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Silver Nanoparticles and PDMS Hybrid Nanostructure for Medical Applications

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Abstract

For many years, people have known about silver's antibacterial qualities. Silver nanoparticles are widely used in consumer products, biomedical equipment, textile products and in other applications. Having a larger surface area to coat or spread over another surface, offers a greater contact area, therefore, increases antimicrobial properties. Also, these nanoparticles can be incorporated into polydimethylsiloxane (PDMS) implants as immobilized or occluded particles to improve their performance in the body. PDMS is commonly used for biomedical applications, including components for microfluidics, catheters, implants, valves, punctual plugs, orthopedics and micro gaskets. It can be manufactured easily in different forms such as fibers, fabrics, films, blocks and porous surfaces. The use of silver nanoparticles for their antimicrobial qualities improves PDMS biocompatibility, because it inhibits microbial growth, thereby making it more attractive for biomedical applications. The presence of metal nanoparticles also helps to reduce the hydrophobic nature of PDMS. This property of PDMS does not encourage cell adhesion, which is a very critical requirement for medical implants. Silver nanoparticles improve the silicone's wettability. The exceptional properties of silver nanoparticles combined with the PDMS have made this hybrid nanostructure applicable to different medical uses.

Keywords: silver, nanoparticles, polydimethylsiloxane, hydrophobicity, hydrophilicity

1. Introduction

Polydimethylsiloxane (PDMS) is a biocompatible material approved by the US Food and Drug Administration in the Biocompatibility Guidelines for Medical Products (Code of

Federal Regulations, 2013). PDMS is odorless, flavorless and resistant to both temperature and chemicals, including acids, oxidants, ammonia and alcohol.

All of these properties are advantages for using this polymer in human body implants. In addition, it can be manufactured easily in different forms. The PDMS is a hybrid compound with a chemical formula $(R_2SiO)_n$, where R is an organic group, such as methyl, ethyl or phenyl, attached to an inorganic chain of silicon and oxygen (Figure 1).

This kind of compound can be synthesized with a wide variety of properties and compositions, allowing the consistency to vary from liquid to gel, or rubber to hard plastic.

PDMS is a good elastomer, because the bonds between the silicon atom and the two oxygen atoms are highly flexible and very strong at the same time, so the angle formed by these links can be opened and closed [1], Figure 2.

The substrate elasticity of PDMS plays an important role in cell adhesion, proliferation and differentiation, thus improving implant integration in the human body.

The porosity, roughness, and surface energy are dominant factors for the material's wettability. These factors are especially valuable in biological environments.

The use of porous materials can be a very practical approach to reaching several goals in current medical applications such as surgical implants. Some chemical compounds, such as sugar or salts with water solubility, can play an important role in the synthesis of porous matrices because these compounds can be removed with water to empty the pores. The pore size, pore connection, and pore density are directly proportional to the crystals size of the chemical compounds and their quantity [2]. Pore size window and accessible void space are critical factors for medical applications [3].

Therefore, optimal pore size and specific surface area are important factors determining migration and cell attachment [4]. Small pores limit the cells' access within the porous matrix, and the diffusion of nutrients and removal of waste products, that can produce necrotic

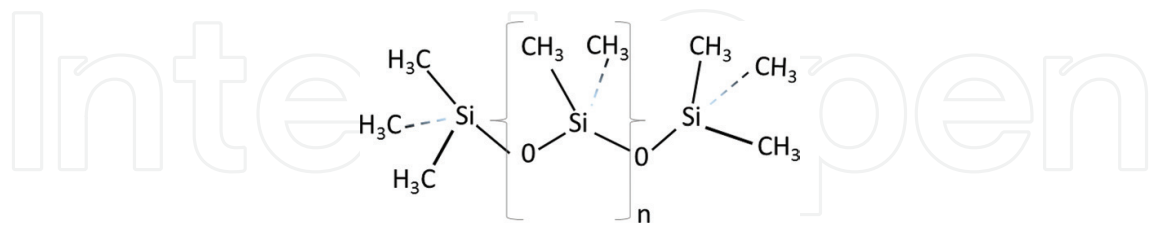


Figure 1. PDMS molecular structure.

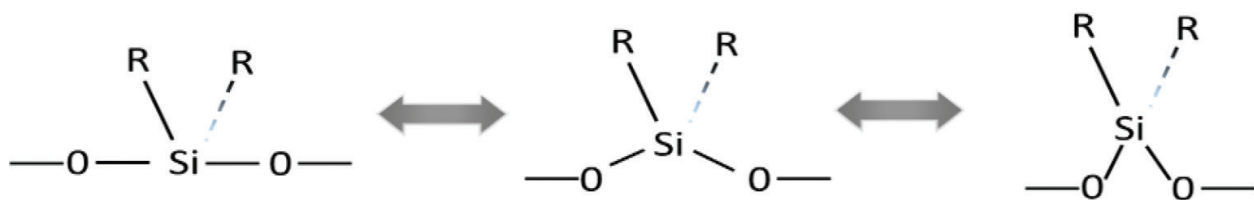


Figure 2. PDMS flexibility and elasticity.

effects in the end. Large pores limit cell attachment in a porous matrix. Composite scaffolds with preserved morphology and microstructure at micrometer scale are promising for tissue engineering. Pore size distribution from $164 \pm 52 \mu\text{m}$ can support the cells' attachment to the porous material for medical applications [5].

Material technologies are critically important for tissue engineering in designing scaffolds or implants, and control of certain properties, such as porosity and pore size distribution, key factors on medical applications [6, 7]. Cell adhesion and growth on a scaffold or implant is controlled by the size and structure of the pore matrix [8, 9].

Sugar or sodium chloride can be used as pore-forming agents. For example, sieved sodium chloride salt (99% purity), with a controlled crystal size can be mixed with polymers to prepare a slurry, then the polymer is cured and the salt removed with water to obtain a porous material with a controlled pore size distribution [10, 11].

The pore structures typically consist of irregularly shaped voids and connecting channels that can be difficult to define. This is due to merging of adjacent cavities in the void walls. Pore size and interconnectivity play key roles in cell interaction with scaffolds and implants [12], see **Figure 3**.

