

## Shear bond strength of composite to primary enamel teeth treated with different concentrations and various molecular weights of chitosan

Sepideh Sorourhomayoun<sup>1</sup>, Homayoon Alaghehmand<sup>2</sup>, Soleiman Mahjoub<sup>3</sup>,  
Soraya Khafri<sup>4</sup>, Maryam Ghasempour<sup>5</sup>✉

1. Assistant Professor, Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
2. Associate Professor, Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
3. Professor, Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
4. Assistant Professor, Social Determination of Health Research center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.
5. Associate Professor, Oral Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran.

✉ **Corresponding Author:** Maryam, Ghasempour, Department of Pediatrics of Dentistry, Faculty of Dentistry, Babol University of Medical Sciences, Babol, IR Iran.

**Email:** m.ghasempour@mubabol.ac.ir

**Tel:** +981132291408

**ORCID** (0000-0002-2075-4438)

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### Abstract

**Introduction:** Today, chitosan has a wide usage in Dentistry. Due to its penetration into tooth tissues, inhibition of the acid penetration and anti-demineralization capabilities, chitosan may interfere with the etching mechanism in the bonding restorations. The aim of this study was to evaluate the effect of chitosan with different concentrations and molecular weight on the shear bond strength (SBS) of composite to deciduous teeth's enamel.

**Material & Methods:** In this in vitro trial, 50 deciduous anterior human maxillary teeth, which extracted because of orthodontic treatment plan, were selected and divide evenly between groups. In the control specimens, Filtek Z250 composite were packed on buccal teeth surfaces after using self-etch primer single bond universal and light-cured. In four next groups the teeth were immersed into chitosan solution with low and high molecular weights and 5 mg/ml and 2.5 mg/ml concentrations for 60 seconds and restored with composite as control groups. The SBS of composite to the teeth enamel were determined with Universal testing machine. Data was analyzed by SPSS-18 software using one-way ANOVA and  $p < 0.05$  was considered significant.

**Results:** The SBS to enamel in the control specimens were  $20.98 \pm 6.31$  MPa. The values were  $17.92 \pm 7.25$ ,  $16.02 \pm 6.03$ ,  $13.26 \pm 5.18$  and  $17.67 \pm 8.95$  MPa in the pretreatment with low molecular weight chitosan in 2.5 mg/ml, low molecular weight chitosan in 5 mg/ml, high molecular weight chitosan in 2.5 mg/ml and high molecular weight chitosan with 5 mg/ml concentrations respectively. No significant differences were found regarding SBS between groups ( $P > 0.05$ ).

**Conclusion:** Despite a slight reduction in SBS of the composite to the enamel of the treated compare to control, use of chitosan in different concentration and molecular weight did not interfere with the SBS of composite to the enamel of deciduous teeth.

**Keywords:** Chitosan, Chitin, Dental enamel, Primary teeth

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## استحکام باند کامپوزیت به مینای دندانهای شیری درمان شده با غلظت ها و وزن های مولکولی متفاوت کیتوزان

سپیده سرورهمایون<sup>۱</sup>، همایون علاقمند<sup>۲</sup>، سلیمان محجوب<sup>۳</sup>، ثریا خفری<sup>۴</sup>، مریم قاسمپور<sup>۵\*</sup>

۱. استادیار، مرکز تحقیقات مواد دندان، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.

۲-دانشیار، مرکز تحقیقات مواد دندان، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.

۳-استاد، مرکز تحقیقات بیولوژی سلولی و مولکولی، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.

۴. استادیار، مرکز تحقیقات عوامل اجتماعی موثر بر سلامت، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.

۵. دانشیار، مرکز تحقیقات سلامت و بهداشت دهان، پژوهشکده سلامت، دانشگاه علوم پزشکی بابل، بابل، ایران.

\*نویسنده مسئول: مریم قاسمپور، گروه دندانپزشکی کودکان، دانشکده دندانپزشکی، دانشگاه علوم پزشکی بابل، بابل، ایران.

