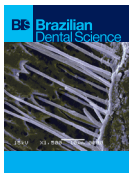
Brazilian Dental Science
Ciência Odontológica Brasileira
unesp
UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos



ORIGINAL ARTICLE

Doi: 10.14295/bds.2020.v23i2.1902

Effect of chitosan nanoparticles on microtensile bond strength of resin composite to dentin: an in vitro study

Efeito de nanopartículas de quitosana na resistência a união por microtração da resina composta à dentina: um estudo in vitro

Amr Mohsen MOHAMED[1], Sameh Mahmoud NABIH[1], Mohamed Ahmed WAKWAK[1]

1 - Faculty of Dental Medicine (Cairo-Boys) - Al Azhar University - Cairo - Egypt.

ABSTRACT

Objective: The purpose of this study was to evaluate the effect of chitosan nanoparticles on microtensile bond strength of resin composite to dentin using self etch adhesive after aging. **Material and Methods:** A total number of 90 freshly extracted, sound human molar teeth. Flat tooth surface was gained after cut of the occlusal surface. Three main groups according to pretreatment of dentin before adhesive application; 0.2 % chitosan, 2.5 % chitosan and no treatment control group. Universal self etch adhesive were applied according to manufacture instruction and 4 mm of Feltik Z250 xt composite. Storage of specimens for 1 day, 3 months and 6 months in 37O C distilled water. After that, the tooth was sectioned to beams of 1 mm x8 mm sticks for microtensile bond strength test using universal testing machine. Scanning electron microscope (SEM) was used to evalute the effect of chitosan nanoparticles on dentin and smear layer. Kruskal-Wallis test was used to compare between the three groups as well as the three aging periods. Dunn's test was used for pair-wise comparisons. The significance level was set at $P \leq 0.05$. **Results:** chitosan 0.2% is statistically significant increase in bond strength than chitosan 2.5% and control in one day group. Three months chitosan 0.2 % groups have statistically significant increase in bond strength than chitosan 2.5%. It was found in 6 months that control and chitosan 0.2 % have statistically significant increase in bond strength than chitosan 2.5%. There was statistically significant difference found between the three studied groups regarding bond strength at different storage times . **Conclusion:** Microtensile bond strength was influenced by different chitosan concentration. Different aging periods had no effect on the microtensile bond strength without application of chitosan and with application of 2.5% chitosan concentration.

KEYWORDS

Chitosan nanoparticles; Microtensile bond strength; MMPs.

RESUMO

Introdução: O objetivo deste estudo foi avaliar o efeito das nanopartículas de quitosana na resistência da microtração de união do compósito de resina à dentina usando adesivo autocondicionante após o envelhecimento. **Material e Métodos:** Foram utilizados um total de 90 dentes molares humanos extraídos e sadios. A superfície plana do dente foi obtida após o corte da superfície oclusal. Os dentes foram divididos em três grupos principais de acordo com o pré-tratamento da dentina e antes da aplicação do adesivo: 0,2% de quitosana, 2,5% de quitosana e nenhum tratamento foi utilizado no grupo controle. O adesivo autocondicionante universal foi aplicado de acordo com as instruções do fabricante e 4 mm de composito Feltik Z250 xt foi inserido. O armazenamento de amostras foi realizado por 1 dia, 3 meses e 6 meses em água destilada a 37 °C. Depois disso, o dente foi seccionado em peças de 1 mm x 8 mm para teste de resistência de união por microtração, utilizando máquina de teste universal. Microscópio eletrônico de varredura (MEV) foi usado para avaliar o efeito das nanopartículas de quitosana na dentina e na camada de smear layer. O teste de Kruskal-Wallis foi utilizado para comparar os três grupos e os três períodos de envelhecimento. O teste de Dunn foi usado para comparação pareada dos grupos. O nível de significância foi estabelecido em $P \leq 0,05$. **Resultados:** A quitosana 0,2% mostrou um aumento estatisticamente significativo na resistência da união quando comparado com a quitosana 2,5% e com o grupo no grupo de 1 dia. Aos três meses, o grupo de quitosana 0,2% apresentou aumento estatisticamente significativo na resistência de união do que a quitosana 2,5%. Verificou-se em 6 meses que o grupo controle e a quitosana 0,2% apresentam aumento estatisticamente significativo na resistência de união do que a quitosana 2,5%. Houve diferença estatisticamente significante entre os três grupos estudados em relação à resistência de união em diferentes tempos de armazenamento. **Conclusão:** A resistência a união por microtração foi influenciada por diferentes concentrações de quitosana. Diferentes períodos de envelhecimento não tiveram efeito sobre a resistência de união a microtração no grupo controle e no grupo de quitosana 2,5%.

