Original Article

Assessment of the New Nanocomposites (PMMA/(HAP, FE2O3) + CROCUS SATIVUS L.) as Dental Fillings and Antifungal.

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Abstract

Background: Polymethyl methacrylate (PMMA) is a cost-effective polymer that exhibits unique properties when combined with organic nanoparticles to create polymer composites with enhanced functionality. **Objectives:** This study seeks to examine the surface roughness of dental fillings constructed from biomaterials using polymethylmethacrylate polymer, and to assess the varying patterns of salivary pH and salivary amylase activity. **Material and Methods:** nanohydroxyapatite prepared from fish bone waste, Fe2O3, and polymethyl methacrylate with the addition of prepared saffron dye was utilized. The proportion of nanohydroxyapatite and iron oxide added was (0.2, 0.4, 0.6) g. The casting method was used to create membranes for the prepared fillings, which were used to investigate the surface roughness. Standardized protocols were to evaluate the pH level and amylase activity using the colorimetric method. **Results:** The surface roughness of Fe_2O_3 nanofillers was noticeably greater than the surface roughness of HAP nanofillers. Although the roughness values varied, they were still below 200 nm. This variation is directly related to the size, diameter, thickness, and relative volumetric loading capacity of the nanomaterials. Salivary alpha-amylase and pH were chosen as biomarkers for changes in the new filling, reflecting the activity of the immune system. The activity was measured in three cases: first, without saliva treatment, and second, when saliva was treated with the addition of different concentrations (0.7%, 8%, 1 g). The results confirmed that the materials used do not affect the activity of saliva enzymes and pH conditions, indicating that the saffron-colored nanomaterials, HAP and Fe2O3, are biocompatible. At (0.6g) of nano-Fe2O3, antifungal activity against Candida albicans was detected. According to the study, saffron-dyed nano-Fe2O3 improve the strength and longevity of composites, and at a concentration of 0.6g, the composite demonstrated antifungal activity against Candida albicans. **Conclusion:** The surfaces of the prepared fillings are smooth crystalline surfaces and non-toxic and biodegradable fillings made of hydroxyapatite, iron oxide and saffron dye based on (PMMA) indicating their efficacy in dental restoration as well as pH and amylase levels in the filling remained stable. It possesses antifungal properties.

Keyword: hydroxyapatite, iron oxide and saffron dye based, PH in filler, Alpha-amylase salivary in filler.

Introduction

In order for oral applications to be successful, it is important to understand the conditions of the oral environment. The interaction between oral tissue and materials plays a crucial role in the success of these applications. The oral environment's primary function is to keep the mouth moist with saliva and aid in swallowing food[1]. The oral environment differs from other parts of the human body, both structurally and functionally. Various types of biological responses to biomaterials are produced[2]. such as Exposure to body fluids can lead to deterioration or corrosion of substances within the oral cavity or the permeation of the teeth or alternatively the oral environment may interact with substance's to release cytotoxic or harmful components[3]. Numerous fillers have the potential to induce various pathological functions, including endocr-

