The Saudi Dental Journal (2023) 35, 692–698

King Saud University

The Saudi Dental Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Microhardness and surface roughness of resin infiltrated bleached enamel surface using atomic force microscopy: An in vitro study

Alaa M. Alsafi, Nadia M. Taher *

Department of Restorative Dental Sciences, College of Dentistry, King Saud University, Riyadh, Saudi Arabia

Received 26 March 2023; revised 23 June 2023; accepted 2 July 2023
Available online 6 July 2023

KEYWORDS

Resin Infiltration;
Bleaching;
Hardness;
Roughness;
White spot lesions;
Atomic force microscopy

Abstract *Aim:* to evaluate the microhardness (VHN) and surface roughness (Ra) of human enamel surface treated with resin infiltration followed by in office bleaching and to study the effect of artificial saliva (AS) storage time on the VHN and Ra of resin infiltrated enamel. *Materials and methods:* Eighty enamel specimens were prepared from extracted human premolar teeth. Specimens were divided into two main groups (I and II) then, they were demineralized to create white spot lesions (WSLs). Group I was divided into A and B sub groups. Group A was treated with in office bleaching material while for group B, resin infiltration (Icon) was applied after 24 h storage in AS followed by bleaching. Group II was divided into (C and D). Group C was treated like group B. Specimens in group D were stored in AS for 14 d after treatment with Icon and before application of bleaching. Atomic force microscopy (AFM) Ra analysis and VHN calculation were done. Independent T Test was used to compare between groups at $P < 0.05$. *Results:* Group B demonstrated a significant increase in VHN compared with group A. Storage time in AS showed no significant difference on VHN or Ra between groups. *Conclusion:* Application of resin infiltration before bleaching significantly increased VHN and slightly improved Ra of WSLs compared with bleaching alone. Application of bleaching material after 24 h or 14 days of the application of resin infiltration did not have a significant effect or surface hardness or roughness of WSLs.

* Corresponding author at: Department of Restorative Dental Sciences, College of Dentistry, King Saud University, PO Box 60169, Riyadh 11545, Saudi Arabia.
E-mail addresses: aalsafi@ksu.edu.sa (A.M. Alsafi), ntaher@ksu.edu.sa (N.M. Taher).

Peer review under responsibility of King Saud University. Production and hosting by Elsevier

ELSEVIER Production and hosting by Elsevier

1. Introduction

White spot lesions (WSLs) is a term used to describe several conditions affecting dental enamel. White discolorations of enamel might be due to developmental enamel defect such as amelogenesis imperfecta (Thylstrup and Fejerskov, 1978) or seen in connection with different disorders such as cerebral palsy (Lin et al., 2011). Trauma to primary teeth or untreated carious lesion may lead to pulpal necrosis and infection that

https://doi.org/10.1016/j.sdentj.2023.07.001
1013-9052 © 2023 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University

could result in enamel defects such as WSLs in the succedaneous permanent teeth (Skaare et al., 2013). WSLs can be seen as localized areas of demineralization resulting from the process of caries formation (Kidd and Fejerskov, 2004).

White spot lesions resulting from enamel demineralization is considered as the first visible evidence of a carious process in the enamel and is characterized by an opaque white appearance (Featherstone, 2008). The opaque white appearance is due to subsurface demineralization with an increase in porosity and consequential alteration of the optical properties of enamel (Chang et al., 1997). Although there is no golden standard for WSLs treatment, several treatment modalities are commonly utilized. Remineralization through application of products containing fluoride or casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) is one of the treatment options (Huang et al., 2013; Beerens et al., 2010). Clinical studies have shown enhancement in the surface remineralization of WSLs with exposure to remineralizing agents (Pandey et al., 2013; Restrepo et al., 2016). However, these agents couldn't completely eliminate WSLs' clinical appearance (Yetkiner et al., 2014). It has been reported that WSLs match better with the remaining tooth structure following bleaching treatment (Kim et al., 2016). Nevertheless, if tooth whitening is applied to decalcified areas, a reduction in enamel microhardness might be expected (Ghanbarzade et al., 2015). Microabrasion was found to be an effective treatment approach for improving the appearance of demineralized enamel lesions (Jahanbin et al., 2015). Conversely, microabrasion was shown to induce changes on enamel surface and negatively affect surface hardness (Mathias et al., 2009). A minimally invasive treatment modality has been introduced in recent years. Resin infiltration has been developed as a conservative method for the treatment of carious WSLs (Paris et al., 2007; Phark et al., 2009). It is a micro-invasive technique developed to close the gap between prevention and restoration by reinforcing and filling the pores of a non cavitated white spot lesion with a light cured resin (Meyer-Lueckel and Paris, 2008; Kielbassa et al., 2009). As a result, the progression of carious lesion is hampered, and mechanical support is provided to the demineralized enamel (Kielbassa et al., 2009). An advantageous effect of resin infiltration is its ability to enhance the chalky white appearance of WSLs, as the utilized resin has a refractive index similar to the healthy enamel (Paris and Meyer-Lueckel, 2009). Few studies have evaluated the combination treatment of bleaching and resin infiltration on WSLs' color and microhardness (Horuztepe and Baseren, 2017; Araújo et al., 2015; Pucci et al., 2012).

