

## Comparison of The Inhibitory Effect of Newer Dentin Bonding Systems Against Cariogenic Bacteria

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**Abstract:** The purpose of this study is to compare and evaluate the antibacterial effects of various newer dentine bonding systems.

*Streptococcus mutans* was used as the test micro-organism and the Agar Diffusion Test was performed to determine the antibacterial property. The dentin bonding agents to be evaluated were grouped as Group A (Clearfil SE Protect Primer), Group B (Prime & Bond NT), Group C

(Single Bond Universal) & Group D (XenoV<sup>+</sup>) and 0.2% Chlorhexidine were used as the positive control. Mueller Hinton blood agar plates were swabbed with *Streptococcus mutans* from BHI Broth and they were divided into five sections. In each section, sterile Whatman no.1 filter paper disks were saturated with 20 µl of each bonding agent and the positive control were placed and incubated at 37 °C for 48 hours in an Anoxomat. The data was collected by measuring the zone of inhibition produced by various study groups. The results obtained were then subjected to statistical analysis using the Randomized block design analysis considering the replications using SPSS 19. Least Square difference analysis was done to do the multiple comparisons. *p* value less than 0.05 was considered as statistically significant.

The various dentin bonding agents evaluated and compared in this present study showed varying degree of antibacterial property against *Streptococcus mutans*. Among them, Clearfil SE Protect showed the maximum antibacterial activity followed by Single Bond Universal. Prime and Bond NT had the least antibacterial property against *Streptococcus mutans*.

**Keywords:** Antibacterial property, blood agar, dental caries, dentin bonding agents, *Streptococcus mutans*

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### I. Introduction

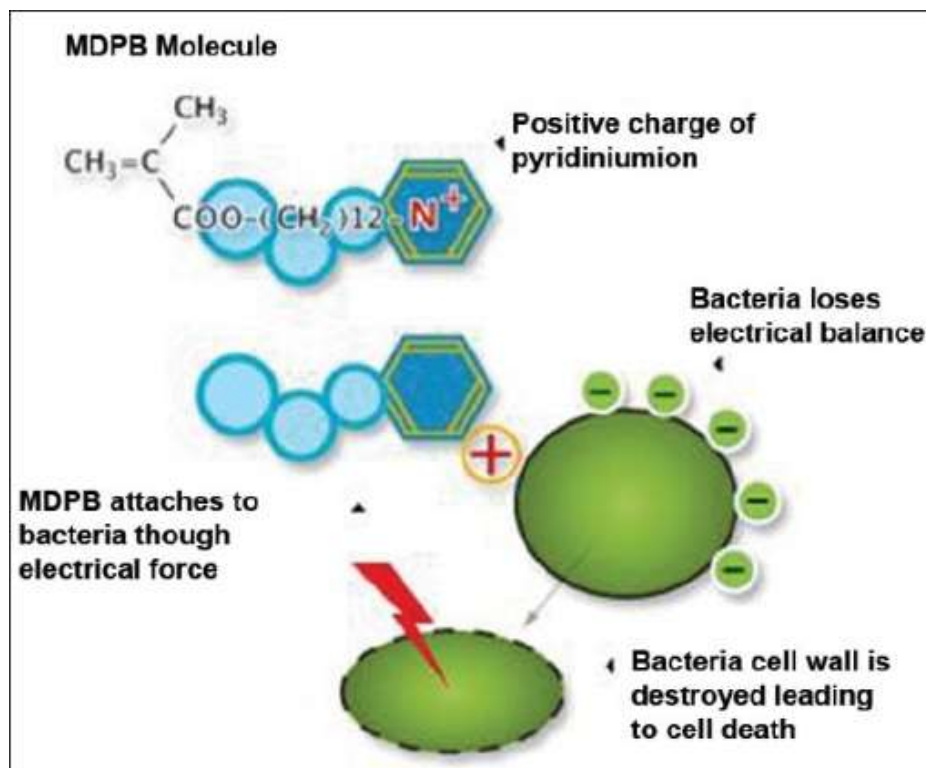
Dental caries is a microbiological disease of the teeth with the primary etiologic agent being *Streptococcus mutans*.<sup>1</sup> It is one of the prevalent diseases seen worldwide. The oral cavity is known to house over 700 different bacterial taxa. The microorganisms present in the oral cavity aid in the defense mechanism of the host by virtue of its role as a barrier. Of them the most important in the causation of dental caries is *Streptococcus mutans*.<sup>2</sup> Thus by virtue of the formation of dental plaque which acts as a substrate for bacterial adhesion, an environment conducive to the development of dental caries occurs. It has been established that the composition of the oral microflora is in a dynamic balance and when this has been hampered, there is the development of dental plaque. Thus the major virulence factors of *Streptococcus mutans* is its acidogenicity (ability to produce acids) and aciduric nature (ability to survive in an environment with low pH). Therefore these factors make it one of the major culprits in the development of dental caries.<sup>3</sup>