Adding to the benefits of porous biomaterials, micro, meso and macro channels can be covered with metallic nanoparticles, to further improve their biocompatibility properties. Recent research has been focused on providing porous materials with interconnected channels and walls. The growing surface functionality is centered primarily on silica-based materials, where their composition arrangement can add different functional groups that interact with metals [13]. Porous materials can be prepared with organic compounds used as templates or pore-forming agents that are later removed with a solvent to empty the pores. Compounds such as sucrose, fructose, d-glucose, d-maltose, dibenzoyl-L-tartaric acid, ascorbic acid, citric acid, etc., can be used as pore-forming agents, then removed these with a solvent extraction process [14]. These pore-forming agents can control the growth of biopolymers into various sizes and shapes to obtain porous matrices for medical applications. Biomaterials of different

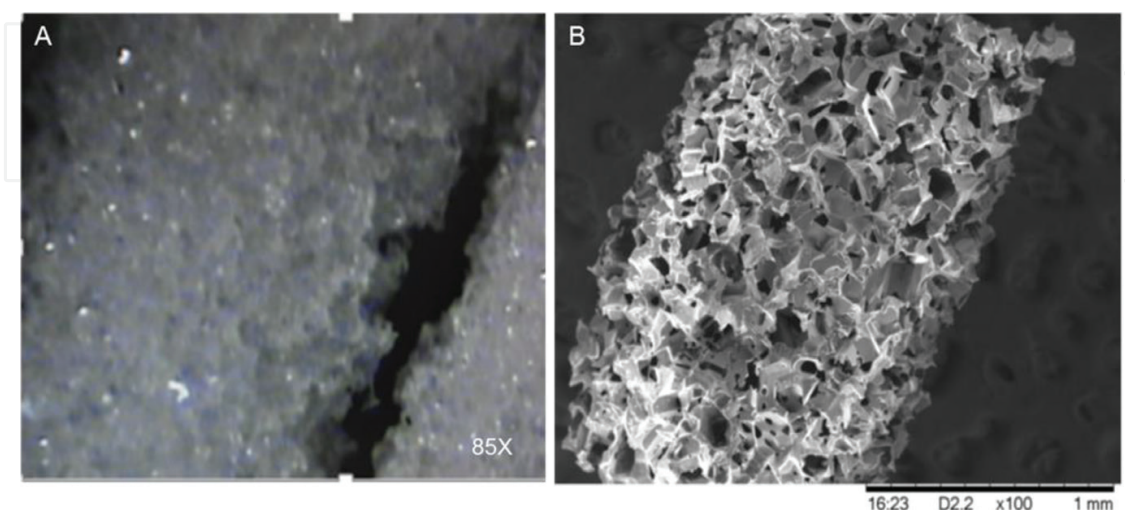


Figure 3. Porous PDMS matrix synthesized with sieved sugar as an agent to form pores: (A) optical micrograph and (B) SEM micrograph.

composition and structures (nanoparticles, nanowires, nanotubes, and nanoporous) can be synthesized through simple wet chemistry [15].

Biocompatible materials (scaffolds and implants) allow the human body to recover biological and mechanical functions, thereby increasing the quality of life. Based on the application or use, the implant must support mechanical loads, and promote long-term biological interaction with the body tissue. Current characteristics from bulk material give the load-bearing capacities for implant applications where this characteristic is required. The interaction with the adjacent tissue is dependent on the scaffold or implant surface [16, 17]. However, the biomaterials, which have micro, meso and macro-porous, show special characteristics, based on pore size, roughness, porosity, and surface energy, that can induce tissue development. The porous matrices with functionalized surfaces can be hosted and allow to leave specific biological molecules that encourage cells to grow inside and over the porous matrix. The hosting and releasing of peptides and proteins is an important key factor, because it opens new design ways for surgical scaffolds and implants. Biomaterials can improve bone rebuilding. In general, organ regeneration, as well as cell and tissue growth where required [18]. Silica porous materials have been used extensively for different medical applications, such as bone regeneration. Ceramic's characteristics, such as large surface area, porosity, and easy functionalization, allow the design biomaterial to enclose active molecules (drugs, peptides, proteins, etc.) [19].

The combination of porous materials with inorganic materials is a developing research area. These products are actually based on the outstanding properties for specific applications of this combination. Direct substitution of component elements in original porous materials, while maintaining structural regularity, provides novel properties that could be applied to surgical implants [20].

A porous PDMS matrix can incorporate silver nanoparticles, and immobilize these particles without affecting pore size distribution or producing a cytotoxic effect [21].

The ability of silver particles to kill microbes by airway blockage or breaking the outer walls of the bacteria has become one of the most important properties or factors for the development of these technologies at nanoparticle level. Application of nanoparticles in medical devices is currently important because some of them have bactericidal and disinfectant effects. The use of biomaterials in implants or scaffolds for medical application is limited by bacterial contamination that introduces infection or disease. Silver nanoparticle coatings or nanoparticle immobilization are currently used as antibacterial additive in poly-methylmethacrylate, the polymer used to manufacture bone implants (prosthetic knees, hips, etc.) [22].

The constantly expanding field of silver nanocomposites has gained significant importance, mainly due to proven antimicrobial properties offering great potential as antimicrobial coatings and agents. Many experimental methods have been proposed, but all of these have advantages as well as drawbacks. In general, identifying the method that allows the preparation of composites with biocompatible, biodegradable, and nontoxic materials (minimize toxic effects after production) is an objective for creating outstanding medical products [23, 24].

Currently, bacterial resistance to antibiotics that have been customarily used poses a challenge. A possible solution is turning to silver nanoparticles, because of their special properties. Antimicrobial uniform or non-uniform films, such as silver nanoparticles immobilized on a biomaterial, may be a viable solution to help in this critical issue [25, 26].

This new research field is not only investigating nanoparticles but also focuses in nanostructures, nanocompounds, nanofilms, and so on. This is a new technology, nanotechnology that has gained widespread acceptance.

2. Experiment

The experimental method used synthesized porous PDMS matrices with pore sizes from 100 to 300 μm and PDMS film, described in previous papers. Silver nanoparticle immobilization was addressed in two different ways:

- Surface silver nanoparticles immobilization
- Occluded silver nanoparticles inside the polymer

The PDMS film is prepared from a solution (30% PDMS, 70% heptane, mass/volume). This solution was applied over a piece of glass, evaporating the heptane for 25 min at room temperature and curing the PDMS film at a temperature of 100°C.

2.1. Chemicals

Poly-dimethylsiloxane (PDMS) Nusil Silicone technology's product (MED-4860), silver nitrate (99.99%, Sigma-Aldrich), disodium ethylenediaminetetra-acetate (EDTA-2Na) ACS reagent (99.4%, powder from Sigma-Aldrich), d-glucose monohydrate (USP grade, Sigma-Aldrich), sodium hydroxide (ACS reagent, $\geq 97.0\%$, pellets, Sigma-Aldrich), ammonia water solution (ACS reagent, 28.0–30.0%, Sigma-Aldrich), anhydrous heptane (99%, Sigma-Aldrich), and anhydrous sodium sulfite ($\geq 98\%$, Sigma-Aldrich).

