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ZEROVALENT BISMUTH NANOPARTICLE AS AN AID FOR TREATMENT OF DENTAL CARIES BY INHIBITING STREPTOCOCCUS MUTANS

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ABSTRACT

Dental caries is a dental biofilm-related disease associated with increased consumption of dietary sugar and fermentable carbohydrates. This biofilm matures to become cariogenic and in some cases the bacteria present in the biofilm turns food starches into acid which erodes teeth. *Streptococcus mutans* (S. Mutans) was found to be the most dominant species in caries initiation having acidogenic and aciduric properties. Glucosyl transferases (Gtfs) have the main role in adhering S. Mutans on tooth enamel which eventually leads to a tooth decay. The adherence of S. Mutans to the surface of enamel or the physiological ability (acidogenicity and aciduricity) in dental biofilms can be reduced, resulting in a decrease caries formation which may be achieved by using a nanostructured material. Nanostructured material are used in many fields, including biological sciences and medicine. Zerovalent bismuth nanoparticles were introduced to resist the resistance caused by microbes to various antibiotics and also showing antimicrobial and antibiofilm activity. However the mechanism of antimicrobial activity and antibiofilm activity is not completely understood, and their precise mechanism of action against bacteria remains to be fully elucidated.

KEYWORDS : Dental caries, *Streptococcus mutans* (S. Multan), Glucosyl transferal

INTRODUCTION

Tooth enamel is the hardest substance in the human body even harder than bone. Guarding of teeth is its prime importance. It can be destroyed by acid and built up of bacteria. Plaque is another part of teeth enamel made up of saliva, food particles, bacteria and other substances. It begins to build up on teeth within 20min after eating. Tooth decay is caused by biofilm (dental plaques) which matures to become cariogenic (causing tooth decay). In some cases the bacteria present in the plaque turns food starches into acid which erodes teeth. This acid takes up healthy minerals from the enamel and causes enamel to wear and tear, and become pitted. Acids of intrinsic (gastrointestinal) and extrinsic (dietary and environmental) origins are the main etiological factors in dental caries. PH plays an important role in tooth decay, the lower you go down the pH scale [0-6] the greater your teeth erodes. The acid is produced from food debris or sugars on the tooth surface. Simple sugars are bacteria's primary energy source they include sucrose, glucose, fructose, lactose and cooked starches.

• Activity of mutans *Streptococci*

Streptococcus mutans was found to be the dominant species in many, but not all, elevated levels of *Streptococcus salivarius*, *Streptococcus sobrinus* and *Streptococcus parasanguinis* were also associated with caries, especially in subjects with no or low level of S.mutans, suggesting this species are alternating pathogens. The most common bacteria associated with dental caries are the hie mutans *Streptococci*, most prominently *Streptococcus mutans* and *Streptococcus sobrinus*. Out of these, *Streptococcus mutans* appears to be the most acid producer in caries initiation. S. mutans is highly acidogenic and aciduric, and considerable clinical and laboratory data implicates this species as the primary pathogen in human dental caries. As caries increases number of bacteria associated with the caries decreases and only 5 species- level taxa were significantly higher as caries stage increased including S. mutans

• Mechanism of action

Steps involved in formation of dental plaque:

- Salivary molecules get adsorbed on the enamel as soon as tooth has been cleared.
- Bacterial interaction with these acquired salivary molecules via several specific cell-to-surface interaction is done by the pioneer species which mainly includes *Streptococcus sanguis* and *Actionomyces viscosus*
- During the third step, other bacterial species like S. mutans

adhere to the pioneer species by cell-to-cell interactions subsequent bacterial growth on tooth surface leads to formation of biofilm called dental plaque.

1.3 Activity of S. mutans in Dental Caries:

Sucrose as a substrate for S. mutans:

Food and beverages are the most common cause of dental caries due to their acidic levels. As we consume this food which contains fermentable carbohydrate like sucrose, fructose, lactose they undergo hydrolysis which facilitates conversion into monosaccharides. S. mutans present on tooth enamel by cell- to- cell interaction with the pioneer species, converts glucose to glucans and fructose to fructans with help of glucosyltransferase (Gtfs). The acidogenic S. mutans are able to form extra cellular polysaccharides (EPS) in presence of sucrose, fructose or glucose. Sucrose is the only dietary carbohydrate that can be transformed into EPS in the plaque and thus considered as the most cariogenic carbohydrate. It helps in adherence of



- mutans on tooth enamel. Gtfs is an enzyme present in S. mutans which split sucrose and use resulting glucose molecule to build long, sticky biofilm chains. This extracellular polysaccharides (EPS) are called glucans.

The glycan appear to be more important in plaque formation by S. mutans than the fructans. Because glucans are less soluble than fructans and are degraded comparatively slowly by oral bacteria. Consequently the glucans appear to persist for a longer period of time in mixed human plaque to maintain the integrity of S. mutans microcolonies. The conversion of dietary sucrose to glucan polymers is an important cariogenic property of S. mutans. In particular, insoluble glucan production has been shown to greatly increase the ability of these organisms to colonize tooth surface. S. mutans strains apparently attach the tooth surface by both adhesion and glucan mediated mechanisms.

