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ASSESSMENT OF ADDING NANO TITANIUM OXIDE PARTICLES ON BIOMECHANICAL BEHAVIOR OF HIGH- TEMPERATURE VULCANIZED MAXILLOFACIAL SILICONE ELASTOMERS

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ABSTRACT

Objective: This study assessed the influence of titanium oxide nanofiller incorporation into MDX4-4210 maxillofacial silicone regarding biomechanical changes.

Methods: Nano titanium oxide powder (P25, Degussa, Germany) was mixed to high-temperature-vulcanized (HTV) silicone (MDX4-4210, Dow Corning, USA) at 0.2 wt%. For testing the cytotoxicity, two groups of silicone specimens, ten specimens each were prepared. Group A: Blank MDX4-4210 silicone (control group); Group B: Nano titanium oxide powder was added to MDX4-4210 silicone; each group was assessed after 24, 48, and 72 hours. For testing mechanical properties 60 specimens were prepared, 20 for testing tear strength, 20 for testing ultimate tensile strength, and 20 for testing hardness. Each group was subdivided into two identical categories: (I) 10 control specimens without nano-titanium oxide, and (II) 10 experimental specimens with nano-titanium oxide powder. The specimens were assessed and one way (ANOVA) test was utilized to analyze the data.

Results: After 24-hour, in the control groups, the cytotoxicity values were higher than those of titanium oxide nanofiller group. There were marked improvements in the mean values of all the tested mechanical properties

Conclusion: incorporating nano-titanium oxide particles improves the biocompatibility and mechanical properties of MDX4-4210 maxillofacial silicone materials.

KEY WORDS: Cytotoxicity, Mechanical Properties, Silicone maxillofacial material, Nano titanium oxide filler.

INTRODUCTION

The demand for prosthetically restoring lost facial parts and tissues congenitally or due to trauma, tumours or disease is dramatically increasing. There are different restrictions for surgical and prosthetic rehabilitations.[1] Surgical reconstruction may be restricted by the accessibility of tissue, the decreased

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blood supply due to radiation, the demand to see the inner portion of the wound, and the patient's age and systemic condition. Prosthetic reconstruction of facial defects is the only alternative if the surgical reconstruction is not possible.[2-4]

The success of a facial prostheses depends on several factors such as support, stability, and retention.[5] Although many maxillofacial materials are available as polyvinyl chloride (PVC), polyurethane, acrylic resin, and silicones, the ideal material for replacing living movable tissues has not been found. Silicone, however, has been judged to be one of the best and most widely used materials due to its permanence, ease of preparation and application, and it is considered innert.[6,7] In the majority of cases facial prostheses should have very thin margins to blend with the skin, thus producing acceptable esthetic results. The tear strength of the material used is therefore of paramount clinical importance. Although the tear strength of silicone has been found acceptable when compared to other materials, it is still considered weak and the material can easily tear with continuous use. Color instability, tearing and cracks of silicone are the most common causes that make the patient ask for changing his maxillofacial prostheses.[8]

Maxillofacial material should be biocompatible and noncarcinogenic.[9] Several researches were carried out to study the effect of adding some reinforcing materials to the silicone maxillofacial material in a trial to improve its physical and mechanical properties.[10,11]

Nano technology can produce new polymers with improved flexibility as a result of reinforcement with nano oxide particles. However, its biological effect is very important to be confirmed. [12,13,14,15]

So, this experimental study aimed to evaluate the effect of incorporating titanium oxide nanofiller on the cytotoxicity, tear strength, ultimate tensile strength, hardness and elongation percentage of maxillofacial silicone elastomers.

