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Evaluation the biological effect of adding Aluminum Oxide, Silver nanoparticles into microwave treated PMMA powder

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Abstract: Many attempts to improve the physical and mechanical properties of acrylic denture base materials by adding nanoparticles or by modifying the polymethyl methacrylate denture base materials. This new denture base product needs to evaluate its biocompatibility because of its application in direct contact with the living tissue. The aim of this study was to evaluate the biocompatibility of acrylic denture base resin without additives prepared from microwave treated polymethyl methacrylate (PMMA) powder group (V), and microwave treated (PMMA) powder grinded with micronizer group (M), and denture base with 1% concentration of Aluminum oxide (Al_2O_3) and Silver (Ag) nanoparticles (NP) that were added separately to untreated group(P) and microwave treated PMMA powder (group V and M) was planned to be evaluated in this study.

Materials and methods: PMMA powder were treated with microwave radiation at a power level of 360watt for ¾ hr. the obtained PMMA powder was then grinded using a domestic blender group(V). The next step is particle size reduction of the microwave treated PMMA powder using micronizer group (M). The particle size and surface area of the obtained microwave treated PMMA powder group (V and M) compared to untreated controlled PMMA powder were measured using Laser Diffraction Particle Size Distribution Analyzer. The mean particle size of groups (V and M) was (52.4408µm, 48.9018µm) with increased surface area to (1387.3cm^2/cm^3, 1539.5cm^2/cm^3) respectively, compared with the control group (P) which had a mean particle size of (111.6329µm) with a decreased surface area to (769.95 cm^2/cm^3). One percent concentration of Al_2O_3 and Ag (NP) were added separately into untreated PMMA powder group (P) and microwave treated PMMA powder group (V and M). Specimens of all the experimental groups, in addition to specimens of unmodified denture base resin as a control group were implanted in the subcutaneous tissue at the back of the Newsland rabbits. Biopsies for histopathological observation were taken after 14days.

Results: According to the scoring criteria used in this study histopathological observation in the subcutaneous tissue of the rabbits showed mild inflammation in the site embedded with samples prepared from microwave treated PMMA powder. No inflammation was observed with the addition of 1% of Ag NP to untreated PMMA powder and mild inflammation when mixed with microwave treated PMMA, while the incorporation of Al_2O_3demonstrate mild to moderate inflammation when added to the tested groups.

Conclusion: It was noted that samples prepared from microwave treated PMMA powder is a biocompatible materials also both types of nanoparticles demonstrated good level of biocompatibility when added to microwave treated and untreated PMMA powder.

Keywords: Biological Effect, Micronizer, Microwave, Nanoparticles, PMMA powder.

Introduction

The development of dental materials is growing every year, as well as their use in many specialties. Among the dental materials, acrylic resins have important application, mainly in dental and maxillofacial prosthesis. These materials need to be exhaustively studied in order to get better knowledge of their biological, physical and chemical properties. This is particularly important when it is considered that these materials can be put in direct contact with the living tissues. In this case, biocompatibility is the goal to be reached [1, 2].This goal is reached when a maximal efficiency and a minimal interference on the local dynamics of the tissues is achieved [1]. Biocompatibility can be defined as the acceptance (or rejection) of artificial material by the surrounding tissues and by the body as a whole [3].

Microwaves are a form of electromagnetic radiation in the frequency range 300-3000 MHz produced by a generator called a magnetron. The way in which a material will be heated by microwaves depends on its shape, size, dielectric

constant and the nature of the microwave equipment used [4, 5]. The microwave irradiation of dentures at a specified setting and exposure time is bactericidal and fungicidal[6], and it has been considered for denture sterilization and disinfection instead of chemical solutions because it requires no special storage, has no expiration date and does not induced resistance to Candida Albicans[7, 8] .

It has been concluded by author[9] that additional treatment with microwave after curing decrease the residual monomer of heat cure acrylic resin materials. Therefore, the reduction of residual monomer content could improve the properties and reduce the cytotoxicity effects of the polymerized acrylic resins. Attempts have been made to improve the physical properties of acrylic resins and to overcome their drawbacks by adding different substances [10]. It has been possible to modify the properties of the polymer by adding specific fillers that are distributed at a nanometric level inside the polymeric matrix. They offer the combined advantages of polymer compounds, flexibility, ductility and processing capacity, as well as completely new properties, stiffness and high thermal stability of the nanostructured materials [11]. Nanoparticles have many remarkable properties because of their small size and very large specific surface areas [12].