The aim of the current research was to evaluate the microhardness and surface roughness of human enamel surface treated with resin infiltration followed by in office bleaching and to study the effect of AS storage time on resin infiltrated enamel surface roughness and microhardness after bleaching.

1.1. Null hypothesis

- Resin infiltration of enamel surface before bleaching does not improve microhardness and surface roughness.
- There is no significant difference in enamel microhardness and surface roughness between 24 h and 14 days artificial saliva storage time of resin infiltrated then bleached specimens.

2. Materials and methods

The materials used in the present study were an in office-bleaching system (Opalescence Boost 40% Hydrogen Peroxide, Ultradent, South Jordan, UT, USA) and a resin infiltration system (Icon; DMG, Hamburg, Germany).

2.1. Specimens' collection

The study was conducted after registration and ethical approval clarification were obtained from College of Dentistry Research Center (CDRC), King Saud University, Riyadh, Kingdom of Saudi Arabia registration No. PR 0063, 27th of April 2017. Eighty enamel specimens were obtained from 40 human premolar teeth that were sound and extracted for orthodontic reasons. Teeth were thoroughly cleaned using an ultrasonic scaler (LM-ProPower UltraLED LM 100741, LMDental, Parainen, Finland). Then, teeth were checked using a light microscope (SWIFT Instruments International, SA, microscope series 80, Tokyo, Japan) at 10× magnification to make sure they were free from caries, white spots, cracks, and other developmental defects. During teeth collection, teeth were stored in 0.1% thymol solution.

2.2. Specimens' preparation

Teeth were embedded from the root side in self cure acrylic resin (Vertex- Dental B.V, Zeist, Netherlands) to stabilize them during the cutting procedure. The teeth cusps were ground flat using 320 grit silicon carbide (SIC) abrasive paper (Buehler,41 Waukegan Road, IL, USA) on a rotary polisher (Automata grinding & polishing unit, Polo 250/3, Jeanwirtz, Germany) under running water for cooling. Teeth were cut vertically in a mesio-distal direction with a diamond cutting saw (Isomet 2000 Precision Saw, 3357, Buehler, IL, 664 USA) with profuse water irrigation. After, teeth were transversely cut at the cementoenamel junction to remove the roots. Two specimens (buccal and lingual surfaces) were obtained from every tooth. The two specimens obtained from the same tooth were marked at the bottom of the specimen with permanent marker to avoid mixing them. A uniform thickness of 4 mm was obtained by grinding the bottom surface (dentin) of the specimens using (320) grit SIC paper under running tap water for cooling. Specimens were then embedded in self cure acrylic resin (Vertex- Dental, Zeist, Netherlands) utilizing custom made silicon molds leaving the top surface (enamel) exposed. Specimens were projected to ensure that the smooth convex surfaces were as parallel to the scanning device as possible. To form the parallel planar surface necessary for the surface microhardness testing and atomic force microscopy reading, the specimen's top (enamel) sides were sequentially ground flat and polished. The previous step was done using (400, 600) SIC paper using running tap water for cooling (Buehler, IL, USA).

2.3. White spot lesion creation

Prepared specimens were immersed in a demineralizing solution. The solution used was a methylcellulose (MEC) acid gel using the following formula: (5% methylcellulose covered with an equal volume of 0.1 M lactic acid, KOH was used to adjust the pH. to 4.6. Specimens were demineralized for 10 days and were stored in an incubator at 37 °C during that period (Nassar et al., 2014). The demineralizing agent was not stirred or replaced throughout the demineralization period.

