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NANO SILVER-COATED POLYPROPYLENE WATER FILTER: II. EVALUATION OF ANTIMICROBIAL EFFICIENCY

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This research will improve our understanding of the microorganism removal effectiveness of the nano silver-coated polypropylene filter used in water purification. Silver nanoparticles were deposited on cylindrical polypropylene water filter by physical vapor deposition method using a modified Balzers machine. The enumeration of bacteria was done by membrane filter method. At a flow rate of 3L/hr and after 5h filtration all of the Escherichia coli cells were killed when the input water had a bacterial load of 10^3 colony-forming units (CFU) per mL. The inductively coupled plasma/mass spectrometry (ICP/MS) was used to determine any trace amount of the silver nano particles left in the water sample after filtration. Results showed that nano particles are stable on the water filter and are not washed away by water flow after 5h filtration. The nano silver-coated filter reported here has the potential to be used as an efficient water treatment technique.

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1. Introduction

Twenty percent of the world's population is currently living without access to safe water for drinking, personal hygiene, and domestic use. The World Health Organization (WHO) Commission on Health and Environment has reported that waterborne diseases have significant negative health impacts world-wide [1]. WHO investigation showed that

80% of human disease is due to contaminated drinking water and recommended that any water intended for drinking should contain fecal and total coliform counts of 0 in any 100mL sample [2,3]. The microorganisms that cause water-associated diseases are classified as bacteria, protozoa, viruses and helminths. However, the presence of bacteria, and in particular Escherichia coli (E-coli), is the main indication of water contamination. E-coli, originally known as Bacterium coli commune, was identified in 1885 by the German pediatrician, Theodor Escherichia, and in 1892 Shardinger proposed the use of this bacteria as an indicator of fecal contamination [4,5].

Nanotechnology is an emerging branch of science used for solving environmental problems including water purification. The major property that makes nano particles attractive is that they are extremely small in size (1-100 nm), which provides higher surface area per unit mass compared to the particles produced by conventional methods. For centuries, silver has been in use for the treatment of burns and chronic wounds [6,7]. It has been known since ancient times that silver has bactericidal properties. As early as 1000 B.C. silver was used to make water potable [8,9]. The silver nano particles show efficient antimicrobial property compared to other salts due

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to their extremely large surface area, which provides better contact with microorganisms [9]. The antimicrobial action of silver nano particles (Ag-NPs) has been widely reported against a broad spectrum of microorganisms [10-14] along with a lack of negative effects such as taste, odour and color [15]. Silver is a safe and effective bactericidal metal because it is non-toxic to animal cells while highly toxic to bacteria such as E-coli and Staphylococcus aureas [15, 16]. Ag-NPs have been shown to effectively inactivate bacteria and inhibit microbial growth [17-20]. Ag-NPs attach to the phosphate and sulphur groups of the cell membranes or membrane proteins [17, 21] and severely damage the cell and its major functions such as permeability, regulation of enzymatic signalling activity and cellular oxidation and respiratory processes [22-25]. Ag-NPs can penetrate the bacterial cell and accumulate to toxic levels that may cause death of the organism [21]. In addition, Ag-NPs can bind to the DNA inside the bacterial cells, preventing its replication [17, 26], or interact with the bacterial ribosome [27].

In this study, nano silver particles were deposited on the cylindrical polypropylene water filters using a modified Balzers machine. The Balzers machine was modified in order to enable coating of the cylindrical filters in a uniform manner, as explained elsewhere [28].

Characterization of the filters was carried out using the scanning electron and atomic force microscopy techniques [28]. The antibacterial property of the filters was evaluated using the membrane filter method. The quantity of silver released from the filters was determined using inductively coupled plasma mass spectrometry (ICP-MS).

2. Experimental

2.1 Materials

E. coli (ATCC strain 8739, USA) was selected as an indicator of fecal contamination of water. A bacterial suspension of E-coli, 1000CFU/mL (CFU = colony-forming units), was diluted to 15mL distilled water. Two hundred grams of Eosin Methylene Blue (EMB) agar (Merck Co, Germany) was used as the growth medium. The silver metal used in this study was of high grade (purity: 99.99%) and was supplied by Merck (Germany). The cylindrical polypropylene filters constructed using multi-layers of the polymer were purchased from Omran Mahab Co. (Tehran, Iran) with an average pore-size of 9.86μm. The polypropylene filter possess high filtering efficiency and can remove dirt, rust, dust, silt, algae and some other particles but not certain microorganisms and bacteria such as coli bacillus. Therefore, it can not be used to remove the water pathogens without proper modification.

