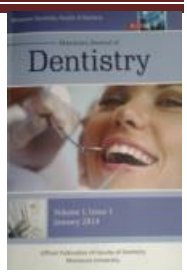

Evaluation of Biologic and Some Physical Properties of Flexible Resin Modified with Antimicrobial Nanostructures

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Abstract:
Objectives:: The present study was designed to evaluate the impact of addition of different antimicrobial nanostructures to flexible resin versus the conventional heat cured acrylic resin type regarding cell cytotoxicity, surface roughness and impact strength.
Methods: Polyamide and heat cured polymethyl methacrylate were used in this study and both were modified with silver vanadate and titania nanorods at different concentrations**.** For cytotoxicity test a total number of 60 disc shaped specimens (30 for each type of resin), were used in cell vitality test. For Surface roughness, a total number of 40 disc shaped specimens for both types of of resin was tested using a two dimensional profilometer. For Impact strength, a total number of 40 rectangular shaped specimens for both types of resin were prepared and measured using impact testing machine using charpy method. Four way ANOVA test was used for cytotoxicity test and two way ANOVA was used for both surface roughness and impact tests. Means and standard deviations (SD) was calculated for the tested groups. Statistical analysis was conducted using SPSS version 16 (Statsitical Packacge for scientific studies). The t test was used for all tests for comparison between groups.
Results: Cytotoxicity of subdivision IAC_4D_2 (heat cured with 1% silver vandate after 48 hrs) was the highest value while $IIBC_4D_1$ (flexible with 1% titania after 24 hrs) was the lowest value. Surface roughness and impact strength of division $IIBC_4$ (Flexible with 5% titania) was the highest while division IBC_1 ((heat cured with 0 % titania) was the lowest.
Conclusions: Silver nanorods have an adverse on cytotoxic effect of both flexible and heat cured acrylic resins. Titania nanorods are biocompatible materials. They gave no cytotoxic effect with flexible resin. Ttitania nanorods increased the impact strength of both flexible and heat cured acrylic resins. Flexible resin had less cytotoxic effect than heat cured acrylic resin. Flexible resin modified with titania nanorods had higher impact strength and surface roughness than those of heat cured acrylic resin.
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Introduction

Loss of teeth is one of the major concern to patient both esthetically as well as functionally, so their replacement by artificial substitutes such as dentures is mandatory. Denture base acts as an intermediate medium between teeth and jaw which transfers all or part of the masticatory forces to the subadjacent tissues.[1]

The perfect denture base material should have several properties as biocompatibility, high bond strength, good esthetics with existing teeth of denture, radioopacity, and should have satisfactory mechanical and physical characteristics.[2] Usage of acrylic resins (ARs) was common owing to their suitable esthetics and advantageous features.[3] A main problem of these materials is the dimensional change during processing, habitually due to the polymerization shrinkage. Consequently, flexible resins (FRs) were presented as a substitute to acrylic resins usage in the fabrication of partial removable appliances and complete ones.[4]

Fitting of prosthetic devices within the oral cavity promotes the deposit of biofilms on dental surfaces and on the prosthetic device and changes the oral conditions.[5] The accumulation of microorganisms favored by the surface irregularities and porosity of acrylic resins, is responsible for oral disorders, such as candidal infection and gingivitis.[6,7] Prosthetic replacement and use of drugs are considered as solutions to these problems. Also, the use of prosthetic appliances with antimicrobial properties may increase the excellence of patients life and reduces discomfort and extra prices.[8,9,10]

Revolution of the nylon based denture base material gives rise to new types of dentures. Flexible dentures are brilliant option over traditional dentures made from methyl methacrylate (MMA), which offer comfort and superior esthetics and adaption to the flexibility and continuous movement in patients which are partially edentulous.[11,12]

Nanotechnology means materials development at the nanoscale, which enables effective control of the matter structure at dimensions of nanoscale, that makes nanoparticles interesting. Nanotechnology includes devices, systems and materials with chemical, biologic and physical properties that differ from those of large-scale structures.[13]

Silver nanoparticles are commonly recognized for their antimicrobial properties.[14] Therefore, compounds containing silver nanoparticles are effective owing to antimicrobial properties against *Streptococcus mutans*, which is the most dangerous cariogenic microorganism found in oral biofilm.[15]

The synthesized titania nanoparticles (TiO_2) were found to be effective against *E.coli, Staph.aureus, C.albicans, and B.subtilis*. Titania nanoparticles may be an acceptable inorganic antimicrobial agents.[16]

So assesment of the impact of addition of silver vanadate and titania nanorods on some biological and physical properties of flexible resin might be significant.

