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Comparative Evaluation of Antibacterial Properties of Fluoride Releasing Pit and Fissure Sealants with Fluoride Releasing Pit and Fissure Sealants Incorporated with Zinc Oxide and Curcumin Nano Particles Respectively: An In-Vitro Study

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KEYWORDS

Fluoride releasing Pit and fissure sealants; Nano particles; antimicrobial analysis; Scanning electron microscopy (SEM)

ABSTRACT:

Aim and Objectives: The study aims to assess the antimicrobial efficacy of fluoride-releasing pit and fissure sealants containing Zinc oxide nanoparticles or Curcumin nanoparticles, and compare them with conventional fluoride-releasing pit and fissure sealants

Materials and Methods: 270 disk shaped fluoride pit and fissure sealant samples (FPFS) 4mm in size were incorporated with nano particles. Curcumin nano particles (CUR NPs) were prepared using antisolvent precipitation and Zinc oxide nano particles (ZNO NPs) were readily obtained. Scanning electronic microscopy (SEM) analysis was performed for nanoparticles and pit and fissure sealant samples. Antimicrobial efficacy of sealants was tested against streptococcus mutans and lactobacillus acidophilus.

Results: SEM Analysis confirmed nanoparticle size in the NPs powders as well as after incorporation into FPFS. Optical density was recorded to evaluate antimicrobial analysis in a microplate reader. Highest optical density of Streptococcus mutans was seen in Group 1 (1% Cur) followed by Group 2 (1% ZnO), Group 3 (0.5% Cur) and Group 4 (0.5% ZnO). Least optical density was seen in Group 6 (negative control). Highest optical density of Lactobacillus acidophilus was seen in Group 4 (0.5% ZnO) followed by Group 2 (1% ZnO), Group 3 (0.5% CUR). Least optical density was seen in Group 6 (negative control). The overall difference in optical density among six groups was statistically significant.

Conclusion: Antimicrobial analysis done using direct contact test and checking optical dentisty (OD) which showed 0.5% ZnO NPs showed highest OD against Lactobacillus acidophilus and while 1% CUR NPs showed highest OD against Streptococcus mutans when compared to positive control which was FPFS.

Journal of Chemical Health Risks

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Introduction:

"Nanotechnology in medicine is going to have a major impact on the survival of the human race"- Bernard Marcus.

Dental caries is a multifactorial disease caused by the alteration of bacterial biofilm composition, leading to an imbalance between the demineralization and remineralization processes. Eventually, it was manifested by the formation of caries lesions in primary and permanent teeth.[1] Overall prevalence of dental caries was 54.16% (CI: 0.4966-0.5866), whereas age-specific prevalence was 62% in patients above 18 years and 52% among 3-18 years of age ($P < 0.0001$). Maximum overall prevalence was noted in mixed dentition (58%).[2]

Pit and fissure caries accounts for about 90% of the caries of permanent posterior teeth and 44% of caries in the primary teeth in children and adolescents.[3]Unique morphology of pits and fissure pattern makes them prone to dental caries in permanent dentition.[4]More effective measures are necessary to protect pits and fissures; these include the use of pit and fissure sealants. Sealant application is a preventive conservative approach involving the introduction of sealants into the pits and fissures of caries prone teeth; this sealant then bonds to the tooth micromechanically, providing a physical barrier that keeps bacteria away from their source of nutrients.[5]

Resin based pit and fissure sealants result in more plaque accumulation and biofilm adherence. Bacterial biofilm growth contributes to secondary caries and failure of resin-based pit and fissure sealants. Secondary caries around the sealed fluoride releasing pits and fissures at the material–tooth interface remains the common problem. Secondary caries may form around the sealed pits and fissures at material–tooth interfaces, either due to partial loss of material or microleakage induced by polymerization shrinkage.[6]

Methods to inhibit biofilm growth on resin composites have been sought for several decades and hence, applications of nanotechnology led to the development of novel strategies in preventive dentistry, especially in the management of bacterial biofilms. Nanoparticles (NPs) provide wider range of interactions with the microorganisms due to their nanoscale dimensions, thereby increasing the antibacterial activity. Nano particles of various materials are been obtained such as metals, metal oxides, polymers, herbs, etc for reinforcement, antimicrobial and therapeutic purposes. [7]

Nanoscience is an emerging domain of science which deals with the study of materials that have very minute dimensions, in the range of nano scale. The word itself is a combination of nano, from the Greek "nanos" (or Latin "nanus"), meaning "Dwarf", and the word "Science" meaning knowledge. Nanotechnology is about to affect almost every field that humans have created. It is an exciting field in technology that will impact medicine, materials and manufacturing. It will revolutionize future world by developing the current using materials in durability and reactivity. We have great opportunities to construct things smaller in size, lighter in weight and stronger. From the present activities going on in the world specifically by no. of conferences, seminars and the funds injected in this field we can say that this rapidly expanding field is going to bring about an innovative transformation in upcoming years. Therefore, scientists and engineers have great interest in this emerging field.[8]Nanoparticles (NPs) dedicated to nanomedical applications ought to have a preferential size of less than 200 nm. [9]

