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3,3'-Diindolylmethane (DIM) as a Natural Agent for Reducing Biofilm and Virulence of Streptococcus mutans

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Abstract

We aimed to investigate the effects of the natural compound 3,3'-Diindolylmethane (DIM) on biofilm formation, microbial growth, and acid production by *Streptococcus mutans*, one of major etiological agents related to dental caries. To determine this, we performed our study and found that the higher the concentration of DIM (100 µg/mL) in comparison to other concentrations the more evident biofilm reduction occurs. A moderate inhibition of bacterial growth at higher concentrations was also noted in our experiments, indicating that DIM is acting mainly by inhibiting bacterial virulence rather than by causing general inhibition of viability. In addition, pH values significantly increased upon treatment with DIM, suggestive of reduction in acid production and reduced cariogenic potential. Overall, results indicate that DIM may act as a potential natural agent with anti-biofilm and anti-virulence activity against *Streptococcus mutans*..

Keywords: *Streptococcus mutans* , 3,3'-Diindolylmethane , Dental caries

Introduction

One critical part of your overall physical condition involves your mouth's wellness; therefore, maintaining good dental hygiene will contribute positively toward enhancing your life experience. One common dental problem caused primarily by poor oral hygiene extends from the transfer of bacteria from one person to another via saliva, which results in cavities (also referred to simply as "caries"). This cavity-forming process happens through the creation of an actual cloud of germs on the tooth itself (the aforementioned "biofilm"), as well as within the mouth (the aforementioned "plaque"). Once formed, the biofilm provides protection to the germs from physical damage and medications designed specifically to kill bacteria; this

often makes it nearly impossible for a dentist to successfully eliminate this infection from within your mouth (Koo et al., 2021; Wang et al., 2022)

Pathogenic micro-organisms are able to grow within an environment created by the biofilm structure, which protects them from harmful conditions and makes them more resistant to antimicrobial agents when tested in vitro, regardless of whether the agent is effective against free organisms or not. (Flemming *et al*., 2023).

List of the most important cariogenic bacteria among oral microorganisms *Streptococcus mutans*. This Gram-positive Facultative Anaerobe (FA), which acts as one of the main dental hyperplasic agents, is capable of adherence to tooth surfaces and metabolizing sugars into organic acids that cause tooth enamel demineralization (Lemos *et al.*, 2023). Moreover, this bacterium produces extracellular polysaccharides which favor biofilm formation and structural stability (Baker *et al*., 2020). Biomaterial-induced infections represent an important challenge in the field of oral healthcare management because of their enhanced tolerance for antibiotics and defenses from host immunity. Long-term use of traditional preventive agents such as mechanical plaque control and chemical antimicrobial agents are proved to have some adverse effects. In addition, the increasing problem of antimicrobial resistance has strengthened the imperative for alternative approaches to manage oral pathogens and prevent dental caries (Daglia *et al*., 2021)

Recently, there has been significant scientific interest in the potential of bioactive compounds from plants to act as antimicrobial agents and anti-biofilms. It is commonly accepted that these compounds are safe for humans and have good compatibility with living cells, as well as being able to act on multiple bacterial pathways at the same time (Daglia et al., 2021). A number of researchers have focused a lot of attention on the compound 3,3'-Diindolylmethane (DIM). It is a naturally occurring compound classified as an indole that forms during digestion of indole-3-carbinol from consuming cruciferous vegetables like broccoli, cabbages (and therefore DIM is plentiful in these foods.)

DIM has a lot of investigation into its anticancer effects but there is much literature to also support its many biological activities, including effects at the antimicrobial, anti-inflammatory, and also as an antioxidant activity. Further to this, several studies have shown that DIM influences bacteriostasis, biofilm formation, regulation of virulence gene expression among specific pathogenic microorganisms (Li et al 2021; Park et al 2023)

Based on the major effects of virulence factors on the development of biofilms and the influence of virulence factors on the pathogenicity of Streptococcus mutans, the use of 3,3′-Diindolylmethane (DIM) as an alternative natural antimicrobial agent may provide an effective mechanism to prevent the formation of dental caries. This research aims to assess the effects of 3,3′-diindolylmethane (DIM) on biofilm development, growth, and acid production by S. mutans, to provide evidence for mechanisms involved in establishing biofilm development and virulence, and to evaluate the potential for DIM to assist in the reduction of these pathogenic properties by demonstrating the importance of oral health..

