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Synthesis and Characterization of Silver Chloride Microwires Using Track Etch Membrane as Template and Evaluation of Antibacterial Activity

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ABSTTRACT

This paper describes the synthesis of AgCl microwires via chemical method at room temperature using track etch membrane (TEM) as template. In this technique, the TEM template was sandwiched between two compartments in a chemical cell in such a way that the template acted as a membrane separating the two chambers. One chamber was filled with silver nitrate solution and other with sodium chloride solution. Synthesized microwires were characterized by scanning electron microscopy (SEM), X-Ray diffraction (XRD), and UV-Vis spectrophotometry. The SEM characterization confirms the formation of cylindrical microwires of diameter and dimensions matching with the morphology of the pores of TEM template. The decrease in energy band gap of AgCl microwires is observed due to the presence of silver along with AgCl and this is also confirmed by XRD peaks. Synthesized samples of TEM impregnated with AgCl microwires were analyzed for their antibacterial activity against bacterial strains Escherichia coli, Staphylococcus aureus and Bacillus subtilis via disc diffusion method and found significantly antibacterial activity against these human pathogens. The antibacterial activity, flexible surface and easy fabrication of the AgCl impregnated TEMs make this material potentially exciting for future investigations to open a door for application in wound dressing, transdermal patches and antibacterial packaging.

Keywords: Silver Halide, Track Etch Membrane (TEM), Microwires, Template, Chemical Synthesis, Antibacterial Activity

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INTRODUCTION

Preparation of materials with low dimensionality and the investigation of their properties have attracted more and more research on account of wide applications ranging from electronics, information and communication industry to medicine and biology or textiles industry [1, 2]. The broad-spectrum antimicrobial properties of silver nano/micro materials encourage their use in biomedical applications, water and air purification, food production, cosmetics, clothing, and numerous household products [3]. Since the ancient times, silver vessels had been used for the storage of water or wine on account of medicinal property of silver metal [4] and silver halides have been exploited for medicinal use as these are antimicrobial and antibacterial materials [5, 6], remerging as a feasible treatment option for wound healing and burn [7]

In recent years, considerable efforts have been focused on the synthesis of silver halide nano/micro structures with well defined dimensions and aspect ratio due to their manifold application potentials in various fields such as catalytic processes, optics and surface-enhanced Raman spectroscopy [4, 8]. Silver halides are also photo-sensitive which make them good candidates for dry printing [9, 10]. Films of silver chloride show high transmission for infra-red radiation and have application as windows for infra-red absorption cells [11]. Applications for silver micro/nano structures predominantly include as an anti-microbial, anti-biotic and anti-fungal (fungicide) agent when incorporated in coatings, plastics, textiles or in biomedical devices for interconnection and reference electrode. One of the most significant uses of silver is as a disinfectant with hygienic for medical purposes especially in burn treatment, wound healing and ulcer treatment. Hence, flexible nano/micro porous host material/template having smooth surface impregnated with silver chloride has a great possibility to be used as photosensitive sheets for dry printing and as patch for wound healing which is one of the motivation behind present work.

Template based synthesis is a versatile and economic technique for synthesizing the variety of nano/micro materials [1, 12-16]. The morphological nano/micro-structures can precisely be determined by selecting the shape and size of the pores in template. TEM is a good alternative for the synthesis of nano/micro structures [16-18]. When energetic heavily ionizing charged particles from a nuclear radiation resource pass through polymers in foil or film shape, they leave tracks of radiation damage in the film which can be selectively etched in a suitable reagent, such as NaOH, resulting into the formation of pores of desired dimensions. The pore size, which is controllable, depends on the nature of incident ions, detector material, etching conditions etc. and may range from a few nanometer to millimeter [19, 20]. The pores can be tailored in the form of cylindrical, conical and funnel-like shapes under controlled etching conditions [21-24].

