Characterization of Silver (Ag) Nanomaterials Synthesized by Horizontal Vapor Phase Crystal (HVPC) Growth Technique for Antimicrobial Applications

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Triangular silver nanoplates, of different orientations, and other nanostructures were successfully synthesized for antimicrobial purposes using the Horizontal Vapor Phase Crystal (HVPC) growth technique. The starting material for the synthesis was 35mg of 99.99% pure silver powder at varying growth temperatures from 800 °C, 900 °C, 1000 °C, and 1100 °C with growth times of 4 hours, 6 hours, or 8 hours at a fixed ramp time of 80 minutes were used as parameters in this study. Results from the SEM micrograph which was supported by EDX analysis showed that nanoparticles, triangular nanoplates, hexagonal nanoplates, nanowires, nanoribbons, nanorods, and nanocubes can be grown in the HVPC technique. The pour plate technique was employed to test the antimicrobial potency of the grown silver nanomaterials. Results revealed that the number of E. coli colonies grown when 10^5 CFU/mL of bacterial solution was exposed to a quartz tube with silver nanomaterials was decreased compared to when it was exposed to a quartz tube with or without silver powder.

Keywords: *nanometerials, antimicrobial, Horizontal Vapor Phase Crystal (HVPC) growth technique, vacuum, pour plate technique*

1. INTRODUCTION

Interest has arisen in the manufacture and the characterization of silver nanomaterials because of their unusually enhanced physicochemical properties and biological activities compared to the bulk parent materials or macro scaled counterparts. These properties provide the wide potential use of silver for numerous applications like antimicrobial applications in medical devices and supplies and in various consumer products (Amendola, Polizzi, & Meneghetti, 2007; Navaladian, S., Viswanathan, Varadarajan, & Viswanath, 2008; Fernandez et al., 2008; Thomas, Yallabu, Sreedhar, & Bajpai, 2007), utilization of fluorescence and surface plasmon resonance (SPR) for sensing applications (Jacob, Mahal, Biswas, Mukherjee, & Kapoor, 2008; Hua, Chen, Ge, & Tan, 2007; Wiley et al., 2007;

Dubas & Pimpan, 2008) and span in electronic applications (Willets & Van Duyne, 2007; Kim, Kim, Ah, Kim, & Jang, 2004; Deshmukh, & Composto, 2007).

Among the properties and applications of silver nanomaterials, its antimicrobial property earns the greatest interest among the existing studies because of its simplicity and importance. The search for a new and stronger antimicrobial agent is unending because the rate at which microorganisms develop resistance is faster than the manufacture of new drugs.

Bulk silver is known for its antimicrobial properties and has been used for several years in the medical field for antimicrobial applications. Silver has even shown to prevent HIV binding to host cells (Nino-Martınez et al., 2008). In fact, colloidal silver or the bulk form of silver was used as an antibiotic before the discovery of penicillin. In addition, silver had been used in water and air filtration to eliminate microorganisms (Chou, Yu, & Yang, 2005; Chen et al., 2008). This antimicrobial property of bulk silver is expected to be carried over and, perhaps, enhanced in silver nanomaterials. However, the hypothesized mechanisms that govern the fate and transport of bulk materials may not be directly applied to materials at the nanoscale and thus, remains to be understood. Several studies proposed that silver nanoparticles may attach to the surface of the cell membrane, disturbing the permeability and the respiration functions of the cell (Kvitek et al., 2008). It is also possible that the silver nanoparticles not only interact with the surface of membrane but can also penetrate inside the cell (Lushnikov & Kulmala, 1998). Thus, the question remains on when and how silver nanomaterials can be a microbicidal or a microbistatic and the effects of different nanostructures to this behavior.

Different synthesizing techniques have been employed to grow silver nanostructures like nanoparticles, nanopowder, nanowires, nanobelts, dentritic nanostructures, nanoplates, nanorings, nanorods, and nanotubes. These techniques includes electromagnetic wave irradiation, template technology, spray pyrolysis technique, surface formation technique, photo reduction method, electron beam evaporation method, polysaccharide method, Tollens method, Langmuir-Blodgett monolayers technique, microwave heating technique, chemical electro-deposition technique, biological method, polyoxometalates method, spinning disk technique, pulse laser ablation technique, and solvothermal technique.