in dysfunctions, cardiovascular ailments, pharyngeal complications, as well as immune and reproductive system disorders[4]. In addition, there are factors that affect the fillings inside the mouth: Surface roughnes[5], pH Changes[6], and Interaction with different food chemicals such as nicotine, caffeine, alcohol and drugs that can also affect the longevity of substances and the effectiveness of enzymes in saliva[7]. The soft tissue and solid tissue are the two groups main of tissues found un the mouth cavity[8]. As this new material aims to replace oral hard tissues (teeth) with fillings of Nanocomposites PMMA/(HAp, Fe2O3) + Crocus sativus L. This study will examine the impact of surface roughness, morphology of dental fillers preparing form the Nanocomposites (PMMA/(HAp, Fe2O3) + Crocus sativus L.), pH of saliva, and alpha amylase. Surface roughness is crucial for characterizing dental composites because it influences bacterial adhesion and plaque accumulation[9]. Surface characteristics are significant in the initial adherence of Candida to the dental resin and offer an opportunity for further bonding and colonization. Understanding the effect of surface roughness in the adhesion of C. albicans to poly (methyl methacrylate) (PMMA). For that A resin material that has been optimized should have the desired physical characteristics and a favorable biologic response. For complete dental fillers to be clinically successful and long-lasting, polymer properties are essential[10]. In addition, the shape and morphology of the fillings have a significant impact on the properties of the filling, as shown to be critical factors in both the loading of the filling and strength[11]. Dietary habit is still the major factor causing dental caries as related with enzyme in oral cavity will induce lowering of pH value[12]. pH quantifies a fluid's acidity, alkalinity, or neutrality. pH values below 7 indicate acidity, whereas values above 7 signify alkalinity[13]. Oral pH significantly influences microbial community formation, particularly molecular microbes linked to ivory tooth decay, including specific bacilli types[14]. Research on fillings and oral pH fluctuates, relying on laboratory conditions. Previous studies have identified the impact of materials on saliva microbial composition, improving understanding of oral microbiota and dental caries, aiding in disease diagnosis and treatment effectiveness.[15]. Amylase, an enzyme found in salivary glands, pancreas, and mammals, hydrolyzes starch and glycogen by cleaving alpha glycosidic bonds, with optimal activity at pH 6.0-7.0 and temperature 37°C.[16]. Amylase, a digestive enzyme found in plants, animals, bacteria, and fungi, is crucial for mucosal immunity and biotechnology. It breaks down carbohydrates in saliva, facilitating digestion. Salivary alpha-amylase, a significant protein component, is a biomarker for stress-induced sympathetic nervous system activation. Recent research explores its intriguing nature.[17]. Use amylase saliva as a vital indicator of stress without taking blood samples[18]. The aim of this study is to investigate and explore the pattern of changes in saliva pH and enzymes after place of different types ratio of fillers sweeteners in persons with high caries risk and low caries risk. The importance of exploring this Prove the quality of fillings in human saliva.

Materials and Methods

Poly (methyl methacrylate) is used as granular form. Provenance of poly (methyl methacrylate) is Shanghai Kaidu Industrial development Co, Ltd, China, saffron dye extraction Through Dehydration: crocus was extracted using According to the method used in the source [19],

Hydroxyapatite (HAP) preperting from wiste fish bone by using Calcination method in the source [20]. Chloroform (IUPAC name, trichloro-methane) has the formula CHCl3, and it is Organic Solvent, clear, colorless, strong-smelling, and dense liquid.

Preparation of (PMMA/HAp+crocus sativus l.) Nano composites. Two grams of PMMA were fully dissolved in 63 ml of chloroform in a glass beaker for thirty minutes under continuous stirring at a temperature of 25°C. Once the pure sample was dissolved, amounts of HAp and Fe2O3 were added in ratios of 0.2, 0.4, and 0.6 grams. The glass beaker was then placed into an ultrasonic device to disperse the nanomaterials for 5 minutes, as shown in Table (1). 10 ml of dye was added to the mixture, and 100% (PMMA-HAp) and diverse samples were formed for each ratio. The composite was first placed in custom molds made by[21] and then treated with LED light curing as in figure (1). After treatment, the composite with the higher ratio (0.6) g of dental filler was ground into powder, dissolved in chloroform, and deposited onto glass surfaces using the casting method.

Table (1) Weight percentages of (PMMA/(Hap, Fe2O3)+ crocus sativus l.) Nanocomposites.

PMMA g	Nano-HAp g	Fe2O3 g
2	0	0
1.8	0.2	0.2
1.6	0.4	0.4
1.4	0.6	0.6

Figure 1: Molds Employed in this Study.