PALAVRAS-CHAVE

Nanopartículas de quitosana; Ristência de união a microtração; MMPs.

INTRODUCTION

The longevity of resin composite restoration is directly related to the stability of the hybrid layer [1]. However, the methacrylate polymers of adhesive systems may undergo chemical hydrolysis and enzymatic degradation by the metalloproteinases [2]. Inhibiting the action of metalloproteinases and preventing degradation of the hybrid layer requires the use of cavity pretreatments, adhesive systems resistant to the action of esterases, or collagenolytic enzyme inhibitors [3].

Chitosan (CNPs) is a non-toxic cationic biopolymer usually obtained by alkaline deacetylation from chitin. The covalent immobilization of chitosan on dentinal collagen has been proposed to induce the remineralization of the exposed and demineralized dentin structure because its functional phosphate groups might bind to calcium ions to form a favorable surface for crystal nucleation, resulting in the formation of a calcium phosphate layer [4]. Chitosan treatment improves the resistance of the dentinal surface to degradation by collagenase [5].

Furthermore, chitosan presents with biocompatibility, chelating capacity and also antimicrobial effects against a broad range of gram-positive and gram-negative bacteria as well as fungi [6]. Previous *in vitro* studies have demonstrated the significant antibiofilm efficacy of chitosan nanoparticles (CNPs) [4]. The natural biopolymer chitosan has the capacity to form a microfibrillar and nanofibrillar network with superior mechanical properties. When this network is associated with bonding agents, it has the potential to show improved resistance to degradation of the mechanical properties of dentin [7].

Chitosan has been used to promote the biomimetic reconstruction of enamel and inhibit biofilm formation on titanium implant surfaces [8]. The incorporation of chitosan in experimental adhesive systems associated with methacrylate monomers has been suggested as a way to improve the biological and mechanical properties of collagen construction and enhance antibacterial activity by means of ionic interactions between chitosan and the bacterial cells [7]. Inhibition of MMPs action and hence collagen degradation has been also attempted to improve the longevity of the resin–dentin interface and the MMP inhibitor has been used as a separate dentin preconditioning step [9]. This study was directed to study the effect of chitosan nanoparticles concentration on microtensile bond strength of resin composite to human dentin using universal self-etch adhesive.

MATERIAL AND METHODS

The materials used in the present study are listed in table I.

Table I - Materials used in this study

Material	Specification	Composition	Manufacturer, website and Batch no.
0.2% chitosan nanoparticles solution	smear layer biomodifier	0.05 gram chitosan were dissolved in 25 ml distilled water contains 0.5ml acetic acid.	Naqa foundation for nanotechnology Cairo, Egypt www.nakaananone-twork.webs.com
2.5% chitosan nanoparticles solution Universal adhesive	smear layer biomodifier One step self--etch adhesive system	0.64 gram chitosan were dissolved in 23 ml distilled water contains 2% v/v acetic acid. MDP* phosphate monomer Dimethacrylate resins, HEMA**, Vitrebond Copolymer, filler, initiators, silane, ethanol, water	Naqa foundation for nanotechnology Cairo,Egypt 3M ESPE St. Paul, MN, USA 692513 http://www.3m.com
Filtek Z250 XT	Nano hybrid filled composite resin	Filler: zirconia/silica (82% by weight (68% by volume) Its matrix is composed BIS-GMA, UDMA, BIS-EMA, PEGDMA and TEGDMA Surface-modified zirconia/silica with a median particle size of approximately 3 microns or less.Non-agglomerated/ non-aggregated 20 nanometer surface-modified silica particles.	3M ESPE St. Paul, MN, USA 692513 http://www.3m.com

** MDP: methacryloyloxydecyl dihydrogen phosphate glycidyl methacrylate.*
*** HEMA: Hydroxy ethyl methacrylate.*

A total number of 90 freshly extracted, sound human molar teeth, free from caries, extracted for pathologic reasons were collected from the Dental Surgery Clinic in the Faculty of Dental Medicine, Al-Azhar University to be used in this study.

Each tooth was cleaned, polished, examined under light microscope to exclude the teeth with morphological defects or cracks. The selected teeth were stored in distilled water at 37°C until use. The distilled water was changed daily.

3 groups (chitosan 0.2%, chitosan 2.5%, and no treatment control). Each tooth was embedded vertically in the mold of acrylic resin to the level of cementoenamel junction of the tooth leaving the occlusal surface projecting above the surface of the mold.