The top-down techniques use silver metal in its bulk form then, mechanically reduce its size to the nanoscale via specialized methodologies (Balaguera-Gelves, M., 2006). The bottom-up (also known as self-assembly) technique involves dissolution of silver salt into a solvent and the subsequent addition of a reducing agent, with the supplemental use of stabilizing agents, if warranted, to prevent agglomeration of nanoparticles.

From the cited techniques employed, the majority suggested that the bottom-up technique is predominantly used in the synthesis of silver nanoparticles relative to the top-down technique (Hatchett & Henry, 1996). However, this majority posed environmental issues associated with the manufacture of silver nanoparticles while taking into account its antibacterial effect. This is due to the production of other chemicals aside from the material of interest, where disposal is a major concern.

It is for these reasons that this research study was undertaken. The synthesized and characterized silver of different nanostructures grown in a simple technique, hopefully, will be helpful as a contribution for building a framework in understanding its antimicrobial property by providing researchers the desired silver nanostructures.

In this study, the HVPC growth technique; a procedure that is similar to Castillon's work; was employed to synthesize the silver nanostructures for antimicrobial purposes. The effects of varying the growth temperature and the growth time on the surface topography and morphology and elemental composition were investigated using the Scanning Electron Microscope (SEM) and Energy Dispersive Xray (EDX). To confirm that the antimicrobial property of bulk silver is carried over and enhanced, the grown silver nanomaterials was tested on E. coli using the pour plate technique.

2. METHODOLOGY

A quartz tube with an inner diameter of 8.5 mm, an outer diameter of 11 mm, and a length of 220 mm was prepared as the container by sealing its one end using a high temperature blow torch fueled by a mixture of LPG and oxygen. It was then cleansed using an ultrasonic cleanser for 30 minutes and was rinsed and dried. Silver powder (approximately 0.035 grams) of high purity (99.99%) from Aldrich Corporation was poured into the tube. The quartz tube with silver powder was attached to a Thermionic High Vacuum System to decrease the pressure at around 10^{-6} Torr and was then fully sealed and detached using the blow torch.

The fully sealed quartz tube containing the silver powder was placed horizontally inside a Thermolyne furnace and was baked. The furnace was programmed at a constant ramp time of 80 minutes, at a certain growth temperature (800 °C, 900 °C, 1000 °C, or 1100 °C), and at a certain growth time (4 hours, 6 hours, or 8 hours). The variation in the growth temperatures was decided based on the melting point of silver at ambient pressure. To achieve the desired temperature gradient necessary for the growth of nanomaterials, the fully sealed quartz tube was inserted halfway through the furnace. This should trigger the silver powder at the hotter end of the tube to evaporate and then condense as it diffuses towards the colder end (Cao, 2004). After the set time for baking, the set up was allowed to cool down on its own to room temperature.

The cooled quartz tube was fully covered with masking tape and was sectioned into four zones. The silver deposits on quartz tube fragments were characterized using SEM (JEOL 5310) and EDX (Oxford with Link ISIS). SEM was used to determine if silver nanomaterials were present on the fragments of the quartz tube and in what structure or morphology. The elemental composition was determined using the EDX. The middle part of the four regions was characterized and only deviated to top or down parts if there was a high suspicion of locating other silver nanostructures. Further characterization was done on grown silver nanoparticles that exhibit different colors using the spectral imaging where the broad blue (460-490 nm) light is the incident light.