The nanomaterials are not only promised to improve the properties and functionalities of dental products but also to lead to the development of innovative, novel products for the benefit of patients [13]. Nano-sized materials show unique properties depending on their size. Especially, metal and metal oxide nanoparticles have been widely investigated because of their potential for many applications [14].

Aluminum oxide commonly referred as alumina with the chemical formula Al_2O_3. As indicated, it is a chemical compound of aluminum and oxygen with strong ionic interatomic bonding, giving rise to its desirable material characteristics. This can exist in several crystalline phases; alpha phase alumina is the strongest and the stiffest of the oxide ceramics. Its high hardness, excellent dielectric properties and good thermal properties make it the material of choice for a wide range of applications. It is also known for its excellent size and shape capabilities with high strength and stiffness too [15].

Resistance of bacteria to bactericides and antibiotic has been increased due to the development of resistant strain. Some antimicrobial agents are extremely irritant and toxic and there is much interest in finding ways to formulate new types of safe and cost effective biocidal materials. Aluminum oxide nanoparticles have wide range application in industrial as well as personal care products. It is known to possess strong antimicrobial properties. With Escherichia coli, alumina NPs showed a mild growth-inhibitory effect, only at very high concentrations [16].

The addition of Al_2O_3 nanoparticles to acrylic resin improved the thermal properties and transverse strength of acrylic resin at the same time this addition decreased water sorption and solubility. On the other hand there was an increase in surface hardness and the surface roughness not significantly changed with increased the concentration of Al_2O_3 nanoparticles [17].

The silver NPs are one of the most commonly used nanoparticles, Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, chemical stability. Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity. Silver nanoparticles have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts [18, 19].

Silver NPs show powerful bactericidal properties even in far lower concentration. Moreover it is seen that, Ag NPs show no significant cytotoxicity against human-derived monocyte cell lines, Silver nanoparticles decrease Candida Albicansadherence of fungi and dental plaque that cause mucosal infections in denture prosthetics. Research in the nanotechnology field promotes the use of silver nanoparticles with antimicrobial benefits and a wide range of applications, preventing the need for infection therapies [16, 20]. Silver nanoparticles can kill all pathogenic micro-organisms, and no report as yet has shown that any organism can readily build up resistance to them [21].

Materials and Methods

A-Preparation of the PMMA Powder That Treated With Microwave Radiation:

Fifty ml of distilled water is added to the 50 gm. of polymethyl methacrylate powder (Vertex-Dental B.V.Johan Van Oldenbamevertlaan, 62, 3705 HJZeist the Netherlands). The PMMA mixed thoroughly with water and left for 1/4 hr. after that, the mixture exposed to microwave radiations (Sunny output 900W 2450 MHz, China) at power level 360 watt for ¾ hr. After completing the exposure, the material was grinded immediately for 5 minutes using a domestic blender (Hanil Grinder, Korea) and sieved by sieve No. 100 micron (Retsch GmbH & Co.KG Germany), the obtained PMMA powder is given the symbol group (V).

The ¾ hr. microwave treated PMMA powder is then grinded in General Company for Drug Industries in Nineveh by using micronizer (Air Pac, India) to produce micro sized PMMA. Micronizer is an air jet mill that is widely utilized and incorporated within the pharmaceutical industries to produce offline powders; the obtained PMMA is given the symbol group (M).HORIBA's LA-300 Laser Diffraction Particle Size Distribution Analyzer was used to estimate the particle size and surface area of the obtained microwave treated PMMA powder group(V and M) compared to untreated controlled PMMA powder. The mean particle size of group(V) was(52.4408µm) with surface area (1387.3 cm^2/cm^3) and the mean particle size of group (M) was (48.9018µm) with increased surface area to (1539.5cm^2/cm^3), compared with the control group (P) which had a mean particle size of (111.6329µm) with a decreased surface area to (769.95 cm^2/cm^3).

