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Effect of Silver Nanoparticles on Some Physical& Biological Properties of Fluid Denture Base Material

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Abstract

Poly methyl methacrylate (PMMA) is one of the most widely used materials in modern prosthodontics. It is widely known due to its simplicity in use and acceptable aesthetic. A new concept of polymerization fluid resin technique was instead of heat and gypsum material. Forty specimens were prepared from two brands of fluid acrylic resin. The samples were divided into two groups, the first one was the control while the other group was incorporated with silver nanoparticles(modified). The tests performed were impact strength, transverse strength, color stability and candida retention ability on the samples. 10 samples for each test were used. The results showed that modified group had significantly higher impact strength than the control group. There was non-significant difference between group of modified fluid acrylic and control group. Regarding color stability, there were highly significant color change after 10 days of immersion in the tea solution for the modified group the results of the biological test showed that the candida retention of the control group was significantly higher than modified group.

Within the limitation of this study it can be concluded that addition of silver nanoparticles has resulted in significant difference between control and modified group for impact strength test. While non-significant difference was seen for transverse strength test. In regard to color stability, showed enhancement in color stability for both before and after placement in tea solution. Addition of silver nanoparticles also caused reduction in candida albicans retention in the added samples.

Keywords: PMMA, fluid resin, Impact strength, Candida albicans.

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الخلاصة

ميثيل ميثاكريليت هي واحدة من أكثر المواد المستخدمة على نطاق واسع بسبب بساطته في الاستخدام والجمالية المقبولة . كان المفهوم الجديد لتقنية راتنج السوائل بلمرة بدلاً من الحرارة ومواد الجبس .تم تحضير أربعين عينة من نوعين من راتينج الأكريليك السائل .تم تقسيم العينات إلى مجموعتين ، الأولى كانت السيطرة بينما تم دمج المجموعة الأخرى مع الجسيمات النانوية الفضية) المعدلة .(كانت الاختبارات التي تم إجراؤها هي قوة التأثير ، والقوة العرضية ، واستقرار اللون ، وقدرة احتباس المبيضات على العينات .تم استخدام ١٠ عينات لكل اختبار .أظهرت النتائج أن المجموعة المعدلة لديها قوة تأثير أعلى بكثير من المجموعة الضابطة .لم يكن هناك فرق معنوي بين مجموعة أكريليك مائع معدل ومجموعة تحكم .فيما يتعلق بثبات اللون ، كان هناك تغير كبير في اللون بعد ١٠ أيام من الغمر في محلول الشاي للمجموعة المعدلة ، أظهرت نتائج الاختبار البيولوجي أن احتباس المبيضات من المجموعة الضابطة كان أعلى بكثير من المجموعة المعدلة.

في حدود هذه الدراسة ، يمكن استنتاج أن إضافة الجسيمات النانوية الفضية قد أدى إلى اختلاف كبير بين المجموعة الضابطة والمعدلة لاختبار قوة التأثير .بينما شوهد اختلاف غير معنوي لاختبار القوة العرضية .فيما يتعلق بثبات اللون ، أظهر تحسنًا في استقرار اللون قبل وبعد وضعه في محلول الشاي .تسبب إضافة الجسيمات النانوية الفضية أيضًا في انخفاض احتباس المبيضات البيض في العينات المضافة.

I. Introduction

Poly methyl methacrylate (PMMA) is one of the most widely used materials in modern prosthodontics. It is widely known due to its simplicity in use and acceptable esthetics [1] PMMA is a thermoplastic polymer, the original material was seen as replacement for glass in a variety of applications, and is currently used extensively in glassing applications, the material is one of the hardest polymer, rigid, glossy finish, and good resistance [2]. Many attempts have been made in dentistry for various purposes like making denture base, artificial teeth, provisional restoration, surgical splints, stents and orthodontic appliances. They are the material of choice for removable complete denture prosthesis [3, 4]. For dental applications PMMA are modified by cross - linking to improve hardness and stiffness (increases molecular weight), increase crazing resistance (small cracks originating at the teeth - denture margin), wear and solvent resistance, but this increases brittleness [5].

However, poly methyl methacrylate is the most commonly used material but it doesn't have optimal property, both denture base material have similar chemical composition except fluid type of denture resins have higher molecular weight powder particles that are much smaller and when mixed with monomer, the resulting mix is very fluid. Therefore, they are referred as fluid resins. They are used with significantly lower powder-liquid ratio [6].