As Vernor Vinge once said, "Even The Largest Avalanche Is Triggered By Small Things." Therefore the complete elimination of carious tissue/dentin during cavity preparation is vital and pertinent to the success of the restorations.<sup>4</sup> But it may not be possible to achieve it completely using traditional methods as residual bacteria are harbored on the affected dentin. Ever since the advent of acid etching by Dr. Michael Buonocore in 1955, there has been a tremendous boom in the field of adhesive dentistry.<sup>5</sup> This has led to emergence and development of adhesive systems and restorative materials which has changed the principles of cavity preparation traditionally advocated by Dr. G.V Black.

In spite of such advancements, microleakage of bacteria through the gap between tooth and restoration and polymerization shrinkage of composites remain one of the main causes of secondary caries and pulpal damage. Therefore the use of restorative materials having antibacterial activity would aid in prolonging the survival of restored teeth.<sup>4</sup> Thus the antibacterial properties of adhesive systems are beneficial in the eradication of residual bacteria from the oral cavity. The anticariogenic property of these adhesive systems involves their

composition and their acidity.<sup>6</sup>These adhesive systems are available as etch and rinse or self –etch bonding systems .

A common method to achieve antibacterial effect is to use an agent-releasing material.<sup>7</sup>Various materials have been incorporated to achieve this goal such as silver nanoparticles, chlorhexidine, 12-methacryloyloxydodecylpyridinium bromide(MDPB) and cetylpyridinium (CPC). Among these MDPB has been used extensively. Imazato et al reported that the unpolymerized MDPB (12-methacryloyloxydodecylpyridinium bromide) shows strong bactericidal activity against residual bacteria in the cavity can be inactivated when an MDPB containing adhesive is applied.<sup>7</sup>



**Fig 1-** Structure of MDPB and its action on bacterial cell<sup>8</sup>

The pyridinium group of MDPB (Fig 1) is positively charged whereas the bacterial cell is negatively charged. Thus, as a result, bacteria lose their electrical balance which destroys the cell membrane of bacteria, leading to bacteriolysis. Therefore the purpose of this study was to compare and evaluate the antibacterial effects of various newer dentine bonding systems against *Streptococcus mutans*.

## II. Materials And Methods

The present study was conducted at the Department of Microbiology, A J Institute of Medical Sciences, Mangalore. Data were collected by recording the zone of inhibition in mm including the diameter of the filter paper disk.

### A) Materials used for testing

Four commercially available dentin bonding agents such as:

- Clearfil SE Protect\*
- Prime & Bond NT\*
- Single Bond Universal\*
- Xeno V<sup>+</sup>\*
- 0.2% chlorhexidine solution
- Mueller Hinton blood agar plates
- Brain Heart Infusion Broth
- Bacterial suspension of *Streptococcus mutans* (MTCC 497)
- Whatman no.1 filter paper disks

(\* composition of the commercially available bonding agents used in this study given in Table- 1)

Bonding agent	LOT number	pH	Manufacturer	Type	Composition
Clearfil SE Protect	00045	2	Kuraray Noritake Dental Inc., Japan	Two bottle-self etch	Primer: 10-MDP 12-MDPB HEMA Hydrophilic dimethacrylates Water Bonding agent: 10-MDP Bis-GMA HEMA Hydrophilic dimethacrylates Camphoroquinone
Prime & Bond NT	1410000965	2.1	Dentsply	Total-etch	Di-&Trimethacrylate resins Functionalised amorphous silica PENTA Photo initiators Stabilizers Cetylaminehydrofluoride Acetone
Single Bond Universal	516620	2.7	3M, ESPE	one bottle, self-etch	MDP phosphate monomer, dimethacrylate resins, HEMA, vitrebond copolymer, filler, ethanol, water, initiators, silane
Xeno V	140800	<2	Dentsply, Germany	One bottle, self-etch	Bifunctional acrylate, acidic acrylate, functionalized phosphoric acid ester, water, tertiary butanol, initiator, stabilizer

