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Antibacterial Effect of Different Nano particle Addition to Fissure Sealants on Streptococcus mutans

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Abstract

Aims: The study was conducted to determine the antibacterial action of two types of resin based fissure sealants on Streptococcus mutans isolated from children's saliva and to evaluate the antibacterial action of both sealants after the addition of different types of nanoparticles (silver, zinc oxide, aluminum oxide and magnesium oxide) at very low concentrations 1-10 %w/w.

Materials and Methods: After Isolation and purification of S. mutans from children's saliva, antibacterial activity of the sealants and the nanoparticles and both of them mixed together was tested by a disk diffusion method.

Results: Showed that both sealants did not exhibit any antibacterial action, the nanoparticles exhibited antibacterial action with varying strength, the addition of the four types of nanoparticles was beneficial and an antibacterial effect was obtained in case of silver and zinc oxide nanoparticle with minimum addition.

Conclusions: Within the limitation of this research, addition of silver and zinc oxide nanoparticles to the sealants resulted in producing zones of inhibition against S. mutans ,while magnesium and aluminium oxide did not produce an effective antibacterial sealant.

Key Words: Caries, Fissure Sealants, Nanoparticles, S. mutans.

Introduction

Dental caries is considered a major public health problem worldwide , it is a chronic non-communicable disease(1). It is a source of pain and suffering for many, it affects children, and its management represents a large proportion of the health resources worldwide(2). Topical and systemic fluorides are effective in reducing smooth surface caries but are less effective in preventing occlusal caries(3). Incorporation of fluoride in pit and fissure sealants has been found to play a promising role in the reduction of pit and fissure caries, thereby reducing overall caries incidence (4). There has been increased emphasis on developing restorative materials with anti cariogenic properties. Inorganic antimicrobials agents were used since ancient times to treat microbial infections(5), prior to the extensive use of chemotherapeutic agents in modern health care systems. According to Sahoo et al (2007)(6) "Nanotechnology is the science and engineering involved in design, synthesis, characterization and application of materials and devices whose smallest unit of measurement in at least one dimension is on the nanometer scale(one billionth of a meter 10^{-9} m)". Nanoparticles have properties unique from their bulk equivalent. With the decrease in the dimensions of the materials to the atomic level, their properties change and can be manipulated in various ways(7).

Aims of this study :Was To determine the antibacterial action of two types of fissure sealant (a fluoridated and non fluoridated) on growth of Streptococcus mutans after isolating it from the saliva. .And to evaluate the antibacterial action of the two fissure sealants after the addition of different types of nanoparticles (Ag ,ZnO, Al_2O_3 and MgO)at different concentrations on S. mutans .

Materials and Methods

1-Media Used Was Prepared According to Manufacturers Instructions:

A-Brain Heart Infusion Broth.(BHIB) (Lab M. limited U.K): Used for the sugar fermentation test.

B-Phospate Buffer Saline (PBS): Prepared from mono basic potasium phosphate (Fluka, Switzerland) and dibasic poatasium phosphate (Fluka, Switzerland) according to Cruickshank et al [8] .

C-Tryptose Phosphate Broth :(TPB) Prepared from Tryptose (Himedia, India).Dextrose (Lab Tech chemicals. India)Sodium chloride ((Lab Tech chemicals. India).Di-sodium hydrogen phosphate (BDH, England) , according to El-Samarrai 2001 [9] used for inoculum preparation.

D-Mitis Salivarius Bacitracin Agar (MSBA): Used for isolation and cultivation of Mutans streptococci ,it was obtained from Mitis Salivaris Agar (Himedia ,India) ,with 1% potassium tellurite (Fluka , Switzerland), bacitracin and sucrose were added to the agar to increase it's specification for isolation of Mutans Streptococci [10] .

E-Mueller Hinton Agar (MHA)(Lab M. limited U.K): Used for antimicrobial susceptibility testing.

2-Fissure Sealants:

Two types of sealants were used, a non fluoridated fissure sealant, Natural Elegance pit and fissure sealant (Henry Schein, Germany) and a fluoridated sealant Conseal F pit and fissure sealant (Southern Dental Industry, Australia).

3-Nanoparticles:

Commercial nanoparticles of Ag, ZnO, Al_2O_3 and MgO were purchased from Alfa Aesar Company (Germany) the characteristics and features of the nanoparticles are presented in Table(1).

