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Influence of Titanium Dioxide and Zinc Oxide Nanoparticles Incorporated in Thermocycled Polymethyl Methacrylate to Improve Flexural Strength, Impact Strength and Antimicrobial Properties: An In-Vitro Study

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ABSTRACT : Polymethyl methacrylate (PMMA) is the most widely used denture base material. However, it has many inherent disadvantages and also it may compromise the physical and mechanical properties while in use and make it vulnerable to fracture under cyclic loading. The main objective of this study is to compare flexural strength, impact strength and anti-microbial properties of polymethyl methacrylate (PMMA) under thermocycling after incorporating titanium dioxide and zinc oxide nanoparticles. N=90 in the ratio of 3:1 after incorporating 1gm titanium dioxide nanoparticles into 30 samples (Group 1) and 1gm zinc oxide nanoparticles into 30 samples (Group 2) and the remaining 30 samples without nanoparticles (Group 3-control group). 10 samples from each group are tested for flexural strength using universal testing machine, impact strength using pendulum impact testing machine and colony formation using scanning electron microscope respectively. The addition of titanium dioxide and zinc oxide nanoparticles shows a significant change in the flexural strength, impact strength and anti-microbial property. P value < 0.001, shows that there is significant difference in the flexural strength, impact strength and anti-microbial property of PMMA after incorporating titanium dioxide nanoparticles and zinc oxide nanoparticles. Within the limitations of the study, nanoparticles can improve the properties of conventional PMMA.

KEYWORDS: Thermocycling, Flexural strength, Impact strength, Anti-microbial property, PMMA, Titanium dioxide nanoparticle, Zinc oxide nanoparticle

I. INTRODUCTION

A wide range of polymers are used for various applications in clinical dentistry[1]. Amongst these, poly methyl methacrylate (PMMA) is a polymer that is most commonly used in dental laboratories to make dentures, maxillofacial prosthesis, splints, orthodontic retainers as well as denture repair, whereas in dental clinics it is used for relining dentures and to make temporary crowns, and on an industrial scale it is used for the fabrication of artificial teeth[2,3,4].

PMMA gained popularity for various dental applications due to its unique properties, including its low density, aesthetics, cost-effectiveness, ease of manipulation, and tailorable physical and mechanical properties. Although there are a number of concerns associated with using PMMA, such as the fracture of dentures due to water sorption, poor impact strength and flexural strength[3]. In addition to these disadvantages, heat-polymerized PMMA resin release residual methyl methacrylate monomer, which affects its dimensional stability[5] and leads to porosity that facilitates the adherence of oral microorganisms such as Candida albicans. Further, monomer leaching can also promote allergic reactions such as stomatitis, oedema, and mucosal irritation leading to ulceration[6]. During mastication, the oral cavity comes in contact with food at different temperatures and masticatory forces which can alter the properties of the denture base materials. One of the main causes of flexural fatigue is due to repeated masticatory load as well as para-functional habits and highimpact forces caused by dropping of denture.[7] Another disadvantage is the ease of deposition of denture plaque on its surface which can lead to increased microbial growth ultimately leading to oral infections. Material ageing can dramatically affect the physical and mechanical properties. Theongoing research has introduced a variety of modifications to overcome these drawbacks and further improve its properties such as its conductivity, water sorption, solubility, impact strength, flexural strength and antimicrobial properties. In the past, different reinforcing agents such as rubbers, macro fibers, and fillers have been employed to improve the mechanical properties of denture base resins. Recent advances in the field of science and technology have led to the development of nanodentistry and introduced new approaches for the better reinforcement of dental materials.[7]

The concept of nanotechnology was introduced by Richard Feynman in 1959.[8] Nanotechnology is “the art and science of manipulating matter at the nanoscale (1-100 nm).” ‘Nano’ is derived from the Greek word which means Dwarf.

