Protection of sound enamel and artificial enamel lesions against demineralisation: caries infiltrant versus adhesive

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Abstract: OBJECTIVE: To compare the protective potential of a conventional adhesive, a caries infiltrant and a combination of both against acidic challenge in vitro. METHODS: One-hundred-and-fifty discs from bovine lower central incisors were fabricated. Seventy-five samples remained untreated, whereas the other half was subjected to a demineralisation process (14 days, acidic buffer, and pH 5) to create artificial enamel lesions. Specimens were then radioactively irradiated, and each 15 sound and demineralised specimens were treated with a caries infiltrant (Icon, DMG), an unfilled adhesive (Heliobond, IvoclarVivadent) or a combination of infiltrant and adhesive. Specimens treated with the adhesive followed by a flowable composite (TetricEvoFlow, IvoclarVivadent) served as positive control, while untreated specimens served as negative control. All samples were then subjected to lactic acid for 3 weeks at pH 4. Loss of apatite was determined using the radiochemical method of liquid scintillation. Data were statistically analysed by Kruskal-Wallis-test, one-way ANOVA and Scheffe's post hoc tests (p .05). RESULTS: In both sound enamel and artificial caries lesions, untreated specimens showed the highest rate of apatite loss, whereas enamel treated with the adhesive and the flowable composite showed almost complete protection surface against dissolution. The caries infiltrant, the adhesive and the combination of both were able to decrease enamel dissolution, but the adhesive and the combination of adhesive and infiltrant were more effective than the infiltrant alone. CONCLUSION: Within the limitations of this in vitro study, the application of an adhesive (alone or in combination with the caries infiltrant) is more effective to protect enamel dissolution than the infiltrant alone.

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Short title: Enamel dissolution protection by infiltrant and adhesive

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Keywords: Enamel; demineralisation; caries infiltration; liquid scintillation; SEM; In vitro

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Results: In both sound enamel and artificial caries lesions, untreated specimens showed the highest rate of apatite loss, whereas enamel treated with the adhesive and the flowable composite showed almost complete protection surface against dissolution. The caries infiltrant, the adhesive and the combination of both were able to decrease enamel dissolution, but the adhesive and the combination of adhesive and infiltrant were more effective than the infiltrant alone.

Conclusion: Within the limitations of this in vitro study, the application of an adhesive (alone or in combination with the caries infiltrant) is more effective to protect enamel dissolution than the infiltrant alone.

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Introduction

Pit and fissure sealing with composite resins bonded to enamel is an effective procedure for caries prevention.[6,32] Based on the good clinical results with sealing of these mentioned predilection sites, approaches have been made to extend this preventive concept to smooth enamel surfaces.[25,26] Among the latter, interproximal areas have the highest risk for caries development.[1,9] The infiltration of caries lesions with low-viscosity light curing resins is considered as treatment option for non-cavitated lesions, which are not expected to arrest or remineralise. In contrast to the conventional sealing concept where a resin layer is created on the surface, caries infiltrants aim to penetrate the porous lesion body thoroughly.[7,14] Compared to conventional dental adhesives, caries infiltrants were optimized for rapid capillary penetration and exhibit a very low-viscosity, low contact angle to enamel and high surface tension.[13] As a consequence, laboratory experiments demonstrated a significantly deeper penetration in the lesion body than conventional adhesives.[11,12] However, in spite of the deeper penetration of caries lesions, it has not be shown yet that caries infiltration as an increased capability to prevent progression of demineralisation than conventional sealing of the lesion.[19] While both infiltrants[11,18,20] and adhesives[16,24] were shown to be effective in reducing progression of artificial enamel lesions, their protective potential on sound enamel was not compared so far. This aspect might of interest in areas neighbouring the lesion, which might be of risk to be affected by demineralisation if the lesion progresses.

Therefore, the aim of the present study was to compare the potential of a conventional adhesive, a caries infiltrant and a combination of both to protect sound enamel and artificial enamel lesion against an acidic challenge in vitro. The hypotheses were that protective capability of the infiltrant and the adhesive do not differ, and that the combination of both is comparable to the respective treatments alone.

