Article

Use of Ytterbium Trifluoride in the Field of Microinvasive Dentistry—An In Vitro Preliminary Study

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Abstract: Background: The aim of this study was an attempt at determining the quantity of the degree of the addition of ytterbium trifluoride as a marker, aimed at facilitating the observation and assessment of the effectiveness of penetration into the decalcified enamel of human teeth of an experimental preparation with the characteristics of a dental infiltrant and a commercially available commercial preparation called Icon. Methods: The test material was 20 decalcified human teeth. The first half of the batch was soaked in Icon, the second half in an experimental preparation with the characteristics of a dental infiltrant and with a component responsible for bacteriostaticity. Ytterbium trifluoride was added to both preparations to facilitate the microscopic observations: 20 mg/1 g in the first phase of the experiment, 60 mg/1 g in the second phase. Results: YbF₃ particles could not be found in the teeth from the first phase of the experiment. Particles rich in ytterbium could be found only in the teeth from the second phase of the experiment, with three times the content of ytterbium. Conclusion: The addition of 6% ytterbium trifluoride (both commercial and laboratory synthesized) facilitates microscopic observation, allowing the conclusion that both Icon and the experimental preparation with the characteristics of a dental infiltrant penetrate the decalcified enamel of a human tooth. The SEM analysis of the preparations in terms of content and particle size of ytterbium trifluoride shows that the distribution is heterogeneous. Large size particles predominate, yet particles with a diameter of less than 1 µm were also found. This may confirm the fact that most of them have probably agglomerated. The method of scattering YbF₃ nanoparticles in the infiltrant resin requires further work so that they do not appear as agglomerates.

Keywords: infiltration; microinvasive dentistry; scanning electron microscopy; ytterbium trifluoride

1. Introduction

Infiltration is an alternative method to the treatment of caries in its initial stage, by filling the gap between prevention and invasive therapy. This method preserves the tooth tissue, which is otherwise removed during the mechanical treatment [1]. The treatment which uses the infiltration method is a relatively new and developing therapy. However, even at this stage, a number of its advantages can be distinguished: the mechanical stabilization of demineralised enamel, the preservation of healthy tooth tissue, the inhibition of the lesion progress, i.e., stopping the caries in its initial stage, minimizing the appearance of secondary caries, lack of possibility of hypersensitivity, and high aesthetic value [2]. The infiltration of early caries lesion means filling the intergranular spaces of demineralised enamel with low viscosity resin. A carious spot sealed in this way is inaccessible to cariogenic bacteria and the acids produced by them, which results in the inhibition of the carious process. The resins used in the infiltration method are characterized by low viscosity and density, low wetting angle and high surface tension, which due to capillary forces allows them to penetrate the material of decalcified enamel prisms [3,4].

Dental infiltrates are used to treat initial caries in both deciduous and permanent teeth. Indications for their use are as follows: stage of caries (from E1 to D1, according to Manji), the improvement of aesthetics; in the case of small and moderate carious changes, at the early stage and on smooth surfaces which indicate no loss of continuity of enamel tissue. Contraindications for infiltration include developmental enamel damage, erosion, injuries and allergies to any component [5]. Orthodontic treatment is a common cause of tooth demineralisation, particularly in vestibular areas. Proper hygiene is difficult to maintain while using permanent braces, which can often result in the development of caries in the form of demineralisation, i.e., a white spot around the braces. The use of a preparation based on polymer resin allows to reduce the white carious spot and prevent the further development of carious lesions [6]. The infiltration technique can also be used in the treatment of fluorosis, which is a condition that develops as a result of excessive tooth exposure to fluorine compounds during enamel development [2].

Icon preparation (DMG, Hamburg, Germany) is currently the only infiltration material available on the market. It is a substance based on methacrylate resins, which has the ability to penetrate deep into demineralised enamel. However, despite its many advantages, the depth of penetration of the preparation into the decalcified enamel cannot be clearly determined, because Icon is transparent and there is no addition of substances that provide contrast in the X-ray examination. Moreover, it does not meet all requirements for infiltrants. One of the features that infiltrants should have is bacteriostaticity, i.e., the ability to inhibit the growth and multiplication of bacteria. Icon does not meet this requirement [5,7].

The permeability of an infiltrant into enamel depends on the morphology of the white spot change, especially the degree of porosity of the tissues. Active spots are more susceptible to infiltration than inactive ones [8]. According to the Turska-Szybka et al.'s study, the method of initial caries treatment with Icon DMG preparation is 100% effective. The study was carried out on a group of 36 children aged 18 to 71 months, in whom earlier changes in the form of white caries spots were found. The observations were conducted for 6 months. The results showed that the caries progression did not occur during the application of the preparation [9].