پست الکترونیکی: m.ghasempour@mubabol.ac.ir تلفن: +۹۸۱۱۳۲۲۹۱۴۰۸

### چکیده

**مقدمه:** امروزه، کیتوزان کاربرد گسترده ای در دندانپزشکی دارد. کیتوزان به دلیل نفوذ در نسج دندان و ممانعت از ورود اسید و نیز دارا بودن پتانسیل ضد دیمیرالیزاسیون؛ احتمال دارد با مکانیسم اچینگ دندانها در ترمیمهای باند شونده تداخل ایجاد کند. این مطالعه با هدف تعیین اثر کاربرد کیتوزان با غلظت ها و وزن های مولکولی متفاوت بر استحکام باند برشی کامپوزیت به مینای دندانهای شیری انجام شد.

**مواد و روش ها:** این مطالعه آزمایشگاهی، ۵۰ دندان قدامی شیری ماگزیلاری انسان که به علت طرح درمان ارتودنسی کشیده شده بودند، بطور مساوی بین گروهها تقسیم گردیدند. گروه کنترل، کامپوزیت Filtek Z250 بدنبال استفاده از باند سلف اچ Single bond universal روی سطح باکال دندانها قرار داده و نوردی گردید. در چهار گروه مطالعه؛ دندانها ابتدا به مدت ۶۰ ثانیه در محلولهای کیتوزان با دو وزن مولکولی کم و زیاد و غلظتهای ۲/۵ و ۵ میلی گرم بر میلی لیتر غوطه ور شدند و سپس ترمیم با کامپوزیت همانند گروه کنترل انجام شد. مقادیر استحکام باند برشی کامپوزیت به مینای دندانها با Universal testing machine تعیین شد. نتایج با نرم افزار SPSS-18 و آزمون آنالیز واریانس یکطرفه مورد آنالیز قرار گرفتند  $p < 0.05$  معنادار بوده است.

**یافته ها:** مقادیر استحکام باند به مینا گروه کنترل معادل  $20/98 \pm 6/31$  MPa و بدنبال آماده سازی با کیتوزان در وزن مولکولی پائین و غلظت ۲/۵ میلی گرم بر میلی لیتر، وزن مولکولی پائین و غلظت ۵ میلی گرم بر میلی لیتر، وزن مولکولی بالا و غلظت ۲/۵ میلی گرم بر میلی لیتر و نیز وزن مولکولی بالا و غلظت ۵ میلی گرم بر میلی لیتر به ترتیب برابر  $17/92 \pm 7/25$ ،  $16/02 \pm 6/03$ ،  $13/26 \pm 5/18$  و  $17/67 \pm 8/95$  MPa بوده است. تفاوت معنی داری از نظر مقادیر استحکام باند برشی در گروههای مختلف دیده نشد ( $P > 0/05$ ).

**نتیجه گیری:** به رغم کاهش اندک استحکام باند برشی، کاربرد کیتوزان در غلظت ها و وزن های مولکولی متفاوت تداخل آشکاری در استحکام باند برشی کامپوزیت به مینای دندانهای شیری نداشته است.

**واژگان کلیدی:** کیتوزان، کیتین، مینا دندان، دندانهای شیری

### Introduction

Different materials have been suggested to control dental biofilm including chlorhexidine (CHX), essential oils, amine fluoride, triclosan and so on. The CHX is the most effective and most commonly administered antibacterial agent for this purpose; however, its use is limited due to its side effects such as distortion in the

sense of taste and pigmentation of oral tissues.<sup>[1]</sup> Studies are ongoing to find new antibacterial materials, and natural compounds like chitosan have gained attention due to their insignificant side effects and promising antimicrobial properties in oral use. It also shows an increase in healing after extraction and its

combination with dental cements can reduce bacterial biofilms.<sup>[2,3]</sup> Chitosan has now extensive applications in different fields of dentistry and has gained popularity due to its several advantages. Chitosan [poly $\beta$ -(1-4)-N-Acetyl-D-glucosamine] is an insoluble biopolymer derived from the deacetylation of chitin.<sup>[3]</sup> It is produced from the shells of Crustaceans including shrimp, lobster and crab or via microbiological and chemical processes.<sup>[4]</sup> The difference in the process of production can cause differences in degree of deacetylation of chitosan, distribution of acetyl groups, length of the chain and structure of chitosan as well as consequently change its solubility, antimicrobial activity or other properties.<sup>[5]</sup>

