococcus aureus(S aureus, CICC 10001, Gram-positive)and Escherichia coliE coli, CICC 10003, Gram-negative were purified and activated for 2 days. The AgNPs/FK/PVA nanofibers membrane was dried at 150°C for 6 h and then cut into a circular shape with a diameter of 10 mm. The two sides of the pieces of nanofibers membrane were sterilized for 1 h by using an ultraviolet lamp, respectively. S aureus and E coli were taken on an agar culture medium plate and coated uniformly, respectively. The as-prepared pieces of AgNPs/FK/PVA nanofibers membrane were put on the culture medium and cultured for 24 h at 37°C, respectively. The diameter of bacteriostatic ring was measured by a Vernier caliper.

## 3 Results And Discussion

### 3.1 AgNPs Characterization

As can be seen from IR of PVP in Fig.1(a), the absorption peak at  $3526\text{ cm}^{-1}$  corresponds to the -OH stretching vibration peak at the end of the polymerization chain in the dispersant PVP, which is attributed to the strong hydrophilicity of PVP<sup>[25]</sup>. The absorption peak at  $2952\text{ cm}^{-1}$  represents the -CH<sub>2</sub>- stretching vibration peak of PVP, the absorption peak at  $1655\text{ cm}^{-1}$  corresponds to the absorption vibration peak of C=O carbonyl group, the absorption peak at  $1287\text{ cm}^{-1}$  is related to the antisymmetric and symmetric stretching vibration peak of C-N bond. It can be seen a significant change from Fig.1(a) compared the IR of the AgNPs modified by the dispersant PVP with the IR of the PVP. After modification, the absorption peak at  $1655\text{ cm}^{-1}$  decreased obviously and has shifted to a lower wavenumber at  $1603\text{ cm}^{-1}$ , which ascribed to the establishment of Ag-O coordination bonds by arc pair electrons on oxygen atoms of PVP carbonyl and outer electron orbitals of Ag particles. The decrease and shift of the absorption peaks at  $1655\text{ cm}^{-1}$  indicate that PVP has been successfully modified on the surface of AgNPs<sup>[26]</sup>. Obviously, the absorption peak of AgNPs modified by the PVP at  $2952\text{ cm}^{-1}$  disappeared and the intensity of other absorption peaks decreased to some extent, which may be due to the black color of the prepared AgNPs. Because the intensity of the absorption peak was weakened attributed the black color during IR test and KBr powder compression<sup>[27]</sup>.

It can be seen from the UV-vis of AgNPs in Fig.1(b) that the sample has an obvious characteristic absorption peak at 409.5 nm, which is the characteristic absorption peak of AgNPs<sup>[28]</sup>. The XRD spectra of as-prepared AgNPs is shown in Fig.1(c). Multiple diffraction peaks at  $2\theta = 38^\circ, 44^\circ, 64^\circ, 77^\circ, 81^\circ$  correspond to the faces of (111), (200), (220), (310), (222) (JCPDS No.87-0720) of face-centered cubic (fcc) type silver (zero valence state), respectively. It shows that silver is successfully prepared<sup>[29-30]</sup>. As can be seen from TEM in Fig.1(d), AgNPs are nearly spherical and uniformly dispersed without agglomeration. The average particle size was  $14.07\pm 2.93\text{ nm}$ , which is conducive to improve the effective contact area between AgNPs and cells, and make it play a strong antibacterial performance<sup>[31]</sup>. The XPS typical absorption peak of silver can be seen in Fig.1(e). The results of XPS show that the absorption peaks of 368.2 eV and 374.3 eV correspond to Ag<sup>0</sup> of Ag3d<sub>3/2</sub> and Ag<sup>0</sup> of Ag3d<sub>5/2</sub>, respectively, with a difference of 6.0 eV. This proves that nano silver exists as silver with zero valence state instead of other silver compounds<sup>[32]</sup>.

In conclusion, the results of Fig.1 suggest that AgNPs has been successfully prepared and dispersed uniformly.

### 3.2 Structural Analysis

Fig.2 presents the TEM images of FK/PVA nanofibers membrane before and after AgNPs modification. Fig.2 (A) showed that the microstructure of FK/PVA nanofibers before AgNPs modification is uniform without any granular impurity, which indicated that FK and PVA had good compatibility. Compared with Fig.2 (A), small black Ag spots can be seen in the FK/PVA nanofibers membrane modified by AgNPs from Fig.2 (B). Because AgNPs is different from FK/PVA nanofibers membrane in composition, structure, and

so on, which result to present different images under TEM. As can be seen from Fig.2, AgNPs particles were successfully modified in the FK/PVA nanofibers membrane.

**Tab. 1** Average diameter of FK/PVA nanofibers membrane with different AgNPs contents

Samples	Average diameter (nm)
0 % -AgNPs	245.00±29.72
0.6%-AgNPs	148.35±37.16
1.2%-AgNPs	112.27±28.89
1.8%-AgNPs	100.11±16.39
2.4%-AgNPs	129.74±33.62
3.0%-AgNPs	150.34±41.46

Fig. 3 suggests the microstructure and the diameter distribution of FK/PVA nanofibers membrane with different AgNPs content. SEM images show that the integral morphology of nanofibers does not change significantly with the addition of AgNPs. It can be clearly seen from Tab. 1 that the diameter of nanofiber decreased gradually with the increase of the content of AgNPs. With the content of AgNPs upto 1.8 wt%, diameter of nanofiber was the smallest approaching to 100 nm. Whereas the diameter of nanofiber increased by raising the content of AgNPs continually. The conductivity of electrospinning solution can be improved by adding AgNPs. Lee Previous study<sup>[33]</sup> have shown that the increase of the conductivity of the spinning solution will bring more charges to the jet, and the fiber diameter will be smaller under the action of the electric field force. Therefore, with the addition of AgNPs, the average diameter of nanofibers gradually decreases.