Kielbassa et al. concluded that the degree of infiltration down to the depth of 60 µm of enamel was sufficient to prevent further enamel demineralisation [2].

The observation of the infiltrant under a microscope is difficult because it is the colour of the tooth tissue, which makes it difficult to determine the extent of its penetration into the area of decalcified tissue.

Ytterbium is a heavy element and can be easily detected using an X-ray microanalyser, therefore ytterbium trifluoride is used in contemporary dentistry as a contrast agent [10]. Attempts have been made to synthesize an experimental preparation with features of a dental infiltrant that uses ytterbium trifluoride as a marker, which will allow to examine the depth of infiltrant penetration. Ytterbium

will be used as a marker during X-ray microanalysis, while the addition of fluoride is intended to have remineralisation and cariostatic effects, protecting the enamel from secondary caries [10–12]. Fluorine also interferes with the adhesion of bacterial plaque to the acquired membrane and weakens the carbohydrate metabolism of plaque bacteria e.g., *Streptococcus mutans* and *Lactobacillus* spp. Fungi also influence the formation of dental plaque such as *Candida albicans*. The questions that remains to be answered is what amount of ytterbium trifluoride addition to low viscosity resin will allow the evaluation of its penetration into the decalcified enamel of a human tooth [12].

There remains an attempt to answer the questions: will the addition of ytterbium trifluoride to the resin of low viscosity enable the assessment of its penetration into the decalcified enamel of human teeth? If so, what amount of ytterbium trifluoride should be added to the resin of low viscosity to allow the analysis of the degree of penetration of the preparation into the enamel of a human tooth?

The aim of this study was an attempt at determining the quantity degree of the addition of ytterbium trifluoride as a marker, aimed at facilitating the observation and assessment of the effectiveness of penetration into the decalcified enamel of human teeth of an experimental preparation with the characteristics of a dental infiltrant and a commercially available commercial preparation called Icon.

2. Materials and Methods

The test material was 20 human teeth—molars and premolars with preserved anatomical crowns, removed for prosthetic and orthodontic reasons. After extraction, the teeth were stored in a chloramine solution. Before the onset of the study, the chloramine solution was removed, and the teeth were rinsed very thoroughly in distilled water and left in it for 24 h. After rinsing and drying, the teeth were chemically decalcified for 4 weeks in an incubator at 37 °C [13]. After the decalcification process was completed, the teeth were rinsed three times in distilled water and left in it for 24 h. Finally, the teeth were dried.

In addition to the commercial Icon preparation, an experimental mixture with features of a dental infiltrant (Table 1) [14] was also used for the purpose of the study. It had a component responsible for bacteriostaticity (metronidazole in the form of PMMA_n-MTZ). Commercial ytterbium trifluoride (YbF₃) (Nanoshell, Salt Lake City, UT, USA) in the form of a fine white powder of 40–80 nm granulation was added to the first half of both preparations, while the second half of ytterbium trifluoride was synthesized under laboratory conditions. Ytterbium trifluoride in the first part of the study was added in the amount of 20 mg/1 g or 2%. Due to difficulties in finding ytterbium trifluoride particles during the microscopic analysis, the experiment was repeated on the next batch of teeth, where preparations were enriched with three times higher content of ytterbium fluoride than before. Ytterbium trifluoride was added in the amount of 60 mg/1 g or 6%. Additionally, after the addition of YbF₃, each of the four preparations was placed in an ultrasound bath for 2 h in order to mix the components better together and to avoid the agglomeration of ytterbium trifluoride particles [12].

Table 1. Composition of the experimental preparation.

Components	Quantity (g)	Percent Content (%)
TEGDMA	3.75	75
HEMA	1.25	25
PMMA _n -MTZ	0.05	1 *
DMAEMA	0.05	1 *
Camphorquinone (CQ)	0.025	0.5 *

* jointly TEGDMA and HEMA.

Four groups of mixtures were used for the tests:

- 1) Experimental preparation + YbF₃ (Nanoshell, Willmington, NC, USA);
- 2) Experimental preparation + YbF₃ obtained in the lab;

- 3) Icon preparation + YbF₃ (Nanoshell, Willmington, NC, USA);
- 4) Icon preparation + YbF₃ obtained in the lab.