Testing of activity Amylase enzyme

The fillings were ground using the mill located in the laboratories of the Department of Geology, Faculty of Science, where they were transformed into fine powder. Then, the weight percentages of the fillers (HAp, Fe2O3) were determined and placed in special laboratory tubes, as shown in Table 2.

Table 2: Weight percentages of fillers added

Filler of Nano-HAP	0.6%gm	8%gm	1gm
Filler of Nano-Fe_2O_3	0.6%gm	8%gm	1gm

Saliva samples are obtained from persons according to the technique followed by Takai [22]. A total of 36 saliva samples were obtained from subjects with dental caries aged between 18-35 years of both sexes during a period of three days in the morning, diagnosed by the dentist, and stored at a temperature of (-5C˚). In this study, we recorded data from (12) subjects, with three samples taken from each subject. First, the mouth was washed with water to remove any food residues. Then, the samples were placed in disposable plastic test tubes, some of which contained filler powder in different percentages. Finally, the samples were divided into three sections:

I. The control group and the number of (12) samples that were not added to the fillers powder.

II. The first comparison group (Comparison Group 1) and its 12 samples are combined with proportions of Nano-HAP filler powder.

III. The second comparison group (Compare Group 2) and its number (12) samples are added with ratios of Nano-Fe2O3 filler powder.

IV. The samples are placed in the centrifuge at 3000 rpm for 5 minutes at a constant

temperature, and the serum is stored at 10°C.

V. After that, a microbead is used to extract the serum, which is then placed in disposable plastic test tubes. Each sample is numbered according to the division.

Amylase activity is determined using the Scandinavian method of Gunatillaka [23]. Using the colorimetric method for estimating amylase activity, the amount of starch compensated by the enzyme under specific conditions of temperature, time and volume using iodine as a reagent, saliva samples were dissolved and diluted in 1/250 of normal saline. The starch solution is prepared by dissolving (0.4 g) of soluble starch paste in a boiling solution containing (200 mg)of dry sodium dihydrogen phosphate, (30mg) of sodium chloride, and (70mg) of sodium benzoate. The mixture is then cooled, and the pH is adjusted to (7). The following procedure is then applied:

I. 20µl of the diluted saliva sample was added to the tube.

II. Contents of the tubes were mixed well and placed in a water bath at 37°C for 7 minutes and 30 seconds.

III. After incubation, 1ml of (5 mg) dissolved iodine solution and 8ml of distilled water were added to the tubes.

IV. The contents were mixed well and the absorbance was measured at (660 nm) using a UV-visible spectrophotometer.

V. Tubes containing all the above contents except the diluted saliva sample were used as a comparison sample.

Amylase activity was calculated using the formula:

Activity Amylase enzyme= (B –T) /B * 1.470

B: Blank Solution (B)

T: Represents the test model

Where B and T are the absorbances for both models and (1.470) is a constant to express the absorbance values. Enzyme activity was expressed in units (U/mL). An enzyme unit is defined as the release of (1mg) of maltose from starch in one minute at pH 7 at 37°C.

Measurement of Physical Parameters and analysis Statistical

The weight of each material was measured after the material was converted to nanoparticles, then the proportions were added to the samples. Body mass index (BMI) was calculated using the weight formula in kg/cm. The enzyme activity of a-amylase was expressed in U/ml. One unit of the enzyme is defined as that liberates 1.0 mg of maltose from starch in 1 min at pH 7.0 at 37C. The enzyme activity was measured for each case at a minimum of three times from the sample collected and presented as ± SD (standard deviation). The student test was used to evaluate the salivary parameters between the normal (pure) condition and additives.