But, when the amount of AgNPs is too much, the concentration of electrospinning solution decreases and the electrospinning solution spray out faster under high voltage, which cause solvent to evaporate slowly and the uniform nanofibers to be formed difficultly. As a result, the diameter of nanofibers becomes larger and the microstructure of nanofibers appears beads.

Fig. 4 shows the EDS of FK / PVA nanofiber membranes with different AgNPs contents. As can be seen from Fig. 4 (a) and (b), the EDS spectra are very similar, showing the expected C and O peaks. The Na peak and Au peak may come from the contamination of NaOH solution after dissolving FK powder and the gold spraying process of pretreatment in SEM test, respectively. It is obvious that there are Ag peaks of FK/PVA nanofibers membrane with adding AgNPs from Fig.4(b), showing that a clear evidence of Ag in nanofibers membrane and the weight fraction of silver is 0.23%. Many obvious bright spots can be observed from Fig. 4(c), indicating the existence of Ag element, and the silver nanoparticles are relatively evenly distributed in the nanofibers. These results are consistent with the TEM shown in Fig. 2.

In Fig. 5, the FT-IR spectra of FK, PVA and FK/PVA nanofibers membrane treated with different AgNPs content were collected. As can be seen from Fig.5 (a), typical adsorption bands of FK was detected<sup>[21]</sup>. The wide and broad adsorption band at 3418 cm<sup>-1</sup> is known as amide A. The amide I band due to C=O stretching vibration were observed in the range of ~1700-1600 cm<sup>-1</sup> and the sharp peak appears at 1648 cm<sup>-1</sup>. The amide II occur at 1549 cm<sup>-1</sup> is related to N-H bending vibration. A peak falls at 1240 cm<sup>-1</sup> is the characteristic adsorption peak of the amide III (1220-1300 cm<sup>-1</sup>)<sup>[34]</sup>.

The absorption peak at 3418 cm<sup>-1</sup> of FK/PVA nanofibers membrane slightly shifted to low wavenumber of 3297 cm<sup>-1</sup> compared Fig.5 (a) with Fig.5 (b). This result indicated that the strong intermolecular hydrogen bond enhances the compatibility between FK and PVA and improves the spinnability of keratin. While the FT-IR spectra of FK/PVA nanofibers membrane with different AgNPs content are similar and the typical characteristic diffraction peaks does not change significantly in Fig. 5 (b). The results show that the addition of AgNPs does not react with the composition of the nanofibers membrane, but it is loaded or embedded in the nanofibers membrane.

### 3. 4 Thermal Analysis

**Tab. 2** Thermogravimetric analysis data of FK/PVA nanofiber with different AgNPs contents

sample	T <sub>d</sub> (°C)	T <sub>max1</sub> (°C)	T <sub>max2</sub> (°C)
0%-AgNPs	194	249	433
0.6%-AgNPs	206	251	436
1.2%-AgNPs	210	253	438
1.8%-AgNPs	211	257	439
2.4%-AgNPs	213	260	442
3.0%-AgNPs	215	261	443

As can be seen from Fig. 6, the weight loss process of nanofibers membrane is mainly divided into three stages. The first stage with decomposition temperature of <100°C was ascribed to evaporation of water and the good hydrophilicity of PVA. The second stage with decomposition temperature of ~250°C may be caused by the decomposition of amino acid residues and the cleavage of peptide bonds in FK<sup>[35]</sup>. The third stage with decomposition temperature of ~430°C was owed to the thermal decomposition of nanofibers membrane. The thermal stability of nanofibers membrane was investigated by using the temperature of 10% mass loss of sample (T<sub>d</sub>) and the temperature of the fastest rate of thermal degradation (T<sub>max</sub>), which corresponding to T<sub>max1</sub> and T<sub>max2</sub> of two degradation stage in this study, respectively. The results are shown more clearly in Tab. 2. It can be seen that T<sub>d</sub>, T<sub>max1</sub> and T<sub>max2</sub> of

FK/PVA nanofibers membrane with AgNPs were improved to a certain extent and gradually increased with the increase of AgNPs content. The results show that the addition of AgNPs increases the initial decomposition temperature of FK/PVA nanofiber membrane and improves the thermal stability of the nanofiber membrane to a certain extent, which is closely related to the good thermal stability of AgNPs.

Fig. 7 is the DSC analysis of FK/PVA nanofibers membrane with different AgNPs content. As can be seen from Fig. 7, the peak near 100 °C is caused by the evaporation of water<sup>[36]</sup>, the peaks obtained at about 200 °C and 250 °C is the melting peak of PVA and endothermic peak of FK, respectively<sup>[37]</sup>. It can be seen that both the melting peak of PVA and endothermic peak of FK moved toward high temperatures after adding AgNPs to FK/PVA nanofibers membrane. And the temperature corresponding to the endothermic peak gradually increased with the increase of AgNPs content. The results suggest that the thermal stability of FK/PVA nanofibers membrane is enhanced after AgNPs modification, which can be attributed to the effect of AgNPs on the strength of hydrogen bond between FK and PVA, and then affects the thermal properties of nanofiber membranes. This result is agreed with TG/TGA analysis results shown in Fig. 6.