Chitosan can be used on prevention of tooth demineralization. It inhibits the release of phosphorus and interferes with the process of demineralization. Chitosan penetrates into the enamel and prevents acid penetration and demineralization. Moreover, due to its anti-demineralization activity, when clinically applied, it may interfere with the mechanism of etching required for bonded resin restorations. It may prevent adequate resin penetration and decrease the bond strength of resin restorations.<sup>[6]</sup>

The use of self-etch adhesives has increased especially in Pediatric Dentistry due to their easy application and low technical sensitivity.<sup>[7]</sup> Strong self-etch adhesive application on dentin dissolves all hydroxyapatite (HA) crystals and causes weak bond strength particularly to dentin. Moderate of acidity self-etch systems does not demineralize dentin more than 1 $\mu$ m. Surface demineralization in these systems is incomplete and HA crystals attached to the collagen fibers are preserved. Preservation of HA crystals beneath the hybrid layer may provide a receptor for additional chemical bonds. Thus, a more favorable bond to dentin is achieved although the bond strength to enamel may be inadequate. Considering the fact that in self-etch systems, rinsing is not performed, these primers remain on the tooth structure, and their low pH may prevent the polymerization of composite resin over the adhesive layer and decrease shear bond strength (SBS).<sup>[7,8]</sup> Since there has been no study evaluating composite bond strength to primary teeth's enamel treated with chitosan, this study aims to evaluate the SBS of composite with a 7<sup>th</sup> generation self-etch adhesive bonding system to primary enamel treated with different concentrations and variable molecular weights of chitosan.

## Materials & Methods

This study was evaluated after obtaining the ethical approval from Babol University of Medical Sciences (IR.Mubabol.HRI.REC.1392.326). In this in-vitro experimental study, chitosan powder (ACROS Organic Co., Morris Plains, NJ, USA) with low (LMW Chi.) and high (HMW Chi.) molecular weight was used. Chitosan solution was prepared by dissolving chitosan biopolymer in 1% acetic acid and constant shaking for 24h at 50°C. Accordingly, 5mg/ml and 2.5 mg/ml concentrations were prepared, and pH of the solution was adjusted at 5.8 using NaOH. Finally, 4 chitosan solutions with different concentrations and molecular weights were prepared:

1. LMW Chi. with 5mg/ml concentration
2. LMW Chi. with 2.5mg/ml concentration
3. HMW Chi. with 5mg/ml concentration
4. HMW Chi. with 2.5mg/ml concentration

Fifty sound primary anterior teeth<sup>[9-11]</sup> with intact buccal surface were collected. Soft tissues were debrided from the tooth surface, and the teeth were put in 0.5% Chloramine T solution for 3 days for disinfection and then stored in saline solution for 1-3 months until the experiment date. The solution was refreshed daily. The teeth were then embedded in self-cure acrylic resin in a prefabricated mold with exposed buccal surfaces. The buccal surfaces were ground flat with 400, 600 and 1200 grit aluminum oxide discs under abundant water irrigation for cooling. This process standardized the orientation of enamel crystals, removed the outer-layer hypermineralized and acid-resistant enamel and simulated the clinical setting where 0.5mm of the labial enamel was removed by beveling or for veneering. Prior to application of bonding agent, the labial surface of the teeth was cleaned by a mixture of water and fluoride-free pumice paste using a rubber cup and low-speed handpiece for 10 seconds. The enamel surface was then washed to eliminate pumice paste and debris as well as dried with oil-free air spray. The teeth were randomly divided into 5 groups of 10 each.

In group 1, composite along with self-etch adhesive was applied to untreated teeth. Single Bond Universal (3M ESPE AG, Germany); which is a 7<sup>th</sup> generation bonding agent was used according to the manufacturer's instructions. The bonding was applied for 20s, the surface was gently air-dried for 5s and light-cured for 10s using LED light-curing unit (Bluephase 8, Ivoclar Vivadent, US) and 800w. Light intensity was measured by radiometer (Bluephase 8, Ivoclar Vivadent, US)