For standardization in all teeth depth cut grooves of 2 mm created at the occlusal surface in the cusps of the molars with high speed handpiece with profuse water coolant. A graduated periodontal probe was used to confirm the depth. These grooves were united together to create a flat tooth surface (the bur was replaced after 3 preparations).

Preparation of chitosan nanoparticles Solutions according to *Sivakami* et al. [10].

For the 0.2 % chitosan solution; 0.05 gram chitosan were dissolved in 25 ml of distilled water which contains 0.5 ml acetic acid, then tripolyphosphate (TPP) solution contains 0.06 gram was dropped into the chitosan beaker at room temperature.

For the 2.5% chitosan solution; 0.64 gram chitosan were dissolved in 23 ml of distilled water which contains 2% v/v acetic acid, then tripolyphosphate (TPP) solution contains 0.1 gram was dropped into the chitosan beaker at room temperature.

After that chitosan solution was magnetically stirred for 45 minutes in order to obtain chitosan nanoparticles solution, these chitosan nanoparticles could be stably stored in refrigerator at 5°C.

Application of chitosan nanoparticles (0.2% and 2.5%) solution

The chitosan nanoparticles were taken from the container by a plastic syringe up to 1millileter (1 ml), for 0.2% chitosan the needle was left on the syringe while for 2.5% chitosan the needle was removed due to the high viscosity of the solution. The chitosan nanoparticles (CNPs) solution was applied on the dentin surface, and left for one minute, followed by rinsing with distilled water using plastic syringe for 15 seconds and dried using air spray at an average distance of 10 cm. Control specimens did not receive any chitosan solution.

Application of the adhesive system

The universal single bond adhesive system was applied in the self etch mode, according to the manufacturer's instructions, using disposable micro brush and rubbed for 20 seconds on the entire dentin surface. Subsequently air thinning was done for approximately 5 seconds until the adhesive layer no longer moves, indicating the complete vaporization of the solvent waiting for 10 seconds for complete infiltration of the adhesive within the dentin, then the adhesive was light cured for 40 seconds with high intensity light curing unit (Elipar LED , 3M USA) as was mentioned by the manufacture. The intensity of the light cure device is 1200 mW/ cm2 and regularly checked afterwards using radiometer. Application of the nanohybrid composite in the mold was done incrementally, where each increment was of 2 mm thickness and was applied using a gold plated composite applicators, then light cured using light curing device for 30 seconds according to manufacturer's instructions. Specimens were stored in distilled water according to each storage period (1 day , 3 months , 6 months) till microtensile bond strength testing.

Microtensile bond strength

Resin-Dentin sticks (1 mm x 1 mm) were formed per tooth using water cooled triple diamond saw blades mounted in a sectioning

machine (Isomet 4000, Buehler, Lake Bluff, IL, USA). Each stick was trimmed into a dumbbell-shaped test specimen producing a round cross-sectional area of 0.5 mm2 , a gauge length of 1 mm, and a radius of curvature or "neck" of 0.6 mm using a diamond bur8 mounted in the Specimen Former machine9 . The dumbbell-shaped specimens were stored in 0.5% Chloramine-T disinfectant solution (0.5% of chloramine-T trihydrate mixed with autoclaved water) for 24 hours.After 24 hours, they were rinsed 5 times using distilled water. Microtensile testing was performed at a crosshead speed of 1 mm/min using a calibrated Universal Testing Machine (Lloyd testing machine, Fareham, England).

Stereo- microscope analysis:

After micro-tensile bond testing all the fractured specimens, were examined using a Stereomicroscope at (25X) magnification. The stereomicroscope device was adjusted to allow for proper vision of the fractured area of the specimens to determine the type of failure mode which could be:

A. Cohesive failure = failure within dentin or resin composite material.

B. Adhesive failure = failure in which no resin composite was seen on dentin surface.

C. Mixed failure = failure in which part of resin composite was seen retained on dentin surface.

Scanning electron microscope examination

Representative specimens from each subgroup were assembled to be ready for scanning electron microscope. Each specimen surface was immersed in 6mm/l hydrochloric acid for 30 sec to remove the smear layer then rinsed with distilled water and immersed in 5, 25% sodium hypochlorite for 10 min to remove the free unencapsulated collagen. Specimens were further rinsed with distilled water and de hydrated through immersion in 99,9 % ethyl alcohol. Resin dentin interface of each disk sample were analyzed using scanning electron microscope at 1500 x magnification.

