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Evaluation of mechanical properties and bond strength to chitosan cross-linked dentin (in vitro study)

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Abstract

The usage of chelating irrigants affects the dentin microhardness and the sealer bond strength with dentin after the RCT. To evaluate the mechanical properties and sealer bond strength of chitosan cross-linked dentin after root canal treatment compared to conventional root canal-treated dentin. This study used 36 single-rooted permanent incisors. All teeth received endodontic treatment initiated by access of the pulp chamber with a round bur, then deroofed to attain straight-line access to all canals. This is followed by cleaning and shaping using the ProTaper system. After this step, the microhardness samples are classified into two subgroups. Group 1A was irrigated with sodium hypochlorite 5.25% only. Group 1B root canal irrigated with sodium hypochlorite 5.25% followed by dentin crosslinking using chitosan nanoparticles, specimens sectioned parallel to the long access of the root using a micro saw machine and copious irrigation. The Vickers test was done on the specimens to evaluate the microhardness from the indentations produced on the root canal surface. While in the push-out test, classified into two subgroups, Group 2A was irrigated with hypochlorite 5.25%, whereas Group 2B received additional dentin crosslinking using chitosan nanoparticles, then obturation was performed in both groups using gutta-percha and resin-based sealer with Cold lateral compaction technique. After this, specimens were sectioned perpendicular to the long access of the tooth. The push-out test was performed by applying load to the root side of the specimen till failure of the bond strength of the sealer. Group 1B (NaOCl 5.25% + 0.2% Chitosan nanoparticles) showed the lowest hardness values compared to group 1A (NaOCl 5.25%) at p=0.02, Group 2A and 2B in Push-out bond strength samples showed an insignificant effect on mean push out at p=0.960. 0.2% chitosan nanoparticles on dentin didn't improve the microhardness but caused a minimal or non-significant microhardness reduction compared to other irrigants with chelation properties. Also, 0.2% chitosan nanoparticles cross-linking didn't improve the bond strength compared to 5.25% NaOCl.

Keywords: Chitosan, dentin, nanoparticles, root canal, tooth

Full length article **Corresponding Author*, e-mail: amiradel@live.com

1. Introduction

Conventional root canal treatment is proven to be successful in pain relief and disinfection of the canal system. Root canal treatment (RCT) using sodium hypochlorite is considered the gold standard in disinfection, still it is deemed to have multiple drawbacks including dentin weakening, irritating the surrounding tissues, being highly toxic and unable to dissolve the smear layer and reduce the modulus of elasticity and flexural strength of dentin but it remains irreplaceable [1]. In the last decades the nanotechnology has been rising in the dental field. To improve mechanical properties and sealer bond strength, Nanotechnology was suggested as part of RCT to enhance quality of the RCT and preserve the intra-fibrillar dentin and collagen. Chitosan nanoparticles are a semi-synthetic molecule derived from amino-polysaccharide and produced by the partial acetylation of chitin. It's a biodegradable, biocompatible, bio-adhesive, strong antibacterial and non-toxic. It's a new alternative for the irrigants in the RCT as it disinfects the canal system such the sodium hypochlorite and chelate the radicular dentin [2]. The various irrigants in root canal treatment affects the radicular dentin, thus compromises the sealing ability and mechanical properties. smear layer removal greatly enhances the irrigant flow, sealer infiltration and bond strength. chitosan nanoparticles have the same chelation effect as EDTA when used as final rinse. Also, the increase in the chitosan application time improves the apical seal and bond strength [4,5]. In contrary to EDTA which causes dentin erosion upon the prolonged application time [3,5]. Thus, improve the bond strength of the resin sealer and penetration of into the deep dentinal tubules [6]. The hydrophilicity of chitosan nanoparticles allows its adsorption and infiltration. Chitosan nanoparticles are cationic in nature which allows the ionic bond with the dentinal calcium ions. On the other hand, the epoxy resin sealer is a hydrophobic sealer that allow increasing of the wettability of sealer material on the dentinal walls which is open freshly cut and irregular after the mechanical instrumentation and irrigation [7]. The multiple irrigants are known to affect the mechanical properties of the treated dentin as they weaken the root structure, which make it more prone to fracture. In addition to the increase of its permeability and adversely affects the sealing and adhesive ability [8]. The evaluation of mechanical properties can be proven by the evidence of mineral loss or gain indirectly through the irrigant action affecting the microhardness like the action of prolonged action of sodium hypochlorite [9, 10,11]. Vickers test is a test used for assessment of the mechanical properties as it's a pyramidal diamond intender with angle 136° that form a square intender on a microhardness testing machine producing indentations on the dentin away from the pulp space and re-evaluated on an analogue image of the specimen of the pyramidal indentation as an impression on the root canal surface [12,13]. The evaluation of the bond strength between the chitosan nanoparticles and cross-linked dentin can be addressed through the push out test [14,15]. The push out test is performed using universal testing machine with 0.9 mm diameter with cold stainless-steel plunger facing the obturating material with crosshead speed with compressive load of 0.5 mm/min until bond failure occur.

