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Effect of incorporating Silver and Copper Nanoparticles on mechanical and antimicrobial properties of maxillofacial silicone elastomer. An in-vitro study

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Abstract

Introduction

Maxillofacial prosthetics play a crucial role in restoring facial defects caused by congenital anomalies, trauma, surgeries, or diseases. Silicone elastomers are widely preferred in these prosthetics due to their desirable properties like biocompatibility, lifelike aesthetics, and ease of fabrication. However, conventional silicone elastomers have limitations. They lack inherent antimicrobial properties, making them susceptible to microbial colonization and potential infections. Additionally, they can deteriorate mechanically over time, compromising the longevity and function of the prosthesis

Aim – The aim of the study was to incorporate Ag nanoparticles and Cu nano-oxides (CuO) in various concentrations into PDMS (HTV Cosmesil M511) and assess the changes in mechanical and antifungal properties

Material and methods

This in vitro study was carried out in the Department of Prosthodontics,–Banaras Hindu University, Varanasi, India Between December 2020 to October 2021.A total of 63 specimens were prepared and were equally divided into each of the 7 study groups. All the samples were subjected to different mechanical and biological tests: Tensile strength, percentage elongation, tear strength, and antifungal activity against C. *Albicans* and C. *Glabrata*. Data collected from quantitative studies of the experimental groups was compared to the control group using one-way analysis of variance (ANOVA) with concentration as the main variable for tensile strength, percentage elongation, tear strength, and antifungal activity. Tukey test was used as a post hoc test to identify differences among the groups at a significance level of $\alpha = 0.05$ for all tests. P values < 0.05 were considered statistically significant. All statistical tests were performed using SPSS.21 statistical software.

Results

There was a significant increase in the tensile strength ($P < 0.05$) in the group Cu1 (1.60 ± 0.13 mpa) when compared with that of the control group (1.37 ± 0.06 mpa). There was an increase in tear strength in all the experimental group. There was a significant increase in the tear strength ($P < 0.001$) in the group Ag1 and Cu1 (15.45±1.1 and 16.38±1.35) respectively when compared with that of the control group (11.46± 1.24). There was a significant increase in the percentage elongation ($P < 0.001$) in the group Ag1 and Cu1 (542±33 and 570±3) respectively when compared with that of the control group (449± 15). As the concentration of nanoparticles increased the absorbance value decreased indicating improved antifungal activity.

Conclusion

Nanoparticles significantly improves wide range of mechanical properties; tensile strength, tear strength, percentage elongation and antifungal activity aagainst C. *albicans* and C. *glabrata*.

Keywords

Silicone elastomers, Maxillofacial prosthetics ,Nanoparticles ,Antifungal activity
Percentage elongation, Candida albicans, Candida glabrata

Introduction

Facial disfigurement can be secondary to congenital anomaly, developmental disorders, trauma and surgical treatment of neoplasm [1]. These anomalies lead to functional insufficiency and aesthetic problems along with psychological distress for patients; hence, rehabilitation is a requirement at all ages. These defects can be managed either surgically by plastic repair or artificially by alloplastic repair. In favourable circumstances plastic repair is the recommended therapeutic method [2]. However, when functional and aesthetic demands cannot be fulfilled with the surgical repair, rehabilitation with a maxillofacial prosthesis fabrication is considered as an optimal option [3]. Prosthetic rehabilitation provides psychological and functional benefits, as it enhances aesthetics, speech, swallowing, self-esteem and overall quality of life.

The earliest description of facial prosthesis was made by French surgeon Ambrose Pare in 1575, and since then, the quest for the perfect material has begun [4]. Silicone elastomer was introduced in 1946, but the first time used by Barnhart for extra-oral prosthesis fabrication in 1960 [5], since then it is the most widely used material for the maxillofacial prosthesis. This is so due to its ease of manipulation, durability, pigmentability, chemical inertness and biocompatibility[5-7]. The silicone elastomer has some limitations. The main problem is its reduced clinical longevity of the prosthesis. Due to low tensile and tear strength, it exhibits modified texture and poorly fitting edges [8].

The silicone elastomer prosthesis surfaces are exposed to soft tissues, saliva and nasal secretions. These secretions may cause diverse microbes to colonise their surfaces, resulting in degradation or infection. Among them are, the most common colonizing fungi are C. *Albicans* and C. *Glabrata* [9,10]. Moisture, body warmth and the nutrient-rich skin secretion residue enhance fungal growth on silicone elastomer surfaces. Facial Skin has a ph in range of 4 to 4.9 and acidic conditions promote C. *Albicans* and C. *Glabrata* growth. candida metabolism also leads to acid production. Acidic pH can promote prosthetic material deterioration, which might result in surface roughness and make it more vulnerable to microbial adhesion, increasing the patient's risk of infection [11,12].

Several strategies have been attempted to address these limitations [13]. Due to advancement in nanotechnology, various Nanoparticles have been tried in maxillofacial silicone elastomers to enhance its properties. Nanosized particles differ in their physical,

chemical, and biological properties compared to their macro-sized counterparts due to their high surface area to volume ratio. Nanoparticles Properties are determined by their size and concentration. Nanoparticles may improve the physical, chemical, mechanical, and biological properties of the material in which they are incorporated depending on the concentration of nanoparticles [14].

The commonly used nanometals in dental material were Ag, Ti, Cu, Au, Zn, and Zr. The Ag and Au were used in their pure form while the nano-oxides of Ti, Zn, Cu, and Zr were employed more often [15]. Silver nanoparticles in their pure form and Copper in their nano oxides (CuO) form have a broad-spectrum antimicrobial property along with improved physiochemical and mechanical properties in reinforced dental materials [15].

The purpose of this study is to incorporate Ag nanoparticles and Cu nano-oxides (CuO) in various concentrations into PDMS (HTV Cosmesil M511) and assess the changes in mechanical and antifungal properties.

Materials and method

This in vitro study was carried out in the Department of Prosthodontics, Banaras Hindu University, Varanasi, India Between December 2020 to October 2021. Before start of study ethical clearance was obtained from institution ethical committee . Sample size was calculated using G Power software.

