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Novel hybrid-glass-based material for infiltration of early caries lesions

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ABSTRACT

Objectives: To assess the effectiveness of two experimental, hybrid-glass-based infiltrants in arresting artificial white spot lesions (WSLs) in vitro, and to compare it with resin-based infiltrant Icon.

Methods: Artificial WSLs were formed on bovine enamel specimens (n = 68). Specimens were divided into four groups according to WSLs treatment: 1) no-treatment control (NTC), 2) infiltration with Icon (Icon), 3) infiltration with experimental hybrid-glass material (EXP), and 4) infiltration with experimental hybrid-glass material containing hydroxyapatite (HAp) nanoparticles (1%) (EXP-HAp). Half of the specimens from each group were subjected to cariogenic challenge using pH-cycling, consisting of a 7-day alternate incubation (37°C) in demineralization (4 h/day, pH=4.6) and remineralization solutions (20 h/day, pH=7.2). Another half of the specimens was incubated in distilled water (control). Caries progression was assessed by measuring surface micro-hardness (SMH), roughness (R_a) and average surface level, and by analyzing WSLs morphology. Non-cycled and pH-cycled specimens were compared with Man-Whitney U test, while different treatment groups were compared with Kruskal-Wallis test with pairwise comparisons ($p < 0.05$).

Results: In all groups (NTC, Icon and EXP-HAp) except EXP, SMH decreased significantly after pH cycling. In addition, SMH increased in EXP upon pH cycling and was significantly higher than in other pH-cycled groups (<0.001). R_a increased considerably, while surface level decreased after pH cycling in all groups except in EXP. Signs of demineralization and roughness increase in NTC, Icon and EXP-HAp were also observed with the SEM.

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Significance: Experimental hybrid-glass-based material without HAp-nanoparticles could completely arrest the progression of WSLs, unlike its version with HAp-nanoparticles and resin-based infiltrant Icon.

1. Introduction

Proximal caries lesions remain a frequently faced problem in daily dental practice, with reported prevalence of up to 77% [1]. Their early detection at a non-cavitated stage, which can be challenging, is highly beneficial, giving the opportunity to employ a non-operative approach in lesion's management. Non-operative treatment, typically consisting of topical fluoride application and improved oral hygiene, aims to arrest and reverse early caries lesions. Its success, however, depends largely on patients' compliance, which is often inadequate. Furthermore, non-operative treatment is typically reserved for non-cavitated, so-called white-spot lesions (WSLs), which are characterized by increased porosity of subsurface enamel. This approach is much less effective in case of cavitated lesions, which are typically treated operatively [2]. Nevertheless, operative treatment of proximal lesions can hardly adhere to the principles of minimally invasive dentistry, since a considerable amount of sound tissue has to be removed to access the lesion, in the presence of an adjacent tooth. Furthermore, this intervention marks the beginning of the so-called "tooth death spiral", leading to a progressive tooth tissue loss, with all its consequences [3]. The operative treatment should, therefore, be postponed as long as possible.

In order to bridge the gap between the non-operative and operative treatment of proximal caries lesions, so-called micro-invasive treatment was introduced about a decade ago. This approach entails lesion sealing with dental adhesive or sealant, or lesion infiltration with a low viscosity resin [4,5]. Both methods were shown to be more effective in arresting proximal caries than non-operative, purely preventive approach [6–8]. Infiltration of porous enamel in incipient lesions disrupts diffusion pathways for bacterial acids, thereby arresting the lesion. In addition, resin infiltration of enamel porosities could mask the whitish appearance of white spot lesions (WSLs), making it applicable in esthetic rehabilitation, although with varying degrees of success [9]. The only commercially available resin-based infiltrant Icon (DMG, Hamburg, Germany) has, therefore, found its place in many dental practices worldwide.