Statistical analysis

The mean and standard deviation values were calculated for each group. Data were collected, tabulated and Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data showed non-normal (non-parametric) distribution. Data were presented as median, range, mean and standard deviation values. Kruskal-Wallis test was used to compare between the three groups as well as the three aging periods. Dunn's test was used for pair-wise comparisons. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

RESULTS

Data were presented as median, range, mean and standard deviation values. Kruskal-Wallis test was used to compare between the three groups as well as the three aging periods. Dunn's test was used for pair-wise comparisons. The significance level was set at $P \leq 0.05$.

Effect of chitosan nanoparticles on microtensile bond strength in table II:

After 1 day aging period

There was a statistically significant difference between different groups (P- value <0.001, Effect size = 0.689). Pair-wise comparisons revealed that Chitosan 0.2% group showed the statistically significantly highest mean micro-tensile bond strength value (38.62 Mpa). On the other hand, chitosan 2.5 % group showed the statistically significant lowest mean microtensile bond strength mean value (14.64 Mpa). However, there was no statistically significant difference between control and Chitosan 2.5% groups.

After 3 months aging period

There was a statistically significant difference between different groups (P- value = 0.009, Effect size = 0.272). Pair-wise comparisons revealed that; there was no statistically significant difference between control and Chitosan 0.2%. Chitosan 0.2% showed the highest microtensile bond strength mean value (25.08 Mpa). On the other hand, Chitosan 2.5% group showed the statistically significantly lowest mean value micro-tensile bond strength (17.79 Mpa).

After 6 months aging period

There was no statistically significant difference between different groups (P-value = 0.287, Effect size = 0.018). Control group showed the highest microtensile bond strength mean value (28.15Mpa). On the other, chitosan 2.5% group showed the lowest microtensile bond strength mean value (20.94Mpa).

Table II - Materials used in this study

Aging	Control	Chitosan 0.2%	Chitosan 2.5%	*P*-value	Effect size (Eta squared)
Median (Range)	20.82 (13.58-29.96)^{B}	39.16 (33.07-47.4)^{A}	15.63 (3.56-24.7)^{B}	<0.001*	0.689
Mean (SD)	21.43 (6.42)	38.62 (5.13)	14.64 (7.32)		
Median (Range)	21.1 (17.58-24.43)^{A}	23.95 (18.24-31.75)^{A}	16.89 (11.24-28.43)^{B}	0.009*	.0272
Mean (SD)	21.03 (2.15)	25.08 (5.45)	17.79 (5.05)		
Median (Range)	28.76 (10.42-40.44)	25.1 (7.93-41.23)	21.36 (7.18-34.11)	0.287	0.018
Mean (SD)	28.15 (9.76)	25.73 (11.11)	20.94 (9.33)		

*: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different

Effect of different aging periods on microtensile bond strength in (Figure 1)

Control group (no chitosan application)

There was no statistically significant difference between the different aging periods in control group (P-value = 0.107, Effect size = 0.091). The highest mean microtensile bond strength values was recorded in 6 months (28.76 Mpa), while the lowest mean microtensile bond strength value was recorded in 3 months (21.03 Mpa). Pair wise comparison showed there was no significant difference between day 1 group and 3 month group. However there was significant difference between day1 and 3 months groups and that of 6 months group.

Chitosan 0.2% group:

There was a statistically significant difference between the different aging periods in Chitosan 0.2% group (P-value = 0.001, Effect size = 0.453). Pair-wise comparisons between the groups revealed that micro-tensile bond strength after 1 day showed the statistically significantly highest mean micro-tensile bond strength value (38.16 Mpa). There was a significant difference between day 1 period group and both the 3 months and 6 months groups .On the other hand, there was no statistically significant difference between micro-tensile bond strength after 3 and 6 months.

Chitosan 2.5% group

There was no statistically significant difference between the aging periods in chitosan 2.5% group (*P*-value = 0.242, Effect size = 0.031). Six months aging period showed the highest microtensile bond strength mean value (20.94Mpa), On the other hand day 1 showed the lowest microtensile bond strength mean value (14.64Mpa). Pair wise comparison revealed that there was no significant difference between day 1 and 3 months group. However, there was significant difference between day 1 and 3 months aging period groups and that of 6 months aging period group.