**Equipment used:**

- Laminar air flow chamber
- Anoxomat
- Incubator

**Instruments used:**

- Micropipette
- Sterile cotton swab
- Test tube

**III. Methodology**

The fresh cultures of *Streptococcus mutans* was obtained by seeding them on Brain Heart Infusion (BHI) agar for 24 hours under anaerobic conditions at 37°C. After incubation, the isolated bacterial colonies were suspended in sterile BHI Broth in a test tube until the turbidity was comparable with 0.5 Mac Farland standard. Mueller Hinton blood agar plate was then swabbed with *Streptococcus mutans* from BHI Broth. Mueller Hinton blood agar plate was then divided into five sections. In each section, sterile Whatman no.1 filter paper disks was saturated with 20 µl of each bonding agent namely Clearfil SE Protect, Prime & Bond NT, Single bond universal & Xeno V<sup>+</sup> and 0.2% of chlorhexidine gluconate as the positive control which are categorized as Group A, B, C, D, and E respectively. With the help of ethanol dipped and flamed forceps, the discs were then aseptically placed over the Mueller Hinton blood agar plates. The above mentioned procedures were performed in the laminar air flow chamber. After this, the MH blood agar plates were incubated at 37 C for 48 hours under anaerobic conditions in Anoxomat.

**IV. Results**

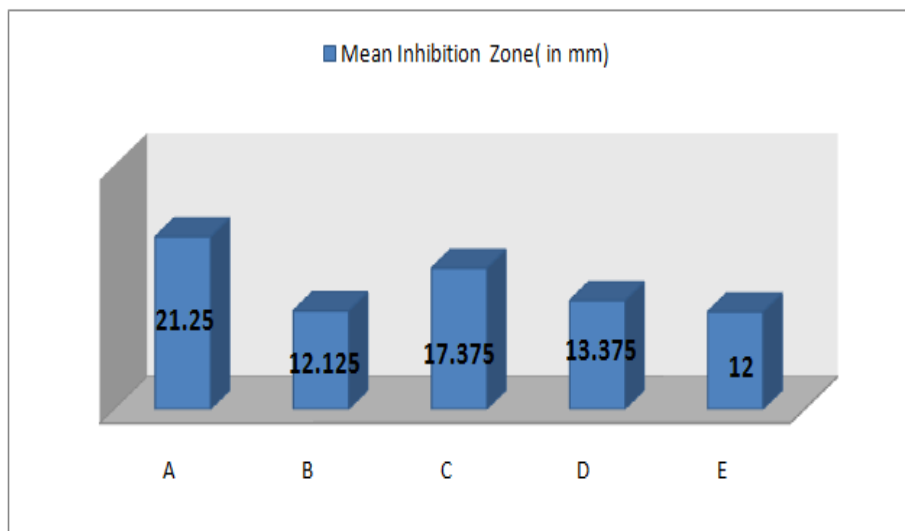
The data was collected by recording the zone of inhibition produced by the respective groups in MH blood agar plates. Statistical analysis was done using Randomized block design analysis considering the replications using SPSS19. Least Square difference analysis was done to do the multiple comparisons. p value less than 0.05 was considered as statistically significant. Group A (Clearfil SE Protect Primer) exhibited the highest mean of zone of inhibition against *S. Mutans*

Group B (Prime and Bond NT) exhibited the lowest amount suppression against S.mutans.