Even though the success of micro-invasive approach is well-documented, at least short-term, potential drawbacks of resin-infiltration method should be addressed [10]. First, resin infiltration of subsurface enamel eliminates every chance of possible lesion remineralization/regression, since it disables the diffusion and deposition of calcium and phosphate ions into the lesion. Second, certain properties of triethylene glycol dimethacrylate (TEGDMA)-based resins, the main component of Icon, raise questions about the suitability of this material for caries infiltration. TEGDMA-based resin is particularly susceptible to water sorption and enzymatic hydrolysis by salivary and bacteria-produced enzymes [11], especially in the absence of inorganic fillers [12]. In the long term, this may lead to the weakening of the diffusion barrier the material forms inside the lesion, and decrease in its ability to arrest caries. In addition, resin-based restoratives are considered more prone to plaque accumulation compared to other dental materials, such as glass-ionomer cement and amalgam, and enamel [13,14], due to their specific surface chemistry, and the lack of antibacterial and acid-buffering abilities [15]. Since proximal lesions develop on surfaces with increased plaque retention, the addition of resinous substrate might boost plaque accumulation in this area, unless patients' oral hygiene is substantially improved. Finally, biocompatibility of resin-based materials is often questioned, since they can release unreacted monomers and other components with potentially toxic effects [16,17]. TEGDMA in particular seems to be one of the most cytotoxic dental resin monomers, due to its relatively high hydrophilicity and elution in aqueous environment [18,19]. In addition, methacrylate monomers, including TEGDMA, are very potent sensitizers. Acrylate allergies, mostly in the form of allergic contact dermatitis, have long been a concern among dental workers and patients [20]. Moreover, the prevalence of this condition increased drastically in the last decade, primarily in the younger female population, as a consequence of the widespread use of acrylate-based nail varnishes [21]. Early sensitization with methacrylates can hinder the later use of methacrylate resin-based dental materials, which are often the first-choice materials for dental restorative treatments. Therefore, the benefits of the preventive application of methacrylate-based materials, such as Icon, in the pediatric population should be questioned and resin-free alternatives should be considered instead.

A highly biocompatible material, with low susceptibility to plaque accumulation and biodegradation, and potential bioactivity or remineralizing ability, would theoretically be a superior alternative to a resin-based caries infiltrant. With the aim of providing such an alternative, we developed a novel, glass-based material for the infiltration of early proximal lesions. It is a low viscosity transparent inorganic-organic hybrid polymer, produced by sol-gel process, which is self-cured shortly after the application. It is considered hybrid-glass material because it contains silica core with an organic side chain, both of which impart specific properties to the material.

The objective of this in-vitro study was to assess the ability of an experimental hybrid-glass infiltrant, both of basic formulation and formulation with added 1% hydroxyapatite (HAp) nanoparticles, to arrest the progression of

artificial WSLs, and to compare it with Icon. The null hypotheses were: 1) there is no difference in WSLs micro-hardness and surface tissue loss between the specimens submitted to caries simulation and the controls, in each treatment group 2) there is no difference in micro-hardness and surface tissue loss among differently treated WSLs.

2. Materials and methods

2.1. Specimen preparation

Freshly extracted bovine incisors (n = 68) were cleaned and cut at cemento-enamel junction with a precision saw (Isomet 1000; Buehler, Lake Bluff, IL, USA) to separate roots from the crowns. The crowns were subsequently embedded in acrylic resin (Vertex, Dentimex, Zeist, the Netherlands) to prepare disk-shaped specimens with vestibular surfaces exposed on one side. The vestibular enamel surfaces were flattened and polished with water-cooled 400-, 600-, 1200- and 2500-grit sandpaper. Right and left parts of enamel surfaces were protected with adhesive tape, leaving a 3 mm-wide experimental window available for the formation of artificial WSLs. These protected areas served as the reference for profilometric measurements of average surface level (see below). Artificial WSLs were formed by incubating specimens for 10 days (37°C) with 8% methylcellulose gel and 0.1 M lactic acid solution (pH = 4.6), following the protocol by ten Cate et al. [22]. Briefly, specimens were first covered with a 5 mm thick layer of 8% methylcellulose gel and left overnight at 4 °C. The specimens were subsequently covered with an equal mass of 0.1 M lactic acid solution with pH adjusted to 4.6 with 1 M KOH, and incubated at 37 °C for 10 days. After the incubation, specimens were washed thoroughly with running deionized water to remove the methylcellulose gel and blow dried to confirm the formation of artificial white spot lesions. Prepared specimens were then stored at 100% relative humidity at 4 °C until use.