### 3.5 Mechanical Analysis

**Tab. 3** Mechanical properties of FK/PVA nanofibers membrane with different AgNPs contents

sample	$s_b$ [MPa]	$e_b$ [%]
0%-AgNPs	3.05±0.19	30.00±3.61
0.6%-AgNPs	3.29±0.12	33.50±2.00
1.2%-AgNPs	4.07±0.26	38.17±5.39
1.8%-AgNPs	4.60±0.07	48.33±1.04
2.4%-AgNPs	3.44±0.17	43.00±5.50
3.0%-AgNPs	3.07±0.23	40.17±2.57

The mechanical properties of nanofibers membranes including tensile strength ( $s_b$ ) and elongation at break ( $e_b$ ) are shown in Table 3. It can be seen that the  $s_b$  and  $e_b$  of FK/PVA nanofibers membrane with no AgNPs are 3.05 MPa and 30.00%, respectively. The  $s_b$  and  $e_b$  of FK/PVA nanofibers membrane by adding AgNPs increase first and then decrease with amount of AgNPs increasing. When the content of AgNPs arrived at 1.8%, the  $s_b$  and  $e_b$  of nanofibers membrane reached the maximum, 4.60 MPa and 48.33%, respectively. Some of the stress of FK/PVA nanofibers membrane is absorbed and dispersed because of the addition of AgNPs, which enhanced and improved the  $s_b$  and  $e_b$ <sup>[38]</sup>.

While the content of AgNPs exceeds 1.8%, the  $s_b$  and  $e_b$  decrease gradually, respectively. That may ascribe to the viscosity of electrospinning solution by adding a great deal of AgNPs. The viscosity of electrospinning solution decreased with the addition of AgNPs, and the degree of entanglement among the macromolecules in the electrospinning solution decreased which finally resulted the  $s_b$  and  $e_b$  degrading.

### 3.6 Bacteriostatic analysis

**Tab. 4** Bacteriostatic effect of FK/PVA nanofibers membranes with different AgNPs content on Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus)

sample	Diameter of E.coil bacteriostatic circle /mm	Diameter of S.aureus bacteriostatic circle /mm
0%-AgNPs	0	0
0.6%-AgNPs	6.03	3.60
1.2%-AgNPs	7.84	3.72
1.8%-AgNPs	8.02	4.46
2.4%-AgNPs	9.46	5.18
3.0%-AgNPs	9.53	6.03

Fig. 8 shows the bacteriostatic circle images of FK/PVA nanofibers membranes with various AgNPs content on Escherichia coli (E.coli) and Staphylococcus aureus (S.aureus). And the diameters of bacteriostatic circle of FK/PVA nanofibers membranes with various AgNPs content are clearly exhibited in Tab. 4. As can be seen from Fig. 8 and Tab. 4, there was no bacteriostatic circle in the culture medium of E.coli and S.aureus of FK/PVA nanofibers membrane without AgNPs, which indicated that FK/PVA nanofibers membrane had no bacteriostatic effect on E.coli and S.aureus. Adding AgNPs, there were obvious bacteriostatic circles in the culture medium of nanofibers membrane, which indicated that AgNPs had bacteriostatic effect on E.coli and S.aureus. The bacteriostatic effect of FK/PVA nanofibers membrane with AgNPs on E.coli was better than that of S.aureus from the comparison of the diameter of bacteriostatic circle. Because S.aureus is a kind of Gram-positive bacteria and have a denser cell wall and peptide layer, which is difficult to be destroyed by AgNPs<sup>[39-41]</sup>.

The bacteriostatic effect of FK/PVA nanofibers membrane with AgNPs on E.coli and S.aureus were improved with the increase of AgNPs content, while the bacteriostatic effect of samples on E.coli was

more obvious and significant than that of *S.aureus*. The diameter of bacteriostatic circle for *E.coli* and *S.aureus* of FK/PVA nanofibers membrane with adding 3.0 wt% AgNPs was 9.53mm and 6.03mm, respectively.

**Tab. 5** Comparison of bacteriostatic effect of keratin-based nanofibers membranes modified by AgNPs between the present study and references

Reference	sample	Diameter of <i>E.coil</i> bacteriostatic circle /mm	Diameter of <i>S.aureus</i> bacteriostatic circle /mm
[18]	AgNPs/Keratin/ PU	1.90	3.10
[20]	AgNPs/Keratin/PA6	>1	>1
[24]	AgNPs/FK/PVA/PEO	8.60	2.96
this study	AgNPs/FK/PVA	9.53	6.03

Tab. 5 shows the comparison of bacteriostatic effect of keratin-based nanofibers membranes modified by AgNPs between the present study and references. It can be seen that the bacteriostatic performance of as-prepared FK/PVA nanofibers membrane with AgNPs in our study was best among the nanofibers membranes listed in Tab. 5 and it is promising for biomedicine materials.

## 4 Conclusion

When the AgNPs content is 1% – 2wt%, the Average diameter of AgNPs/FK/PVA nanofibers is about 100nm. Thermal analysis indicated that the addition of AgNPs increases the initial decomposition temperature of FK/PVA nanofiber membrane and improves the thermal stability of the nanofiber membrane to a certain extent, which is closely related to the good thermal stability of AgNPs. Similarly, mechanical properties of FK/PVA nanofibers membrane effectively enhanced by adding AgNPs. And when the content of AgNPs arrived at 1.8%, the  $\sigma_b$  and  $\epsilon_b$  of nanofibers membrane reached the maximum, 4.60 MPa and 48.33%, respectively. Furthermore, antibacterial activity suggested that the diameter of bacteriostatic circle for *E. coli* and *S. aureus* of FK/PVA nanofibers membrane with adding 3.0 wt% AgNPs was 9.53mm and 6.03mm, respectively. The results showed that the antibacterial effect of AgNPs/FK/PVA bio-membrane was better than that of other AgNPs loaded keratin based nanofiber membranes in prior studies. To improve the comprehensive performance of FK-based bio-membrane, the best adding amount of AgNPs is about 1–2%. This study gave full play to the biocompatibility of keratin and the high-efficiency antibacterial property of AgNPs, and provided a new idea for the preparation of new medical materials with strong antibacterial and biological non-toxic. Next step, further studies in-depth are urgent to develop the biomedical and cytological research using AgNPs/FK/PVA bio-membrane.

# Declarations

**Ethics approval and consent to participate:** Not applicable

**Consent for publication:** All authors agree to the publication of this paper.

**Availability of data and materials:**

All data generated and analyzed in this research are included in this manuscript.