2.2 Methods

A 55.0nm silver layer was coated on cylindrical polypropylene water filters by a modified Balzers 760 machine (Germany) using the electron beam gun system [28]. The Balzers machine was modified in order to enable coating of the cylindrical filters in a homogenous manner as explained before [28]. Bacterial attachment to the surface of the filters was visualised using a JEOL JSM-6400 scanning electron microscope (Japan). For this, square-shaped samples with the approximate size of 1cm by 1cm were cut from the filter surfaces and visualized by the SEM. Prior to SEM analysis of the filters containing bacteria, the samples were treated with 4% glutaraldehyde for 24h at 4ºC following by two rounds of washing with 0.1M sodium cacodylate buffer and a post-fixation in 1% osmium tetroxide (2h, 4ºC). The samples were then dehydrated using a series of different concentrations of acetone (35- 100%) following by drying and sputter-coating the samples with gold.

To examine the bactericidal effect of the nano silver-coated polypropylene water filters, a custom-made pilot plant was used as shown in Fig. 1. Initially 15L of distilled water was inoculated with 10^3cfu/mL E-coli bacteria. A Stainless steel centrifugal pump (1–phase, 1/2 suction/discharge, Max Head 20m), was used for feed and recirculation. The flow of water was measured by rotameter and the temperature was controlled by a cooling device inserted into the feed tank. Two gauges were used to measure the pressure at the entrance and exit to the housing of

the silver coated polypropylene water filter (Fig. 1). The flow rate was adjusted to 3L/hr and the pressure difference before and after the water filter was 0.1bar.

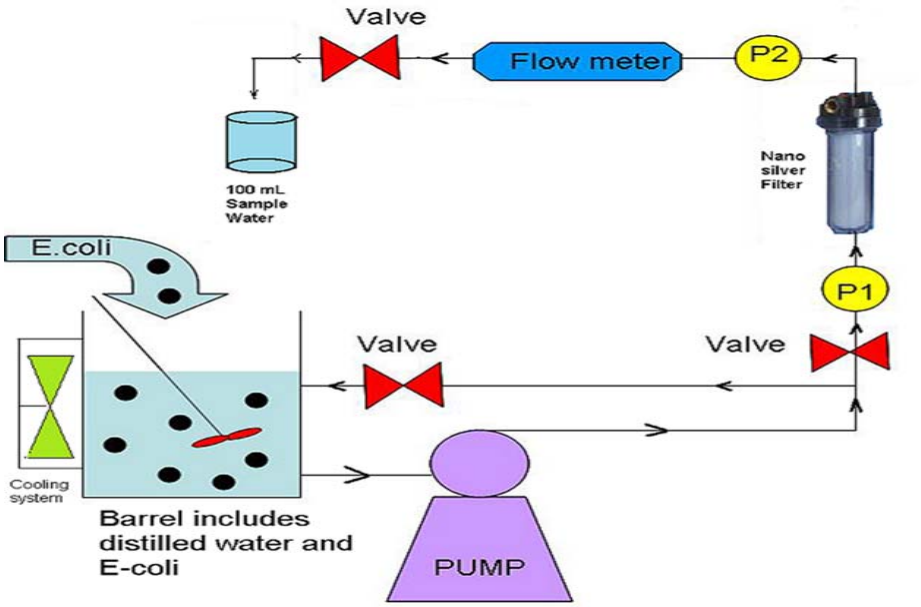

Fig 1. Experimental set-up. Distilled water includes 10^3cfu/mL and was circulated by a centrifugal pump. P1 and P2 are manometers.

The antibacterial property of the filters was evaluated using the membrane filter method [29]. The bacteria-loaded distilled water was circulated for 15min before filtration. An aliquot of 100mL of this water was taken for the bacterial assay and then the nano silver coated filter was put in place in the pilot plant (Fig. 1). After filtration for certain time intervals, appropriate volumes of the water samples (100mL) were taken and passed through 0.45μm pore size cellulose ester membrane filters (Millipore, USA) that retain the bacteria present in the samples. The membrane filters were placed on the 5mL plates of EMB agar and incubated at 35°C for up to 24 hours. The plates were then inspected for the presence of red-violet color which indicates presence of the E-coli cells. All red violet colonies were counted and recorded.

