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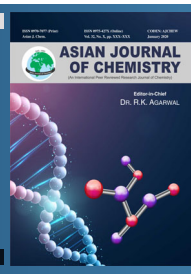

# Preparation and Characterization of PMMA-AgNPs Polymer Composite as a Dental Prosthesis

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Silver nanoparticles (AgNPs) have been used for centuries in the field of medicine due to the antimicrobial properties. AgNPs has been synthesized and incorporated in different aspects of biomaterials. It is reported that AgNPs as a result of its small size, it provides sufficient antimicrobial effect at lower filler level, thus can be used in dentistry for prevention and reduction of biofilm formation on a surfaces of dental prosthesis. The purpose of this study is to develop AgNPs antimicrobial acrylic resin for dental prosthesis. The effect of AgNPs incorporated into acrylic resin poly methyl methacrylate (PMMA) on the bacterial biofilm was studied in terms of bacterial growth and the incorporating effect on the thermal stability of these polymeric biocides was evaluated. Silver nanoparticles in colloidal form was added to PMMA(ONDA-CRYL) using microwave and make four dental prosthesis at the different concentration. The specimens were delivered to the four toothless patients for 21 days. The formed biofilm was tested for microbiological study (taxonomic profile). After setting, the specimens were characterized to determine the spatial distribution of AgNPs on the PMMA matrix through scanning electron microscope and the thermal stability was examined using TGA and DSC. The modified PMMA prosthesis base containing AgNPs, which exhibited good *in vivo* antimicrobial properties without altering their thermal properties of degradation as well as their mechanical properties and minimize the maximum infectious signs by reducing the formation of microbial biofilm forming on the surfaces of dental prostheses. As the modification of PMMA with AgNPs improved the anti-biofilm properties without altering its mechanical and thermals properties to the degradation, it could be used as a dental prosthesis.

**Keywords: Silver nanoparticls, Biofilm, Polymere biocid, Microwave incorporation, Dental prosthesis.**

## INTRODUCTION

The major objective of a dentist is to protect the oral cavity, which is the gateway to the whole body. Fungal and bacterial biofilms are known to cause public health problems. Majority of the oral diseases are caused by dental plaque [1]. Nanoparticles have become useful tools for various dental applications in endodontic, periodontics, restorative dentistry, orthodontics, and oral cancers. Among them, silver nanoparticles (AgNPs) because of its antimicrobial and restorative properties of the oral mucosa have been used in medicine and dentistry [2]. To prevent or reduce biofilm formation, AgNPs have been incorporated into biomaterials. Because of small particle size, they have excellent antimicrobial action without affecting the mechanical properties of the materials. This unique property of AgNPs makes them appropriate as prime fillers in different biomaterials in which they play a vital role in enhancing properties [3-5].

Currently, a synthetic resin used in dentistry is based on acrylic resin poly methyl methacrylate (PMMA). Hence, this study shows that there are no other materials that have been found to match the appearance of the oral soft tissue and have the same high fidelity as acrylic resins [6]. Due to its satisfactory overall performance, it is widely used in the construction of full dentures. However, many researchers have demonstrated that PMMA can serve as a repository for many microorganisms and may support the formation of biofilms [7]. By colonizing the prosthesis with various microorganisms, the insertion of dental prosthesis causes a drastic change in the oral environment. It isolates the underlying mucosa from the mechanical cleaning of the tongue and the free flow of saliva [8]. In addition, the porous surface of PMMA and the irregularities on the anatomical surface of prosthesis contribute to the accumulation of microorganisms. This mainly causes problems with denture stomatitis or candidiasis [9].

With the advent of nanotechnology, silver nanoparticles (AgNPs) have been synthesized and shown a potent antimicrobial properties [10]. Silver nanoparticles (AgNPs) have demonstrated unique interactions with bacteria and fungi species [11]; thereafter, are widely used in medical area, such as in wound sutures [12], endotracheal tubes [13], surgical instruments [14], and bone prostheses [15].

The antimicrobial mechanism of AgNPs has been extensively investigated but it remains unclear [16,17]. It seems that silver ions interact with the peptidoglycan cell wall [18] causing structural changes, increased membrane permeability and finally, cell death [19]. Further, AgNPs could interact with the exposed sulfhydryl groups in bacterial proteins, avoiding DNA replication [19].

