ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
Index Copernicus Value: 49. 23
PubMed - National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 3, Issue – 5, October - 2018, Page No. : 148 - 157

The Antimicrobial and Mechanical Properties of Acrylic Resins with Al_2O_3 and SiO_2 Nanoparticles

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Type of Publication: Original Research paper

Conflicts of Interest: Nil

Abstract

The purpose of this study was to evaluate the effects of the incorporation of Al_2O_3 and SiO_2 nanoparticles on heat-activated acrylic resin polymerized at different times and their influence on resin's surface roughness and antimicrobial capacity against *Streptococcus mutans* and *Candida albicans*. One-hundred twenty specimens in 10 mm diameter and 2 mm thickness were divided into two groups as A (polymerized for 30 min. at 100 ºC)and B (polymerized for 8 hours at 100 ºC).Group A and B were divided to five subgroups according to type and concentration of reinforcing nanoparticles (n=12). 1:Without nanoparticles (control group), 2:%3 wt Al_2O_3,3:%5 wt Al_2O_3, 4: %3 wt SiO_2, 5: %5 wt SiO2. Antimicrobialanalysis of specimens were performed against*Streptococcus mutans and Candida albicans.* Surface roughness of specimens was measured by AFM system in non-contact mode. The data were statistically analyzed using Kolmogorov–Smirnov, Two-way ANOVA and Tukey's HSD test.The incorporation of both nanoparticles significantly affected the antimicrobial capacity against *Streptococcus Mutans and Candida Albicans*($p<0.05$). Group A specimensshowed higher bacterial and fungal accumulation than group B specimens. Group A specimens showed higher surface roughness than group B specimens. Al_2O_3 and SiO_2 nanoparticles increased surface roughness of A and B groups.

Keywords: Acrylic resin, Al_2O_3 and SiO_2 nanoparticles,Surface roughness,*Streptococcus mutans*,*Candida albicans*.

Introduction

One of the commonly used polymers in dental field is polymethylmethacrylate (PMMA), which is used as either heat polymerized or self-polymerized acrylic resin. The popularity of acrylic resin is related mainly to its ease in manipulation, ease in finishing and polishing, as well as it needs inexpensive equipment[1].The removable appliances are mostly made of poly (methyl methacrylate) (PMMA)[2].

The use of resin materials for dental treatments exacerbates the presence of plaque as they favor plaque formation. To counter and circumvent this problematic association between dental resins and dental plaque,

manystudies have focused on endowing resin-based dental materials with antibacterial properties[3].

The major problems when patients used acrylic dentures for a long time that have the accumulation of *Candida albicans* and *Streptococcus mutans* especially at the border of the dentures[4].Therefore one of the goals in acrylic dentures used should be to induce antimicrobial capability in prosthodontic and orthodontic appliances [5].

However, the long-term existence of removable orthodontic acrylic appliances with their porous surfaces in the mouth may lead to biofilm formation, with a negative effect on oral microbiota and consequently result in tooth decay, gingivitis, and periodontitis Insertion of acrylic appliances may also affect the metabolic activity and pathogenicity of the biofilm [6].The development of dental acrylic resins capable of inhibiting biofilm formation is therefore critical in the control of oral disease[7].These factors also limit the longevity of rehabilitative treatment and constitute a risk of opportunistic infections, reducing quality of life and generating additional costs to patients [8].

Four kinds of oral bacteria (*Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus gordonii*, and *Actinomyces naeslundi*) have been shown to contribute to the initial attachment and mature architectural development of biofilms. Among microbial populations, *Candida albicans*is a fungus commonly detected on acrylic resin denture surfaces [9].In the clinical application dentures with antimicrobial properties could improve the oral health of elderly patients and prevent denture stomatitis.*Candida albicans* is suggested as major causative organism for denture stomatitis, which is most common amongst complete denture wearers. The inner surface of the prosthesis is rough, and in addition to local and systemic factors contributes to the proliferation of *Candida albicans*, as well as to adherence of this pathogen in 60% of patients with removable prostheses[10]However, various other bacteria were suggested to play role in denture stomatitis, of all candida albicans has high capacity to adhere to denture base resins and form structured biofilm.There are other factors that favour the develop of oral candidiasis, such as denture base fit, metabolic disorder, patient's age, mucosa conditions, epithelial changes, poor diet, appropriate denture hygiene, xerostomia and salivary flow[11]