The color change of a polymeric material may be caused by intrinsic and extrinsic factors. Intrinsic factors involve resin discoloration itself and matrix changes, occurring during the aging process of the material due to many physical and chemical conditions, furthermore, extrinsic factors such as thermal changes, stain accumulation, artificial dyes used in food, cleaning procedures, and handling by the patient can also cause discoloration [7].

Nanotechnology has opened up new avenues of research and offers many applications in human health [8, 9]. Nanotechnology can be defined as using materials and structures with nanoscale dimensions usually the range 1-100 nm [10]. The effect of silver nano particles (Ag-NPs) are one of the most commonly used antimicrobial nano materials in range of consumer products and for medical applications [11]. This study was conducted to assess some physical properties and the antifungal activity of silver nanoparticles against candida when they are added to fluid denture base material powder in 0.4% concentration by volume [11].

II. Materials and Methods

Forty specimens were prepared from two brands of fluid acrylic resin, the first controlled group 20 specimens were prepared from the fluid acrylic resin (Mega press NV+ JFT) and the second group was modified by adding silver nanoparticles to the fluid acrylic (0.4/100gm of fluid resin), These specimens were divided into four testing groups according to the type of the test to be done later on. Then each group was subdivided into two groups. For each test, five specimens for two control and the other five specimens for the modified resin as shown in Table 1 [12]. Table 2 shows the composition of each material.

Table 1: Verifying the specimen number and type of testes

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Table 2: Verifying the composition and properties of fluid resin and silver nanoparticles

Fluid acrylic resin	**Liquid**	**Powder**
	- Megadental GmbH - Mega press NV + JET X. - Cold cure and contain 500 ml	- Mega press NV. - Kaltpolymerisat - Cold cur and contain 1000g.
	- Seeweg 20.D 63654Budingen. - Made in Germany. - Normal working and verarbeitungsdauer time: 7-10 min	
Silver nanoparticles	- Part number: MKN- Ag- 020. - Silver nanopowder (Ag 99.95% pure, APS 20nm, SSA: ~m2/g. - Lot # SN0710. - Appearances: black nanopowder, Morphology: spherical - Synthesis process: wet chemistry. - True dentistry: 10.5 g/cm3 and expire date: 2017 – 2020.	

III. Specimen preparation

According to manufacturer instruction in a mixing ratio by volume / parts by weight were 1 ml liquid/ 1.5 g powder, the (polymer) powder of required quantity is poured into the liquid (monomer) in a clean dry jar and mixed manually thoroughly for 20 second the 0.4 g silvernano particles then added to the mixing as shown in Table 3.

Testes	Specimen No.	Control group	Modified group
		Polymer/monomer ratio 1ml/1.5gm	Polymer/monomer ratio1ml/1.5gm/0.4%g silver nanoparticles
Impact	10	5	5
Transvers strength	10	5	5
Color stability	10	5	5
Candida retention	10	5	5

Table 3: Shows the mixing ratio

Sample NO.	Type of tests		Study groups	
			Control	Modified
			Polymer - Monomer Ratio	Adding silver particles
10	Impact		1ml /1.5gm	0.4%g
10	Transvers strength		1ml/1.5gm	0.4%g
10	Color stability	B	1ml/1.5gm	0.4%g
		A	1ml/1.5gm	0.4%g
10	Candida retention		1ml/1.5gm	0.4%g

The dough stage is reached after 4 minutes and remains till 6 minute then after that the resin was poured in to a mould prepared by metal specimens invested by a silicone gel material in to a special flask (Casta fiask) which is a clear plastic, high resistance to the temperature and easy to control. This flask possess 3 openings, from one opening the fluid resin was poured and the air exuded through the other openings and when the fluid resin exudes through these opening means that the mould has been filled, it composed of two parts, can be opened and joined together by two metal clips. The three plastic reservoirs placed at the upper part of the flask to close the reservoirs after filling the flask then cured by pressure curing device, the mold placed in pressure curing device that was filled with water at room temperature, the mold is completely immersed in water then the temperature gradually raised up to 120 °C at 20psi pressure for 15-30 minute [13].

The temperature of the device was 0-95 °C that permitted to increase the water temperature to 120 °C. The higher temperatures of water above boiled point could only be reached under pressure. In order to avoid that hot water vapor of 120 °C escapes from the pressure pot and in order to reduce the risk of scalding when opening the lid, this device was equipped with a safety lock which only allows the lid to open when the operated pressure in the unit [13]. When using a pressure pot, it is not necessary to pressurize it with air to more than 20 psi

(pounds per square inch), the cooling last from 5–8 minutes from time expiration to the opening of the lid. When the flask completely cooled removed from the chamber and opened for removing the specimens [13].