2.2. WSLs infiltration and cariogenic challenge

The specimens were first randomly assigned to four groups, according to the WSLs treatment: 1) no-treatment control (NTC) specimens (n = 16), 2) infiltration with Icon (Icon) (n = 18), 3) infiltration with experimental infiltrant with 1% HAp nanoparticles (EXP-HAp) (n = 17), and 4) infiltration with experimental infiltrant without HAp nanoparticles (EXP) (n = 17). Composition and application procedure of the tested materials are shown in Table 1. Half of specimens from each group were then exposed to cariogenic challenge in form of 7-days long pH cycling (pH-cycled subgroup), while the second half was placed in distilled water for the same period of time (non-cycled control subgroup). pH cycling consisted of alternate immersion of the specimens in demineralization solution (2 mM $CaCl_2$, 2 mM KH_2PO_4, 75 mM acetic acid, 0.1 mM Tris buffer, pH adjusted to 4.6 using 1 M KOH) for 4 h, and remineralization solution (1.25 mM $Ca(NO_3)_2$, 0.90 mM KH_2PO_4, 129.91 mM KCl, 59.93 mM Tris buffer, pH 7.4) for 20 h per day, at 37°C. Between demineralization and remineralization cycles, specimens were rinsed with deionized water.

2.3. Surface micro-hardness, tissue loss and morphology assessment

Before the analyses, protective tapes were removed from the specimens. Surface Knoop micro-hardness was measured with micro-hardness-testing machine (Mitutoyo HM-124; Mitutoyo, USA) at five points in each specimen, with a diamond indenter (pressing load: 0.1 N, dwell time: 30 s). Tissue loss in WSLs was assessed by measuring surface roughness (R_a) and surface average level, with stylus profilometer (Mitutoyo S-J 400; Mitutoyo America, Aurora, IL, USA). R_a was measured along five lines in the middle of WSLs (cutoff length: 0.25 mm). Surface average level was measured relative to the areas of sound enamel control on both sides of WSLs. Profilometer stylus moved across reference areas and WSL between them (speed: 0.5 mm/s, vertical sensitivity: 0.23 nm, distance between recording points: 0.69 nm), whereby three line-scans were recorded per specimen, and average depth of WSL surface was calculated for each scan with custom-made software. Finally, three randomly selected specimens from each group and subgroup were analyzed with SEM. Specimens were dried, sputter-coated with gold (S150B, Edwards, Burgess Hill, England) and WSLs surface morphology was observed with SEM (EVO® Scanning Electron Microscope, Carl Zeiss Microscopy GmbH, Jena, Germany) at the acceleration voltage of 10 kV.

2.4. Statistical analysis

Statistical analyses were performed using SPSS software (IBM, Armonk, New York, USA). Normality of data distribution was assessed with Saphiro-Wilk test, and equality of variances across the groups with Levene's test. The effect of factors 'treatment' and 'pH cycling' on SMH, R_a and surface average level data, as well as the interaction between these two factors, was estimated with two-way ANOVA. Differences in SMH, R_a and surface average level between non-cycled and pH-cycled specimens were tested with Man-Whitney U test, while the differences in these variables among different treatment groups were tested with Kruskal-Wallis test with pairwise comparisons. The level of significance for all tests was set at 0.05.

3. Results

According to two-way ANOVA, all three measured variables, Knoop SMH, R_a and average surface level of WSLs, were significantly affected by factors 'treatment' and 'pH cycling'. In addition, there was an interaction between these two factors in all three data sets.

There was a significant difference in SMH among different treatment groups both in non-cycled and pH-cycled specimens ($p < 0.001$ and $p < 0.000$, respectively) (Table 2). In non-cycled specimens, Icon group had significantly higher hardness compared to all other groups. In pH-cycled specimens, however, hardness was significantly lower compared

Table 1 – Composition and the application procedure of the tested materials.

Materials	Composition*	Application procedure
Icon (DMG, Hamburg, Germany)	**Icon-Dry**: Ethanol (99%) **Icon-Infiltrant**: Triethylene glycol dimethacrylate (TEGDMA) (70–95%); Camphorquinone (CQ) (< 2.5%); additives	Acid etching with 37% orthophosphoric acid gel (5 s); rinsing with deionized water (15 s) and air drying; Application of Icon-Dry for 30 s and air drying; First application of Icon-Infiltrant and letting it set for 3 min; removal of excess material with a cotton pellet; light-curing for 40 s (Elipar™ S10, 3 M ESPE, St. Paul, MN, USA); Second application of Icon-Infiltrant and letting it set for 1 min; Removal of excess material with a cotton pellet; light-curing for 40 s
EXP (Hybrid Glass Poland Sp. z o. o., Wroclaw, Poland)	Hybrid-glass monomer/oligomer (15%); ethanol	Acid etching with 37% orthophosphoric acid gel (5 s); rinsing with deionized water (15 s) and air drying; Pre-warming of the specimens for 10 min at 37°C; First application of EXP and letting it set and cure for 1 min; Pre-warming of the specimens for 10 min at 37°C; Second application of EXP and letting it set and cure for 1 min
EXP-HAp (Hybrid Glass Poland Sp. z o. o., Wroclaw, Poland)	Hybrid-glass monomer/oligomer (15%); 1% HAp nanoparticles; ethanol	Same as in the EXP group.