In the present work, microwires of silver chloride were synthesized via chemical method using TEM as template by placing the TEM in between two chambers, one filled with $AgNO_3$ and other filled with NaCl solution. The morphological, material and potical characterization of the synthesized microwires has also been carried out and it is observed that synthesized microwires have dimensions and shape matching with that of pores acting as molds in the template used. Synthesized samples of TEMs impregnated with AgCl microwires were also analyzed for their antibacterial activity against bacterial strains via disc diffusion method and found significantly antibacterial these human pathogens.

MATERIALS AND METHODS

Materials

Hydrophilic polycarbonate track etch membranes (manufactured by Sartorius Stedim Biotech) of high grade polycarbonate film using track etch technology was used as template. These TEMs have uniform capillary pore structures with precise narrow pore size distribution. The specified pore size is 0.2 µm and porosity is less than 15%. The pores are symmetrical and cylindrical in shape. Double distilled water was used to prepare the solutions of silver nitrate and sodium chloride. Dichloromethane was used to dissolve polycarbonate matrix after synthesis of microwires. All the chemicals and regents of GR grade were used and procured from Molychem, Mumbai, India. Antibacterial activity of AgCl microwires impregnated TEM was studied against three model bacteria *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* using disc diffusion method. Media used to check the antimicrobial activity is nutrient agar and the experiment was carried out in aseptic conditions.

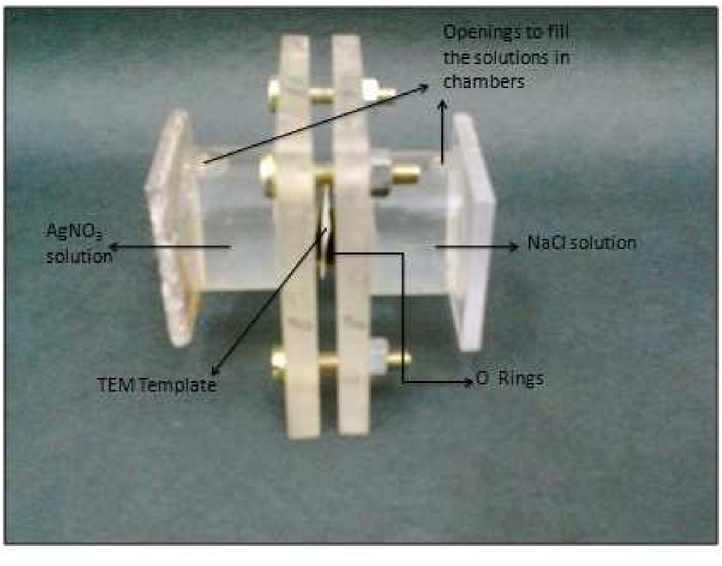


Fig. 1. Paired cell for synthesis of microwires

Method
The polycarbonate TEM with pore diameter 0.2 µm was used as template for the fabrication of microwires of silver chloride. The TEM template was placed in a paired cell in such a way that it separated the cell in two chambers [13, 17]. The O rings were placed on the both sides of the template at the aperture of the chambers for sealing, so that solution does not spill out or leak from the chambers. The experimental set-up is shown in the Figure 1. For the deposition of wires, one chamber was filled with 1.5 M solution of $AgNO_3$ and other one was filled with 1.5 M solution of NaCl and the cell was left for 12 hours at room temperature in dark environment because $AgNO_3$ is photosensitive. After 12 hours, the cell was opened, the deposition of pale white colour was observed on template in the area of aperture which indicated that AgCl microwires were formed within the pores of TEM. The sample was rinsed with double distilled water and dried in the dark environment.

Characterization
For the morphological characterization of the silver chloride microwires by means of Scanning Electron Microscopy (SEM), the cleaned and dried samples were mounted on the specially designed aluminum stubs with the help of double sided adhesive tape. The matrix of polycarbonate was dissolved by pouring the dichloromethane drop by drop for about 20 minutes. After that the sample on the stub was again dried and coated with a layer of gold using JEOL, Fine Sputter JFC-1100 sputter coater and viewed under JEOL, JSM 6100 scanning electron microscope at an accelerating voltage of 15KV. Images were recorded on the photographic film in the form of negatives at different magnifications.

