Evaluating the Antimicrobial Activity and Physical Properties of Acrylic Resins Which are Reinforced with Three Different Nanoparticles – An in Vitro Study

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Abstract: ***Purpose**: To evaluate the effect of antimicrobial activity and effect on physical properties of acrylic resins which are reinforced with three different nano particles. **Materials and Methods**: The nanoparticles were incorporated into the acrylic resins according to the corresponding percentages 1%,3%,5%. A total of 300 specimens were fabricated in which 150 are disc shaped and 150 are rectangular bar shaped. All the disk shaped samples were subjected to laboratory analysis for bacterial growth and rectangular disks were subjected to test the physical properties (hardness and fracture resistance). Here the control group PMMA resin which were prepared without any mixture of nanoparticles and testing groups were PMMA incorporated with silver nanoparticles, zirconia nanoparticles and carbon nanotubes. The mean values of obtained results were tabulated and send for statistical analysis. **Results**: There is significance difference seen between all the groups and the control group in both antibacterial and physical properties (hardness and fracture resistance). **Conclusion**: In bacterial growth inhibition silver group is best followed by zirconia and carbon group. In hardness and fracture resistance zirconia group is best followed by carbon and silver. They have improved the biologic and physical properties of denture bases.*

Keywords: PMMA, antimicrobial activity, silver nanoparticles, carbon nanotubes, zirconia nanoparticles, hardness, fracture resistance.

1. Introduction

Acrylic resins are most commonly and extensively used materials as they are able to provide essential properties and have necessary characteristics for their use in diverse functions such as fabricating various dental prosthesis, temporary crowns, denture liners and orthodontic appliances. PMMA is the most popular denture base material currently available. Almost all the dentures are fabricated with this type of polymer. Although the characteristics of this material are not ideal in every aspect, it has many desirable features that make it very favourable(1).

Additionally, poor or inappropriate denture hygiene results in scratches, accumulation of debris and biofilm formation contributing to malodour and growth of microrganisms causing inflammatory changes in the clinical use of dentures and the maintenance of oral health and a rough surface could be induced in resin based dentures due to the common methods of cleansing such as tooth brushing. Thus acrylic resin based dentures must be appropriately processed and polished to improve mechanical properties and achieve a smooth surface.(2)

In prosthodontics acrylic resins are most commonly used material for various dental treatment modalities, where thin biofilm of microorganisms are formed on acrylic resins when they are kept in mouth(3). Taking this into consideration the purpose of the study is to evaluate the ability to inhibit the growth of the biofilm and to increase efficiency of physical properties of acrylic resins when they are added with nano particles of silver, Carbon Nano Tubes and zirconia nano particles.The smaller the diameter of the particle, the better the particle distribution and dispersion in the polymer mass. So these nanoparticles were chosen for the study.

2. Materials and Methodology

All the three materials silver nanoparticles carbon nanotubes and zirconia nanoparticles were provided in powder form by the manufacturer.

Sample fabrication

A total of 300 samples were fabricated in which 150 were disc shaped, used for antimicrobial testing and other 150 were rectangular bar shaped used for physical property(hardness and fracture resistance) testing.DPI heat cure material was selected. To obtain the wax patterns of the specimens, stainless steel dies were used measuring about 30mm diameter and thicknesses about 3.2mm for disc form and bar form about 10.5mm x 3.2mm x 30.2mm. This excess 0.2mm in each die is to compensate for finishing and final adjustment.(ISO/DIS 1576). The obtained wax patterns were flasked using a two pour technique. After elimination of wax, separating medium is applied to both upper and lower chambers.

Processing of Specimens

A recommended mixing ratio of 3:1 (powder an monomer) according to manufacturer's instructions (DPI), was used for specimen preparation. The control group was prepared without any mixture of nanoparticles into monomer. The testing groups were prepared with mixing silver nanoparticles into monomer for silver group, zirconia nanoparticles into monomer for zirconia group, carbon nanotubes into monomer for carbon group. For each sample, 5ml of monomer was taken. For 1% - in 5ml of monomer 0.05mg of nanoparticles were agitated for each sample. For 3% - in 5ml of monomer 0.015mg of nanoparticles were agitated for each sample. For 5% - in 5ml of monomer 0.025mg of nanoparticles were agitated for each sample. the material was ready and packed at dough stage and it was bench cured for 30 min and ready for polymerization.