Lactate buffer was used in the production of WSLs to mimic clinical situation since lactic acid is produced when sucrose or glucose get fermented by streptococci (Nyvad and Kilian, 1987). Following creation of WSLs, specimens were divided into two main groups (I and II) 40 specimens each. Groups were then subdivided into two subgroups (A & B) and (C & D) each containing 20 specimens. Since natural teeth show considerable variability, each tooth was sectioned into two equal parts, to serve as control or experimental specimens in the subgroups of the same group. The groups received the following treatments: Group IA: Application of in-office bleaching only. Group IB: Application of resin infiltration followed by 24 h immersion in AS then, application of in-office bleaching. Group IIC: Treated as group B. Group IID: Application of resin infiltration followed by 14 days immersion in AS then, application of in-office bleaching.

2.4. Application of resin infiltration material

Specimens were treated with resin infiltration system: (Icon; DMG, Hamburg, Germany) following manufacturer instructions (Table 1).

2.5. Application of in office bleaching material

Enamel surfaces were treated with a chemically activated in office-bleaching system (Opalescence Boost 40% Hydrogen Peroxide, Ultradent, South Jordan, UT, USA) following manufacturer instructions (Table 1).

2.6. Storage in artificial saliva

After the application of resin infiltration, specimens of groups B and C were stored in AS for 24 h in an incubator at 37 °C while specimens of group D were stored for 14 days. Then, in office bleaching was applied. The following formula was used to prepare the AS: 1 g sodium carboxymethylcellulose, 4.3 g xylitol, 0.1 g potassium chloride, 0.1 g sodium chloride, 0.02 mg sodium fluoride, 5 mg calcium chloride, 5 mg magnesium chloride, 5 mg calcium chloride, 40 mg potassium phosphate, 1 mg potassium thiocyanate, and 100 g distilled deionized water, at pH 7 at 37 °C (Han et al., 2014). Specimens of all groups were stored in AS for 24 h then placed in distilled water at a room temperature until the tests were performed.

2.7. Measurement of enamel surface hardness

Hardness measurements were conducted using Vickers hardness tester (NOVA 130/240, serial #13002178648, INNOVATEST Europe, The Netherlands). A load of 300 g was applied to the specimens for 15 s. Load and time were constant for all specimens throughout the study. Three indentations were made on the flattest surface of each treated specimen.

2.8. Measurement of enamel surface roughness

An atomic force microscope (AFM) (Multimode 8, Bruker, Serial # 3C119, Santa Barbara, CA, USA) was utilized. This device required the specimens' dimensions to have a maximum thickness of 4 mm (height) and a maximum width of 15 mm. The noncontact AFM scan mode was used where the specimens were placed on an isolation table and connected to an optic microscope with a personal computer. The roughness parameter analyzed was the average roughness, which is the arithmetic mean of the height of peaks and depth of valleys from a mean line in the measuring length.

2.9. Statistical analysis

The Statistical Package for Social Science (SPSS version 22.0 for Windows; IBM SPSS, 2013, Armonk, NY, USA) was used to perform the statistical analysis. The statistical comparison between the study groups was performed using independent *t*-test. All tests were performed at a significance level of ($P < 0.05$).

3. Results

3.1. Surface microhardness

The mean and standard deviation of Vickers Microhardness value (Kg/mm^2) for each group are presented in Table 2. Statistical analysis of the mean revealed a statistically signifi-

Table 1 Composition and manufacturer instructions of used materials.

Material	Manufacturer	Composition	Direction of use
Icon caries infiltration	DMG Hamburg, Germany	Icon-Etch (HCl 15%)Icon-Dry (99% ethanol) Icon-Infiltrant (methacrylate-based resin matrix, initiators, additives)	1. Clean surface2. Apply Icon-Etch. Let set for 2 min3. Rinse off with water for 30 s. Air dry4. Apply Icon-Dry. Let set for 30 s. Air dry5. Apply Icon-Infiltrant. Let set for 3 min6. Light-cure for 40 s7. Apply Icon-Infiltrant. Let set for 1 min8. Light-cure for 40 s
Opalescence Boost 40% Hydrogen Peroxide	Ultradent South Jordan, USA	Activator (1.1% sodium fluoride, 3% potassium nitrate, unique chemical activator)Bleaching gel (40% hydrogen peroxide)	1. Mix the two syringes together2. Apply the activated gel as a 0.5–1-mm-thick layer to the specimen.3. Leave gel on specimen for 20 min4. Remove bleaching agent using high volume suction with an endo tip5. Apply again for another 20 min (total of 40 min).6. Clean and wash with water

cant difference at P = 0.000 between group A and group B as shown in Table 2. The resin infiltration prior to bleaching group demonstrated a significant increase in the microhardness levels when compared with the bleaching alone group.