Dentistry is considered to be a branch of medicine, such as orthopedics or physiology or neurology. In fact, while based on common foundations of medicine, dentistry is a large stand-alone area, which has its own grasps to the different fields mentioned above (American Dental Association, 2014). As a result, within the dental area several specialized disciplines exist. [10]Materials used in dentistry have higher scope in applications of nanotechnology, such as incorporating nano particles in different restorative or preventive materials. This extension of nanotechnology in the field of dentistry is termed 'Nanodentistry.' It is revolutionizing every aspect of dentistry. It consists of therapeutic and diagnostic tools and supportive aids to maintain oral hygiene with the help of nanomaterials. Research in nanodentistry is evolving holistically but slowly with the advanced finding of symbiotic use of novel polymers, natural polymers, metals, minerals, and drugs.[11] And in a such a way different nano particle can be incorporated in different materials and can be subjected to different physical and chemical tests to evaluate their properties.

Journal of Chemical Health Risks

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JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

In this study nano particles chosen are Zinc oxide (ZnO) and Curcumin(Cur) nano particles are being chosen . Antibacterial activity of zinc oxide nanoparticles (ZnO-NPs) has received significant interest worldwide particularly by the implementation of nanotechnology to synthesize particles in the nanometer region. Many microorganisms exist in the range from hundreds of nanometers to tens of micrometers. ZnO-NPs exhibit attractive antibacterial properties due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity.. Interestingly, ZnO-NPs are reported by several studies as non-toxic to human cells(Colon G et al 2006), this aspect necessitated their usage as antibacterial agents, noxious to microorganisms, and hold good biocompatibility to human cells[12] Hence, as a functional inorganic important material, ZnO nano particles is increasingly being developed for use in research and bio medical material application.

Curcumin is a natural occurring organic active constituent of Curcuma longa (turmeric root stem) that shows many different pharmacological effects such as anticancer, antioxidant, anti-inflammatory, antimicrobial and antiviral activity. Curcumin inhibits the growth and proliferation of many bacterial strains such as staphylococci, lactobacilli and streptococci. [13] Curcumin can be used in the form of micro- and nanoparticles. Nanoparticles have a high potential for use against pathogenic bacteria since they can well pass the bacterial cell membrane due to their small size.[14] Application of this biomolecule in the form of nano particles in dentistry is not being researched widely and hence considering its properties its incorporation in dental materials and its antimicrobial efficacy should be evaluated.

Hence, addition of these two nano particles to commercially available fluoride releasing pit and fissure can help us evaluate antimicrobial activity and also let us have evaluation of organic and inorganic nano particles in resin based fluoride releasing pit and fissure sealant.

Materials and Methods:

Methodology to prepare curcumin nano particles: Antisolvent precipitation

Curcumin (100 mg, 0.27 mmol; Sigma Chemical Company, St. Louis, MO, USA) was obtained in dichloromethane (20 mL), and 1 mL of this solution was added to boiling water (50 mL) dropwise with a flow rate of 0.2 mL/minute within five minutes under ultrasonic conditions, with an ultrasonic power of 100W and a frequency of 30kHz. After sonication for 10 minutes, the contents were stirred at 200 rpm at room temperature for about 20 minutes until a clear orange-colored solution was obtained. The solution was concentrated under reduced pressure at 50°C and was then freeze-dried to obtain a pale orange powder and then subjected to scanning electron microscopy to ensure its optimal particle size.[15](Figure 2)

Zinc oxide nano particles were readily available in 30-40 nm size (Sigma Chemical Company, St. Louis, MO, USA)(Figure 1) *After preparation of curcumin nano particles; zinc oxide nano particles and curcumin nano particles were subjected to SEM analysis to check the particle size.*

Categorization of samples

- Group-1 Fluoride releasing pit and fissure sealants with 1%CUR NPs
- Group-2 Fluoride releasing pit and fissure sealants with 1% ZNO NPs
- Group-3 Fluoride releasing pit and fissure sealants with 0.5%CUR NPs
- Group-4 Fluoride releasing pit and fissure sealants with 0.5% ZNO NPs
- Group-5 [Positive Control] Fluoride releasing pit and fissure sealants (FPFS)
- Group-6 [Negative Control]

Preparation of samples of fluoride releasing pit and fissure sealants incorporated with nano particles for SEM analysis

- CUR NPs and ZNO NPs were weighed using digital analytical balance and dispensed on weigh boat and the onto which FPFS were added according to ratios for respective groups. (Fig
- The mixed FPFS were dispensed on glass slide and with help of another slide they were spread to form a thin layer on the slide. (fig

Journal of Chemical Health Risks

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JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

- Then each slide was cured separately for 40 secs using led curing light.
- FPFS with CUR NPs took 60 secs to cure completely
- With help of a cutter square samples were cut and then stored in tubes and then subjected to SEM analysis.
- SEM analysis was done after gold coating the samples and then placed in high vaccum chamber.
- They were analysed with 500x magnification to 100000x magnification and the size of particles were evaluated.