MATERIALS AND METHODS

The materials used in the present study are shown in Table 1.

Specimen preparation

A total number of 140 Specimens were prepared and divided into two main groups:

Group I: 70 specimens constructed of heat cured acrylic resin

Group II: 70 specimens specimens constructed of flexible resin.

SubgroupA: addition of silver vanadate nanorods.

*Subgroup B:*addition of titania nanorods.

Then Subgroups divided into 2 divisions: *Division 1:* 0 wt%, *Division 2:* 1 wt% then subdivided into two subdivisions. *Subdivision 1: 24 hrs, subdivision 2: 48 hrs.*

For surface roughness and impact strength the subgroups divided into 4 divisions: *Division 1:* 0 wt%, *Division 2:* 1 wt%, *Division 3:* 2.5 wt%, *Division 4:* 5 wt%..

Materials / Nanorods			I	II
A	C_1	D_1	IAC_1D_1	$IIAC_1D_1$
	C_4		IAC_4D_1	$IIAC_4D_1$
	C_1	D_2	IAC_1D_2	$IIAC_1D_2$
	C_4		IAC_4D_2	$IIAC_4D_2$
B	C_1	D_1	IBC_1D_1	$IIBC_1D_1$
	C_4		IBC_4D_1	$IIBC_4D_1$
	C_1	D_2	IBC_1D_2	$IIBC_1D_2$
	C_4		IBC_4D_2	$IIBC_4D_2$

Materials / Nanorods		I (Heat cured)	II (Flexible)
A	C_1	IAC_1	$IIAC_1$
	C_2	IAC_2	$IIAC_2$
	C_3	IAC_3	$IIAC_3$
	C_4	IAC_4	$IIAC_4$
B	C_1	IBC_1	IBC_1
	C_2	IBC_2	IBC_2
	C_3	IBC_3	IBC_3
	C_4	IBC_4	IBC_4

Preparation of silver vanadate nanorods

100 ml of equal concentrations (0.005M) of both ammonium vanadate (NH_4VO_3) and silver nitrate($AgNO_3$) were prepared in deionized water. Prepared solution of $AgNO_3$ was added and mixed vigorously using magnetic stirrer to solution of NH_4VO_3 drop by drop at room temperature and fixed pH (~ 4.6). During fraternization process, the pH of the product varied from 4.6 to 5.8 and yellow colored precipitate was synthesized at a final pH of 5.8.[17]

Preparartion of Titania nanorods

Hydrothermal process was employed to the synthesis of TiO_2 nanorods using a chemical process. In a typical preparation procedure,[83] anatase TiO_2 white powders was

placed into a teflon lined autoclave. Then, NaOH aqueous solution addition up to 80% of the total volume of a sealed stainless steel tank maintained at 200 °C for 24 hrs in the autoclave without shaking or stirring during the heating. When the autoclave was obviously cooled to temperature of the room, the achieved specimens was successively washed with aqueous solution of dilute HCl, deionized water and absolute ethanol for several times. Drying of the specimens was done at 70°C for 6 hrs. Lastly, soft white colored fibrous powder was produced.[18]

Examination of nanorods using transmission electron microscope

The morphologies of silver and titania nanorods were analyzed with transmission electron microscope (TEM). Figure 1 (A and B).

Mixing of heat cured acrylic resin with the nanorods

Heat cured resin specimens were prepared by mixing different concentrations of silver vanadate and titania nanorods with the acrylic resin polymer. After mixing the two powders, the monomer was added following the manufacturer instructions. Once it reached the dough phase, the resin was adjusted to the muffle mold using a hydraulic press. It was cured via short cured cycle. the flask was put in a water bath and the temperature was increased gradually from room temperature to 65 °C within 30 minutes and was kept at 75 °C for one and half hour, then it was kept at boiling for 30 minutes and the excess material was removed, followed by polishing with water sandpaper.[19]

Mixing of flexible resin with the nanorods

All cartridges containing grains of flexible resins were subjected to a ball mill technique that grind grains to fine particles, then nanorods with different concentrations have been added to grains of flexible resin cartridges.[20]

Cytotoxicity test (Cell viability test)

The cytotoxicity test was performed in Nile Center for Science and Technology, Mansoura, Egypt, under the supervision of professional staff. A total number of 60 disc shaped specimens 8mm x 2 mm have been prepared (30 for each resin).