Nanotechnology represents the ability to image, manipulate and model functionalities on the nanometre scale. This discipline includes the study of nanoparticles, which can be classified as particles with a size no greater than 100 nm. Those particles with an antimicrobial function have received considerable attention within a range of diverse fields, including medicine and dentistry. These include spherical, cubic and needle-like nanoscaled particles (ca. 5-100 nm) and near-nanoscaled devices (up to micrometres) [12].Resistance of bacteria to bactericides and antibiotic has been increased due to the development of resistant strain. Some antimicrobial agents are extremely irritant and toxic.By the way,there is much interest in finding ways to formulate new types of safe and cost effective biocidal materials[13].

Aluminum oxide commonly referred as alumina with the chemical formula Al_2O_3. As indicated, it is a chemical compound of aluminum and oxygen with strong ionic interatomic bonding, giving rise to its desirable material characteristics. This can exist in several crystalline phases; alpha phase alumina is the strongest and the stiffest of theoxide ceramics. Its high hardness, excellent dielectric properties and good thermal properties make it the material of choice for a wide range of applications. It is also known for its excellent size and shape capabilities with high strength and stiffness too[14].Aluminum oxide nanoparticles are alsoknown to possess strong

antimicrobial properties.Alumina nanoparticles showed a mild growth-inhibitory effect on *Escherichia coli*, only at very high concentrations[13]Among compounds as inorganic carriers such as apatite, zeolite and phosphate, SiO_2 is more promising due to its porous structure and absorption properties. Nano SiO_2 particles possess extremely high surface activity and adsorb various ions and molecules[15]

The purpose of this study was to evaluate theantibiofilm activity of acrylic resins polymerized at different times containing Al_2O_3 and SiO_2 nanoparticles against *Streptococcus mutans* and *Candida albicans*which are the mainmicroorganisms associated with dental prostheses.The null hypothesis tested was that 3% wt and 5% wt Al_2O_3 and SiO_2nanoparticles incorporated into PMMA acrylic resin would decrease adherence of *Candidaalbicans*and *Streptococcus mutans* and effect the surface roughness of resins.

Materials and methods

In this study heat-cured acrylic resin(Meliodent,Hereaus Kulzer,Germany)were used. 3% wt and 5% wt Al_2O_3 and SiO_2 nanoparticles were added to acrylic resins.Firstly specimens were divided into two groups as A and Baccording to polymerization time respectively for 30 minutes and 8 hours at 100 ^oC. Then each group were divided into five subgroups(Group A1-A5) according to nanoparticle type and %weight ratio. Specimen groups were shown in Table 1.Every group consisted of twelve specimens. One-hundred twenty specimens were prepared in 10 mm diameter and 2 mm thickness. Al_2O_3 and SiO_2 nanoparticles (MKNano,Canada) were added to the monomer of acrylic resins and they were mixed for 30 min. Used acrylic resin and nanoparticles were given in Table 2.

One-hundred twenty wax specimens were prepared. Specimens were invested with dental plaster. Flasks were placed to dewax in conventional water bath. They were opened and cleaned to remove traces of wax. Specimens were prepared by hand mixing 2.2 g of PMMA powder with 1.1 mL of methyl methacrylate monomer using a powder to monomer ratio of 2:1. Hydraulic pressure was maintained for 5 minutes before placing the assembly in to boiling water. The specimens of Group A and B were conventionally molded, heat-cured under compression in 100 ^oC water respectively for 30 minutes and 8 hours. The specimens were removed from the flasks after curing.