SEM procedure for analysis of nano particles

The samples of NPs were in powdered form and were subjected to SEM Analysis.

Step 1 - The sample was fixed on a SEM-stub using double-sided adhesive tape

Step 2 - The powdered samples were spread on the stub as thinly and evenly as possible so as to ensure electrons have path to the stub base for proper conductivity.

Step 3 - Sample stub kept in SEM chamber and operated at 3 kV.

Step 4 - Run the sample and capture the images as different magnification.

The SEM analysis was done to check the particle size of the NPs.

SEM procedure for analysis of FPFS incorporated with NPs.

Step 1 - The sample was fixed on a SEM-stub using double-sided adhesive tape

Step 2 - Sample coated with thin layer of gold under vacuum

Step 3 Sample stub kept in SEM chamber, operate at 3 kV

Step 4 - Run the sample and capture the images as different magnification

Preparation of samples of FPFS incorporated with nano particles for anti-microbial analysis

- 270 FPFS samples were made. (135 each for testing antimicrobial properties against Streptococcus mutans and Lactobacillus acidophilus
- CUR NPs and ZNO NPs were weighed using digital analytical balance and dispensed on weigh boat and onto which FPFS were added according to ratios for respective groups.
- Each sample was made in flat disc shape with diameter ≈4mm to accommodate into the microtiter plate well which is 5mm in diameter.
- The standardization was maintained using 4mm diameter circles drawn beneath the glass slab and then FPFS were dispensed to accommodate the circle and light cured immediately for 40 secs.
- The prepared samples of each group were placed in the wells of four 96-well microtiter plates
- The rows and columns marking was recorded to for identification of samples of each group.
- 4 microtiter plates were required for samples to accommodate (2 each for 1 bacterial strain)

Anti microbial analysis of PFS against streptococcus mutans and lactobacillus acidophilus

A volume of 200 ml of a bacterial suspension (3 x10^6 CFU/well of S.mutans and 3 x 10^6CFU/well of L. acidophilus) was added into each well. This volume was sufficient to cover the entire sample of FPFS. The antibacterial activities of the tested materials were measured as inhibition of bacterial growth compared to that in untreated control wells (Negative Control) as determined by measuring change in optical density [OD (biomass)] at 650 nm using Erba Lisa Scan Microplate Reader (Erba Mannheim) after incubation for 72 hrs at 37^OC. [16]

Results:

SEM analysis of ZnO NPs

The observable average particle size estimated for ZnO NPs 53.65nm at 200000x magnification

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

SEM analysis of CUR NPs

The average particle size estimated for CUR NPs was 31.36 nm at 200000x magnification.

Preparation of FPFS samples for anti-microbial analysis. (a) FPFS and FPFS with ZnO nanoparticles

SEM analysis for FPFS incorporated with nano particles.

The nanoparticles were evenly distributed in the FPFS. The particles visible on the surface were measured and the incorporation in the nano particle form was confirmed.

The average nano particle size retained in each group and was confirmed after measurement of the particle size.

The following was the average particle size visible on the surface of the FPFS in each group at 100000x magnification

Group 1: 1% CUR NPs – 45.93nm

Group 2: 1% ZnO NPs – 27.36nm

Group-3: 0.5% CUR NPs - 32.44nm

Group-4: 0.5% ZNO NPs – 37.16nm

Antimicrobial analysis.

Antimicrobial analysis was done using Direct contact test using microplate reader. Increase in optical density refers to increase in inhibition of bacteria.

Table no.1 shows the comparison of optical density of Lactobacillus among six groups. Highest optical density of Lactobacillus was seen in Group 4 (0.5% ZnO) followed by Group 2 (1% ZnO), Group 3 (0.5% CUR). Least optical density was seen in Group 6 (negative control). The overall difference in optical density of Lactobacillus among six groups was statistically significant. Table no.2 shows the pairwise comparison of optical density of Lactobacillus among six groups. Group 1 showed significantly lower optical density as compared to Group 2, Group 3 and Group 4 and no difference with Group 5 and Group 6. Group 2 showed significantly higher optical density as compared to Group 1, Group 5 and Group 6 and no difference with Group 3 and Group 4. Group 3 showed significantly higher optical density as compared to Group 1, Group 5 and Group 6 and no difference with Group 2 and Group 4. Group 4 showed significantly higher optical density as compared to Group 1, Group 5 and Group 6 and no difference with Group 2 and Group 3. Group 5 showed significantly lower optical density as compared to Group 2, Group 3 and Group 4 and no difference with Group 1 and Group 6. Group 6 showed significantly lower optical density as compared to Group 2, Group 3 and Group 4 and no difference with Group 1 and Group 5.