B-Preparation of Polymer with Nano Particles Additive:

Unmodified acrylic resin control samples were processed according to the manufactures specifications. Nanoparticles Aluminum oxide (Al_2O_3) (α-phase) with an average diameter of 20-30nm and silver (NP) with an average diameter of 80 nm were obtained from (Beijing Dk nanotechnology co., ltd). For samples that contained nanoparticles, 1% of Al_2O_3 and Ag (NP) were added separately in to the obtained microwave treated PMMA powder (group V and M) in addition to the untreated PMMA as a control group (P). In order to get uniform distribution of these nanoparticles inside the polymer the capsule of the amalgamator (Dentomat, Degussa, Type600,Germany) was modified by attaching small covered plastic bottle into which the PMMA powder and nanoparticles are placed and the amalgamator turned-on for 1 minute to ensure homogenize distribution of nanoparticles inside the polymer, to this uniform mixture of nanoparticles and PMMA powder, monomer was later added according to the ratio recommended by the manufacture.

Biocompatibility Test:

Biocompatibility was tested by animal implantation in vivo. Nine white male (Newsland) rabbits, 4-6 months old with an average weight 1.250-1.350 Kg were included in this study. The animals were housed in an animal house in the University of Kufa/ College of Veterinar. Twenty seven discs samples with 6mm in diameter and 2mm thickness [2] were prepared to perform this test. These samples were divided into three groups, as follows:

First group:
1-Unmodified control heat cure acrylic samples, group (P).
2- Heat cure acrylic samples mixed with 1% Al_2O_3, group ($PAl_2O_3$1%).
3- Heat cure acrylic samples mixed with 1% Ag, group (PAg1%).

Second group:
1- Samples prepared from PMMA powder that are treated with microwave radiation for ¾ hr., group (V).
2- Samples prepared from PMMA powder that are treated with microwave radiation for ¾ hr. mixed with 1% Al_2O_3, group (VAl_2O_3 1%).
3- Samples prepared from PMMA powder that are treated with microwave radiation for ¾ hr. mixed with 1% Ag, group (VAg1%).

Third group:
1-Samples prepared from PMMA powder that are treated with microwave radiation for ¾ hr. followed by grinding with micronizer, group (M).
2- Samples prepared from PMMA powder that are treated with microwave radiation for ¾ hr. followed by grinding with micronizer mixed with 1% Al_2O_3, group($MAl_2O_3$1%).
3- Samples prepared from PMMA powder that are treated with microwave radiation for ¾ hr. followed by grinding with micronizer mixed with 1% Ag, group(MAg1%).

All specimens were finished and sterilized in sodium hypochlorite (0.5%) for 10min [2]. After that, the specimens conditioned in distilled water for 24 hours at room temperature. Rabbits were anesthetized by intramuscular injection 1ml/kg ketamin hydrochloride mixed with xylezine 0.1ml/kg. The fur skin was shaved manually over the lower part of the back. The shaved area was divided by vertebral column into right and left side. The control specimen was implanted in the right side. In the left side, the test specimen was implanted. An incision of 1cm length was made through the skin. The subcutaneous supra muscular tissues were separated with blunt end instrument to create a pouch for the specimen then the specimen was held with a pair of tweezers and inserted in the pouch, 5mm away from the incision line. The incisions were sutured and the area was cleaned and disinfected with povidoniodion disinfectant solution. After 14 days of the implantation period the rabbits were anesthetized again. A two cm excisional biopsy was taken, which involved the skin, the embedded specimen with some of supra muscular tissue. This biopsy placed in 10% buffered formalin for one-day fixation. The specimens were removed and the tissues were conventionally processed (paraffin inclusion), and stained by haematoxylin and eosin (H.E), for light microscopical observation.

Criteria of Examination

The criteria of examination include the type and numbers of inflammatory cells which in turn reflect the severity of the inflammatory reaction. The scoring criteria [2] for the severity of inflammatory reaction which were used in this study are listed in table (1).

Table (1): scoring criteria for the severity of inflammatory reaction [2].

Score	Severity of inflammation	Number of cells
0	**No inflammation**	**0-15**
1	Mild inflammation: Thickness of reaction zone similar of only slightly wider than normal tissue	16-60
2	Moderate inflammation: Increased reaction zone presence of macrophages and/or plasma cells	61-105
3	Severe inflammation: Increased reaction zone presence of macrophages and plasma cells occasional foci of neutrophils, granulocytes, and /or lymphocytes	106-150
4	Extreme inflammation: Focal areas of necrotic tissue densely infiltrated by inflammatory cells	< 150

Result and Discussions

The results of clinical findings revealed that all subcutaneous implant sites appeared to heal satisfactory at 14 days interval. Also, all the implants were palpable and visible under the skin, besides that, no gross inflammatory signs were detected in all experimental animals. No rejection to implants was observed. The tissue supporting specimens ofunmodified heat-cured resin as a control showed moderate inflammation with a thick fibrotic capsule formation this reaction may be due to surgical trauma and foreign body reaction. The fibrous capsule showed dense fibrous tissue with fibroblast cell, few plasma cells, lymphocyte cells can be detected with underlying congested blood vessels and edema as shown in Figure (1-A).