Testing of the Antimicrobial Nanocomposites

`A study was conducted to examine the mycoses effects of Nanocomposite (PMMA/Fe2O3+ saffron dye) on mycoses candida albicans which involved the type of candida albicans commonly found on human skin. that were obtained from mycoses Laboratory / University of Babylon / College of Science. Agar disc diffusion test (Potato Dextrose Agar was used as a negative control, and fungi were used as a positive control) was used to verify the antifungal activity of nanocomposite (PPMMA/Fe2O3+saffron dye) with the help of cotton buds used to prepare the Petri dish. To prepare the Petri dish, 50 µl of a suspension solution of 0.5 C. albicans was inoculated. The nanocomposite disk samples grown with the fungi were placed. After that, the agar plates were incubated for 48 hours at

(°C).The antimycoses properties of the Nanocomposite(In the form of tablets) were evaluated through a susceptibility test, The agar-well diffusion method was employed to assess the candida albicans' activity of the Nanocomposite against the mycoses candida albican. Using the sizes of the areas were measured Count the damping formed around the samples using a digital caliper three times. cork porers with a diameter of 6 mm, wells were created. Potato dextrose agar was employed as the negative control, and mycoses were used as the positive control.

Ethical consideration

The University of Babylon at College Science Ethical Committee gave its approval to this project. Before any samples were collected, verbal consent was obtained from each individual who was involved in this study.

Results

Surface Roughness and Morphology for the Dental Fillers

Analyzing the membrane surfaces of fillings prepared using casting method and reinforced by AFM microanalysis is essential for creating an illustrative image and calculating the surface roughness[142]. In Figure (3) and Tables (3), the AFM image analysis of both types of inserts is illustrated. It is observed that the surface roughness of the Fe_2O_3 nanofillers (41.5 nm) is greater than that of the HAP nanofillers (30.8 nm).

Table 3: illustrates the surface roughness coefficients of the filler composite after additions of nano-hydroxyapatite and iron oxide.

Samples	Average roughness (nm)	RMS (nm)
Filler of Nano HAP	30.8	37.2
Filler of Nano Fe_2O_3	41.5	48.2

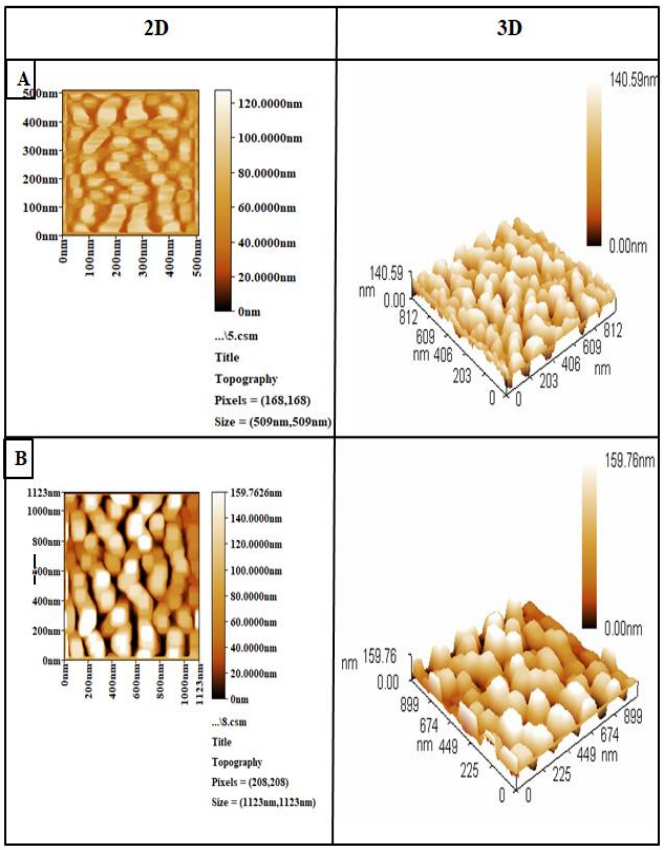

Figure 3: AFM images of the surface roughness coefficients of the filler composite in 2D, 3D (A) At the addition of HAP, (B) At the addition of Fe_2O_3.