## EXPERIMENTAL

A series of solutions of $AgNO_3$ were prepared using 0.036, 0.05 and 0.0964 g of silver nitrate dissolved in 50 mL of demineralized water separately and the solution was stirred with magnetic stirrer for 8 min. Similarly, 0.0194 g of $KBH_4$ was also dissolved in 150 mL of demineralized water for 8 min in a magnetic stirrer. In same way, 1 g of polyvinyl pyrrolidone (PVP) was dissolved in 33 mL of demineralized water for 8 min using a magnetic stirrer. Now, 30 mL of $KBH_4$ solution was placed in an ice bath for 20 min and stirred while the temperature of the solution was checked not to exceed beyond 2 ºC. Then, 3 mL of $AgNO_3$ was thus added a drop/second after that 2 drops of PVP (3%) was added at 1 °C. The characterization of silver nanoparticles solutions was done using X-ray diffraction and UV-Vis spectrophotometer. After the incorporation of silver AgNPs into PMMA, an obtained biofilm was characterized by TGA, DSC and SEM.

During patient appointments, the biofilm that has been formed on the surfaces of the dental prosthesis was sampled for the four patients who have acrylic resin-based prostheses modified by the addition of AgNPs with different concentrations (0% control sample, S1, S2 and S3). Then bacterial culture was made using the following protocol:

Firstly, a sample was taken from the dental wall using sterile stick or cotton swab. Sterilize the test tube containing distilled water by passing it near the flame and place the sample inside, the test tube was closed and the solution was shaken. Pipette was used to measure the solution and few amount was dropped on the agar. The steps were repeated 4 to 6 times for each agar. The petri dishes were placed in an oven for 5 days at a constant temperature of 37 ºC [20].

**Incorporation of silver nanoparticles in acrylic resin:** The preparation of resin consists of a rapid one-minute mixing, with a spatula, in a glass container, containing polymethyl methacrylate powder with methyl methacrylate monomer liquid. AgNPs were incorporated through adding a monomer, usually 2-(*tert*-butylamino)ethyl methacrylate, in order to improve silver salt solubility in the resin solution. For the incorporation of silver nanoparticles into polymethyl methacrylate resin, 3 mL of methyl methacrylate monomer and 1 µg/mL of AgNPs were mixed with a magnetic stirrer for 2 min in a clean glass under agitation of magnetic stirrer until the saturation of the monomer and controlled by the movement of the barraux magnetic, which indicates that the solution is saturated by the slowing down of the agitation *i.e.* the content appeared as a paste.

This study used the same protocol for different concentration of colloidal silver solutions (S1, S2 and S3) then put to the application in the plastic micro-wave muffle which contains the negative dental prosthesis and after the PMMA-silver nanoparticles biofilms reaches the plastic phase, it was charged to 500 W for 3 min. The muffle was opened and loosen the prosthesis and finish with polishing to prevent injury to the oral mucosa of patient.

## RESULTS AND DISCUSSION

Silver is a benign bactericidal metal as it is non-toxic to animal cells although it is very toxic to bacteria [21]. It has appropriate antibacterial activity *i.e.* improved when transformed to nanoparticles throughincreasing their surface-to-volume ratio [22]. For this study, silver nanoparticles with increasing concentrations S1, S2 and S3 were incorporated into PMMA, the bacterial density values disclosed that the bacterial colonies decreased with increasing AgNPs concentration.

**SEM analysis:** The scanning electron micrographs (SEM) of the biofilm samples had a compact and homogeneous structure without any sign of pores. The topography in cross-section is visualized in Fig. 1. The section appeared smooth but had cracks and roughness distributed along the thickness of film.