The maxillofacial silicone elastomer Cosmesil M511 HTV was procured from Technovent Ltd, UK. Cu and Ag nanoparticles were incorporated into elastomer in concentrations of 1wt%, 2wt%, 3wt%. A total of 63 specimens were prepared and were equally divided into each of the 7 study groups. All the samples were subjected to different mechanical and biological tests: Tensile strength, percentage elongation, tear strength, and antifungal activity against C. *Albicans* and C. *Glabrata*.

The details of materials used in this study are listed in Table1.a.

Samples were grouped into a total of 6 experimental groups and 1 control group to study the mechanical properties study and 6 experimental groups, 1 control group and 1 blank control group for antifungal activity.

Study groups are given in table 1. (b)

Sample preparation

Initially, an aluminum die of dimension (10cm×1cm×1cm) was designed with autocad and processed by the computer numerical control machine (CNC).

The experimental group were made by combining the silicone elastomer (Cosmesil M 511) with various concentrations of 2 different nanoparticles (Ag, Cu); 1wt%, 2wt%, 3wt%. Silicon elastomer samples without nanoparticles acted as control group.

For the experimental groups, Cu and Ag nanoparticles were weighed by a digital electronic weight balance (Shimadzu corp, Japan). Next, the pre-weighed silicone base (part A) was mixed with preweighed nanoparticles. Nanoparticles powder was hand mixed with the base for 20 minutes with a clean, stiff, flat-ended spatula. As per the manufacturer's instructions, the base with nanoparticles (part A) was hand-mixed with the catalyst (cross-linking agent, Part B) in the proportion of 10:1 for further 10 minutes. The mixture was poured into a 20-ml disposable plastic syringe for injection into the moulds.

Next, the matrix part, bottom part and upper part of the die were coated with petroleum jelly. The metallic matrix was placed over the bottom part and secured with screws. The prepared silicone was then injected in excess into the metal moulds and then transferred to a vacuum oven at 60 degree Celsius for 15 minutes to eliminate all air bubble. Afterwards, the Upper part was placed over the metal moulds, secured with the screws. Then, the die was transferred to a hot air oven at 100 degree Celsius , and cured for 1 hr. After curing, the moulds were carefully separated, specimens were removed, and the flush and excess material was removed by a scalpel and blade no. 11. The control group was prepared in the same way as described for experimental groups except for adding the nanoparticles. Samples were kept away from light and heat until tested.

Mechanical properties

All specimens were evaluated for tensile strength, percentage elongation, and tear strength using the universal testing machine (Tinius Oslen, USA).

A) Tensile strength.

Nine specimens were prepared for each study group. The thickness and width of each specimen were measured at three different locations using a digital calliper with digital readout. The mean value was entered as input data which was used in calculating the specimen cross-sectional area through the computer software. The specimen was kept under tension in the grips of the universal testing machine. The lower member of the universal testing machine remained fixed, while the upper member moved at a constant rate of 1000 mm/min cross-head speed. The maximum amount of force immediately before breaking and

elongation measurements were recorded by the computer software. The stress-strain curves were constructed. Tensile strength was recorded by computer software.

B) Percentage Elongation Testing

The percentage elongation was calculated concurrently with the tensile strength testing. The original length was measured before testing using the digital calliper. Percentage elongation was recorded by the computer software.

C) Tear Strength Testing.

Tear strength was calculated concurrently with tensile strength testing. The thickness of each specimen was measured at three different locations using a digital calliper with digital readout and the average value was calculated. The force required to break the specimen was recorded by the computer software. From these measurements, tear strength (N/mm) was calculated using the following equation:

$$T = F/ D$$

Where T is the tear strength (N/mm); F is the maximum force (N); D is the thickness of the specimen (mm).

Anti-fungal properties

C. *Albicans* and C. *Glabrata*

C. *Albicans* strain (ATCC90028), and C. *Glabrata* strain (ATCC90030) was grown on Potato Dextrose Agar (PDA) plate overnight at 37 degree Celsius . Then, colonies were suspended into PBS, and vortex to achieve a suspension of 0.5 mcfarland turbidity ($1.5x\ 10^8$ CFU/ml). This suspension act as a primary biofilm inoculum.

Biofilm Formation

Test samples consisted of circular disks extracted from the remnants of silicone elastomer sheets that were used to make samples for tensile tests. A total of 56 disks were extracted which were equally divided into 8 study groups; 6 experimental groups (Ag and Cu nanoparticles), 1 control group (without nanoparticles) and a blank control group (without nanoparticles and fungal inoculum). Disks were sterilized in an autoclave at 121 degree Celsius and 15 psi for 15 minutes. The biofilm formation protocol employed in this study

was based on the work described by Chandra et al [16]. All discs were preconditioned with PBS in 96-well flat-bottom tissue culture plates (TCPs) (Tarsons, Kolkata, India) and incubated at room temperature for 90 minutes. Preconditioned disks were removed and placed in another 96-well tissue culture plate. 200 µl of primary biofilm inoculum was added to each well containing disk and incubated at 37 degree Celsius for 90 minutes. 200 µl of fresh Yeast Nitrogen Base (YNB) medium was added to each well of the new 96-well TCP. Disks were removed carefully and placed in TCP containing 200 µl of fresh YNB and incubated at 37 degree Celsius for 48 hours. Blank controls were prepared in parallel, which contained control sample disks and all the same procedure was carried out except the addition of primary biofilm inoculum.

Two methods were used for the quantitative assessment of the biofilm matrix: Crystal violet assay and MTT colorimetric assay. The crystal violet staining (CV) assay is simple and reproducible assay and is based on the growth rate reduction refected by the colorimetric determination of the stained cells. MTT assay is a versatile and popular assay to assess mitochondrial succinic dehydrogenase enzyme activity which reduces MTT dye to water-insoluble formazan product.