In the first phase of the experiment, half of the decalcified teeth (10) were used, which were divided into two zones A and B by a red line along the long tooth axis (Figure 1). In 5 teeth, infiltration was performed in zone A with the use of the experimental preparation with commercial YbF₃ by Nanoshell (2%). In zone B, an experimental preparation with laboratory YbF₃ (2%) was applied. In the next 5 teeth, infiltration in zone A was performed with the use of the Icon preparation with commercial YbF₃ from Nanoshell (2%). In Zone B, it was Icon with laboratory YbF₃ (2%). In the second phase of the experiment, the second batch of 10 decalcified teeth was used and 6% ytterbium trifluoride was added. The preparations were applied according to the recommendations of the manufacturer of Icon. Initially, the tooth enamel was etched with Icon-Etch, i.e., 15% hydrochloric acid for 2 min, then the whole surface was thoroughly rinsed with a torrent of water for 30 s and dried with air without oil and water. Subsequently, Icon-Dry (99% ethyl alcohol) was applied to the already dried surface and left for 30 s, after which it was dried again with a stream of clean air. The application of experimental preparations and Icon was carried out in two stages. The first layer of each preparation was applied to the respective tooth zones and left for 3 min. After that time, the applied layer was cured with a polymerization lamp for 40 s. Then, the second layer was applied and left for 1 min and then cured for 40 s with a polymerisation lamp. A LEDEX WL-070 lamp (Dentmate, New Taipei City, Taiwan) was used during the polymerisation process. After soaking, the teeth were cut perpendicularly to the pre-determined line with the use of a diamond saw and pre-polished (Figure 2). The pre-prepared specimens were immersed in chemically hardened plastic (Duracryl Plus, SpofaDental, Ostrava, Czech Republic) and then finally polished using Optidisc Soft-lex abrasive discs (Kerr, Brea, CA, USA) with an increasingly fine grain size. The polished specimens were cooled with distilled water. The test material was then polished with pure wool discs slightly moistened with distilled water (TDV, Pomerode, Brazil). The last stage was polishing them with pastes (Chema Polish I, Chema Polish II, CHEMA-ELEKTROMET, Rzeszów, Poland) (Figure 3). Finally, the specimens were sprayed with a layer of carbon and thus prepared for observation under a Hitachi S4200 scanning electron microscope (Tokyo, Japan).

Figure 1. A cut tooth with marked zones—the outside.

Figure 2. A cut tooth with marked zones—the inside.

Figure 3. A sample of a decalcified tooth prepared for microscopic observation.

In order to conduct this research, an application was filled with the Bioethics Committee of the Medical University of Silesia in Katowice. The Committee issued an approving motion no. KNW/0022/KB/310/18/19 on the day of 14.01.2019.

3. Results

The research conducted with the use of a scanning electron microscope was focused on the determination of the degree of penetration of the tested preparations into the structure of decalcified tooth enamel. The addition of YbF₃ should, as assumed, facilitate this task.

At first, the samples with 2% ytterbium trifluoride were tested and after a long search, only single YbF₃ particles were found (Figure 4). This was probably due to the small amount of YbF₃ in these preparations. Therefore, the tests were repeated, this time on the sample labelled as 4.1.1, where YbF₃ was at 6%. On the basis of microscopic observations, it can be concluded that the YbF₃ content is not high, but certainly no particles of YbF₃ can be seen on the surface of the dental enamel, probably since these particles penetrate the tubules. The SEM analysis of the preparations in terms of content and particle size of ytterbium trifluoride shows that the distribution is heterogeneous. Large size particles predominate, yet particles with a diameter of less than 1 μm were also found. This may confirm the fact that most of them have probably agglomerated. Selected examples are shown in Figure 5a–c, where ytterbium-rich particles are marked with arrows.

Figure 4. Image showing the distribution of ytterbium in tooth tissues infiltrated by a preparation with 2% YbF₃ content.

Figure 5. (a–c) Images of tooth sample 4.1.1 with marked particles that contain ytterbium trifluoride in tooth tissues infiltrated by the preparation with 6% YbF₃ content.

In the part of the tooth covered with Icon, no particles containing ytterbium were observed. Even at high magnification, it is difficult to see the differences in the structure of the enamel layer near the edge of the tooth and at a considerable distance from it. Therefore, just on the basis of the differences in the morphology of these areas alone, it is not possible to determine the depth of penetration of the tested preparations.