3.2. Surface roughness

A quantitative evaluation of the surface roughness values from the AFM images was performed by calculating the mean surface roughness (nm). The mean and standard deviation of surface roughness (Ra) for each group are presented in Table 2. The data revealed a lower Ra value for group B in comparison to group A. However, the difference was not statistically significant ($P > 0.05$) *(*Table 2*)*. No statistically significant difference was evident between groups C and D ($P > 0.05$). Nonetheless, increasing the duration of the specimen's storage in AS from 24 h (group C) to 14 d (group D) resulted in a slightly higher Ra.

3.3. Descriptive analysis of AFM images

Demineralized and bleached specimens of group A showed wide and deep enamel grooves between enamel prisms *(*Fig. 1*)*. AFM images of resin infiltrated specimens showed penetration of resin infiltration into demineralized spaces *(*Fig. 2*)*. Resin infiltration with Icon showed a thin non-homogenous surface, where resin was visible on enamel surface in an irregular pattern showing column gaps *(*Fig. 3*)* or honey-comb pattern *(*Fig. 4*)* filling in areas of porosity and leaving hole gaps between adjacent enamel prisms. The effect of AS storage is evident in the AFM images where resin infiltrated surface appears rougher with wrinkled appearance for the specimens of group D as compared with group C *(*Figs. 3 & 4*)*.

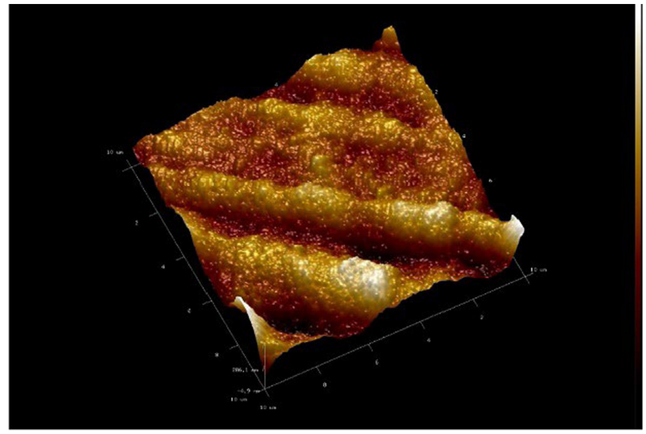

Fig. 1 Atomic force microscopy obtained images of group A (Bleached alone). Wide and deep enamel grooves are evident between enamel prisms.

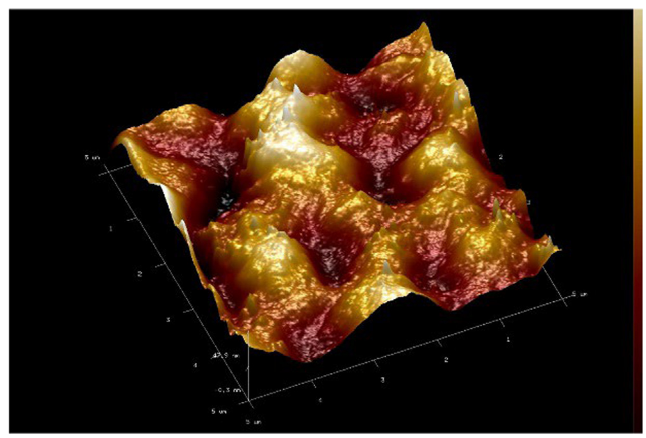

Fig. 2 Atomic force microscopy obtained images of group B showing penetration of resin infiltration into demineralized spaces.

4. Discussion

The outcomes of the present study showed that resin infiltration prior to bleaching significantly increased the surface microhardness of enamel WSLs when compared with bleaching alone but did not have a significant effect on the surface roughness. Hence, the first null hypothesis was partially rejected.