**Competing interests:**

The authors declare that they have no competing interests.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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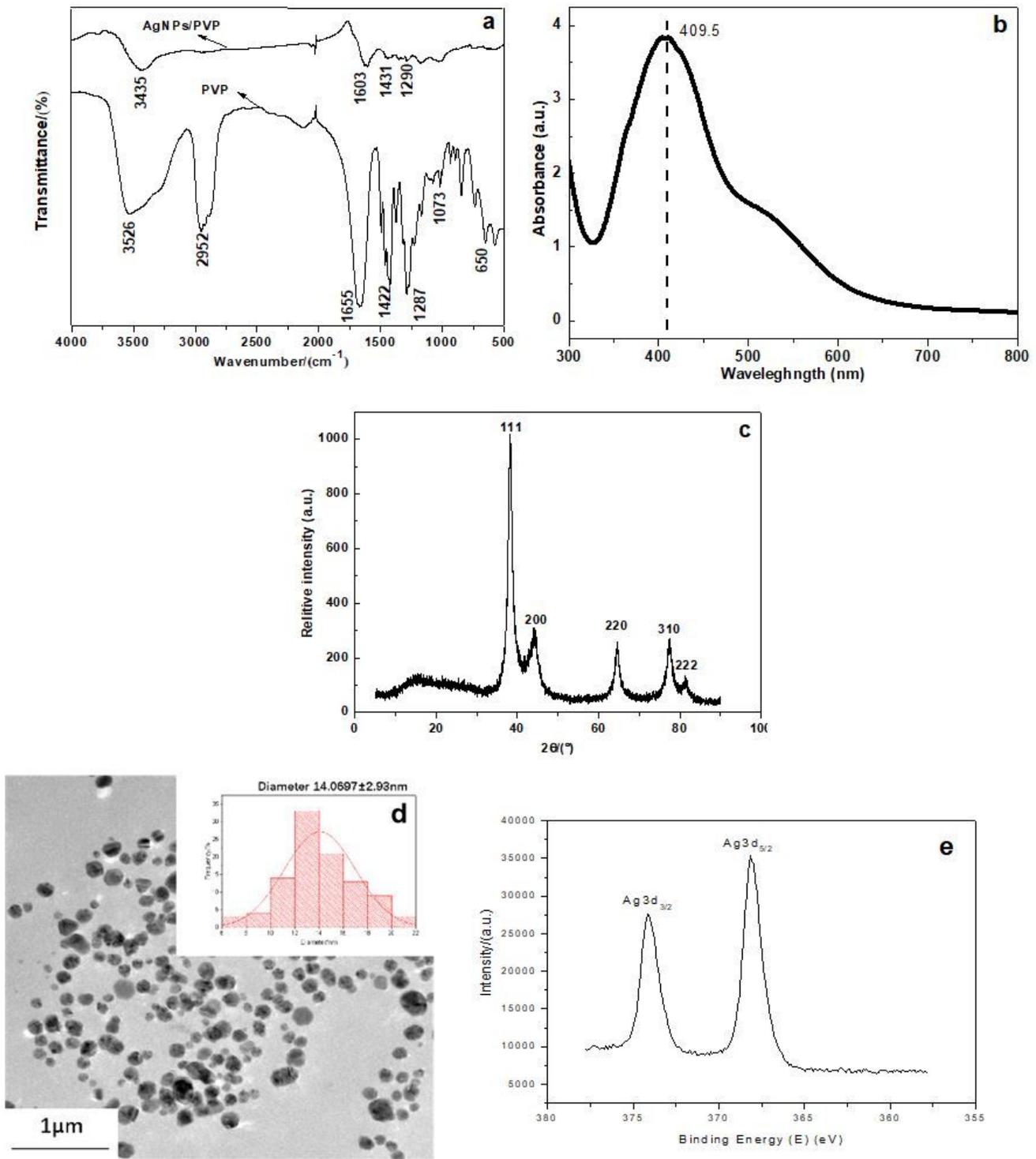
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## Figures



**Figure 1**

Characterization of AgNPs: (a) FTIR; (b) UV-vis; (c) XRD; (d) TEM and diameter distribution; (e) XPS.

