

# INVESTIGATING THE ANTIBACTERIAL EFFECT OF CHITOSAN IN DENTAL RESIN COMPOSITES: A PILOT STUDY

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## ABSTRACT

*The tendency of (resin-based composites) RBCs to allow more plaque formation results in secondary caries. Chitosan (CS) inhibits the growth of Streptococcus mutans (S.mutans); which is most frequently caries causing bacteria. Therefore, CS was incorporated into RBCs to determine its in vitro antibacterial activity against S.mutans.*

*CS was added to flowable and microhybrid RBCs at various concentrations of 0 (control) 0.25, 0.5 & 1.0 % by weight. Five disc shaped specimens (15mm diameter, 2mm thickness) were prepared for each experimental and control group. Interaction of cured chitosan modified RBCs with S. mutans biofilms was evaluated using agar diffusion test method. The results of ADT were negative. The experimental RBCs containing CS did not show growth inhibition zone around lawn growth of S.mutans compared to control group. Further experiments are in progress to determine CS modified RBCs antibacterial efficacy by direct contact test. Within the limitations of this study Antibacterial activity of CS modified RBCs is comparable to that of commercial RBCs when evaluated by agar diffusion test method.*

**Key Words:** Chitosan antibacterial effect, composites.

## INTRODUCTION

The concerns over mercury toxicity of amalgam and increased demands of patients for tooth matching restorations have led to a greater use of dental resin-based composites (RBCs) worldwide. However, in vitro<sup>1</sup> and in vivo<sup>2</sup> studies have reported that more plaque accumulation occurs on RBCs compared to other restorative materials for instance amalgam and glass ionomers<sup>3</sup> or dental hard tissues such as enamel.<sup>4</sup> In vitro enamel demineralization owing to plaque accumulation has been observed around different RBCs.<sup>5</sup> Growth of plaque adjacent to the restoration margins

may lead to secondary caries in vivo and limit the longevity of RBCs.<sup>6</sup>

Mechanical properties of RBCs have been improved substantially since their development,<sup>7</sup> however their antibacterial properties are still considered unsatisfactory by dentists as well as investigators.<sup>8</sup> As a result, various antibacterial agents such as antibiotics,<sup>9</sup> silver ions,<sup>10</sup> iodine and quaternary ammonium compounds<sup>11</sup> were added to RBCs. These agents gradually released from RBCs<sup>12</sup> and their release into the surrounding location at different releasing rates had several disadvantages such as a decrease in the mechanical properties, short-term effectiveness, and possible toxicity if the release is not properly controlled.<sup>11</sup>

Chitosan (CS) is a copolymer of N-acetyl-d-glucosamine (Glc-NAc) and d-glucosamine (GlcN) which is present in insects' exoskeletons, crustaceans' shells and fungi cell walls. The major properties of this polymer CS include biodegradability, biocompatibility, non-toxicity, high bioactivity, selective permeability, antimicrobial activity, ability to form gel and film, chelation ability and absorptive capacity. Therefore, it has been proposed as a safer material for use in biomedical applications.<sup>13</sup>

There is evidence that addition of CS in dental materials, increases the biocompatibility of materials, inhibits the adsorption of oral bacteria to the tooth surface.<sup>14,15</sup> The CS has peculiar properties that has been reported in the literature such as: drug carrier

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of controlled release,<sup>16</sup> anti-bacterial,<sup>17,18</sup> prevention of decalcification of dental enamel<sup>19</sup> by inhibiting bacterial plaque formation and these properties reveal its potential in numerous commercial products.

The mechanism of antimicrobial activity of CS is yet to be explored thoroughly; however mechanisms suggested in literature state that the amino groups of the CS when come in contact with physiological fluids are protonated and if bind to anionic groups of the microorganisms, resulting in the agglutination of the microbial cells and inhibition of growth.<sup>20</sup> Although use of CS in different formulations, such as dental adhesives, restorative materials, toothpastes (Chitodent®), mouthwash solutions and chewing gums, is stated in literature. In all forms the CS has shown antibacterial activity for Streptococcus bacteria groups. The CS inhibits the bacterial plaque formation and stimulates salivation in vivo.<sup>21</sup> These effects suggest the application of CS as preventive and therapeutic agent to control dental caries.<sup>21,22</sup> Since one of most common cause of RBC restorations failure is secondary caries,<sup>6</sup> therefore, the CS was incorporated into RBCs, to enhance their antimicrobial activity which might aid the development of antimicrobial RBCs without compromising physical and mechanical properties.

## METHODOLOGY

Two commercially available RBCs, one conventional microhybrid and one flowable (3M ESPE, USA) and CS coarse ground flakes and powder (Sigma Aldrich, MO USA) with > 75% degree of deacetylation were used. Culture media namely Mitis Salivarius Agar (MSA), Brain heart infusion broth (BHI) and Phosphate buffer saline (PBS) buffer supplemented with Bacitracin (Sigma Aldrich, MO USA) were utilized for the growth of streptococcus mutans ATCC culture # 25175 of Microbiologists USA.