All the specimens were grounded with 400 grit size silicon carbide paper. Before test procedure the storage of specimens in distilled water at 37 ^oC for 48 hours was carried out. 20 mL tryptic soy broth media containing *Streptococcus mutans* RSHM 676 (10^8 CFU/mL)and *Candida albicans*ATCC 10231 (10^8 CFU/mL)strains were prepared. The specimens were placed in the tryptic soy broth media and incubated for 15 minutes at room temperature. After taken out from the medium, allowed to dry 30 minutes on sterile paper in a sterile cabinet. Subsequently, the specimens were put into tube containing 2 mL sterile water and vortexed for the passage of microorganisms on the surface of specimens to the water. 0.01ml of water was taken from tubes with a micropipette and inoculated in Columbia Agar with 5% sheep blood (Biomerieux, France) for*Streptococcus mutans* and Sabouraud dextrose agar (Biomerieux, France) for*Candida albicans*. After incubation at 37° C for 24 hours, colony counts were performed.

Surface roughness of specimens was measured by SPM-9600 AFM system (SHIMADZU, Kyoto, Japan) in non-contact mode with a silicon nitride tip of NSG11 (NT-MDT, Moscow,Russia). One specimen from each group was imaged at three randomly selected sites to provide a three-dimensional perspective of the surface, from which the mean surface roughness (Ra) was calculated by AFM

systemic software (VectorScan 3.3.1). Ra represents the averagedistance from the roughness profile to the center plane of the profile.

Kolmogorov-Smirnov test was used to test the normal distribution of the variables.The results were analyzed by two-way ANOVA followed by Tukey's honestly significant difference (HSD) test with a general linear model procedure in SSPS 17.0 (SPSS Inc., Chicago, USA). A significance level of 0.05 was used for statistical tests.

Results

A. Microbial analysis results for *Streptococcus mutans*

There were significant differences between groups and within groups according to polimerization cycle (*P*=.000). Table 3 showed the mean and standard deviation of microbial accumulation for groupson*Streptococcus mutans*. Group A specimens (30 min. polymerized acrylic resin) showed higher microbial accumulation than group B specimens (8 hours polymerized acrylic resin) against *Streptococcus mutans*. So as polymerization time increased, microbial accumulation decreased. The nanoparticle type did not significantly effect the antimicrobial capacity of the resins. %3 Al_2O_3 nanoparticles showed more antibacterial activity than %5 Al_2O_3 nanoparticles in Group A. The least mean value for microbial accumulation was observed in the long time polymerized acrylic resin with SiO_2 nanoparticles.

B. Microbial analysis results for *Candida albicans*

There were significant differences between groups and within groups according to polimerization cycle (*P*=.000).Table 4 showed the mean and standard deviation of microbial accumulation for groups on*Candida albicans*. Generally, group A(30 min polymerized acrylic resin) specimens showed higher microbial accumulation than group B(8 hours polymerized acrylic resin) specimens.As *Streptococcus mutans*,the higher the polymerization time, the more antibacterial performance of resins was observed for *Candida albicans*. The nanoparticle weight and type did not significantly effect the antimicrobial capacity of the resins.The nanoparticle incorporation did not decrease the fungal accumulation for resins. The least mean value for microbial accumulation was observed in the long time polymerized acrylic resin with %5 wt Al_2O_3nanoparticles.

C. Surface roughness results

Representative AFM images of Group A(30 min polymerized acrylic resin) and Group B(8 hours polymerized acrylic resin) specimens were shown in Fig. 1 and Fig. 2. One specimen were selected randomly for surface roughness and AFM images in groups. Groups containing %5 SiO_2 and Al_2O_3nanoparticles showed more surface irregularities than other groups. Especially, SiO_2 nanoparticles effect surface texture more than Al_2O_3nanoparticles. Moderately high number of irregularitiessuch as peaks and valleys were visible, which were favorable for adhesion. In contrast, no surface texturing could be seen for control group. The control group specimen surfaces were significantly smoother than surfaces of specimens with nanoparticles. The mean surface roughness values (Ra) for specimenswere shown in Table 5 and Table 6. Al_2O_3 and SiO_2 nanoparticles increased surface roughness of acrylic resin. Surface roughness decreased as polymerization time increased. The groups with nanoparticles were respectively different from the control group. The highest values were observed in groups containing %5 SiO_2 nanoparticles. Especially, groups containing SiO_2 nanoparticles showed higher surface roughness values than groups containing Al_2O_3 nanoparticles.