Methods

Specimen preparation

Specimens were prepared from 150 extracted bovine permanent incisors. Only tooth material free of defects or cracks was selected. The teeth were cleaned and sectioned at the enamel-cementum junction using a water-cooled cutting wheel (Isomet, Buehler, Illinois, USA). The labial surface of the teeth was cleaned by brushing during 25 min. Brushing was performed in a custom-made brushing machine at 2.5 N with a manual toothbrush (ParoM39, ESRO, Thalwil, Switzerland) and a toothpaste-slurry (6 g Depurdent, Wild SA, Basel, Switzerland; 10 g artificial saliva; 8 drops of a silicone antifoam, Fluka, Art. Nr. 85390, Switzerland). Discs with a diameter of 7 mm were cut from the mid-labial aspect of each tooth using a custom-made diamond-coated trephine (80µm, Intensiv SA, Lugano-Grancia, Switzerland). The discs were then flattened from the bottom to approximately 2 mm in height (Struers, Birmensdorf).

Then, half of the specimens were immersed for 14 days in an acidic buffer containing 3 mM $CaCl_2$ x 2 H_2O, 3 mM KH_2PO_4, 50 mM acetic acid, 6 µM MHDP, KOH to adjust the initial pH to 5.0 and traces of thymol.[4] This solution was renewed each second day to keep the pH constant.

The Specimens were irradiated at the "Atominstitut der Österreichischen Universitäten" (Vienna, Austria) with an exposure time of 85 min to a neutron flow of 1.02 x 10 e^{12} neutrons/cm^2.s, resulting in a ß⁻-activity of 0.56 Gbq/p.

Treatment procedure

Before the enamel treatment, all surfaces except the top enamel test surface were carefully sealed with unfilled bonding agent (Heliobond, Ivaoclar-Vivadent, Schaan, Liechtenstein) and a flowable composite resin (Tetric EvoFlow, Ivoclar Vivadent, Schaan, Liechtenstein). Therefore, the lateral cutted enamel was carefully etched with 35% phosphoric acid (UltraEtch 35%, Ultradent Products, South Jordan, USA) and rinsed with distilled water for 60 s before the sealing procedure.

Then, 75 sound and 75 demineralised samples were randomly assigned to five groups (15 samples each) and treated as follows:

1. The enamel surface was etched for 60 s with 35% phosphoric acid (UltraEtch 35%, Ultradent Products, South Jordan, USA), and then rinsed distilled water for 60 s. After air drying of the surface, an unfilled adhesive (Heliobond, Ivoclar Vivadent, Schaan, Liechtenstein) was applied for 20 s with a microbrush, then thinned with mild air (1-2 s) and light cured for 20 s (3M Espe Elipar S10, 3M Espe, Seefeld, Germany).
2. The enamel surface was etched for 2 min with 15% hydrochloric acid (Icon Etch, DMG, Hamburg, Germany) and then rinsed with water spray for 30 s. Air drying of the surface was followed by application of ethanol (Icon Dry, DMG, Hamburg, Germany) for 30 s and additional air drying. Then, the low-viscosity resin infiltrant (Icon Infiltrant, DMG, Hamburg, Germany) was applied on the surface for 3 min by means of the sponge applicator provided with the resin infiltration system. After light-curing for 40 s, the infiltrant was applied for further 60 s and again light-cured for 40 s.
3. Specimens were first treated with the caries infiltrant as described under point 2. Then, the adhesive was applied and light cured for 20 s.
4. Same treatment as under point 1 but additional application of a flowable composite resin material (Tetric EvoFlow, Ivoclar Vivadent, Schaan, Liechtenstein), which was light cured for 40 s (3M Espe Elipar S10, 3M Espe, Seefeld, Germany); positive control
5. No sealing; negative control

The composition of the adhesive systems and materials based on the manufacturers' instructions are listed in Table 1.

Acidic challenge and evaluation of mineral loss

All specimens were immersed at 37 °C under constant motion for up to 21 days in 5 ml of artificial saliva or 5 ml lactic acid (15 µmol/l, pH 4).

After 1, 2, 4, 7, 11, 14 and 21 days, immersion solutions were collected, the weight of each sample was measured and 1 ml 2N HCl plus 4 ml distilled water were added to an end volume of 10 ml. ^{32}P was assessed by determining the Cherenkow radiation. For calculation of mineralised tissue loss standard solutions of ^{32}P and apatite, together with a background sample were prepared, as described elsewhere.[27] In short, the resulting counts per minute (CPM) were calculated to the date of irradiation, resulting in decays per minute (DPM). The arithmetic mean of the samples was calculated and the amount of dissolved mineralised tissue was calculated with the aid of the apatite standard.

