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MICROBIOLOGICAL EVALUATION OF ACRYLIC RESIN MODIFIED WITH SILVER AND GOLD NANOPARTICLES

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ABSTRACT

Purpose: The objective of the study was to assess the antimicrobial activity of acrylic denture base containing nanosilver and nanogold to; *S. aureus, E. coli* and *C. albicans*. **Methods:** Three concentrations of silver and gold nanoparticles (0.01 %, 0.1%, 1% of polymer weight) were added to MMA monomer, cured samples of modified PMMA were evaluated for their antimicrobial activity to three micro-organisms using Broth Microdilution Assay. **Results:** Both nanosilver and nanogold showed antimicrobial activity to the tested micro-organisms. **Conclusion:** Addition of nanogold and nanosilver to acrylic bases could improve their antimicrobial activity. **Key words:** Nanosilver, Nanogold, Antimicrobial activity.

INTRODUCTION

Although poly methyl methacrylate (PMMA) is not ideal in every respect, it is still the most popular denture base material [(1)]. It is a well received biomaterial by dental profession due to its acceptable advantages, however its mechanical performance is not ideal [(2)].

Denture stomatitis is an oral pathology that affects a large number of patients using complete or partial dentures. The main etiologic factors related to denture stomatitis are trauma, poor oral hygiene and infection with *Candida* species [(3)]. There are evidences that denture stomatitis is not a result of *C. albicans* solely, but rather it is an outcome of multispecies biofilms that may include *Strept. mutans, Staphy. aureus, E. coli* and *Klebsiella spp* [(4)]. Resistance and recurrent of denture stomatitis infection is a major problem facing denture wearers treated with systemic or local treatment modalities [(5)].

Nanoparticles are antimicrobial agents that incorporated into denture bases with the possibility of preventing or reducing bacterial and fungal contamination. Nanomaterials have many unique physicochemical properties, such as ultra-small size, large surface area to mass ratio, extensive thermal stability, and high reactivity[(6)]. These properties can be used to overcome some of the limitations found in some traditional therapeutic and diagnostic agents [(7)]. The properties of polymer nanocomposites depend on the type of incorporated nanoparticles, their size and shape, as well as the concentration and interaction with the polymer matrix [(8)].

Mendieta I et al [(9)] concluded that silver nanoparticles (AgNPs) when added to a PMMA formulation, resulted in successful reduction of adherence of *C. albicans*. Similarly, Nam K 2012[(10)] concluded that tissue conditioner modified with nanosilver showed antimicrobial activity toward *S. aureus* when the concentrations of nanosilver was above 0.1% and toward *C. albicans* when the concentration of nanosilver was above 0.5%.

Eid A et al [(11)] used gold nanoparticles (AuNPs) having sizes ranging from 5-20 nm to study their antifungal activity. The results that obtained lend

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strong evidence that could warrant the consideration of gold nanoparticles as antifungal agent that could circumvent the side and passive immune effects of other biocidal medications.

It is desirable for dental materials to have a low susceptibility to oral microorganism adhesion [12]. Previous studies implicated that Nanosilver and Nanogold added to PMMA might have antimicrobial activity that could overcome the problem of recurrence of denture stomatitis after stoppage of treatment, this activity depended on many factors including nanoparticles content, size and shape, way of nanoparticles loading, method of nanoparticles construction and even the type of acrylic resin to be modified [12].

MATERIALS AND METHODS

Silver nanoparticles and gold nanoparticles were synthesized in a colloidal solution in an alcohol medium, AgNPs were triangular prisms in shape while AuNPs were spherical in shape. The average nanoparticles size was 20 nm. Three different concentrations were prepared (0.01%, 0.1%, 1%) of the polymer weight. Solutions were kept in a properly sealed bottles at room temperature till be added to the monomer [13].

Disk shaped metal patterns were constructed with a diameter of 20mm and thickness of 2mm according to ADA specification no.12,1999 for denture bases [14] and were invested in flasks with dental stone. After setting of stone, the flasks were opened and the patterns were removed, leaving cavities that were used as matrixes for the fabrication of acrylic resin specimens. Polymer and the modified monomer with nanoparticles were mixed according to the manufacturer's instructions and packed into the previously prepared cavities and allowed to be polymerized. The specimens were autoclaved and then immersed in sterile artificial saliva at 37±2C° for 7 days [15]. Three standard strain organisms were used in the current study; *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739) and *C. albicans* (ATCC 10231).