A) Crystal violet assay

C. Albicans and C. Glabrata biofilm formation on test samples were measured using a CV assay as suggested by Singh et al [17]. Residual media was removed from each well, and then the wells were washed thrice with 200 µl of PBS to remove unattached cells. Fixation of the biofilm was done by heat fixation at 60 degree Celsius for 30 minutes. For staining, 200 µl of 0.5% CV solution was added to all wells for 5 minutes. Excess CV was removed, and the plates were washed with running tap water. For elution, 150 µl of ethanol: acetone mixture (80:20) was added and leftover for 30 minutes at room temperature. The elute was then resuspended in wells of new TCP. The absorbance was measured at 570 nm by using a scanning multiwell spectrophotometer (Elisa Reader, Thermo scientific, USA).

B) MTT Colorimetric Assay

C. Albicans and C. Glabrata biofilm formation on test samples was quantified using a tetrazolium salt based 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) colorimetric ass, as suggested by Hawser et al [18]. This method measures enzyme activity that reduces MTT dye to water-insoluble formazan dye. The MTT assay is rapid, reproducible, non-invasive, non-destructive and requires minimal post-processing of samples.

The reduction of MTT to formazan crystals can only take place in the presence of viable cells and the necessary reductase enzymes. Therefore, the MTT assay measures the optical density of formazan crystals in solution as an indication of the metabolic activity of viable C. Albicans and C. Glabrata cells. Immediately after biofilm maturation, 50 µl of MTT solution (a stock solution containing 5 mg of MTT per ml of PBS, diluted 1:5 in pre-warmed 0.15 M PBS before application) was added to each well. Media plus MTT was removed after incubation for 5 h at 37°C, the and the wells were washed three times with 0.15 M PBS (2 ml) to eliminate the traces of MTT. Dimethyl sulfoxide (1 ml) was then added to solubilize the MTT formazan product. MTT formazan formation was measured at 570 nm by using a scanning multiwell spectrophotometer (Elisa Reader, Thermo scientific, USA).

Statistical analysis

Data collected from quantitative studies of the experimental groups was compared to the control group using one-way analysis of variance (ANOVA) with concentration as the main variable for tensile strength, percentage elongation, tear strength, and antifungal activity. Tukey test was used as a post hoc test to identify differences among the groups at a significance level of $\alpha = 0.05$ for all tests. P values < 0.05 were considered statistically significant. All statistical tests were performed using SPSS.21 statistical software.

Results

Mechanical properties

Tensile strength

The mean values of tensile strength (mpa) of all the studied groups are given in Table 2.(a) and shown graphically in Graph 1.(a). There was a significant increase in the tensile strength ($P < 0.05$) in the group Cu1 (1.60 ± 0.13 mpa) when compared with that of the control group (1.37 ± 0.06 mpa). There was no significant difference ($P > 0.05$) in the tensile strength between group Ag1 and Cu1. There was decrease in tensile strength as the concentrations of nanoparticles were increased. There was slight increase in tensile strength in group Ag2 and Ag3, but not statistically significant (P>0.05) when compared to control group. Cu2 and Cu3 group showed reduced tensile strength (1.22±0.08mpa and 1.18±0.05) which was statistically significant (P<0.05) when compared to Ag2 and Ag3 group (1.51±0.06 and 1.46±0.04) although, not statistically significant in comparison to control group (P>0.05).

Tear strength

The mean values of tear strength (N/mm) of all the studied groups are given in Table 2.(b) shown graphically in Graph 1(b). There was an increase in tear strength in all the experimental group. There was a significant increase in the tear strength ($P < 0.001$) in the group Ag1 and Cu1 (15.45±1.1 and 16.38±1.35) respectively when compared with that of the control group (11.46± 1.24). There was no significant difference ($P > 0.05$) in the tear strength between group Ag1 and Cu1. There was decrease in tear strength as the concentrations of nanoparticles were increased. Increase in tear strength in Ag2, Ag3 and Cu2 group compared to control group was found but not statistically significant (P>0.05).

Percentage elongation

The mean values of percentage elongation (%) of all the studied groups are given in table 2.c and shown graphically in Graph 1(c). There was a significant increase in the percentage elongation ($P < 0.001$) in the group Ag1 and Cu1 (542±33 and 570±3) respectively when compared with that of the control group (449± 15). No significant difference ($P > 0.05$) in the percentage elongation between group Ag1 and Cu1 was found. A decrease in percentage elongation was observed as the concentrations of nanoparticles were increased. There was slight increase in percentage elongation in Ag2, Ag3, Cu2, Cu3 group compared to control group but not statistically significant (P>0.05).

Antifungal activity

Crystal violet assay

The mean values of the optical density (O.D.) of all the study groups are given in Table 3.(a) and 3.(b) and shown graphically in Graph 2.(a) and Graph 2.(b) for C. *Albicans* and C. *Glabrata* respectively. All the experimental groups shows significant decrease (P<0.01) in absorbance compared to control group 0.139±0.02 (C. *Albicans*) and 0.151±0.01 (C. *Glabrata*) indicating improved antifungal activity of nanoparticles against both the strain of C. *Albicans* and C. *Glabrata*. As the concentration of nanoparticles increased the absorbance value decreased indicating improved antifungal activity.

MTT Colorimetric assay

The mean values of the optical density of all the studies groups are given in Table 4.(a) and 4.(b) and shown graphically in Graph 3.(a) and Graph 3.(b) for C. *Albicans* and C. *Glabrata* respectively. Groups; Ag3, Cu1, Cu2, and Cu3 shows significant decrease ($P<0.01$) in absorbance value compared to control group, against C. *Albicans (*0.143±0.02). There was no significant difference ($P>0.01$) in absorbance value between the above mentioned groups. Reduce value of absorbance indicate better antifungal activity. As the concentration of nanoparticles increased the absorbance value decreased indicating improved antifungal activity.

With regard to C. *Glabrata*, groups; Ag2, Ag3, Cu1, Cu2, Cu3 showed significant decrease ($P<0.01$) in absorbance value compared to control group (0.224±0.01). There was no significant difference ($P>0.01$) in absorbance value between; Ag2, Ag3, Cu1, Cu2, Cu3 groups. Ag1 group showed improved antifungal activity against C.*glabrata* but not statistically significant ($P>0.01$) in comparision to control group. There was also no significant difference ($P>0.01$) present in between Ag1, Ag2, Ag3, Cu1, Cu2, Cu3 groups against C. *Glabrata*.