From the characterized silver nanomaterials grown using the HVPC growth technique in various growth temperature and growth time, the optimum growth conditions were deduced. The desired dimension of silver nanomaterials for antimicrobial purpose was then synthesized. Pour plate technique was employed to confirm that the antimicrobial property of bulk silver is carried over or perhaps enhanced in the grown silver nanomaterials. A 10^5 CFU/mL of E coli was prepared through serial dilution of the original 10^8 CFU/mL bacterial solution. This was done to avoid overcrowding of grown colonies. Four sealed quartz tubes were prepared for this test: tube 1 - a quartz tube that doesn’t contain silver but was placed inside the furnace, tube 2 - a quartz tube that contains 35 mg of silver powder but was not placed inside the furnace, and tubes 3 and 4 - two quartz tubes that contain silver nanomaterials (mostly triangular nanoplates). The ends of the tubes, the ones placed outside the furnace, were cracked and served as the entry points of the bacterial solution.

Five milliliters of the bacterial solution was poured into the quartz tubes and was then shaken for 30 minutes using an orbital shaker. One mL of the bacterial solution from each quartz tube is poured in separate sterile petri dishes to which is then poured 9 mL of sterile and cold (at 45 °C) nutrient agar medium. The contents were thoroughly mixed and allowed to solidify. The dishes were then incubated at 35 °C for 24 hours before comparing the colonies grown on each petri dish.

3. RESULTS AND DISCUSSION

Presented in Table 1 is a summary of the structures formed on the zones of the quartz tube at growth temperatures of 800 °C, 900 °C, 1000 °C, and 1100 °C and at growth times of 4 hrs, 6 hrs, and 8 hrs. Table 1 reveals silver nanostructures formed in the region of quartz tube placed inside the furnace. The optimum size and the number of nanoplates were best grown at low growth temperature and at short growth time at zones 1 and 2. Spherical nanoparticles were best grown at the end of zones 2 and 4. The desired size can be achieved by increasing the growth time regardless of the growth temperature. Nanowires and nanorods were best grown at high growth temperature and at short growth time.

Table 1
Summary of the structures formed on the quartz tube at various growth temperature and growth time.

Temp. (in °C)	Zone	Time (in hours)		
		4	6	8
800	1	✓ microstructures ✓ faceted nanocrystals ✓ hexagonal nanoplates ✓ triangular nanoplates (side length: 300 nm–1000nm)	✓ microstructures ✓ faceted nanocrystals ✓ hexagonal nanoplates ✓ triangular nanoplates (side length: 500 nm – 1500nm)	✓ microstructures ✓ faceted nanocrystals ✓ hexagonal nanoplates ✓ triangular nanoplates (800 nm – 5000nm)
	2	✓ nanoparticles ✓ nanowires ✓ nanoribbons ✓ hexagonal nanoplates ✓ triangular nanoplates (side length: 300 nm – 1000nm)	✓ nanoparticles ✓ nanowires ✓ nanoribbons ✓ hexagonal nanoplates ✓ triangular nanoplates (side length: 500 nm – 1000 nm)	✓ nanoparticles ✓ nanowires ✓ nanoribbons ✓ hexagonal nanoplates ✓ triangular nanoplates ✓ microplates (side length 1000nm – 5000nm)
	3	✓ none	✓ nanocubes	✓ nanocubes
	4	✓ nanoparticles	✓ nanoparticles	✓ nanoparticles
900	1	✓ microstructures ✓ faceted nanocrystals ✓ microspheres	✓ microstructures ✓ faceted nanocrystals ✓ microspheres	✓ microstructures ✓ faceted nanocrystals ✓ microspheres
	2	✓ nanoparticles ✓ nanowires ✓ nanoribbons ✓ faceted nanocrystals ✓ hexagonal nanoplates ✓ triangular nanoplates	✓ nanoparticles ✓ nanowires ✓ nanoribbons ✓ faceted microcrystals ✓ hexagonal nanoplates ✓ triangular nanoplates (side length: 500nm-1100 nm)	✓ nanoparticles ✓ nanowires ✓ nanoribbons ✓ faceted microcrystals ✓ hexagonal nanoplates ✓ triangular nanoplates ✓ microplates
	3	✓	✓ Nanocubes	✓ nanocubes
	4	✓ nanoparticles	✓ nanoparticles	✓ nanoparticles
1000	1	✓ microspheres	✓ microspheres	✓ microspheres
	2	✓ microstructures ✓ nanoparticles ✓ microwires ✓ faceted microcrystals	✓ microstructures ✓ nanoparticles ✓ microwire ✓ faceted ✓ microcrystals ✓ microplates (side length: 700nm-1300nm)	✓ microstructures ✓ nanoparticles ✓ microwires ✓ faceted microcrystals ✓ microplates
	3	✓ none	✓ nanoparticles	✓ nanoparticles
	4	✓ nanoparticles	✓ nanoparticles	✓ nanoparticles