*As disclosed by the manufacturer

Table 2 – WSLs Knoop hardness, surface roughness (R_a) and surface average level relative to the sound enamel, expressed as median and interquartile range (between square brackets) values, for all groups and subgroups.

Knoop hardness (KHN)			
Group	Non-cycled (control)	pH-cycled	p value (Mann-Whitney U test)
NTC	20.6 [9.5] A	4.8 [6.5] a	< 0.001
Icon	32.1 [23.5] B	9.3 [3.9] b	< 0.001
EXP-HAp	20.3 [17.6] A	6.0 [2.4] a	< 0.001
EXP	18.1 [8.2] A	25.9 [10.5] c	< 0.001
p value (Kruskal-Wallis test)	< 0.001	< 0.001	
Surface average roughness (R_a) (μm)			
NTC	0.04 [0.01] B	0.24 [0.39] a	< 0.001
Icon	0.46 [0.68] C	0.75 [0.93] b	0.021
EXP-HAp	0.29 [0.20] A	0.60 [0.45] b	< 0.001
EXP	0.19 [0.20] A	0.16 [0.20] a	0.017
p value (Kruskal-Wallis test)	< 0.001	< 0.001	
Surface average level relative to sound enamel (μm)			
NTC	0.3 [2.5] B	-2.6 [4.1] b	< 0.001
Icon	-8.8 [5.4] A	-12.7 [4.4] a	0.010
EXP-HAp	-3.1 [4.5] A	-12.5 [7.2] a	< 0.001
EXP	-0.1 [3.4] B	0.7 [2.3] c	0.099
p value (Kruskal-Wallis test)	< 0.001	< 0.001	

Values designated with same letters were not significantly different according to pairwise comparisons. Significance values in pairwise comparisons have been adjusted by the Bonferroni correction for multiple comparisons. Level of significance was set at 0.05 in all statistical tests.

to non-cycled counterparts in all treatment groups, except in EXP group, where it was significantly higher. In addition, among pH-cycled specimens, EXP group had the highest SMH, followed by Icon group, while EXP-HAp group had a significantly lower SMH, comparable to that of NTC group (Fig. 1a).

There was a significant difference in WSLs R_a among different treatment groups both in non-cycled and pH-cycled specimens ($p < 0.001$) (Table 2). Among non-cycled specimens, NTC group had significantly lower R_a compared to all other groups, followed by EXP and EXP-HAp groups, while Icon group had significantly higher R_a than all other groups. In pH-cycled specimens, R_a was significantly higher compared to non-cycled counterparts in all treatment groups, except in EXP group, where it was lower (Fig. 1b).

There was a significant difference in WSLs average surface level (relative to the surrounding sound enamel) among different treatment groups both in non-cycled and pH-cycled specimens ($p < 0.001$) (Table 2), with Icon group having the lowest surface level, followed by EXP-HAp group, in both subgroups. In all treatment groups, WSLs surface level relative to the surrounding sound enamel significantly decreased in pH-cycled specimens compared to non-cycled

