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The Effect of Denitrification in the Human Dental Biofilm on Oral Tissues

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Authors' contributions

This work was carried out in collaboration between all authors. Author LTY designed and wrote the review. Authors MIAH and TBTA managed and edited the versions of the review. All authors read and approved the final manuscript.

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ABSTRACT

Global oral health surveys reveal that periodontal disease and dental caries affect human populations throughout the world. Denitrification plays both positive and negative roles in these disease processes by producing by-products that alter the oral environment, change the oral ecological balance, and the oral tissue immune response. Nitrate reduction may have a detrimental effect on the tooth supporting structures if the by-products of reduction are not neutralized. However, the nitrogen oxide by-products are beneficial in reducing dental caries. In this paper, we review the process of denitrification and its effect on the oral tissues.

Keywords: Denitrification; salivary nitrate; dental caries; periodontal disease.

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1. INTRODUCTION

Severe periodontitis and caries incidence have been increasing over the last two decades. [1] They are most globally prevalent diseases and are ranked among the top 100 causes in the "disability-adjusted life-years" metric. Dental biofilm causes periodontal disease and dental caries that affect a wide range population and negatively affect the quality of life of middle-aged and older adults. According to the statistics, periodontal problems are currently more prevalent and denitrification may be part of the pathological process that takes place in this disease [2-5].

Denitrification is a process of nitrate reduction primarily performed by facultative anaerobic or microaerophilic organisms: however aerobic denitrification can also take place [6]. Denitrification results in the formation of dinitrogen (N_2) from nitrate (NO_3^-) via nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) intermediates.

In the oral cavity, nitrate reduction is conducted by nitrate reducing bacteria that reside in the dental biofilm on tooth surfaces and surrounding periodontium. [7]. Nitrate from dietary sources is absorbed by the intestine and then re-absorbed from the bloodstream and secreted into the oral cavity as a component of saliva [8]. Nitrate-reducing bacteria in the dental biofilm reduce salivary nitrate to nitrite [6,9-11], which is further reduced to NO by nitrite reducing bacteria [12,13]. Veillonella spp. was found to be the most prevalent nitrite reducer and thus may play a major role in the nitrate reduction process within the oral cavity [11].

Nitrate secretion in saliva would enhance the survival and growth of bacteria that possess the ability to utilize nitrate. Hence, this creates a symbiotic relationship between the host and microbiota; the host providing an environment and nutrients in return for nitrite production [6].

Availability of denitrification by-products in the oral cavity depends also on the factors that affect the oral ecology, such as the level of dental plaque pH, salivary flow rate, saliva buffering components and types and amount of food intake [14,15]

2. DENITRIFICATION EFFECT ON THE PROGRESSION OF PERIODONTAL DISEASE

Periodontitis is an inflammatory disease resulting in the destruction of the supporting structures of the teeth (the periodontal ligament and the alveolar bone). It results in the formation of pockets between soft tissue of the gingiva and the tooth that can eventually cause tooth loss [16].

Nitrogen oxide is the important denitrification product that tends to aggravate the damage of tooth supporting tissues by periodontitis [17]. The amount of nitrogen oxide accumulated in the dental biofilm depends on the amount of nitrate in food consumed. Nitric oxide oxidation is another source for nitrogen oxides production in the saliva [18]. If the plaque pH level remains low for an extended time period, this would result in the accumulation of a high percentage of nitrogen oxide in the dental biofilm. The inner layer of the dental plaque is separated from saliva tending to neutralise and scavenge the surface layer of the plaque. Viscous saliva will not be able to penetrate the plaque to scavenge the deeper layers resulting in the accumulation of nitrogen oxide within the plaque. If the by-product scavenging process is delayed or diminished, nitrogen oxide diffuses into the surrounding periodontal tissues aggravating nitrogen oxide-induced damage to the gingival tissues [7].