4-Saliva Collection:

Stimulated saliva was collected from healthy children attending for dental treatment at the Pedodontic Orthodontic and Preventive Dentistry Department, College of Dentistry , University of Mosul, Collection was performed according to Tenovuo and Lagerlöf (1996)[11] . After diluting, 0.1 ml was taken and spread in duplicate on Mitis Salivarius Bacitracin Agar, the plates were incubated anaerobically using a candle jar for 24 hr. at 37°C then aerobically for 24 hrs [12] .

Table (1) Nanoparticles Used in the Study

Formula	Form	Purity	Particle size (nm)	LoT
Ag	powder	99.9%	20-40	C09Y011
ZnO	powder	99%	20-30	I07W013
Al_2O_3	powder	99.5%	40-50	B18Z014
MgO	powder	99+%	100	H16X041

5-Isolate Identification:

After estimation of positive samples, the following methods were used for initial characterization of the isolates[13]

a. Colonial shape and form on Mitis Salivarius Bacitracin Agar.
b. Gram-staining and microscopic examination.
c. Catalase test.
d-Sugar Fermentation test. Fermentation of mannitol (BDH. England), sorbitol (Duchefa. Netherlands), raffinose (Merk . Germany).

6-Inoculum Preperation:

The inoculum of the S. mutans was prepared with turbidity equivalent to 0.5 McFarland scale (1.5 x 10^8 CFU/mL)[14] in test-tubes containing 5 ml of TPB , and incubated for 18 hours.

7-Bauer-Kirby Disk Diffusion Test(Disk-Diffusion Antimicrobial Test) :

Agar diffusion technique [15]was used to study the antimicrobial effect of the fissure sealants mixed with different concentrations of nanoparticles. The addition of nanoparticles was gradually performed 1% w/w addition, a sensitive three digital electrical balance (Kern and Sohn, Germany)was used, and the nanoparticles were added to the two types of fissure sealants by mixing them with a plastic spatula and ultrasonication (JGC Co,Germany) for five minutes [16] at each time after each addition, the procedure involved seeding 0.1ml of S. mutans inoculum on Mueller Hinton Agar , that was left at room temperature for 10 minutes. Wells of equal size and 2mm depth were punched in the agar. Each well was filled with the fissure sealant after mixing it homogenously with different nanoparticles .

The sealant was then polymerized from the top according to manufacturers instruction using LED light for 40 seconds(Coxo medical instrument, China). Plates left for 15minutes at room temperature then incubated anaerobically for 24hrs with a candle jar at 37°C. For this experiment ,the control negative were the two types of sealants (non fluoridated and fluoridated) without the addition of nanoparticles, the control positives were obtained by outlining a 6mm diameter circle on to the surface of the agar and filling it with 0.1 gm of the four types of the nanoparticles Ag, ZnO, Al_2O_3 and MgO that were applied directly on the agar surface(equivalent to 10% w/w addition). The diameters of the halos of bacterial growth inhibition were measured and photographed The experiment was repeated three times to minimize the error.

Statistical Analysis

Means& standard deviations were calculated. Duncans Multiple Range Test was used to test the significance between different concentration of nanoparticles mixed to both fissure sealants. Results were considered significant when $P \leq 0.05$.

Results

Identification of S mutans was carried out by : -

A-Examination of Colony Morphology: On the selective agar plates, S. mutans colonies appeared ovoid or spherical in shape with a raised or convex surface, light blue in colour about 1-2mm in diameter, with smooth surfaces similar to " gum drops" [17] Figure (1and 2).

B-Microscopic Examination : Showed S. mutans cells were gram positive, non motile, spherical or ovoid in shape, arranged in non spore forming chains, short or medium in length as seen in Figure (3).

C-Catalase Test: S. mutans isolates were catalase negative [13].