Discussion

The formation of biofilm on the surfaces of removable prostheses plays an important role in thedevelopment of caries, periodontal disease and mucositis[16,17]The

colonization process on the surface of dental prostheses is characterized by various steps[17] and occurs because acrylic resins have porosity, absence of ionic charge on the surface, roughness, and a capacity to absorb fluids, all of which lead to the accumulation of micro-organisms[18,19].The null hypothesis of our study was accepted for *Streptococcus mutans* but rejected for *Candida albicans* for antimicrobial capacity of nanoparticle incorporated resins.The null hypothesis related with surface roughness was also accepted for *Streptococcus mutans* and *Candida albicans* for nanoparticle incorporated resins.

Infection of the mucosa under prosthesis is a common problem in these patients. Denture related stomatitis is the most frequent problem in patients with complete denture (especially in maxilla) which is caused by an ill-fitting denture, trauma during mastication poor oral hygiene and presence of opportunistic organisms such as *Candida albicans*in oral cavity[20]. Our experiment was done on *Candida albicans* as the most prevalent strain in denture stomatitis and on *Streptococcus mutans*, which is the leading etiological factor in dental carries and frequently found in oral cavity[20,21].

Kiriyama *et al*.[22]also found that there was a significant decrease in residual viable cell count of *Streptococcus mutans*when compared to exposure to resin without antibacterial agent like our study.*Streptococcusmutans* appears to be more sensitive to the nanoparticles in our study as depict in Fig. 1 similarly with Kiriyama *et al*.The nanoparticleshad noinhibitory effect on *Candida albicans*.They found that the correlation coefficient for *Candida albicans* was lower than those of the other four bacteriaas we found in our study for *Streptococcus mutans* and *Candida albicans.Candida albicans* produces quantitatively more biofilm than other *Candida* species.We also support the thesis of Kiriyama *et al* about the *Candida Albicans'* lower antibacterial effect that the proportion of proteins is lower in fungus than in bacteria. Therefore, compared with bacteria, fungus has a lower amount of amino acids to react with silver ions. It was also probable that the cell components and microstructure of microorganisms caused the difference in antimicrobial effects.Matsunami *et al*.[15]also found that *Streptococcus mutans* exhibit a high anti-microbial efficacy, even if the content of silver nanoparticles in NanoAg-IS-PMMA-BD is as low as 1% w/w all test bacteria.We also foundthe least microbial accumulation was in the long time polymerized acrylic resin with SiO_2 nanoparticles.Silicon oxides are consideredto be more appropriate carriers because of their porousstructures and better adsorption properties. Nano-silicondioxide (nano-SiO2) has the advantage of having extremelyhigh surface activity, which enables it to absorb various ionsand molecules[15].

Recently, nanotechnology has become increasingly important in the biomedical and pharmaceutical areas as alternative antimicrobial strategy due to re-emergence infectious diseases and the appearance of antibiotic-resistant strains especially within Gram-negative microorganisms[23].Various nanoparticles (NP)have been added to different dental materials (TiO_2, SiO_2, ZnO, CeO_2, Ag, CuO etc.) in order to induce antimicrobial property[24].In this study 3% wt and 5% wt Al_2O_3 and SiO_2 nanoparticles were added to heat-cured acrylic resins. Sodagar *et al*.[25] evaluated the antimicrobial activity of acrylic resins containing TiO_2 and SiO_2 nanoparticles and their mixture (TiO_2/SiO_2 nanoparticles). Antimicrobial properties were determined against planktonic *Lactobacillus acidophilus* and *Streptococcus mutans.*Percentage of bacterial reduction in % 1 SiO_2 nanoparticles containing acrylic resin ranged from 19% to 51%. In our study 8 hours polymerized acrylic resin with

% 5SiO_2 nanoparticles' bacterial reduction was %48 similarly. As increasing %wt ofnanoparticle,microbial accumulation of acrylic resin decreased. The groups exhibited strong antimicrobial activity against *Streptococcus mutans* as it was in our study.