Discussion

Properties that are considered essential for the maxillofacial silicone elastomers are, high tear resistance, high tensile strength, good level of elongation at break. None of the commercially available material has all these properties. The addition of fillers is necessary for achieving a certain degree of reinforcement that leads to improvement of the mechanical properties[19].

This study aimed to develop an improved maxillofacial prosthetic material to achieve enhanced mechanical properties and antifungal activity. For this purpose, formulations were developed by incorporating two different nanoparticles (Ag, Cu) with varying concentrations (1wt%, 2wt%, 3wt%). These formulations were then evaluated for mechanical properties and antifungal activity.

The results from this study indicate that incorporation of 1wt% of silver and copper nanoparticles in silicone elastomer (Cosmesil M511) showed the maximum improvement of mechanical properties; Tensile strength, tear strength, percentage elongation. This study is also supported by various studies with different nanoparticles, **Han et al** [20] , found that incorporating Ti, Zn, or Ce at concentrations of 2.0% to 2.5% by weight into a silicone-based

elastomer improves hardness, tear strength, tensile strength, and elongation. When the concentration was increased to 3.0%, the tear strength, tensile strength, and elongation decreased, indicating that recommended concentration should not exceed 2.5%. **Shakir et al [21],** also found that the addition of 0.25wt% and 0.2wt% of Ti-O_2 nanoparticles showed overall improved mechanical properties in RTV silicone elastomer and HTV silicone elastomer respectively. **Tukmachi et al [22]** , also suggested that addition of SiO_2 nanoparticles in 4%,5%, and 6% concentration showed overall improvement in mechanical properties compared to control group but the highest improvement was shown by 5% SiO_2 nanoparticles. But **Sonnahalli et al [23] ,** in their study found that addition of silver nanoparticles in 20ppm concentration decreased the hardness although it had no effect on the tear strength and colour stability. The reason for improved mechanical properties in our study may be due to the different concentration of silver used compared to the concentration used by **Sonnahalli et al[23]**.

The results from this study indicate that incorporation of nanoparticles at concentrations of 1% by weight into a maxillofacial silicone improves tear strength, tensile strength, and elongation. Although nanoparticles can reinforce the silicone elastomer matrix, the reinforcement relies on the polymer properties, filler characteristics (particle size or specific surface area, structure and surface activity), filler loading and processing condition. It is critical to maintain the filler content at a reasonable level otherwise, the nanoparticles may agglomerate because of their higher surface energy and chemical reactivity; [19]. This fact helps to explain the observed decrease in tensile strength, percentage elongation, and tear strength when the concentration of nanoparticles increased to 2wt% and 3wt%

Regarding to antifungal activity Results from both MTT colorimetric assay and crystal violet assay showed that both silver and copper nanoparticles demonstrated improved antifungal activity against C. *albicans* and C. *glabrata* compared to control group.

In concern with CV assay, Antifungal activity increases as the concentration of both the nanoparticles (Ag and Cu) increased from 1% to 2% although no significant difference was found when the concentration of the nanoparticles increased from 2wt% to 3wt% in the silicone elastomer (Cosmesil M511). Antifungal activity of silicone elastomer (Cosmesil M511) incorporated with 2wt% and 3wt% concentration of the nanoparticles (Ag and Cu) was similar to the blank control group.

With regard to MTT colorimetric assay, silicone elastomer (Cosmesil M511) reinforced with copper nanoparticles showed better antifungal activity in comparison to silicone elastomer (Cosmesil M511) reinforced with silver nanoparticles against both; C. *albicans* and C. *glabrata* strains. Silicone elastomer (Cosmesil M511) reinforced with 3wt% silver nanoparticles had significant antifungal activity against C. *albicans*. Copper nanoparticles reinforced Silicone elastomer (Cosmesil M511) had equivalent antifungal activity in all three concentrations (1wt%, 2wt%, 3wt%) against C .*albicans.*

In respect of antifungal activity against C. *glabrata*, copper nanoparticles reinforced silicone elastomer (Cosmesil M511) in all the three concentrations (1wt%, 2wt%, 3wt%) were significantly effective. Regarding silver nanoparticle reinforced silicone elastomer (Cosmesil M511) antifungal activity, 2wt% and 3wt% concentration nanoparticles reinforced silicone elastomer had significant effectiveness.

Previous studies have reported the antimicrobial properties of silver and copper nanoparticles in various dental materials like composite resins, acrylic denture base resins, endodontic materials, dental implants, restorative cements, and orthodontic brackets and adhesives and in maxillofacial silicone elastomer [15,24,25].

The mechanism by which Ag and Cu may control the growth of C. albicans and C. glabrata is not fully understood. Early studies have shown that free reactive radicals block microbial DNA replication, inactivate vital enzymes necessary for ATP production and oxidation of glucose, and damage microbial cell walls, resulting in cell death[26].

To the best of our knowledge, this was the first study describing the mechanical properties and antifungal activity of copper nanoparticles in silicone elastomer (Cosmesil M511) in various concentrations and also the first study describing the antifungal activity of silver nanoparticles in silicone elastomers in various concentrations. Further, studies are needed in future regarding this aspect.

Conclusion

Under the conditions of this study and with the specific materials used, the following conclusions can be derived.

1) Reinforcement of Cosmesil M-511 HTV maxillofacial silicone elastomer with 1wt% of silver and copper nanoparticles significantly improves wide range of mechanical properties; tensile strength, tear strength and percentage elongation.
2) Reinforcement of Cosmesil M-511 HTV maxillofacial silicone elastomer with silver and copper nanoparticles significantly improved the antifungal activity against C. *albicans* and C. *glabrata*.