2.2. Nano-film coating

Silver nitrate (AgNO_3) was dissolved in a minimum amount of DI-water, later ammonia water solution was added, and the chemical mixture produced a silver ammoniacal complex. The silver concentration was adjusted to 0.0135 M. Next, ethylenediaminetetraacetic disodium salt solution was prepared, and this solution was added to the silver solution, molar ratio 1:1 respect to AgNO_3 . Next the porous silicone matrices or PDMS film was combined with the silver solution, and mixed. Then, d-glucose solution was prepared and added to the mixture, and heated to reach at a temperature of 50°C for 1 h. The molar ratio from silver nitrate to d-glucose monohydrate was 1:5, final pH = 9.0.

For more details on preparation of a porous PDMS matrix, silver immobilization, and PDMS's film see previous papers.

2.3. Occluded silver nanoparticles inside the polymer

The AgNO_3 was dissolved in DI-water, added to the PDMS solution (30% PDMS, 70% heptane) mass/volume, placed in a magnetic mixer hot plate, mixed and heated from 35 to 45°C. Heptane was added to maintain the volume.

Later the solution was applied over a piece of glass, the heptane was evaporated for 25 min at room temperature and the PDMS film was cured at a temperature of 100°C.

2.4. Characterization method

2.4.1. Optical inspection

An optical microscope, Smart Scope Flash 200, model CNC200, serial SVW2003849 was used to perform a visual inspection. Smart Scope Flash is an automatic dimensional piece of equipment, with a measurement system, and optical metrology.

2.4.2. Scanning electron microscope (SEM)

SEM images were obtained, using a JEOL JSM-6390LV Scanning Electron Microscope, pores and particle size were verified.

2.4.3. Wettability test

A contact angle goniometer was used to measure PDMS's wettability, some film samples with and without silver immobilized nanoparticles were used to measure the wettability. The silicone film was mixed with the silver solution as described in Section 2.3. Then the contact angle was measured.

2.4.4. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image and observe small components such as metallic nanoparticles.

2.4.5. Fourier transform infrared (FTIR)

FTIR was used to show some changes in the functional groups on the silicone surface.

3. Results

The PDMS can be oxidized to produce silanol. Silanol is a functional group with the bond $\text{Si}-\text{O}-\text{H}$, as the functional group ($\text{C}-\text{O}-\text{H}$) found in alcohols.

Silanol compounds are more acidic than corresponding organic alcohols. This behavior contrasts with its electronegative property. Silicone is less electronegative than carbon (1.90 vs. 2.55), Et_3SiOH 's pKa is 13.6 vs. 19 for tert-butyl alcohol. Because of their greater acidity, silanol can be fully deprotonated in an aqueous solution [27]. Also this group $\text{Si}-\text{O}-\text{H}$ gives a little polar characteristic to the polymer that contributes to the surface energy and improves the wettability.

Poly-dimethylsiloxane (PDMS) product, from Nusil Silicone technology (MED-4860), can be oxidized with hydrochloric acid and sodium hydroxide solution treatment (etching).

Figure 4 shows PDMS ATR FTIR spectrum from the elastomer without treatment and **Figure 5** shows PDMS ATR FTIR spectrum after the etching process and silver immobilization.

PDMS spectrums, **Figures 4** and **5** show the symmetric and asymmetric stretching peaks of the methyl groups at 2963 and 2906 cm^{-1} , along with the deformation vibration peak of this group, too, at 1412 and 1258 cm^{-1} . Also, the Si—O—Si asymmetric stretching peaks are present, between 930 and 1200 cm^{-1} , and the Si—C vibrations and methyl rocking peak at around 800 cm^{-1} .

Comparing **Figure 4** to **Figure 5**, a weak broad peak approximately from 3200 to 3700 cm^{-1} is present only in **Figure 5**, and it corresponds to the —OH functional group present on the PDMS surface representing Si—OH bonding after the oxidation process (etching). Also, the characteristic peaks shown by the presence of methyl (—CH₃) functional group in **Figure 5** show a gradual decrease in intensity.

The different and bright colors observed on the porous PDMS matrices after silver immobilization are produced by the nanoparticles' plasmon effect (the quantum of plasma oscillation produced by the vibration of noble metals, such as silver and gold, and free electrons that is the consequence of the formation of a dipole in the material due to exposition of electromagnetic