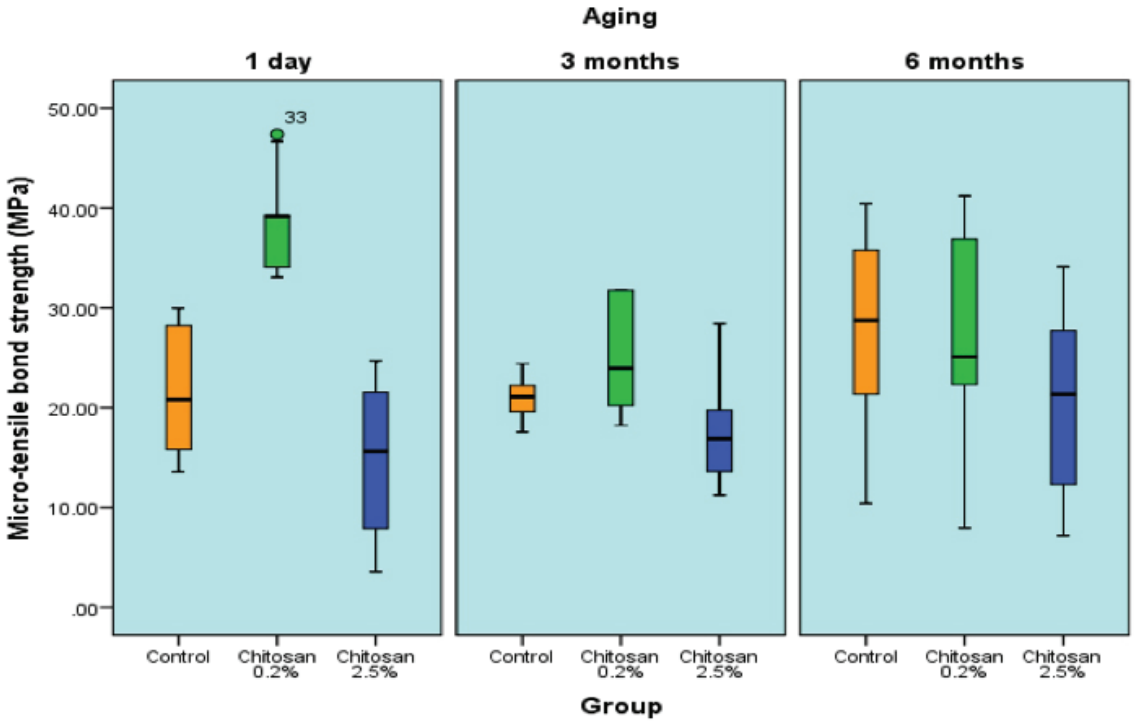


Figure 1 - Box plot representing median and range values for micro-tensile bond strength of the three groups.

Scanning electron microscope

The scanning electron microscope was done to reveal the amount of impregnation of the resin tags within the dentin and the presence of chitosan nanoparticles within the dentin surface. (Figure 2 and 3) Showing specimen treated with chitosan nanoparticles 0.2% and 2.5% at day 1, 3 m and 6m period illustrate long resin tags and chitosan particles entrapped within dentin surface.

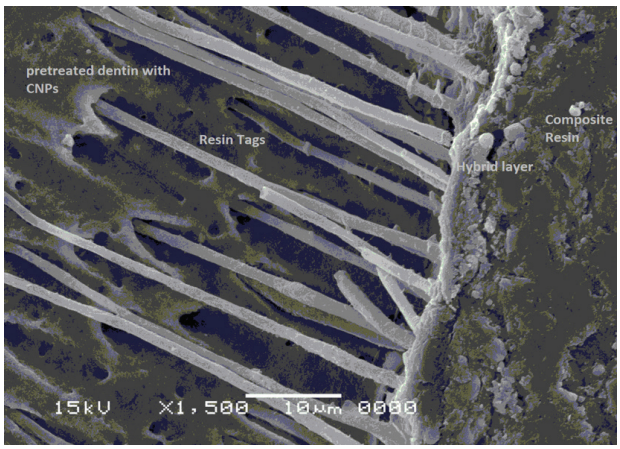


Figure 2 - Scanning electron microscope of specimen treated with chitosan nanoparticles 0.2% at day 1 period showing long resin tags chitosan particles entrapped within dentin surface.

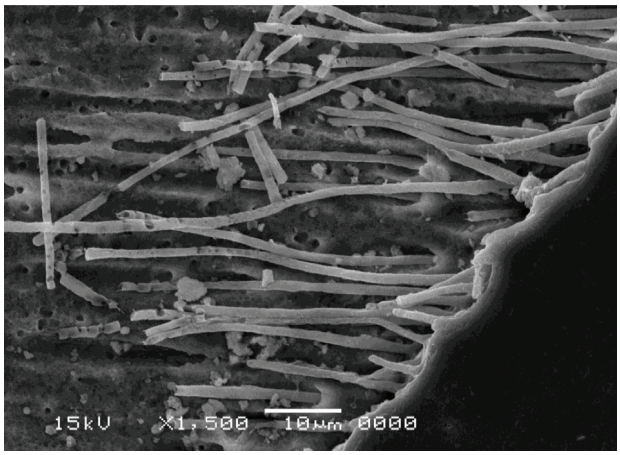


Figure 3 - Scanning electron microscope of specimen treated with chitosan nanoparticles 0.2% at 3 months aging period showing long resin tags chitosan particles entrapped within dentin surface.