National Nanotechnology Initiative defined nanotechnology as the “Research and technology development at the atomic, molecular and macromolecular levels in the length scale of approximately 1-100 nm range, to provide a fundamental understanding of phenomena and materials at the nanoscale and to create and use structures, devices and systems that have novel properties and functions because of their small and/or intermediate size.” The future trend in dentistry is nanotechnology, aptly termed as Nano dentistry.[9] The small size of nanoparticles provides larger surface area and hence increase the effectiveness of nanoparticles. The most common nanometals used in dental materials are titanium dioxide (TiO_2), and zinc oxide (ZnO), gold, silver, copper oxide, magnesium oxide, iron oxide, cerium oxide, zirconium, aluminium oxide.[10] TiO_2 nanoparticles and ZnO nanoparticles (NPs), with their unparalleled properties such as high selectivity, enhanced cytotoxicity, biocompatibility, and ease of synthesis, these materials were utilized in the field of dentistry.

The improvements of denture base mechanical properties should be accompanied by improvements in surface properties. Although, the effects of TiO_2 nanoparticles and ZnO nanoparticles addition on denture base strength have been reported, there is a lack of comparative investigations of nanoparticle effect on surface properties. Therefore, this study was done to assess and compare the effects of adding TiO_2 nanoparticles and ZnO nanoparticles on the flexural strength, impact strength and antimicrobial properties of PMMA denture base material.

II. METHODOLOGY

90 number of samples of polymethyl methacrylate blocks are made and this is divided into 3 main groups with 30 samples each. Out of which 1gram titanium dioxide nanoparticles are incorporated into 30 samples (Group 1) and 1gram of zinc oxide nanoparticles incorporated into 30 samples (Group 2) and the remaining 30 samples without nanoparticles (Group 3- control group). All the samples are thermocycled and based on the tests to be done it is again subdivided into group A, B and C with 10 samples each for testing flexural strength, impact strength and anti-microbial growth respectively.

PMMA specimen preparation

(a) Preparation of metal dies for sample preparation

• Rectangular standardized specimens (65 mm × 10 mm × 3 mm) were prepared, as per the ISO 1567 for measuring flexural and impact strength. (Fig 1)

• Circular standardized specimens were prepared with diameter 10mm and height 3mm (10 mm × 3 mm) for testing microbial growth. (Fig 1)

(b) Preparation of mould

Metal dies are coated with a thin layer of petroleum jelly before being invested with dental plaster. Plaster and water are mixed and poured in the inferior part of the flask. Then the metal dies are placed into unset plaster. Once plaster sets, separating medium is applied and allowed to set. Next, the superior part of the flask was placed on the inferior part, plaster poured in it and pressed under hydraulic pressure device. After setting, the flask is opened and the metal dies are carefully removed from the investing material. The moulds are evaluated for any porosities and roughness and then apply separating medium.

Fabrication of the Heat cure PMMA specimens

All ingredients were measured using standardized weighing machine and glass measuring cylinders.

1gram of titanium dioxide nanoparticle was added into 10gram PMMA polymer and mixed thoroughly using manual method. Then it is mixed with 4.3 ml liquid monomer and allowed to reach dough stage. During the dough stage, it is kneaded

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and packed in the mould. The trial closure was done using cellophane sheet. The flask was then placed in hydraulic press and pressure was applied incrementally until the flask was firmly closed. Flask was reopened and cellophane sheet was removed. The flash was teased away gently with round end of wax knife. The flask assembly was closed and placed in hydraulic press for 30 minutes (bench curing) and then transferred to the flask carrier. Similarly, heat cure PMMA specimens incorporated with 1gm of zinc oxide nanoparticle (Group2) and control group(Group 3) was fabricated.

The PMMA heat cure resin was processed by a short curing cycle according to the manufacturer's recommendation. After bench cooling for 30 minutes, the flasks were immersed in the cold water until they were cooled to room temperature and deflasking was done. Samples with defects and not fulfilling dimensional specifications were discarded and new samples were fabricated. Finishing of all the specimens was done with tungsten carbide bur and polished using the conventional laboratory polishing method: coarse pumice, water, lathe bristle brush, and soft leather polishing wheel. The finished and polished specimens were stored for wet conditions in distilled water at room temperature.