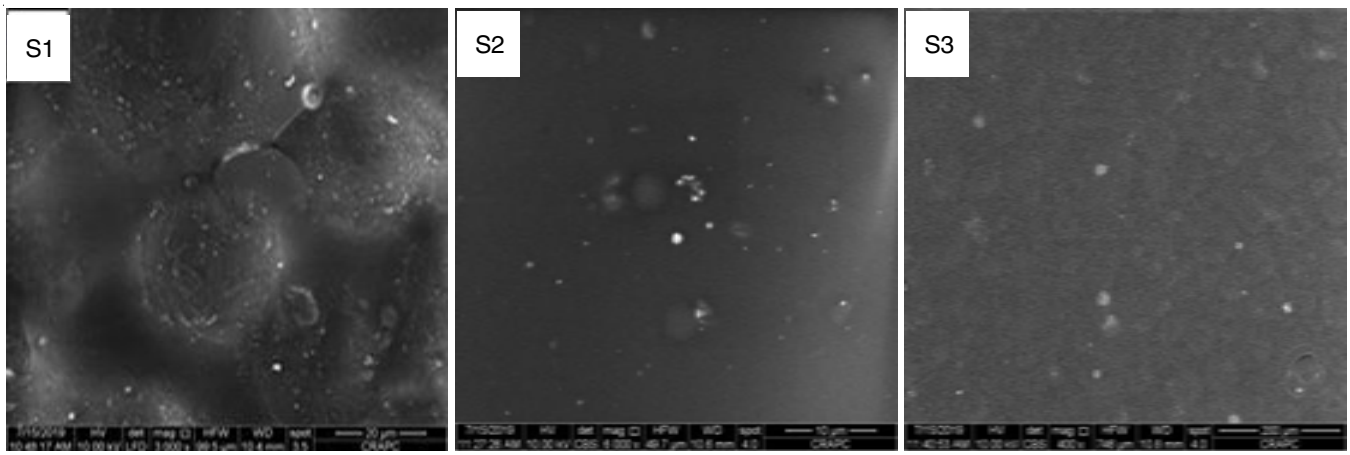


Fig. 1. Scanning electron microscope of AgNPs-PMMA

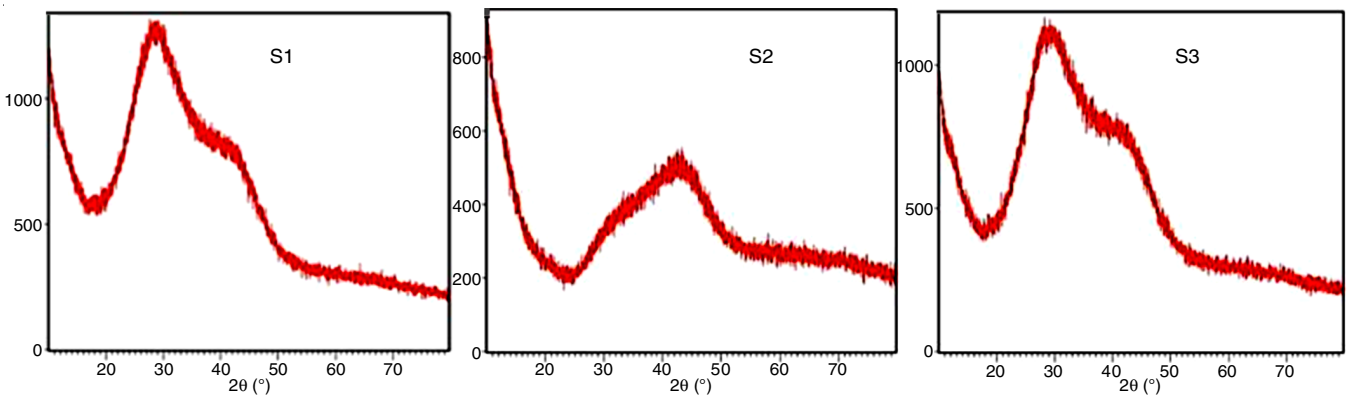


Fig. 2. X-ray diffraction analysis of AgNPs solutions

This behaviour could be resulted due to the lack of interactions that stabilize the structure. The samples consisted of highly polydisperse particles in size and very small particles were comparable to an illuminating point appeared in all the samples [4].

The SEM results were therefore confirmed by scanning electron microscopy (SEM) that a variation in UV irradiation time during the preparation of silver nanoparticles in PMMA results in a change in shapes and morphologies of synthesized AgNPs. It should be noted that almost spherical and cubic particles were more or less presented in all the samples, which is at the origin of the appearance of surface plasmon resonance bands of these particles at 419 nm [9].

**XRD analysis:** X-ray diffraction analysis was used to characterize the AgNPs after centrifugation of the solutions S1, S2 and S3 of the protocol using $KBH_4$ as a reducing agent. PANalyticalX'Pert Pro MPD Diffractometer was used to analyze the samples. The X-ray diffraction spectra of AgNPs of three samples are shown in Fig. 2. On the three spectra, it was noted that there is a presence of two diffraction peaks at the angular positions of 38.11° and 44.7°, respectively. These peaks were at (111) and (200) of the cubic structure silver. These results confirmed the formation of AgNPs in colloidal suspension in three samples. A Gaussian fit of the diffraction peaks was used to determine the width at half height (w) of peak and estimate the size of crystallites. The average size of nanoparticles was 32.6 nm. Similarly, the presence of face-centered cubic crystal structure of the AgNPs was confirmed by the diffraction peaks at $2\theta = 38.11$ and 44.7° attributed to the (111) and (200) planes, respectively. The characteristic peak width ofAgNPs confirmed the formation of nanoscale particles [1].