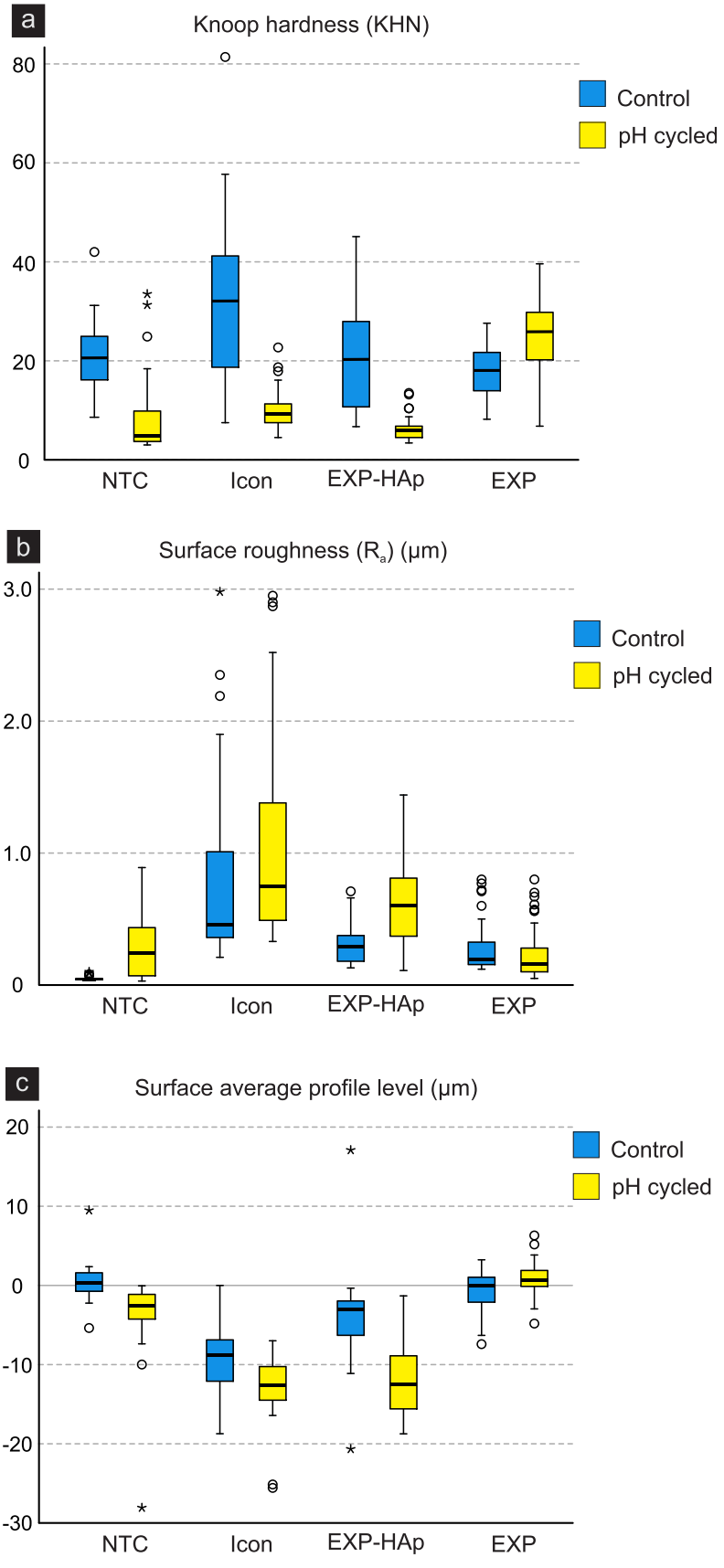
a
Knoop hardness (KHN)
80
60
40
20
0
Control
pH cycled
NTC
Icon
EXP-HAp
EXP
b
Surface roughness (Ra) (µm)
3.0
2.0
1.0
0
Control
pH cycled
NTC
Icon
EXP-HAp
EXP
c
Surface average profile level (µm)
20
10
0
-10
-20
-30
Control
pH cycled
NTC
Icon
EXP-HAp
EXP

Fig. 1 – Knoop hardness (KHN) (a), surface roughness (R_a) (μm) (b) and surface average level relative to the surrounding sound enamel (μm) (c) in all groups (NTC, Icon, EXP-HAp and EXP) and subgroups (non-cycled and pH-cycled). Box-and-whisker plots show the medians (black central lines) with 25% and 75% quartiles (lower and upper box edges) and interquartile range (IQR) between them; whiskers represent the lowest value within 1.5 IQR from the lower quartile, and the highest value within 1.5 IQR from the upper quartile; the outliers are shown as dots and asterisks (extreme outliers).

controls, except in EXP group, where it remained at a similar level (Fig. 1c).