Control and modified acrylic resin samples were made for each performed test so that (n =10) according to international organization for standardization [16].

Broth Microdilution Assay

Each disc sample of nanosilver and nanogold particles and control discs were placed on flat bottom of separated 12-well cell culture plate and 100µL (equivalent to the turbidity of 0.5 McFarland standard) of initial microbial suspensions in 1.0 ml of Mueller Hinton broth and Sabouraud dextrose, broth for bacteria and Candida respectively, were inoculated to each well. Three wells containing microbial suspension without tested discs as (Growth control) were included in the plate and the plates were incubated at 35°C for 48 h. After incubation period 200µL from each well was taken under aseptic condition and placed on sterile 96-well flat-bottomed microtiter plate. Optical densities were measured at (620nm) using ELISA microplate reader (Sun Rise–TECAN, Inc. ®, USA) [17].

Finally, cell concentrations were transformed to a mean growth inhibition percentage (%). The percentage of microbial growth reduction (GR %) was estimated using as reference the control treatment (growth without discs) as: GR% **=**

Where, *C* is the cell concentrations under the control treatment and *T* is the cell concentrations under the disc treatment. Three replicates were considered. The results were recorded as means ±SD of the triplicate experiment [18].

RESULTS

Post Hoc Test (LSD) (table 1, figure 1, 2 and 3).

No antimicrobial activity of the control group to any of the tested microbial strains. There was a significant increase in the antimicrobial activity of nanosilver 0.1% subgroup (95.0±0.74) and nanosilver 1% subgroup (100.0 ± 0.50) to *E. coli*. A statistically significant increase was found in Nanogold

0.1% subgroup (55.0±0.98) and Nanogold 1% subgroup (100.0±0.42) to *E. coli*.

There was a statistically significant increase in antimicrobial activity of all nanosilver subgroups to *S. aureus*. A significant increase was found in nanogold 0.1% subgroup (100.0 ± 0.69) and nanogold 1% subgroup to *S. aureus* (100.0±0.96).

There was a statistically significant increase in nanosilver 0.1% subgroup (100.0±0.63) and Nanosilver 1% subgroup (100.0±0.50) to *C. albicans*. A statistically significant increase was found in all nanogold subgroups to *C. albicans*.

TABLE (1) Comparison between studied groups according to antimicrobial activity to pathogenic organisms.

Tested organisms	Control growth	Control discs	Discs with different conc. Of Nanosilver (O.D.620)			Discs with different conc. Of Nanogold (O.D.620)		
			0.01%	0.1%	1%	0.01%	0.1%	1%
E. coli	2.1 ± 0.31	0.0 ± 0.0	0.0 ± 0.0	95.0 ± 0.74	100.0 ± 0.50	0.0 ± 0.0	55.0 ± 0.98	100.0 ± 0.42
$P_{Control\ growth}$		-	-	<0.001*	<0.001*	-	<0.001*	<0.001*
$P_{Control\ discs}$			-	<0.001*	<0.001*	-	<0.001*	<0.001*
S. aureus	1.8±0.22	0.0 ± 0.0	100.0 ± 0.39	100.0 ± 0.45	100.0 ± 0.60	0.0 ± 0.0	100.0 ± 0.69	100.0 ± 0.96
$P_{Control\ growth}$		-	<0.001*	<0.001*	<0.001*	-	<0.001*	<0.001*
$P_{Control\ discs}$			<0.001*	<0.001*	<0.001*	-	<0.001*	<0.001*
C. albicans	2.3 ± 0.44	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.63	100.0 ± 0.50	100.0 ± 0.44	100.0 ± 0.86	100.0 ± 0.76
$P_{Control\ growth}$		-	-	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
$P_{Control\ discs}$			-	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

$P_{Control\ growth}$: P value for comparing between Control growth group and each other group

$P_{Control\ discs}$: P value for comparing between Control discs group and each other group

Significance between groups was done using Student t-test. * Considered Statistically significant at $P \leq 0.05$

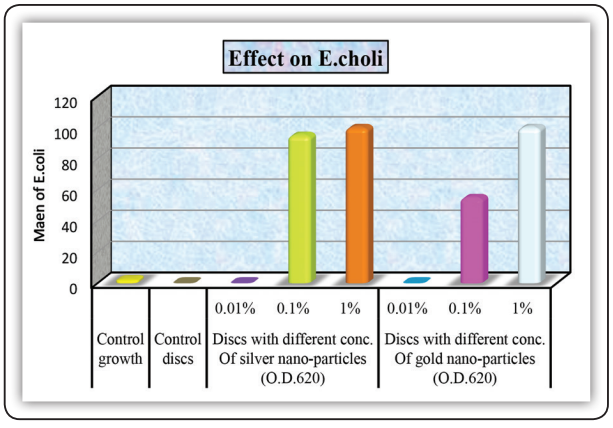

Fig. (1) Bar chart showing comparison between the studied groups according to antimicrobial activity to *E. coli*.