1100	1	✓ microspheres	✓ microsphere	✓ microspheres
	2	✓ microstructures ✓ nanoparticles ✓ microwires ✓ adhered microstructures ✓ microplates	✓ microstructures ✓ nanoparticles ✓ microwires ✓ adhered microstructures ✓ microplates (side length : 800nm -13800 nm)	✓ microstructures ✓ nanoparticles ✓ mircowires ✓ adhered microstructures ✓ microplates
	3	✓ none	✓ nanoparticles	✓ nanoparticles
	4	✓ nanoparticles	✓ nanoparticles	✓ nanoparticles

Figure 1 is a representative EDX spectra and EDX analyses of a triangular nanoplate. The unlabeled peaks correspond to gold (Au), which was used in coating the samples for EDX characterization. The Si and O peaks are attributed to the glass substrate (SiO). The representative spectra of the structure reveal that the structure found was indeed made up of silver.

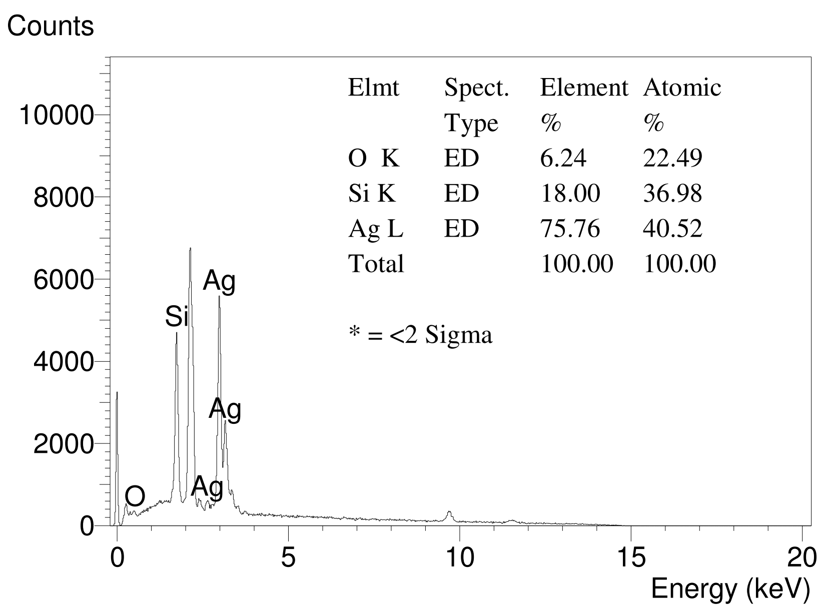
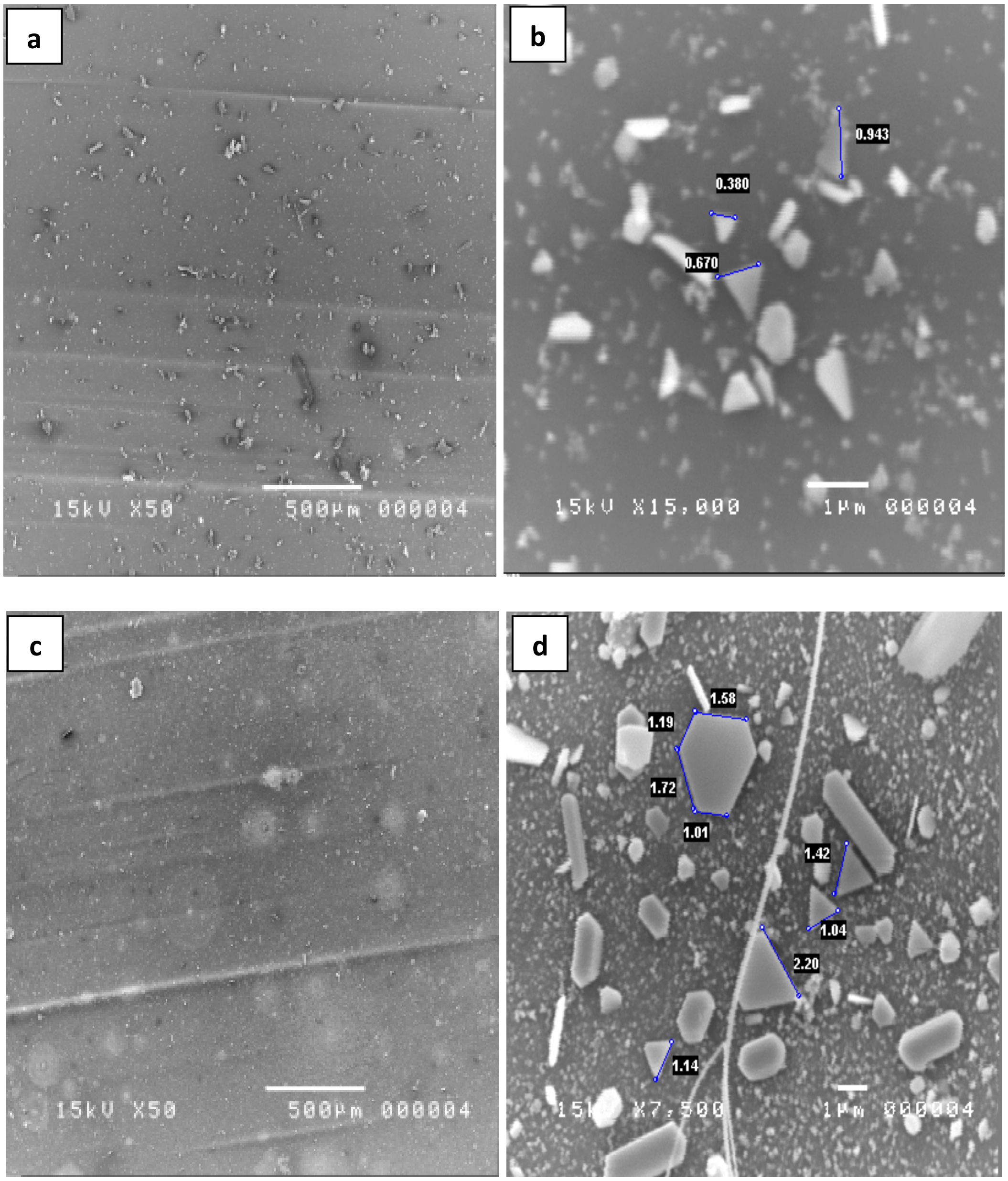


***Figure 1*. EDX spectrum of triangular nanoplates synthesized at 1100°C and 6 hours.**

Figure 2 shows the SEM micrographs of the silver nanomaterials grown at a fixed temperature of 800 °C and at various growth times, 4 hrs, 6 hrs, and 8 hrs.


a
15kV X50
500μm 000004
b
0.943
0.380
0.670
15kV X15,000
1μm 000004
c
15kV X50
500μm 000004
d
1.58
1.19
1.72
1.01
1.42
1.04
2.20
1.14
1μm 000004