Product	Size	Batch number	CAS number	Company
Silver nanoparticles	10nm	1033	7440-22-4	Amnium Technologies private limited, Pune, India
Copper Oxide Nanoparticles	10nm	1029	1317-38-0	Amnium Technologies private limited, Pune, India
HTV (Cosmesil M511				Technovent ltd, UK

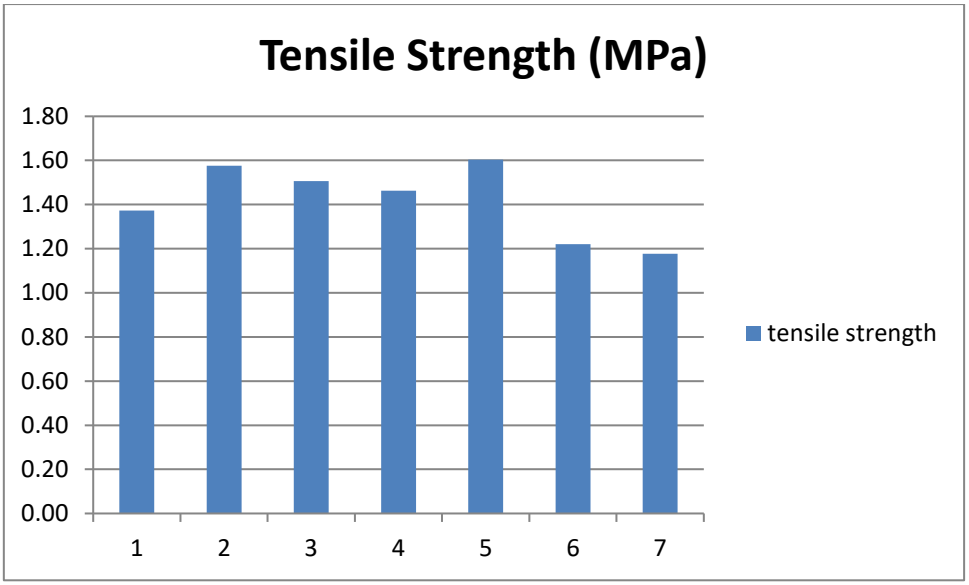
Table1. (a) Details of the material used in this study.

Study Groups	
C	Specimen without any nanoparticle
Ag1	Specimen containing 1% Ag Nanoparticle
Ag2	Specimen containing 2% Ag Nanoparticle
Ag3	Specimen containing 3% Ag Nanoparticle
Cu1	Specimen containing 1% Cu Nanoparticle
Cu2	Specimen containing 2% Cu Nanoparticle
Cu3	Specimen containing 3% Cu Nanoparticle

Table1.(b)Study groups that were studied in this study

Group	Mean± S.D	P value	Tukeys post hoc test
C	1.37±0.06	P<0.001	(Cu1 ≥ Ag1) > (Ag1 ≥ Ag2 ≥Ag3)
Ag1	1.58±0.11		
Ag2	1.51±0.06		(Ag2 ≥ Ag3 ≥ C ≥ Cu2≥Cu3)
Ag3	1.46±0.04		
Cu1	1.60±0.13		(Cu1 ≥ Ag1) > (Ag2 ≥ Ag3 ≥ C ≥ Cu2 ≥ Cu3)
Cu2	1.22±0.08		
Cu3	1.18±0.05		

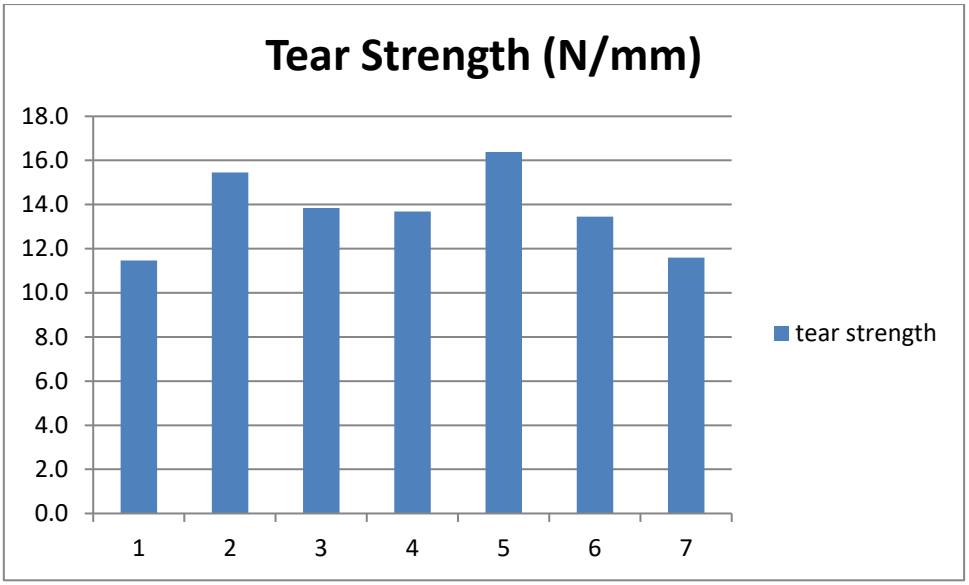
Table2. (a) Means ± SD of tensile strength (MPa) and Tukeys post hoc test of all the study group



Graph1. (a) Bar graph depicting Means ± SD of tensile strength (MPa) of all the study group.

Group	Mean (N/mm)± S.D	P value	Tukeys post hoc test
C	11.46±1.24	P<0.001	(Cu1 ≥ Ag1) > (Ag2 ≥ Cu2 ≥ Ag3 ≥ Cu3 ≥ C)
Ag1	15.45±1.11		
Ag2	13.84±0.40		
Ag3	13.69±0.47		
Cu1	16.38±1.35		
Cu2	13.45±0.26		
Cu3	11.60±0.87		