The inductively coupled plasma/mass spectrometry (ICP/MS) was used to determine any amount of nano silver particles in the water sample at the end of a 5h filtration process. An Optima 7300Dv instrument (Perkin Elmer Corporation, Norwalk, CT, USA) was used according to the reported procedure [30-32].

3. Results and discussion

Nano silver-coated polypropylene water treatment filters were prepared by a previously described methodology [28]. The polypropylene filters are not capable of removing certain microorganisms and bacteria such as E-coli. Therefore, nano silver coating was used as a mean to improve the efficiency of the filters with respect of water disinfection.

The mechanism of the antimicrobial action of silver ions is not completely known. However, the effect of silver ions on bacteria is linked with its interaction with thiol group compounds found in the respiratory enzymes of the bacterial cells. Silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process [24]. In case of E-coli, silver acts by inhibiting the uptake of phosphate and releasing phosphate, mannitol, succinate, proline and glutamine from the E-coli cells [9]. In addition, it was shown that Ag+ ions prevent DNA replication by binding to the polynucleotide molecules, hence resulting in bacterial death [25].

After circulating the distilled water inoculated with 10^3cfu/mL E-coli through the pilot plant for 15min, a 100mL sample was assessed for the presence of the bacteria. Figure 2 depicts

presence of equal to or more than 1600cfu/ml of E-coli in the water sample before filtration (control). Figure 3 shows bacterial count for the water sample after 2h filtration. As shown in Figure 3, after 2h filtration, the number of E-coli colonies reduced to 282. The number of E-coli in the water treated for 2h is significantly reduced when compared to the number of the bacteria in the control, untreated, water sample

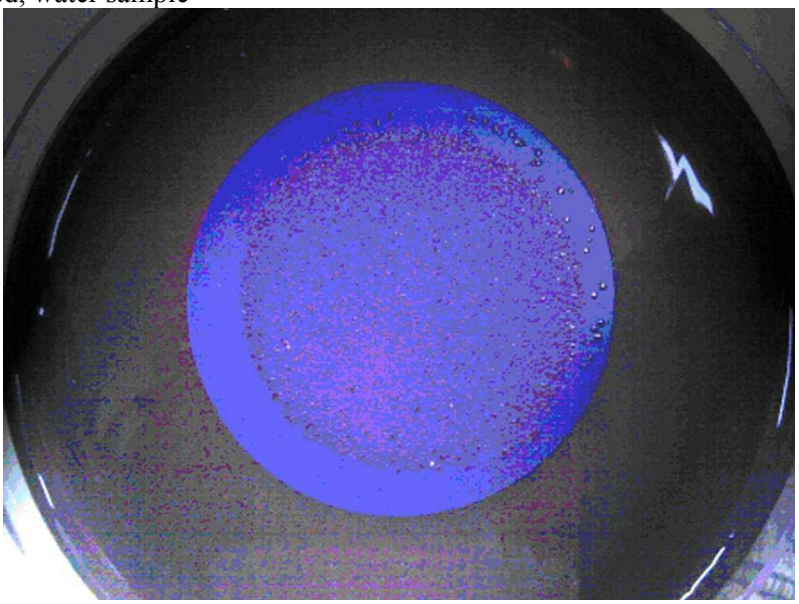

Fig 2. Membrane filter assay for detection of E-coli in the water sample before filtration. The number of bacteria is equal to or more than 1600cfu/mL. Water input had a bacterial load of 10^3cfu/mL and a flow rate of 3L/hr.

Fig 3. Membrane filter result for E.coli (1000cfu/ml) after 2 hour filtration. There are about 282 colonies in this image.

After 4h filtration, the number of bacterial contamination in the filtered water was reduced further and 20 colonies of E-coli remained in the tested water sample (Fig. 4). When filtration was continued for another 30min, only 2 colonies of E-coli remained in the water sample (Fig. 5).

However, at the end of 5h filtration there was no bacterium detected in the treated water (Fig. 6). The results are in line with the WHO requirements for drinking water [1].