Figure 4 - Scanning electron microscope of specimen treated with chitosan nanoparticles 0.2% at 6 months aging period showing medium sized resin tags chitosan particles entrapped within dentin surface.

DISCUSSION

Chitosan is a natural polysaccharide, which has gained popularity in the field of dentistry because of its properties (biocompatibility, bioadhesion, and no toxicity) [4]. Chitosan nanoparticles (CNPS) was used as a biomodification material for smear layer before the usage of dental adhesive, so, alter the smear layer that increases the durability of bond strength [11]. Chitosan is a natural polysaccharide, which has gained popularity in the field of dentistry because of its properties (biodegradability, biocompatibility, bio-adhesion, and no toxicity) Due to its acidic pH; it shows significant chelating capacity for different metal ions, which validates its use in various industries. Due to these properties, chitosan was used in various dental treatments such as in cases of direct pulp capping, in the treatment of dentinal tubule infection, and in tissue regeneration in pulp wounds [12]. The use of chitosan in different concentrations was able to remove or modify the smear layer. Chitosan is known to remove the inorganic content of the smear layer on the dentin surface. Despite the working mechanism of chitosan is not fully understood, it is assumed that adsorption, ionic exchange and chelation are responsible for the formation of reaction between the substrate and the metallic ions. This type of interaction depends highly on the ions involved, the chemical structure of chitosan, and the pH of the solution. The chitosan polymer is formed of a chain of several dimers of chitin [4].

In our study, the results showed that the highest value of bond strength was recorded at chitosan 0.2 % and the lowest value was recorded by chitosan 2.5 %. These results show that when the concentration of chitosan increases the bond strength value decrease while the bond strength increased by time after six months.

This was attributed to the fact that Chitosan, along with its biocompatibility, has the ability to form fibrillar arrangements within the protein matrix with improvement in mechanical properties and degradation resistance The significant improvement in μTBS could be partially attributed to the inforcement effect of chitosan on universal self etch adhesive bonding to dentin. Chitosan might play a role in opening interfibrillar spaces and significantly affect resin infiltration and hybrid layer formation. It is well-known that for successful bonding to dentin, open interfibrillar spaces and proper resin infiltration of the demineralized Intertubular dentin collagen fibrils network to form a hybrid layer are required. While, the decrease μTBS found with specimens of 2.5% CNP concentration could be explained by the obliteration of the interfibrillar spaces due to aggregation of the chitosan inside demineralized dentin collagen network. The obliteration of the interfibrillar spaces with rough textured hybrid layer , poor resin infiltration [13].

Moreover this result may be also attributed to the presence of a chemical link by means of covalent bonds between the universal adhesive and chitosan and resin, and a physical bond to the organic part of dentin through electrostatic interactions, thus increasing the stability of the link and the hybrid layer and increase the μTBS value for

0.2 % CNP concentration [14].

These results are in agreement with Elsaka S and Elnaghy A (2012) chitosan application has been proposed and presented a less hybrid layer degradation and a stability of resin dentin adhesion long term indicating that the MMP inhibition can preserve the interface integrity [6] .

Moreover, the ultramorphology of the demineralized dentin substrate was changed after pretreatment of dentin with chitosan according to Paulo C et al (2017). Under scanning electron microscope, the increase in the diameter of the dentinal tubules, resembling a funnel, the microporosity, presence of secondary tubules, anastomosis, due to the loss of peritubular and reduce of intertubular dentin [15].

The result of this study was in Disagreement with Shrestha A [16] who stated that Photomicrographs of dentin beams showed that chitosan was deposited on the surface of demineralized dentin after 7 days in water instead of being incorporated into the dentin matrix. It could explain why chitosan was not able to prevent dentin dry mass loss and collagenolytic activity. Another important fact to be considered is that during the analysis of collagen degradation, the demineralized beams were phosphoric acid-etched and, then, chitosan was applied. It has been reported that the endogenous enzymes present in dentin can be previously inactivated by phosphoric acid, and, subsequently, be reactivated by etch-and-rinse adhesives [17]. The adhesive system was not applied during this analysis since dentin beams were not restored, unlike beams submitted to the microtensile bond strength test. This could be one of the reasons why chitosan did not influence the hidroxiproline release, but preserve the composite resin bond strength to dentin after long-term storage [18].

