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ANTIBACTERIAL EFFICACY OF A MOUTHWASH CONTAINING HYDROXYAPATITE NANOPARTICLES ALONE OR IN COMBINATION WITH CHLORHEXIDINE/FLUORIDE

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ABSTRACT

Aim: This study was carried out to evaluate the antibacterial activity of a mouthwash containing clusters of zinc-carbonate hydroxyapatite nanoparticles in combination with chlohexidine or fluoride.

Materials and methods: Antimicrobial agents used in this study were Chlorhexidine diacetate, Sodium fluoride powder and Dr.Wolff's Biorepair against *Streptococcus mutans* (MS). In this study, the relative antimicrobial efficacy of newly introduced zinc-carbonate hydroxyapatite nanoparticels containing mouthwash, chlorhexidine, sodium fluoride and their combination against MS was tested. The MS was inoculated onto its specified culturel media. Agar diffusion test is done. The inhibition zone diameters were measured. Mean inhibition zone diameters were recorded and statistically analyzed

Result: For the effect of the tested antibacterial on the *Streptococcus mutans*, results showed that the mean diameter and standard deviation of inhibition zone (mm) for chlorhexidine (CHX) was 23.9 (2.04) mm, sodium fluoride (NaF) was 12.2 (0.75) mm, hydroxyapatite microclusters containing mouthwash (HAPMC) was 18.2 (1.3) mm, HAPMC+CHX was 22.5 (2.6) mm and HAPMC+NaF was 18.7 (1.8) mm.

Conclusion: Hydroxyapatite microclusters containing mouthwash had a potential antimicrobial activity against MS alone or in combination with other antimicrobials.

INTRODUCTION

Clinical decision-making and the balance between preventive and surgical intervention have become an important part of daily dental practice [(1)]. In the era of these medical models of caries treatment renewed interest in developing an antimicrobial approach for the management of dental caries. In conjunction with this concept, control and prevention of caries has been sought by reducing the number of colonizing bacteria. Reducing their

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level in the oral cavity will provide an additional rationale for the prevention of dental caries [2]

Chlorhexidine (CHX) has been known as an excellent broad spectrum antibacterial compound[3]. Nevertheless, the reported frequent side effects including taste perception alteration and an increase in tooth discoloration [4,5] makes the use of CHX in dental practice questionable.

Also, fluoride is one of the most important and effective component in dental caries prevention programs [6].The mechanisms by which fluoride prevents dental caries may involve at least two major routes [7]; inhibition of tooth mineral dissolution by acid, and enhancement of remineralization in carious lesions. In addition, many studies have also shown that fluoride can affect the biological ability of *Streptococcus mutans* which is the one of the causative factor of dental caries [8,9].

Recently, hydroxyapatite microclusters containing mouthwash is proved as remeneralizing and desensitizing agents by many studies [10]**.** It was reported that it contains some components which might have antibacterial effect [11]. These newly biological and biomimetic approaches are required to optimize the prevention of caries [12,13].

The combination between the antibacterial agents and hydroxyapatite nanoparticles in one mouthwash appearing attractive solution. Therefore, a study focusing on the potential antibacterial activity of an accredited mouthwash containing clusters of zinc-carbonate hydroxyapatite nanoparticles in combination with chlorhexidine or fluoride might be of benefit.

MATERIALS

Streptococcus mutans (MS) (ATCC 25175) Strain was obtained from microbiological resources center (Cairo MERCIN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt).Antimicrobial agents used in this study were Chlorhexidine diacetate (Serva Electrophoresis, GmbH-69115 Heidelberg, Germany), Sodium fluoride powder (Sigma-Aldrich chemistry, St. Louis, Missouri, USA) and Dr.Wolff's Biorepair (Clusters of zinc-carbonate hydroxyapatite nanoparticles) (Dr. Kuret Wolff, Bielefeld, Bologna-Italia)

METHODS

Determenation of bacterial concentration

Serial dilutions of each bacterial strain were done. Swab of the cultivated bacteria was mixed in 100 ml of broth of the specific selective media then the suspension was placed in a shaker for three hours.The optical density (OD) was determined by a spectrophotometer at 600 nm.

Antibacterial preparation

0.1% of chlorhexidine diacetate (CHX) and sodium fluoride (NaF) antimicrobial solutions were prepared. For Dr.Wolff's Biorepair it was diluted by distilled water to get 1:1 concentration. To prepare the combination between zinc-carbonate hydroxyapatite nanoclusters containing mouthwashes and CHX or NaF; sterilized solutions are used in a ratio 1:1

Assessment of the antibacterial activity

Six sterile glass petri dishes containing the selective media; were prepared. The agar was poured into each petri dish. After setting of the agar, six equidistant wells were made using sterile glass cylinder (cork borer). Then, the punched agar were removed leaving the wells which were later filled with the tested antimicrobials. The 100 ml from each antimicrobial solution were dispensed in the wells. The plates were left for 30 minutes at room temperature to allow diffusion of the tested antimicrobials through the agar; afterwards they were incubated at 37˚C for 24 hours. For the negative control group, distilled water was used. After 24 hours, all the plates were removed from the incubator and observed for zones of inhibition of microbial growth around the wells containing the tested antimicrobials.