Marra *et al.*[26]investigated antimicrobial activity of acrylic resin combined with PTBAEMA (10% and 25%) against *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans*. Acrylic resin combined with 10% and 25% PTBAEMA showed significiant antimicrobial activity against *Staphylococcus aureus* and *Streptococcus mutans*as we found for *Streptococcus mutans* in our study. They also found no antifungal activityof resinsagainst *Candida albicans* like we found in our study[26].

Adams *et al.*[27]demonstrated that adding nanoparticles to acrylic specimens could reduce bacterial growth and population. Regarding the nanoparticles, it may be concluded that the higher the concentration, the higher the antimicrobial activity. They found that a concentration of 1% turned out to be more bactericidal than 0.5%, when nano-SiO_2 are employed.In our study,8 hours polymerized acrylic resin with % 5SiO_2 nanoparticles'*Streptococcus mutans* accumulationwas the lowest similarly with this study.

Sodagar *et al.*[25]found that nano-SiO_2 was not as effective as nano-TiO_2 under UVA.As in our study,this study showed that in contrast to previous studies, nano-sized SiO_2 was not an inert substance and had some antibacterial effects.Adding TiO_2 and SiO_2 nanoparticles to PMMA can impart antimicrobial activity against *Streptococcus mutans* to the resins.

The addition of Al_2O_3 nanoparticles to acrylic resin improved the thermal properties and transverse strength of acrylic resin at the same time this addition decreased water sorption and solubility.In supporting this,we foundthe least mean value for fungal accumulation that was observed in the 8 h polymerized acrylic resin with %5 wt Al_2O_3 nanoparticles.

Jasim *et al.*[28]found that there was an increase insurface hardness as we found in our study but the surface roughness was not significantly changed with the increased concentration of Al_2O_3nanoparticles. Consani *et al.*[29]investigated gloss, roughness, hardness and impact strength of conventional and boiled polymerized acrylic resins having different polymerization cycles. There was statistically significant difference in the impact strength for denture base resins polymerized using long cycle and short curing cycle in each technique, with better surface roughness results for the long curing process as we found in our study.This result can be related with the reduction in the residual monomer content and reduced porosity may have occurred during the long polymerization cycle, which proved to be better than the short polymerization cycle.[30]

We also evaluated the effect of Al_2O_3 addition on the surface roughness of the acrylic resin material as Vojdani *et al.* [31].The surface roughness of denture material is important, because it affects the oral health of tissues in direct contact with the dentures. The surface roughness threshold for acrylic resin is 0.2 mm, below which no significant decrease in bacterial colonization occurs. Dramatic colonization would be expected to occur on surfaces with a roughness value of 2.2 mm [31].The surface roughness values of our study is below this threshold for long polymerized resin except the group containing %5 SiO_2 nanoparticles. In agreement with the study of Saad-Eldeen[32],the results of our study showed that incorporating Al_2O_3 at two different concentrations did not adversely affect the roughness of the denture base resin.The highest values were observed in groups containing %5 SiO_2 nanoparticles. Especially, groups containing SiO_2 nanoparticles showed higher surface

roughness values than groups containing Al_2O_3 nanoparticles.

Conclusion

According to test results the following results were found;

1. The polymerization timeprovided an antimicrobial activity for all acrylic resins.So aspolymerization time increased, microbial accumulation decreased for both microorganisms.
2. The Al_2O_3 and SiO_2nanoparticles provided an antimicrobial activity for *Streptococcus mutans*.The least mean value for microbial accumulation was observed in the long time polymerized acrylic resin with%5 wt SiO_2 nanoparticles.
3. The nanoparticle incorporation did not decrease the fungal accumulation for resins. The least mean value for fungal accumulation was observed in the long time polymerized acrylic resin with %5 wt Al_2O_3 nanoparticles.
4. Al_2O_3 and SiO_2 nanoparticles increased surface roughness of acrylic resin for both of groups. SiO_2 nanoparticles showed higher surface roughness values than groups containing Al_2O_3 nanoparticles.