Materials and Methods

Bacterial Culture Conditions

A strain of *Streptococcus mutans* A microbiology lab culture supplies this isolate. Brain Heart Infusion Broth (BHI) and BHIAgar were utilized for growing this isolate, as both are nutrient-rich media that allow for the growth of fastidious oral streptococci (Atlas, 2010; Forbes et al., 2017). Sucrose was included to aid in developing a biofilm. The compound being tested was 3,3′-diindolylmethane (DIM), which was prepared in a DMSO (dimethyl sulfoxide) solvent. All washing processes utilized PBS (phosphate buffered saline). Biofilms were stained with crystal violet dye. All reagents were of analytical grade (O'Toole, 2011)

Streptococcus mutans colonies were moved into Brain Heart Infusion (BHI) broth and placed in an anaerobic environment at 37°C for 24 hours. These growth conditions have been shown to continue the metabolism of S. mutans and preserve the most important virulence factors of the organism during its growth. (Bowen & Koo, 2011; Lemos *et al.*, 2019).

Preparation of DIM Solutions

The DIM compound was solubilized with 100% dimethyl sulfoxide (DMSO) to allow preparation of a stock solution of DIM. The stock solution was diluted with BHI to achieve a range of working concentrations. On the final plate, DMSO concentration did not exceed 1%, which may inhibit bacterial growth. (CLSI, 2022).

Biofilm Assay

Biofilm development was evaluated with a microtiter plate evaluation. In this instance, BHI broth with added sugar was incrementally mixed into sterile 96-well polystyrene plates using previously prepared liquid numbers from each category of bacteria. Each of the prepared liquid samples had a different concentration of DIM to form an experimental category; however one category did not contain any DIM and constituted the negative control category. Following an incubation period of 24 hours at 37°C, the test wells were gently rinsed with phosphate buffered saline (PBS) to remove any non-adhering cells, stained (using crystal violet) and then assessed for biofilm (biomass) using spectrophotometric measurement (absorbance 570 nm). (O'Toole, 2011; Stepanović *et al.*, 2007).

Effect of DIM on Bacterial Growth

The effect of DIM on bacterial growth was assessed by measuring optical density at 600 nm using a spectrophotometer. Control samples without DIM were included for comparison (Madigan *et al*., 2018).

Assessment of Acid Production

The pH of the culture supernatant was measured after incubation to evaluate acid production by Streptococcus mutans. Changes in pH were used as an indicator of bacterial acidogenic activity (Lemos *et al*., 2019).

Statistical Analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation. Differences between groups were known statistically significant at $p < 0.05$ (Motulsky, 2014).

Results

Effect of DIM on Biofilm Formation

The results in table(1) show that DIM had a significant effect on reducing biofilm formation. The control group, which was not treated with DIM, recorded the highest mean optical absorption value (OD570 = 1.25 ± 0.08), indicating high biofilm formation. Upon adding DIM at a concentration of 25 µg/mL, the absorption value decreased to 0.98 ± 0.07, compared to the control group. A greater decrease was observed at a concentration of 50 µg/mL, where the absorption value reached 0.72 ± 0.06). At the highest concentration of 100 µg/mL, the absorption value decreased significantly to 0.45 ± 0.05, with high statistical significance ($p < 0.01$).