2. Materials and methods

2.1. Samples classification grouping

Thirty-Six Teeth were selected for this study. The Sample classification was divided into two groups, Group 1 of 18 tooth to evaluate microhardness while group 2 of 18 tooth to evaluate sealer bond strength. The microhardness group divided into 2 groups 9 tooth each, Group 1A where tooth was subjected to root canal treatment with sodium hypochlorite irrigation while group 1B was subjected to root canal treatment irrigated with sodium hypochlorite following by chitosan crosslinking. The push out group contain 2 groups, group 2A was subjected to root canal treatment with sodium hypochlorite irrigation then obturated while group 2B was subjected to root canal treatment irrigated with sodium hypochlorite following by chitosan crosslinking then obturated.

2.2. Sample preparation and evaluation

36 single root incisors teeth were subjected to endodontic treatment starting with access preparation of the pulp chamber with a round bur, deroofing to achieve straight line access, Canals were then negotiated using K-File 15 to achieve full working length that was calculated and verified using Radiographs. Cleaning and shaping was done using protaper next sequentially in a crown down manner using E-connect pro endomotor with torque on 200gcm and speed of 350rpm according to the manufacturer guidelines. Manual Apical Preparation was done using K-file 40 where irrigation with 2ml sodium hypochlorite 2.5% concentration was used between each file using a 30- gauge needle in a plastic syringe for 30 seconds.

2.3. Chitosan nanoparticle crosslinking

After irrigation with sodium hypochlorite a final rinse with nanoparticle chitosan 0.2% concentration to crosslink the dentin for 3 minutes for group 1 and 2.

2.4. Tooth sectioning for evaluation of microhardness

Group 1A (n=9) and 1B (n=9) were cross sectioned longitudinally labio-lingually using IsoMed 4000 microsaw Buehler, USA. A mounting diamond disk 0.6mm thickness at speed of 2500rpm and a feeding rate of 10mm/min under water cooling.

2.5. Evaluation of microhardness using Vickers Test

The evaluation of the dentin microhardness is done using Vickers test (Tukon 1102 Wilson microhardness tester, Buehler, Germany). The load is applied smoothly (from 50-100gram force) without impact, forcing the indenter into the test specimen. The indenter is held in place. After the load is removed, the indentation made on the dentin is done 30-50micron away the canal lumen for each specimen 3 indentations were made where the distance between each indentation is 0.5mm. The indentation focused with the magnifying eye piece and the two impression diagonals are measured, usually to the nearest 0.1-μm with a micrometer minimum three indentations and averaged. The Vickers hardness (HV) is calculated using:

$$HV = 1854.4L/d2$$

Where the load L is in gf (gram force) and the average diagonal d is in μm (this produces hardness number units of gf/μm2. in practice the numbers are reported without indication of the units).

2.6. Tooth sectioning for evaluation of push out

Teeth were obturated using cold lateral compaction technique. Group 2A (n=9) and 2B (n=9) were cross sectioned perpendicularly mesio-distally in 2mm slice using IsoMed 4000 microsaw Buehler, USA. A mounting diamond disk 0.6mm thickness at speed of 2500rpm and a feeding rate of 10mm/min under water cooling.