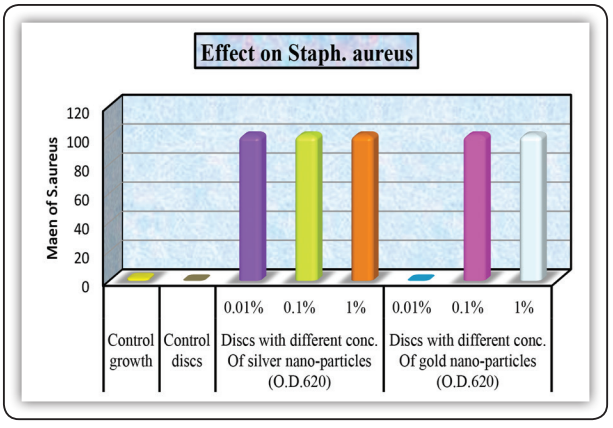

Fig. (2) Bar chart showing comparison between the studied groups according to antimicrobial activity to *S. aureus*

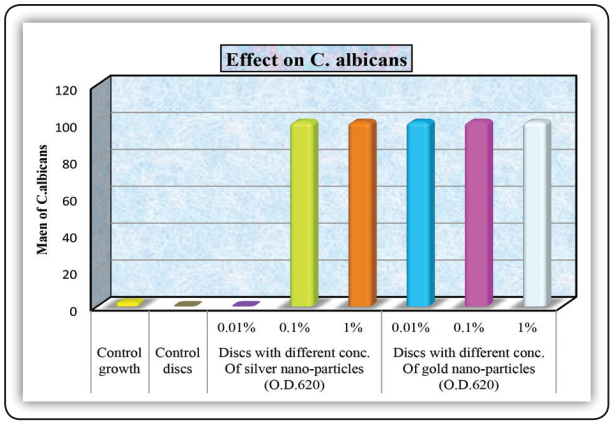

Fig. (3) Bar chart showing comparison between the studied groups according to antimicrobial activity to *C. albicans*

DISCCUSION

The Broth Microdilution Assay, revealed that nanosilver 0.1% subgroup, Nanosilver 1% subgroup, nanogold 0.1% subgroup and nanogold 1% subgroup showed highly significant antimicrobial activity to the three reference strains, as compared to the control group. Nanosilver 0.01% subgroup showed antimicrobial activity only to one strain which was *S. aureus* while Nanogold 0.01% subgroup showed antimicrobial activity only to *C. albicans*.

Silver nanoparticles were reported as antimicrobial agents against bacteria, and their mechanism of action was found to be multifactorial. These nanoparticles preferably bind to Sulphur-containing proteins, thereby forming pores in the cell wall and membrane, leading to loss of intracellular contents. Furthermore, nanoparticles attack respiratory chain enzymes, causing cell disintegration, and interact with phosphate in the DNA, preventing cell division [(19)].

It has been generally believed that the mechanism of the antibacterial effects of gold nanoparticles involves their absorption and accumulation by the bacterial cells that would lead to shrinkage of the cytoplasm membrane or its detachment from the cell wall [(11)].

Another suggestion for the antimicrobial mechanism of action of nanoparticles was related to their catalytic action, oxygen is converted into active oxygen (including hydroxyl radicals) by the action of light energy and/or H_2O in the air or water only at polar surfaces. These active oxygen radicals cause the structural damage in bacteria and lead to the damage or even the death of the microorganisms, the so called "oligodynamic of metal ions "[(20)].

The results of the present study implicate that nanosilver and Nanogold added to PMMA might act like latent antimicrobial material and it could provide the additional benefit of antimicrobial effect even if dentures are worn at night, therefore, could be used as one of the alternative therapy for denture stomatitis resistant to conventional treatment or geriatric denture bearing patients under medically compromised status.

CONCLUSIONS

Modifying PMMA with nanosilver or nanogold significantly increased the antimicrobial activity specially with the higher concentrations. The results suggested that PMMA containing nanosilver and nanogold could be antimicrobial dental materials in denture plaque control.

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