Table no.3 shows the comparison of optical density of S. mutans among six groups. Highest optical density of S mutans was seen in Group 1 (1% Cur) followed by Group 2 (1% ZnO), Group 3 (0.5% Cur) and Group 4 (0.5% ZnO). Least optical density was seen in Group 6 (negative control). The overall difference in optical density of S. mutans among six groups was statistically significant. Table no.4 shows the pairwise comparison of optical density of S. mutans among six groups. Group 1 showed significantly higher optical density as compared to Group 3, Group 4, group 5 and Group 6 and no difference with Group 2. Group 2 showed significantly higher optical density as compared to Group 5 and Group 6 and no difference with Group 1, Group 3 and Group 4. Group 3 showed significantly higher optical density as compared to Group 6, significantly lower optical density as compared to Group 1 and no difference with Group 2, Group 4 and Group 5. Group 4 showed significantly higher optical density as compared to Group 6, significantly lower optical density as compared to Group 1 and no difference with Group 2, Group 3 and Group 5. Group 5 showed significantly lower optical density as compared to Group 1 & Group 2 and no difference with Group 3, Group 4 and Group 6. Group 5 showed significantly lower optical density as compared to Group 1, Group 2 Group 3, and Group 4 and no difference with Group 5

Discussion:

Dental caries is the most prevalent chronic disease worldwide. It's an infectious disease characterized by a multifactorial etiology and slow evolution that leads to the destruction of dental hard tissues. The extensive measures for preventive dental care, is a key to the awareness of population of its existence and to the decline of its prevalence.[17]

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

Pit and fissure sealants technique can be used as part of primary prevention, ante ceding the development of dental caries, or as a secondary prevention measure stopping the disease progress. It is a tool for caries prevention on an individual basis or as part of a public health measure for at-risk populations.[18] With capability to reduce the global burden of the disease an effective, long-lasting pit and fissure sealant with enhanced physical and chemical properties is the need of the hour.

There are 2 types of materials in pit and fissure sealants being used that are glass ionomer and resin-based pit and fissure sealants. Most widely used are resin based because of better physical properties. Biofilm accumulation on resin restorations results in high prevalence of secondary caries.[19] Resin materials do not have inherent antibacterial property and it is learned that microorganisms can metabolize set resin matrix.[20] Antibacterial activity of the resin-based materials with fluoride and chlorhexidine was investigated and found that they initially possess strong antibacterial activity, but their release rates do not last long.[2122]

Nanoscale-based approaches are being widely used and have been proven to be more effective in elimination of biofilm and inhibition of dental caries.[23] NPs are generally classified into organic (dendrimers, micelles, liposomes, or polymers), inorganic (metal or metal oxide based), or carbon = based (fullerenes, graphene, or carbon nano tubes).[24] The large surface area and high charge density of NPs enable them to interact with the negatively-charged surface of bacterial cells to a greater extent resulting in enhanced antimicrobial activity . Moreover, NPs combined with polymers or coated onto biomaterial surfaces was found to exhibit superior antimicrobial properties in the oral cavity [25]

Advancements in nanotechnology have enabled the fabrication of nanoparticles with improved properties.[26] In this study 2 nano particles; Zinc Oxide (ZnO NPs) and Curcumin (CUR NPs) were added to fluoride releasing pit and fissure sealants to check their antibacterial properties and if the antibacterial properties are enhanced post addition into the FPFS.

The current caries paradigm holds that dental caries is caused by acidogenic bacteria that produce lactic acid as a result of the anaerobic fermentation of carbohydrates, coupled with their aciduric properties that allow their survival in a low pH milieu. The ability of lactobacilli and MS to ferment a variety of carbohydrates and to survive in a low pH environment is the major hallmark of the caries paradigm. Before the discovery of MS, lactobacilli were considered the major etiological agent of dental caries because of the high correlation between the Lactobacillus salivary counts and the caries scores (Loesche et al. 1984).

The supragingival dental biofilm constitutes an ecosystem of bacteria that exhibits a variety of physiological characteristics. In particular, the acid production resulting from carbohydrate metabolism by these bacteria and the subsequent decrease in environmental pH are responsible for the demineralization of tooth surfaces (Marsh and Nyvad, 2008). Much research has identified mutans streptococci (MS) as the major pathogens of dental caries. This is because, first, MS are frequently isolated from cavitated caries lesions; second, MS induce caries formation in animals fed a sucrose-rich diet; third, MS are highly acidogenic and aciduric (Hamada and Slade, 1980; Loesche, 1986); and fourth, MS are able to produce surface antigens I/II and water-insoluble glucan, which promote bacterial adhesion to the tooth surface and to other bacteria (Hamada and Slade, 1980).

A systematic literature review by Tanzer et al. (2001) confirms a central role for the MS in the initiation of dental caries on tooth surface. As adherence is one major factor in the formation of dental plaque, some authors have investigated in vitro the correlation between the presence of lactobacilli in dental plaque and their capacity to coaggregate with other species.[27] Hence the antibacterial properties of FPFS and those with incorporated nanoparticles were tested against S. mutans and L. acidophillus.