2.7. Evaluation method for the sealer bond strength push out test with dentin after crosslinking

The evaluation of the sealer bond strength is done using push out testing machine. The filling material was then loaded with a 0.9 mm diameter stainless steel plunger selected. The plunger was mounted on the upper part of a universal testing machine (Instron universal testing machine model 3345 England data recorded using computer software Blue hill 3 (version 3.3). The samples were aligned over a support jig in an apical to coronal direction to avoid any constriction interference. The tests were conducted at a cross head speed of 0.5 min-1 using a 500N load cell. The highest value recorded was taken as the push-out bond strength. The area under load was calculated by:

Area = circumference of restoration × thickness.

The push-out value in MPa (Mega Pascal) was calculated from force (N) divided by area in mm2.

3. Results

3.1. Evaluation of microhardness of sodium hypochlorite group and 0.2% chitosan nanoparticles group

3.1.1. Two-Way ANOVA

Different groups showed a significant effect on mean hardness, NaOCl 5.25% + 0.2% (1B)Chitosan showed the lowest hardness vales compared to NaOCl 5.25% at p=0.02. Different root sections showed insignificant effect on mean hardness on mean hardness (p=0.172). The interaction between both groups and root section was insignificant at p=0.735 (Table 1).

3.2. Evaluation of bond strength of sodium hypochlorite group and 0.2% chitosan nanoparticles group

3.2.1. Two-Way ANOVA

Different groups showed insignificant effect on mean push out at p=0.960. Different root sections showed insignificant effect on mean push out on mean push out (p=0.664). The interaction between both groups and root section was insignificant at p=0.190 (Table 2).

4. Discussion

The success of root canal treatment hinges on various factors, such as operator skill, material quality, and achieving optimal disinfection. Preserving tooth functionality and structural integrity post-treatment is vital. Root canal treatment may render dentin more susceptible to fracture [16]. Ensuring coronal and apical sealing is mandatory, influenced by factors like coronal restoration quality, isolation, and bonding. Achieving apical sealing depends on thorough canal disinfection, precise gutta-percha cone fitting, sealer type, and dentin pretreatment to remove the smear layer blocking dentinal tubules [17]. The presence of the smear layer may cause endodontic failure on the long term due to the leakage through the canal. Preserving mechanical properties, especially microhardness, is essential post-treatment. EDTA are commonly used to remove the smear layer, The combination of Sodium hypochlorite and EDTA irrigation can negatively impact mechanical properties and microhardness, making the tooth more prone to fracture. Chitosan nanoparticles offer potential but lack comprehensive documentation of their chelating mechanism on dentin. Two theories describe this mechanism: the bridge model and the pendant model. These nanoparticles, derived from amino-polysaccharide through partial chitin acetylation, possess advantages like biodegradability, biocompatibility, antibacterial properties, and non-toxicity [18]. Chitosan nanoparticles serve as alternatives for root canal irrigants, disinfecting the canal system and chelating radicular dentin. They efficiently remove the smear layer, affecting sealer bonding, preserving collagen fibers [19]. Crosslinking methods (chemical, physical, or photo) induce intra- and intermolecular crosslinks in collagen, stabilizing biological tissues and constructs. Chemical cross-linking with chitosan nanoparticles is an efficient, low-cytotoxicity method for collagen-based biomaterials. A Study conducted by Shresta found that chemical crosslinking and photo dynamic crosslinking had similar effects on dentin collagen toughness and mechanical stability, as evaluated by Transmission Electron Microscopy [20]. The time required of treatment for chemical crosslinking for collagen is much longer than the photo dynamic method for the mechanical stabilization of the collagen. The short treatment time is desirable but the preparation for photo dynamic crosslinking through using the photo-oxidizable amino acid and the crosslinking procedure using light activated photosensitizers which limits its clinical use in routine dental work. Nanotechnology enables the creation of advanced biomaterials with unique biological, chemical, and physical properties. Nano-irrigants effectively eliminate germs, treat dentin before obturation, and enhance root canal workability and fluidity. Recent modifications to root canal irrigants aim to improve physicochemical properties, enhance bioactivity, and boost antibacterial effectiveness. Nanomaterials, as defined by the EPA and USFDA, exhibit unique properties in nanoscale dimensions and display altered mechanical and chemical reactivity compared to their larger counterparts. [21]. Vickers testing is widely used for microhardness evaluation across various materials, covering the full hardness range. In comparison to the Knopp microhardness test, the Knoop diamond indenter penetrates specimens only half as deep as the Vickers diamond indenter, making it suitable for testing thin layers, like aluminum foil. In our study, we assessed microhardness at three points (coronal, middle, and apical) in radicular dentin due to differences in anatomy, diameter, and direction of dentinal tubules in each third. We conducted tests on longitudinally sectioned samples to measure microhardness in the superficial dentin layer from the canal lumen. Conversely, horizontal sectioning evaluated microhardness from the superficial dentin layer toward the canal lumen to the cementum [22]. The comparison of microhardness between 5.25% NaOCl and 0.2% chitosan nanoparticles after dentin crosslinking for 3 minutes using the Vickers test with a two-way ANOVA showed no significant difference between the two groups (0.2% chitosan nanoparticles and 5.25% NaOCl). Similarly, no variation in microhardness was observed among the root sections (coronal, middle, and apical). Regarding the concentration of chitosan nanoparticles for dentin treatment and its impact on microhardness, the results are debatable. Our study aligned with the use of 0.5% chitosan nanoparticles, which exhibited greater microhardness resistance. In contrast, a study by Ratih found that 0.5% chitosan nanoparticles used for 1 minute and 3 minutes showed no significant difference in microhardness. In our study, 0.2% chitosan nanoparticles might not have produced significant effects on dentin's mechanical properties, possibly due to the lower concentration used, as higher concentrations led to greater dentin erosion [23, 24]. A different study observed that 0.2% chitosan led to improved dentin microhardness compared to 17% EDTA and 2.5% sodium hypochlorite. In this case, EDTA resulted in the lowest microhardness, attributed to its removal of not only inorganic materials in the smear layer but also the dissolution of the calcium hydroxyapatite matrix.