Comparing the results of the microscopic images of teeth with 2% YbF₃ content with teeth containing three times higher concentration, i.e., 6% of YbF₃, it can be concluded that in teeth with 2% YbF₃, particles are poorly visible. In teeth with 6% of YbF₃ content, the amount of ytterbium particles is more satisfactory, but it is not possible to compare the depth of penetration of the applied preparations. There are no nanoparticles on the surface of the teeth, only under the surface of enamel, which indicates that ytterbium trifluoride penetrates deep into the enamel without remaining on its surface. However, at this stage of observation, it is difficult to clearly determine the degree of penetration of preparations into the decalcified enamel. No differences were observed between the preparations with commercial YbF₃ when compared to the compound synthesized at a laboratory.

4. Discussion

For the infiltration effect of the change to be effective, and for the white stain to be no longer noticeable, the micropores in the enamel structure should be filled (soaked) with low viscosity resin penetrating to the appropriate depth, so that the difference in the refractive index in decalcified tissue areas is eliminated. Healthy enamel has a refractive index RI = 1.62, whereas the micropores of enamel filled with water have RI = 1.33 and altered (dried) enamel have RI = 1.0. Therefore, differences in the refractive index in different areas of enamel are clinically noticeable as white spots or cloudiness. If micropores of the enamel are filled with resin (RI = 1.52), which cannot evaporate after curing,

in contrast to an aqueous environment (e.g., saliva), the difference in refractive indexes between healthy enamel in filled pores is negligible and the change is no longer clinically noticeable [15].

The use of infiltrants in dentistry is also effective during procedures aimed at improving the aesthetics of dentition, e.g., during treatments of lesions caused by fluorosis. Todorova VI et al. treated two patients (women, 13 and 24 years old) with white spots caused by medium stages of fluorosis. Both the application and finishing procedures were carried out according to the manufacturer's recommendations. The researchers showed a stable visual effect of Icon application after 12 and 18 months. They also emphasized the need for regular follow-up visits to observe the discolorations of the infiltrated area and to re-polish the applied layer of the preparation. [16].

Some researchers pointed out the need to determine the degree by which the infiltrant penetrates decalcified tissues. Experiments in microinvasive dentistry, within the area of initial caries, are difficult to carry out because the surface layer of enamel is strongly mineralised. The decalcification area is sub-surface, so the surface layer makes it difficult, or even impossible, for the resin to penetrate the changed tissue. For this reason, the first step to prepare tissues for the use of an infiltrant is to etch them with a 15% hydrochloric acid solution for 120 s. In the infiltration method, the use of hydrochloric acid as an etching agent is more effective than the use of 37% orthophosphoric acid (as a classic etching agent), as proved by Paris et al. and Meyer-Lueckel H et al. in their studies. [15–19].

Schnabl et al. attempted to determine the degree by which an infiltrant penetrates tooth tissue in the case of developmental changes by demonstrating the effectiveness of the infiltration method in the treatment of molars in a 17 year-old patient. The treatment was performed on four partially impacted third molars with congenital enamel hypomineralisation. The patient's dentition demonstrated symptoms specific for MIH (Molar incisor hypomineralisation), such as yellowish-brown discolouration, the loss of translucency and surface roughness [20]. Clinically, the teeth showed defects classified as code 2–3 according to ICDAS (International Caries Detection and Assessment System). After surgical tooth extraction, infiltration was performed with the Icon preparation enriched with fluorescent dye (Lumogen Orange; BASF, Ludwigshafen, Germany), following the manufacturer's recommendations. After analysis with bright field, fluorescent and confocal laser scanning microscopy, the penetration depth of the preparation was recorded to be 2 mm. The significant depth of penetration into the tissues results from the reduction of enamel density and its structure disturbance, related to the structure typical for teeth affected by MIH. In addition, teeth with cavities covering a third of the external part of dentin were qualified for the study. The methodology of research on teeth with MIH was related to the control group of human teeth without congenital hypomineralisation, affected by initial caries of the fissures classified by the same researcher as code 1 according to ICDAS. In the control group, the depth of penetration was much shallower, including only infected enamel tissue. The authors of the study described the efficacy of infiltration as satisfactory and indicated it as one of the possible methods of treatment of tooth enamel lesions with developmental hypomineralisation. [21].

In an in vitro study, Meyer-Lueckel et al. compared the depth of resin penetration in extracted human teeth with the use of confocal double fluorescence microscopy and obtained the following results: 159 μm after 30 s, 152 μm after 1 min, 414 μm after 3 min, and 407 μm after 5 min. This proved that the optimal resin penetration time is 3 min, i.e., as recommended by the manufacturer [17].