Table2: Mean diameters and standard deviation values of antibacterial inhibition zones

**BAR GRAPH REPRESENTING MEAN OF ZONE OF INHIBITION OF THE TEST MATERIALS**

Groups	Sample Size (N)	Mean (in mm)	Standard Error
A	8	21.250	1.025
B	8	12.125	1.025
C	8	17.375	1.025
D	8	13.375	1.025
E	8	12.000	1.025



**Table 3:** inter group comparison of mean zones of inhibition of various dentin bonding agents using adt.

Group comparison		Mean difference	Sig.
A	B	9.12500	<b>0.000 &lt; 0.05, HS</b>
	C	3.87500	0.083
	D	7.87500	<b>0.000 &lt; 0.05, HS</b>
	E	9.25000	<b>0.000 &lt; 0.05, HS</b>
B	A	-9.12500	<b>0.000 &lt; 0.05, HS</b>
	C	-5.25000	<b>0.008 &lt; 0.05, HS</b>
	D	-1.25000	0.912
	E	0.12500	1.000
C	A	-3.87500	0.083
	B	5.25000	<b>0.008 &lt; 0.05, HS</b>
	D	4.00000	0.069
	E	5.37500	<b>0.007 &lt; 0.05, HS</b>
D	A	-7.87500	<b>0.000 &lt; 0.05, HS</b>
	B	1.25000	0.912
	C	-4.00000	0.069
	E	1.37500	0.880
E	A	-9.25000	<b>0.000 &lt; 0.05, HS</b>
	B	-0.12500	1.000
	C	-5.37500	<b>0.007 &lt; 0.05, HS</b>
	D	-1.37500	0.880

From the Table 3, it is clear the mean differences between group A with B, C, D, and E were statistically significant. B with A and C were statistically significant. C with A, B, D and E were significant. D with A and C were significant. E with A and C were significant.

## V. Discussion

Dental caries is one of the most common infections of bacterial origin seen in humans.<sup>9</sup> Shafer (1993) defined dental caries as “an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation.”<sup>10</sup> Mutans streptococci are the most cariogenic pathogen.<sup>11</sup> Basically, *S. mutans* derives its name to a group of seven closely related species collectively referred to as the mutans streptococci.<sup>12</sup> Dental caries is basically caused by the acid that is produced by the cariogenic bacteria in the presence of carbohydrates.<sup>13</sup>

Therefore *Streptococcus mutans* was chosen as the test microorganism for the present study as it is considered to being the most significant organism in the causation of primary and secondary caries.<sup>14</sup> Due to the aesthetic demands of the patient, minimally invasive composite restorations are being increasingly performed. The major goal in the treatment of dental caries is the complete removal of carious dentin during cavity preparation.<sup>15</sup> Resin composites are presently one of the most popular restorative materials used in the field of dentistry. Adequate adhesion between the tooth and the restoration is needed which determines the success of the restoration. It has been reported that as much as 70% of composite restorations are replaced due to failed restorations.<sup>13</sup> It has been reported mainly due to recurrent caries followed by fracture.<sup>16</sup>

According to Federation Dentaire Internationale (1962), secondary caries is defined as “a positively diagnosed carious lesion which occurs at the margins of an existing restoration.” Secondary caries could be caused due to inadequate oral hygiene, bacterial microleakage, residual bacteria in cavity preparation or a combination of these causes. Since resin composites are hydrophobic in nature, an intermediate layer of dentin bonding agents needs to be applied to aid in the adhesion to the tooth structure. The etch and rinse adhesion strategy (formerly known as total etch) involves two types of adhesives based on the number of steps involved such as:

- a) Three-step etch-and-rinse adhesives- In these, after the application of the phosphoric acid etchant and rinsing with water, a solvent-rich primer is applied (hydrophilic functional monomer) and air-dried, followed by an adhesive resin which is polymerized. Coming to the two-step etch-and-rinse adhesives, after the phosphoric acid etching and rinsing it off with water, dentin and enamel are simultaneously primed and bonded which is followed by air-drying and polymerization. To overcome the difficulties associated with acid-demineralized dentin depth and the subsequent resin infiltration of the etch-and-rinse adhesives, a much more user-friendly and less technique sensitive method was introduced namely, the self-etch adhesive systems. This adhesion strategy involves two types of adhesives based on the number of steps involved:
  - a) Two-step self-etch adhesives, in which enamel and dentin are simultaneously conditioned and primed using a self-etching primer which is acidic in nature, followed by the application of an adhesive resin (hydrophobic resin), which is polymerized.
  - b) One-step, self-etch adhesives, in which the acidic primer and the hydrophobic adhesive resin come all together in one self-etching solution. The uniqueness is the occurrence of conditioning, priming and infiltration of the substrate prior to polymerization.<sup>17</sup>