**UV-Vis spectrophotometer analysis:** The samples were characterized using a UV-Vis spectrophotometer (Specord 210 Plus) and the absorption graphs of each concentration were plotted. Fig. 3 represents an absorption spectrum of the different solutions prepared, it is noted that, there are three curves having a peak at 494 nm (UV). It is therefore interesting to note a slight shift to the right of the order of 3 nm, between the peaks of three curves. This is explained by the increasing number of AgNPs (final solution), which evolves proportionally to the concentration of the starting solutions. So, a direct impact on the solution/light interaction. The higher the concentration, the higher the absorbance. The absorbance of nanoparticles evolves towards blue [7].

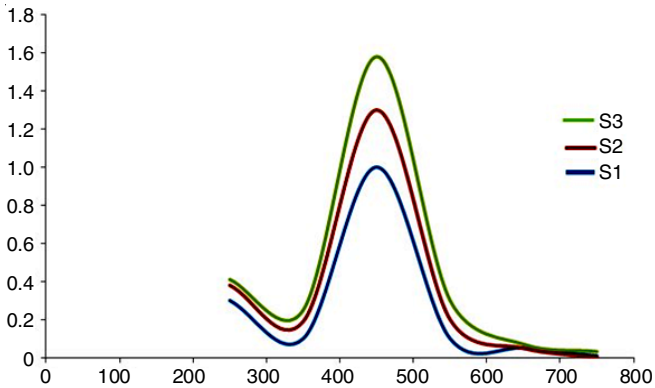


Fig. 3. Absorption spectrum (UV-VIS) of AgNPs solutions

The analysis were also carried out after one month of preparation of the solutions in order to study the stability of the prepared nanoparticles. All solutions have a broad absorption band from 300 to 500 nm (Fig. 3). The maximum and the rate of these spectra depend on the volume of the added precursor. There was a shift in the maximum optical absorption recorded at long wavelengths with an increase in the volume of $AgNO_3$ solution. This result indicates an increase in the size of AgNPs with the increase volume of $AgNO_3$ solution, which means that a large amount of the stabilizer promotes the formation of small nanoparticles. It was also noted that the nanoparticles prepared with an $AgNO_3/KBH_4$ ratio, the absorption bands become wider after one month. This enlargement can be explained by increasing the size distribution [18].

**Thermal analysis:** The thermograms were obtained from the first heating ramps. In each case, at least three identical samples having the same composition were prepared and used to verify the reproducibility of the results. The glass transition temperature of the polymer was determined from the midpoint of the thermogram transition range.

The degradation patterns of PMMA-Ag nanocomposites was studied by TGA. Fig. 4 shows the results obtained for the acrylic resin containing 35 ppm of silver nanoparticles. These results were identical for 15 and 60 ppm of silver. It can be noted that whatever the material considered, the degradation of PMMA-AgNPs was always occured in two stages. In case of AgNPs resin, an offset of thermal degradation can be observed at slightly lower temperature (30 ºC). A more pronounced shift on the step corresponds to the degradation of the soft segments. This could mean a predominance of silver nanoparticles in

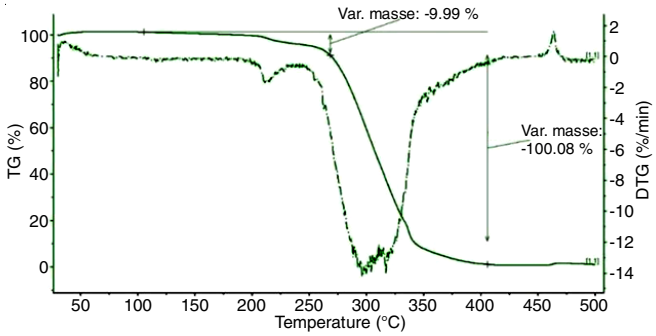


Fig. 4. Thermogravimetric analysis of AgNPs-PMMA

the flexible phase of polymer [23]. Fig. 4 also shows a thermal behaviour indicating a perfect dispersion of AgNPs within the chains of the polymethacrylate resin. Finally, in case of nanocomposite, the thermal degradation of material does not seem to be affected by the presence of silver nanoparticles.