Table (1): The Effect of DIM on Biofilm Formation

DIM Concentration (µg/mL)	Mean OD570	SD	p-value
0 (Control)	1.25	0.08	-
25	0.98	0.07	<0.05
50	0.72	0.06	<0.05
100	0.45	0.05	<0.01

Effect of DIM on Bacterial Growth

Table 2 shows the impact of DIM on the growth of bacteria. At 25 µg/mL, the mean optical density decreased to 1.02 ± 0.06 and was not statistically different from that of the control group. Bactericidal rates using 50 µg/mL fell significantly compared to controls, with OD600 measured at 0.90 ± 0.05. This trend continued with the maximum concentration tested of 100 µg/mL; bacteria measured OD600 was measured at 0.78 ± 0.04. Therefore, these data corroborate that DIM demonstrates concentrations to inhibit bacteria growth..

Table (2): The Effect of DIM on Bacterial Growth

DIM Concentration (µg/mL)	Mean OD600	SD	p-value
0 (Control)	1.10	0.05	-
25	1.02	0.06	NS
50	0.90	0.05	<0.05
100	0.78	0.04	<0.05

Effect of DIM on Acid Production

As shown in Table (3), DIM has an anti-acidic effect through the measured pH levels. The average pH of the control was 4.5 ± 0.2, which indicates high levels of acid production, and when a 25 µg/mL concentration of DIM was applied, the average pH increased to 4.9 ± 0.2 (higher than the control). The average pH will continue to increase to 5.3 ± 0.1 with a 50 µg/mL concentration and then reach the highest pH measured at 5.8 ± 0.1 with a concentration of 100 µg/mL (the highest concentration tested). Thus, from these results, we can see that DIM decreases the amount of acid produced because the higher the amount of DIM applied, the higher the pH value becomes according to the amount of DIM applied..

Table (3): The Effect of DIM on Acid Production

DIM Concentration (µg/mL)	Mean pH	SD	p-value
0 (Control)	**4.5**	**0.2**	**-**
25	**4.9**	**0.2**	**<0.05**
50	**5.3**	**0.1**	**<0.05**
100	**5.8**	**0.1**	**<0.01**

Discussion

The development of caries is an intricate process involving interactions of carbohydrates (mainly sugars), microbes (bacteria), and the body (host factors). During an individual's lifetime, bacteria will use sugar to produce acid. This acid will eat away at the enamel on the teeth, resulting in a condition known as "demineralisation of the dental enamel." This process can be very rapid, especially when bacteria are in the form of a biofilm, and are attached to their substrates, providing protection from environmental stress (Featherstone, 2000). Dental caries affects people throughout their lifetime globally, as it remains a significant public health problem. Many studies have reported that 60-90% of children between the ages 6-12 years experience dental caries, and a similar finding has been reported among adults (Petersen, 2003). Both socioeconomic status and nutrition influence the prevalence of caries (Beltran-Aguilar et al., 2005). The development of caries occurs when cariogenic bacteria metabolize sugar from the diet, producing acids, which decrease the pH of dental plaque to below the critical level that is needed for the demineralization of the enamel (Featherstone, 2000). MinR is actually favoured by multiple acid attacks, because of mineral loss and the advancement of the lesions (Fejerskov, 2004.(

The reduction of biofilm formations seen is consistent with studies that report the anti-biofilm activity of indole-derived materials against oral pathogens (Li et al., 21; Park et al., 2023). By targeting the formation of biofilm, the potential for dental caries to develop could be reduced while maintaining the total balance of the existing microbiota and not completely eliminating the microorganisms (Koo et al., 2017). Baruch et al., (2023) study supports our findings that the DIM compound has potent inhibitory effects on biofilm formation and on the generation of extracellular polymeric substances (EPS); therefore, their results suggest that DIM may be highly effective in preventing the infection caused by S. mutans. Specifically, due to the action of DIM, which can inhibit the biological processes of quorum sensing of bacteria involved in regulating biofilm formation, DIM has been shown to have a significant impact on the production of extracellular polymeric substances or EPS by biofilm. EPS represents the majority of the structural component of biofilm and provides the primary mechanism by which biofilm will adhere to surfaces and survive in the face of environmental hostile conditions such as low pH. A reduction in the amount of EPS being produced will diminish the cohesive ability of a biofilm and make bacteria more vulnerable to the effects of external stressors than when EPS levels are at their normal limits. The present data suggests that DIM will exhibit a moderate inhibitory effect on the growth of bacteria at the higher concentrations of DIM as compared with the control. Control samples will not exhibit any statistically significant reduction at the lowest concentration of DIM; however, samples in the higher concentration of DIM showed a marked reduction in growth as a result of a clear concentration-dependent inhibitory effect..