1. Impact strength test

The specimens used for impact strength test was prepared according to the ISO no. 179-1 [14]. with dimensions (80mm X 10mm X 4mm) length, width and thickness respectively, the specimens was tested after being conditioned in distilled water at 37°C for 48 hours using an incubator and the testing procedure done by a Charpy type digital impact tester (Electric Charpy impact tester, LY-XJJD-5, China), according to the procedure given by ISO 179 [14]. The speed of fall of the striker will be 2.9 m/s, and the striker has an energy range of 0.5,1,2, 4, and 5 joules, a striker of one joule testing capacity was used.

Impact strength = (E / b.d) X 103 (ISO 179-1, 2000) where

E : is energy absorbed to break the specimen

b: is width of the specimen in millimeter.

d: is thickness of the specimens in millimeter

The test specimen, supports near its ends as a horizontal beam, is impacted by a single blow of a striker, with the line of impact midway between the supports and strikes at a high, nominally constant, velocity [14].

2. Transverse strength test

Ten specimens five as a control and the other five are the modified, that prepared from fluid acrylic resin denture bas material measuring (65 x 10 x 3) mm ± 0.03mm in length x width x depth, respectively. The transverse deflection and transverse strength of the specimens were measured in air by three points bending on a computerized universal testing machine. The device was supplied with a central loading plunger and two supports with polished cylindrical and projection in the center of the cylindrical, the two support are apart from each by the 100mm. The supports were parallel to each other and perpendicular to the longitudinal central

line, the force and deflection were sensed by a sensor which was linked directly to the computed program that gave directly force-elongation table and record it in sequence from the starting of the test till the end.

The test specimens were held at each end of the two supports which away from each other by 50mm, and the loading plunger placed midway between supports, the tests were carried out with a constant crosshead speed of 0.5mm/ minute. The specimens were deflected till the fracture is occurring, then the transverse strength was calculated using the following formula:

Transverse strength = (3PL) / (2B.H2) MPa

Where:

P: is the peak load

L: is the span length (50mm)

B: is the width of the specimen (10mm)

H: is the thickness of the specimen (3mm).

3. Color stability

For the color stability test, rectangular metal specimens were constructed with the dimensions (25 X 4 X 0.5) mm length, width and thickness respectively to fit into the specimen carrying chamber of the spectrophotometer, and the color stability is the property of a material that allows color to be maintained over a period of time in a given environment, it is considered an important physical property of dental materials [15].

All spectrophotometer instruments designed to measure the absorption of radiant energy have the basic components [15].

1. A stable source of radiant energy (Light).

2. A wavelength selector to isolate a desired wavelength from the source (filter or monochromator).

3. Transparent container (cuvette) for the sample.

4. A radiation detector (phototube) to convert the radiant energy received to a measurable signal and a readout device that displays the signal from the detector.

The energy source is to provide a stable source of light radiation, whereas the wavelength selector permits separation of radiation of the desired wavelength from other radiation, the light radiation passes through a glass container with specimen and the detector measures the energy after it has passed through the species, the readout device calculates the amount of light absorbed by the specimen displays the signal from the detector as absorbance or transmission. [16].

The light absorption for each specimen was measured before immersion of the specimens in the solutions, the standard solution of tea prepared from 4 grams of dry tea boiled in 500 ml of distilled water for 4 minutes, and allowed to cool at room temperature, and the solution would decanted from tea leaves, this solution was used for staining the test specimens and the specimens measured by spectrophotometer, then immersion of it in the fresh tea solutions (that prepared prior to use) for 10 days [17].

4. Candia test specimens

Polymethyl methacrylate squares (10 × 10 × 0.5 mm) length, width and thickness. were fabricated, fluid resin acrylic samples processes according to manufacturers, specimens were equivalent in size and surface finish. No surface modification were making to the processed squares following recovery and then placed in distilled water for 24 h and stored dry until used [18].

The acrylic specimens containing silver nanoparticles was eluted with culture medium for 1, 2, and 5 days, the samples were then fixed with glutaraldehyde and Gram stained and adhered Candidial cells were examined by light microscope [19].

Preparation of C. albicans.