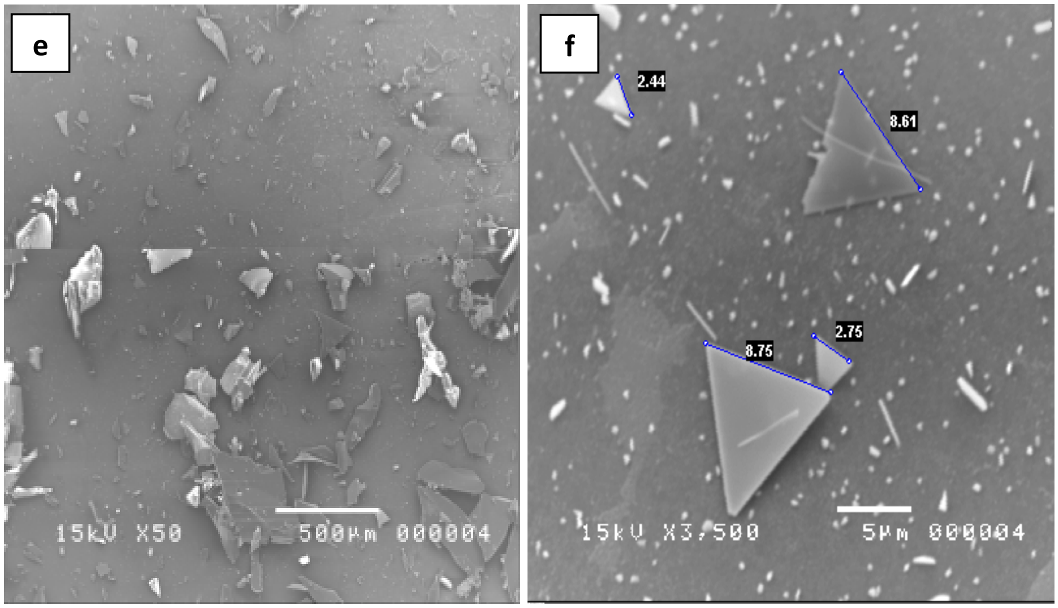


***Figure 2*. Particle-size distribution on quartz tube and triangular structures of silver grown at 800 °C with growth times of (a-b) 4 hours, (c-d) 6 hours, and (e-f) 8 hours.**

Figure 2 presents the comparison of the sizes of grown nanomaterials at a growth temperature of 800 °C at various growth times, 4 hours, 6 hours, and 8 hours. The figure reveals that the size of triangular nanoplates became larger when the growth time was increased. This is further supported by micrographs taken at x50 magnification that show increase in size of microstructures at longer growth time. Thus, it was determined that 4 hrs is the best growth time for growing silver triangular nanoplates. However, there were remnants of undeposited silver that can be retrieved. Thus, to maximize the silver powder, 6 hrs is preferred to be the best growth time for growing the most number of silver triangular nanoplates for antimicrobial purposes.

Figures 3-5 show details of the grown colonies in each petri dish. It can be seen from the pictures that the number of colonies in plates 3 and 4 are lesser, compared to plates 1 and 2. Comparing Figure 3 and 4 does not show any significant difference. This indicated that the 35 mg of silver powder was not enough to kill E. coli in a 10^5 CFU/mL bacterial solution in 30 minutes. However in Figure 5, there was a distinct difference from Figures 3 and 4 since there is lesser number of grown colonies. This indicated that the antimicrobial property of bulk silver was not only carried over but was enhanced on the grown silver nanomaterials counterpart. Since, the most number of triangular nanoplates were synthesized and that they have high {111} active facets, such enhancement was due to the increase in surface contact of the silver atoms to the bacteria (Pal, Tak, & Song, 2007).