Sterilization of the heat cured and flexible specimens

All the specimens were washed firstly with ethanol 70%, then phosphate buffered saline (PBS), before placing in 6 well plate. Such plates were further opened and the discs were exposed to UV light for 60 min to ensure for complete eradication of any microorganisms on the discs before performing the cytotoxicity test.[21]

Preparation of cells for cytotoxicity

A cryotube (1.5 ml) of cell line from human adipose tissue was taken from -80˚C freezer and dispensed in 8.5 ml Dulbecco's Modified Eagle's Medium (DMEM) that contains 10 % fetal bovine serum (FBS). The cell suspension was transferred to 10 cm^2 cell culture plate and incubated at 37°C, and 5% Co_2 to allow for growth of the cells. The confluence of the cells was checked daily using inverted microscope and when 80-90 % confluence was reached, the cells were trypsinized and splitted.[21,22]

Trypsinization of cells

Before splitting, the media was aspirated and the adhered cells were washed with sterile PBS, followed by addition of 1 ml trypsin EDTA (Ethylene diamine tetraacetic acid) (0.5 wt%) to initiate detachment of adherent cells, and finally 4 ml of the medium was added. The suspension of the detached cells was pipetted up and down several times to destroy cell clumps followed by counting of the cells in the suspension.[21,,22]

Counting of the percentage viable cells

Ten-fold serial dilutions (100 µl) of the cell suspension were prepared and mixed gently with 400 µl 0.4% trypan blue. Each trypan blue-treated cell dilution was applied to a glass hemocytometer and examined under microscope. Living cells do not take up trypan blue, while dead cells will appear blue-stained. The appropriate dilution was selected for determination of the number of the viable cells in the initial suspension according to the following equation: .[21,,22]

% Viable cells = [number of viable (non-stained cells)/ total number of viable and dead cells] x 100

Incubation of the discs with cells

After counting of the cells, 2 x 10^5 trypsinized cells were transferred to each well of 6-well plate. The volume in each well was completed to 2 ml with DMEM + 10 % FBS medium. Using sterile forceps. Each acrylic resin disk was placed into the corresponding labeled well. [107,108] For each disc, duplicate wells were investigated 24 and 48 hrs post incubation of the disc with cells. Duplicate wells without discs were included in the experiment as control. Incubation of each 6-well plate was done at 5% Co_2 and 37 °C. .[21,,22]

Determination of cytotoxicity effect

After 24 and 48 hrs of incubation of the discs with cells, the cells were trypsinized and the percentage of viable cells was determined in each well as previously discussed. The cytotoxic effect of each disc was expressed as percentage (%) of dead cells after subtracting the percent of viable cells from the cells in the control well incubated for the same period of incubation. .[21,,22]

Surface roughness

A total number of 40 disk shaped specimens (8 mm diameter x 2 mm thickness) were prepared (20 specimens for each resin). Surface roughness was performed using a two dimensional profilometer (Mitutoyo SJ-201-Japan). The specimens were fixed under the stylus in 3 different sites for each specimen. The average roughness (Ra) was measured by moving the stylus along the surface (0.25 mm cut-off). Data was calculated numerically by computer program in micrometer. Ra was calculated according to the following equation: [23] $\mathbf{Ra=\Sigma Rt/n}$ Where, **Rt** is the peak to valley height,, **n** is the number of peaks.

Impact strength

A total number of 40 rectangular specimens measuring 55 mm x 6 mm x 4 mm were prepared (35 specimens for each material type). The impact strength was evaluated using a test machine (Zowek Roell Amsler /RKP 450- Germany). A charpy method with a pendulum of 15 joules testing capacity was used to strike the specimens, in which the specimens were horizontally positioned with a distance of 40 mm between the two fixed supports, the charpy impact strength of un notched specimens was measured in KJ/m^2.[24]

The data obtained were tabulated for statistical analysis. Four way ANOVA test was used for cytotoxicity test and two way ANOVA was used for both surface roughness and impact tests. Means and standard deviations (SD) was calculated for the tested groups. Statistical

analysis was conducted using SPSS version 16 (Statsitical Packacge for scientific studies). The t test was used for comparison between groups for all tests.