Icon utilizes triethylene glycol dimethacrylate (TEGDMA) for lesion infiltration. TEGDMA is an unfilled low viscosity light curing resin. Compared to other resins such as urethane dimethacrylate (UDEMA) and bisphenol A-glycidyl methacrylate (bis-GMA), TEGDEMA has a lower tensile and flexural strength (Gonçalves et al., 2008). The high hardness obtained with Icon might be attributed to the infiltrant resin's ability to penetrate and fill the voids present in WSLs, regardless of the resin's mechanical properties. As a result of infiltration, it appears that a consistant resin-hydroxyapatite complex with a high surface hardness is obtained (Zhao and Ren, 2016). Furthermore, the recommended protocol by Icon's manufacturer is to apply the infiltrant resin twice. It was

Table 2 Mean, standard deviation (SD) and *P* value of tested groups for both measurements.

Groups tested(n = 20)	**Surface hardness (Kg/mm^2)**		**Surface roughness (nm)**	
	Mean VHN ± SD	***P* value**	**Mean Ra ± SD**	***P* value**
A	120.54 ± 35.47	0.000*	26.35 ± 8.43	0.663
B	279.39 ± 52.42		25.10 ± 9.50	
C	275.80 ± 46.47	0.923	33.28 ± 8.25	0.126
D	277.34 ± 53.76		38.44 ± 12.21	

* Significant difference at $P < 0.05$.

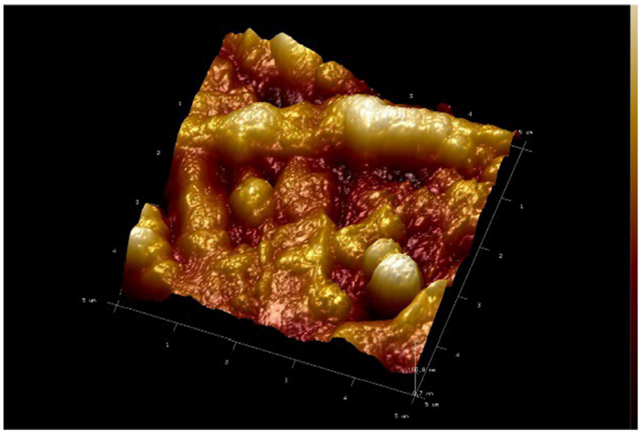

Fig. 3 Atomic force microscopy obtained images of group C (24 h storage in artificial saliva). Resin infiltration with Icon shows a non-homogenous surface where resin was visible as an irregular pattern showing column gaps.

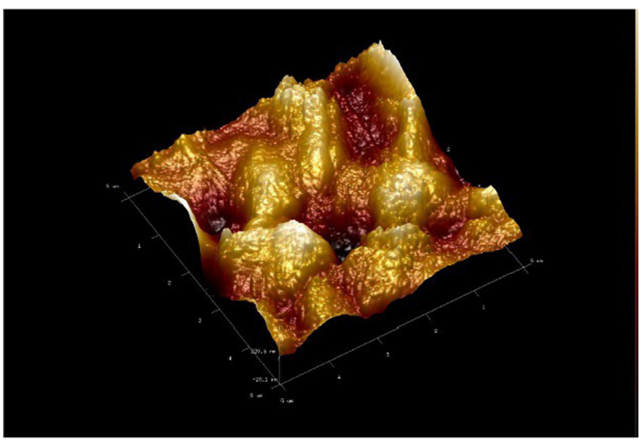

Fig. 4 Atomic force microscopy obtained images of group D (14 days storage in artificial saliva). Resin infiltration with Icon shows a non-homogenous surface where resin was displaying a honey-comb pattern leaving hole gaps between adjacent enamel prisms.

reported that that twice application of resin could compensate for polymerization shrinkage and fill voids within the infiltrated lesion which leads to increase in hardness (Paris et al. 2013). Furthermore, ethanol (Icon Dry) which is used to prepare the surface before resin application, have been shown to increase penetration coefficients of resins thus, improving infiltration depth (Paris and Meyer-Lueckel, 2010). On the contrary to the present study, Horuztepe and Baseren (2017) treated their samples with resin infiltration after bleaching. They concluded that bleaching before resin infiltration resulted in lower microhardness values compared with resin infiltration alone and bleaching negatively influenced penetration of resin infiltration. The result of the current investigation is consistent with that of Ghanbarzade et al. (2015). In their study, they demonstrated a significant reduction in the WSL's microhardness following home and in-office bleaching. Their result indicated a possible alteration in the mineral content of demineralized enamel caused by bleaching. The reduction in hardness could be the result of the alteration of bleached enamel surfaces. Studies showed that enamel composition could be modified by the removal of some components such as calcium and phosphate. Tezel et al. (2007) concluded that 35% and 38% hydrogen peroxide might cause significantly more loss of Ca^{2+} from the enamel surfaces than 10% carbamide peroxide.