These findings are in agreement with those reported by Baruch et al. (2023), who showed that treatment of S. mutans with 3,3′-Diindolylmethane (DIM) significantly reduced bacterial viability compared to untreated controls. This supports the notion that DIM interferes with bacterial survival. Notably, S. mutans contributes to caries development through acid production from carbohydrate metabolism, yet it is able to withstand acidic conditions via its acid tolerance response (ATR), which may influence its persistence in the oral environment.

In dental plaque, pH fluctuates rapidly from 7 to below 3 within 20 minutes due to sugar ingestion. To adapt to these shocks, *S. mutans* possesses both stable and acid-induced mechanisms that work together to promote: protection and repair of macromolecules, modification of metabolic pathways, secondary metabolism, increased cell density and biofilm formation, and regulation of intracellular pH (Matsui& Cvitkovitch, 2010).

The inhibition of acid production observed in the groups treated with DIM indicates lower cariogenic potential and is in agreement with other studies reported by Lemos *et al*.(2019) .Our investigation found that the treated agent DIM significantly inhibited biofilm formation and acid generation of *Streptococcus mutans*, with a moderate effect on bacterial growth. These findings suggest that DIM is an anti-biofilm and anti-virulence compound.

Conclusions

Under the conditions of this study, we found that 3,3′-Diindolylmethane (DIM) inhibited biofilm formation and acid production of Streptococcus mutans with only a modest inhibitory effect on bacterial growth. According to the results, DIM inhibits the virulence characteristics of S. mutans rather than abolishing the growth, which can be beneficial for inhibiting dental caries without upsetting oral microbial community. DIM exhibited concentration-dependent antibiofilm activity. Yet more molecular as well as clinical studies remain to be performed for understanding its mechanism of action and for exploring its potential in oral health applications.

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References:

- Baker, J. L., Faustoferri, R. C., & Quivey, R. G. (2020). Acid tolerance mechanisms of oral streptococci. *Current Oral Health Reports, 7*(2), 101–108. https://doi.org/10.1007/s40496-020-00252-6
- Baruch, Y., Golberg, K., Sun, Q., Yew-Hoong Gin, K., Marks, R. S., & Kushmaro, A. (2023). 3,3'-Diindolylmethane (DIM): A Potential Therapeutic Agent against Cariogenic *Streptococcus mutans* Biofilm. *Antibiotics (Basel, Switzerland), 12*(6), 1017. https://doi.org/10.3390/antibiotics12061017
- Beltrán-Aguilar, E. D., Barker, L. K., Canto, M. T., Dye, B. A., Gooch, B. F., Griffin, S. O., … Wu, T. (2005). Surveillance for dental caries, dental sealants, tooth retention, edentulism, and enamel

fluorosis—United States, 1988–1994 and 1999–2002. *Morbidity and Mortality Weekly Report: Surveillance Summaries, 54*(3), 1–43.