RESULTS

Regrading cytotoxicity test, four way ANOVA (Table 2) showed the significant difference ($P<0.05$) between all specimens and the interaction between them.. Table 3 showed that the highest mean value (82%) was for subdivision IAC_4D_2 while the lowest mean value (0%) was for subdivision IBC_4D_1. There was significant difference between Subgroup A and B in all specimens.

As for surface roughness test, two way ANOVA (Table 4) showed the significant difference ($P<0.05$) between all specimens and the interaction between them. Table 5 showed that the highest mean value (0.956 µm) was for division $IIBC_4$ and the lowest mean value (0.45 µm) was for division IBC_1. There was significant difference between all specimens except between divisions $IIBC_1$ and $IIBC_2$.

In impact test, two way ANOVA (Table 6) showed the significant difference ($P<0.05$) between all specimens and the interaction between them. Table 7 showed that the highest mean value (4.03 kJ/m^2) was for division $IIBC_4$ and the lowest mean value (0.93 kJ/m^2) was for division IBC_1. There was significant difference between all specimens, except between divisions IBC_1 and IBC_2, and also between divisions IBC_3 and IBC_4 and between divisions $IIBC_1$ and $IIBC_2$.

DISCUSSION

For denture fabrication polymethyl methacrylate (PMMA) has been the most used material from the time when they were introduced. Polymethyl methacrylate PMMA posses many benefits such as an outstanding esthetic, low sorption of water and solubility, sufficient strength, low toxicity, ease of repair and simplicity of technique of molding. However, it has some drawbacks as polymerization shrinkage, lower impact strength, flexural strength and resistance to fatigue which bring about denture failure during chewing or dropping from hand of the patient. So enhancement of some properties of PMMA has been done as metal wires addition, fibers, and chemical structure modification. Nylon polymer has paying attention as a material of denture base in the current years. Polyamide resin was introduced as a material of denture base.[25]

Nanotechnology is now the most encouraging field to generate new applications in health care including dentistry. Nanoparticles of metal oxide are one of the most used applications of nanotechnology in dental materials such as dental restorative materials, denture bases and dental implants .[26]

One of the most applied nanoparticles are silver nanoparticles (AgNPs) that have been found to be effective due to their antimicrobial properties.[27] A main problem with AgNPs usage is the difficulty of homogeneously dispersing and incorporating the resin. Accordingly, nanostructured silver vanadate is an option material for health care usage as it prevents the agglomeration of nanoparticles, giving high surface contact with the microorganisms and greater antibacterial activity.[10]

TiO_2 nanoparticles are progressively used due to its good properties as lack of toxicity, chemically inertness, not expensive, antibacterial effect, high microhardness and resistance to corrosion.[28] Nanoparticles of TiO_2 have been used as additives to biomaterials so as to prompt antimicrobial properties.[29]

Cytotoxicity

The results of the current study showed that cytotoxicity of heat cured acrylic resins specimens had higher values than those of polyamide. It has been stated that the acrylic resins used for denture bases fabrication have showed many degrees of in vivo allergic responses and in vitro cytotoxicity. This cytotoxicity might be as a result of components that did not react and remained after the polymerization process.[30] Polymethyl methacrylate cytotoxicity in general is claimed by residual methyl methacrylate, that could be released from denture base. The released MMA could cause cell toxicity in vitro after incubation for 24 hours. Products of biodegradation of acrylic resins have been supposed of being a causal factor for chemical irritation, sensitization and pain of the oral mucosa, ulceration and oral diseases such as burning mouth syndrome and denture stomatitis. Allergic reactions related to acrylic resins revealed that MMA monomer and other additives were also related as benzoyl peroxide.[30]

Since thermoplastic resins were generated by addition polymerization reaction. This reaction involves long linear chains that were held together by forces of van der Waals that are weak. The long linear chains could move freely at high temperature without experiencing degradation.[31] Meanwhile, conventional heat cured acrylic resins are three-dimensional networks that were produced by condensation polymerization and hence high temperatures could degrade them. So, thermoplastic polymers were suggested to be more biocompatible biomaterial (compared with conventional heat-polymerized acrylic resin) as a result of their basic structure and the method of polymerization used.[31] Also polyamide is a material that is free of monomer. Currently, no enough knowledge about the causes of cytotoxicity of polyamide, what mechanism lead to the death of the cell and how it can be reduced. [30]