Table 1: Two-way ANOVA for the effect of different groups and tooth sections on the mean hardness.

Hardness	Type III Sum of Squares	f	Mean Square	F	Sig.
Groups	265.420		265.420	6.229	0.02*
Section	161.646		80.823	1.897	0.172
Groups * Section	26.622		13.311	0.312	0.735
Error	1022.585	4	42.608		
Total	83684.968	0			
Corrected Total	1476.273	9			

NS= Non-significant, *= significant

Table 2: Two-Way ANOVA for the effect of different groups and tooth section on the mean push out.

Pushout	Type III Sum of Squares	DF	Mean Square	F	Sig.
Groups	0.015	1	0.015	0.003	0.960
Section	4.978	2	2.489	0.417	0.664
Groups * Section	21.254	2	10.627	1.780	0.190
Error	143.249	24	5.969		
Total	1076.305	30			
Corrected Total	169.496	29			

NS= Non-significant, *= significant

On the other hand, 0.2% chitosan nanoparticles minimally affected dentin's structure, effectively removing the smear layer without inducing demineralization. Additionally, 0.2% chitosan promoted remineralization of demineralized dentin, possibly due to the presence of phosphate groups that attract calcium ions, facilitating crystal nucleation and forming a calcium phosphate layer. This remineralization ability may explain the higher microhardness and lower surface roughness observed with 0.2% chitosan [25]. Conversely, a study by Pimenta evaluated dentin microhardness after chitosan crosslinking using the Knoop hardness test. Comparing 0.2% chitosan nanoparticles, 17% EDTA, and 10% citric acid as final irrigants, no significant differences in microhardness reduction were found. The 0.2% chitosan nanoparticles with a 5-minute application time appeared to be the most viable option for root dentin. The similar chelating effect of 0.2% chitosan compared to the other solutions, combined with its advantageous properties and low concentration, suggests that it is a preferred chelating solution for dentin decalcification. EDTA efficiently reduced dentinal microhardness due to its chelating properties, and the effectiveness of chelating agents depends on several factors, such as application time, pH, concentration, and volume, our study showed no significant difference between 0.2% chitosan nanoparticles and the control group (distilled water), which differed from the Pimenta study, possibly due to the application time of 0.2% chitosan nanoparticles being