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Figure Captions

Fig. 1. Atomic force micrographs of groups. (A) Control group, (B) %3 wt Al_2O_3 nanoparticles group, (C) %5 wt Al_2O_3 nanoparticles group, (D) %3 wtSiO_2nanoparticles group, (E) %3 wt SiO_2nanoparticles group.

Fig. 2. Atomic force micrographs of Group B specimens. (A) Control group, (B) %3 wt Al_2O_3 nanoparticles, (C) %5 wt Al_2O_3 nanoparticles, (D) %3 wt SiO_2 nanoparticles, (E) %5wt SiO_2nanoparticles.

Table 1. Specimen groups

Table 2. Properties of acrylic resin and nanoparticles

Table 3. Results of two way ANOVA for *Streptococcus mutans*

Table 4.Mean and standard deviation of microbial accumulation for groupson *Streptococcus mutans*

Table 5. Results of two way ANOVAfor *Candida albicans*

Table 6. Mean and standard deviation of microbial accumulation for groupson *Candida albicans*

Table 7. Meansurface roughnesses (Ra) of groups

Table 8. Meansurface roughnesses (Ra) of group B specimens.

Table 1 Specimen groups

Groups	Description
Group A	Acrylic resin polymerized for 30 minutes at 100 °C.
Group B	Acrylic resin polymerized for 8 hours at 100 °C.
Subgroups	
1	Acrylic resin without nanoparticles (control group)
2	Acrylic resin containing %3 wt Al_2O_3 nanoparticles
3	Acrylic resin containing %5 wt Al_2O_3 nanoparticles
4	Acrylic resin containing %3 wt SiO_2 nanoparticles
5	Acrylic resin containing %5 wt SiO_2 nanoparticles

Table 2 Properties of acrylic resin and nanoparticles

Material	Manufacturer
Acrylic resin Polymethyl methacrylate (PMMA) Meliodent	Heraeus Kulzer, Germany
Al_2O_3 nanopowder (99.5% pure, powder size 40-50 nm)	MKNANO, Canada
SiO_2 nanopowder coated with silane coupling agent (99.5% pure, powder size 15 nm)	MKNANO, Canada

Table 3 Mean and standard deviation of microbial accumulation for groups on *Streptococcus Mutans*

Groups	N	Means ± SD
Groups A1	12	137,83±33,9^{ab}
Groups B1	12	101,66±18,48^{c}
Groups A2	12	83,83±12,06^{cd}
Groups B2	12	57,41±12,13^{e}
Groups A3	12	96,08 ±21,3^{a}
Groups B3	12	71,41±21,52^{d}
Groups A4	12	71,58±16,46^{d}
Groups B4	12	59,33±12,92^{bc}
Groups A5	12	70,50±12,55^{de}
Groups B5	12	48,41±10,03^{c}

Table 4 Mean and standard deviation of microbial accumulation for groups on *Candida Albicans*

Groups	N	Means ± SD
Groups A1	12	21,58±8,15^{bc}
Groups B1	12	9±3,12^{e}
Groups A2	12	26,25±6,76^{c}
Groups B2	12	9,16±4,69^{e}
Groups A3	12	33,58±8,17^{ed}
Groups B3	12	8±4,53^{ab}
Groups A4	12	30,91±12,21^{d}
Groups B4	12	12,33±4,11^{a}
Groups A5	12	39,75±13,16^{a}
Groups B5	12	12,91±4,88^{c}

Table 5. Meansurface roughnesses (Ra) of Group A specimens

Group	Ra(nm)
(A1) Control	196.408
(A2) %3 Al_2O_3	232.431
(A3) %5 $Al_2O_3$256.4	
(A4) %3 SiO_2	287.772
(A5) %5 SiO_2	330.586