D-Sugar Fermentation Test: Colonies of S. mutans have the ability to ferment sorbitol, mannitol and raffinose[13]. A positive reaction was indicated by the change in color of bromocresol purple indicator (BDH, England) from purple to yellow .Figures (5 and 6) display sensitivities of S. mutans to the two control negative and four control positive, Both fissure sealants (negative controls) exerted no antibacterial effect, For control positives, the different types of nanoparticles displayed varying degrees of sensitivity with the silver being superior, with a zone of inhibition of 18.47 mm , zinc oxide having 17.92 mm, then magnesium oxide having less antibacterial activity, and finally aluminium oxide which exhibited the least sensitivity. Tables (2,3,4&5) demonstrate the sensitivity of S. mutans to different concentrations of nanoparticles mixed with the fissure sealants, it can be seen that silver nanoparticles, when mixed with 1 % w/w addition showed an immediate antibacterial effect that increased with increasing concentrations, at 3% w/w it showed a zone of inhibition of more than 13mm for both types of sealant that only increased to slightly higher than 14mm at 10% w/w addition, for zinc oxide an antibacterial effect began to appear with 4% w/w addition that also increased with increasing concentration of the zinc oxide nanoparticles in the sealants. Small zones of inhibition were seen with magnesium oxide when it was mixed with (9& 10%) w/w addition, while aluminum oxide did not exhibit any antibacterial activity. Figure (6) displays sensitivity of S. mutans to 3%w/w addition of nanoparticles to the fluoridated sealant .

Discussion

Dental caries belongs to a group of common but complex or multifactorial diseases, such as cancer, cardiovascular diseases and diabetes in which many genetic environmental and behavioral risk factors interact[18] . Clinical studies have shown that caries initiation is associated with an increase in the proportions of oral acidogenic and aciduric bacteria, so this study was performed using S. mutans that was isolated on its selective agar media, this bacteria is a known cariogenic bacteria and is considered as a good indicator bacteria for elevated risk of getting new caries[19]. Sealant materials provide a physical barrier between the oral habitant and deep pits and fissures, which are highly vulnerable to the initiation of caries[20]. Preventive as well as therapeutic sealants are often applied soon after eruption of teeth[21]. With recent advances in material science, the mechanical properties of dental materials have been extensively studied. However, little effort has been made to revise their biological and antimicrobial properties. Thus in this study, examination of the antibacterial properties of the fissure sealants against S. mutans was conducted. Antibacterial testing can be done either by diffusion or by dilution technique.

A diffusion technique was used here, because nanoparticles mixed with composite fillings, exert their antimicrobial effect by direct surface contact with the oral microorganisms through diffusion[22]. Resin-based sealants are the most popular sealing materials in contemporary dentistry, because they are highly esthetic and exhibit the best retention of all sealant materials, have less microleakage and are easy in application [20], so it was decided to use two types of resin based sealants in this study, a fluoridated and non fluoridated one. Figure (5) displays that both types of the used sealants did not exert any antibacterial effect against S. mutans demonstrated by the absence of zones of inhibition, in previous studies, fluoride products exerted an antibacterial effect [23,24]. This might be attributed to the reason that according to a study the amount of fluoride released from conseal f fissure sealants is 9.39+/-2.17 microg F/mm^2 in saline[25] and this is less than the amount of fluoride required to exert an antibacterial effect and produce sufficient inhibition growth of the bacteria, that should range between 20-300 ppm[26,27].

The current study demonstrated that silver and zinc oxide nanoparticles, when added to both sealants produced an antibacterial effect against S. mutans , that increased with increasing concentration of the nanoparticles that were mixed with both sealants, while aluminium oxide did not and magnesium oxide produced small zones of inhibition, it was not possible to increase the addition more than 10% because as the sealants became thicker, they began to loose their flowing ability, which is essential to be able to fill the pits and fissures and seal them, in addition to affecting other properties of fissure sealants. According to caries risk assessments, high risk for development of dental caries have a higher percentage of S. mutans and Oral Lactobacilli with values exceeding 10^5 CFU/ml of saliva[28], in addition to that the polymerization shrinkage of composite resin-based sealants might facilitate the formation of gaps between the resin and tooth surface, providing space for bacterial invasion and proliferation, thereby leading to caries and the failure of restorations. Therefore, if sealants could provide additional antibacterial protection, and not merely act as a physical barrier especially against cariogenic bacteria, then the CFU/ml saliva of the cariogenic bacteria would be reduced to less than 10^5 in the high risk group, reducing their susceptibility for the development of caries.

Conclusion

Within the limitation of this research, the addition of silver and zinc oxide nanoparticles to both types of sealants had a beneficial effect regarding bactericidal activity, but further investigations are required to evaluate other properties before they can be used in a clinical trial on high risk children.

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Table (2): Antimicrobial Activity of Different Concentrations of Silver Nanoparticles Mixed with the Two Types Of Sealants Against S. mutans.