To investigate crystal structure of AgCl microwires, the XRD pattern of prepared samples were recorded using EXPERT-PRO X-ray diffractometer equipped with a Gonio-meter PW0350/60 using Cu-Kα radiation, $\lambda = 1.54060$ A^o. In order to study fundamental optical property of microwires, the reflection and absorption spectra were also taken at various wavelengths using UV-Vis spectrophotometer Lameda-650 from Perkin-ELMER.

Antibacterial activity of the TEM impregnated with AgCl microwires was evaluated using disc diffusion method. The standard zone of inhibition (ZOI) assay was carried out against three model bacterial strains *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*. For it, Nutrient Agar media was autoclaved at 120º C for 15 minutes and about thirty ml of media was poured into each petri plate and then left it to cool to about 45º C so that media get solidified. The bacterial strains were spread over the sterilized plate using a spreading loop and left allowed to dry. Two TEM discs (size 4.5 mm diameter) were placed in each plate and were incubated at 37^0C for 24 hours. After incubation, the antibacterial activity indicated by ZOI surrounding the discs was recorded. The experiment was carried out in aseptic conditions.

RESULTS AND DISCUSSION

Synthesis and Characterization of AgCl Microwires
The mechanism of formation of AgCl microwires with in pores of TEM from aqueous solutions in two chambers of the cell, shown in Figure 1 in experimental details, might be represented as

$$Na^+_{(aq)}+Cl^-_{(aq)}+Ag^+_{(aq)}+NO_3^-{}_{(aq)} \rightarrow Na^+_{(aq)}+NO_3^-{}_{(aq)}+AgCl_{(s)}$$

In one chamber, silver nitrate dissociates into silver ions (Ag^+) and nitrate ions (NO_3^-) in solution, and in other chamber, sodium chloride dissociates into sodium ions (Na^+) and chloride ions (Cl^-). Both of these solutions were originally colorless before the start of chemical reaction. Within the pores of the template, sodium chloride reacts with silver nitrate, the silver ions combine with the chloride ions, forming silver chloride (AgCl) insoluble white compound that precipitates out of solution. These solid precipitates have very low solubility in water and therefore are not easily converted back into its reactants. The cell is left for adequate time of 12 hours, the above process continues till the pores are completely filled with the AgCl resulting into the formation of silver chloride microwires.