Morphological assessment

For the SEM analysis, two additional specimens of each group were prepared for examination of the surfaces directly after application of the test materials and after 21 d storage in lactic acid. Specimens were dehydrated in a desiccator device (Optivac, König Physik, Diemelstadt, Germany) using blue silica gel, sputter-coated with gold and examined at 10 kV (Zeiss Supra 50 VP, Zeiss, Oberkochen, Germany).

Data presentation and analysis

Statistical analysis was performed with StatView (Version 5, Abacus Concepts Inc., Berkley, USA).

Cumulative mineral loss in µg apatite was calculated for each group. Normal distribution was tested using Kolmogorov-Smirnov and Shaprio-Wilk tests.

As data were not normally distributed, non-parametric statistics (Kruskal-Wallis test) were applied within sound specimens and within specimens with artificial lesions to analyse possible differences between the groups at the different time points. Non-parametric statistics were followed by one-way ANOVA, separately for sound and demineralised enamel, and Scheffe`s post-hoc tests. The level for statistical significance was set at $p < 0.05$.

Results

Mineral loss

In both sound enamel and enamel exhibiting artificial lesions, Kruskal-Wallis tests revealed significant differences between the groups at each time point. Cumulative enamel loss at the evaluated time points is presented in Figures 1 and 2.

Untreated specimens showed the highest rate of apatite loss over the whole observation period, whereas enamel treated with the adhesive and the flowable composite showed almost complete protection surface against dissolution at all time points. The caries infiltrant, the adhesive and the combination of the infiltrant and the adhesive reduced apatite dissolution significantly, but the adhesive and the combination of infiltrant and adhesive were more protective against acid dissolution than the infiltrant alone, irrespective whether sound or demineralised enamel was treated.

Morphological aspects

The morphological appearances of the sound and predemineralised specimens before and after storage in lactic acid for 21 days are presented in Figure 3, respectively.

Untreated enamel exposed to lactic acid showed only minute surface changes (Figure 3 B). Application of the caries infiltrant system induced a severe demineralisation with a honeycomb appearance of enamel (Figure 3 I). In contrast, the enamel surface appeared smooth after application of the adhesive or the combination of caries infiltrant and adhesive, even after storage in lactic acid for 21 days (Figure 3 M/N).

Artificial-caries like lesions revealed a demineralised surface (Figure 3 C). While application of the caries infiltrant increased/modified demineralisation (Figure 3 K), the adhesive induced a sealing of the surface (Figure 3 G), which was still present after 21 d storage in lactic acid (Figure 3 H/P).

Discussion

This study demonstrated that both a caries infiltrant system and a conventional adhesive protect sound enamel and artificial enamel lesions from further demineralisation. However, the adhesive and the combination of infiltrant and adhesive were more effective than the caries infiltrant alone.

In the present study, bovine teeth were used, which are widely used in resin infiltration tests.[16,19] Following the same irradiation protocol as in previous studies,[28,29] the cumulative mineral loss of untreated samples comparing the results of the present investigation revealed remarkably comparable results with median losses of 580 and 660 µg apatite, respectively. The samples had the same pretreatment and geometry, but the embedding was slightly different. In the present study, we used an adhesive embedding of the discs, which revealed a more reliable lining and sealing of the irradiated samples during the dissolution process. The median mineral loss in the former study was still 26 µg apatite as compared to nearly zero in this investigation.

As in previous studies lactic acid was used for the demineralisation regime as it represents the main organic acid produced by dental plaque bacteria.[5,28,29] However, it should be acknowledged that the study conditions differed from the in vivo situation in that there was no protective salivary pellicle and that enamel surfaces were in continuous contact with the acidic challenge.