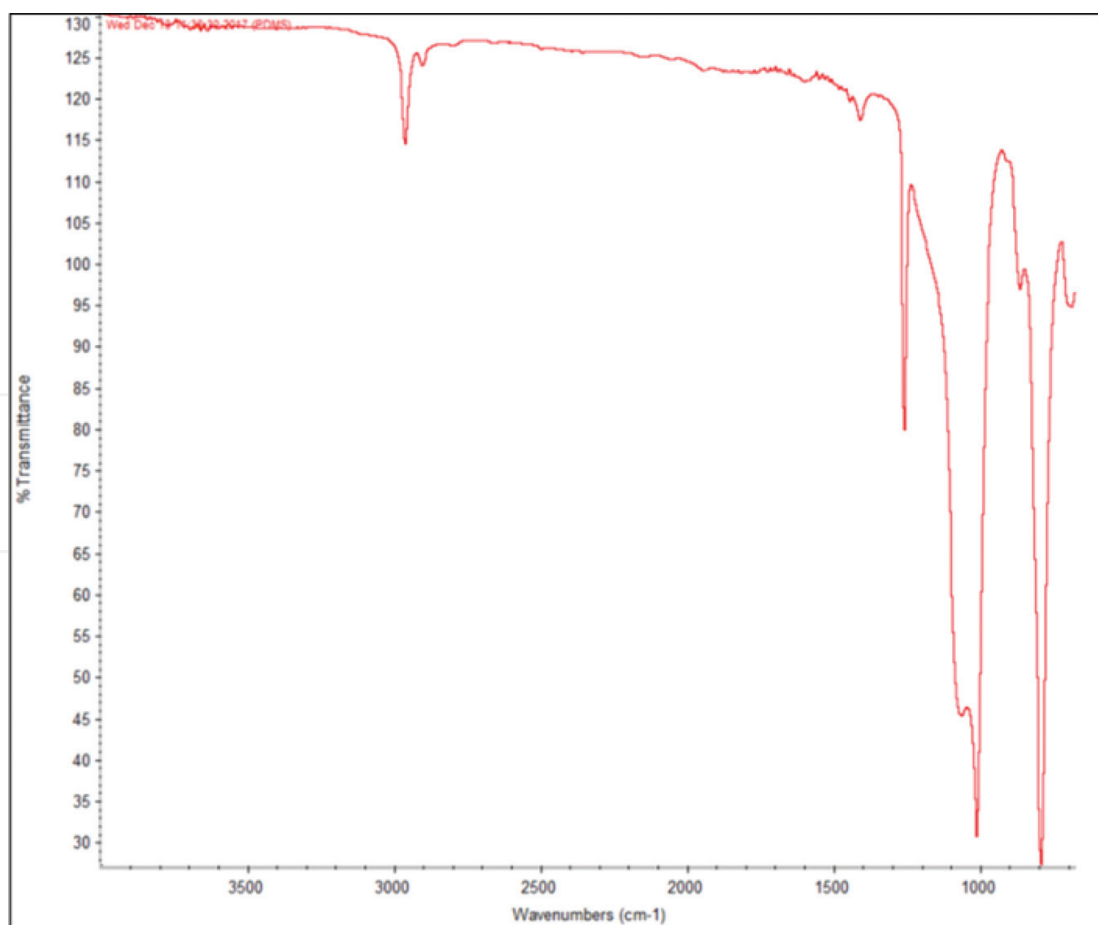


Figure 4. PDMS, ATR FTIR spectrum, sample without treatment.

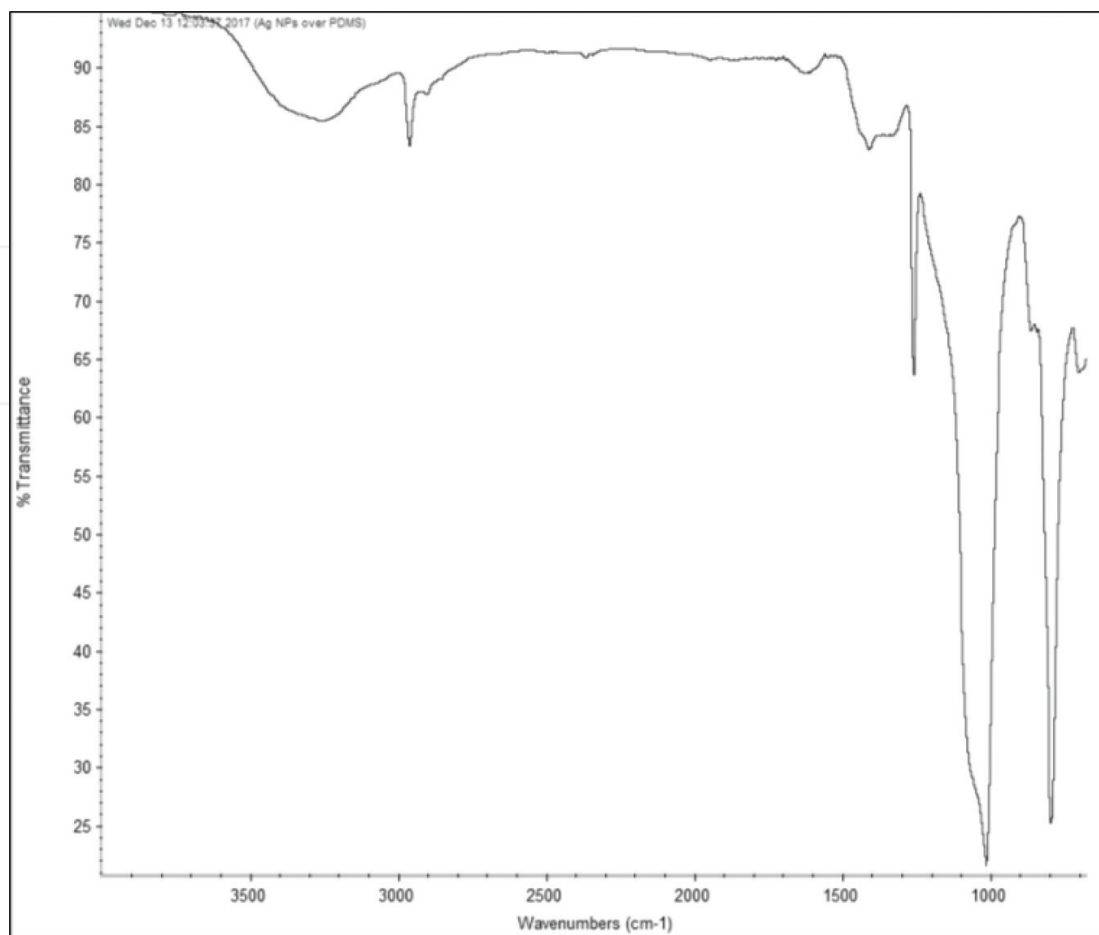


Figure 5. PDMS, ATR FTIR spectrum from a sample after the etching treatment and silver nanoparticle immobilization.

waves). Generally metallic silver nanocrystals show typical optical absorption due to their surface plasmon resonance, showing a bright color (**Figure 6**).

The silver nanoparticles' immobilization on PDMS has an important medical application improving the PDMS surface properties. Therefore, these particles potentially have antimicrobial activity toward many microbes. Along with this antimicrobial activity, silver nanoparticles show unacceptable toxic effects on human health. In addition, chronic exposure to silver causes adverse effects such as permanent bluish-gray discoloration of the skin (argyria) and eyes (argyrosis). Exposure to soluble silver compounds may produce other toxic effects. Some of these effects are liver and kidney damage, eyes, skin, respiratory and intestinal tracts irritation, and changes to blood cells [28].

Despite the toxicity data, silver nanoparticles have many medical applications. During recent years outbreaks of re-emerging and emerging infectious diseases have been a significant burden on the global economy and public health. Population and urbanization growth, poor water quality and lack of environmental hygiene are the main reasons for increased outbreak of infectious pathogens. Comprehensive treatments using advanced disinfectant nanomaterials have been proposed for prevention of such outbreaks. Among these nanomaterials, silver nanoparticles (Ag-NPs) with unique properties of high antimicrobial activity have attracted much interest from scientists and technologists for the development of nanosilver-based products [29].