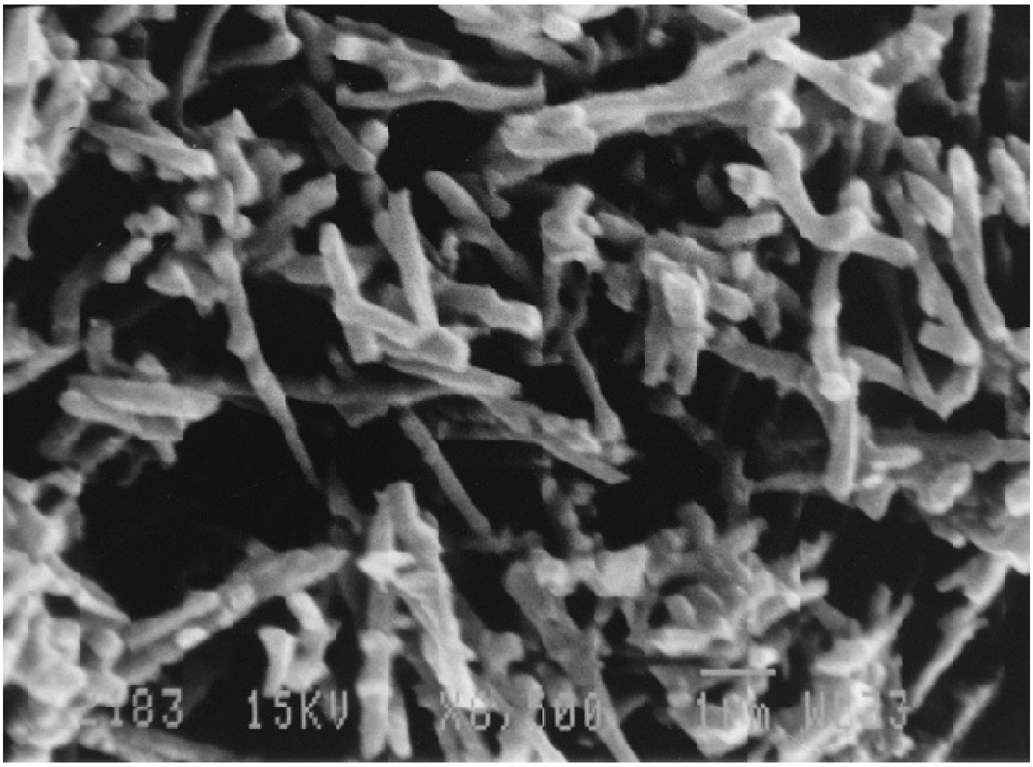


Fig. 2. SEM image of AgCl microwires from top view

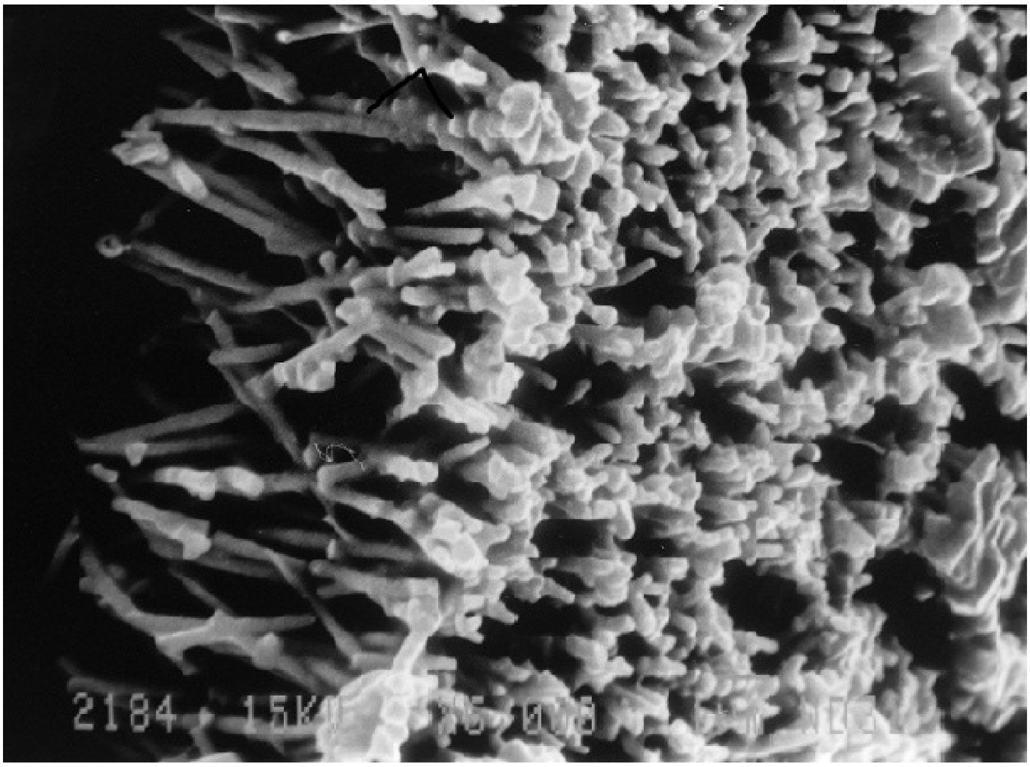


Fig. 3. SEM side view image of AgCl microwires

Figures 2 and 3 illustrate scanning electron micrographs of synthesized silver chloride microwires from top and side views respectively. The pale white color of deposited sample turned into grayish color when it was exposed to light which also confirms that deposited material is silver chloride. It is observed from SEM micrographs that diameter of wires is about 200 nm that closely corresponds to the diameter of cylindrical pores of polycarbonate TEM template. It is also seen that orientation and direction of growth of all the AgCl microwires is uniform (except some stray alignment caused on account of dissolution of the host template) because of the confined growth of microwires with in the ordered and symmetric pores of TEM template. The length of the microwires also matches with the thickness of the TEM which is about 10 µm. From this it is inference that desired shape and size of micro/nano structures can be obtained by changing the morphology of pores as molds in the host TEM as template [17].