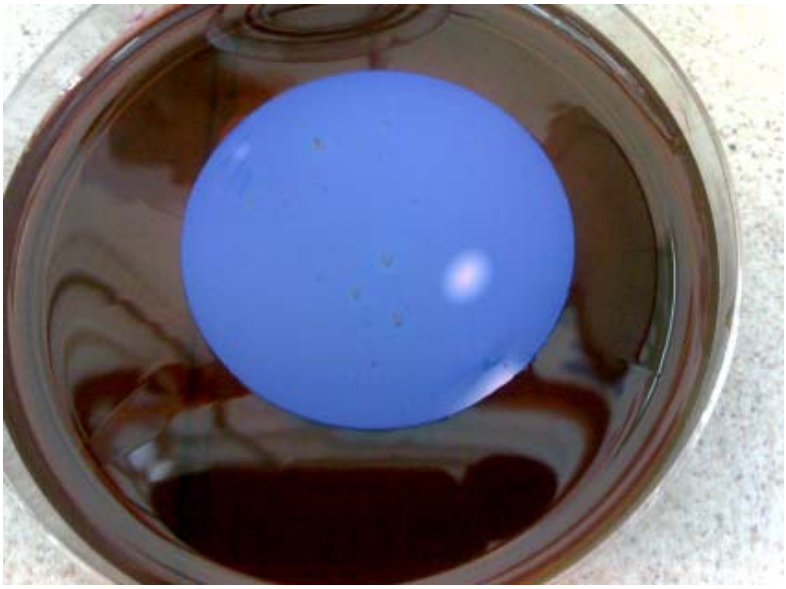

Fig 4. Membranes filter result for E-coli (1000cfu/ml) after 4 hour filtration. There are about 20 colonies in this image.

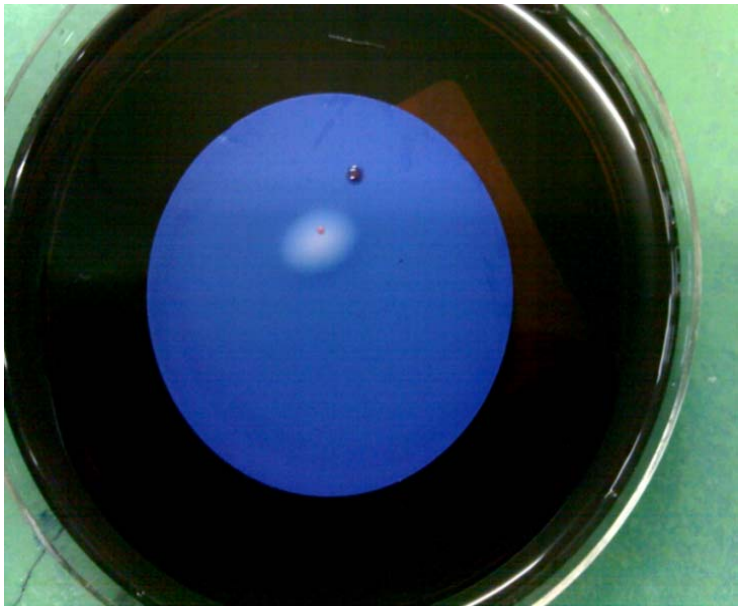

Fig 5. Membrane filter assay for detection of E-coli in the treated water sample after 4.5h filtration. There are 2 bacterial colonies in this image.

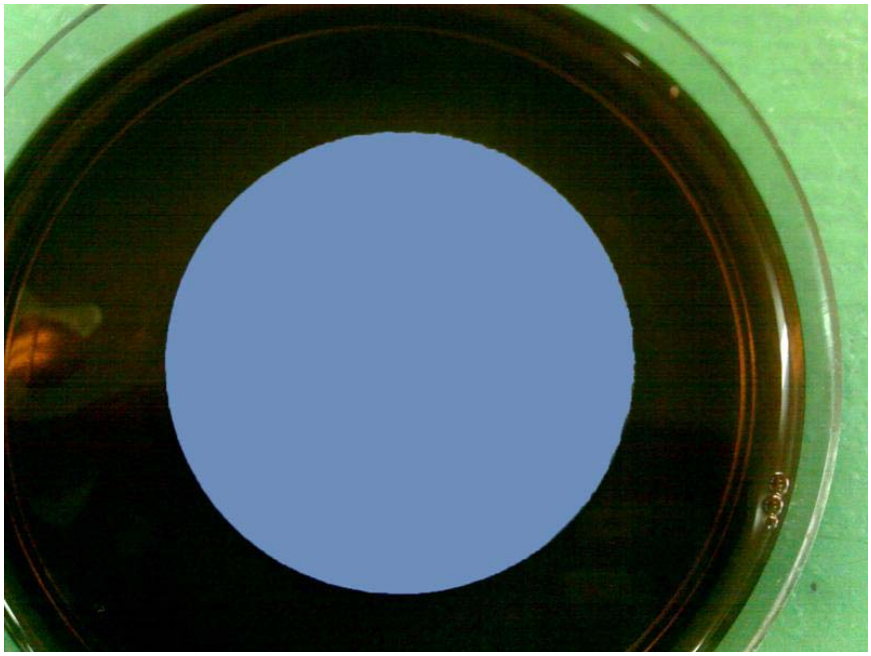

Fig 6. Membranes filter assay for detection of E-coli in the treated water sample after 5h filtration. There is no bacterium in this image.