SEM analysis showed that the WSLs surface of non-cycled controls appeared the smoothest in NTC and EXP group, followed by EXP-HAp group, while the surface of Icon-infiltrated specimens appeared somewhat rougher (Fig. 2a, c, e and g). In pH-cycled specimens, WSLs surface was damaged with signs of demineralization and disintegration, compared to non-cycled counterparts, in all treatment groups (Fig. 2b, d and f), except in EXP, where it remained intact (Fig. 2 h).

4. Discussion

This in-vitro study aimed to assess the efficacy of experimental hybrid-glass-based infiltrant, and its variant with 1% HAp nanoparticles, in arresting artificial WSLs. The material was developed in order to provide a more biocompatible and biostable alternative to currently only commercially available, resin-based infiltrant, Icon. Based on the presented results, which demonstrated a significant change in WSLs SMH and tissue loss following a cariogenic challenge, the first null hypothesis was rejected. In addition, due to observed significant differences in the measured variables among differently treated WSLs, the second null hypothesis was rejected as well.

Infiltration with the experimental material without HAp nanoparticles was able to completely arrest WSLs, as no decrease in SMH and profile level and increase in R_a, were observed in pH-cycled compared to non-cycled specimens. SMH of WSLs infiltrated with the experimental material was even higher in the pH-cycled specimens compared to the controls kept in deionized water for the same period of time. This suggests that acidic environment might have enhanced material's curing reaction, which is no surprise since acids or bases are typically used to catalyze the polycondensation reaction of this material. As the largest portion of the material is cured within one minute after application at temperature above 30°C, pH cycling accelerated the subsequent, final phase of curing, which resulted in increased SMH. The hardening reaction of this hybrid-glass polymer is based on the condensation of Si-OH bonds and formation of Si-O-Si moiety and H_2O molecule, and is triggered by evaporation of ethanol-based solvent upon the application, which allows diluted monomers/oligomers to come in close proximity. Temperature sensitivity of the hardening reaction was the reason for the pre-warming of specimens at 37°C before material application.

In addition to acid-catalyzed SMH increase, no tissue loss was observed at the surface of WSLs infiltrated with the experimental infiltrant. This not only confirms the absence of tissue demineralization, but also suggests that our material is resistant to acid degradation, at least in the short term, which enables it to form an efficient diffusion barrier. The smooth, glassy surface of the infiltrated lesion, is also advantageous because it contributes to a lower plaque accumulation, which is one of the main culprits for the formation of caries lesions in the first place.

Conversely, resin-based infiltrant Icon was not able to stop further WSLs demineralization in this study, as considerable decrease in WSL SMH was observed in pH-cycled resin-infiltrated specimens, together with the increase in roughness, decrease in average lesion level and alterations of surface morphology, suggesting the diffusion of acids into the lesions. On the one hand, our findings differ from some previously reported in-vitro [23–25], in-situ [26] and short-term clinical data [27–29], which have demonstrated the ability of Icon to arrest or slow down the WSLs progression. On the other hand, our results corroborate the findings of previously reported work, in which a considerable decrease in microhardness and mineral loss of artificial resin-infiltrated WSLs, was observed after a cariogenic challenge, suggesting the lesion progression [30–33].

Possible reasons for the inefficiency of Icon to prevent acid diffusion in the mentioned studies, including ours, are related to the inherent drawbacks of TEGDMA-based resin. Due to its relatively high hydrophilicity, TEGDMA is susceptible to water sorption, elution in water-based medium, and hydrolysis [11,34]. These processes are accelerated by the lack of fillers [12] and relatively low degree of conversion of Icon, which is shown to be around 50% [30,35], most likely due to formation of thick oxygen inhibition layer. It has indeed been shown that monomer elution from Icon-infiltrated WSLs was significantly higher compared to other resin-based materials such as sealants, adhesives, and composites [36]. Only after the removal of oxygen inhibition layer by polishing, which was not performed in our study, was monomer elution comparable with other groups.