Compared with etch-and-rinse adhesives, self-etching adhesives have the following advantages. Firstly, self-etching adhesives have a less technique-sensitive procedure as the etch-and-rinse procedure is not needed which causes the collapse of demineralized collagen network after acid etching. Secondly, due to the simultaneous demineralization and resin infiltration an optimally infiltrated hybrid layer is formed. However, recent observations of nanoleakage beyond hybrid layer have led to some doubt on complete resin infiltration. Thirdly, mild self-etching adhesives produce less post-operative pain due to the use of the smear layer as the bonding substrate, leaving residual smear plugs that cause less dentinal fluid flow than etch-and-rinse adhesives. Finally, the mild self-etching adhesives leave hydroxyapatite crystals available for chemical bonding of functional monomers to calcium, which may contribute to interface stability.<sup>18</sup> The all-in-one system involves a single step.

In the self-etching adhesives, due to the absence of the rinsing procedure, the existence of bacteria may occur at the tooth-restoration interface. This interface is most prone to the passage of irritants leading to microleakage and finally pulpal pathosis.<sup>17</sup> Moreover, due to the simultaneous occurrence of etching and priming in these self-etching adhesives, there is an integration of the smear layer into the adhesive interface. The incorporated smear layer may interfere with the demineralising process of the self-etching adhesives leading to interfacial gaps.<sup>19</sup> This interface is most prone to passage of irritants leading to microleakage and finally pulpal pathosis. Therefore with the vast strides that are occurring in the field of aesthetic dentistry, usage of self-

etching adhesives with antibacterial properties would be beneficial in eliminating secondary caries and failure of the restoration.

In the present study, the materials were tested for their antibacterial activity against *S. mutans*, the main pathogen causing initiation and development of secondary caries included Clearfil SE Protect, Prime & Bond NT, Single Bond Universal and Xeno V<sup>+</sup>. Prime & Bond NT is a two-step etch and rinse adhesive whereas Clearfil SE Protect, Single Bond Universal and Xeno V<sup>+</sup> are self-etching adhesives. Various methods have been formulated to determine the antimicrobial activity of dental materials. Clearfil SE Protect is self-etching primer/adhesive system by Kuraray, Japan. The pH of its primer is 2. In the present study, the antibacterial activity of its primer was tested against *S. mutans*. In this study, Clearfil SE Protect self-etching primer exhibited highest mean of zone of inhibition against *S. Mutans* whereas Prime and Bond NT exhibited the lowest amount of suppression against *S. mutans* (Table-5). Addition of MDP and MDPB has been shown to have antibacterial properties.<sup>20</sup> Clearfil SE Protect is an adhesive having antibacterial monomer MDPB. Methacryloyldodecylpyridinium bromide (MDPB) is a quaternary ammonium derivative synthesized from dodecylpyridinium bromide and methacryloyl group.

MDPB contains a positive charge which causes the loss of electrical imbalance in bacterial cell, leading to cell wall destruction and therefore cell death as depicted in Fig 1.<sup>8</sup> According to Imazato et al, unpolymerized MDPB demonstrates antibacterial activity and aids to inactivate the residual bacteria. The present study confirms such a bactericidal activity of the Clearfil SE Protect Primer containing MDPB. Similar results with Clearfil SE protect was observed by Korkmaz in 2008 and Ozel et al 2016 in which the antibacterial activity was attributed to the low pH of 2 of the primer.<sup>6</sup> According to studies by Imazato et al, the primer of Clearfil Protect Bond containing 5% MDPB was successful in eliminating *Streptococcus mutans* within 30 seconds of contact time.<sup>20</sup> Esteves et al have evaluated the antibacterial property of various self-etch adhesives against *Streptococci* and found that Clearfil Protect Bond had the highest antibacterial activity among the dentin bonding agents tested.<sup>8</sup>

Prime and Bond NT is a dentin bonding agent wherein the primer and the adhesive are present in the same bottle. Therefore it involves a total etch concept wherein a separate etching step is needed prior to the use of the primer-adhesive solution.<sup>21</sup> Prime and Bond NT did not show any significant antibacterial property which is in contrast to a study by Sampath et al (2011) which stated its antibacterial activity due to presence of fluoride. In a similar study by Ambikathanaya et al (2013), Prime and Bond NT exhibited the highest antibacterial property and this was attributed to its lower pH and the presence of fluoride followed by Xeno V.<sup>19</sup> The addition of fluoride in the dentin bonding agents augments the demineralization protective effect of them. The mechanism of action of fluoride is believed to occur due to the following mechanism such as direct binding of fluoride/HF to enzymes and other bacterial proteins, binding of metal fluoride complexes and its action as a transmembrane proton carrier.