before curing. Filtek Z250 Filtek Z250 (3M ESPE, Germany) was applied to the enamel surface by packing the composite into the cylindrical plastic matrices with an internal diameter of 2mm and height of 2mm. Excess composite was removed using an explorer tip and light-curing was done according to the manufacturer's instructions using LED light-curing unit. In the four other groups that were previously mentioned, the teeth were immersed into chitosan solutions with different molecular weights and concentrations for 60<sup>s</sup>, and then composite along with the self-etch primer was applied as in group 1. All specimens were subjected to thermocycling (1000 cycles at 5-55°C and 60s of dwell time) and were then transferred to universal testing machine (Zwick GmbH & Co. KG, Germany). Shear loads were applied with a knife edge jig at a crosshead speed of 0.5 mm/min in dental-composite interface until failure. Data (newton) were divided to interface area (□□12.56), and the SBS values were recorded in MPa. The SBS values were recorded in MPa. Difference in the SBS values of 5 groups was analyzed by SPSS 18 using one-way ANOVA, and p<0.05 was considered statistically significant.

## Results

The mean SBS of Filtek Z250 in conjunction with Single Bond Universal to primary enamel in the control group (no treatment) and in four study groups treated with different concentration and molecular weights of chitosan is demonstrated in table 1.

**Table1. Shear bond strengths of composite to primary enamel teeth treated with different concentrations and molecular weights of chitosan (Mpa)**

Groups (n=50)	Mean+SD
Control	20.98+6.31
LMW & concentration 2.5mg/ml	17.92+7.35
LMW & concentration 5mg/ml	16.02+6.03
HMW & concentration 2.5mg/ml	13.26+5.18
HMW & concentration 5mg/ml	17.67+8.95

LMW: low molecular weight .HMW: high molecular weight

Despite the small decrease in the SBS values in the study groups compared to the control, according to one-way ANOVA, the difference between groups was not significant (p=0.17). In other words, application of chitosan in different concentrations and molecular weights had insignificant effects on the SBS of composite to primary tooth enamel.

## Discussion

Chitosan is capable of penetrating into the tooth structure and prevents acid penetration. Moreover, due to its anti-demineralization properties, it may interfere with the mechanism of etching and prevent resin penetration, resulting in a decrease of the bond strength of resin restorations.<sup>[6]</sup> The aim of this study was to assess the effect of different concentrations of chitosan with different molecular weights on the SBS of composite to primary tooth enamel. Based on the results, the SBS of Filtek Z250 composite in conjunction with Single Bond Universal (7<sup>th</sup> generation) to untreated primary tooth enamel was slightly higher than that to enamels treated with HMW and LMW 2.5 and 5mg/ml concentrations of chitosan. However, the difference was not statistically significant.

No study has evaluated the effects of chitosan's application on composite's SBS to primary tooth enamel. However, few studies have reported conflicting findings on permanent teeth in this respect. Mohsen et al. in 2020 in their in vitro study on the effect of chitosan nanoparticles on microtensile bond strength of resin composite to permanent molar dentin have shown that the chitosan 0.2% has increased bond strength compared to chitosan 2.5% and control groups in one day-period and three months period but after 6 months, control and chitosan 0.2 % had higher bond strength than chitosan 2.5%. Therefore, the microtensile bond strength was influenced by different chitosan concentration.<sup>[9]</sup> Abuelenain in 2018 evaluated the effect of chitosan treated dentin surface on dentin SBS using self-etch adhesives. They indicated a significant higher SBS of resin composite on untreated dentin surface (control group).<sup>[10]</sup> As it stated previously, the lower bond strength to chitosan treated surface could be inferred to the residual traces of chitosan within tooth structure that could prevent the adhesive penetration. Mohamed Ata in 2019 evaluated the effects of chitosan pretreatment on demineralized dentin surface and suggested that it significantly improved SBS for both 24-h and 6-month storage in distilled water by using total etch adhesive system.<sup>[11]</sup> These results are in contrary to those of the present study, probably due to our results being achieved on enamel rather than dentin and a difference in bonding system. The crosslinking of collagen contributes to the tensile properties of the dentine matrix. Dentine's collagen matrix can be degraded in a number of ways such as demineralization during dental caries causing the fibers collagen to

become denuded and fragile. Exposed collagen fibers damaged by endogenous protease and extrinsic factors can lead to permanent damage.<sup>[12]</sup> The reason for this contrast can be that the collagen crosslinking ability of chitosan does not work on enamel structure; hence, the chitosan pretreatment of enamel has no effect on the SBS of self-etch adhesive