3 minutes rather than 5 minutes [26]. The idea of improvement dentin microhardness is opposed in a study conducted by Bastawy when used 0.2% chitosan compared to 2% chitosan and 17% EDTA. The results indicated that irrigating root canals with 2% chitosan or 17% EDTA solutions significantly reduced the microhardness of root canal dentin compared to the control group, whereas 0.2% chitosan resulted in less reduction in dentin microhardness than 2% chitosan or 17% EDTA. This discrepancy is likely attributed to the concentration of the solution and its pH. Higher concentrations and lower pH intensified the demineralizing action, as seen with 2% chitosan. This suggests a balance between pH decrease and viscosity increase with rising solution concentration. [27, 28]. The bond strength between sealer and dentine is crucial for root canal treatment success. Achieving a hermetic seal for the canal space and apex involves adequate bond strength between the sealer and dentin. Several tests were used to assess sealer bond strength, with the push-out test being the most common. However, these test models can't replicate exact clinical conditions due to variations in radicular dentin and canal wall surfaces. Root sections are categorized into coronal, middle, and apical thirds due to differing dentin anatomy. Sealer penetration varies between these areas, with the coronal third showing the most penetration due to wide dentinal tubules. Following crosslinking chitosan nanoparticles, the canal is obturated with a resin-based sealer, which recorded the highest bond strength results in a study by Kaur using the push-out test. This choice is also supported by a study comparing different sealer types, concluding that resin-based sealers have the lowest solubility [29,30]. The comparison of bond strength between using 5.25% NaOCl to dentin and 0.2% chitosan nanoparticles after dentin crosslinking for 3 minutes using push out test showed that there is no difference in sealer bond strength between the test group (0.2% chitosan nanoparticles) and the control group (5.25% NaOCl) as the results between the two groups was insignificant. Also the test showed that there is no difference in sealer bond strength between the root sections (Coronal, middle and apical). In our push-out test, the insignificant results may be linked to root dentin roughness since bonding in the resin-dentine interface relies on surface roughness. Increased dentin roughness results in a lower contact angle, favoring better bonding due to surface irregularities.

Another study by Choudhury suggested that 0.2% chitosan nanoparticles may be a weak chelator. In their study, they evaluated push-out bond strength between 0.2% chitosan nanoparticles and 17% EDTA for 1 minute as a final irrigant. The results showed higher bond strength with 17% EDTA, indicating that chitosan nanoparticles might not be the most effective chelating agent for final irrigation. However, the contact time of 1 minute may not have been sufficient for chitosan nanoparticles to function optimally. In our study, we used 0.2% chitosan nanoparticles for 3 minutes, as suggested by Silva's study, which found that 0.2% chitosan used for the same duration resulted in better smear layer removal and opened tubules [31, 32]. In contrast, another study by Raith reported high bond strength with chitosan nanoparticles. They compared push-out bond strength between 17% EDTA and chitosan nanoparticles at 0.5% for 1 minute and 3 minutes. The results showed that using 0.5% chitosan nanoparticles for 3 minutes resulted in significantly higher bond strength than other samples, which might be attributed to the higher concentration. Our study used 0.2% chitosan nanoparticles for 3 minutes [33]. Chitosan yielded better results in the push-out test when used with bioceramic sealers compared to 17% EDTA. The study found significantly higher bond strength in the apical third for the 0.2% chitosan nanoparticle group, possibly due to changes in the Calcium: Phosphorus ratio during dentin treatment and the flowability of the bioceramic sealer to reach the apical third, which resin sealers may not access. In terms of failure mode, resin-based and bioceramic sealers predominantly exhibited cohesive failures due to stable bonding with radicular dentin compared to gutta-percha [34].

5. Conclusions

Within the limitation of this study the following can be concluded:

- using 0.2% chitosan nanoparticles on dentin didn’t improve the microhardness but caused a minimal or non-significant microhardness reduction in comparison to other irrigants with chelation properties.
- 0.2% chitosan nanoparticles didn't improve the bond strength in comparison to 5.25% NaOCl

References

[1] F.M. Pascon, K.R. Kantovitz, P.A. Sacramento, M. Nobre-dos-Santos, R.M. Puppin-Rontani. (2009). Effect of sodium hypochlorite on dentine mechanical properties. A review. Journal of Dentistry. 37(12): 903-908.

[2] K. Divya, M. Jisha. (2018). Chitosan nanoparticles preparation and applications. Environmental chemistry letters. Journal of Dentistry 16: 101-112.