In this study artificial lesions of about 150 µm depth were used.[2] However, although these lesions were shown to exhibit some of the typical histological structure of intact enamel caries including the intact surface layer,[8] the histological condition of the surface und subsurface layers is unpredictable in the clinical situation. The surface layer is thicker in natural (~ 40 to 50 µm and) compared to artificial (~ 20 µm) lesions,[3] but it was shown that hydrochloric acid etching is required not only for natural[23] but also for artificial caries lesions[2] to remove the surface layer completely and allow for successful penetration of the infiltrant. It was therefore decided to use the infiltration material as provided in the commercially

available kit, i.e. using hydrochloric acid conditioning material. Heliobond was chosen as a representative of an unfilled enamel bonding agent as it exhibits potential to penetrate at least early enamel lesions.[10,13,30] However, it has to be considered that natural enamel lesions might be much deeper than the artificial lesions created in the present study. Therefore, an incomplete penetration under natural caries conditions is possible.

While resin infiltrants led to a complete, but partially inhomogenous penetration of artificial lesions, Heliobond was shown to induce the formation of a homogenous surface layer, but penetrated only the outer part of the lesion.[13] The results of the present study indicate that the superficial penetration and surface coating of the adhesive might be more effective in protecting enamel dissolution than the penetration of the infiltrant. This observation is also evident in the morphological evaluation of this study. While the surfaces treated with the caries infiltrant exhibited a demineralised appearance, the other test groups revealed a dense adhesive layer, respectively. While the amount of TEGDMA in the resin infiltrant promotes the penetration of the resin,[22] it also increases the susceptibility to degradation compared to resins containing less TEGDMA [17,31]. Moreover, the surface leakage might be a result of polymerization shrinkage and polymerization stress of the resin.[21] However, surprisingly, the infiltrant alone showed some protection against the acid dissolution even in sound enamel, which does not represent a substrate for the infiltrant.

However, in both substrates, the combination of both materials was not more effective than the adhesive alone. At least for artificial enamel lesions it was assumed that the infiltration of the demineralised subsurface layer and the sealing of the surface might have an additive effect on the dissolution protection. As the bonding of the adhesive is not impaired on infiltrated enamel surfaces,[33] this observation cannot be explained yet. However, covering infiltrated lesions with an adhesive layer might be beneficial in terms of surface properties, as surface roughness of infiltrated lesions is comparatively high [15].

Conclusion

The application of an adhesive (alone or in combination with the caries infiltrant) is more effective to protect enamel dissolution than the infiltrant alone within the limitations of this in vitro study.

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Captions

Figure 1

Results of the sealing ability after different treatments of sound enamel specimens determined as loss of apatite in µg (box-plot illustration; horizontal bars: medians; boxes: inter-quartile areas; error bars: 10^{th} and 90^{th} percentiles; dots: extreme values). Identical superscript capitals at the different time points represent no statistically different values.

Figure 2

Results of the sealing ability after different treatments of artificial enamel lesion specimens determined as loss of apatite in µg (box-plot illustration; horizontal bars: medians; boxes: inter-quartile areas; error bars: 10^{th} and 90^{th} percentiles; dots: extreme values). Identical superscript capitals at the different time points represent no statistically different values.

Figure 3

SEM images at 5'000x of representative samples after different treatments of sound enamel and artificial caries lesions before (baseline) and after 21 days lactic acid exposure.