Histopathological examination of biopsy involved heat-cured acrylic mixed with 1%Al_2O_3 (NP) illustrated moderate inflammation with a thick edematous fibrous capsule formation surrounding disc space. This capsule is a well-organized fibrotic tissue showing collagen fibers with fibroblast cells, lymphocyte cells, eosinophil cells, and few plasma cells scattered within the fibers, as shown in Figure (1-B).While tissue sections surrounding implanted pellets of heat cured acrylic resin containing 1% Ag(NP) showed thin capsule which was formed mainly of fibroblasts and scanty inflammatory cells were detected in the section and according to the scoring criteria there is no tissue reaction, no inflammation recorded in Figure (1-C). This result could be attributed to the bactericidal effect of nano silver by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall, leading to increased cell permeability and cell death [16]. In case of histopathological examination of heat-cured acrylic treated with microwave for ¾ hr. showed few plasma cells, lymphocyte cells, fibroblast cells, and eosinophil cells, this histological examination illustrated mild inflammation, Figure (2-A). These findings indicate that samples prepared from PMMA powder treated by microwave showed mild inflammation in relation to control group with moderate inflammation results. These results could be due to sterilization and disinfection effect of microwave irradiation of PMMA powder [7, 8].

Microscopical field of sections obtained from biopsies contained heat-cured acrylic treated with microwave irradiation for ¾ hr. and containing 1% Al_2O_3 nanoparticles revealed mild inflammation with the area of disc implantation showed thick fibrous capsule with edema and fibroblast cells scattered within fibrous tissue. Few eosinophil cells and moderate infiltration of lymphocyte cells, plasma cells also can be detected in this section as shown in Figure (2-B) this reaction might be due to surface charge interactions between theAl_2O_3 nanoparticles and cells. Free radical scavenging properties of the particles prevented cell wall disruption and drastic antimicrobial action. This laboratory scale study suggests that alumina NP may only exhibit mild toxicity toward microorganisms in the environment [16].Regarding this group, heat-cured acrylic treated with microwave irradiation for ¾ hr. and containing 1% Ag NP results revealed mild inflammation. The histopathological findings showed the fibrotic capsule as a thin fibrotic band surrounds the disc implant. This capsule infiltrated by few plasma cells, scanty eosinophil cells also still fibroblast formed in the section, as shown in Figure (2-C).

The biopsy taken after 14days of implantation of samples prepared from microwave treated PMMA for ¾ hr. and grinded with micronizer, showed that the disc space was lined by thick fibrous tissue capsule. On histological examination of capsule, it shows plasma cells, lymphocyte cells, eosinophil cells, and fibroblast cells. According to the number of inflammatory cells and the thickness of reaction zone the histopathological findings revealed mild inflammation in relation to control group as shown in Figure (3-A). These findings could be due to the effect of reduction in size of the structure of material PMMA powder after grinding by micronizer (nanotechnology), and agreed with Orellana, et al.[11] who stated that reduction in size of structure of material allows developing customized plastic materials for specific applications and optimizing the properties of a polymer. The biopsy examination of heat-cured acrylic group (M), containing 1%Al_2O_3 NP showed mild inflammation with a thick fibrous capsule formation surrounding disc space. This capsule is a well-organized fibrotic tissue showing collagen fibers with fibroblast cells scattered within the fibers, lymphocyte cells, and plasma cells as shown in Figure (3-B).While the biopsy taken after 14days of implantation of heat-cured acrylic group (M), containing 1%Ag NP showed that the disc space was lined by thin fibrous tissue capsule. On histological examination of capsule, it showed plasma cell, lymphocyte cells, eosinophil cells, and fibroblast cells. The number of inflammatory cell revealed a mild inflammation but the thickness of reaction zone was greater than the control group as shown in Figure (3-C). Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the Nano-scale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the Nano scale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material. The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material [22].

Conclusion

In all experimental groups, the body reaction to the implanted material show good response since there was no clinical signs of inflammation. The presence of fibroblast cells and collagen fibers in the histological slides may directly indicate that the experimental materials allow cell proliferation without a cytotoxic effect, and they are biocompatible with cells.