2. Introduction to GTF and its role

1) In formation of insoluble Glucans:

Multiple GTFs were involved in synthesis of glucans, involved in sucrose enhanced colonization on teeth. S. mutans harbours three GTFs : Gtf B, Gtf C and Gtf D. Gtf B synthesizes predominantly insoluble glucans, Gtf D only produces water soluble glucans, and Gtf C can synthesize both soluble and insoluble glucans. Both products of the Gtf B and C genes are important for sucrose enhanced

colonization and cariogenicity. Unlike most strains of *S. mutans* examined, this strain contains only two *Gtf* genes on its chromosomes: one expressing an enzyme synthesizing insoluble glucan, *Gtf BC*, and other corresponding to the *Gtf D* gene. Studies have demonstrated that glucan produced by *Gtf B* and *Gtf C* are essential for the assembly of *S. mutans* biofilms. While glucans produced by *Gtf D* serve not only as a primer for *Gtf B*, but also as a source of nutrient for *S. mutans* and other bacteria

2) In formation of Lactic acid (Glycolysis Pathway)

When sucrose is encountered by *S. mutans* it enters glycolysis pathway which produces pyruvate and eventually produces lactic acid (anaerobic respiration). Due to production of acid there is a fall in pH which leads to demineralization of tooth by solubilizing calcium phosphate causing dental caries.

Use of zerovalent bismuth nanoparticle as an innovative approach. Despite continuous efforts, the increasing prevalence of resistance among pathogenic bacteria to common antibiotics has become one of the most significant concerns in modern medicine. So to resist the resistance caused by microbes to different antibiotics an innovative approach of synthesizing Zerovalent Bismuth Nanoparticles was introduced. Nanostructured materials are used in many fields, including biological sciences and medicine. Nanoparticles have an increased surface area and therefore have increased interaction with biological targets and hence are applied as coating materials, as well as in treatment and diagnosis. The objective of this approach was to analyze the antimicrobial activity of bismuth nanoparticles against oral bacteria and their antibiofilm capabilities.

1. *mutans* as target with antimicrobial and antibiofilm activity

The antimicrobial effect of the bismuth nanoparticles on growth of *S. mutans* was determined using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Biotium, Hayward, CA), 10, 11 following the instructions of the manufacturer. *S. mutans* was grown in trypticase soy broth (BD Difco, Sparks MD) at 37°C overnight in aerobic conditions. The bacteria were counted using a Neubauer chamber, and 1×10^4 cells were inoculated in 100 µL of trypticase soy broth medium in a 96-well polystyrene plate. Three wells with only trypticase soy broth medium were used as controls for growth of *S. mutans*. Chlorhexidine 0.12% (Ultradent Products, South Jordan, UT) was used as a positive antimicrobial control. We used 2 mM of zerovalent bismuth nanoparticles to interfere with bacterial growth. The 96-well plate was incubated at 37°C overnight. Next, 10 µL of MTT was added to each well, and the plate was protected against light and incubated at 37°C for 2 hours. Next, 200 µL of dimethyl sulfoxide was added to dissolve the reduced MTT. The amount of live cells was determined using a microplate absorbance reader (Biorad, Philadelphia, PA) at 595 nm. The experiment was repeated three times, and the measured optical density was analyzed by descriptive statistics.

The antibiofilm activity of the bismuth nanoparticles was determined by fluorescence microscopy, following the methodology described above. To observe the biofilm, SYTO 9 green dye (Invitrogen, Carlsbad, CA) was added at a final concentration of 20 µM. The 96-well plate was incubated for 30 minutes at room temperature and protected against light. The *S. mutans* biofilm was visualized using a Carl Zeiss Z1 Axio Inverter microscope (Thornwood, NY) at 485 nm.

Comparison of Zerovalent Bismuth Nanoparticles with chlorhexidine

As Bismuth nanoparticles were compared with chlorhexidine results

showed that these nanoparticles reduced the number of bacteria (i.e. antimicrobial activity) by 69% as compared to chlorhexidine which achieved a 63% reduction in number of bacteria. (Bismuth Nanoparticles 10 mM, Chlorhexidine-0.12%). While zerovalent bismuth nanoparticles had antibiofilm activity which was as effective as that of chlorhexidine. By testing the inhibitory action of Bismuth nanoparticles on biofilm at 6 and 18 hours post inoculation similar results were obtained as compared to chlorhexidine

3. Results

The mechanism of antimicrobial activity for nanoparticles is not completely understood, and their precise mechanism of action against bacteria remains to be fully elucidated. Several studies have shown that a positive charge on the metal ion is critical for antimicrobial activity, allowing for electrostatic attraction between the negative charge on the bacterial cell membrane and the positive charge on the nanoparticle. As 69% of cells were inactivated by these nanoparticles, the cells which have survived were not sufficient to form a biofilm. The minimal inhibitory concentration of bismuth nanoparticles that interfered with *S. mutans* growth was 0.5 mM.

CONCLUSION

Zerovalent bismuth nanoparticles could be an interesting antimicrobial agent to be incorporated into an oral antiseptic preparation.

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