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Evaluation of antimicrobial property of self etch adhesive incorporated with green synthesized tio2 nanoparticles: An invitro study

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***Abstract*---**Since its introduction about fifty years ago, adhesive technology has advanced rapidly. In this current era,nanoparticles has gained attention in restorative dentistry because of its advantages like improving bond strength and stability of restoration.As opposed to the chemical process, the green synthesis of titanium oxide nanoparticles has more advantages one of which is improved antimicrobial activity. Antibacterial dental adhesives can help to prevent recurrent or secondary caries. The aim of this invitro study was to evaluate the antimicrobial property of self etch adhesives incorporated with Tio2 nanoparticles. The extracts of Piper longum herb was used to make titanium dioxide nanoparticles (TiO2NPs) in a green way.Its aim is to create a tight bond between tooth dentin and restorative material.Failures in this process could result in microleakage, allowing bacteria, fluids, and ions to infiltrate.The antimicrobial property of self etch adhesives incorporated with Tio2 nanoparticles were tested against standard strain of S.mutans, lactobacillus and E.faecalis using the Zone of inhibition test. The diameter of inhibition zones were measured in millimeters.Nano-TiO2 incorporated dental adhesive has efficient antibacterial effect when compared to regular dental adhesive. Tio2 incorporated self etch adhesives shows significant antimicrobial effect compared to

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conventional self etch adhesives. Self-etch adhesive systems are promising because they are simple to use, chemically bind to tooth structure, and retain dentin hydroxyapatite, which is critical for bonding durability. Additionally the use of the TiO2 nanoparticle containing adhesive systems tested in this study may have no risk of developing secondary caries as a result of bacterial microleakage.

Keywords---dentin adhesive, microleakage, secondary caries, titanium dioxide nanoparticles.

Introduction

Microorganisms are normal commensals of oral cavity. Plaque biofilm is a complex population of bacteria or fungi that causes infection by shielding pathogenic microorganisms and eluding host defence mechanisms. It is a major cause of caries, periodontitis, and other dental diseases[1]. Although there have been numerous studies aimed at developing antimicrobial agents to address this problem, the majority of these efforts have failed due to the rapid degradation and release of antibacterial agents, resulting in low efficacy and safety concerns. Secondary caries is a significant determinant of how long dental restorations last. Secondary caries is caused mainly by mutans streptococci. Due to the high prevalence of recurrent caries following restorative therapy, the therapeutic results uncovered by direct filling materials have received a lot of attention[2]. Composite restorations are made up of two main parts: a resin composite for filling the cavity and a dentin bonding system (DBS) that is added to the cavity before the filling materials are placed. To avoid bacterial contamination, restorations must be sealed. Even the most advanced dentin bonding systems are not capable of providing a full seal in clinical settings, despite the fact that they were designed to seal cavity preparations and prevent marginal leakage. Resin-based materials accumulate more dental biofilm than other restorative materials, according to Svanberg et al 2002.

Nanomaterials are small stable objects with a diameter of 1–100 nanometers. Nanoparticles (NPs) may be a potential treatment and prevention method for dental infections[3]. Because of their enhanced and unusual physicochemical properties, such as ultra-small sizes, high surface-area-to-mass ratios, and increased chemical reactivity, nanomaterials hold promise in antimicrobial therapies. Because of their high charge density, NPs can interact more effectively with the negatively charged surface of bacterial cells, resulting in increased antimicrobial activity[4]. Furthermore, in the oral cavity, NPs mixed with polymers or coated onto biomaterial surfaces were found to have superior antimicrobial properties. Also NPs have superior antibacterial activity against bacteria that are resistant to antibiotics. As a result, the use of nanoparticles in dentistry may be especially beneficial[5].