Sealant Type	Mean Zones of Inhibition in mm(w/w Addition) + SD (Duncan's Grouping)											
	C (–)	C (+)	**1%**	**2%**	**3%**	**4%**	**5%**	**6%**	**7%**	**8%**	**9%**	**10%**
Fluoridated	0.0 + 0.0 (e)	18.47 + 0.45 (a)	4.31 + 0.26 (a)	8.47 + 0.22 (d)	13.6 + 0.26 (c)	13.73 + 0.75 (bc)	14.05 + 0.18 (bc)	14.13 + 0.21 (bc)	14.17 + 0.35 (bc)	14.20 + 0.30 (bc)	14.22 + 0.35 (bc)	14.33 + 0.25 (b)
Non Fluoridated	0.0 + 0.0 (g)	18.47 + 0.45 (a)	4.03 + 0.21 (f)	8.17 + 0.15 (e)	13.3 + 0.40 (d)	13.53 + 0.25 (cd)	13.67 + 0.26 (bcd)	13.83 + 0.25 (bcd)	13.9 + 0.10 (bc)	13.97 + 0.38 (bc)	14.03 + 0.42 (bc)	14.13 + 0.42 (b)

Duncan's Multiple Range Test
Means with different letters are statistically significant $P \leq 0.05$.

Table (3): Antimicrobial Activity of Different Concentrations of Zinc Oxide Nanoparticles Mixed with the Two Types Of Sealants againstS. mutans.

Sealant Type	Mean Zones of Inhibition in mm(w/w Addition) + SD (Duncan's Grouping)											
	C (–)	C (+)	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
Fluoridated	0.0 + 0.0 (g)	17.92 + 0.38 (a)	0.0 + 0.0 (g)	0.0 + 0.0 (g)	0.0 + 0.0 (g)	4.04 + 0.14 (f)	7.27 + 0.21 (e)	10.10 + 0.2 (d)	13.50 + 0.17 (c)	13.60 + 0.36 (bc)	13.63 + 0.35 (bc)	13.93 + 0.15 (b)
Non Fluoridated	0.0 + 0.0 (f)	17.92 + 0.38 (a)	0.0 + 0.0 (f)	0.0 + 0.0 (f)	0.0 + 0.0 (f)	3.83 + 0.57 (e)	6.93 + 0.21 (d)	9.30 + 0.70 (c)	13.33 + 0.31 (b)	13.40 + 0.5 (b)	13.43 + 0.55 (b)	13.8 + 0.62 (b)

Duncan's Multiple Range Test
Means with different letters are statistically significant P≤0.05.

Table(4)Antimicrobial Activity of Different Concentrations of Magnesium Oxide Nanoparticles Mixed with the Two Types Of Sealants against S. mutans.

Sealant Type	Mean Zones of Inhibition in mm (w/w Addition) + SD (Duncan's Grouping)											
	C (–)	C (+)	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
Fluoridated	0.0 + 0.0 (c)	8.00 + 0.50 (a)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	2.07+ 0.15 (b)	2.60 + 0.44 (b)
Non Fluoridated	0.0 + 0.0 (c)	8.00 + 0.50 (a)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	0.0 + 0.0 (c)	1.90 + 0.46 (b)	2.13 + 0.38 (b)

Duncan's Multiple Range Test
Means with different letters are statistically significant P≤0.05.

Table (5) Antimicrobial activity of Different Concentrations of Aluminium Oxide Nanoparticles Mixed with the Two Types Of Sealants against S. mutans.

Sealant Type	Mean Zones of Inhibition in mm(w/w Addition) + SD (Duncan's Grouping)											
	C (–)	C (+)	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
Fluoridated	0.0 + 0.0 (b)	4.87 + 0.42 (a)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)
Non Fluoridated	0.0 + 0.0 (b)	4.87+ 0.42 (a)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)	0.0 + 0.0 (b)

Duncan's Multiple Range Test
Means with different letters are statistically significant P≤0.05.