Turska-Szybka et al. examined the depth of resin penetration in deciduous tooth enamel in an analysis with the use of a scanning electron microscope. They determined that the degree of resin penetration increased with the depth of carious lesion in deciduous teeth and is $\sim 182.2 \pm 119.4 \mu\text{m}$, proving that the resin penetrates at least half the depth of a carious lesion in enamel (64.2% of the carious spot depth) [3]. Microscopic observations carried out on 20 removed human teeth by Subramaniam P et al. showed that the depth of Icon penetration into the enamel of removed human teeth varies between $1.96 \pm 1.00 \mu\text{m}$ and $6.06 \pm 3.32 \mu\text{m}$. The obtained results show that the fluctuation of penetration depth by the infiltrating resin is therefore significant. This is probably due to the fact that different methods of observation and measurement were used to assess the degree of penetration. A standard procedure of enamel infiltration with the Icon commercial preparation was carried out to

prepare the teeth, then the samples were incubated for 24 h in methylene blue at 37 °C, after which the teeth were cut in half. Forty samples prepared in this way were observed with the use of an optical microscope with 80× magnification by taking pictures of the infiltrated areas, then the depth of penetration was measured with the use of Motic software [22].

The research group of Silva et al. used 60 extracted human premolar and molar teeth to evaluate the degree of improvement in the aesthetics of decalcified tissues. Teeth with defects on the chewing surface were classified with codes 1, 2 and 3 according to ICDAS with the use of QLF, i.e., quantitative light-induced fluorescence. Teeth were infiltrated with Icon according to the manufacturer's recommendations. Then, the QLF tests were repeated. A statistically significant decrease in the observed lesion area, a decrease in fluorescence ΔF and a decrease in fluorescence in the carious lesion area ΔQ were noticed when compared to the measurements taken before the infiltration with Icon. In addition, the researchers analysed the depth of penetration of the infiltrant and its relation to the ICDAS code: for code 1 the penetration was 0.23 ± 0.17 , for code 2 it was 1.25 ± 1.08 , and for code 3 1.20 ± 0.82 , respectively [23]. The obtained results are similar to those recorded by Min et al., who analysed changes of the QLF value in infiltrated carious defects in smooth tooth surfaces of bovine teeth [24].

In the experimental research on an infiltrant, its penetration coefficient (PC) plays an important role. The research carried out by the team lead by H. Meyer-Lueckel shows that the higher the value of the penetration coefficient, the deeper the preparation penetrates the decalcified enamel tissue and the better it seals it. [25].

The difference in the values of the penetration coefficient between our own experimental preparation synthesized for the purpose of the study in comparison to the commercially available Icon preparation is small, which was noticed by M. Skucha-Nowak et al. [13].

Problems with the visualization of infiltrant penetration while using the scanning electron microscope may be caused by particle sizes of ytterbium trifluoride (YbF₃) while compared to decalcified enamel ducts, which are about 2.5 µm in diameter. Ytterbium trifluoride (YbF₃) particles tend to aggregate and form larger structures, which may prevent infiltration. The presence of YbF₃ particles may suggest that it was not the result of infiltration by enamel preparations but rather their mechanical displacement during sample preparation, or more precisely during polishing. In the case of the tested teeth, the presence of YbF₃ grains was found both on the tooth surface and in deeper layers. Therefore, it is difficult to determine the method of infiltration [13,26]. On the other hand, the penetration depth values obtained for both preparations are very low as the depth of etched enamel canals with Icon-Etch is 30–40 µm and the dental infiltrant should fully seal them to block the access for carious bacteria and the products of their metabolism. [27].

During experiments, some researchers assess the degree of infiltrant penetration into the tissues with the use of bovine teeth [24,28], whereas the current study was performed on extracted human teeth. This is essential because bovine teeth feature higher porosity as compared to human teeth, which significantly facilitates and promotes penetration. Nevertheless, bovine teeth are frequently used in studies as they are easier to obtain and have a similar composition to human teeth [29–31].

5. Conclusions

The addition of 6% ytterbium trifluoride (both commercial and laboratory synthesized) facilitates microscopic observation, allowing the conclusion that both Icon and the experimental preparation with the characteristics of a dental infiltrant penetrate the decalcified enamel of a human tooth.

Based on the SEM analysis of the preparations in terms of the content and particle size of ytterbium trifluoride, it was concluded that their distribution was heterogeneous. Large size particles predominate, yet particles with a diameter of less than 1 µm were also found. This confirmed that most of them have probably agglomerated.

The method of scattering YbF₃ nanoparticles in the infiltrant resin should be refined so that they do not appear as agglomerates.

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