MATERIALS AND METHODS

Nano titanium oxide powder (P25, Degussa, Germany) was mixed to MDX4-4210 high- temperature-vulcanized (HTV) silicone, (Dow Corning, USA Factor II, Inc., Lakeside, AZ, USA) at 0.2 wt%. For testing the cytotoxicity, two groups of silicone specimens, ten specimens each were prepared. Group A: Blank MDX4-4210 silicone (control group); Group B: Nano titanium oxide powder was added to MDX4-4210 silicone. All the maxillofacial Silicone elastomers were prepared by the same investigator. Each group was assessed after 24, 48, and 72 hours. A metal mold with dimensions of 2 mm height x 10 mm diameter was prepared. In order to improve the viscosity and avoid voids entrapment during mixing nanoparticles and silicone, a thixotropic agent was added to the mix following the manufacturer's instructions. Silicones were manually mixed and poured onto the mold cavity. A flask press was used to press the mold at 1 mm/bar pressure and then inserted in a polymerization oven at a 135°C for 30 minutes. To prevent bacterial contamination, the specimens were sterilized in an autoclave (Charisma vacuum TD, Italy) Prior to the microbiological evaluation,

Evaluation of Biocompatibility

An MTT assay (Cat no.: 1-0011; Immuno Biotech, Stillwater, OK) was used to assess Cell viability. L-929 mouse fibroblast cells were taken from stock-frozen cell lines and cultivated in Dulbecco's Modified Eagle's Medium (DMEM) (BiochromAG, Berlin,Germany), with 10% fetal bovine serum (FBS) and 0.2% penicillin/streptomycin at 37°C in 5%CO2 in air. The cells were sat on in 96-well plates (100 µL/well) with a density of 4×104 cells/mL. The cell culture medium was detached after 24 hours, and then add a fresh medium of the experimental materials. Crude untreated cells act as controls. The cells were incubated for 3 days. According to the manufacturer's protocol, MTT assay was carried out each day. An inverted microscopy (IX70; Olympus, Tokyo, Japan) was used to see the

cell morphology. 10 µL of MTT reagent were added after 24, 48, and 72 hours of incubation, and incubated for 4 hours. Then, 100 µL of solubilization solution were added and incubated overnight. After that, a UV visible spectrophotometer (EZ Read 400 Microplate reader; Biochrom, Cambourne, UK). was used to evaluate the absorbance at 560 nm.

SPSS (Version 23) software package (SPSS, Chicago, IL) was used to analyze the collected data. A paired sample t-test was utilized to analyze the difference after 24, 48, and 72 hours in each experimental group. To evaluate the group differences in cytotoxicity after 24 hours, one-way ANOVA test were used. A difference was considered statistically significant at $p < 0.05$.

To evaluate the mechanical properties, a total of 60 MDX4-4210 specimens were prepared, 20 for tear strength, 20 for tensile strength, and 20 for hardness. Each group was again divided into two identical categories: (I) 10 control specimens without nano-titanium oxide, and (II) 10 experimental specimens with nano- titanium oxide powder

Adding of nano-titanium oxide powder to the silicone base (part A)

At first, a clean mixing container was meditate on by a digital scale and then nano titanium oxide powder was putted in the container and weighed again and then gradually put previously weighed silicone base and mix them by clean hand spatula for 1min. then mixing continued for 2 min in an automatic mixer without turning the vacuum on to prevent suction of the nano particles.[16, 17] Then turn on vacuum at 28 in Hg. to avoid formation of air bubbles and continue mixing for 7 min. [18,19] the mix was left a side for 2 minutes as mixing produce heat that affect the working time of silicone.[20]

Adding of the catalyst (part B)

The catalyst (Part B) is added to the base (part A) following the manufacturer's instructions at a humid atmosphere at 23 ± 2 °C and mixed by automatic mixing machine with vacuum for 5 min. and then the mix is loaded into a syringe for packing it into the molds.