Test Activity Amylase enzyme for the dental fillers

The distribution of filling ratio, amylase enzyme activity and level of PH was analyzed using descriptive statistics. A comparison was made between a blank (B) and a modified (t-test model) to study the relationship between amylase activity and dental filling additive for both types of fillings. Three sections were utilized, each with a specific filling ratio, and standard errors were calculated to accommodate the age groups of 18-38 year-olds. The results for the age groups (18-38 years) were analyzed using statistical software (SPSS, version 22, Repeated-Measures-ANOVA) .

The results in the table (4)and(4) figure show the pH level of saliva samples from dental caries patients, indicating that the pH level did not significantly change in the three cases (normal saliva solution for the patients) and that the pH level did not change significantly in the three conditions (normal saliva solution for the patients). Also, The results in the table(5) and figure(5) illustrate the amylase activity of saliva samples from dental caries patients, indicating that the level of amylase activity did not significantly change in the three cases (normal saliva solution of the patients).

Table 4: Descriptive Statistics of the level of PH in saliva in its third condition indicates its treatment without or with the addition of the substance

Number of Group	Mean±SD	Sig.
7% gm	7.245 ±0.19257	0.181
0.8 gm	7.2342 ±0.25207	0.17
1 gm	6.2740 ±0.15748	0.548

Table 5: Descriptive Statistics of The level of the enzyme amylase activity in saliva in its third condition indicates its treatment without or with the addition of the substance

Number of Group	Mean±SD	Sig.
7% gm	523.23±289.396	0.921
0.8 gm	495.0734±394.31906	0.74
1 gm	560.7495±332.50432	0.934

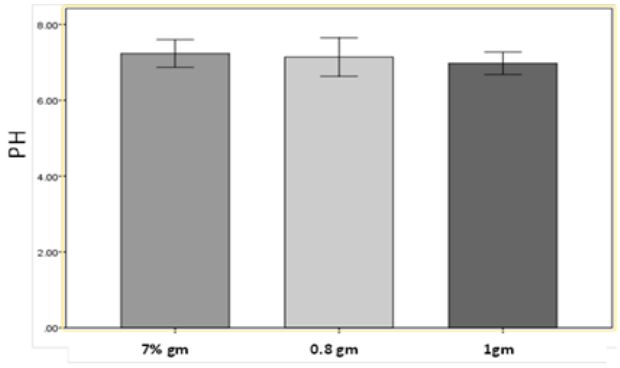

Figure 4: The level of PH in saliva in its third condition indicates its treatment without or with the addition of the substance.

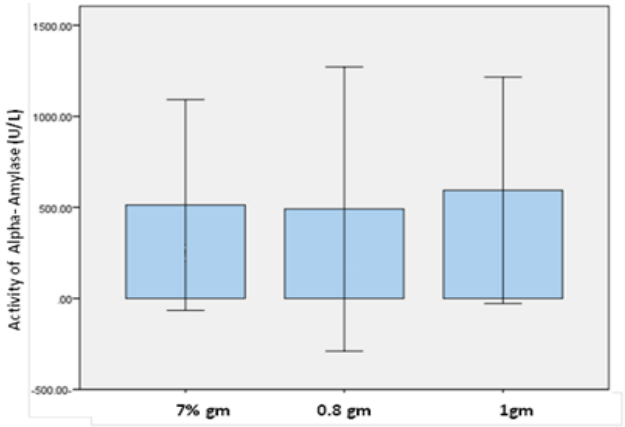

Figure 5: The level of the enzyme amylase activity in saliva in its third condition indicates its treatment without or with the addition of the substance.

The efficiency of anti mycoses activity of Nanocomposite(PMMA/Fe2O3+saffron dye):

A study was conducted to determine the fungicidal effects of nanocomposites (PMMA/Fe2O3+ saffron dye) on Candida albicans fungi obtained from the Mycology Laboratory / University of Babylon / College of Science and complementary to the previous studiesof the composite(PMMA/HAP+ saffron dye). Figure (6-A) shows a picture of the Petri dish poured into it after pouring PDA into it, while Figure (6-B) shows a picture of the dish after the process of transplanting colonies of Candida albicans fungi into it. Figure (6-C). The following figure illustrates the effectiveness of samples formulated with various proportions of nanomaterials on Candida albicans fungal isolates. As illustrated in Figure (6).