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Figures Used

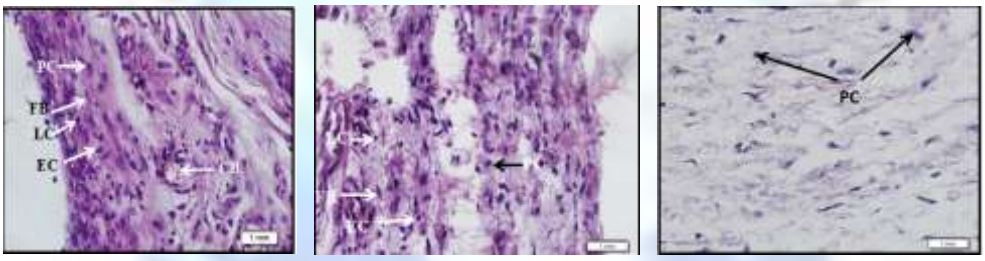


Figure1: Microphotograph views for the first group of the tested materials.
A: Unmodified control heat cure acrylic.
B: Heat cure acrylic mixed with 1% Al_2O_3 (NP).
C: Heat cure acrylic mixed with 1% Ag (NP).

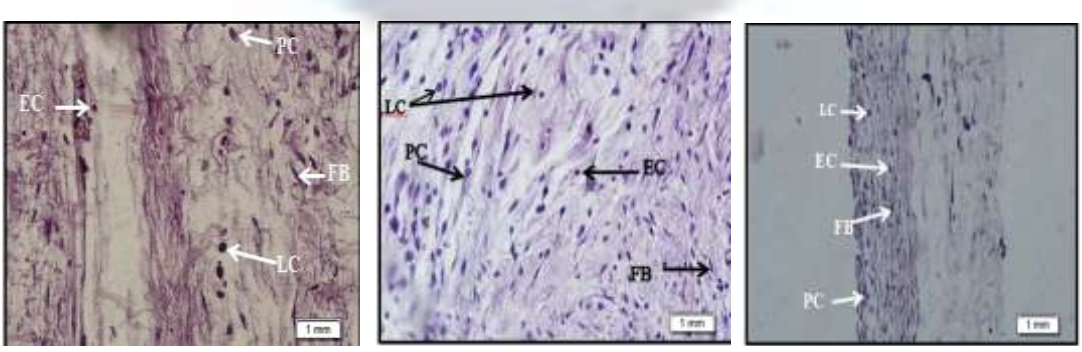
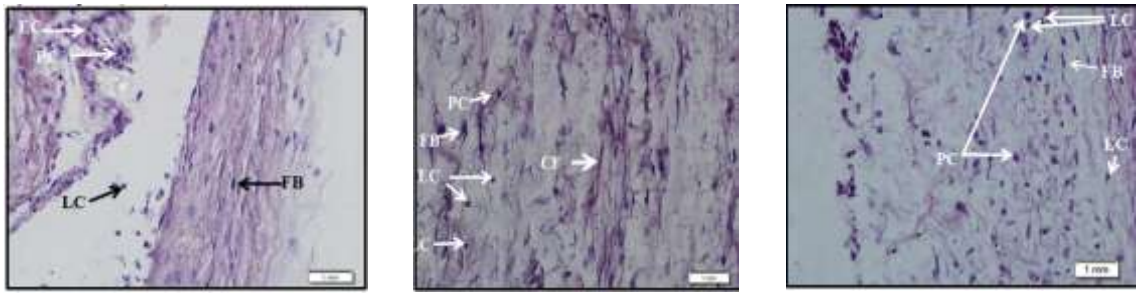


Figure 2: Microphotograph views for the second group of the tested materials .
A: Samples prepared from microwave treated PMMA powder, group (V) .
B: Samples prepared from microwave treated PMMA powder mixed with 1% Al_2O_3 (NP).
C: Samples prepared from microwave treated PMMA powder mixed with 1% Ag (NP).



A B C

Figure 3: Microphotograph views for the third group of the tested materials.
A: Samples prepared from microwave treated PMMA powder grinded with micronizer.
B: Samples prepared from microwave treated PMMA powder grinded with micronizer and mixed with 1% Al_2O_3 (NP).
C: Samples prepared from microwave treated PMMA powder grinded with micronizer and mixed with 1% Ag (NP).