AFM provides a detailed qualitative and quantitative data about surface topography. Its main advantage over the other technologies is that it provides higher resolution images at the nano scale. The AFM technique has emerged as the most reliable in the evaluation of surface roughness (Kakaboura et al., 2007). In the present study, results showed that the group which received bleaching alone recorded higher (but not significant) roughness values compared with resin infiltrated group prior to bleaching. On the other hand, surface roughness was slightly higher for specimens that were incubated for 14 days when compared with 24 h period of incubation.

The demineralization process that was done in this study to produce WSLs might also have resulted in increased surface roughness. It would be expected that the application of resin infiltration material to fill WSL's porosities would result in improved roughness values if we compare the roughness value to demineralized as well as bleached enamel surfaces. However, no significant difference was evident between groups A and B. Several studies reported inferior roughness values related to resin infiltration with Icon. In a study carried by Taher et al. (2012) using profilometry, resin infiltration with Icon showed significantly higher roughness values compared with fissure sealant. Their result was further confirmed by AFM and computed tomography when Taher (2013) compared surface roughness of Icon with that of fissure sealant and sound enamel. Similar findings were reported by Gurdogan et al. (2017) as well as Ulrich et al. (2015), where their data revealed that Icon's roughness values were significantly higher than healthy enamel and described Icon surface as having an inhomogeneous surfaces showing small granular particles. The description of Icon surfaces in the previous articles applies to AFM images obtained in the present study, where resin infiltrated specimens presented inhomogeneous surfaces with resin that can be seen in the enamel grains *(*Fig. 2*)*.

The current study showed that increasing the storage time from 24 h to 14 d did not have a negative impact on surface microhardness of the resin infiltrated specimen. Conversely, the surface roughness was slightly higher for specimens that were incubated for 14 d compared with those incubated for 24 h. The difference between the two groups in surface microhardness and roughness was not significant which made us accept the second null hypothesis. The literature lacks articles evaluating the effect of AS storage time on resin infiltrated enamel. Zhao and Ren (2016) reported that microhardness of resin infiltrated enamel was slightly reduced after 180 h of water storage, but this reduction was not statistically significant while surface roughness was significantly increased after water storage.

Compared with other resins like UDMA and Bis-GMA, TEGDMA showes the highest degree of water sorption due to the presence of hydrophilic ether linkages (Ferracane, 2006). This might affect the mechanical properties of resin based materials. Nonetheless, the hardness of resin infiltrated specimens was not greatly affected by storage time in AS probably because infiltration resin was able to surround the hydroxyapatite crystals in the WSL and make a relatively uniform resin-hydroxyapatite complex (Zhao and Ren, 2016). The increase in roughness with the increase of storage time might be attributed to the loss of the oxygen inhibited layer. This layer could be washed away or worn-off by salivary flow or mechanical action which might lead to re-exposure of the enamel substance and affect the roughness values (Mueller et al., 2011). Further research is recommended for longer period of observation.

As with other laboratory reports, our study faced some limitations. First, artificial lesions created on the specimens might not completely represent clinical white spot lesions. However, this problem is common with in vitro caries studies since there is no standardized characterization of white lesions. Second, artificial saliva is used in laboratory studies to mimic remineralization capacity in clinical situations. However, it is important to note that the dynamics of saliva - enamel interaction is a difficult to fully replicate in laboratory research.

5. Conclusions

Based on our observations and considering limitations of this study, application of resin infiltration prior to bleaching is recommended since it significantly increased the surface microhardness of WSLs. Delaying bleaching procedure for 14 days after resin infiltration of WSLs did not have a significant effect on surface microhardness or surface roughness as compared with bleaching after 24 h of resin infiltration.

Funding

This study was supported by Prince Naief health research center (Research institution).