Silver vanadate nanorods have an adverse cytotoxic effect on both polyamide and heat cured acrylic specimens due to indirect involvement of silver nanorods in the mitochondrial toxicity and DNA damage. Mitochondrial respiratory chain disruption by silver nanoparticles may be a possible mechanism of toxicity resulting in production of reactive oxygen species (ROS) and also interruption of the adenosine triphosphate (ATP) synthesis, which in turn causes DNA damage.[32] This was in contrast with **Acosta-Torres et al.**,[33] who investigated cytotoxicity of acrylic resin containing silver nanoparticles, TiO_2 and Fe_2O_3 nanoparticles and found that the compound of PMMA-silver nanoparticle was not found to be cytotoxic or genotoxic, according to mitochondrial enzymatic activity and estimation of DNA replication.

Polyamide and heat cured specimens modified with titania nanorods exhibited lower cytotoxicity effect. This finding was in accordance with **Tsuji et al.**,[34] who investigated biocompatibility of denture base acrylic resin modified with titania coating and found that TiO_2-coated denture base resin has no irritation to the oral mucosa, nor

does it cause skin sensitization. Any leaching from the coating components has no toxic effects for tissues.

Since the cytotoxicity test of silver vanadatr modified heat cured acrylic resin reached (82%) after 48 hrs for heat cured acrylic resin, and (42%) after 48 hrs for that of flexible resin. Meanwhile the cytotoxicity of titania with flexible resin was (0%) which is reasonable to be used with vital tissues, Accordingly concentrations (1%, 2.5%, 5%) of titania was estimated to be investigated for its effect on physical properties.

Surface Roughness

Rougher surfaces may also lead to microbial colonization, formation of biofilm, discoloration of the prosthesis and can cause discomfort to patients. Different bacteria and fungi species have more ability for adhesion on rough surfaces of denture bases.[35]

The results of the current study showed that, titania nanorods have increased surface roughness of both heat cured and flexible resin specimens and this might be attributed to filler incorporation which increased roughness of denture base materials.[36] Heat cured acrylic resin specimens showed lower values of surface roughness than those of polyamide ones. This is due to higher finishing and polishing which could be easily done.[37] This result was in agreement with **Eghtedar et al.**,[38] who evaluated surface roughness of two polyamide material kinds used in the fabrication of denture base and compared them with a kind of heat cured polymethyl methacrylate denture base material and found that polyamide denture base material had higher surface roughness than that of polymethyl methacrylate. **El-Din et al.**[39] compared between heat cured poly methyl-methacrylate, thermoplastic polyamide and thermoplastic acetal resins regarding their surface roughness and found that polyamide demonstrated higher surface roughness than that of PMMA.

The higher surface roughness values of polyamide specimens were affected by the mold surface disintegration that was heated to a higher temperature than with the heat cured acrylic resin and also as a result of the pressure throughout injection molding. Difficult finishing and polishing of polyamides due to its low melting temperature have been reported.[35] During polishing of polyamide specimens, fraying at the margins was observed infrequently which occurred due to overheating and exposure of fibers.[37] **Menaka et al.,**[35] evaluated surface roughness of a polyamide denture base material in comparison with poly methyl methacrylate and found that PMMA acrylic denture base resin group exhibited significantly smoother surface than that of polyamide. Also **de Freitas Fernandes**, **et al.**[37] evaluated denture cleansers efficiency on biofilm of *Candida* formed on polyamide and PMMA resins and concluded that PMMA acrylic denture base resins group has significantly smoother surface than that of polyamide.

Impact strength

Impact strength is of an importance in dentures since dentures are subjected to sudden force which lead to their fracture if their impact strength isn't sufficient to resist theses forces. Fracture of dentures is a problem frequently meeted by removal of it by its wearers and dentists.[40]

Results of the current study showed that polyamide specimens showed higher impact values than that that of heat cured acrylic resins. This could be related to the properties of the chemical structure of polyamide, allowing it to absorb forces in a better way that is unlike those of PMMA. The finding of this study was in agreement with **Koray, et al.,**[41] who studied polyamide mechanical and thermal properties versus those of reinforced denture base of PMMA materials and found that injection molded thermoplastic resins had higher impact strength than the conventional heat polymerized acrylic resin.