The differential enthalpic analysis of nanocomposite containing 35 ppm of silver revealed only one thermal response to about 31 ºC corresponding to the glass transition of soft segments. The presence of silver nanoparticles does not influence the thermal properties of composite materials regardless of the silver concentration in the composite. On the other hand, the DSC analysis of the material showed the presence of a spike melting at 150 ºC (Fig. 5). It was also observed that thermograms of all the systems mount a similar behaviour, a slow increase of heat flux followed by a stronger increase, causing a passage corresponding to the vitreous transition temperature, which believes with increasing concentration of nanoparticles [12].

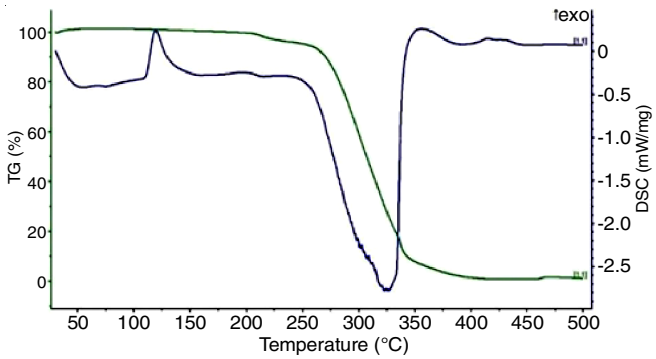


Fig. 5. Differential scanning calorimetry (DSC) of the mixture of AgNPs-PMMA

***in vitro* Bioactivity:** After bacterial culture on simple blood based nutrient gelose (a polysaccharide), we proceeded to the visual description of 4 samples taken from the biofilm formation on the patient's 4 prosthetic surfaces, after which a fresh observation was made under the microscope. The following results were obtained:

**Control sample (S0):** After culturing for 48 h in a 37 ºC plant, colour colonies varied between white and yellow and a diameter from 0.3 to 0.8 mm circular and sometimes amorphous, clusters and isolate colonies.There was presence of a strong bacterial density and the polymicrobial flora was very abundant. The proliferation of several bacterial species was variable and Gram short forms can be observed associated with *Arcuate bacilli* and possibly other very diverse forms (anaerobic bacteria). Frequent presence of small bacilli Gram-+ve or -ve were abundant. Presence of Gram-+ve and Gram-ve *cocci* and absence of bacterial mobility were noticed.

**Sample S1:** Fig. 6 shows a culture of 48 h on blood agar. After cultured for 48 h at 37 ºC, flask colour colonies varies between white and yellow of a diameter between 0.3 to 0.6 mm, with a circular and sometimes amorphous. Also there was an appearance of clusters and colonies isolated with a smooth appearance.There was presence of a strong bacterial density than in the S0 sample and the polymicrobial flora was very abundant.There was a proliferation of several bacterial species that can be observed associated with *Arcuate bacilli* and possibly other very diverse forms (anaerobic bacteria).

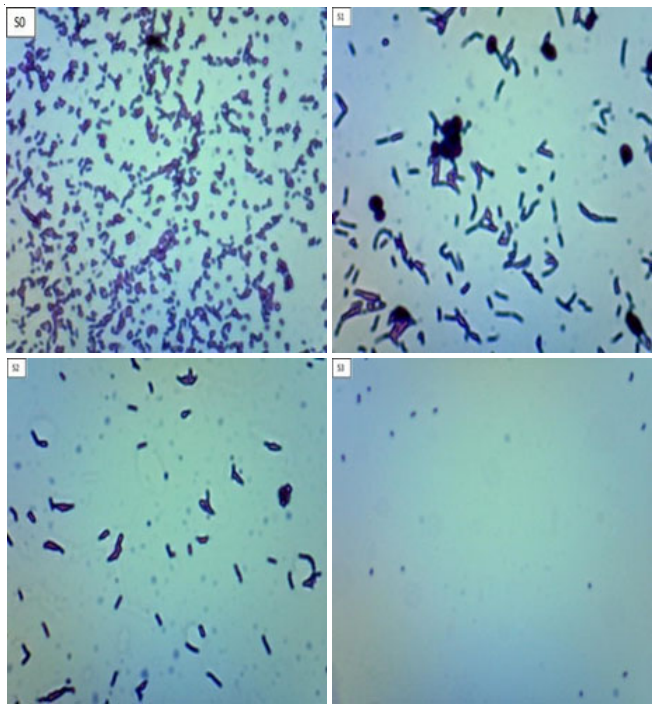


Fig. 6. Microstructural analysis of biofilm samples on the surfaces of dental prosthesis