Single Bond Universal & Xeno V<sup>+</sup> are both one bottle self-etch adhesives wherein the etchant, primer and finally the adhesive are all combined in the same bottle thus, simplifying the adhesive system. In the present study Single Bond Universal adhesive exhibited the better antibacterial property than Xeno V<sup>+</sup>. As confirmed by previous studies, it is the cytotoxic nature of the monomers and the acidic pH of the self-etching primer that is mainly responsible for the inhibition of the growth of bacteria. Thus the antibacterial property of the adhesive systems can be attributed either due to their low pH or to the presence of certain antibacterial components such as glutaraldehyde/ MDPB.<sup>6,22</sup> Since bacteria cannot thrive in an acidic environment, the acidic nature of the adhesives plays a major role in influencing the antibacterial activity of the material.<sup>6</sup> Xeno V adhesive system has a pH of 1.38 as reported by a previous study leading to its antibacterial activity.<sup>19</sup> So therefore, the antibacterial property of Xeno V<sup>+</sup> having a pH < 2 (Table 1) in this current study can be attributed to its lower pH value. Single Bond Universal contains the acidic MDP monomer rendering it antibacterial (Table 1). Thus the antibacterial activity of Single Bond Universal & Xeno V<sup>+</sup> can be attributed to their lower pH. There has not been any study in the literature regarding the antibacterial activity of Xeno V<sup>+</sup> and Single Bond Universal.

Chlorhexidine is an antiseptic with a wide mode of action. It has been used in the control of bacterial plaque and disinfection of therapeutic cavities by virtue of its ability to denature bacterial cell.<sup>23</sup> It is also effective in reducing the levels of *Streptococcus mutans* found on exposed carious root surfaces. For this reason, it is used as a positive control for studies on bacterial growth or antibacterial activity.<sup>19</sup> Therefore 0.2% of chlorhexidine was used in this study as a positive control.

In this present study, the agar diffusion test was used to evaluate and compare the antibacterial property of the various dentin bonding agents. The various adhesives used in this study have demonstrated antibacterial action to varying degrees. The agar diffusion test has been widely used to determine and compare the antibacterial property of various dental materials.<sup>24,25</sup> The advantage of agar diffusion test is that it aids in direct comparisons of test materials against the test microorganisms. Moreover, the results obtained by the ADT can indicate the existence of diffusible components into an aqueous medium. But, ADT has certain drawbacks like its ability to measure only the water-soluble components and the solubility and the diffusibility of the test agent

affect the inhibition zone. The results of this method not only depend on the toxicity of the material for a particular microorganism but also on the diffusability of the material across the medium. A material that diffuses more easily would be capable of providing larger zones of microbial growth inhibition. Moreover, other factors like the inoculum size, the material/agar contact and incubation time may also affect the results.

Observations from the present study revealed that all the tested dentin bonding agents had antibacterial activity against *Streptococcus mutans* but not to the same degree. Further studies both in vivo and in vitro are needed to determine the long-term antibacterial effect of the dentin bonding systems. Moreover, the depth of bacterial invasion into the dentinal tubules needs to be investigated.

## VI. Conclusion

The present in vitro study evaluated and compared the antibacterial property of newer adhesive systems on *Streptococcus mutans* using Agar Diffusion Test.

Under the limitations of this study, the following conclusions were drawn:

1. The dentin bonding systems had different inhibitory action on *Streptococcus mutans* during the incubation period.
2. Inhibition of the growth of *Streptococcus mutans* is due to the direct contact of the test bacteria with the adhesive system.
3. In this study, Clearfil SE Protect self-etching Primer had the maximum antibacterial property and Prime and Bond NT had the least antibacterial activity.

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Conflict of interest: None

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