CURING CYCLE-Short curing cycle: the temperature was raised to 74^0c and maintained for 90 min and temperature raised to 100^0c for additional 30 min. After the polymerization, samples were obtained from the flasks, the fins and excess of the polymerized specimen was trimmed and polished.

Antimicrobial Effects

One standard strain organism was used (C. albicans). For any susceptibility testing, standard inoculums must be employed. The standard inoculums were prepared according to 0.5 McFarland. This is a referenceto adjust turbidity of yeast suspensions so that number of yeast will be within a given range i.e1.5 x 108colony forming units/ml by transferring 1-2 colonies of 48 hours culture to brain heart broth then they were incubated at 350c until the turbidity of media was equal to 0.5 McFarland.

In a 5ml of suspension the sample was made to contaminated and were placed in the incubator for 48 hours. After 48 hours they were taken out and on the top surface of the each sample, sterile distal water was placed, then inoculation loop was heated on the bunsen burner and loop was cooled for 10 to 15 seconds for every sample. Loop was placed on the drop of distal water and it was agitated, then a loop full of solution was collected from the sample and streaked on the labelled agar plates(the agar plates should be labelled for the purpose of identifying). After diffusing the microorganism suspension on the solid agar plates these plates were incubated for 24 hours and the bacterial colonies were counted. The obtained values of colonies were tabulated and were statistically analysed.

Testing of Hardness

Another rectangular bar shaped samples (n=150)were used for hardness evaluation. Macro hardness tester future tech FM – 700 was used for hardness test. The sample was pressed into plate specimen then the indentations were formed on the samples and digital reading was seen on the display of the hardness machine. Three values were obtained for each sample, the mean of three values obtained on each sample were calculated, tabulated and statistically analysed.

Testing of Fracture Resistance

The rectangular bars obtained after hardness test evaluation were subjected for testing fracture resistance. Each sample strip was tested in mechanical testing device. A universal testing machine (Instron, UK) performed a three point bending test to measure fracture resistance. A center load was applied at a crosshead speed of 1mm/min. The maximum load for each specimen of all the groups were tabulated separately and their corresponding flexural strength were calculated and statistically analysed.

3. Statistical Analysis

Mean and standard deviation were employed to describe the data**.** The obtained antibacterial efficiency, fracture resistance, and macrohardness (Rockwell) values were tabulated and statistically analysed between the study groups by using one way ANOVA test and Tukey Post Hoc Test.

4. Results

Silver showed the best compared to the control group followed by zirconia nanoparticles and carbon nanotubes in antibacterial activity(table1) and zirconia showed the best in hardness and fracture resistance(table 2 and 3) followed by silver and zirconia groups. According to parameter 1%,3%,5% ofantibacterial and hardness group in silver group 5% silveris best (63.2 &32.92) in zirconia group 5%zirconia(79.3 & 95.06) is best and incarbon group3%carbon(93.3& 33.67) is best. According to the each level in 1%,3% and 5% of all the three individual groups silver is best.(1%-91.0),(3%-69.7),(5%-63.2) in antibacterial activity and zirconia is best in hardness group(1%-32.98) (3%-34.29)(5%-95.06). (table 1 and table 2) where as In fracture resistance zirconia, according to parameter in 1%,3%,5% of silver group 1% silver(137.9) is best, in 1%,3%,5% of zirconia group 3% zirconia(150.1) is best and in 1%,3%,5% of carbon group 5% carbon(134.1) is best. According to the each level in 1% and 3% of all the three individual groups zirconia is best and in 5% of all the three groups carbon group(134.1) is present.

Table 1: Comparision of mean values of anti bacterial activity

Parameter	Mean	SD	F-Value	P- Value
Silver	74.62	17.028	207.26	0.000
Zirconia	89.22	19.539		
Carbon	100.67	14.947		
Control	162.33	20.41		

The p- value of the ANOVA test is significant (0.000), reveals that there is significant difference between all the groups.

Table 2: Comparision of mean values of hardness

Parameter	Mean	SD	F-Value	P- Value
Zirconia	54.11	31.57	19.96	0.000
Carbon	32.80	2.66		
Silver	31.77	4.54		
Control	31.32	3.50		

The p- value of the is 0.000, that reveals that there is significant difference between all the groups.