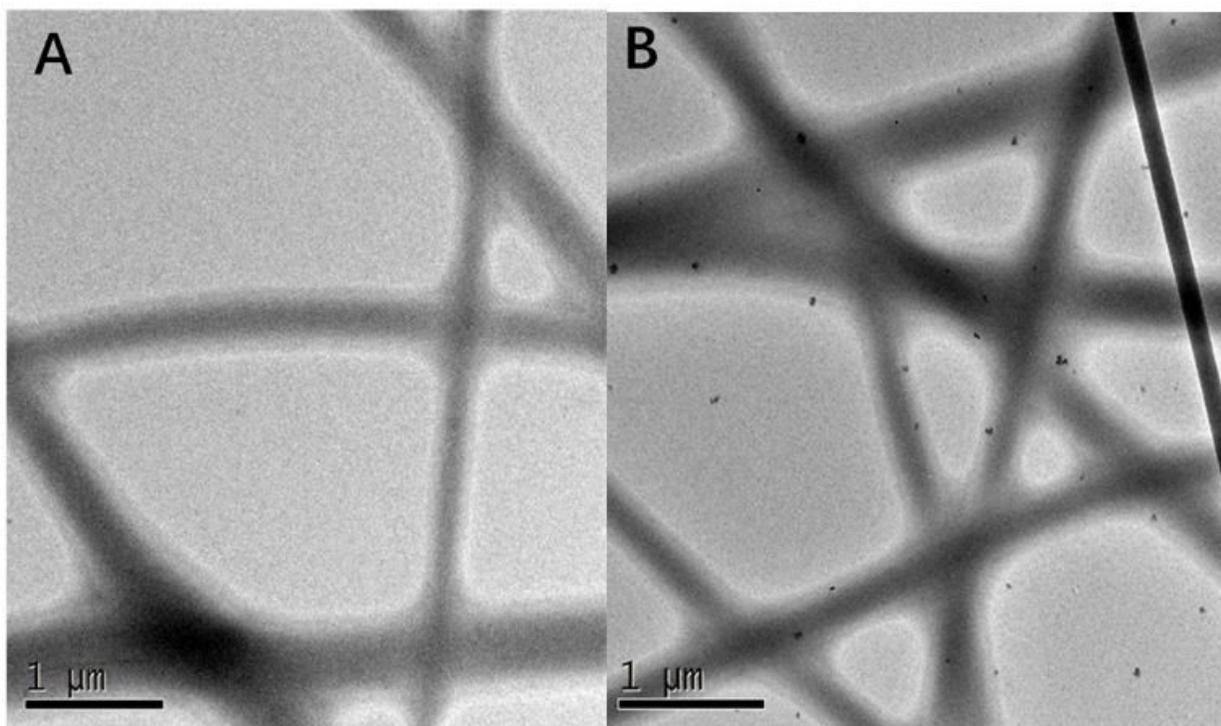


Figure 2

TEM images of FK/PVA nanofibers membrane before and after AgNPs modification (A) 0%-AgNPs; (B) 3%-AgNPs

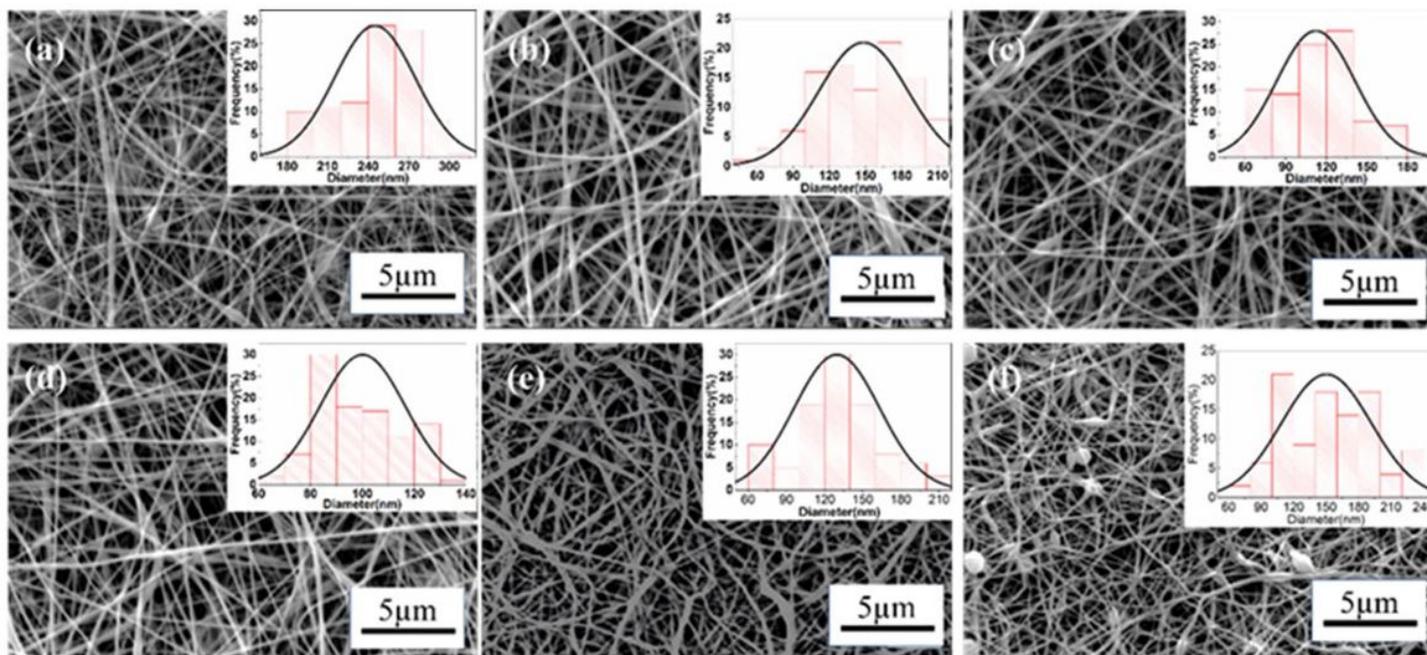


Figure 3

SEM images and the diameter distribution of FK/PVA nanofibers membrane with different AgNPs content (a) 0%-AgNPs; (b) 0.6%-AgNPs; (c) 1.2%-AgNPs; (d) 1.8%-AgNPs; (e) 2.4%-AgNPs; (f) 3.0%-AgNPs.