- Bowen, W. H., & Koo, H. (2011). Biology of *Streptococcus mutans*-derived glucosyltransferases: Role in extracellular matrix formation of cariogenic biofilms. *Caries Research, 45*(1), 69–86. https://doi.org/10.1159/000324598
- Daglia, M., Di Lorenzo, A., Nabavi, S. F., Talas, Z. S., & Nabavi, S. M. (2021). Antibacterial activity of plant polyphenols against foodborne pathogens: A review. *Food Chemistry, 350*, 129267. https://doi.org/10.1016/j.foodchem.2021.129267
- Daglia, M., Papetti, A., Grisoli, P., Aceti, C., Spini, V., Dacarro, C., & Gazzani, G. (2021). Antibacterial activity of plant polyphenols and their role in oral health. *Journal of Applied Microbiology, 130*(3), 701–716. https://doi.org/10.1111/jam.14876
- Featherstone, J. D. B. (2000). The science and practice of caries prevention. *The Journal of the American Dental Association, 131*(7), 887–899. https://doi.org/10.14219/jada.archive.2000.0307
- Fejerskov, O. (2004). Changing paradigms in concepts on dental caries: Consequences for oral health care. *Caries Research, 38*(3), 182–191. https://doi.org/10.1159/000077753.
- Flemming, H. C., van Hullebusch, E. D., Neu, T. R., Nielsen, P. H., Seviour, T., Stoodley, P., Wingender, J., Wuertz, S., & Wilderer, P. A. (2023). The biofilm matrix: Multitasking in a shared space. *Nature Reviews Microbiology, 21*(2), 70–86. https://doi.org/10.1038/s41579-022-00791-0.
- Koo, H., Bowen, W. H., & Lemos, J. A. (2021). The influence of biofilm structure on the pathogenicity of oral bacteria. *Journal of Dental Research, 100*(7), 738–748. https://doi.org/10.1177/00220345211004594
- Koo, H., Falsetta, M. L., & Klein, M. I. (2021). The exopolysaccharide matrix: A virulence determinant of cariogenic biofilms. *Journal of Dental Research, 100*(9), 906–914. https://doi.org/10.1177/00220345211005794.
- Koo, H., Xiao, J., Klein, M. I., & Jeon, J. G. (2017). Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. *Journal of Dental Research, 96*(12), 1414–1421.
- Lemos, J. A., Palmer, S. R., Zeng, L., Wen, Z. T., Kajfasz, J. K., Freires, I. A., Abranches, J., & Brady, L. J. (2019). The biology of *Streptococcus mutans*. *Microbiology Spectrum, 7*(1), GPP3-0051-2018. https://doi.org/10.1128/microbiolspec.GPP3-0051-2018
- Lemos, J. A., Zeng, L., Burne, R. A., & Abranches, J. (2023). Biology of cariogenic biofilms: Pathways to disease. *Microbiology Spectrum, 11*(1), e03847-22. https://doi.org/10.1128/spectrum.03847-22
- Li, Y., Wang, J., Zhang, X., & Liu, H. (2021). Antibacterial and antibiofilm activity of indole derivatives against oral pathogens. *Frontiers in Microbiology, 12*, 728345. https://doi.org/10.3389/fmicb.2021.728345
- Marsh, P. D., & Martin, M. V. (1999). *Oral microbiology* (4th ed.). Wright.
- Matsui, R., & Cvitkovitch, D. (2010). Acid tolerance mechanisms utilized by Streptococcus mutans. Future microbiology, 5(3), 403–417. https://doi.org/10.2217/fmb.09.129

- Park, S. Y., Kim, J. H., Lee, J. H., & Kim, Y. G. (2023). Natural indole compounds inhibit biofilm formation and virulence gene expression in oral bacteria. *International Journal of Molecular Sciences, 24*(4), 3567. https://doi.org/10.3390/ijms24043567
- Petersen, P. E. (2003). *The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century*. World Health Organization.
- Wang, Y., Ren, Z., Zhou, X., Hu, M., & Li, J. (2022). Targeting biofilm formation in *Streptococcus mutans*: Current strategies and future perspectives. *Frontiers in Cellular and Infection Microbiology, 12*, 915432. https://doi.org/10.3389/fcimb.2022.915432.
- Weng, J. R., Tsai, C. H., Kulp, S. K., & Chen, C. S. (2019). Indole-3-carbinol and its dimer 3,3′-diindolylmethane: Mechanisms of action and pharmacological effects. *Biochemical Pharmacology, 162*, 185–198. https://doi.org/10.1016/j.bcp.2019.01.022