[3] S. Mai, Y.K. Kim, D.D. Arola, L.-s. Gu, J.R. Kim, D.H. Pashley, F.R. Tay. (2010). Differential aggressiveness of ethylenediamine tetraacetic acid in causing canal wall erosion in the presence of sodium hypochlorite. Journal of Dentistry. 38(3): 201-206.

[4] K. Zhang, F.R. Tay, Y.K. Kim, J.K. Mitchell, J.R. Kim, M. Carrilho, D.H. Pashley, J.-q. Ling. (2010). The effect of initial irrigation with two different sodium hypochlorite concentrations on the erosion of instrumented radicular dentin. Dental Materials. 26(6): 514-523.

[5] M.D. Morris, K.-W. Lee, K.A. Agee, S. Bouillaguet, D.H. Pashley. (2001). Effects of sodium hypochlorite and RC-prep on bond strengths of resin cement to endodontic surfaces. Journal of Endodontics. 27(12): 753-757.

[6] M. Marending, H. Luder, T. Brunner, S. Knecht, W.J. Stark, M. Zehnder. (2007). Effect of sodium hypochlorite on human root dentine–mechanical, chemical and structural evaluation. International Endodontic Journal. 40(10): 786-793.

[7] A. del Carpio-Perochena, C.M. Bramante, M.A.H. Duarte, M.R. de Moura, F.A. Aouada, A. Kishen. (2015). Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restorative Dentistry & Endodontics. 40(3): 195.

[8] A. Hashmi, X. Zhang, A. Kishen. (2019). Impact of dentin substrate modification with chitosan-hydroxyapatite precursor nano complexes on sealer penetration and tensile strength. Journal of Endodontics. 45(7): 935-942.

[9] L. Gu, X. Cai, J. Guo, D. Pashley, L. Breschi, H. Xu, X. Wang, F. Tay, L. Niu. (2019). Chitosan-based extrafibrillar demineralization for dentin bonding. Journal of Dental Research. 98(2): 186-193.

[10] W. Kong, X. Gu, J. Sun. (2020). Progress in biomodification of dentin type I collagen by different types of cross-linkers. Chinese Journal of Stomatology. 55(2): 135-138.

[11] S.F. Massoud, S.M. Moussa, S.A. Hanafy, R.M. El Backly. (2017). Evaluation of the microhardness of root canal dentin after different irrigation protocols (in vitro study). Alexandria Dental Journal. 42(1): 73-79.

[12] Y.-R. Zhang, W. Du, X.-D. Zhou, H.-Y. Yu. (2014). Review of research on the mechanical properties of the human tooth. International journal of oral science. 6(2): 61-69.

[13] A.S. Fawzy, L.I. Nitisusanta, K. Iqbal, U. Daood, L.T. Beng, J. Neo. (2013). Chitosan/Riboflavin-modified demineralized dentin as a potential substrate for bonding. Journal of the Mechanical Behavior of Biomedical Materials, 17, 278-289

[14] D.N. Ratih, N.I. Sari, P. Santosa, N.M.N. Kaswati. (2020). Time-dependent effect of chitosan nanoparticles as final irrigation on the apical sealing ability and push-out bond strength of root canal obturation. International Journal of Dentistry 3(4) 123-133.

[15] K. Pivatto, F.L.M. Pedro, O.A. Guedes, A.F.d. Silva, E. Piva, T.M. Pereira, W.L.d.O.d. Rosa, A.H. Borges. (2020). Cytotoxicity of chelating agents used in endodontics and their influence on MMPs of cell membranes. Brazilian Dental Journal. 31: 32-36.

[16] F.M. Pascon, K.R. Kantovitz, P.A. Sacramento, M. Nobre-dos-Santos, R.M. Puppin-Rontani. (2009). Effect of sodium hypochlorite on dentine mechanical properties. A review. Journal of Dentistry. 37(12): 903-908.

[17] M. Maslamani, M. Khalaf, A.K. Mitra. (2017). Association of quality of coronal filling with the outcome of endodontic treatment: A follow-up study. Dentistry Journal. 5(1): 5-14.

[18] A. del Carpio-Perochena, C.M. Bramante, M.A.H. Duarte, M.R. de Moura, F.A. Aouada, A. Kishen. (2015). Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restorative Dentistry & Endodontics. 40(3): 195.