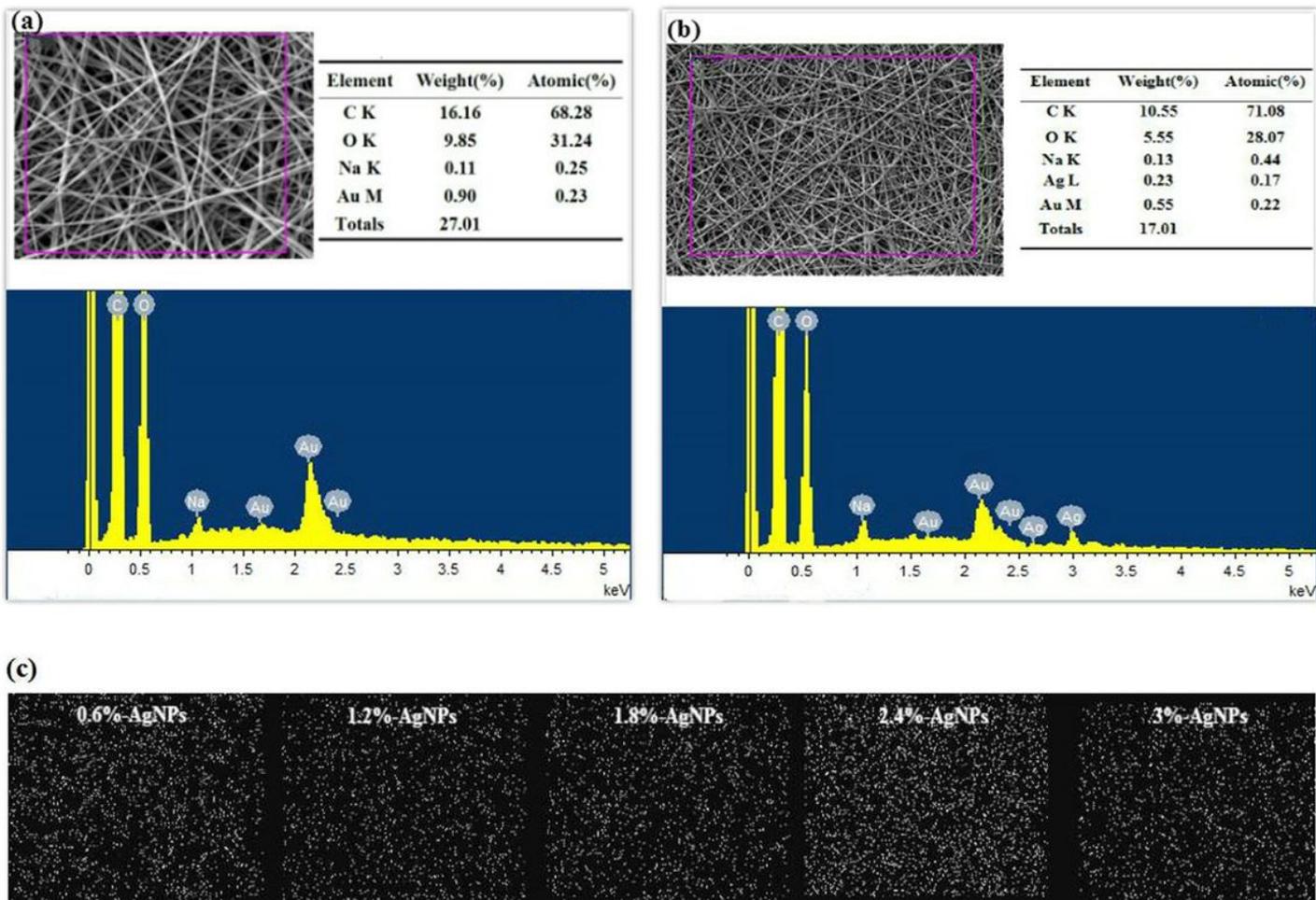
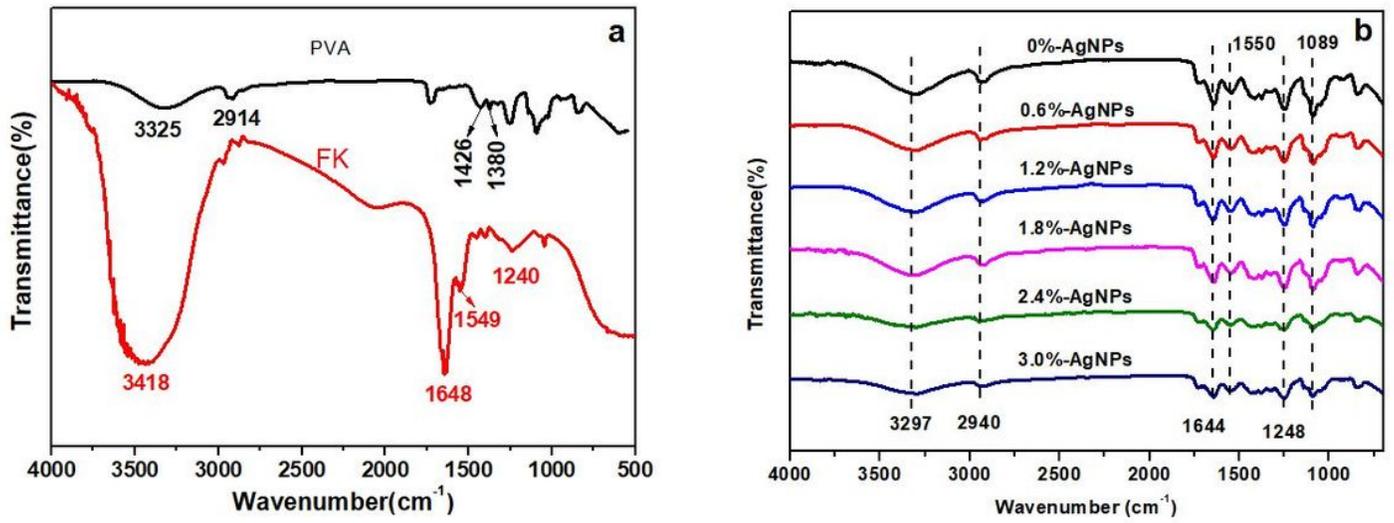


Figure 4

EDS(Energy-dispersive X-ray spectra) of FK/PVA nanofibers with various weight fractions of AgNPs (a) 0%-AgNPs (b) 3%-AgNPs (c) silver elemental distributions