Concerning chitosan nanoparticles 0.2 % at 3 months aging period, there was an increase in the microtensile bond strength value compared to that of control at the same aging period and at the same time there was no statistically difference between 3 months and 6 months aging period. Moreover there was an increase in the mean microtensile bond values concerning 2.5 % chitosan at 3 months and 6 months aging periods.

This agrees with Gu L et al. [19] who concluded that chitosan- based extrafibrillar demineralization reduces endogenous MMP-initiated collagen degradation, prevents water permeation within hybrid layers. Resin-dentin interface should be optimally sealed to prevent water movement. When water channels are created in resin infiltrated dentin during bonding, intrapulpal pressure replenishes intrinsic water from the pulp chamber [20]. Reduction in water permeability in chitosan conditioned dentin, attributed to the retention of the smear plugs, also fosters long-term bond stability.

Matrix metalloproteinases (MMPs) present in dentin can be activated at an acidic pH and initiate the degradation of collagen fibrils. The highly alkaline pH of the phosphorylated chitosan solution may have inhibited the degradation by MMPs in the organic matrix of dentin [21].

Moreover this was in agreement with Ururahy M et al. [22] who stated that the use of chitosan on dentin has shown favorable results in regarding to enabling the formation of a calcium phosphate layer on the demineralized dentin. The use of chitosan promoted dentin structure to be more resist to hydrolytic degradation challenges, as well, collagen-degrading enzymes, which can favor an increase in the durability of adhesive restorations.

The results also are in agreement with Elsaka S and Elnaghy A [6] who stated that chitosan application proposed and presented a less hybrid layer degradation and a long term stability of resin dentin adhesion indicating that the MMP inhibition can preserve the interface integrity. Also, this result was in agreement with Balaji H et al. [13] who

revealed that the modification of the bonding substrate with chitosan and riboflavin increases the mechanical properties, enhanced the mechanical stability of demineralized dentin substrates against hydrolytic and collagenolytic degradation of the MMP. It is difficult to entirely correlate laboratory findings with the clinical behavior is the limitation of this study. In natural teeth, pulp pressure and inter-tubular fluid have great influence on moisture level thus affecting tooth/restoration interface. Therefor, Further in vivo studies should be done to evaluate the durability of the bond strength between dentin and resin composite more than six months.

Conflict of interest: no conflict of interest

CONCLUSION

Under the circumstances of this study, the following conclusions were suggested:

1-Microtensile bond strength was influenced by different chitosan concentration.

2-Different aging periods had no effect on the microtensile bond strength without application of chitosan and with application of 2.5% chitosan concentration.

3-Application of chitosan nanoparticles affect the microtensile bond strength of resin composite to dentin positively.