The pH of the self-etching primer solution in self-etching adhesive systems is low enough to demineralize the smear layer and the underlying dentin surface, allowing etching and priming of the cavity to be done simultaneously[6]. Residual bacteria can remain at the interface between the tooth and the restorative

material as a result of the non rinsing procedure. In an adhesive system, the dentin primer is the part that comes into contact with and interacts with the dentin substrate during the first stage of restoration. These bacteria could be removed if tooth conditioners, such as primers have antibacterial activity thereby preventing secondary caries[7]. As a result, the antibacterial function of these adhesive systems that are directly applied to the dentin is critical to the restoration's durability.

Brannstrom et al 1973 found that even in the presence of a strong seal from the oral cavity, residual bacteria in the cavity preparation multiply within the smear layer. Polymerizable cationic monomers, which can covalently bond within the polymer matrix, can currently be used to provide antibacterial activity to resin-based dental materials. DBS containing cationic monomers such as methacryloyloxy dodecyl pyridinium bromide (a quaternary ammonium compound) and methacryloxylethyl cetyl dimethyl ammonium chloride (a quaternary ammonium compound) inhibited biofilm formation on their surface. Previous research has suggested that reducing the amount of bacteria at the tooth-restoration interface can have an effect on the occurrence of dental caries[8]. Therefore, antibacterial activity is an important property of materials for successful restoration. Nanoparticles can bind to and penetrate the cell walls of Gram-positive and Gram-negative bacteria, causing cell function to be disrupted by the release of related ions[9]. As a result, NPs are useful in the prevention and treatment of diseases caused by drug-resistant microorganisms, as well as the prevention and treatment of biofilm formation. Although the exact mechanism of action of NPs is not yet known, the antibacterial mechanism can be loosely divided into three forms. The antibacterial pathways are as follows: Interacting with peptidoglycan cell walls and membranes to induce cell lysis; interacting with bacterial proteins to stop protein synthesis; and interacting with bacterial (cytoplasmic) DNA to stop DNA replication[10,11]. No other studies have proposed synthesis of TiO_2 nanoparticles from Piper longum and further evaluation of antimicrobial property after incorporating into self etch adhesive. So the aim of the study is to evaluate antimicrobial property of self etch adhesives incorporated with Tio2 nanoparticles. The null hypothesis is there is no significant difference in antimicrobial effect of n-Tio2 incorporated self etch adhesives & conventional self etch adhesives.

Materials and Methods

Piper longum 1gm was taken in 100ml distilled water and boiled for 5 minutes at 100 Cusing a heating mantle. The extracts were then filtered through Whatman N.1 filter paper before being processed and supernatant was used for further analysis.0.38gm of Tio_2 powder is added to 50ml of distilled water. Now 50ml of filtered piper longum extract is added with 50ml ofTio2 mixed distilled water. The solution is kept in orbital shaker and magnetic stirrer with hot plate for further mixing for 2 hours. The absorption spectra of the produced nanoparticles in the range of 300 nm and 800 nm were measured using a UV-vis spectrophotometer. The solution is then mixed in magnetic stirrer for 2 days. This is followed by centrifugation at 8000 rpm for 10 minutes to remove supernatant and thus TiO_2 nanoparticles were isolated. Self etch adhesive used in this study was Restorite Bond 7G(PRIME Dental products Pvt Ltd).The prepared TiO_2 nanoparticles were

measured and 1 wt% was directly incorporated into the bonding agent in the dark and mixed using vortex mixer for 10 minutes and then stored in a light proof bottle .Thus the experimental solution is prepared. Remaining bonding agent was left without any addition of nanoparticles that serves as control solution. Two study groups are given below:

- Group 1- Conventional Self etch Adhesive
- Group 2- Tio_2 nanoparticle incorporated Self etch Adhesive

The antibacterial properties of Tio_2 nanoparticles were investigated using the agar well diffusion method. Both conventional and TiO_2 nanoparticle incorporated self etch adhesives were screened against common cariogenic bacteria such as Streptococcus mutans, Enterococcus faecalis, and Lactobacillus acidophilus. Finally, the zone of inhibition was measured to evaluate the antimicrobial activity.