Figure 1

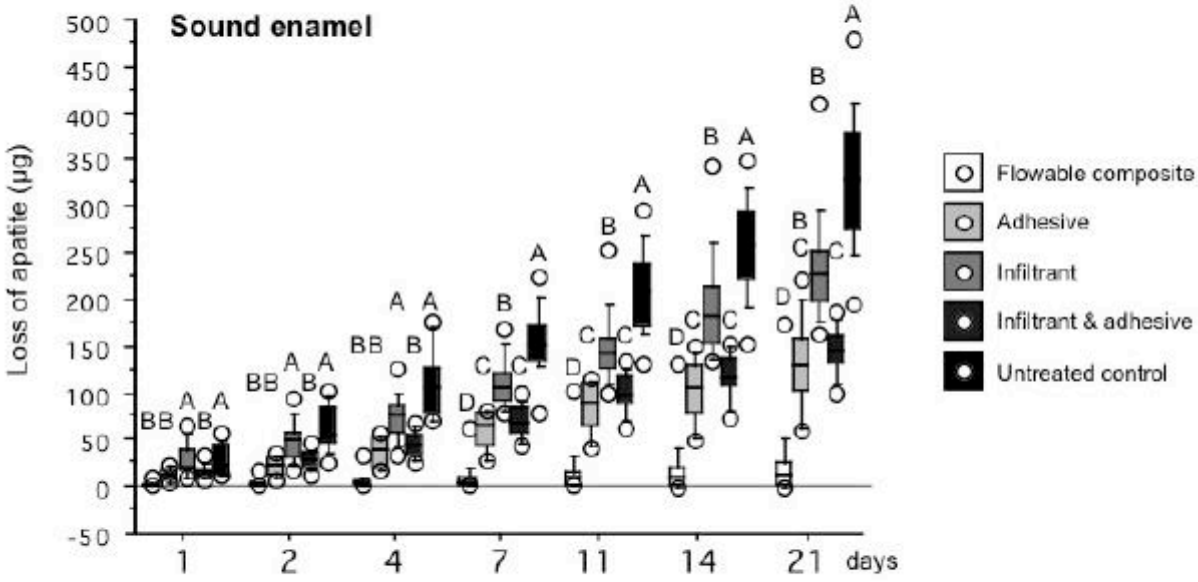
Sound enamel
Loss of apatite (µg)
500
450
400
350
300
250
200
150
100
50
0
-50
1
2
4
7
11
14
21
days
Flowable composite
Adhesive
Infiltrant
Infiltrant & adhesive
Untreated control

Figure 2

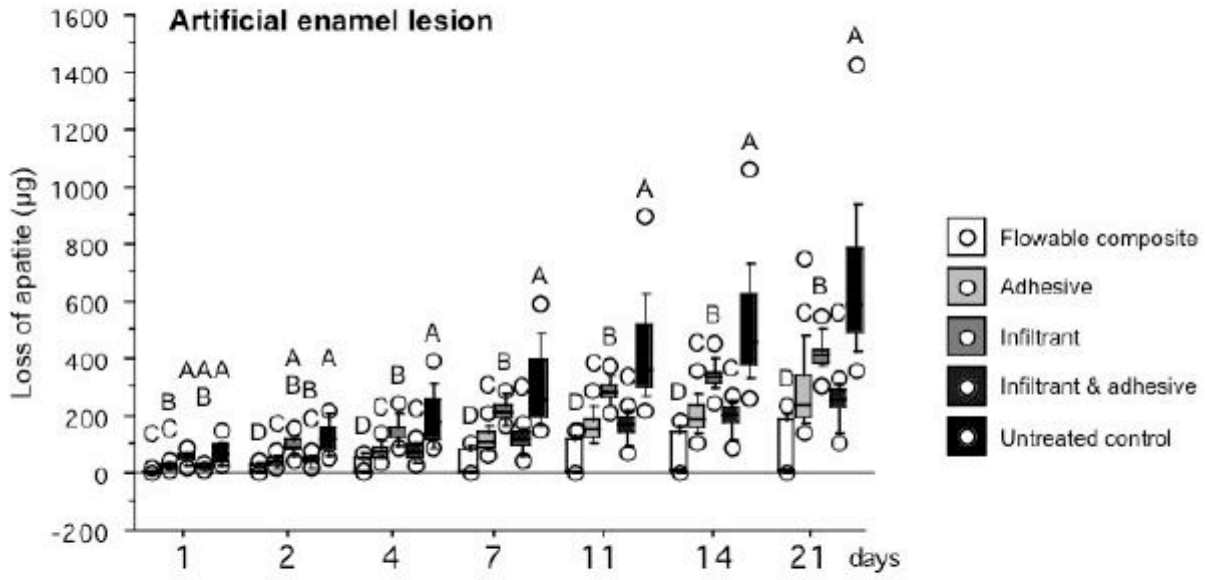
Artificial enamel lesion
Loss of apatite (µg)
1600
1400
1200
1000
800
600
400
200
0
-200
1
2
4
7
11
14
21
days
Flowable composite
Adhesive
Infiltrant
Infiltrant & adhesive
Untreated control