Fig. 7 is a scanning electron micrograph of a section of the surface of the nano silver coated filter, after 5h filtration process, and depicts bacterial attachment to the filter surface. This image is a further proof that the manufactured filter is efficient in removing E-coli from drinking water.

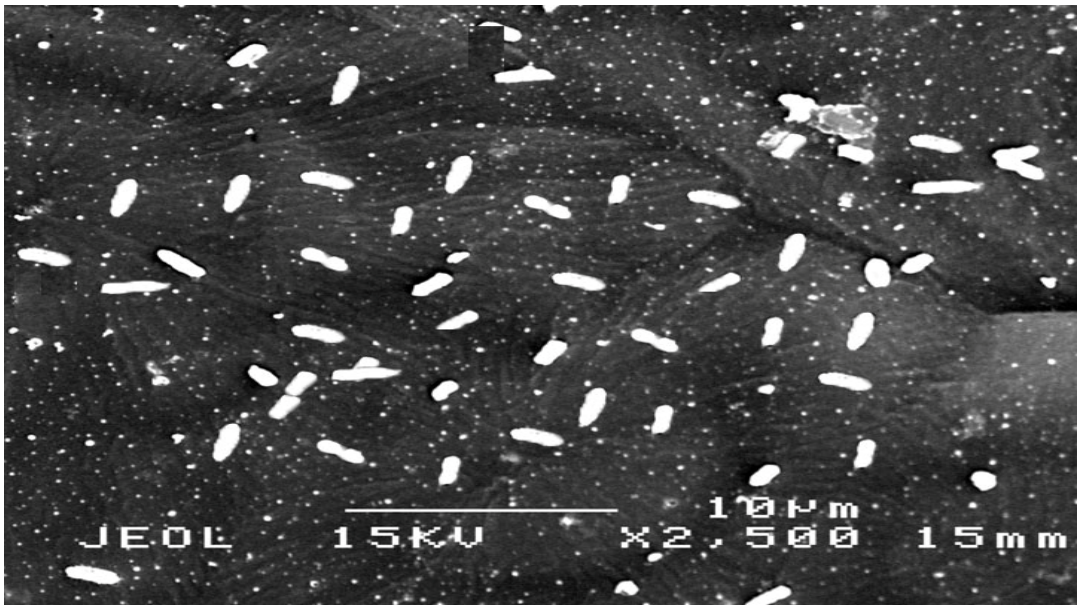

Fig 7. Representative scanning electron micrograph of E-coli cells attached to the surface of the nano silver-coated polypropylene water filter.

After proving the antibacterial efficiency of the nano silver-coated filters, the next step was to evaluate the amount of silver particles released from the filters to the water. The inductively coupled plasma/mass spectrometry (ICP/MS) was used to determine any amount of silver nano

particles in the water sample after 5h filtration. The output count of nano silver particles in the filtered water sample was nil, indicating the stability of the manufactured filters and their ability to retain the silver nanoparticles on their surface. According to the literature, the average abundance of silver in the U.S. drinking waters is 0.23μg/L [27]. Consequently, the nano silver-coated filter technology can offer a completely efficient and safe solution for the treatment of drinking water.

4. Conclusions

Cylindrical polypropylene water filters were coated by a 55.0nm layer of nano silver particles using a modified Balzers machine. The antibacterial efficiency of the filters were evaluated in a custom-made pilot plant. After 5h filtration, the nano silver-coated filters were able to remove 100% of the E-coli contamination when the input water had a bacterial load of 10^3cfu/mL and a flow rate of 3L/hr. The inductively coupled plasma/mass spectrometry examination revealed that there was no nano silver particle in the filtered water sample. These results are in agreement with the WHO requirements for drinking water and suggest the possibility of the use of the nano silver-coated filter in drinking water purification.

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