Table 6 Mean surface roughnesses (Ra) of Group B specimens

Group	Ra(nm)
(B1) Control	96.454
(B2) %3 Al_2O_3	135.43
(B3) %5 Al_2O_3	166.48
(B4) %3 SiO_2	178.72
(B5) %5 SiO_2	235.56

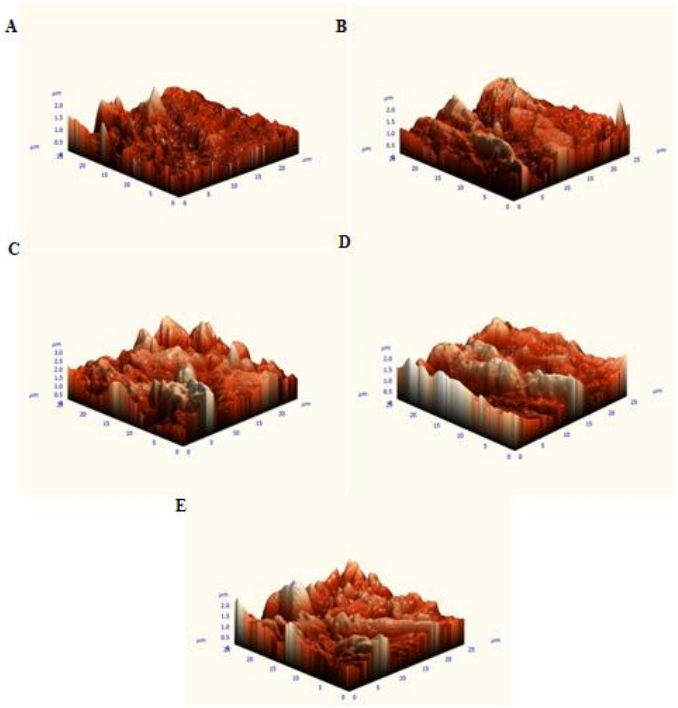

Fig. 1. Atomic force micrographs of Group A specimens. (A) Control group, (B) %3 wt Al_2O_3 nanoparticles, (C) %5 wt Al_2O_3 nanoparticles, (D) %3 wt SiO_2 nanoparticles, (E) %5 wt SiO_2 nanoparticles.

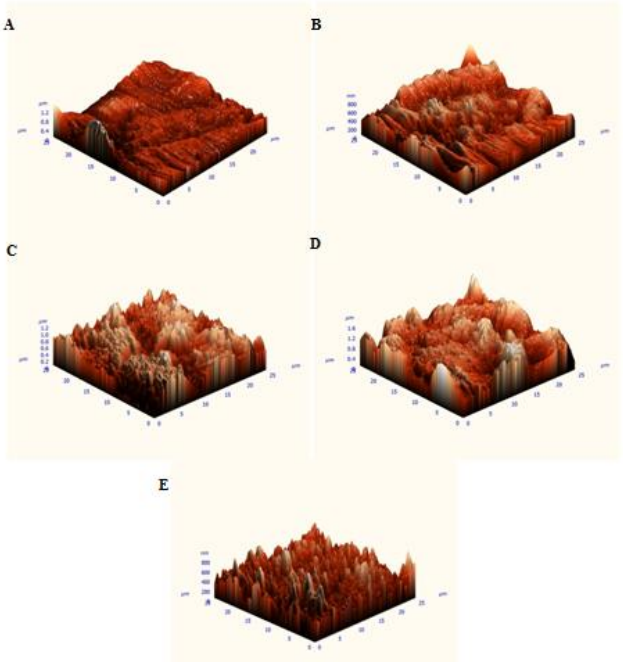

Fig. 2. Atomic force micrographs of Group B specimens. (A) Control group, (B) %3 wt Al_2O_3 nanoparticles, (C) %5 wt Al_2O_3 nanoparticles, (D %3 wt SiO_2 nanoparticles, (E) %5 wt SiO_2 nanoparticles.