Figure 3

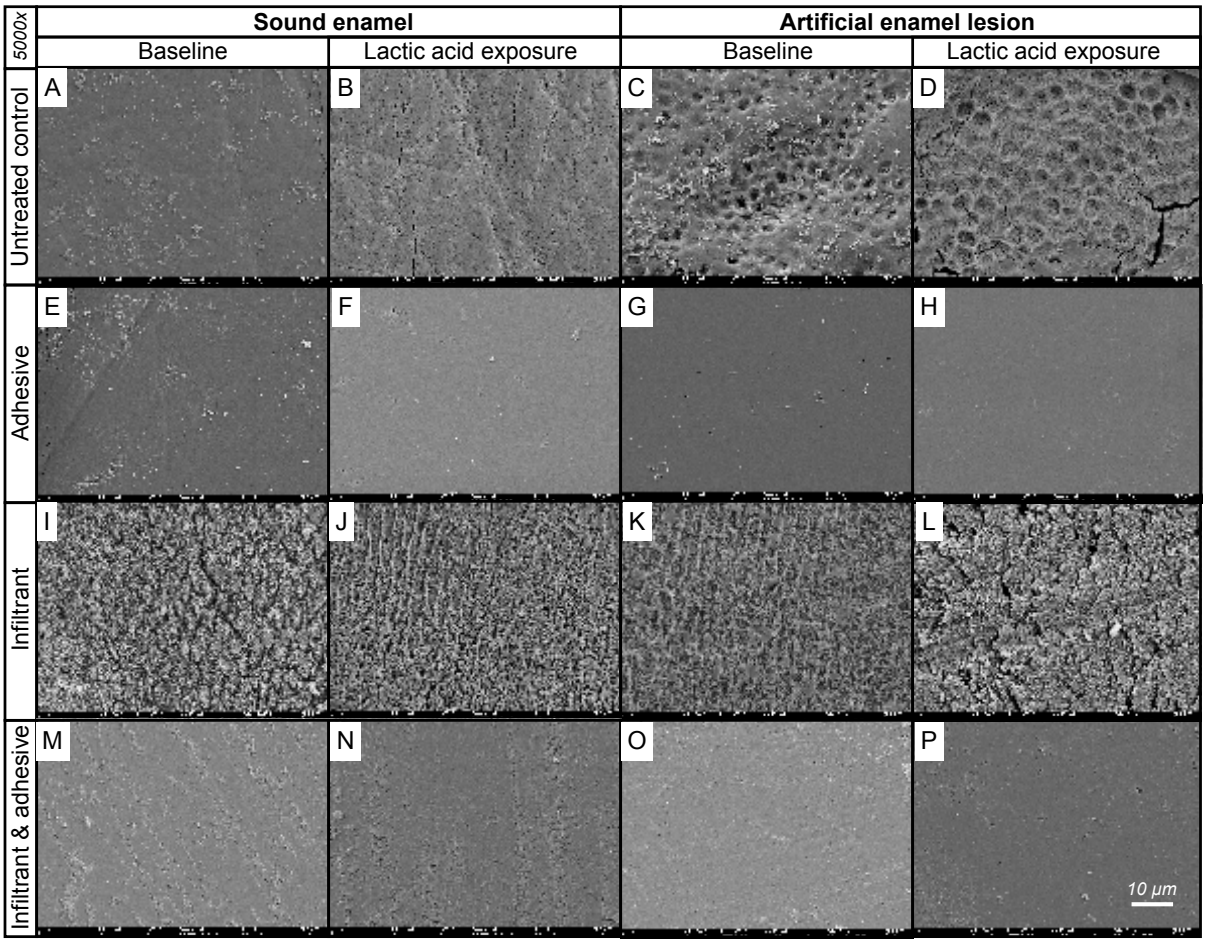
5000x
Sound enamel
Artificial enamel lesion
Baseline
Lactic acid exposure
Baseline
Lactic acid exposure
Untreated control
Adhesive
Infiltrant
Infiltrant & adhesive
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
10 µm

Table 1

Composition of the unfilled bonding resin (conventional adhesive) and the low viscosity infiltrant system accordingly to the manufacturers' information.

Product	Composition	Lot number	Manufacturer
Adhesive (Heliobond)	Bis-GMA, TEGDMA, initiators, stabilizers	L24292	Ivoclar Vivadent, Schaan, Liechtenstein
Infiltrant (Icon)	Icon etch: 15% hydrochloric acid, water, pyrogenic silica, tenside, pigments	632178	DMG, Hamburg, Germany
	Icon Dry: ethanol		
	Icon Infiltrant: TEGDMA-based resin		