[19] A. Shrestha, S. Friedman, A. Kishen. (2011). Photodynamically crosslinked and chitosan-incorporated dentin collagen. Journal of dental research. 90(11): 1346-1351.

[20] W. Zakrzewski, M. Dobrzyński, A. Zawadzka-Knefel, A. Lubojański, W. Dobrzyński, M. Janecki, K. Kurek, M. Szymonowicz, R.J. Wiglusz, Z. Rybak. (2021). Nanomaterials application in endodontics. Materials. 14(18): 5296-5320.

[21] E. Roduner. (2006). Size matters: why nanomaterials are different. Chemical Society Reviews. 35(7): 583-592.

[22] Baena, E., Cunha, S. R., Maravić, T., Comba, A., Paganelli, F., Alessandri-Bonetti, G., ... & Mazzoni, A. (2020). Effect of chitosan as a cross-linker on matrix metalloproteinase activity and bond stability with different adhesive systems. Marine drugs, 18(5), 263-278.

[23] V.T. Perchyonok, S. Zhang, S.R. Grobler, T.G. Oberholzer. (2013). Insights into and relative effect of chitosan-H, chitosan-H-propolis, chitosan-H-propolis-nystatin and chitosan-H-nystatin on dentine bond strength. European journal of dentistry, 7(04), 412-418.

[24] L. Hardan, U. Daood, R. Bourgi, C.E. Cuevas-Suarez, W. Devoto, M. Zarow, ... & M. Lukomska-Szymanska. (2022). Effect of collagen crosslinkers on dentin bond strength of adhesive systems: A systematic review and meta-analysis. Cells, 11(15), 2417.-2444.

[25] A. Shrestha, S. Friedman, A. Kishen. (2011). Photodynamically crosslinked and chitosan-incorporated dentin collagen. Journal of Dental Research, 90(11), 1346-1351.

[26] J.A. Pimenta, D. Zaparolli, J.D. Pécora, A.M. Cruz-Filho. (2012). Chitosan: effect of a new chelating agent on the microhardness of root dentin. Brazilian Dental Journal. 23: 212-217.

[27] H. Bastawy, R. Ezzat. (2016). Impact of chitosan as chelating agent on microhardness and mineral content of intraradicular dentin. Al-Azhar Dental Journal for Girls. 3(1): 1-10.

[28] J.D. Sterrett, B. Delaney, A. Rizkalla, C.H. Hawkins. (1991). Optimal citric acid concentration for dentinal demineralization. Quintessence international. 22(5) 223-225.

[29] N. Kaur, S. Bhandari, P. Mahajan, P. Monga, S. Mahajan, N. Bajaj. (2019). In vitro evaluation of bond strength of sealers after using various irrigants and Erbium-Yttrium-Aluminum-Garnet laser irradiation on radicular dentin surface: A SEM study. Endodontology. 31(1): 110-116.

[30] S.S. Chandra, P. Shankar, R. Indira. (2012). Depth of penetration of four resin sealers into radicular dentinal tubules: a confocal microscopic study. Journal of Endodontics. 38(10): 1412-1416.

[31] S. Choudhury, S. Shivanand, A.C. Patil, S.A. Patil, P.K. Doddwad, C. Patil. (2020). Evaluation of push-out bond strength of a resin sealer to dentin after a final flush of three irrigants. The journal of contemporary dental practice. 21: 982-985.

[32] P. Silva, D.F.C. Guedes, F.V. Nakadi, J.D. Pécora, A.M.d. Cruz-Filho. (2013). Chitosan: a new solution for removal of smear layer after root canal instrumentation. International Endodontic Journal. 46(4): 332-338.

[33] U. Daood, H. Omar, J.K.H. Tsoi, A.S. Fawzy. (2018). Long-term bond strength to dentine of a chitosan-riboflavin modified two-step etch-and-rinse adhesives. International Journal of Adhesion and Adhesives, 85, 263-273.

[34] A.M. Mohamed, A.G. Ghoneim, N. N. Roshdy. (2021). Evaluation of the effect of final rinse with chitosan nanoparticles and EDTA on the push-out bond strength of resin-based and bioceramic-based root canal sealers (a comparative in vitro study). Acta Scientific Dental Scienecs. 5(4):66–75.