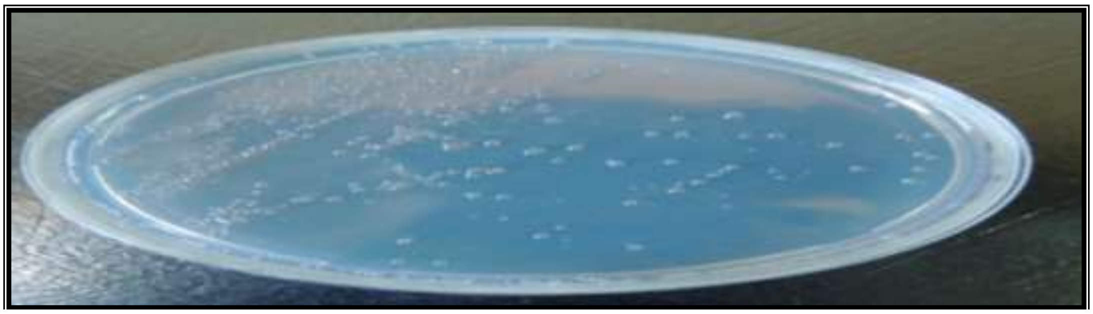

Figure (1) Streptococcus mutans Colonies on MSBA

Figure (2) Streptococcus mutans Colonies on MSBA(Gum Drops)

Figure (3) Gram Stain of S. mutans Chains Isolated from MSBA

Figure (4) Disk Diffusion Antimicrobial Susceptibility test. Control Negative A- Fluoridated sealant B- Non Fluoridated Sealant

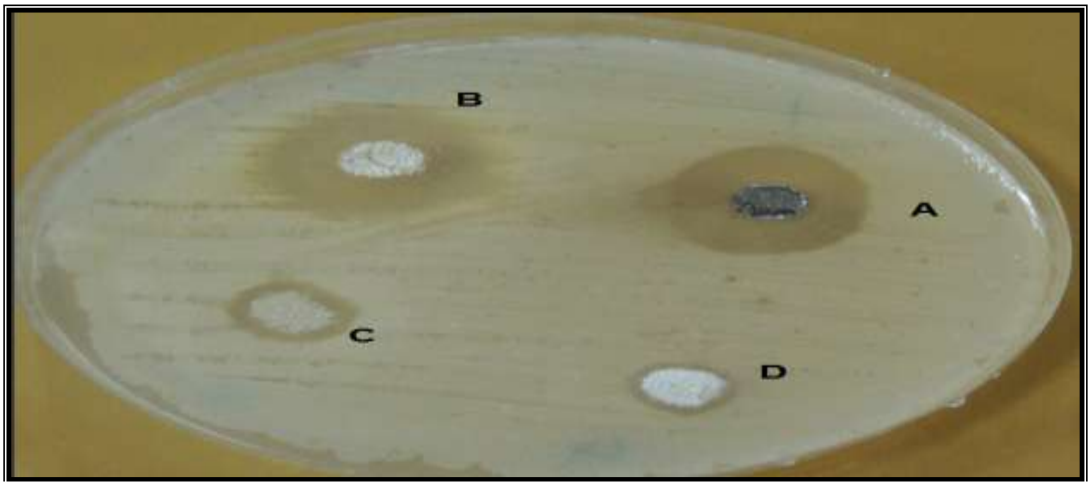

Figure (5) Disk Diffusion Antimicrobial Susceptibility test Control Positive A-AgNP , B- ZnONP , C-MgONP , D-Al_2O_3NP

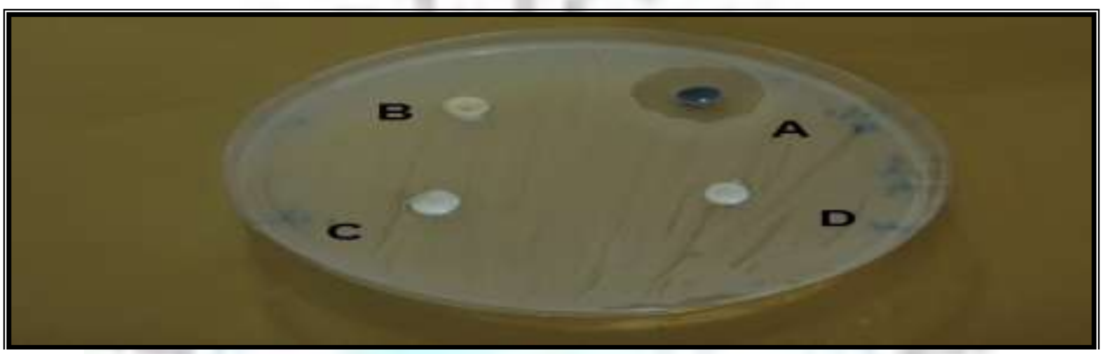

Figure (6) Disk Diffusion Antimicrobial Susceptibility Test(Sensitivities of Streptococcus mutans to 3% w/w Addition of Nanoparticles to Fluoridated Fissure Sealants). A-Ag B-ZnO C-MgO D-Al_2O_3