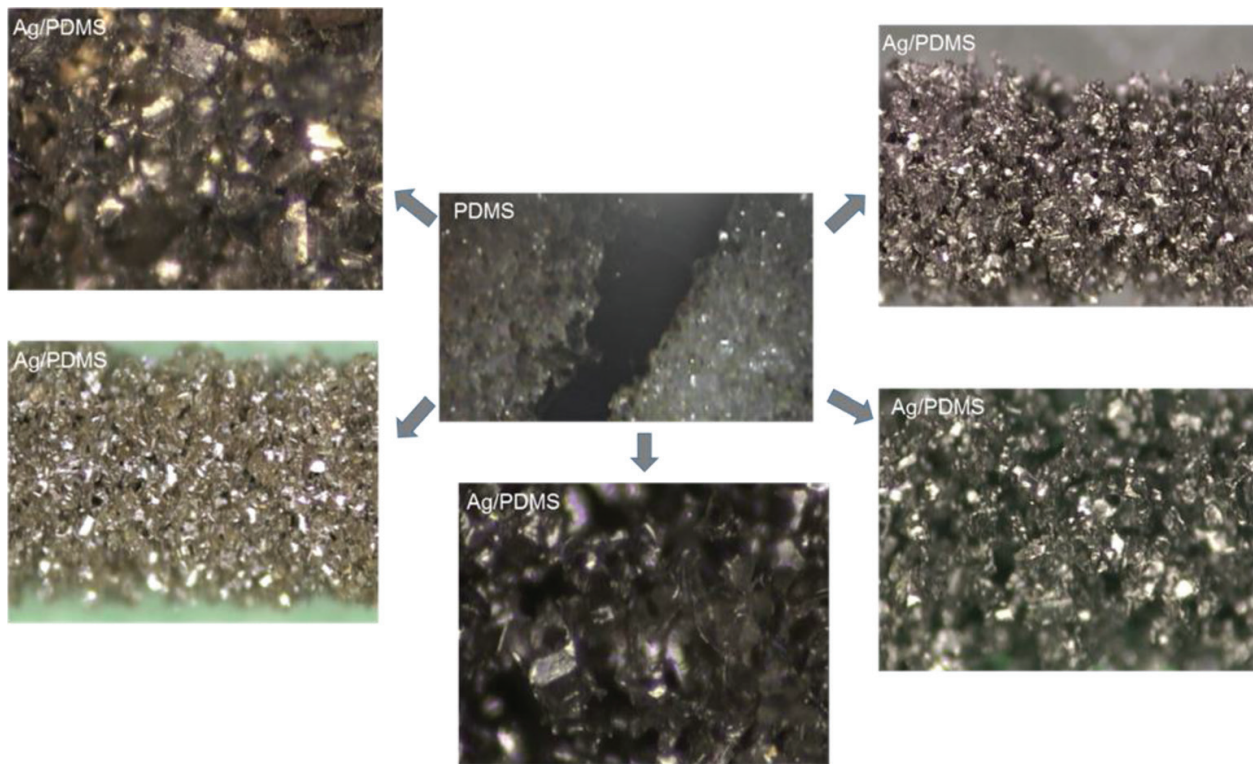


Figure 6. PDMS before and after silver nanoparticle immobilization, optical micrograph from different positions.

Silver nanoparticles have been demonstrated to be an effective biocide with a broad-spectrum, including Gram-negative and Gram-positive bacteria, in which there are many highly pathogenic bacterial strains [30, 31].

Silver nanoparticles biocide activity is based on three mechanisms:

1. Nanoparticles can attach to the surface of the cell membrane and drastically disturb its proper functions, such as permeability and respiration.
2. Nanoparticles are able to penetrate inside the bacteria and cause further damage by interacting with sulfur and phosphorus containing compounds.
3. Silver nanoparticles release silver ions. Silver ions are predominantly responsible of silver nanoparticles' bactericidal activity. Silver ions can kill bacteria cells [32].

Silver nanoparticles' toxic responses are related to their chemical characteristics and their aggregation; their toxicity depends on their composition [33]. There are mechanisms devised to nullify any toxicity caused by silver nanoparticles to humans and the environment so that their unique properties can be used to increase human quality of life without any negative effects [34].

Antimicrobial materials with immobilized or occluded silver nanoparticles are of considerable interest because those applications avoid all issues associated with the negative impact from the nanoparticles' toxicity. There is a significant debate on the mode of bactericidal action of silver nanoparticles. Both contact killing and/or ion-mediated killing have been proposed. Contact killing is the predominant bactericidal mechanism when silver nanoparticles are immobilized in or on a substrate, and these show great efficacy. Silver-silica-based hybrid

nanostructures are becoming more and more common as silica surface satisfies different functionalities for silver nanoparticles' immobilization, which makes possible the nanostructure surface modification. The silica's surface terminates in siloxane groups (—Si—O—Si—) with oxygen atoms on its surface as silanol groups (—Si—OH) that make the silver nanoparticles' immobilization possible. The antibacterial surface was found to be extremely stable in an aqueous medium; no significant leaching was observed. Thus, immobilization of silver nanoparticles on a silica surface may promote reuse, reduce environmental risks associated with leaching of AgNPs and also enhance cost-effectiveness [35].

PDMS as was mentioned has (—Si—O—Si—) too and it can perform a chemical reaction with different compounds to produce (—Si—OH) groups, the same functional group used on the silver nanoparticle immobilization over silica. The research consists of silver nanoparticle immobilization in PDMS. In this application, silver ion release decreases because silver nanoparticles are not free, and their big surface area is not totally exposed based on the silver's interaction with the oxygen groups (silver immobilization over PDMS surface). This effect is reflected by the non-toxicity of this nanostructure as reported in previous papers.

As shown in **Figure 7**, silver nanoparticles were immobilized over the PDMS surface and some of them formed clusters near to the pores. Also, the EDX spectrum shows the presence of silver, **Figure 8**.

When silver nitrate solution is mixed with the PDMS solution, the silver ions are reduced to silver nanoparticles and the PDMS is oxidized to silanol. This reaction is illustrated by the color change in the mixture, **Figure 9**. Later the solvent evaporated, and the silver nanoparticles were occluded inside the polymer structure, **Figure 10**.

Figure 11, as in **Figures 4** and **5**, demonstrates strong characteristic peaks from the PDMS, the methyl symmetric and asymmetric stretching respectively, and the deformation vibration of the same group, the Si—O—Si asymmetric stretching, Si—C vibrations and methyl rocking. Also, as in **Figure 5**, the spectrum shows a weak broad flat peak ranging from approximately 3200 to 3700 cm^{-1} that correlates to Si—OH bonding after the redox chemical reaction between the silver ions and the PDMS. On this spectrum (**Figure 7**) the characteristic peaks from methyl (—CH_3) functional groups also show a decrease in intensity.