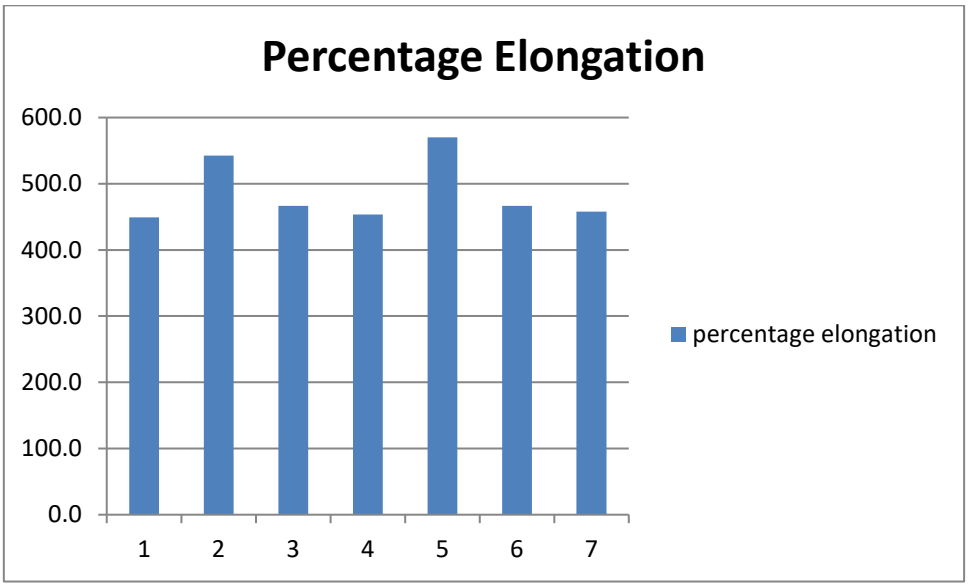
Table2 (b) Means ± SD of tear strength and Tukeys post hoc test of all the study group.



Graph1.(b) Bar graph depicting Means ± SD of Tear strength of all the study group.

Group	Mean ± S.D	P value	Tukeys post hoc test
C	449.3±15.1	P<0.001	(Cu1 ≥ Ag1) (Ag2 ≥ Ag3 ≥ C) (Cu2 ≥ Cu3 ≥C) (Cu1 ≥ Ag1) > (Ag3 ≥ Ag2 ≥ C ≥ Cu2 ≥ Cu3)
Ag1	542.3±33.4		
Ag2	466.7±5		
Ag3	453.7±4.5		
Cu1	570±3		
Cu2	466.7±11.6		
Cu3	458±12.5		

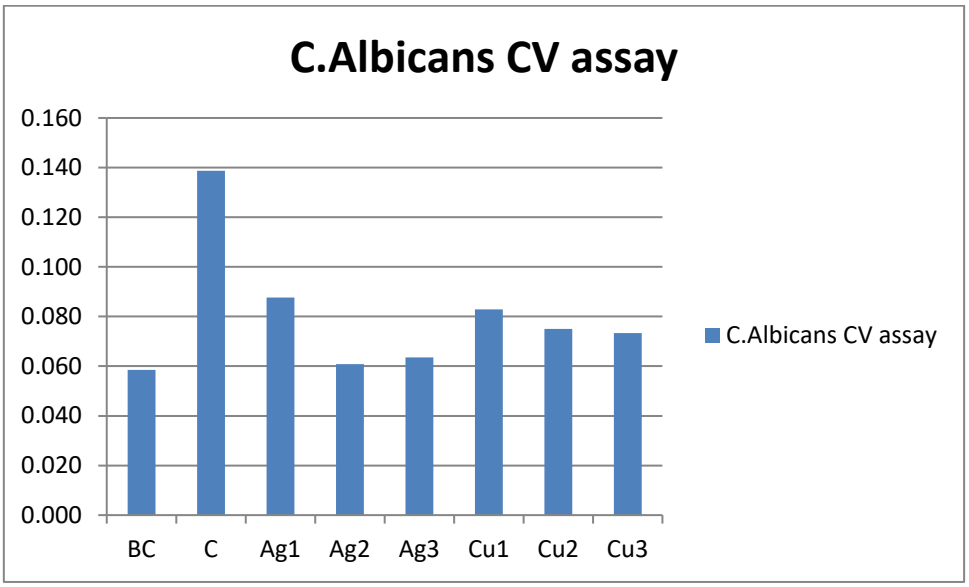
Table2.(c)Means ± SD of percentage elongation and Tukeys post hoc test of all the study group



Graph1.(c) Bar graph depicting Means ± SD of percentage elongation of all the study group

Groups	Mean ± S.D	P value	Tukeys post hoc test
BC	0.059±0.00	P<0.01	(BC ≥ Ag2 ≥ Ag3 ≥ Cu3 ≥ Cu2 ≥ Cu1) (Ag3≥ Cu3 ≥ Cu2 ≥ Cu1 ≥ Ag1) (BC ≥ Ag2) > Ag1 >C
C	0.139±0.02		
Ag1	0.088±0.01		
Ag2	0.061±0.01		
Ag3	0.064±0.01		
Cu1	0.083±0.02		
Cu2	0.075±0.02		
Cu3	0.073±0.01		

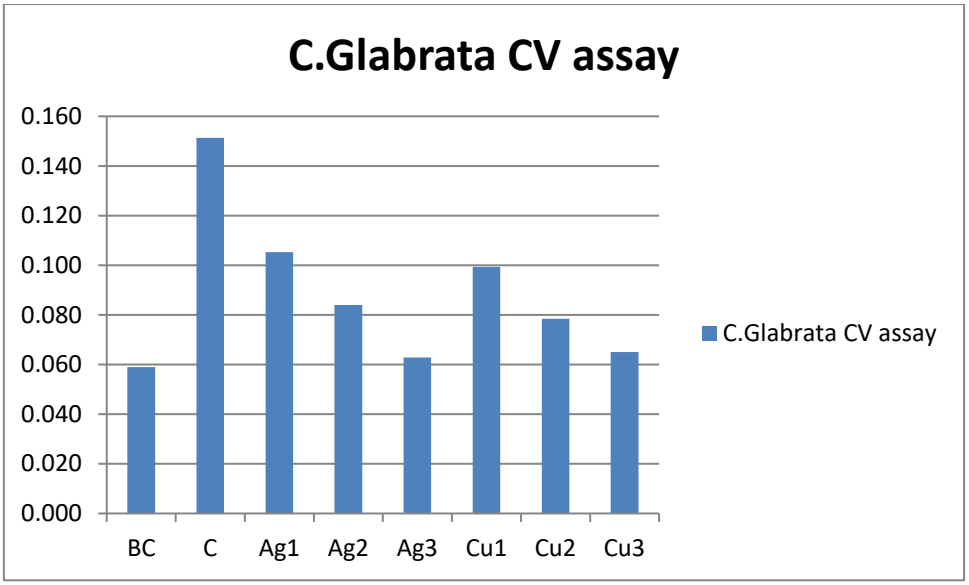
Table3.(a) Means ± SD of O.D of CV assay against C .albicans and Tukeys post hoc test of all the study group



Graph2. (a) bar graph depicting Means of O.D of CV assay against C. albicans of all the study groups.