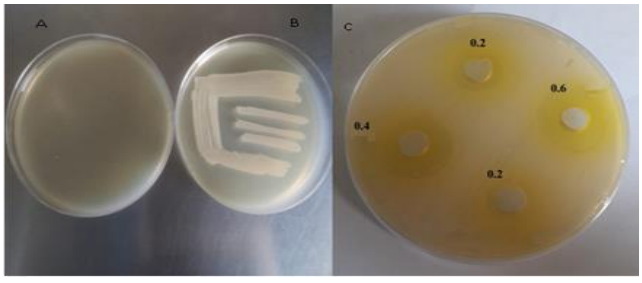

Figure 6: Antifungal inhibition zone of (PMMA\ Fe_2O_3+saffran dye) (0.2, 0.4, and 0.6 ratios) against C. albicans.

Discussion

The AFM image analysis of both types of inserts is illustrated in figure (3). It is observed that the surface roughness of the Fe_2O_3 nanofillers is greater than that of the HAP nanofillers. This difference is directly related to the size, diameter, thickness, and relative volumetric loading capacity of the nanomaterials, as well as the dispersion of nanoparticles within the polymer matrix.. As a result, there are areas with weak, soft, low-filling resin, leading to lower surface roughness[24][25][26]. The white areas in the figures represent grains of material clustered one on top of the other. For these areas, assume that the neighboring grains combine to form large clusters. Therefore, the grains in the white areas are larger in size compared to the others .So the roughness of the nano iron oxide filling is due to the thickness, diameter, and size of the nano Fe_2O_3, which is larger than the nano HAP. Although roughness values varied, the values were still lower than (200nm) which has been reported as an initial point for bacterial plaque accumulation and risk of caries and gingivitis Thus, it can be assumed that the surfaces evaluated in this study have a smooth surface, which does not present any risk for plaque accumulation[5]. As for the results in Table (4,5) for saliva samples from dental caries patients indicated that the level of PH level, amylase activity did not significantly change in the three cases. In contrast, the level of amylase activity did not change significantly in the three cases of normal saliva solution of the patients. This was because the saffron-dyed nanomaterials used, HAP and Fe2O3, are biocompatible, non-toxic, and harmless to dental tissues and cells after applying the filling powder composed of HAP and Fe2O3 to the PMMA matrix [27-30, 19]. The associations between dental caries and amylase activity also with PH levels for all people and the three age groups were not statistically significant. For 18-38 year old older adults. As illustrated in Figure 6, the percentage of Fe2O3 nanoparticle added to the dye and PMMA was observed to affect the inhibition of fungi. This effect is due to the nanoparticles and also to the presence of saffron dye which contains biologically active terpenoids [31,32].

Conclusion

There have been life indicators discovered for psychological and clinical physiological practice. The use of salivary biomarkers has become increasingly popular over the past decade. Over the course of two years, there has been concern that dental amalgams could be used for tooth restoration because they were available at a time when their use was significantly reduced for several reasons, including toxicity[15]. The objective of the study was to analyze and investigate the changes in filling roughness, salivary pH, and enzymes after varying types of filler proportions in subjects with high caries risk and low caries risk. The proximity of the average surface roughness (Roughness) to the root mean square (RMS) values indicates that the surface of the formed fillings is smooth, crystalline, and very smooth, as indicated by the atomic force microscopy (AFM) measurement of the prepared dental fillings. The filler produced from hydroxyapatite, iron oxide, and saffron dye has been proven to be non-toxic and biodegradable. This was determined through testing with the amylase enzyme, which remained unchanged and at the same level. It possesses antifungal properties.

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