Pit and fissure sealants may not reach the bottom of pits and fissures and hence a space remains under the polymerized sealant material.[28] Secondary caries or recurrent caries often occurs around sealed pits and fissures between the tooth structure and material due to either microleakage induced by the materials' polymerization shrinkage or partial detachment from the tooth.[29]

Many studies have focused on only calcium and phosphate releasing properties to inhibit dental caries,

Journal of Chemical Health Risks
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JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

which prevented demineralization and simultaneously promoted remineralization. Therefore, a material with antibacterial properties and with continuous acid neutralization which can be effectively utilized in oral environments in patients at a high risk of exposure to dental caries must be developed.

In this study, ZnO NPs used were commercially available (Sigma Aldrich USA, 30-40nm range) and were subjected to SEM analysis to confirm nanoparticle size. CUR NPs were formulated in pharmaceutics lab and subjected to SEM analysis to confirm nano particle size. To our knowledge no study confirms with NPs size with SEM analysis before incorporation in the dental material and later confirms the retention of the same in the pit and fissure sealants.

Zinc is an essential trace element which is found in the muscle, bone, skin and also in the hard tissues of the tooth. Zinc Oxide Nanoparticles (ZnO NPs) are white colored, odorless powder and have a molecular weight of 81.38 g/mol. FDA considers it as a generally recognized as safe (GRAS) substance. Its extensive applications in dentistry are credited to the unique optical, magnetic, morphological, electrical, catalytic, mechanical, and photochemical properties which can be easily altered as per the requirements: by modifying the size, doping with supplementary compounds, or adjusting the conditions of synthesis. As the size of the particles decrease, the desirable characteristics improve. [30]

ZnO showed significant antibacterial activity over a wide spectrum of bacterial species when particle size is reduced to nanometer range.[31] Nanoscale dimensions of ZnO materials allow considerable broader gamut of interactions with microorganisms increasing their antibacterial property.[32] Hence due to such properties ZnO NPs was added to the FPFS to evaluate the antimicrobial properties. Zinc oxice has been used in many resin based dental materials such as composites. Composites containing nano zinc-oxide particles or silver nanoparticles exhibited higher antibacterial activity against Streptococcus mutans and Lactobacillus. The effect of zinc-oxide on Streptococcus mutans was significantly higher than that of silver ($p < 0.05$).[33]

The antibacterial activity of resin composites containing ZnO nanoparticles against Streptococcus mutans using the direct contact test demonstrates that by increasing the nanoparticle content, the bacterial growth is significantly diminished ($p < 0.05$)**Error! Bookmark not defined.** A systematic review mentioned studies that demonstrated a significant improvement of antibacterial properties in composites containing at least 1% ZnO-NPs (wt %), but they mentioned they are unlikely to present any clear clinical advantage due to the short lifetime of observed antibacterial properties.[34]

A total of 196 fissure sealant samples were divided into six test groups and a control group. The test group samples were prepared by incorporating two concentrations (0.5 wt% and 1 wt%) of ZnO and CaF_2 NPs into the sealants. Sealants containing 1 wt% ZnO and CaF2 NPs and their mixture exhibited significantly higher antibacterial activity against Streptococcus mutans and Lactobacillus acidophilus when compared to control group ($P < 0.001$). Samples with ZnO NPs exhibited similar mechanical properties as conventional sealant (control group); however, the samples with CaF2 NPs showed inferior mechanical properties ($P < 0.05$).[35]

. The possible explanations that could be responsible for antimicrobial behavior of ZnO NPs are (1) production of active oxygen species such as H_2O_2 which inhibit the growth of bacteria by internalization into the bacterial cell membrane causing destruction of cellular components such as lipids, DNA, and proteins;[36] (2) zinc ions interfere with the bacterial enzyme systems by displacing magnesium ions which are essential for enzymatic activity of the dental plaque;[37] and (3) interaction between the NPs and bacteria caused by electrostatic forces which are produced by light exposure.[38] These properties add to the antimicrobial activity of fluoride present in the fluoride releasing pit and fissure sealant.