The titania nanorods increased the impact strength of both polyamide and heat cured acrylic specimens. These results were in agreement with **Mohamed et al.,**[42] who studied the effect of incorporation of nano particles of TiO_2 on mechanical and physical properties of two dissimilar kinds of denture base acrylic resin and concluded that 1% TiO_2 could improve the impact strength of the conventional acrylic resin material. Besides, the incorporation of TiO_2 improved the properties of PMMA both mechanically and thermally.[43] **Asar et al**.,[40] evaluated the effect of different metal oxides on PMMA properties mechanically and physically and found that titania nanoparticles increased the impact strength of PMMA at both 1and 2wt%.

CONCLUSIONS

Based on the results and within the limitations of this study, the following conclusions can be drawn:

1.Silver nanorods have an adverse cytotoxic effect on both flexible and heat cured acrylic resins

2.Titania nanorods are biocompatible materials. They have no cytotoxic effect with flexible resin.

3.Titania nanorods increased impact strength of both flexible and heat cured acrylic resins.

4.Flexible resin modified with titania nanorods has higher impact strength and surface roughness than that of heat cured acrylic resin.

Table 1: Materials used in the study.

Material (product name)	Type	Lot no.	Manufacturer
Vetrex	Heat cured acrylic resin	LN114H03	Vertex-Dental.Johan van. Olden-bamevelten 623705 HJ Zeist. The Neteherlands.
Dentiflex Multipress injection system	A polyamide (nylon) thermoplastic polymer.	433206D	Rokodent. Bór 177, 42-200 CzęstochowaPoland
Silver vanadate nanorods			Prepared in national research center
Titania nanorods			

Table 2: Four-way ANOVA showing the effect of nanoparticles, materials, concentrations, time and the interaction on cytotoxicity.

Source	DF	Anova SS	Mean Square	F Value	Pr> F
Nano	1	0.5591162	0.5591162	6193.21	0.0001
Mater	1	0.2826702	0.2826702	3131.08	0.0001
Conc	1	0.0658971	0.0658971	729.93	0.0001
Time	1	0.3568163	0.3568163	3952.37	0.0001
nano*mater	1	0.0916563	0.0916563	1015.26	0.0001
nano*conc	1	0.5591162	0.5591162	6193.21	0.0001
mater*conc	1	0.111120	0.1111206	1230.86	0.0001
nano*mater*conc	1	0.0916563	0.0916563	1015.26	0.0001
nano*Time	1	0.0648637	0.0648637	718.48	0.0001
mater*Time	1	0.0047501	0.0047501	52.62	0.0001
nano*mater*Time	1	0.0011850	0.0011850	13.13	0.0010
conc*Time	1	0.0011165	0.0011165	12.37	0.0013
nano*conc*Time	1	0.0648637	0.0648637	718.48	0.0001
mater*conc*Time	1	0.0074127	0.0074127	82.11	0.0001
nano*mater*conc*Time	1	0.0011850	0.0011850	13.13	0.0010
Error	32	0.0028889	0.0000902		
Corrected total	47	2.2663155			

Table 3: Means and standard deviations (SD) of cytotoxicity (%) of both groups I and II.

Time	Subgroups	I		II		T value	P value
		Mean	SD	Mean	SD		
24 hrs	C_1	0.095^{A}	0.0001	0.082^{B}	0.00057	36.25	0.0001
	AC_4	0.539^{A}	0.0015	0.090^{B}	0.00057	476.5	0.0001
	BC_4	0.060^{A}	0.0015	0.000^{B}	0.00000	127.2	0.0001
48 hrs	C_1	0.320^{A}	0.0026	0.220^{B}	0.02650	6.64	0.0001
	AC_4	0.820^{A}	0.0005	0.420^{B}	0.00500	137.5	0.0001
	BC_4	0.091^{A}	0.0010	0.001^{B}	0.00020	149.4	0.0001

Means with similar superscript capital letter in one raw are not significantly different (P >0.05).

Table 4: Two-way ANOVA showing the effect of nanoparticles, materials concentrations and the interaction on roughness.