Previous studies showed that over production of NO was implicated in the pathogenesis of periodontal disease [19,20]. NO, at normal concentrations, is a messenger molecule that organizes the functions of immune system cells that are part of the inflammatory–process. [21]. Lipopolysaccharides from the plaque bacteria can stimulate production of pro-inflammatory cytokines, that induce the immune cells to release high amount of NO. Subsequently, neutrophils produce matrix metalloproteinases that causes further damage to the tissues surrounding the tooth. [22]. Using selective inducible nitric oxide synthase inhibitor or scavenger has been shown to be protective against the progression of alveolar bone loss. Subgingival local delivery of NO inhibitors might be useful in the treatment of periodontal tissue inflammation; whereas, systemic NO inhibitor delivery was shown to decrease bone resorption in a periodontitis animal model [18].

Denitrification by-products may initiate gingival inflammation that attracts and triggers human neutrophils to use myeloperoxidase for conversion of nitrite into the oxidants nitryl chloride and nitrogen dioxide resulting in cellular toxicity [23,24]. Production of peroxynitrite – a redox derivative of NO – seems to augment the inflammatory response by sustaining the nuclear

localization of nuclear factor-κB and causes higher toxicity, lipid peroxidation, and damage to proteins and nucleic acids in host tissue [25,26]. Inflammation will escalate the intensity of nitric oxide synthesis in periodontal tissues. Previous studies have shown increased salivary concentrations of nitric oxide in periodontitis patients as compared to healthy individuals [27,28].

3. DENITRIFICATION EFFECT ON DENTAL CARIES FORMATION

Salivary nitrate reduction by the oral bacteria contributes to the major nitrite exposure in the human body [29]. In the oral cavity, salivary nitrate is utilized by bacteria that are capable of rapidly reducing nitrate to nitrite as part of their respiratory process. Mature dental biofilm is relatively thick and gelatinous thereby limiting the diffusion of oxygen to its deeper layers. Therefore, the microbes that survive in the deeper parts of the biofilm are facultative anaerobes. This would be the reason why nitrogen oxides are significantly higher in those with poor oral hygiene compared to those with good oral hygiene who have a thinner dental biofilm [30]. Denitrification and nitrate ammonification is shown to be increased in dental plaque samples from individuals with enamel and dentin caries [31]. This would be a mechanism to neutralize or buffer the acid produced by cariogenic bacteria and may limit the caries process. Neutral pH environment is favorable for the remineralization of enamel hydroxyapatite crystals [7,30].

Many anaerobic facultative bacteria synthesize nitrate reductase in low oxygen tension [9,11,32,33], hence, reduction can take place in the thick biofilm or deep tongue crypts [32]. In thick cariogenic plaque, the low pH level allows the production of acidified nitrite. It was reported that pH below 7 would promote the conversion of *L*-arginine to NO and its compounds [34]. NO is one of the by-products and it is recognized for its antibacterial effect that inhibits the acidogenic bacteria that are responsible for caries formation [30,35].

Nitric oxide antibacterial effects are thought to include DNA modifications and interactions with other reactive species. For example, reaction with superoxide produces the highly reactive molecule peroxynitrite [36]. Therefore, nitrate recycling and the availability of large amounts of nitrite and microbial nitrate reductase in the oral cavity would result in bacteriostatic and possibly bacteriocidal effects that subsequently limits the survival of acidogenic bacteria and decreases caries formation [11,35]. Production of nitrite from salivary nitrate by nitrate reducing bacteria may limit the growth of cariogenic bacteria as a result of the production of antimicrobial oxides of nitrogen, including nitric oxide.

4. CONCLUSION

Human dental biofilm hosts many types of bacteria that include the nitrate reducing bacteria. These bacteria are utilize the salivary nitrate for respiration. Salivary nitrate is a reservoir from which a variety of nitrogen oxides are formed, most notably nitric oxide. Denitrification can have a dual effect, either detrimental by aggravating the damage of tooth supporting structure or beneficial by relatively limiting the dental caries formation.

Denitrification by-products have a relative impact on the ecology and pH level of the oral cavity. If the pH level is low, the denitrification will help to neutralise the pH level and diminish tooth structure demineralization. If the denitrification by-products (oxides) are produced in high amounts, these oxides can damage the tooth supporting tissues (alveolar bone and periodontal ligament).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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