Thermocycling of the test samples: (Fig 3)

In the present in vitro study thermal cycling was done to stimulate the intraoral conditions. All samples subjected to thermal cycling for 5000 cycles respectively in distilled water bath between 5˚ Celsius and 55˚ Celsius with a 30 sec dwell time in a thermocycler apparatus. This is based on the assumption that oral cavity may be subjected to maximum of 10 times extreme hot and cold temperatures during eating of food per day. Upon completion of thermocycling the samples were stored in distilled water in their respective container at room temperature, until they were subjected to flexural strength, impact strength and anti-microbial growth testing.

To obtain the flexural strength (MPa) the following formula was used: $FS = 3\ WL/2bd^2$, considering FS as the flexural strength, W the maximum load before the fracture (N), L the distance of 50 mm between the supports, b the width of the specimen (mm), and d the thickness of the specimen (mm).

In this study, the Charpy method with unnotched specimens was used to evaluate the

Mean and standard deviation of the three groups are presented in table 1, 2 and 3. One way ANOVA test is used for analysing the data.

impact strength of acrylics tested because it was reported that the methods used to apply the notch can set up stresses and be difficult and time consuming, and not reproducible. Impact strength (IS) was calculated using the following formula; $IS=E/wt$ where E is the energy required to break the specimen (J), w is the width (6 mm) and t is the thickness of the specimen (4 mm).

Specimens were prepared with standard dimensions of 10 mm x 3 mm. An inoculum of the yeast strain, obtained from Sabouraud Dextrose Agar (SDA) plates, was suspended in 30 ml of Sabouraud Dextrose Broth (SDB) and incubated at 37 ºC for 24 hours under agitation (120 rpm). After this time, the microorganisms were harvested by centrifugation at 8000 rpm for 5 min at 15 ºC, washed twice and resuspended with phosphate-buffered saline (PBS) of pH 7.0 to a concentration of 107 cells/mL by adjusting the optical density (OD) of 0.951 at 530 nm using a spectrophotometer.

Each sterile specimen of PMMA incorporated with nanoparticles or PMMA without nanoparticles (control) was carefully placed into a glass tube, 0.1 ml of cell suspension (107 cells/ml) obtained was added and the volume adjusted to 10 ml with SDB. After 24 hours of incubation at 37ºC, the specimens were aseptically removed and rinsed 5 times in phosphatebuffered saline to remove weak adherent yeasts. Each specimen was then immersed in 10 mL of PBS and vortexed for 1 min to remove the biofilm from the acrylic surface. Serial decimal dilutions of the microorganism suspensions were immediately prepared, inoculated on SDA and incubated for 48 hours at 37 ºC. Thereafter, resulting colonies were counted, transformed to Log10 CFU and standardized according to the area of acrylic specimens (Log/cm2). Biofilm structure formed on the surface of specimens of PMMA incorporated with nanoparticles and PMMA (control) were analysed by SEM.

III. RESULTS AND STATISTICAL ANALYSIS

The present study was undertaken to compare the flexural strength, impact strength and anti-microbial properties of titanium dioxide and zinc oxide nanoparticles incorporated in PMMA after thermocycling.

Table 1: Summary of flexural strength

Groups	*Count*	*Mean*	*Standard deviation*	*P value*
GROUP1	10	125.3	2.45	<0.001
GROUP2	10	66.19	1.56	
GROUP3	10	115.39	2.22	

Table 2: Summary of impact strength

Groups	*Count*	*Mean*	*Standard deviation*	*P value*
GROUP1	10	4.03	0.20	<0.001
GROUP2	10	7.09	0.38	
GROUP3	10	22.7	1.05	

Table 3: Summary of antimicrobial growth

First dilution

Groups	Count	Mean	Standard deviation
GROUP1(10^1)	10	0	0
GROUP2(10^1)	10	0	0
GROUP3(10^1)	10	245.7	2.71

Second dilution

Groups	Count	Mean	Standard deviation
GROUP1(10^3)	10	0	0
GROUP 2(10^3)	10	0	0
GROUP3(10^3)	10	3.2	0.63