Results

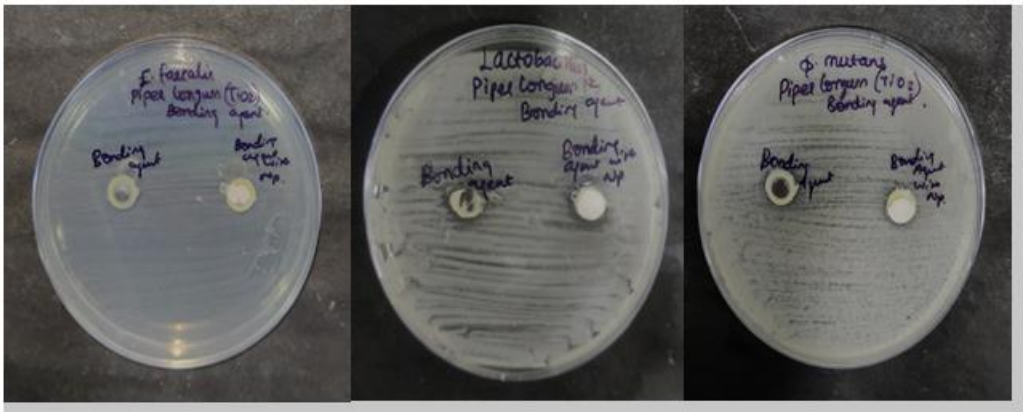

Figure 1a: ZOI against E.faecalis, 1b:ZOI against L.acidophilus, Figure 1c:ZOI against S.mutans

Table 1

Zone of Inhibition	SE adhesive	n-Tio_2 incorporated SE adhesive
E.faecalis	**12mm**	**12mm**
S.mutans	**13mm**	**14mm**
Lactobacillus acidophilus	**12mm**	**15mm**

Discussion

Many attempts have been made to produce dental materials that inhibit bacterial growth. The current study evaluated the inhibition of bacterial growth by agar diffusion test of self etch adhesives incorporated with Tio2 nanoparticles against three microorganisms. The bacterial species used in this study are

cariogenic.Among them, *S mutans* and *L acidophilus* are commonly found colonizing the healthy oral cavity and are related to the initial colonization of dental surfaces or to fissures and smaller caries lesion[12].Based on this study, nano-TiO2 incorporated dental adhesive has efficient antibacterial effect and can be used effectively which is in accordance with previous study done by (Haghi et al 2012), who has evaluated the antimicrobial effect of TiO2 NPs on pathogenic strains of E. colis, S.mutans and showed that the TiO2 NPs cause little pores in bacterial cell walls, leading to increased permeability and cell death. Thus it can prevent secondary caries formation[13].The aetiology and pathogenesis of human dental caries have been linked to S. mutans colonisation on tooth surfaces. S. mutans' ability to produce acid through sugar fermentation, as well as its acid tolerance, are important virulence factors in the production of dental caries. Acid development by S. mutans causes low pH levels in dental plaque, which contributes to tooth enamel demineralization and the onset of caries[14]. S. mutans' acidogenicity is accompanied by its acid-tolerance, or aciduricity. Also at pH 4.4, which is growth-inhibitory, S. mutans retains glycolytic capabilities. In response to a low pH, S. mutans increases the activity of the proton translocating F-ATPase, which protects it from environmental acid stress. This mechanism causes the intracellular pH to be higher than the extracellular pH which is essential in the development of dental caries.

There are several studies that describe the bonding system incorporating antibacterial components. Bapna et al.1992 investigated the antibacterial activity of Scotchbond adhesive resin that had been treated with different chemical agents. Cured resins containing sodium fluoride, dodecylamine, or bipyridine prevented the growth and attachment of S. mutans, despite the fact that their research was not aimed at developing an antibacterial bonding system. Kudou et al.2000 attempted to create an antibacterial bonding mechanism for direct pulp capping by incorporating vancomycin or metronidazole into a 4-META/MMA-TBB resin[15]. They found that the resin containing 1 to 5% vancomycin inhibited both streptococci and actinomycetes studied, and that the vancomycin-containing resin had no effect on the tensile bond strength.Imazato et al.2002 investigated the effectiveness of 5 percent MDPB in combination with a self-etching adhesive system (Clearfil Protect Bond) against S. mutans, Lactobacillus casei, and Actinomyces naeslundii against S. mutans, Lactobacillus casei, and Actinomyces naeslundii. The residual bacteria in the cavity can be inactivated when an MDPB-containing bonding system is used because unpolymerized MDPB has high bactericidal activity. Furthermore, after light activation, the bacteria can be inhibited by the immobilised agent, as seen on the surface of MDPB-added composites. As a result of the addition of MDPB, a bonding mechanism with antibacterial properties can be achieved both before and after setting[16].