Elsaka et al. in 2012 assessed the effect of addition of chitosan to self-etch primer on the antimicrobial activity and push-out bond strength to radicular dentin and demonstrated that addition of chitosan had no unfavorable effect on bond strength to radicular dentin.<sup>[13]</sup> This finding is in accordance with our results. The same authors in another study evaluated the effects of calcium hydroxide combined with chitosan solutions on dentinal walls of the root canals infected with *Enterococcus faecalis* and reported that this new intracanal medicament had no adverse effect on the bond strength of Real Seal self-etch sealer to radicular dentin.<sup>[14]</sup> Thus, considering the multiple advantages of chitosan, it may be used for tooth surface treatment before the bonding procedure. Chitosan and its oligosaccharides have different functional properties and are a topic of interest for researchers due to their biological activities and possible applications in food, pharmaceutical, agricultural and environmental industries. The antimicrobial activity of chitosan and its derivatives is an important property of these materials and is directly related to their biological applications.<sup>[15-18]</sup>

In the current study, the SBS test was used since it was a simple, reproducible, conventional and highly efficient technique to test the adhesion of dental adhesives. This technique uses shear loads to replicate masticatory forces to restorations in order to simulate the clinical setting and oral cavity environment with relatively high accuracy.<sup>[19]</sup> The multifactorial environment of the oral cavity cannot be exactly simulated by the current in-vitro techniques because numerous variables such as saliva, patient behaviors and so on play a role in this environment. That is why almost all research protocols in this regard use the process of artificial aging to better simulate the clinical setting. Application of thermal cycles is among the most commonly used techniques to simulate the process of aging and thermal changes occurring in the oral environment. Some studies have demonstrated that the bond strength of dental adhesives decreases following thermo cycling.<sup>[20]</sup> In the present study, all specimens were subjected to 1000 thermal cycles between 5-55°C

with 60s of dwell time to simulate the clinical setting. Lower post-operative tooth hypersensitivity is an important clinical benefit of self-etch systems which is mainly attributed to their less invasiveness. Self-etch adhesives react with superficial dentin and close the dentinal tubules with smears. Self-etch adhesives may have one-step or two-step application based on whether the self-etch primer and adhesive resin are packed in separate bottles or are combined in a single bottle.<sup>[21]</sup>

The mechanism of action of strong self-etch adhesives shares some similarities with etch and rinse adhesives. It means that, when applied, they remove almost all the mineral content of HA crystals from the superficial collagen layers and chemical reactions between the minerals and HA, and also functional monomers are eliminated. This mechanism is another reason explaining the decreased bond strength of self-etch systems. In other words, when self-etch bonding is applied, the existing bond is exclusively micromechanical and the absence of chemical bonds definitely results in the reduction of bond strength.<sup>[22, 23]</sup> The crosshead speed in the ongoing study was 0.5 mm/min in the SBS testing, and the shear loads were applied to the specimens in the Zwick/Roell universal testing machine until failure.<sup>[24]</sup> Most relevant studies apply a crosshead speed of 0.5-1 mm/min for SBS testing which is not in accordance with the in-vivo conditions in the oral cavity. Because the speed of mastication is within the range of 81-100 mm/s or 4860-6000 mm/min with a frequency range of 1.03-1.2 Hz.<sup>[25,26]</sup> However, considering the fact that the speed of loading affects the fracture load and also in order to be able to compare the results of the current study with those of previous ones, the conditions of the present were set according to those of similar studies. It is advised that the research should be done on dentin of primary teeth with different generation of bonding. Limitation of this study was low number of similar studies for comparison.

## Conclusion

Chitosan's application in different concentrations/ molecular weights does not compromise the SBS of composite to primary enamel.

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**Conflict of interest:** There is no conflict of interest to declare.

## Authors' Contribution

The study designed by Maryam Ghasempour and Homayoon Alaghehmand. The study data were collected by Sepideh Sorourhomayoun. Soleiman Mahjoub cooperated us in laboratory process. Statistical analysis and interpretation of data were performed by Soraya Kafri. Manuscript preparation was done by Sepideh Sorourhomayoun. Study supervision was performed by Maryam Ghasempour

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