Third dilution

Groups	Count	Mean	Standard deviation
GROUP1(10^5)	10	0	0
GROUP 2(10^5)	10	0	0
GROUP3(10^5)	10	0	0

The statistical analysis by one way ANOVA test regarding flexural strength of PMMA incorporated with nanoparticles like titanium dioxide and zinc oxide after thermocycling shows that there is a significant difference in the flexural strength between control and test groups of both materials. P value <0.001, so there is strong evidence against the null hypothesis. Titanium dioxide showed more flexural strength (mean: 125.3 N/mm^2) compared to other groups.

The statistical analysis by one way ANOVA test regarding impact strength of PMMA incorporated with titanium dioxide and zinc oxide after thermocycling shows that there is a significant difference in the impact strength between control and test groups of both materials. P value < 0.001, so there is strong evidence against null hypothesis. Zinc oxide showed more impact strength (22.7KJ/m^2) compared to other groups.

Regarding anti-microbial growth in PMMA control sample, the yeast has shown considerably good growth. In the final dilution, colonies are absent. The TiO_2 and ZnO nanoparticles incorporated PMMA samples

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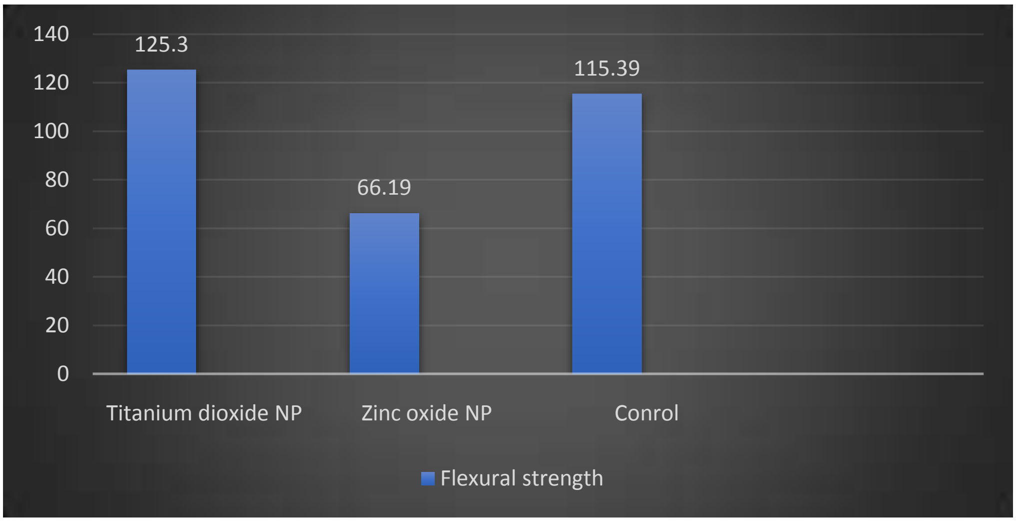
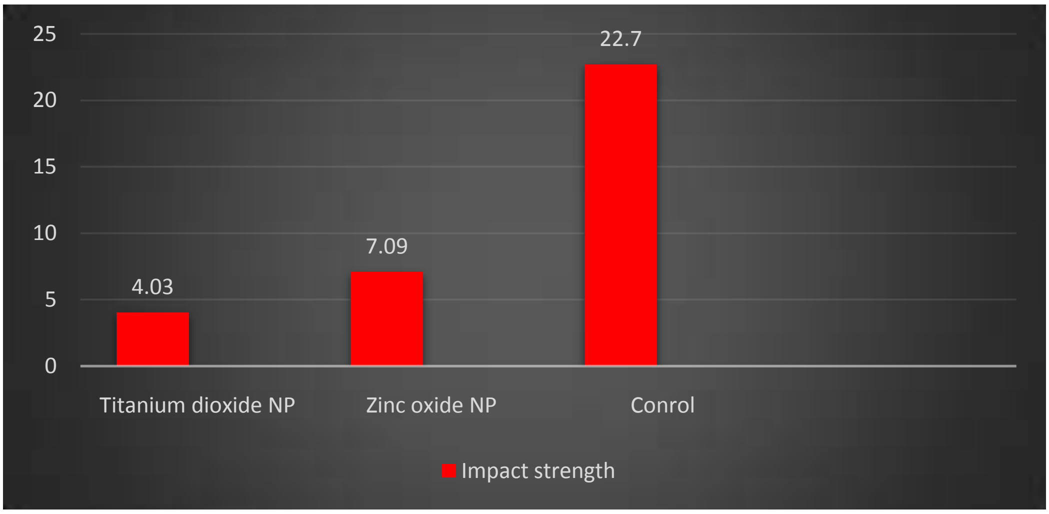
strongly inhibit the growth of yeast species even at the first dilution. Statistical analysis by one way ANOVA test shows that there is a significant difference in the microbial growth between control and test groups of both materials. TiO_2 and ZnOnanoparticles have excellent anti-microbial property when incorporated in PMMA as they prevent the colony formation.