CRediT authorship contribution statement

Alaa M. Alsafi: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Nadia M. Taher:** Conceptualization, Methodology, Investigation, Resources, Visualization, Supervision.

Acknowledgments

The authors would like to thank the College of Dentistry Research Center (CDRC) and Deanship of scientific research at King Saud University. Their thanks also extend to Prince Naief Health Research Centre for funding this study, to Eng. Majed Aljomah from the National Nanotechnology Center, King Abdulaziz City for Science and Technology for his assistance with the Atomic Force Microscopy (AFM) device and Dr. Nasser El.Meflehi for performing the statistical analysis.

References

Araújo, G., Naufel, F., Alonso, R., Lima, D., Puppin-Rontani, R., 2015. Influence of staining solution and bleaching on color stability of resin used for caries infiltration. Oper. Dent. 40, 250–256.

Beerens, M., Van Der Veen, M., Van Beek, H., Ten Cate, J., 2010. Effects of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up. Eur. J. Oral Sci. 118, 610–617.

Chang, H., Walsh, L., Freer, T., 1997. Enamel demineralization during orthodontic treatment Aetiology and prevention. Aust. Dental J. 42, 322–327.

Featherstone, J., 2008. Dental caries: a dynamic disease process. Aust. Dent. J. 53, 286–291.

Ferracane, J., 2006. Hygroscopic and hydrolytic effects in dental polymer networks. Dent. Mater. 22, 211–222.

Ghanbarzade, M., Ahrari, F., Akbari, M., Hamzei, H., 2015. Microhardness of demineralized enamel following home bleaching and laser-assisted in office bleaching. J. Clin. Exp. Dent. 7, 405–409.

Gonçalves, F., Pfeifer, C., Ferracane, J., Braga, R., 2008. Contraction stress determinants in dimethacrylate composites. J. Dent. Res. 87, 367–371.

Gurdogan, E., Ozdemir-Ozenen, D., Sandalli, N., 2017. Evaluation of surface roughness characteristics using atomic force microscopy and inspection of microhardness following resin infiltration with Icon. J. Esthet. Restor. Dent. 29, 201–208.

Han, Y., Mo, S., Jiang, L., Zhu, Y., 2014. Effects of antioxidants on the microleakage of composite resin restorations after external tooth bleaching. Eur. J. Dent. 8, 147–153.

Horuztepe, S., Baseren, M., 2017. Effect of resin infiltration on the color and microhardness of bleached white-spot lesions in bovine enamel (an in vitro study). J. Esthet. Restor. Dent. 29, 378–385.

Huang, G., Roloff-Chiang, B., Mills, B., Shalchi, S., Spiekerman, C., Korpak, A., et al, 2013. Effectiveness of MI Paste Plus and PreviDent fluoride varnish for treatment of white spot lesions: a randomized controlled trial. Am. J. Orthod. Dentofac. Orthop. 143, 31–41.

Jahanbin, A., Ameri, H., Shahabi, M., Ghazi, A., 2015. Management of post-orthodontic white spot lesions and subsequent enamel discoloration with two microabrasion techniques. J. Dent. 16, 56–60.

Kakaboura, A., Fragouli, M., Rahiotis, C., Silikas, N., 2007. Evaluation of surface characteristics of dental composites using profilometry, scanning electron, atomic force microscopy and gloss-meter. J. Mater. Sci. - Mater. Med. 18, 155–163.

Kidd, E., Fejerskov, O., 2004. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J. Dent. Res. 83, 35–38.

Kielbassa, A.M., Muller, J., Gernhardt, C.R., 2009. Closing the gap between oral hygiene and minimally invasive dentistry: a review on the resin infiltration technique of incipient (proximal) enamel lesions. Quintessence Int. 40, 663–681.

Kim, Y., Son, H., Yi, K., Ahn, J., Chang, J., 2016. Bleaching effects on color, chemical, and mechanical properties of white spot lesions. Oper. Dent. 41, 318–326.

Lin, X., Wu, W., Zhang, C., Lo, E., Chu, C., Dissanayaka, W., 2011. Prevalence and distribution of developmental enamel defects in children with cerebral palsy in Beijing, China. Int. J. Paediatr. Dent. 21, 23–28.