Packing the mix inside the metal mold cavities

The metal molds and glass slabs were painted with a separating solution and wait to dry.[19] Glass slab bottom had been secured to the metallic matrix. The metal mold was loaded by silicone mix and left for 1 min to guarantee bubbles free mold then the mold was covered by the cover glass slab and one kg weight placed over the mold. The slab was now placed into a preheated oven and left for 1 h, following the manufacturer's instructions.[21,22] The specimens were cautiously demolded[23] and inspected for defects, which were excluded.[24] The perfect specimens were left in a modified box in temperature of 10-30 °C. according to the manufacture instruction.[25] 24 h before testing ,The specimens were placed at a temperature of 23 ± 2 °C for a minimum of 3 h after removing the flash with a surgical blade and scalpel.[26, 27]

Evaluation of the Mechanical properties

Tear strength

It calculates of the resistance of a subject to tearing forces. Tear strength test was conducted according to the ASTM NO D624 procedure using unnicked 90-degree angle shaped specimen with the dimensions given in (Fig.1). Specimen thickness was 1.8 mm.The specimens were stretched at a constant rate of 50 cm / min in an Instorn Testing Machine (Universal Testing Machine, Instron 1195, USA.) [22] in the research lab of the Faculty of Dentistry, Taif University.

Tear strength was calculated by the following equation:

$$T = F / D \quad Kg / mm.$$

Where: T = tear resistance, F = breaking force, and D = thickness of the specimen.

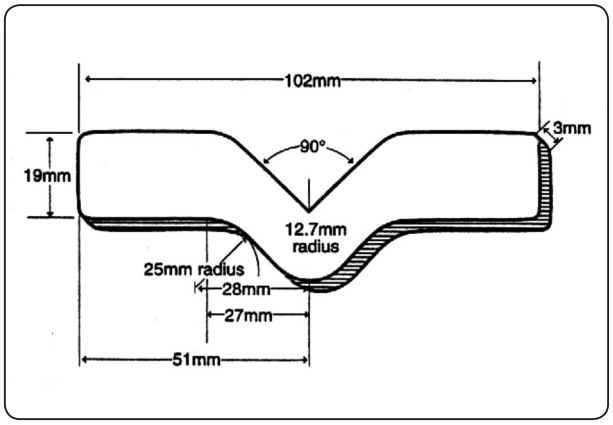

Fig. (1): ASTM NO D624 specifications for trouser-shaped specimens.

Hardness test

25 x 25 x 6 mm^3 cube specimens were prepared according to ASTM D2240. The hardness of the specimens was tested at five separate points. Each point was located 6 mm away from each other and away from the border. Type A Shore hardness digital tester was used for assessment.[27, 28]

Tensile strength test

Dumbbell shaped specimens was designed according to the ISO 37 test using a computer-controlled universal testing machine (Instorn Testing Machine, Instron 1195, USA.). 6mm wide, 115mm long, and 3mm thick were prepared according to manufacturer's instructions (Fig.2). The specimens selected for testing were ideal without cracks, voids or irregularities on the surface. The test specimens were vertically aligned in the grip holder. The upper part of the apparatus moved at a standard rate of 5 cm/min while the lower part remains fixed.[29]

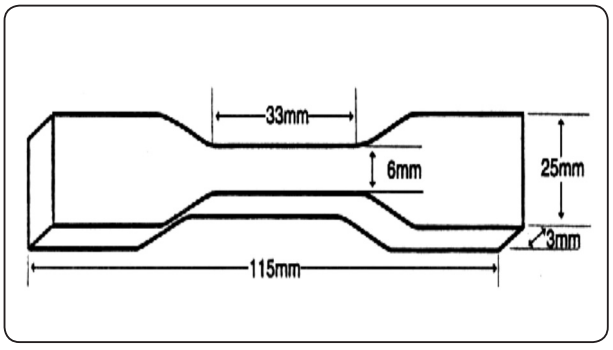

Fig. (2): Specifications for dumbbell-shaped specimens

Tensile strength was determined by dividing the maximum load (L) by the original cross sectional area (A), where L was the force needed to break the specimen while A was the cross sectional area of the unstretched specimen. (width x thickness of unstretched specimen) .[(29)]

$$\text{Tensile Strength} = L / A \quad Kg/cm^2$$

Elongation percentage test

The deformation that results from the application of a tensile force is elongation. The percentage elongation (% EL) was calculated as follows:

% Elongation = increase in length / original length x 100

Statistical analysis

The data was collected and transferred into SPSS (Version 23) and the statistical analysis was performed using one way ANOVA test. Pair wise comparison between groups was made with the Mann Whitney U test at 0.05 level of significance.