Groups	Mean ± S.D	P value	Tukeys post hoc test
BC	0.059±0.00	P<0.01	(BC ≥ Ag3 ≥ Cu3 ≥ Cu2) > Ag2 (Ag3 ≥ Cu3 ≥ Cu2 ≥ Ag2) (Cu1 ≥ Ag1) > C
C	0.151±0.01		
Ag1	0.105±0.01		
Ag2	0.084±0.01		
Ag3	0.063±0.01		
Cu1	0.99±0.01		
Cu2	0.079±0.01		
Cu3	0.065±0.01		

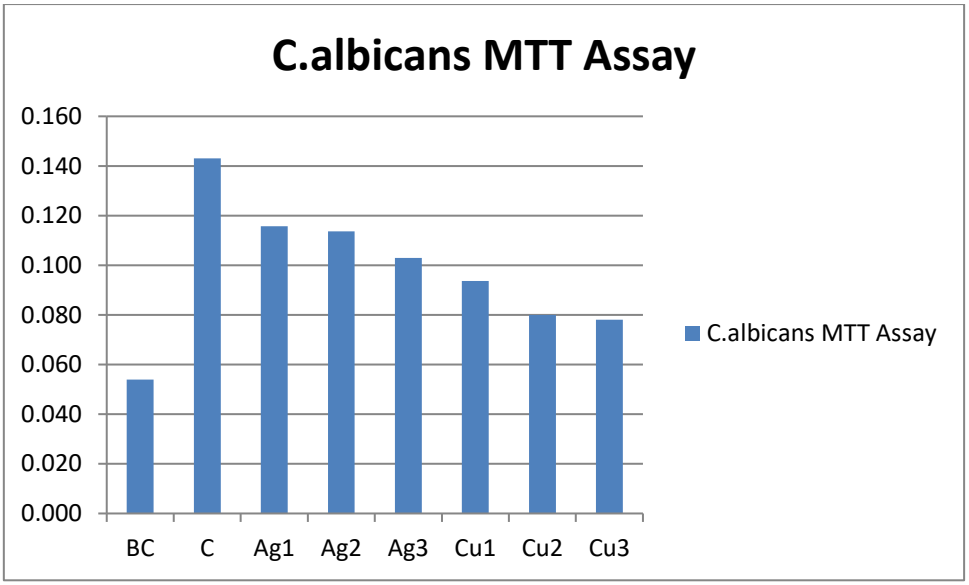
Table3.(b) Means ± SD of O.D of CV assay against C. glabrata and Tukeys post hoc test of all the study group



Graph2.(b) bar graph depicting Means of O.D of CV assay against C. glabrata of all the study group.

Groups	Mean ± S.D	P value	Tukeys post hoc test
BC	0.054±0.01	P<0.01	(BC ≥ Cu3 ≥ Cu2)
C	0.143±0.02		
Ag1	0.116±0.00		(Cu3 ≥ Cu2 ≥ Cu1 ≥ Ag3) > C
Ag2	0.114±0.01		
Ag3	0.103±0.02		(Ag2 ≥ Ag1 ≥ C)
Cu1	0.94±0.02		
Cu2	0.080±0.01		
Cu3	0.078±0.01		

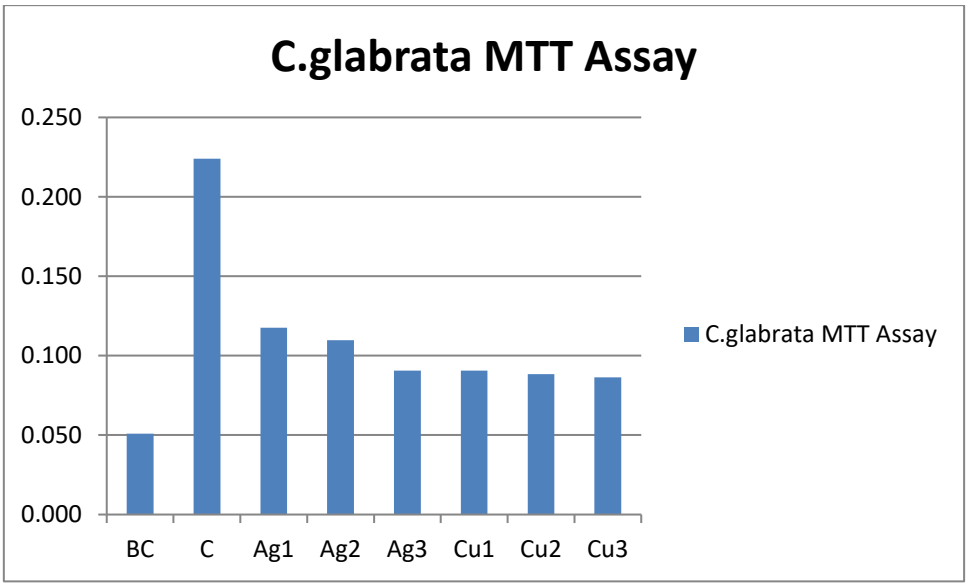
Table4.(a) Means ± SD of O.D of MTT colorimetric Assay against C. albicans and Tukeys post hoc test of all the study groups.



Graph3. (a) Bar graph depicting Means of O.D of MTT colorimetric assay against C. albicans of all the study groups.

Groups	Mean ± S.D	P value	Tukeys post hoc test
BC	0.051±0.00	P<0.01	(BC ≥ Cu3 ≥ Cu2 ≥ Cu1 ≥Ag3) (Cu3 ≥ Cu2 ≥ Cu1 ≥ Ag3 ≥ Ag2) > C
C	0.224±0.1		
Ag1	0.118±0.01		
Ag2	0.110±0.02		
Ag3	0.091±0.01		
Cu1	0.091±0.00		
Cu2	0.088±0.04		
Cu3	0.086±0.01		

Table4.(b) Means ± SD of O.D of MTT colorimetric Assay against C. glabrata and Tukeys post hoc test of all the study groups.