Statistical analysis

All the collected data were analyzed using two-way ANOVA system to determine the effect of the antimicrobials and the bacterial strain as well as their interaction on the inhibition zone. One-way ANOVA was used to test the significant difference among the antimicrobials.

RESULT

For the effect of the tested antibacterial on the *Streptococcus mutans* bacterial strains, results showed that the mean diameter and standard deviation of inhibition zone (mm) for chlorhexidine (CHX) was 23.9 (2.04) mm, sodium fluoride (NaF) was 12.2 (0.75) mm, hydroxyapatite microclusters containing mouthwash (HAPMC) was 18.2 (1.3) mm, HAPMC+CHX was 22.5 (2.6) mm and HAPMC+NaF was 18.7 (1.8) mm.

One way ANOVA showed that the tested antimicrobials had a statistically significant difference in their effects against Streptococcus *mutans* (Table 1). Chlorhexidine (CHX) and Hydroxyapatite microclusters containing mouthwash with cholrhexidine (HAPMC+CHX) showed the highest antibacterial effect against *Streptococcus mutans* this was followed by HAPMC alone and Hydroxyapatite microclusters containing mouthwash with NaF (HAPMC +NaF) then the NaF alone.

DISCUSSION

In the present study, 0.1% chrlorhexidine showed the highest inhibitory effect against MS. The superiorly have been reported in studies conducted by (**Nakamoto et al., 1995**) and (**Bruschi et al., 2006**). Chlorhexidine affects the bacteria using the following mechanism, The bacterial cell wall is negatively charged and contains sulphates and phosphates then dicationic positively charged chlorhexidine is attracted to the negatively charged bacterial cell wall with specific and strong adsorption to phosphate containing compounds that alters the integrity of the bacterial cell membrane and chlorhexidine is attracted to the inner cell membrane by increasing the concentration of chlorhexidine there is progressive damage to the membrane. Chlorhexidine binds to the phospholipids in the inner membrane and there is leakage of low molecular weight compounds like potassium ions then cytoplasm of the cells is chemically precipitated [16].

The efficacy of the sodium fluoride alone or in combination with any tested antimicrobial was less than chlorhexidine against all the tested microorganisms. These were similar results to those obtained by (**Malhotra et al., 2011**). As mentioned earlier, the main mechanism of the action of fluoride is to maintain equilibrium between the demineralization and remineralization of dental hard tissues rather than antimicrobial action.

	Chlorhexidine (CHX)	**Sodium fluoride (NaF)**	**Hydroxyapatite microclusters containing mouthwash (HAPMC)**	**HAPMC + CHX**	**HAPMC + NaF**	**Distilled water**	***P*-value**
Streptococcus mutans	**23.9 (2.04)A**	**12.2 (0.75)B**	**18.2 (1.3) C**	**22.5 (2.6) A**	**18.7 (1.8) C**	**Negative D**	**<0.001**

Different letters denoted statistical significance within rows at P<0.01

Most of the *in vivo* studies have shown a cariostatic effect of fluoride gels or mouthwashes at 1% or 2% fluoride [18]. Thus, the observed lower efficacy of sodium fluoride could also be attributed to the lower percentage of fluoride (0.2% of NaF) used in this study. This raised concerns for the effective concentration of NaF as antibacterial versus the human health hazard.

In the present study, the mouthwash containing hydroxyapatite nanoparticles alone or in combination with any tested antimicrobial showed relatively comparable results against the tested bacteria. The mouthwash containing hydroxyapatite nanoparticles from its composition contain ZnO nanoparticles which had antibacterial properties. It contains sugar alcohols, such as sorbitol and especially xylitol, which are known to have antimicrobial properties[19]. In addition to these well-accepted effects, the antiadherent but not the antimicrobial effects can be attributed to the size of the adopted biomimetic hydroxyapatite nano particles mimicking the smallest building units of the dental enamel, the enamel crystallites; the respective microclusters of Biorepair are composed of nano sized crystallites[20]. These particles might fill the defects caused by dental caries. So, we recommend the combination between CHX and a mouthwash containing hydroxyapatite nanoparticles to gain benefit of the powerful antimicrobial effect of CHX as well as the remeneralizing effect of mouthwash containing hydroxyapatite nanoparticles.

The combination of sodium fluoride with mouthwash containing hydroxyapatite nanoparticles was more effective against MS compared to sodium fluoride alone. This might be due to the possible synergetic effect of their combination on the MS. Still we need further investigations with highly sophisticated tools to detect the direct action of these combinations on the virulence microorganisms.

This study raised many questions among them; can the mouthwash containing hydroxyapatite nanoparticles replace chlorhexidine? Is there a biomimetic mouthwash that could completely eradicate causative microorganisms of dental caries? Still many studies need to be conducted; *in vivo* and *in vitro*; to answer these questions.

CONCLUSIONS

In the light of this study, the following conclusions were drawn:

1- Hydroxyapatite microclusters containing mouthwash had a potential antimicrobial activity against the tested bacterial strains alone or in combination with other antimicrobials.

2- Chlorhexidine is still surpassing the tested antimicrobial agents; however, its combination with hydroxyapatite microclusters containing mouthwash was advantageous against *Streptococcus mutans*

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