Mathias, J., Kavitha, S., Mahalaxmi, S., 2009. A comparison of surface roughness after micro abrasion of enamel with and without using CPP-ACP: an in vitro study. J. Conserv. Dent. 12, 22–25.

Meyer-Lueckel, H., Paris, S., 2008. Improved resin infiltration of natural caries lesions. J. Dent. Res. 87, 1112–1116.

Mueller, J., Yang, F., Neumann, K., Kielbassa, A.M., 2011. Surface tridimensional topography analysis of materials and finishing procedures after resinous infiltration of subsurface bovine enamel lesions. Quintessence Int. 42, 135–147.

Nassar, H., Lippert, F., Eckert, G., Hara, A., 2014. Dentifrice fluoride and abrasivity interplay on artificial caries lesions. Caries Res. 48, 557–565.

Nyvad, B., Kilian, M., 1987. Microbiology of the early colonization of human enamel and root surfaces in vivo. Scand. J. Dent. Res. 95, 369–380.

Pandey, H., Agarwal, A., Choudhary, G., Pandey, L., 2013. Effect of fluoridated toothpaste on white spot lesions in postorthodontic patients. Int. J. Clin. Pediatric Dent. 6, 85–88.

Paris, S., Meyer-Lueckel, H., Kielbassa, A.M., 2007. Resin infiltration of natural caries lesions. J. Dent. Res. 86, 662–666.

Paris, S., Meyer-Lueckel, H., 2009. Masking of labial enamel white spot lesions by resin infiltration–a clinical report. Quintessence Int. 40, 713–718.

Paris, S., Meyer-Lueckel, H., 2010. Infiltration of natural caries lesions with experimental resins differing in penetration coefficients and ethanol addition. Caries Res. 44, 408–414.

Paris, S., Schwendicke, F., Seddig, S., Müller, W., Dörfer, C., Meyer-Lueckel, H., 2013. Micro-hardness and mineral loss of enamel lesions after infiltration with various resins: Influence of infiltrant composition and application frequency in vitro. J. Dent. 41, 543–548.

Phark, J.H., Duarte Jr, S., Meyer-Lueckel, H., Paris, S., 2009. Caries infiltration with resins: a novel treatment option for interproximal caries. Compend. Contin. Educ. Dent. 30, 13–17.

Pucci, C., Torres, C., Borges, A., Santos, L., Rêgo, H., 2012. Efficacy of bleaching treatment on demineralized enamel treated with resin infiltration technique. World J. Dent. 3, 279–283.

Restrepo, M., Bussaneli, D., Jeremias, F., Cordeiro, R., Raveli, D., Magalhães, A., et al, 2016. Control of white spot lesions with use of fluoride varnish or chlorhexidine gel during orthodontic treatment a randomized clinical trial. J. Clin. Pediatr. Dent. 40, 274–280.

Skaare, A., Maseng, A.A., Wang, N., 2013. Enamel defects in permanent incisors after trauma to primary predecessors: inter-observer agreement based on photographs. Dent. Traumatol. 29, 79–83.

Taher, N.M., 2013. Atomic force microscopy and tridimensional topography analysis of human enamel after resinous infiltration and storage in water. Saudi Med. J. 34, 408–414.

Taher, N., Alkhamis, H., Dowaidi, S., 2012. The influence of resin infiltration system on enamel microhardness and surface roughness: an in vitro study. Saudi Dental J. 24, 79–84.

Tezel, H., Ertaş, O.S., Ozata, F., Dalgar, H., Korkut, Z.O., 2007. Effect of bleaching agents on calcium loss from the enamel surface. Quintessence Int. 38, 339–347.

Thylstrup, A., Fejerskov, O., 1978. Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. Commun. Dent. Oral Epidemiol. 6, 315–328.

Ulrich, I., Mueller, J., Wolgin, M., Frank, W., Kielbassa, A.M., 2015. Tridimensional surface roughness analysis after resin infiltration of (deproteinized) natural subsurface carious lesions. Clin. Oral Invest. 19, 1473–1483.

Yetkiner, E., Wegehaupt, F., Wiegand, A., Attin, R., Attin, T., 2014. Colour improvement and stability of white spot lesions following infiltration, micro-abrasion, or fluoride treatments in vitro. Eur. J. Orthodontics 36, 595–602.

Zhao, X., Ren, Y., 2016. surface properties and color stability of resin-infiltrated enamel lesions. Oper. Dent. 41, 617–626.