Graph 1: Comparison of flexural strength of PMMA incorporated with titanium dioxide and zinc oxide nanoparticles and control group after thermocycling



Graph 2: Comparison of impact strength of PMMA incorporated with titanium dioxide and zinc oxide nanoparticles and control group after thermocycling



IV. DISCUSSION

This study compared the effect of flexural strength, impact strength and anti-microbial property of PMMA incorporated with titanium dioxide and zinc oxide nanoparticles after thermocycling.

The numerous advantages of poly methyl methacrylate (PMMA) make it the most dominant polymer used as denture base material. The ease of processing, low cost, light weight, stability in the oral cavity, and aesthetic properties are of these advantages.[11] However, this material is not ideal in every aspect. PMMA resin denture base material has poor surface properties and weak mechanical properties including impact and flexural strength.[12] Therefore, resins should be reinforced using different materials to enhance their properties. Recently, nanotechnology invaded the dental field and initiated investigative research projects to explore the possible applications and expected

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benefits within dentistry. It is of paramount importance to know the science behind this nanotechnology to know how to utilize it to our advantage.

In dentistry, many attempts have been made to create an improved version of PMMA with the addition of different nanosized fillers.[13] The properties of the new composite material depend on the nature of the added nanoparticles, their size, and morphology.[14]In this study, 1gm titanium dioxide and 1gm zinc oxide nanoparticles are incorporated to improve the properties of PMMA under thermocycling to stimulate the intraoral conditions.

TiO_2 nanoparticle is a biocompatible material, chemically stable, free of toxicity, resistant to corrosion with high strength, high refractive index and economically feasible material. Even the slight addition of TiO_2 nanoparticle reinforcing agent to a polymeric material affects the electrical, optical, chemical, and physical properties of the resulting hybrid material.

Properties of the hybrid material (PMMA incorporated with TiO_2 nanoparticle) will depend on the dispersion of the nanoparticles. within the matrix, which is directly related to the amount of nanoparticles added. To achieve good dispersion of nanoparticles within polymers, different methods of TiO_2 nanoparticle addition were suggested. It can be either added to acrylic powder or monomer. The addition of TiO_2 nanoparticle to acrylic powder was suggested where the required percentages of titanium dioxide nanoparticles were weighed and thoroughly mixed with acrylic powder. To attain a uniform and homogenous distribution of TiO_2 nanoparticle, the use of ultrasonic mixer, mortar and pestle, high-energy ball milling and silanization of particles were all employed in which ball milling seemed to be the most effective method.[15,16,17] In another method, TiO_2 nanoparticles were mixed with the acrylic powder up to 20 min in an amalgamator to obtain a homogenous mix.[18-19] In this study, manual method of mixing the nanoparticles with resin powder by hand to create the desired filler/powder ratio.[20]