After preparing the agar medium, broth medium, yeast nitrogen base(YNB) then we prepared the *Cahyndida albicans* were grown on Brain Heart Infusion (BHI)agar over night at 37 °C. One colony of candida strain was inoculated in 5 mL of 30 % BHI and 70 % YNB and

incubated at 37 °C for 24 h. The concentration of the yeast culture 3 × 107 cells/ mL in BHI/YNB. Then, acrylic samples were placed in each well of a 12-well plate. Inoculate the wells with 2 mL of suspension in BHI/YNB media and incubate for 3hr at 37 °C under aerobic condition. After 3hrs incubation, the samples were washed with sterile phosphate buffer saline and then move the samples to a new sterile well plate filled with 2 mL fresh BHI/YNB. The biofilm will be allowed to grow for 24 h at 37 °C under aerobic conditions [20].

Colony forming unit CFU

Microscope observation epithelial cells and coupons with adherent yeasts were treated as described above, but without acetic acid. A duplicate of each plate was performed. Candida attached to crystal violet was quantified using an inverted light microscope. Ten fields were randomly observed in each well. As the samples were set up in triplicate for each experiment, the mean number of yeasts per 10 fields was expressed as number of cells per unit area of the well [21].

Results

Table 4 shows t-test results for assessing the effect of nanosilver on the impact, transverse strength, color stability and Candida retention of fluid acrylic resin denture base material. The results showed that comparison of controlled group and modified group of fluid acrylic resin, reveals that the modified group had significantly higher impact strength than the control group ($P < 0.01$).

The results of bending test for the 10 specimens of fluid acrylic resin denture base showed that the 5 modified fluid acrylic resin was the highest mean value for bending test (148.4N/mm2), followed by the control fluid acrylic resin (129.0 N/mm2) statistically there was non-significant difference between the two groups.

Regarding Color stability, t- test was done for the two groups control and modified also before tea immersion in tea and revealed that there was non statistically significant difference ($P>0.05$) between all the two groups of fluid acrylic resin denture base as shown in Table 4

but there were highly significant color change after 10 day immersion in the tea solution for the modified group (P< 0.01).

Regarding Candida retention, t-test was revealed that the mean of control group was significantly higher than modified group (P<0.01).

Table 4: Effect of different tests, impact, transvers strength, color stability and candida retention on both control and modified study groups.

Sample NO.	Type of tests		Study groups		Probability T- test
			Control	Modified	
			Mean ± SE NO. 5	Mean ± SE NO.5	
10	Impact		4.90±0.69	10.23±0.62	P<0.01
10	Transvers strength		129.0±4.56	148.4±11.68	p>0.05
10	Color stability	B	1.70±0.03	1.79±0.02	p>0.05
		A	4.90±0.69	10.23±0.62	P<0.01
10	Candida retention		30.0±4.64	10.80±2.24	(
B:before; **A:After**					

Discussion

There were high significant differences between means of impact strength among control and modified groups that disagree with results of [22], when they found that the highest impact stress for the conventional fluid acrylic resin without silver nanoparticles, this may be due to the sample size, or the amount of the additives, also the type of the used resin.

Transverse strength that widely used for evaluation of quality of acrylic denture base material was non significantly increased in modified group this agree with result of [23] when they found the mean compressive strength of acrylic reinforced with nanosilver were higher than the unmodified one, reverse result was found by [22] who reported that the addition of silver nanoparticles had no effect on flexural strength and elastic modulus of acrylic resin.

The means of color stability in before(B) and after(A) groups were increased in modified compared with control (P>0.05), (P<0,01) respectively, that agree with [24] they reported that acrylic resin exhibited the least color alteration, and the addition of pigments to the resin enhanced its color stability, in addition of nanoparticle materials partially blocks the passage of ultraviolet rays because of its size and gray color, reducing color degradation of other pigments and overall color change of the denture. Indeed, the color of the specimens was changed by adding the silver nanoparticles to dark gray and increased by the specimen's thickness.

The silver nanoparticle cause reduction of the Candida retention and that agree with [25] who observed that the bioactivity and biomass of *C. albicans* biofilm successively decreased with increasing nano-silver solution concentration also the thickness and live/dead cell ratio were successively reduced with increasing nano-silver concentrations [25].

Conclusion

Within the limitation of this study the authors can conclude that addition of silver nanoparticles has resulted in significant difference between control and modified group for impact strength test. While non-significant difference was seen for transverse strength test. In regard to color stability, showed enhancement in color stability for both before and after placement in tea solution. Lastly the addition of silver nanoparticles caused reduction in candida albicans retention in the added samples.

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