**Figure 5**

FT-IR spectra of FK/PVA nanofibers membrane with different AgNPs content

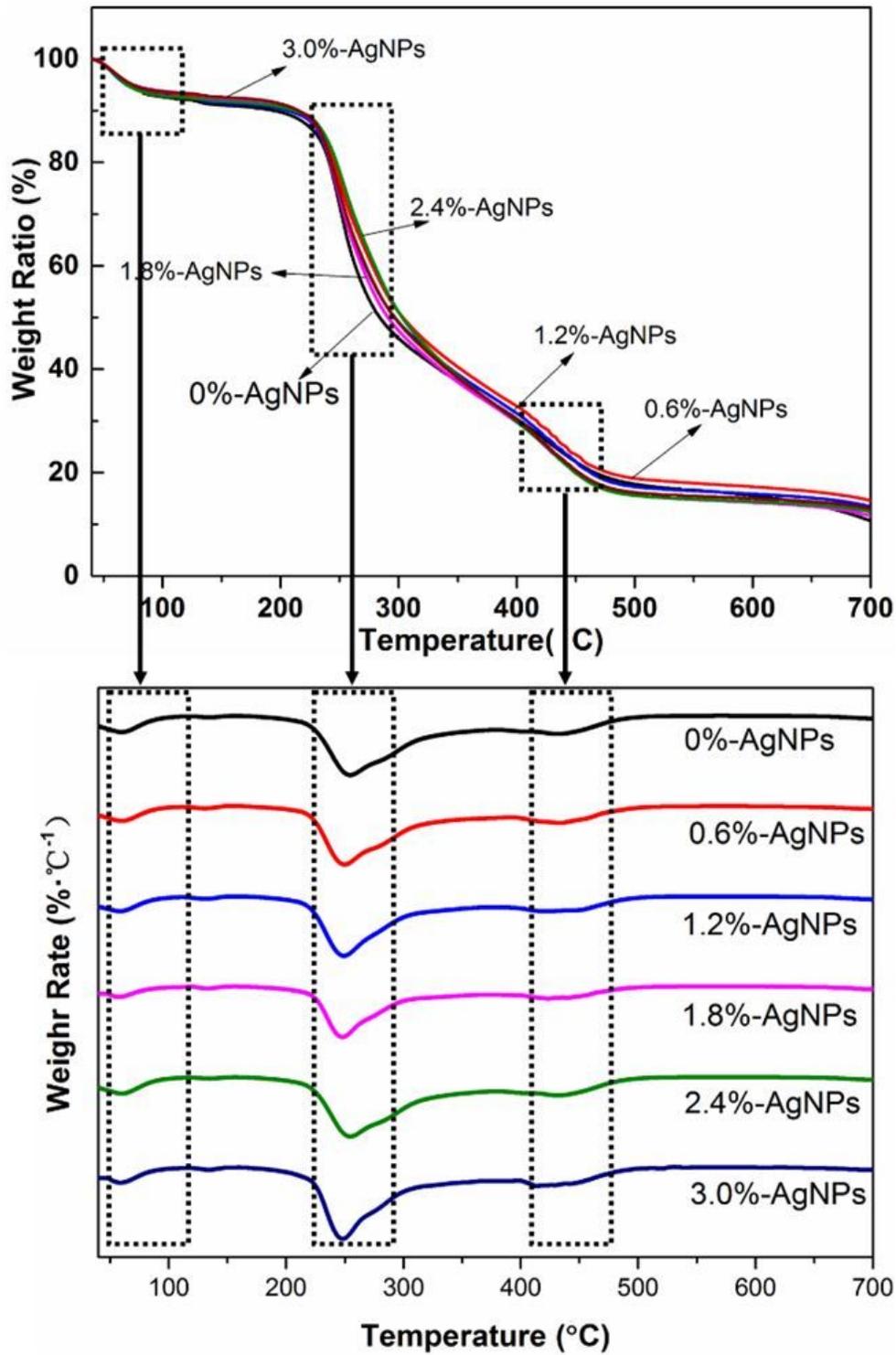


Figure 6

TG and DTG curves of FK/PVA nanofibers membranes with different AgNPs content

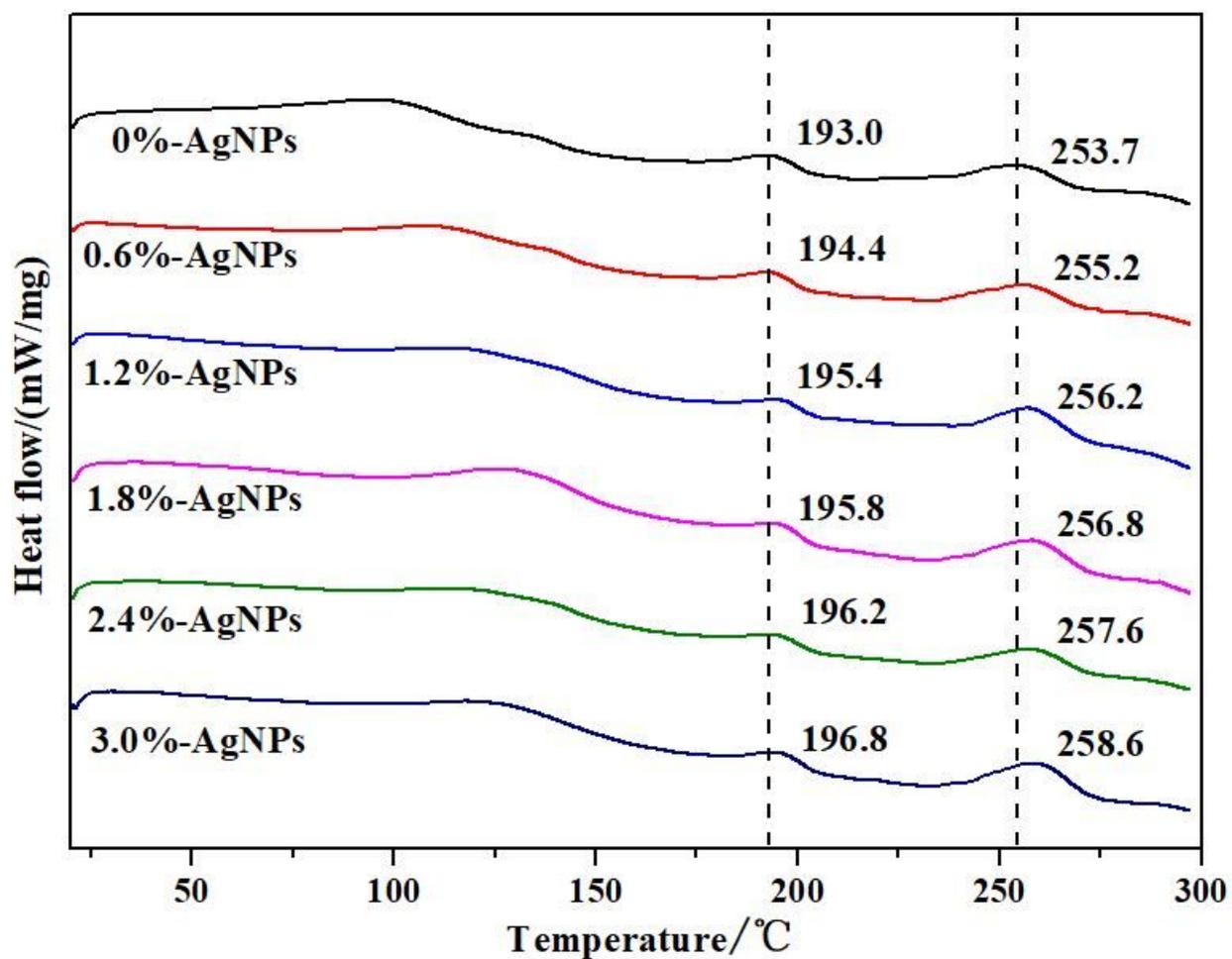
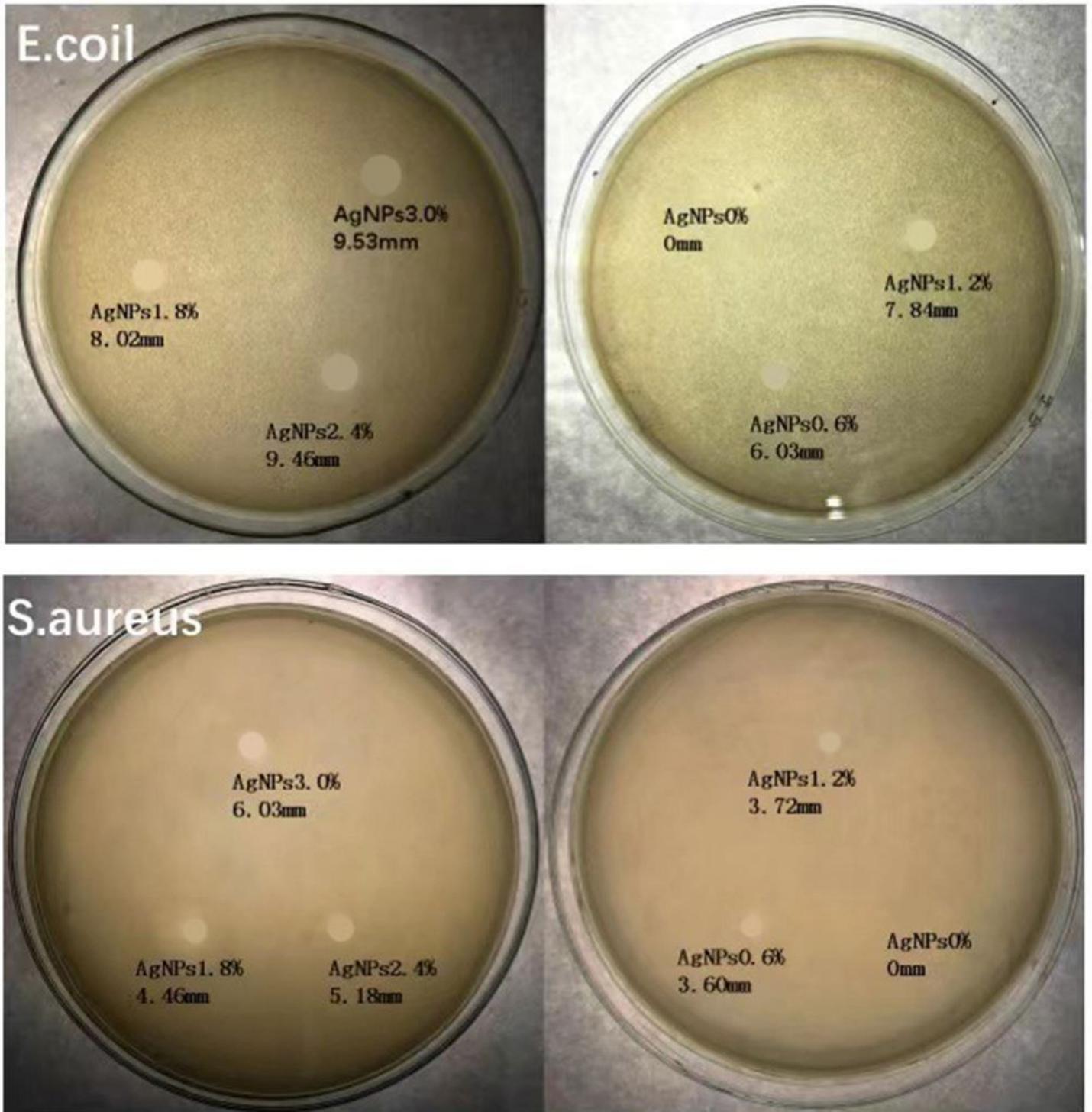


Figure 7

DSC curves of FK/PVA nanofibers membranes with different AgNPs content



**Figure 8**

Bacteriostatic circle images of FK/PVA nanofibers membranes with various AgNPs content on different bacteria: *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*)

## Supplementary Files

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- [GraphicalAbstract.png](#)