RESULTS

Cytotoxicity

Table 1 shows results of viability test after 24-hour incubation period, the MTT assay revealed that there was a high cell viability (1.02) for both the control group A (MDX4-4210 silicone) and experimental Group B (silicone reinforced with titanium oxide powder). After 24 hours, there was statistically significantly difference between the two groups. The cell viability test for both groups showed marked increase by time from 24 - 48 hours which was statistically significant in the two groups, while the results of the cell viability from 48 - 72 hours was statistically insignificant (Table 2).

TABLE (1) Results of viability test after 24 hours

MDX4-4210 Silicone	n: 20	Group A (Control)	1.02 NS
		Group B (TiO2 addition)	1.09 NS

The results indicate insignificant difference at (p > 0.05).

NS: Not significant

TABLE (2) Cell viability after different periods of incubation

Test groups	*n*	24 hours	48 hours	72 hours
Group A	10	1.02	1.22	1.33
Group B	10	1.09	1.36	1.30

The results indicate significant difference.

Mechanical Properties

The mean values and standard deviations of tear strength, hardness, ultimate tensile strength, and percent elongation of both groups are shown in table (3). Pair wise comparison between groups was made with the Mann Whitney U test at a 0.05 level of significance.

Tear strength test (kg/mm)

Regarding tear strength, group II (MDX4-4210 silicone with titanium oxide nanofiller incorporation) had the higher tear strength values (mean = 3.29 ± (0.19)) than group I (control group) (mean = 1.21 ± (0.09)). The Mann- Whitney U test showed that there was also a significant difference among the groups for tear strength. (G I – G II = -1.698). There was highly statistically significant difference with P > 0.05.

Hardness test (Kg/cm2)

The mean values and standard deviation of the Shore A hardness of the control group was 2.37 ± (0.28) for and 6.94 ± (0.79) for the experimental group. The Mann- Whitney U test revealed that there was also a significant difference among the groups for modulus of elasticity (G I-G II = -1.760). There was highly statistically significant difference between the two groups with $p < 0.05$.

Tensile test (Kg/cm2)

Group II exhibited higher tensile strength (mean = 10.84 ± (0.20(), than group I (control) was (mean = 7.80 ± (0.19)) (P<0.05). The Mann- Whitney U test revealed that there was also a significant difference among the groups for ultimate tensile strength. (G1-G II = -2.170).A highly significant difference was found among both the experimental and control groups for ultimate tensile strength. (P> 0.05)

Elongation percentage (%) test

The mean values and standard deviation of the Elongation percentage (%) test of the (HTV) MDX4-4210 silicone groups which were 457.70 ± (6.52) for the control group and 400.43 ± (8.04) for the experimental group. There was highly statistically significant difference between the two groups with p < 0.05.

TABLE (3) Mean values and standard deviations of the mechanical properties studied for the both groups.

Groups		**Mechanical Properties**			
		Tear strength (kg/mm)	**Hardness (Kg/cm2)**	**Ultimate tensile strength (Kg/cm2)**	**Percent elongation (%)**
I	**Mean ± (SD)**	1.21 ± (0.09)	2.37 ± (0.28)	7.80 ± (0.19)	457.70 ± (6.52)
II	**Mean ± (SD)**	3.29 ± (0.19)	6.94 ± (0.79)	10.84 ± (0.20)	400.43 ± (8.04)
G I - G II	**Mann Whitney P-value**	-1.698 0.0014*	-1.760 0.0013*	-2.170 0.0010*	-2.611 0.0007*