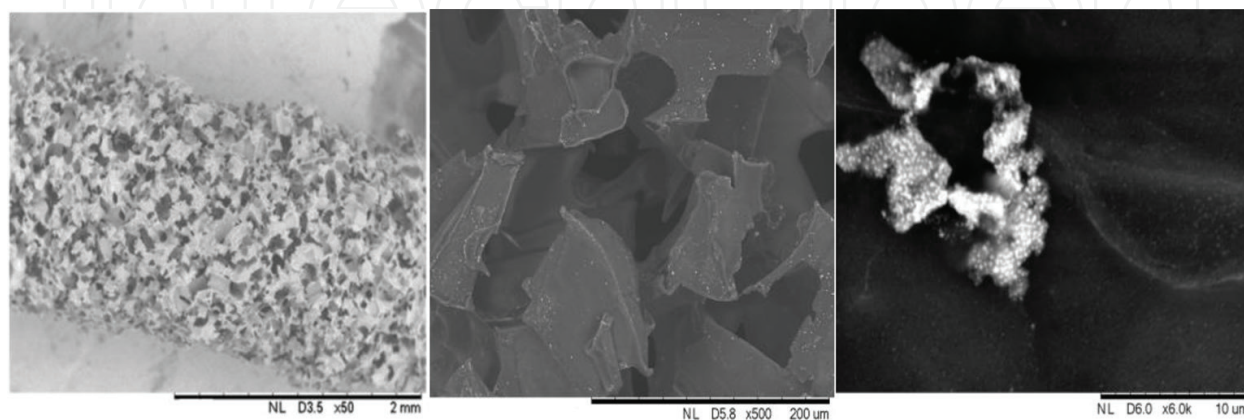


Figure 7. Silver nanoparticles immobilized on PDMS, SEM micrograph. (A) $\times 50$, (B) $\times 500$, (C) $\times 6000$.

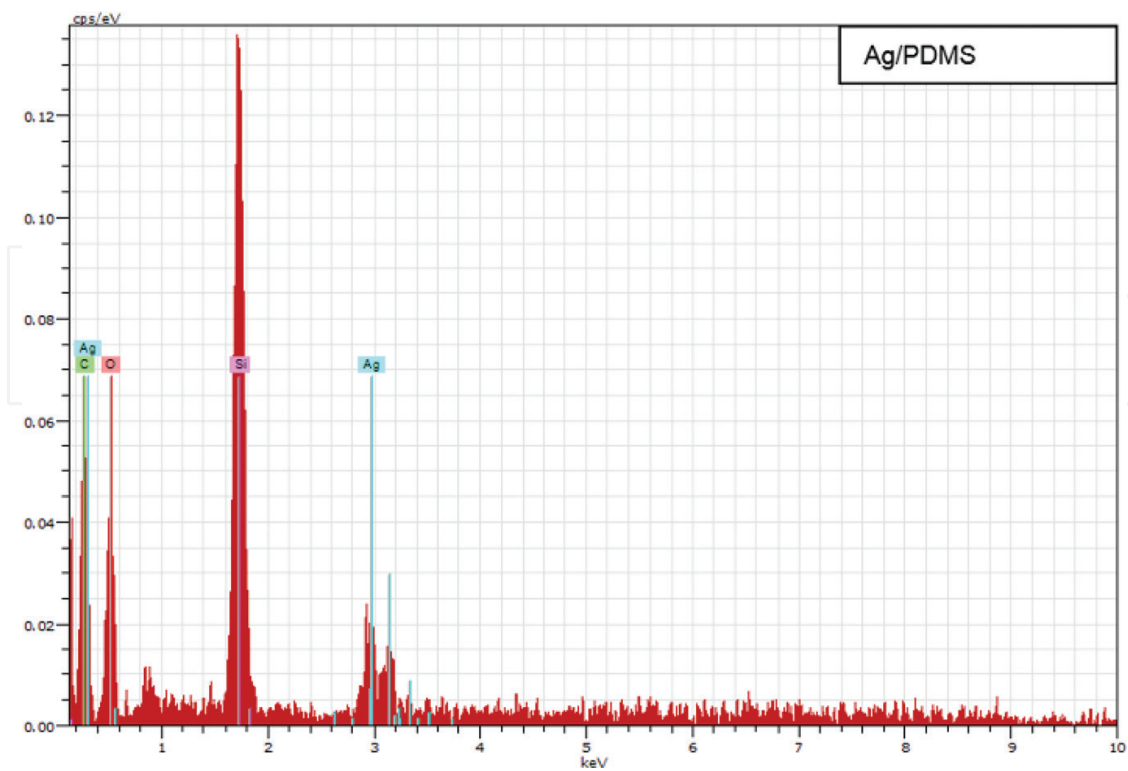


Figure 8. Silver nanoparticles immobilized on PDMS, Energy-dispersive X-ray spectroscopy (EDS) spectrum.

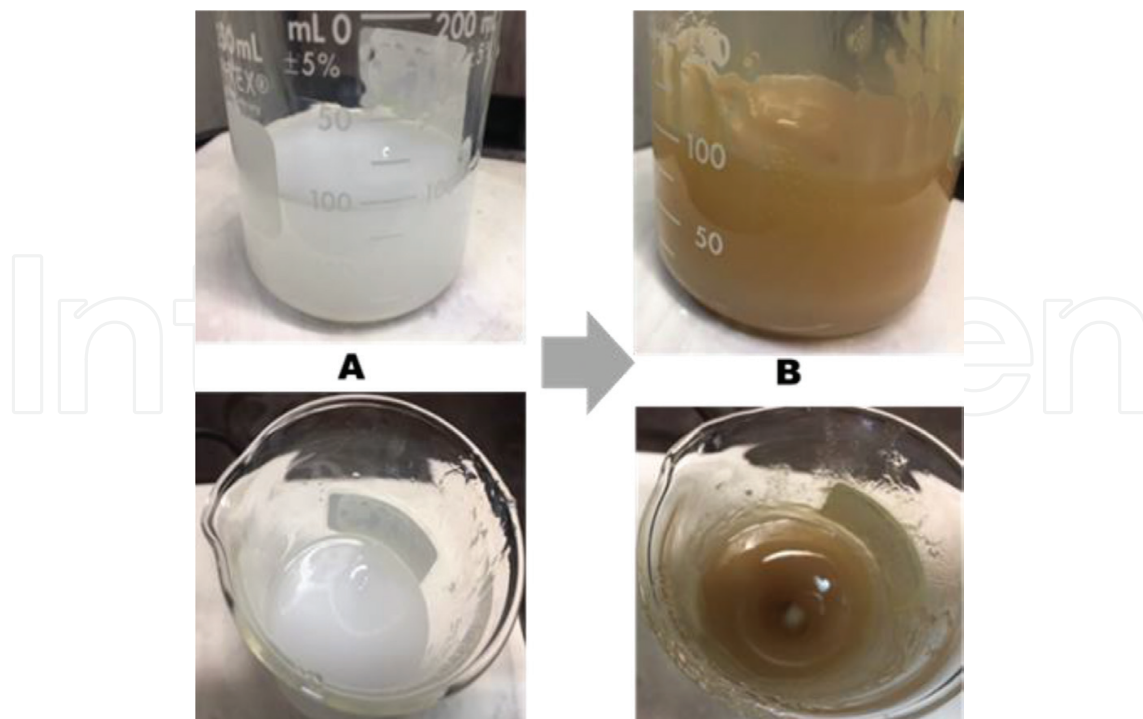


Figure 9. Silver nanoparticles synthesized inside PDMS' dispersion: (A) PDMS heptane solution and (B) PDMS heptane solution and silver nanoparticles synthesized inside.