X-Ray Diffraction (XRD) of microwires was carried out between 20^o to 90^o for 2θ values and pattern was obtained as shown in the Figure 4. Strong reflections at 2θ= 27.838^0, 32.390^0, 46.343^0, 54.872^0, 57.708^0, 67.572^0, 74.621^0, 76.841^0, 85.915^0 corresponding to (111), (200), (220), (311), (222), (400), (331), (420), (422) planes confirms the presence of crystalline AgCl and two peaks at 2θ= 44.475^0, 64.715^0 corresponding to (200), (220) planes indicates presence of Ag clusters.

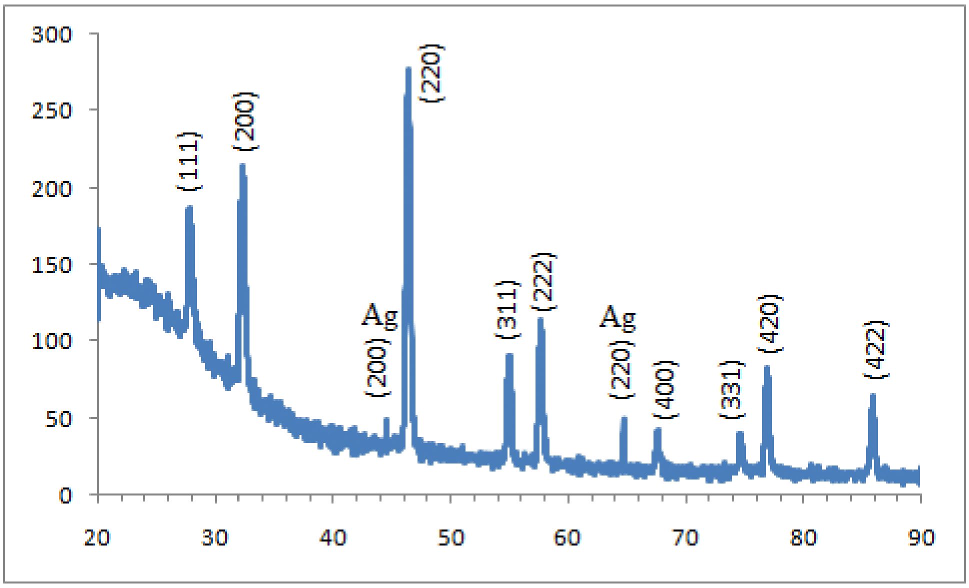


Fig. 4. XRD pattern of AgCl microwires impregnated TEM

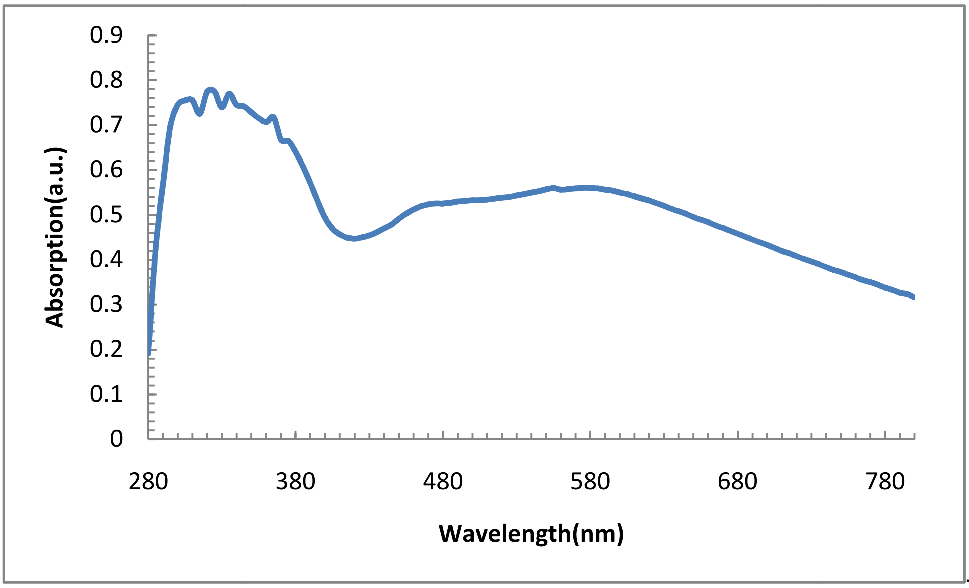


Fig. 5. Plot of absorption (α) vs wavelength (λ) for synthesized AgCl microwires

The absorption spectrum is shown in the Figure 5. Sudden fall in reflectance is observed due to absorption of light by the material. The energy band gap of AgCl microwires is determined from absorption spectra using Tauc relation. The Graph between $(\alpha hv)^2$ vs. hv is plotted