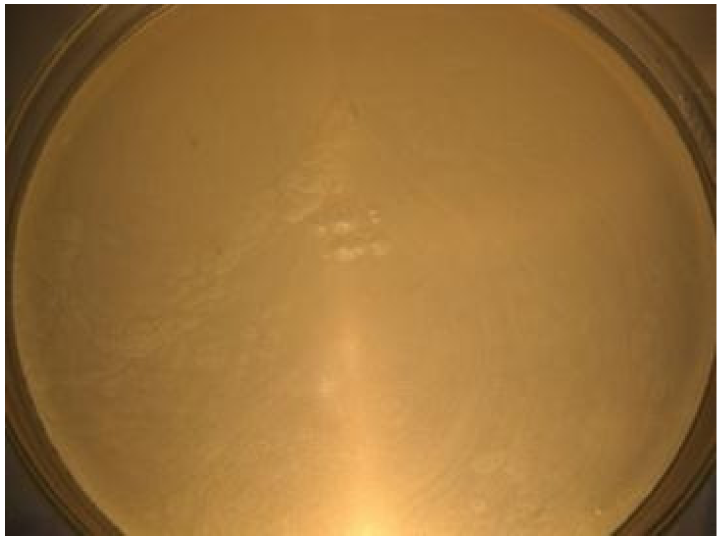


***Figure 3*. E. coli colonies grown at 10^5 CFU/mL of bacterial solution and was exposed to plain quartz tube.**

***Figure 4*. E. coli colonies grown at 10^5 CFU/mL of bacterial solution and was exposed to quartz tube with 35 mg of silver powder.**

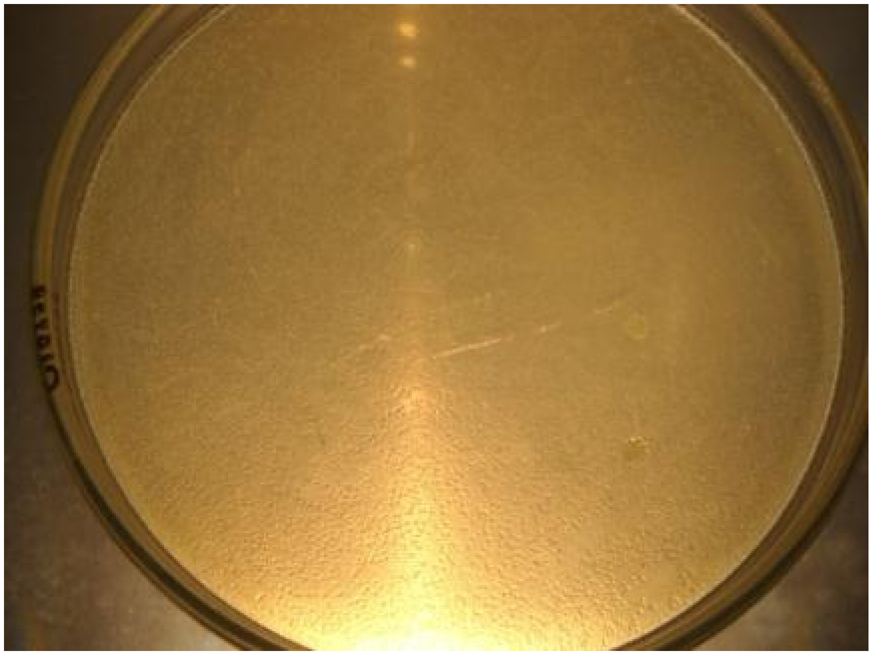


***Figure 5*. E. coli colonies grown at 10^5 CFU/mL of bacterial solution and was exposed to quartz tube with silver nanomaterials.**

4. SUMMARY AND CONCLUSION

SEM micrographs, supported by EDX analysis, showed that nanoparticles, triangular nanoplates, hexagonal nanoplates, nanowires, nanoribbons, nanorods, and nanocubes can be grown in the HVPC technique. Optimum size and number of nanoplates (triangle and hexagon) were grown best at a low growth temperature (800°C) and at a short growth time (4 hours and/or 6 hours) at zones 1 and 2. Spherical nanoparticles were optimally grown at the end of zones 2 and 4. The desired size could be achieved by increasing the growth time regardless of the growth temperature. Nanowires and nanorods were best grown at a high growth temperature (1100°C) and at a short growth time (4 hours). Furthermore, observed triangular plates were found to grow in different orientation. The antimicrobial test, pour plate technique, revealed that the number of E. coli colonies grown in a 10^5 CFU/mL of bacterial solution, which was exposed to a quartz tube with silver nanomaterials, decreased compared to other setups.

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