Table 3: Comparision of mean values of fracture resistance

Parameter	Mean	SD	F-Value	P- Value
Zirconia	133.45	6.26	1.93	0.130
Carbon	127.10	26.22		
Silver	129.32	15.21		
Control	124.44	21.23		

The p- value of the ANOVA test is 0.130, that reveals that there is no significant difference between all the groups.

5. Discussion

On bases of their properties which possess biocompatibility with the oral tissues, excellent esthetics, superior mechanical properties like modulus of elasticity, impact strength, flexural strength, bond strength ability to repair and dimensional accuracy. Clinical failures of complete or partial denture prosthesis caused from poly(methyl methacrylate) are most likely in the form of fracture either due to fatigue or impact forces of mastication.[(4)] Several researches have tried to improve the physical and mechanical properties of the denture base materials.[(5)]

Poly(methyl methacrylate) has been used in dental prosthetic devices for almost 70 years.[(5)] Although it is the most widely used in dentistry for fabrication of denture bases, this material is still insufficient tofulfill the perfect mechanical requirements for dental applications. This issue was attributed mainly to its low fracture resistance and plaque accumulation.[(6)] Candida albicans, Staphylococcus aureus and Streptococcus mutans are the major microorganism species which colonises the surface and oral mucosa of denture wearers and showed increased resistance to the antimicrobial agents.[(7)]

Now a days nanotechnology has tremendous potential, but social issues of public acceptance, ethics, regulation and human safety must be addressed before molecular nanotechnology can be seen as the possibility of providing high quality dental care to the 80% of the world's population that currently receives no significant dental care.[(8)] Nanoparticles have become an attractive material not only for technical but also commercial applications.[(9)]

Today exclusively used nanoparticles are titanium nanoparticles, copper nanoparticles, silver nanoparticles, iron oxide, magnesium nanoparticles, carbon nanotubes, nitric oxide nanoparticles and polyethylenimine and quaternary ammonium oxide compounds.[(10)]

In this study, three materials were chosen silver nanoparticles, carbon nanotubes and zirconia nanoparticles. Silver nanoparticles were picked out for its best antibacterial activity and they have attracted increased interest due to their unique inertness, physical, chemical and biological properties compared to their macro scaled counter parts.[(11)]

Carbon nanotubes were chosen for its well-known physical and mechanical properties. They are strong, resilient and lightweight and usually form stable cylindrical structure and have a flawless structure, nanotubes were used as they have great surface tension than the nanoparticles.[(12)]
Zirconium nanoparticles powder has been selected to improve the properties of PMMA, it is well known for their mechanical properties. Zirconia has three well-defined crystal phases, they are cubic (c-$ZrO2$), tetragonal (t-$ZrO2$) and monoclinic (m-$ZrO2$) under normal atmosphere and at different temperature.[(13)]

As the heat cured acrylic resin material is used upto greater extent in dentistry. Many prior studies were conducted to analyse antibacterial activity and physical properties of the heat cure material. the main aim of the study is to evaluate the effect of antimicrobial activity and effect on physical properties (hardness and fracture resistance) of acrylic resins reinforced with silver nanoparticles, carbon nanotubes and zirconia nanoparticles.

Three different materials silver nanoparticles, carbon nanotubes and zirconia nanoparticles in three different percentages 1%, 3%, 5% with heat cure acrylic resin were evaluated for determining the antibacterial activity and physical properties like hardness and fracture resistance. Objective of using 1%, 3%, 5% is to provide their physical and chemical properties within the biological limits.[(14)]

The tests which are most frequently used to test the antimicrobial tests were Antimicrobial gradient method, Broth dilution test or tube dilution test, disc diffusion test.[(15)]

By usingspread plate technique colony forming units were counted and values were obtained by applying Broth dilution test in the present study.

In antibacterial efficacy silver(74.2) showed a greater mean than the control group(162.33) followed by zirconia group(89.22) and carbon group(100.67) (table 1) It shows that bacterial colony count insilver group in all 3 percentages(1%,3%,5%) has been decreased when compared to otherthree groups.

This is may be due to catalytic action of silver, oxygen is converted into active oxygen by the action of light energy or H_2O in 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 so called "oligodynamic action of silver'. Another reason is silver nanoparticles were shown to cause pits in the cell wall by increasing the membrane permeability and inactivating the respiratory chain causing the cell damage.