### Experimental RBCs preparation

Three experimental RBC groups were prepared by adding 0.25%, 0.5%, and 1.0% (w/w) CS to each commercial microhybrid and flowable RBCs. The RBCs and CS coarse ground flakes and powder were weighed using analytic balance (Ay-Z20 Japan) accurate to 0.001 gram. In a 50 ml glass beaker CS was incorporated into the RBCs and homogeneously mixed in a dark room with glass rod.<sup>23</sup> Commercial microhybrid and flowable RBCs without CS served as control groups.

### Specimen preparation

For each RBC group, five disc-shaped specimens (15 mm diameter, 1 mm thickness) were prepared using aluminum mold as specified by ISO 4049, 2000.<sup>26</sup> The top and bottom surfaces of each specimen were covered with cellulose acetate strip (0.1 mm thickness) to decrease the oxygen inhibition effects. All specimens were light polymerized from top surface by Elipar LED curing unit (3M ESPE, Germany). The curing tip was placed in contact with the acetate strip and specimen was cured in an orbital sequence four times for 40 seconds each. Then each specimen was taken out of mold and flash cut away using a blade. Specimens were sterilized by

immersion in 70% (by Volume) ethanol for 30 minutes followed by same period immersion in 100 % ethanol. Then specimens were ready for antibacterial activity testing.

### Preparation of bacterial suspension

The pure culture of *S. mutans* ATCC # 25175 kwik-stik was inoculated by gently rolling the swab over one-third of the MSA plate. Using a sterile loop, streaking was performed to facilitate colony isolation. The inoculated primary culture plate was immediately incubated at 37°C in 5% CO<sub>2</sub> Incubator (Thermo Scientific CO<sub>2</sub> Incubator) for 24 hours. In order to make bacterial suspension few colonies of *S. mutans* was transferred to 5 ml of broth [BHI, Difco, and Detroit, MI]. Following overnight incubation, the top 4 ml of the broth was transferred to a new test tube and centrifuged for 10 minutes at 3175×g. The resultant bacterial pellet was suspended in 5ml of phosphate-buffered saline [PBS, Sigma, St. Louis, MO] and vortexed gently for 10 seconds prior to use.<sup>24</sup>

### Antibacterial test

The antibacterial test (ADT) was conducted following the procedures stated in literature.<sup>24</sup> The *S. mutans* bacterial suspension (200 µl volume) turbidity equivalent to 0.5 Mc Farland standard was spread on a Petri dish of MSA Agar (Sigma St. Louis, MO, USA) supplemented with bacitracin 0.0625 g/ml (Sigma St. Louis, MO, USA). The test and control group specimens (n=5) were placed on the surface then these plates were incubated for 48 hours at 37°C in 5% CO<sub>2</sub> Incubator (Thermo Scientific CO<sub>2</sub> Incubator). After 48 hours of incubation the diameter of inhibition zone around each specimen disc was measured in mm using a metric ruler, by placing the ruler on the bottom of the plate.<sup>24</sup>

## RESULTS

According to agar diffusion test method there was no growth inhibition zone in the lawn growth of *S. mutans* around the samples of RBCs supplemented with 0.25, 0.5 or 1.0% w/w chitosan or in the control RBCs (Table 1).

## DISCUSSION

There is evidence that addition of CS in dental materials increases the biocompatibility of materials, inhibits the adsorption of oral bacteria to the tooth surface.<sup>14,15</sup> Since one of most common cause of RBC restorations failure is secondary caries.<sup>6</sup> Therefore, in this study, CS coarse ground flakes and powder was added as antibacterial agents in RBCs.

The ADT was used as a gold standard as this method has been widely used in most of studies testing antimicrobial activity of various commercial and experimental RBCs. CS was added at different weight percents into RBCs. Concentration of CS in RBCs was above minimum inhibitory concentration of CS reported against *S. mutans*. However, no inhibition zone in lawn growth of *S. mutans* was found around experimental groups in present study. No inhibition zone may be attributed to immobilization and non release behavior of CS into

TABLE 1: EFFECT OF CHITOSAN BASED RBCS ON GROWTH OF *S. MUTANS*

Tested material	Content of CS wt %	ADT Inhibition Zone(mm)
Flowable RBCs	0	0
	0.25	0
	0.50	0
	1.0	0
Conventional RBCs	0	0
	0.25	0
	0.50	0
	1.0	0

RBCs matrix. Since ADT is capable of determining antibacterial activity of only soluble agents which diffuse into agar gel and inhibit the bacterial growth, but is not appropriate for non soluble antibacterial agents with surface contact inhibition properties. Therefore, to further confirm antibacterial activity of chitosan modified RBCs by surface inhibition, more experiments are in progress using direct contact test method. The findings of this study support previous studies according to which when antibacterial agents incorporated into RBCs such as ZnO nano particles, PEI nanoparticles, MDPB get immobilized into RBC matrix no inhibition zone was detected.<sup>24-25</sup>

More experiments using various latest methods i.e. SEM, DCT via optical density determination and colony forming unit count are necessary in order to further explore antibacterial activity of newly developed CS added RBCs. Moreover, effect of CS on physical, mechanical and aesthetic properties of RBCs needs to be evaluated.

## CONCLUSION

Within the limitations of current pilot study, the absence of inhibition zone in lawn growth of *S. mutans* is well evident in all RBCs tested, which may possibly be due to immobilization and non release behavior of CS into RBCs matrix.

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