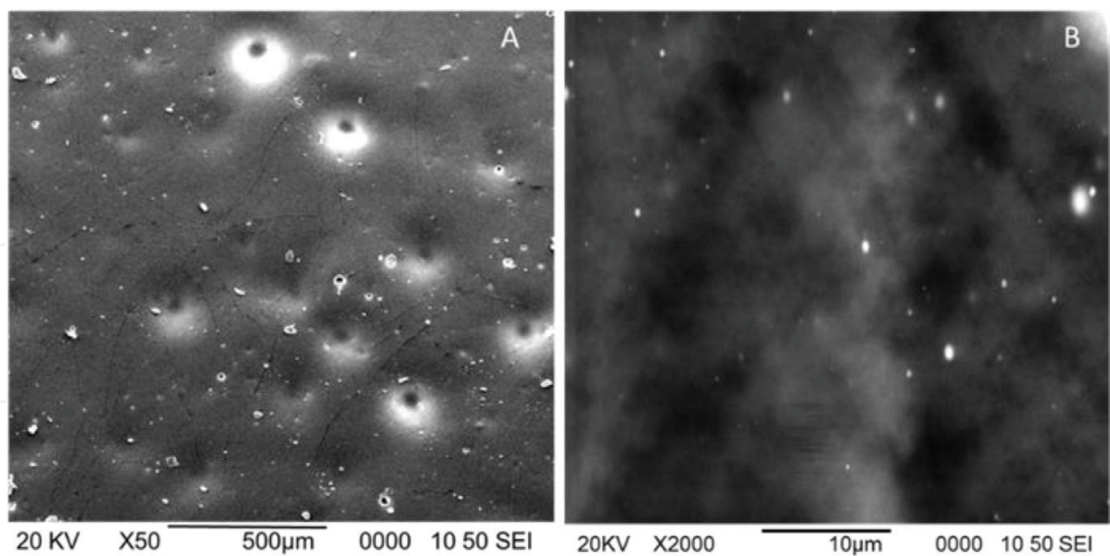


Figure 10. Silver nanoparticles occluded inside PDMS' film, SEM micrograph: (A) ×50 and (B) ×2000.

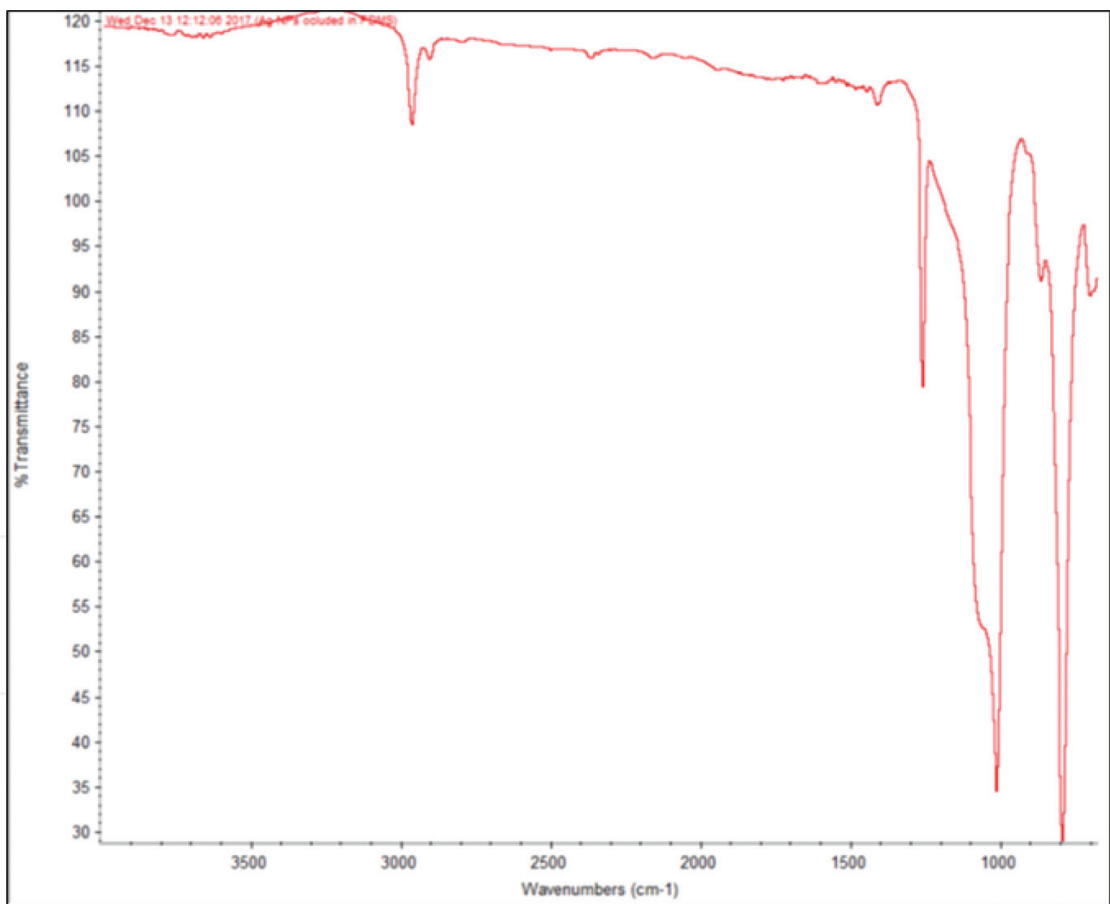


Figure 11. PDMS, ATR FTIR spectrum from a sample with occluded silver nanoparticles.

PDMS material in general comprises of repeated units of $\text{—O—Si(CH}_3\text{)}_2\text{—}$, which on exposure to oxygen plasma or corona treatment develops silanol groups (—OH) at the expense of methyl groups (—CH_3). The surface oxidation layer increases the concentration of hydroxyl groups. As the

hydroxyl groups are polar, they turn the hydrophobic exposed PDMS surface to a hydrophilic surface [36]. This effect can be shown by the contact angle change of deionized water or the changes in ATR FTIR spectrum peaks; the OH peak appears and the methyl peaks' intensity is reduced.