REFERENCES

1. Spencer P, Jonggu Park QY, Misra A, Bohaty BS, Singh V, Parthasarathy R, et al. Durable bonds at the adhesive/dentin interface: an impossible mission or simply a moving target? Braz Dent Sci. 2012 Jan;15(1):4-18.
2. Matos AB, Trevelin LT, Silva BTFD, Francisconi-Dos-Rios LF, Siriani LK, Cardoso MV. Bonding efficiency and durability: current possibilities. Braz Oral Res. 2017 Aug 28;31(suppl 1):e57. doi: 10.1590/1807-3107BOR-2017.vol31.0057.
3. Cheung RC, Ng TB, Wong JH, Chan WY. Chitosan: an update on potential biomedical and pharmaceutical applications. Mar Drugs. 2015 Aug 14;13(8):5156-86. doi: 10.3390/md13085156.
4. Del Carpio-Perochena A, Bramante CM, Duarte MA, de Moura MR, Aouada FA, Kishen A. Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restor Dent Endod. 2015 Aug;40(3):195-201. doi: 10.5395/rde.2015.40.3.195.
5. Kishen A, Shrestha S, Shrestha A, Cheng C, Goh C. Characterizing the collagen stabilizing effect of crosslinked chitosan nanoparticles against collagenase degradation. Dent Mater. 2016 Aug;32(8):968-77. Doi: 10.1016/j.dental.2016.05.005.
6. Elsaka S, Elnaghy A. Effect of addition of chitosan to self-etching primer: antibacterial activity and push-out bond strength to radicular dentin. J Biomed Res. 2012 Jul;26(4):288-94. doi: 10.7555/JBR.26.20120042.
7. Lobato MF, Turssi CP, Amaral FL, França FM, Basting RT. Chitosan incorporated in a total-etch adhesive system: antimicrobial activity against Streptococcus mutans and Lactobacillus casei. Gen Dent. 2017 Jan-Feb;65(1):62-66.
8. Daood U, Iqbal K, Nitisusanta LI, Fawzy AS. Effect of chitosan/riboflavin modification on resin/dentin interface: spectroscopic and microscopic investigations. J Biomed Mater Res A. 2013 Jul;101(7):1846-56. Doi: 10.1002/jbm.a.34482.
9. Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, Cenci MS. MMP Inhibitors on dentin stability: a systematic review and meta-analysis. J Dent Res. 2014 Aug;93(8):733-43. doi: 10.1177/0022034514538046.
10. Sivakami MS, Gomathi T, Venkatesan J, Jeong HS, Kim SK, Sudha PN. Preparation and characterization of nano chitosan for treatment wastewaters. Int J Biol Macromol. 2013 Jun;57:204-12. doi: 10.1016/j.ijbiomac.2013.03.005
11. Nunes RA de C, Amaral FLB do, França FMG, Turssi CP, Basting RT. Chitosan in different concentrations added to a two-step etch-and-rinse adhesive system: influence on bond strength to dentin. Brazilian Dent Sci. 2017;20(4):55-62. doi: 10.14295/bds.2017.v20i4.1461
12. Mittal A, Dadu S, Yendrembam B, Abraham A, Singh NS GP. Comparison of new irrigating solutions on smear layer removal and calcium ions chelation from the root canal: an in vitro study. Endodontology. 2018;30(1):55–61.
13. Balaji H. MMP inhibitors-A review. Int J Pharm Sci Heal Care Issue. 2017;2(7):45–56.
14. Münchow EA, Bottino MC. Recent advances in adhesive bonding: the role of biomolecules, nanocompounds, and bonding strategies in enhancing resin bonding to dental substrates. Curr Oral Health Rep. 2017 Sep;4(3):215-227. doi: 10.1007/s40496-017-0146-y.
15. da Cruz-Filho AM, Bordin ARV, Souza-Flamini LE, Guedes DFDC, Saquy PC, Silva RG, et al. Analysis of the shelf life of chitosan stored in different types of packaging, using colorimetry and dentin microhardness. Restor Dent Endod. 2017 May;42(2):87-94. doi: 10.5395/rde.2017.42.2.87
16. Shrestha A, Friedman S, Kishen A. Photodynamically crosslinked and chitosan-incorporated dentin collagen. J Dent Res. 2011 Nov;90(11):1346-51. doi: 10.1177/0022034511421928.
17. Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res. 2011 Aug;90(8):953-68. doi: 10.1177/0022034510391799.
18. Gajjela RS, Satish RK, Sajjan GS, Varma KM, Rambabu T, Vijaya Lakshmi BH. Comparative evaluation of chlorhexidine, grape seed extract, riboflavin/chitosan modification on microtensile bond strength of composite resin to dentin after polymerase chain reaction thermocycling: An in vitro study. J Conserv Dent. 2017 Mar-Apr;20(2):120-124. doi: 10.4103/0972-0707.212241.
19. Gu LS, Cai X, Guo JM, Pashley DH, Breschi L, Xu HHK, et al. Chitosan-based extrafibrillar demineralization for dentin bonding. J Dent Res. 2019 Feb;98(2):186-193. doi: 10.1177/0022034518805419.
20. Cuevas-Suárez CE, da Rosa WLO, Lund RG, da Silva AF, Piva E. Bonding performance of universal adhesives: an updated systematic review and meta-analysis. J Adhes Dent. 2019;21(1):7-26. doi: 10.3290/j.jad.a41975.

21. Buzalaf MA, Kato MT, Hannas AR. The role of matrix metalloproteinases in dental erosion. Adv Dent Res. 2012 Sep;24(2):72-6. doi: 10.1177/0022034512455029.

22. Ururahy MS, Curylofo-Zotti FA, Galo R, Nogueira LF, Ramos AP, Corona SA. Wettability and surface morphology of eroded dentin treated with chitosan. Arch Oral Biol. 2017 Mar;75:68-73. doi: 10.1016/j.archoralbio.2016.11.017

Mohamed Ahmed Wakwak
(Corresponding address)
2 Makram Ebid, Nasr City, Cairo, Egypt. Postal code: 11765
E-mail: drwakwak2006@azhar.edu.eg

Date submitted: 2019 Oct 13

Accept submission: 2019 Nov 26