Curcumin (1,7-bis4-hydroxy-3-methoxyphenyl-1,6-heptadiene-3,5-Dione) is the most active polyphenolic constituent, powerful ingredient in the traditional herbal practices.[39]Optimal antimicrobial activity of curcumin has been documented against Enterococcus faecalis and S. mutans.[40] The cariostatic effect of curcumin is mediated by prevention of bacterial adhesion to enamel and destruction of bacterial cell wall via disrupting the peptidoglycan layer.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

Moreover, despite its strong antimicrobial activity, curcumin is non-toxic and safe; Fernandes et al, reported that cell viability of human gingival fibroblasts was not affected by exposure to curcumin in short or long-term. Yen et al, showed improved physicochemical properties of CUR NPs compared to curcumin. They added that CUR NPs has higher solubility and bioavailability than curcumin extract emulsion and can better pass through the cell membrane; as the result, CUR NPs has greater efficacy in lower dose.[41]

The use of curcumin serves two roles in that, it gives a color tint to the pit and fissure sealant. Furthermore, being antibacterial, curcumin would also serve to prevent caries. These formulations contain a resin and in addition may contain extracts of other agents apart from curcumin.[42]

Hence in this study CUR NPs were added to FPFS to check their anti-microbial activity

An *in vitro* experimental study, 1%, 5% and 10% curcNPs were added to Transbond XT composite. 36 discs were fabricated of the four composites which were used for disc diffusion test and 36 were used for biofilm test to assess their antimicrobial activity against Streptococcus mutans and sanguinis and Lactobacillus acidophilus where 1% CUR NPs showed increased antibacterial properties but rest groups did not show any antibacterial activity where solubility of CUR NPs was said to be the issue. [15]

An vitro study used 48 acrylic discs containing 0.5%, 1%, and 2% CUR NPs. The antimicrobial properties of the discs against Streptococcus mutans, Streptococcus sanguinis, Lactobacillus acidophilus, and Candida albicans were evaluated using disc agar diffusion (DAD), eluted component, and biofilm inhibition tests. DAD test showed that none of the curcumin nanoparticle concentrations caused growth inhibition zones for any microorganisms. All the concentrations were effective against all four microorganisms in the biofilm inhibition test except 0.5% for L. acidophilus. In the eluted component test, solutions containing 2% concentration had maximum growth inhibition of all the groups at all time intervals.[43]

The antibacterial effect of curcumin can be explained by following mechanism (1) Studies show that curcumin inhibited bacteria by damaging bacterial membrane. A membrane permeabilization assays confirms that the addition of curcumin results in membrane leakage in both Gram-negative and Grampositive bacteria (2) Curcumin significantly bacteria by inducing significant production of ROS, including singlet oxygen and hydroxyl radicals (3) Curcumin is reported to function as an efflux pump inhibitor in a multi drug resistant pathogenic bacteria (4) Curcumin has been shown to inhibit bacterial cell proliferation by perturbation suggesting that it inhibits bacterial cytokinesis[44]

In this study the evaluation of antimicrobial property of FPFS incorporated with ZnO NPs and CUR NPs was evaluated using direct contact test. Direct contact test was preferred over agar diffusion test for the evaluation of antibacterial activity because studies have shown that in agar diffusion test, sufficient amount of ZnO could not leach to the surrounding environment due to its insolubility.[4546]

We found that ZnO NPs and CUR NPs could endow the FPFS with good antibacterial activity. ZnO NPs and CUR NPs and their mixture in both the concentrations (0.5% and 1%) exhibited stronger antibacterial activity against S. mutans and L. acidophilus.

In this study ZnO NPs (1% and 0.5%) showed higher antimicrobial activity against L. acidophilus compared to FPFS and FPFS with CUR NPs (1% and 0.5%) and also exhibited antimicrobial activity against S.mutans but less compared to FPFS with 1% CUR NPs

In our study FPFS with 1% and 0.5% CUR NPs show increased antimicrobial properties as compared to FPFS against L.acidophilus and S. mutans but less as compared to FPFS with 1% and 0.5% ZnO NPs.

To summarize, the addition of ZnO and CUR NPs to sealants could significantly inhibit the growth of S. mutans and Lactobacillus. Addition of these NPs may alter the flow and color of the material; hence, the present study gives further impetus to investigate these physical properties and also the other mechanical properties such as compressive strength, flexural strength, marginal integrity and wear resistance, etc

Conclusion:

Journal of Chemical Health Risks

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JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

Findings from the present study suggest both nanoparticles improve the antimicrobial efficacy of FPFS. Antimicrobial analysis done using direct contact test and checking optical dentisty (OD) which showed 0.5% ZnO NPs showed highest OD against Lactobacillus acidophilus and while 1% CUR NPs showed highest OD against Streptococcus mutans when compared to positive control which was FPFS.