The mix of strong acids like H_2SO_4 and HNO_3 in an H_2O solution can induce the PDMS oxidation. Wet surface chemical oxidation produces a rough oxidase surface [37]. A different wet surface chemical oxidation (etching) used to treat the PDMS is piranha solution, followed by a dip in KOH solution. The piranha solution has hydrogen peroxide (H_2O_2) and sulfuric acid (H_2SO_4) in 1:1 ratio. The above-mentioned PDMS surface activation processes involve cleavage of the nonpolar hydrophobic methyl ($-CH_3$) group of the siloxane polymer chain and oxidation of the cleaved sites to polar hydrophilic silanol ($Si-OH$). The result is the increase of the polymer surface energy, thereby rendering it wettable [38].

The silver nanoparticles immobilized over the surface or occluded on PDMS film (**Figures 10 and 12**) were visualized with a SEM and TEM micrograph. The TEM micrograph shows an agglomerated or cluster when the nanoparticles are immobilized over the surface, **Figure 12A**. When these nanoparticles are occluded, these are more spread out, **Figure 12B**.

PDMS film samples with immobilized and occluded silver nanoparticles were tested to check their wettability and compare them with pure PDMS film, see **Figure 13**.

The obtained results are:

- Water drop contact angle measured of the pure PDMS sample 107°
- PDMS with immobilized silver nanoparticles over their surface 61°
- PDMS with occluded silver nanoparticles 76°

The data obtained reflect the different wettability (surface energy) between the samples. Pure PDMS' wettability is lower than occluded silver nanoparticles in PDMS' wettability, but silver

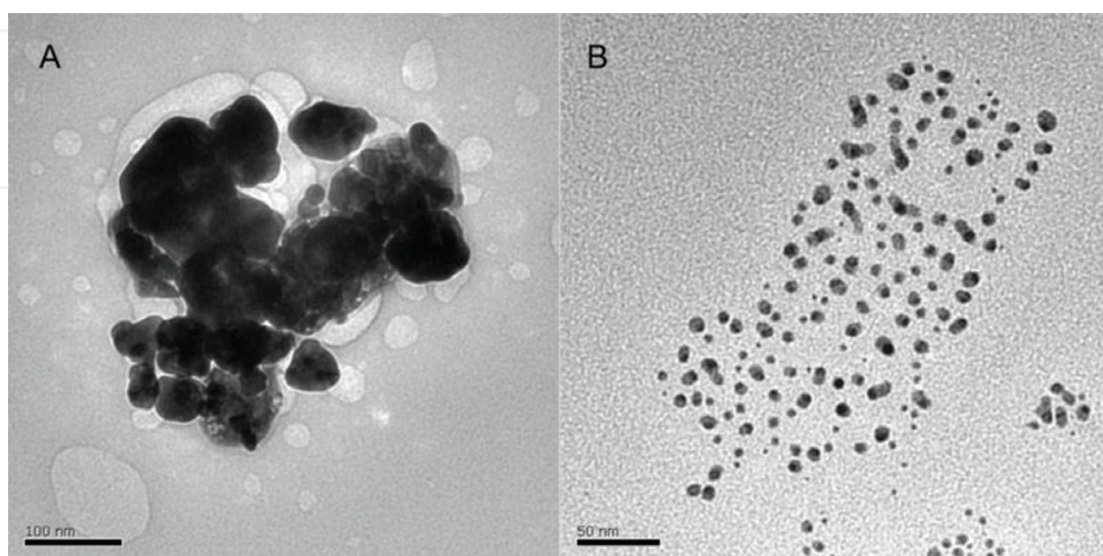


Figure 12. TEM micrograph, silver nanoparticles on PDMS. (A) Immobilized over the surface and (B) occluded.

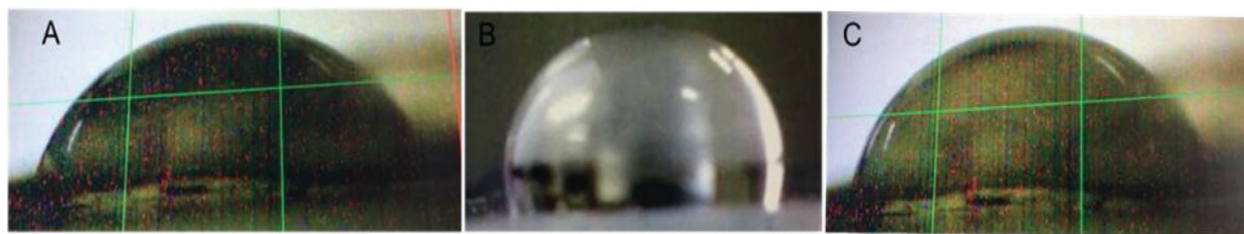


Figure 13. Water drop placed on different PDMS hybrid structures. (A) Silver nanoparticles immobilized over PDMS surface, (B) pure PDMS, and (C) occluded silver nanoparticles inside PDMS.

nanoparticles immobilized over PDMS surface's wettability is stronger than occluded silver nanoparticles in PDMS' wettability. These data demonstrate how the silver nanoparticles turn the hydrophobic PDMS surface into a hydrophilic surface. Also the decrease in the water drop contact angle coincides with the results obtained in **Figures 4, 5** and **7**. **Figure 5** shows stronger Si—OH peak than **Figure 11**. Si—OH peak intensity is related to wettability properties, because silanol groups contribute to the wettability too, just as the silver nanoparticles.

4. Conclusion

The PDMS chain, although it has oxygen groups (Si—O—Si) on its structure, is hydrophobic, because the methyl groups (—CH₃) bond to the chain are non-polar compounds with hydrophobic properties.

PDMS oxidation can turn a hydrophobic surface into a hydrophilic surface, because in this process the methyl group is changed by the hydroxyl group. The hydroxyl group is polar and gives hydrophilic characteristics to the surface, so then surface energy increases.

Hydrophilic PDMS can be addressed with a special treatment such as plasma, corona or other treatments, but it can also be done with wet chemical treatment (etching). In this process the strong acids following the alkaline solution produce the PDMS oxidation, as is shown in these research data. This etching process permits the silver nanoparticle immobilization on a PDMS surface, and wettability increases. Also, the silver nitrate can react directly with a PDMS/heptane solution to produce a PDMS nanostructure with occluded silver nanoparticles. During the process a little PDMS oxidation is produced and it improves PDMS wettability, too.

Production of PDMS' matrices with silver nanoparticles to form hybrid nanostructures, in addition to giving bactericidal properties to the surface, fosters cellular growing, associated with wettability.

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