Source	DF	Anova SS	Mean Square	F Value	Pr> F
Mater	1	2.12941875	2.12941875	5774.69	0.0001
Conc	3	0.10160625	0.03386875	91.85	0.0001
mater*conc	3	0.00185625	0.00061875	1.68	0.1913
Error	32	0.01180000	0.00036875		
Corrected total	47	2.25739792			

Table 5: Means and standard deviations (SD) of roughness (μm) of I and II related to subgroup B.

Subgroup divisions	I		II		T value	P value
	Mean	SD	Mean	SD		
BC_1	0.450^{Bd}	0.0100	0.856^{Ac}	0.015	38.58	0.0001
BC_2	0.470^{Bc}	0.01	0.876^{Ac}	0.005	61	0.0001
BC_3	0.503^{Bb}	0.005	0.910^{Ab}	0.01	61	0.0001
BC_4	0.536^{Ba}	0.005	0.956^{Aa}	0.011	56.35	0.0001

Means with similar superscript capital letter in one raw and similar superscript small letter in one column are not significantly different (P >0.05).

Table 6: Two way ANOVA showing the effect of nanoparticles, materials, concentrations and the interaction on impact strength.

Source	DF	Anova SS	Mean Square	F Value	Pr> F
Mater	1	43.56735208	1.50062431	5501.8	0.0001
Conc	3	1.79312292	0.59770764	75.48	0.0001
mater*conc	3	4.50187292	1.11029097	140.21	0.0001
Error	32	0.25340000	0.00791857		
Corrected total	47	74.3561979			

Table 7: Means and standard deviations (SD) of Impact (kJ/m^2) of I and II related to subgroup B.

Subgroup Divisions	I		II		T value	P value
	Mean	SD	Mean	SD		
BC_1	0.93^{Bb}	0.11	3.53^{Ac}	0.05	34.88	0.0001
BC_2	1.03^{Bb}	0.05	3.66^{Abc}	0.11	35.33	0.0001
BC_3	1.13^{Ba}	0.05	3.83^{Ab}	0.15	28.64	0.0001
BC_4	1.16^{Ba}	0.05	4.03^{Aa}	0.05	60.81	0.0001

Means with similar superscript capital letter in one raw and similar superscript small letter in one column are not significantly different (P >0.05).

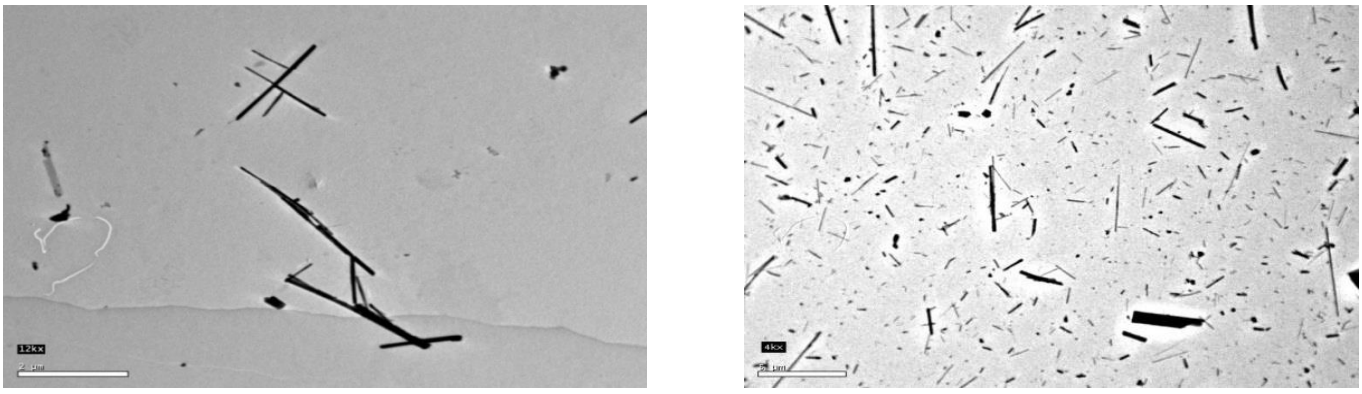

Figure 1: A) Silver nanorods under TEM with 12 kx (12,000) magnification. B) Titania nanorods under TEM with 4 kx (4,000) magnification.

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