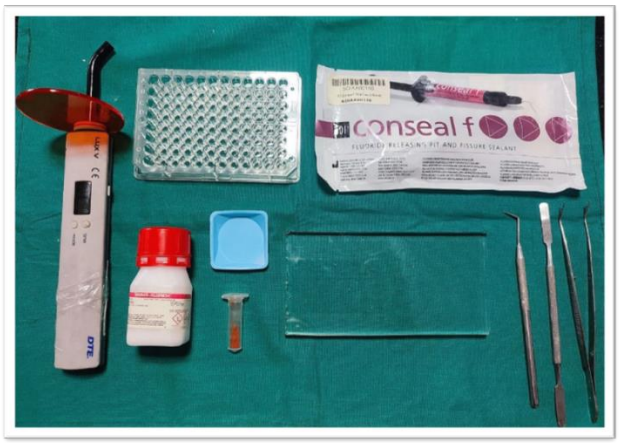

Figure 1: **Armamentarium and materials.(**a) LUX V Woodpecker LED Light curing light, (b.) 96-well Microtiter plate, (c.) Conseal- F Fluoride releasing pit and fissure sealants. (FPFS), (d.) Zinc oxide nano particles 30-40 nm (ZnO NPs) Sigma Chemical Company, St. Lou is, MO, USA, (e.) Weigh boats (Heathrow Scientific), (f.) Curcumin nano particles (CUR NPs) made from curcumin (100 mg, 0.27 mmol;Sigma Chemical Company, St. Louis, MO, USA, (g.) Glass slab, (h.) Probe, (i) Spatula, (j) Tweezer.

Figure 2: Preparation of curcumin nano particles in ethanol**.**

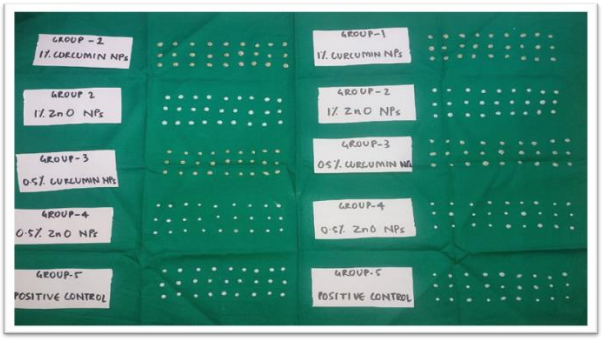

Figure 3: 270 samples of FPFS and FPFS incorporated with Zinc oxide and Curcumin Nano particles.

Journal of Chemical Health Risks
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JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

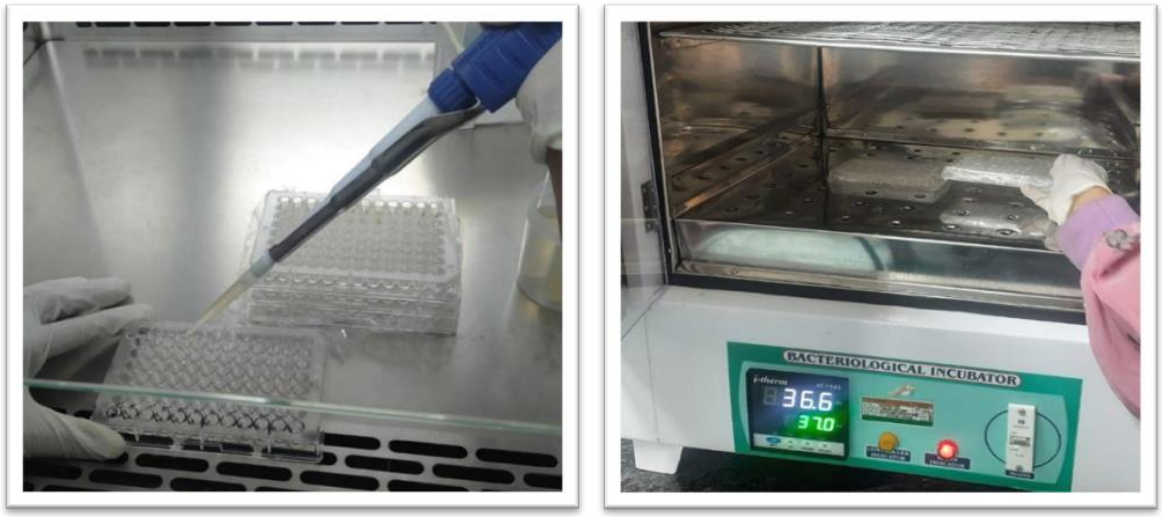

Figure 4: Inoculation of micro titer plates with 200ml bacterial suspension with 3 x 10^6 CFU/well and incubation

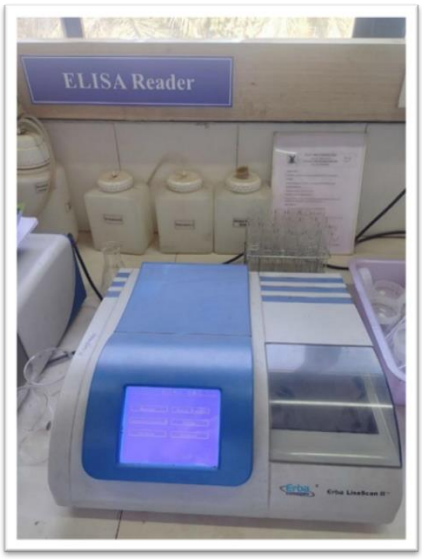

Figure 2: Microplate reader to obtain optical density for antimicrobial analysis.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