Graph3.(b) bar graph depicting Means of O.D of MTT colorimetric assay against C. glabrata of all the study groups.

References

1. Chang TL, Garrett N, Roumanas E, Beumer III J. Treatment satisfaction with facial prostheses. The Journal of prosthetic dentistry. 2005;94(3):275-80.
2. Fatihallah AA, Alsamaraay ME. Effect of Polyamide (Nylon 6) Micro-Particles Incorporation into RTV Maxillofacial Silicone Elastomer on Tear and Tensile Strength. Journal of Baghdad college of dentistry. 2017;325(5593):1-6.
3. Kurunmäki H, Kantola R, Hatamleh MM, Watts DC, Vallittu PK. A fiber-reinforced composite prosthesis restoring a lateral midfacial defect: a clinical report. The Journal of prosthetic dentistry. 2008;100(5):348-52.
4. Gibson T. The prostheses of ambroise pare. British journal of plastic surgery. 1955;8:3-8.
5. Barnhart GW. A new material and technic in the art of somato-prosthesis. Journal of Dental Research. 1960;39(4):836-44.
6. Bal BT, Yılmaz H, Aydın C, et al: In vitro Cytotoxicity of maxillofacial silicone elastomers: effect of accelerated aging. J Biomed Mater Res B Appl Biomater 2009;89:122-126
7. Zayed SM, Alshimy AM, Fahmy AE. Effect of surface treated silicon dioxide Nanoparticles on some mechanical properties of maxillofacial silicone elastomer. International journal of biomaterials. 2014;2014, Article ID 750398, 07
8. Kiat-Amnuay S, Mekkayarajjananonth T, Powers JM, Chambers MS and Lemon JC. Interaction of pigments and opacifiers on colour stability of MDX4-4210/type A Maxillofacial elastomers on artificial ageing. J Prosthet Dent 2006;95:249-57.

9. Kamikawa Y, Mori Y, Nagayama T, Fujisaki J, Hirabayashi D, Sakamoto R, Hamada T, Sugihara K. Frequency of clinically isolated strains of oral Candida species at Kagoshima University Hospital, Japan, and their susceptibility to antifungal drugs in 2006–2007 and 2012–2013. BMC Oral Health. 2014 Dec;14(1):1-9.
10. Coenye TK, De Prijck K, Nailis H, J Nelis H. Prevention of Candida albicans biofilm formation. The Open Mycology Journal. 2011;5(1):09-20.
11. Spielmann N, Wong DT. Saliva: diagnostics and therapeutic perspectives. Oral diseases. 2011;17(4):345-54.
12. Mandracci P, Mussano F, Ricciardi C, Ceruti P, Pirri F, Carossa S. Low temperature growth of thin film coatings for the surface modification of dental prostheses. Surface and Coatings Technology. 2008;202(11):2477-81.
13. Mouzakis DE, Papadopoulos TD, Polyzois GL, Griniari PG. Dynamic mechanical properties of a maxillofacial silicone elastomer incorporating a ZnO additive: The effect of artificial aging. J Craniofac Surg 2010;21:1867-71
14. El-Nour KM, Eftaiha AA, Al-Warthan A, Ammar RA. Synthesis and applications of Silver Nanoparticles. Arab J Chem 2010;3:135-40.
15. Agnihotri R, Gaur S, Albin S. Nanometals in dentistry: Applications and toxicological implications—A systematic review. Biological trace element research. 2020;197(1):70-88.
16. Chandra J, Mukherjee PK, Ghannoum MA. In vitro growth and analysis of Candida biofilms. Nature protocols. 2008;3(12):1909-24.
17. Singh AK, Prakash P, Achra A, Singh GP, Das A, Singh RK. Standardization and classification of in vitro biofilm formation by clinical isolates of Staphylococcus aureus. Journal of global infectious diseases. 2017;9(3):93.
18. Hawser SP, Douglas LJ. Biofilm formation by Candida species on the surface of catheter materials in vitro. Infection and immunity. 1994 Mar;62(3):915-21.
19. G. Liu, Y. Li, F. Yan, Z. Zhao, L. Zhou and Q. Xue. Effect of Nanoscale SiO2 and TiO2 as the Fillers on the Mechanical Properties and Aging Behaviour of Linear Low-Density Polyethylene/Low-Density Polyethylene Blends,J Polym Environ 2005;13(4): 339- 348.
20. Han Y, Kiat-amnuay S, Powers JM, Zhao Y. Effect of nano-oxide concentration on the mechanical properties of a maxillofacial silicone elastomer. The Journal of Prosthetic Dentistry. 2008;100(6):465-73.

21. Shakir DA, Abdul-Ameer FM. Effect of nano-titanium oxide addition on some mechanical properties of silicone elastomers for maxillofacial prostheses. J Taibah Univ Med Sci 2018;13:281-90
22. Tukmachi M, Moudhaffer M. Effect of nano silicon dioxide addition on some properties of heat vulcanized maxillofacial silicone elastomer. IOSR-JPBS. 2017;12(3):37-43.
23. Sonnahalli NK, Chowdhary R. Effect of adding Silver nanoparticle on physical and mechanical properties of maxillofacial silicone elastomer material-an in-vitro study. Journal of Prosthodontic Research. 2020;64(12)431-435.
24. Govindankutty D. Applications of nanotechnology in orthodontics and its future. Inter J App Den 2015
25. Ramazanzadeh B, Jahanbin A, Yaghoubi M, Shahtahmassbi N, Ghazvini K, Shakeri M, Shafaee H. Comparison of antibacterial effects of ZnO and CuO nanoparticles coated brackets against streptococcus mutans. J Dent (Shiraz) 2015;16:200–205
26. Allaker RP. The use of nanoparticles to control oral biofilm formation. Journal of Dental Research 2010;89(11):1175-1186.