as shown in the Figure 6 and the extrapolation of straight line to hv axis gives the energy band gap of AgCl microwires, which comes out to be 2.9 eV against the known value of 3.25 eV.

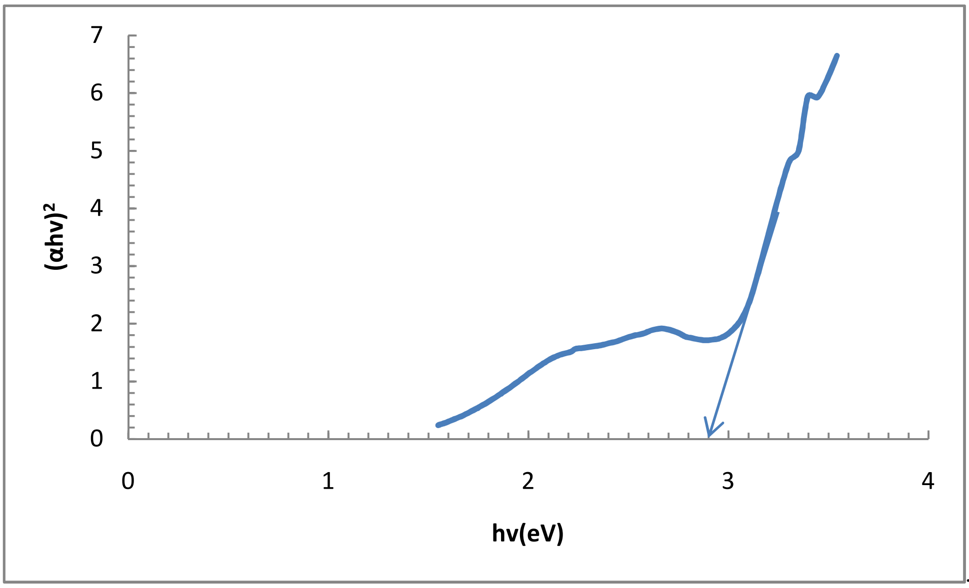


Fig. 6. Plot of $(\alpha h\nu)^2$ vs hv for AgCl microwires from absorption spectra

The decrease in band gap is observed due to formation of silver clusters along with AgCl during deposition process; new electronic transition level exists due to presence of silver which requires less energy than an AgCl valence–conduction band transition and can be induced by visible light [25].

Antibacterial Activity of TEM Impregnated With AgCl Microwires

Significant antibacterial activity exerted by samples against both Gram-negative and Gram-positive bacteria is observed as illustrated in Figures 7 (a, b and c). The ZOI was measured and found to be 11 mm, 12 mm and 14 mm for *Escherichia coli, Staphylococcus aureus and Bacillus subtilis* respectively as depicted in Table 1.