** Statistically significant difference (p> 0.05)*

DISCUSSION

There are so many techniques used to test the biologic effects of different dental materials namely, the MTT assay, agar overlay test, and Millipore filter test.[32] MTT assay, is a good and widely used method to test the biocompatibility of dental materials so it had been used in the present study. The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.[32]

The MTT is decreased by mitochondrial succinate dehydrogenase to give a blue formazan product, which accumulates in cells as it does not pass through the plasma membrane. Then adding isopropanol acid will lead to lysis of the cell membranes and release of the solubilized formazan product. The yellow tetrazolium MTT salt is changed by the action of dehydrogenase into a purple dye, MTT-formazan in the presence of live cells. This purple dye can be easily measured by a spectrophotometer.[33]

The MTT results show the cell number and the intensity of cell metabolism which allow simple, rapid, and repeated tests and the absence of radioisotopes so it is considered a sensitive index of the cytotoxicity of dental materials.[33,34] in this research there were a direct relation between the duration of the incubation period (from 24 to 72 hours) and the cell survival.

Srivastava et al. concluded that Nano-titanium oxide has been used in a lot of industries owing to its good physical and chemical properties. On the other hand its usage leads to pulmonary heart disease. Oxidative stress, Genotoxicity, lung cancer and skin injury. These serious health problems are mainly associated with inhalation routes of exposure. [35,36]

Cytotoxicity of silicone elastomers reinforced with titanium oxide nanoparticles were assessed by El-Fray et al. who found that these elastomers were nontoxic.[37] the outcome of current work were in harmony with those of El-Fray et al, in spite that silicone elastomers used in both studies were different.

Nano titanium oxide powder (P25, Degussa, Germany) were added to improve the mechanical properties of high- temperature-vulcanized (HTV) silicone (MDX4-4210, Dow Corning, USA) at 0.2 wt%. It was applied following the safety data sheet instructions.[38, 39]

Although numerous advances in materials for facial prostheses have been made in the past several years, the need for improvement continues. The materials should stay stable and not affected by ecological factors like ultraviolet rays, oxygen, secretions (salivary, nasal, sebaceous), and adhesives and their solvents, It must be non toxic, non carcinogenic, non allergic, biocompatible, It must resist stains. It should have high tear strength, high ultimate tensile strength, acceptable elongation percentage and satisfactory hardness.[38] The improvement of mechanical properties depends on the filler amount, particle size, polymer properties, and processing conditions.[39]

There was a highly significant improvement ($p < 0.05$) when nano-titanium oxide was incorporated

(HTV) MDX4-4210. The titanium oxide powders enhance in the development of cross-linked arrangement in the silicone mix resulting in improvement of tear strength of the tested material. So adding titanium oxide strengthen the maxillofacial silicone elastomers.[40, 41] Tear strength improved with the slightly reduction in time of curing.[42] The findings of current study is parallel to those of earlier studies.[10, 20]

There was a significant elevation in Shore A hardness when 0.2 wt% of nano titanium oxide was added to HTV silicone. Results revealed improvement of the hardness but within limits. Adding of nano particles to silicone improve the cross-linkage density and elastic modulus of silicone. [43, 44, 45]

These findings are in accordance with the results other studies which recommended adding of nano particles to improve the hardness of silicone elastomers.[20,46] There was a significant improvement in the ultimate tensile strength and elongation values of cured silicone when nano titanium oxide particles were incorporated in concentrations of 0.2wt% to HTV silicone materials. These results can be explained that nano particles enhance the cross-linkage and density of the polymer making it more stiff and strong and improving the tensile strength.[47,48] The tensile strength and percentage elongation results of this study were in agreement with other studies.[10,18]

CONCLUSION

Adding titanium oxide nanoparticles to maxillofacial silicone elastomer as a reinforcing material is considered biologically accepted. However, further studies the cytotoxicity should be carried out prior to clinical usage. Also, nano titanium oxide incorporation into MDX4-4210 maxillofacial silicone elastomers improves tear strength, the hardness, tensile strength, and elongation percentage.

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