In order to improve mechanical properties and remineralizing effect of our material, 1% of HAp nanoparticles was admixed to the basic formulation and tested in this study. To our surprise, HAp-containing version failed to provide any protection against cariogenic challenge, as significant decrease in SMH was observed in this group, together with surface tissue loss and deterioration, similar to that of untreated WSLs. HAp nanoparticles are often added to different dental materials to enhance their biocompatibility, bioactivity and mechanical properties [37–39]. The achieved effects, however, depend, on the particle morphology, crystallinity, size, concentration, etc. [40]. Amorphous HAp nanoparticles, such as those used in this study, are more soluble in acidic environment, compared to highly crystalline, rod-shaped HAp nanoparticles [30]. Their dissolution during pH cycling left the porosities in the material behind, which allowed acid diffusion and further tissue demineralization. Similar to our results, when amorphous HAp

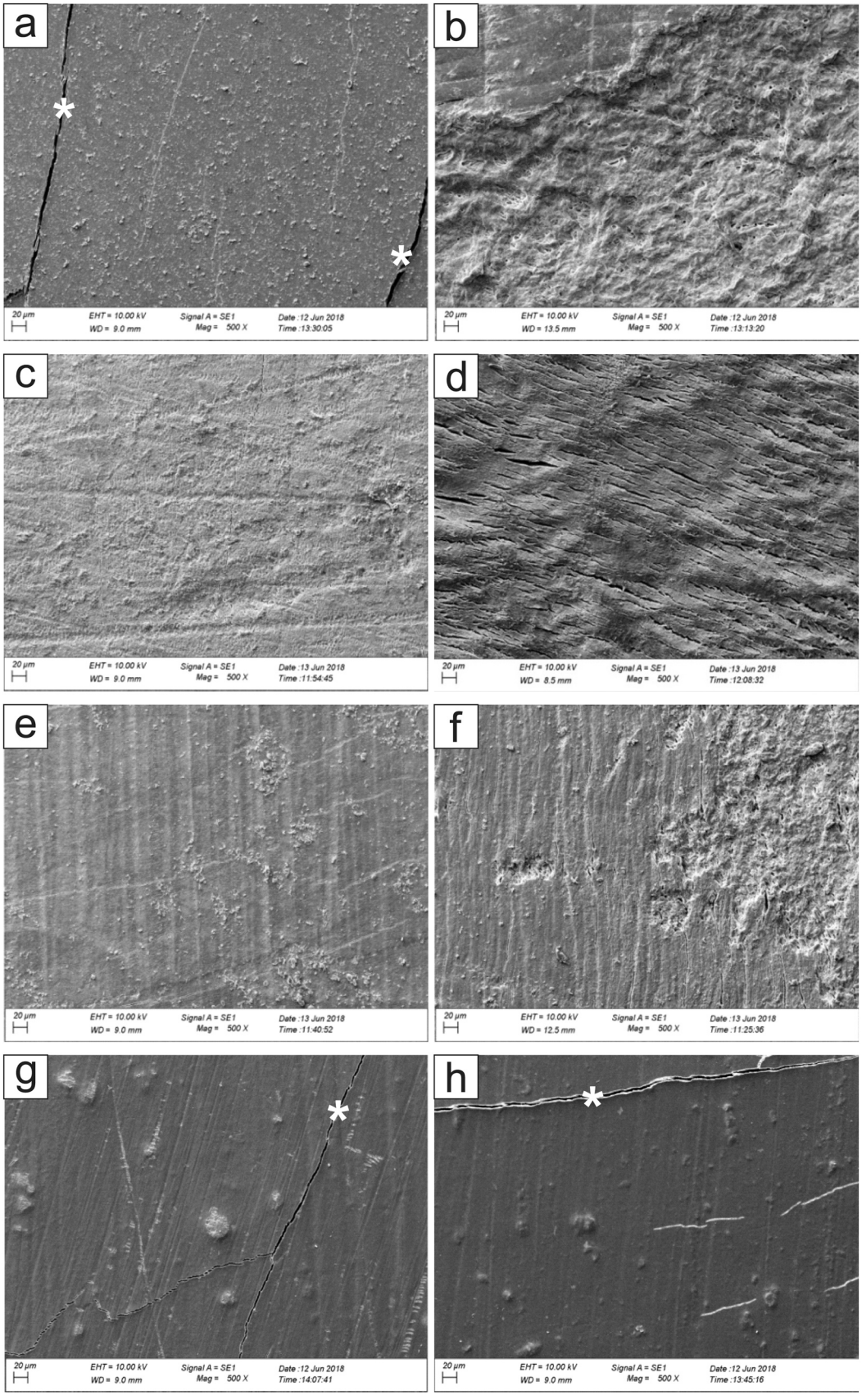
a
20 µm EHT = 10.00 kV WD = 9.0 mm Signal A = SE1 Mag = 500 X Date :12 Jun 2018 Time :13:30:05
b
20 µm EHT = 10.00 kV WD = 13.5 mm Signal A = SE1 Mag = 500 X Date :12 Jun 2018 Time :13:13:20
c
20 µm EHT = 10.00 kV WD = 9.0 mm Signal A = SE1 Mag = 500 X Date :13 Jun 2018 Time :11:54:45
d
20 µm EHT = 10.00 kV WD = 8.5 mm Signal A = SE1 Mag = 500 X Date :13 Jun 2018 Time :12:08:32
e
20 µm EHT = 10.00 kV WD = 9.0 mm Signal A = SE1 Mag = 500 X Date :13 Jun 2018 Time :11:40:52
f
20 µm EHT = 10.00 kV WD = 12.5 mm Signal A = SE1 Mag = 500 X Date :13 Jun 2018 Time :11:25:36
g
20 µm EHT = 10.00 kV WD = 9.0 mm Signal A = SE1 Mag = 500 X Date :12 Jun 2018 Time :14:07:41
h
20 µm EHT = 10.00 kV WD = 9.0 mm Signal A = SE1 Mag = 500 X Date :12 Jun 2018 Time :13:45:16