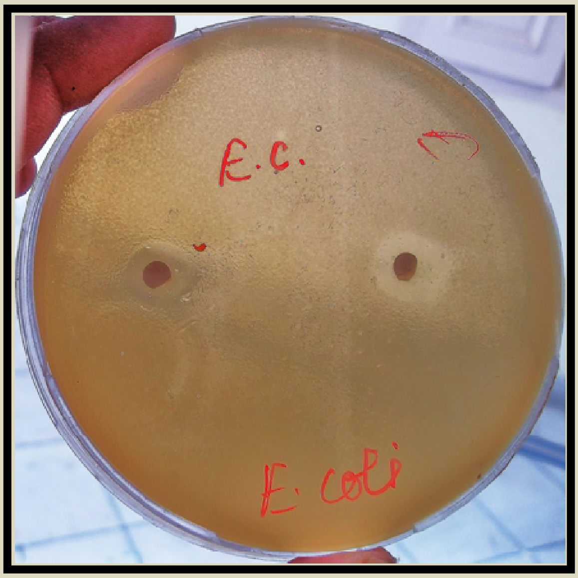
In the literature the antibacterial activity of silver and its compounds are reported to a large extent [26-30], but the mechanism of antimicrobial activity of silver and silver based mico/nonomaterials against any bacteria is not well explained [31, 32]. A cell wall, constituted by polysaccharides and peptides collectively named *peptidoglycan*, exists outside of the bacterial cell which is essential for the survival of bacteria [33]. There are two different types of cell walls in bacteria called Gram-positive and Gram-negative. Gram-positive bacteria possess a thick cell wall containing many layers of *peptidoglycan* and Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of *peptidoglycan*.

Table 1. Antibacterial activity of the TEM impregnated with AgCl microwires

Name of Bacteria	Class of Bacteria	Zone of Inhibition(ZOI)
Escherichia coli, and	Gram-negative	11 mm
Staphylococcus aureus	Gram-positive	14mm
Bacillus subtilis	Gram-positive	12mm

Surfaces of silver chloride microstructures affect/interact directly with the bacterial outer membrane, causing the membrane to rupture and results in killing of bacteria. Apparently, the silver ions interfere with the respiratory metabolism of the bacteria and therefore, exhibit antibacterial activity [27]. The enhanced bactericidal effect has been attributed to high aspect ratio of micro/nano structures which allows them to interact closely with outer cell membrane of microbes due to increased surface area and is not merely due to the release of silver ions. It is also reported in earlier studies that DNA loses its replication ability, cellular proteins become inactivated and protein denaturation occurs on interaction with Ag^+ ions [32, 235, 35]. The interaction between silver halide structures and constituents of the outer membrane of bacterial membrane causes structural changes and degradation leading to cell death [36-41]. Extensive investigations are still required to better understand the interaction between silver mico/nanostructures and bacterial components which would shed light on the mode of action as bactericidal material.

It is also observed that surface of the polycarbonate TEMs samples after deposition of AgCl microwires in the pores is smooth and flexible, not stiff, and hence can take shape of other surface on which it is pasted or laminated. AgCl is an established photosensitive and antimicrobial material, which is impregnated in the pores of TEMs, making these samples suitable as substrate for dry printing, antimicrobial pakaging and as patch for wound healing.



(a) Antimicrobial activity against *E.coli*

(b) Antimicrobial activity against *S. aureus*

(c) Antimicrobial activity against *B. subtills*

Fig. 7. Antibacterial activity of the TEM impregnated with AgCl microwires

CONCLUSION

Chemical synthesis of silver chloride microwires has been performed at room temperature using the polycarbonate TEM as template. Using this method, ordered and uniform polycrystalline microwires of cylindrical shape, with dimensions matching the morphology of the pores in TEM template acting as molds, have successfully been prepared. Synthesized samples of TEM impregnated with AgCl microwires were analyzed for their antibacterial activity against model bacterial strains *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* via disc diffusion method and found significantly antimicrobial against these human pathogens. After deposition, the TEMs impregnated with AgCl microwires have potential for application to be used as substrate for dry printing due to its photosensitivity and also as a patch for wound healing due to antimicrobial activity of silver chloride. The different shapes and sizes of nano/micro structures can be synthesized using chemical method by changing the morphology of pores in TEM templates.

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