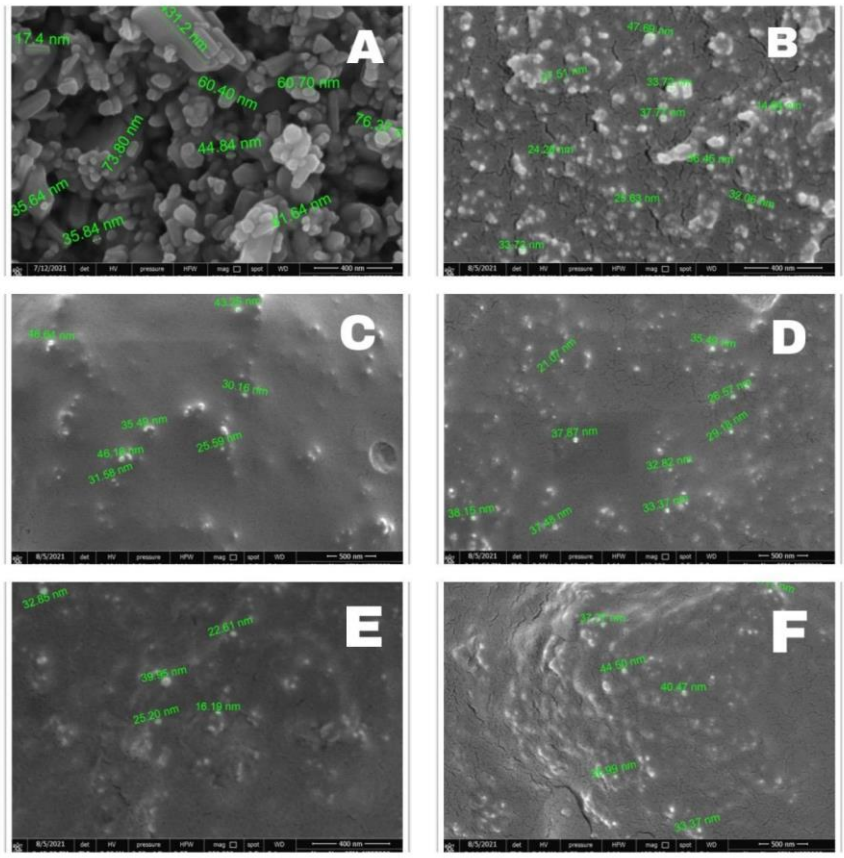

Figure 6: (A) CUR NPs; (B) ZnO NPs; (C) 1% CUR NPs IN FPFS; (D) 1% ZnO NPs in FPFS; (E) 0.5% CUR NPs in FPFA; (F) 0.5% ZnO NPs

TABLE 1: COMPARISON OF OPTICAL DENSITY OF LACTOBACILLUS AMONG SIX GROUPS

Groups	Mean	SD	p value
Group-1 (1%CUR)	0.46	0.16	<0.001*
Group-2 (1% ZnO)	0.88	0.12	
Group-3 (0.5%CUR)	0.84	0.17	
Group-4 [0.5%ZnO]	0.89	0.04	
Group-5 [Positive Control] FPFS	0.50	0.13	
Group-6 [Negative Control]	0.30	0.08	

One-way ANOVA test; * indicates significant difference at p≤0.05

TABLE 2: PAIRWISE COMPARISON OF OPTICAL DENSITY OF LACTOBACILLUS AMONG SIX GROUPS

Group	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group-1	--	0.016*	0.028*	0.013*	0.999	0.593
Group-2	--	--	0.999	1.000	0.030*	0.001*
Group-3	--	--	--	0.997	0.050*	0.002*
Group-4	--	--	--	--	0.025*	0.001*
Group-5	--	--	--	--	--	0.390

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 364-377 | ISSN:2251-6727

Group-6	--	--	--	--	--	--

Post Hoc Tukey test; * indicates significant difference at p≤0.05

TABLE 3: COMPARISON OF OPTICAL DENSITY OF S. MUTANS AMONG SIX GROUPS

Groups	Mean	SD	p value
Group-1 (1%CUR)	0.73	0.02	<0.001*
Group-2 (1% ZnO)	0.67	0.05	
Group-3 (0.5%CUR)	0.44	0.20	
Group-4 [0.5%ZnO]	0.43	0.12	
Group-5 [Positive Control] FPFS	0.30	0.04	
Group-6 [Negative Control]	0.11	0.04	

One-way ANOVA test; * indicates significant difference at p≤0.05

TABLE 4: PAIRWISE COMPARISON OF OPTICAL DENSITY OF S. MUTANS AMONG SIX GROUPS

Group	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group-1	--	0.972	0.038*	0.028*	0.002*	<0.001*
Group-2	--	--	0.128	0.097	0.008*	<0.001*
Group-3	--	--	--	1.000	0.568	0.015*
Group-4	--	--	--	--	0.665	0.019*
Group-5	--	--	--	--	--	0.226
Group-6	--	--	--	--	--	--

Post Hoc Tukey test; * indicates significant difference at p≤0.05

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