A synthetic route using plants has been used in the biosynthesis of nanoparticles in a wide variety of applications. The use of less chemicals in the green synthesis of titanium oxide nanoparticles has a distinct benefit. Particle size, surface morphology, crystalline phase, and dimension can all affect the physical and chemical properties of Tio_2 nanoparticles[17]. In our present study, Tio_2 nanoparticles were green synthesized from herbal plant piper longum. The use of Piper longum plant extract in the green synthesis of TiO_2 metal oxide was a cost-effective and environmentally friendly process[18]. The simplest closed system

model for bacterial growth is the agar plate. Petri dishes, which often ensure bacterial growth due to the presence of proteins and carbohydrates, have the same workability. In this present study, Zone of inhibition is seen in all study groups and comparatively more inhibition is seen in Lactobacillus acidophilus. As discussed above,Lactobacillus acidophilus bacteria live in the pits and fissures of occlusal surfaces of teeth. These bacteria can cause cavities in both deciduous teeth and the first permanent molars. In caries lesions, a few Lactobacillus species are present, but they are mostly absent in caries-free infants. Lactobacilli found in caries lesions are both a significant contributor to caries development and a preventative measure. Streptococcus mutans and Lactobacillus have been identified as the key pathogenic bacteria in previous studies[19]. As a result, Lactobacillus is regarded as the oral flora's second most cariogenic bacteria. It is not the caries initiator, but it does play a part in the progression of caries[20]. In this study inhibition is seen for both the microorganisms. The current study's approach has some limitations in terms of reproducing physiological conditions of the pulpo-dental complex, such as the presence of intratubular fluid and intra-pulpal pressure. Because of these limitations, in vitro findings cannot be directly extrapolated to an in vivo situation because they do not represent the actual impact of the material when used in clinical settings. Certain limitations of Nanoparticle initiated antimicrobial efficacy is, despite the fact that NPs have substantial antibacterial efficacy, there are still drawbacks to their use, such as inconsistent antibacterial concentrations against micro-biofilm and slight toxicity. Further examination of bond strength,cytotoxicity and also invivo studies are needed.

Conclusion

Within the limitations of the study, it can be concluded that TiO_2 incorporated self etch adhesives produce significant anitimicrobial effect compared to conventional self etch adhesives. It can be beneficial in preventing caries progression and even secondary caries formation due to microleakage. Thus the use of the TiO_2 nanoparticle containing adhesive systems tested in this study may have no risk of developing secondary caries as a result of bacterial microleakage.

References

1. Dental Caries: Diagnosis, Prevention and Management. BoD – Books on Demand; 2018. 176 p.
2. Enan ET, Ashour AA, Basha S, Felemban NH, Gad El-Rab SMF. Antimicrobial activity of biosynthesized silver nanoparticles, amoxicillin, and glass-ionomer cement against Streptococcus mutans and Staphylococcus aureus. Nanotechnology. 2021 Mar 3;32(21):215101.
3. Asiri AM, Inamuddin D, Mohammad A. Applications of Nanocomposite Materials in Dentistry. Woodhead Publishing; 2018. 364 p.
4. Soltaninejad V, Ahghari MR, Taheri-Ledari R, Maleki A. Bifunctional PVA/ZnO/AgI/Chlorophyll Nanocomposite Film: Enhanced Photocatalytic Activity for Degradation of Pollutants and Antimicrobial Property under Visible-Light Irradiation. Langmuir. 2021 Apr 20;37(15):4700–13.
5. Kiomarsi N, Zamani P, Bahador A, Hashemikamangar SS, Pourhajibagher M, Kharazifard MJ. Effect of Addition of Nano-TiO, Nano-SiO, and a