Fig. 2 – Representative scanning electron micrographs (mag. 500x) of WSL surfaces in non-cycled (a, c, e and g) and pH-cycled specimens (b, d, f and h). WSL surface in NTC group was smooth in non-cycled specimens (a) due to the presence of mineralized surface layer, which was mostly disintegrated in pH-cycled specimens (b) due to demineralization. Icon-infiltrated WSLs had slightly rougher surface in non-cycled specimens (c), and noticeably rougher surface with signs of demineralization and degradation in pH-cycled specimens (d). WSLs in EXP-HAp group presented a smooth surface in non-cycled specimens (e), while large parts of the surface were disintegrated in pH-cycled specimens (f). There was no difference in the surface morphology of WSLs between non-cycled (g) and pH-cycled specimens (h) in EXP group, since in both subgroups lesion surface remained smooth. Asterisks (*) denote the cracks originating from the specimen drying procedure and vacuum present in SEM chambre.

nanoparticles were added to a resin infiltrant, its ability to prevent WSL progression diminished, while the addition of crystalline HAp nanoparticles resulted in a significantly improved material [30].

In this study, progression of artificial WSLs was estimated primarily based on the change in their SMH. This approach was chosen because it is rather simple, fast and robust, and it has often been used to assess WSL progression [30–32]. It should be, however, kept in mind that SMH of infiltrated WSLs reflects not only the hardness of affected enamel but also of the infiltrating material itself. Nevertheless, the comparison with the untreated WSLs allows estimating these effects separately. Next to the SMH, WSLs surface tissue loss, and changes in surface morphology, were also assessed in this study, to obtain a more complete picture of the effect of acidic environment. Another frequently used method for WSL progression assessment is transverse microradiography (TMR) [23,24,26]. Although destructive and time-consuming, this radiographic technique is considered the gold standard in cariology for the assessment of lesion depth and mineral loss. However, this method cannot assess the alterations of the infiltrating material by acids, which can compromise its caries arresting ability.

Artificial WSLs created on bovine enamel were used in this study. Although this is a standardized and commonly used method in in-vitro studies, it has limited external validity due to the differences between artificial and natural WSLs. Artificial WSLs have much smaller depth (approximately 80–100 µm) than natural WSLs (300–900 µm) [41]. Consequently, the thickness of the infiltrant is much lower, and the effect of its degradation may be more pronounced in artificial lesions. Also, artificial WSLs have proportionally thinner mineralized surface layer. Therefore, WSLs were briefly (5 s) etched with orthophosphoric acid to dissolve surface layer and improve infiltration in this study, instead of prolonged etching with hydrochloric acid, as recommended for the clinical use of Icon [42].

5. Conclusions

Within the limitations of this study, we conclude that infiltration with experimental, hybrid-glass-based material, could prevent the progression of WSLs. Mostly thanks to its higher stability in acidic/cariogenic environment, our material performed significantly better than resin-based infiltrant Icon, and could be considered its viable alternative in future.

The unique formulation of this material could potentially impart it with other desirable properties, such as good biocompatibility, low bacterial accumulation and even bioactivity, which will be assessed in future studies.

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