Zinc oxide nanoparticles (ZnO NPs) have been widely investigated over the past two decades because of their superior antibacterial, antifungal, electrical, chemical and optical properties. It is biocompatible, biosafe, and nontoxic with considerable bactericidal properties for the broad range of bacteria (Gram positive and Gram negative) and fungi. ZnO holds a unique optical, chemical sensing, semiconducting, electric conductivity, and piezoelectric properties. ZnO nanoparticles can easily be synthesized by numerous techniques. Therefore, ZnO has a potential biocompatibility over many other metal oxides and has explored many pronounced applications in current antiviral, antimicrobial, biomedical, and environmental areas.[16]

ZnO nanoparticles can be prepared by several methods, resulting in nanostructures of different shapes such as solvent-based ultrasonic irradiation, hydrothermal, micro-emulsion, physical vapor deposition, arc plasma, thermal evaporation, solvothermal, microwave synthesis, wet chemical, solgel, and green methods like plant extracts. In this study, manual method of mixing the nanoparticles with resin powder by hand to create the desired filler/powder ratio.[16]

The addition of titanium dioxide and zinc oxide nanoparticles to PMMA improve both biological and mechanical properties. In this study, commercially available titanium dioxide and zinc oxide nanoparticles are added to polymer and mixed uniformly to compare the flexural strength, impact strength and anti-microbial growth under standard conditions. Rectangular specimens of standard dimensions were fabricated for accommodating the universal testing machine for flexural strength and impact strength. Circular specimens were fabricated for studying anti-microbial growth.

In this in-vitro study, the samples were prepared in standard dimensions, thus care was taken to design and carryout this study with utmost accuracy. However, the present study has got certain limitations. The monomer polymer ratio of PMMA specimen were not uniform among the samples.Only a specific amount of nanoparticles are added and compared the properties. The study does not compare the properties at different concentration.Colony formation is studied using yeast species, commonly causing denture stomatitis. No other species was included in the study.The nanoparticles used in this study have an average particle size of 30-50 nm for ZnO and 10-20 nm for TiO_2..Incorporation of nanoparticles affected the color of the heat-polymerized PMMA denture base resin.

V. CONCLUSION

Within the limitations of the study, addition of titanium dioxide nanoparticles and zinc oxide nanoparticles into PMMA can significantly improve the flexural strength, impact strength and anti-microbial properties after thermocycling.This study considered only the above-mentioned properties which is requisite for a denture base material and further studies are inevitable to examine the effects on remaining properties.

Furthermore, studies which intend to assess the mechanical properties on a long-term basis and examine the consistent presence of titanium dioxide nanoparticles and zinc oxide nanoparticles over a period of time especially when used by patients are essential.

Figure 1. Metal dies for fabrication of sample

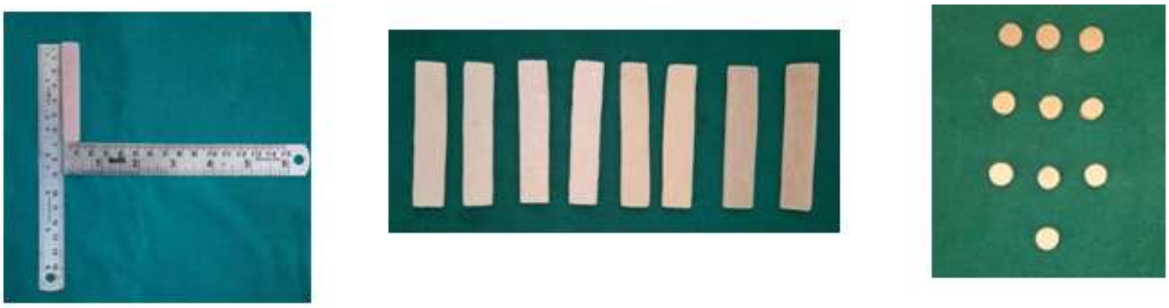

Figure2 : Samples of standard dimension
(a)For flexural and impact strength(b)For anti-microbial growth

Figure 3: Samples during Thermocycling

Figure 4:Samples mounted on Universal Testing Machine

Figure 5:Pure colonies of Candida albicans (ATCC 10231)

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