**Sample S2:** After cultured for 48 h in a strawberry tree at 37 ºC, colonies of varying colour appeared between white and yellow with diameters ranging from 0.2 to 0.4 mm circular and sometimes amorphous and the appearance of clusters and isolated colonies. However, presence of an average bacterial density and the polymicrobial flora was scanty. The proliferation of several bacterial species such as variable Gram short forms can be observed associated with *Arcuate bacilli* and possibly other diverse forms (anaerobic bacteria).

**Sample S3:** After cultured for 48 h in a strawberry tree at 37 ºC, colonies of varying colour appeared between white and yellow with diameters ranging from 0.2 to 0.4 mm circular and sometimes amorphous, there was an appearance of clusters and isolated colonies with presence of a low bacterial density. Polymicrobial flora was less abundant and the proliferation of very few bacterial species like variable short Gram forms may be observed, possibly associated with other very diverse forms (anaerobic bacteria). It was found that in the presence of inflammation of oral mucosa, the salivary pH was acidic. An increase in pH and relatively proportional to the increase in the concen-

tration of silver nanoparticles in PMMA. The following pHs were reported as S0 control: pH acid 5; S1: pH acid 5; S2: pH acid 6; and S3: relatively neutral pH 7.

### Clinical significance

**S0 control:** Mucus of edente (severe stomatitis), presence of generalized inflammation of the palatal arch with severe erythema and burning sensation during chewing.

**S1:** Stomatitis, a scratch at the level of reflection line with a yellowish background and a painful red lacing were reported.

**S2:** Slight inflammation, a slight inflammation at the level of palatal vault with modification of colour of mucosa towards red was also observed.

**S3:** Absence of inflammation, mucosa appears clinically healthy in the absence of almost any sign of inflammation. When there was an increase in concentration of AgNPs there is practically an absence of inflammatory signs of an oral mucous membrane. Thus, when one increases the concentration at the fide and to the extent, there was a decrease of the severity of inflammation until the disappearance of inflammatory signs (Fig. 7).

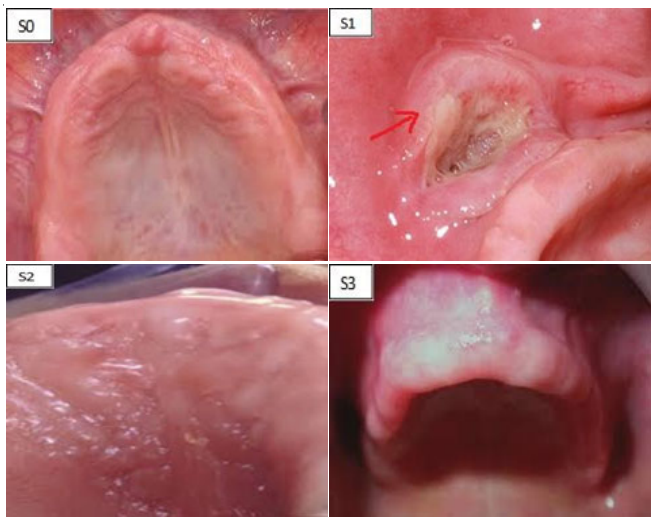


Fig. 7. Clinical significance of the effect of silver nanoparticles on oral mucosa (S0) control showing severe stomatitis, (S1) stomatitis, (S2) slight inflammation and (S3) absence of inflammation

### Conclusion

Scanning electron microscopic studies confirmed that the variation in UV irradiation time during the preparation of silver nanoparticles in PMMA results in a change in the shapes and morphologies of the synthesized silver nanoparticles. According to this study on the total toothless patient, which carry prosthesis made with a polymer resin modified by the additions of silver nanoparticles in colloidal form, it was found that no major modifications of salivary pH except in the case of control sample which is an acid pH following the inflammatory acidosis and in the case of S3 sample which contained a high concentration of silver nanoparticles, the pH was relatively neutral. An increase in pH was relatively proportional to the increase in the concentration of silver nanoparticles in PMMA.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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