Suganya et al(16), evaluated anti candida effect of heat cure denture base resin reinforced at the ratio of 4:1, 3:1, 2:1 by weight and suggested that the nano silver with its rapid & broad spectrum efficacy and sustained silver release appears to be more effective antimicrobial activity. Yoshiaki et al(17) demonstrated the anti candidiasis action of silver nanoparticle and concluded that denture base materials processed by silver nanoparticles is one of the way to prevent the denture associated oral candidiasis. This antifungal effects by silver are attributable to the disruption of the structure of the fungal cell membrane due to the destruction of membrane integrity, resulting in leakage of intercellular ions and other materials and to the inhibition of the normal budding processes by affecting the cell cycle.

Hardness is the quality of firmness produced by cohesion of particles composing a substance, as evidence by resistance to penetration, abrasion, scratching, cutting or shaping. It denotes strength, stiffness, brittleness, resilience, toughness or combination of these qualities. Hence this property was tested in this study. In this study applying Rockwell test, values were obtained.

Mean of hardness test values showed greater in zirconia nanoparticles. when compared with control group. It shows that hardness of samples have been increasedin an order of zirconia(54.11) carbon nanotubes(32.80)and silver nanoparticles group(31.77) (Table 2). There is no much variation between the carbon nanotubes and silver nanoparticles and control group(31.32) even though carbon is better than the silver nanoparticles. In According to parameter 5% zirconia(95.06) had shown greater hardness in zirconia group, 5% of silver(32.92) shown greater hardness in silver group and 3% carbon(33.67) shown greater hardness in carbon group. According to level zirconia is best in all three individual groups of 1%, 3% and 5%. On an overall hardness is more in zirconia this increase in hardness in zirconia may be due to high interfacial shear strength between the nanoparticles and resin matrix as a result of formation of cross-links or supra molecular bonding which cover or shield the nanofillers which in turn prevent propagation of crack, which lead to increase in flexural strength, fracture toughness and hardness.

TaherehGhaffari et al,(18) evaluated a study on PMMA mixed with silver nanoparticles amalgometer at 0.2% and 2% weight and concluded that proper concentrations in PMMA can improve its mechanical characteristics without any adverse effects. Due to high concentrations, it results in agglomeration sites, which function as impurities and decreases tensile strength and increases the compressive strength.

Some of the materials which give high fracture resistance were the aluminium alloys, titanium, zirconia, iron, copper alloys, steel etc for all classes ofmaterials, the fracture resistance does not simply depend upon the maximumstress or strain to cause fracture but also on the ubiquitous presence of crack-likedefects and their size, since the pre existing defect distribution is rarely known instrength test.

Three point bend test was used on mechanical testing machine in the study. Mean of fracture resistance test values showed greater in zirconia nanoparticle when compared to control group. It shows that fracture resistance of samples have been increased than the control group in an order of zirconia nanoparticle group, silver nanoparticles group and carbon nanotubes group(Table 3) there is no much variation between the carbon nanotubes and silver nanoparticles and control group even though silver is better than the carbon nanotubes.

According to parameter in 1%,3%,5% of silver group 1% silver(137.91) is best, in 1%,3%,5% of zirconia group 3% zirconia(150.8) is best and in 1%,3%,5% of carbon group 5% carbon(134.11) is best. According to the each level in 1% and 3% of all the three individual groups zirconia is best and in 5% of all the three groups carbon group is present.

This is may be due to when sufficient stress develops and crack begins to propagate, a transformation of zirconia from metastable crystal phase to the monoclinic phase occurs which depletes the energy of crack propagation and expansion zirconia occurs and places the crack under a state of compressive stress and crack propagation is arrested this may improve fracture resistance.

Naveen M. Ayad et al(19), carried out study to compare effect of reinforcement of high impact acrylic resin with zirconia on physical and mechanical properties and showed increased transverse strength and fracture resistance due to expansion zirconia occurs and places the crack under a state of compressive stress and crack propagation is arrested, this may improve fracture resistance.

6. Conclusion

It was observed that acrylic resins reinforced with nanoparticles have showed decrease in bacterial adherence and increase in hardness and fracture resistance.Silver showed the best role in antibacterial activity followed by zirconia and carbon and zirconia is best in hardness and fracture resistance followed by carbon and silver.

The only disadvantage is the colour change observed. Light blue colour in silver group, pinkish white pale colour observed in zirconia group and black colour in carbon group. Furthermore long term studies are required to enhance the optical properties and methods to prevent colour change of denture base.

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