Combination of Both, on Antimicrobial Activity of an Orthodontic Composite. J Contemp Dent Pract. 2020 Aug 1;21(8):857–62.
6. Zhang L, Xu Y, Jin X, Wang C, Li M, Zhou Z, et al. Biomimetic fabrication and application of fibrous-like nanotubes. Life Sci. 2021 Apr 1;270:119126.
7. Oz FD, Ergin E, Canatan S. Twenty-four-month clinical performance of different universal adhesives in etch-and-rinse, selective etching and self-etch application modes in NCCL - a randomized controlled clinical trial. J Appl Oral Sci. 2019 Apr 11;27:e20180358.
8. United States. General Accounting Office. Reducing Tooth Decay, More Emphasis on Fluoridation Needed: Report to the Congress. 1979. 52 p.
9. Butrón Téllez Girón C, Hernández Sierra JF, DeAlba-Montero I, Urbano Peña M de LA, Ruiz F. Therapeutic Use of Silver Nanoparticles in the Prevention and Arrest of Dental Caries. Bioinorg Chem Appl. 2020 Aug 12;2020:8882930.
10. Richards MN, Johnson GR, Lum JS, McDonald R, Salter WB, Simpson K, et al. Investigating Surface Binding Effects: Antibacterial Efficacy of Bound 8-Hydroxyquinoline against Staphylococcus aureus and Escherichia coli. J Appl Microbiol [Internet]. 2021 Apr 16; Available from: http://dx.doi.org/10.1111/jam.15105
11. Anderson R, Groundwater PW, Todd A, Worsley A. Antibacterial Agents: Chemistry, Mode of Action, Mechanisms of Resistance and Clinical Applications. John Wiley & Sons; 2012. 376 p.
12. Dhanasekaran D, Thajuddin N. Microbial Biofilms: Importance and Applications. BoD – Books on Demand; 2016. 524 p.
13. Lin Z, Wu S, Liu X, Qian S, Chu PK, Zheng Y, et al. A surface-engineered multifunctional TiO based nano-layer simultaneously elevates the corrosion resistance, osteoconductivity and antimicrobial property of a magnesium alloy. Acta Biomater. 2019 Nov;99:495–513.
14. Pulbutr P, Rattanakiat S, Phetsaardeiam N, Modtaku P, Denchai R, Jaruchotikamol A, et al. Anticariogenic Activities of Derris reticulata Ethanolic Stem Extract Against Streptococcus mutans. Pak J Biol Sci. 2018 Jan;21(6):300–6.
15. Kishen A. Nanotechnology in Endodontics: Current and Potential Clinical Applications. Springer; 2015. 199 p.
16. Rudawska A. Adhesives: Applications and Properties. BoD – Books on Demand; 2016. 398 p.
17. Shukla AK, Iravani S. Green Synthesis, Characterization and Applications of Nanoparticles. Elsevier; 2018. 548 p.
18. Karthik L, Vishnu Kirthi A, Ranjan S, Mohana Srinivasan V. Biological Synthesis of Nanoparticles and Their Applications. CRC Press; 2020. 284 p.
19. Ibrahim MS, Balhaddad AA, Garcia IM, Hefni E, Collares FM, Martinho FC, et al. Tooth sealing formulation with bacteria-killing surface and on-demand ion release/recharge inhibits early childhood caries key pathogens. J Biomed Mater Res B Appl Biomater. 2020 Nov;108(8):3217–27.
20. Liu A, Wang Y, Song Y, Du Y. Treatment with compound Lactobacillus acidophilus followed by a tetracycline- and furazolidone-containing quadruple regimen as a rescue therapy for Helicobacter pylori infection. Saudi J Gastroenterol. 2020 Mar;26(2):78–83.