

ORIGINAL PAPERS

Adv Clin Exp Med 2016, 25, 6, 1293–1301
DOI: 10.17219/acem/63795

© Copyright by Wrocław Medical University
ISSN 1899–5276

MAŁGORZATA SKUCHA-NOWAK^{1, A-F}, ANNA MERTAS^{2, B}, MARTA TANASIEWICZ^{1, E}

Using an Electron Scanning Microscope to Assess the Penetrating Abilities of an Experimental Preparation with Features of a Dental Infiltrant: Preliminary Study

¹ Department of Conservative Dentistry with Endodontics, Medical University of Silesia in Katowice, Bytom, Poland

² Chair and Department of Microbiology and Immunology, School of Medicine with the Division of Dentistry, Medical University of Silesia in Katowice, Zabrze, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. The resin infiltration technique is one of the micro-invasive methods whose aim is the penetration of demineralized enamel with a low viscosity resin. This technique allows the dentist to avoid the application of mechanical means of treatment.

Objectives. The objective of this preliminary study was to attempt to determine the possibilities of using an electron microscope to assess the penetrating abilities of an experimental preparation with features of a dental infiltrant and to compare the depth of infiltration of the designed experimental preparation with an infiltrant available on the market.

Material and Methods. A bioactive methacrylate monomer based on PMMA with built-in metronidazole was synthesized. The commercially available Icon solution (with contrast agent YbF₃) and the experimental solution were applied to the relevant parts of teeth. The dissected sections along the long tooth axis and polished surfaces were then examined with use of an electron scanning microscope.

Results. The backscattered electron technique gives much better results than the secondary electron method as it makes it possible to localize even very small YbF₃ particles.

Conclusions. The authors concluded that the backscattered electron technique gives much better results than the secondary electron method as it makes it possible to localize even very small particles of the contrast agent. In order to prevent blockage of decalcified enamel tissue by ytterbium trifluoride (YbF₃) grains, a nanoparticle form of that compound should be used (that is, particles with sizes in the range of 10⁻⁹ m) (*Adv Clin Exp Med* 2016, 25, 6, 1293–1301).

Key words: microinvasive dentistry, ytterbium trifluoride, electron scanning microscope, experimental infiltrants.

Micro-invasive dentistry aims at stopping dental caries in its early stage. It assumes elimination of a bacterial infection at an early stage and tight sealing of a decalcified spot on the surface of enamel using a material with adhesive chemical properties. The resin infiltration technique is one of the micro-invasive methods which allows the dentist to avoid application of mechanical means of treatment [1, 2].

Enamel prisms are the basic structural element of human enamel. They are long, rod-shaped structures, aligning in vertical and horizontal lines

from the enamel-dentine boundary to the surface of the crown. The maximal length of a prism, which is also the maximal thickness of enamel, is approx. 2500 µm (2.5 mm), while the minimal length in the cervical region amounts to tens of micrometers. There are several million prisms in a single tooth with a width of approximately 5 µm near dentine and approximately 9 µm near the surface [3]. The space between prisms is filled with an interprismatic substance, which contains mainly water and organic compounds. Prisms are basophilic, while the interprismatic substance is acido-

philic [3, 4]. Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is the main component of the hard tooth tissue. Its crystals are stable in alkaline environments, with pH value over 7 and destabilize in acidic environment, with a pH value below 5. The development of dental caries is related to the notion of cariostasis, which is the result of equilibrium between the subsequent processes of demineralization and remineralization that occur at the surface of enamel. Fluorides play an important role in the process of cariostasis as they increase absorption of calcium and phosphorus ions and facilitate creation of fluorohydroxyapatite, which features increased resistance to acids [3, 4]. Healthy enamel never changes its color, while a pale-white spot on its surface is a sign of ongoing demineralization. This is a so-called white carious spot (*macula alba*) and it is a result of the decreased content of mineral components in the subsurface region of enamel [1, 4]. The structure of enamel is shaped during the process of odontogenesis and depends on the organic and inorganic components which are provided to the body (proteins, A, D and C vitamins, amino acids, and trace elements, e.g. fluorine, phosphorus, calcium and iron) [3]. Enamel is a homogeneous structure, and its surface is the hardest as it contains the highest concentration of phosphate ions, calcium, fluorine and chlorine. The ability to exchange the hydroxide ion for a fluoride ion is one of the most important features of hydroxyapatites. This allows for creation of fluorapatite which is more resistant to dissolution [3]. As teeth become older, the level of calcium, sulfur, potassium and zinc increases, therefore the susceptibility to dental caries depends on the age of a given tooth [4].

A dental infiltrant is a polymer resin-based substance which has the ability to penetrate the demineralized parts of a tooth and to seal enamel canals [5]. The phenomenon of infiltration is defined in medicine as permeation of a liquid into pores or grooves in a substance. This notion is used for treatment methods which use low-viscosity resins with high permeability to penetrate subsurface carious lesions. Infiltration with a resin is a type of micro-invasive dental treatment as it does not require any interference with the hard tooth tissue. This makes it possible to reduce and then inhibit the development of a white spot carious lesion and is used in those cases where the loss of enamel has not yet occurred [2, 5]. In order to allow a polymer resin to be used as a dental infiltrant, it should have the following features: the ability to polymerize to a solid state, a resistance to chemical and mechanical influence, no interaction with food and medicine, appropriate cosmetic and aesthetic appearance at the tooth surface, no toxic influence on the

oral cavity, and bacteriostaticity (inhibition of bacterial development) [5]. Such features of an infiltrant as: hydrophilicity, low viscosity, low contact angle and high surface tension allow it to penetrate the inner parts of enamel [2]. Bacteriostaticity, which is the ability to inhibit the growth and stop the multiplication of bacteria, is a very important feature of an infiltrant. Icon (Icon caries infiltrant; DMG; Hamburg, Germany) is the first, and so far the only, preparation used as an infiltrant. It is a substance based on methacrylic resins which has the ability to infiltrate the demineralized parts of enamel. After polymerization in the canals of decalcified enamel, it creates a network of hardened resin which cuts off the cariogenic (caries-inflicting) bacteria and their metabolic by-products, therefore preventing the development of dental caries. As mentioned above, bacteriostaticity is a very important feature dental infiltrants should have [5], whereas Icon does not. This was the basis for the idea of creating an experimental solution which would supplement that feature with the addition of metronidazole [6, 7].

The objective of the preliminary study was to attempt to determine the possibilities of using an electron microscope to assess the penetrating abilities of an experimental preparation with the features of a dental infiltrant and to compare the depth of infiltration of the designed experimental preparation with an infiltrant available on the market.

Material and Methods

Laboratory Materials and Methods

An analysis of the ^1H NMR spectra of the commercially available infiltrant (Icon) and a review of available literature made it possible to establish the composition of the experimental preparation (composed of triethylene glycol dimethacrylate, 2-hydroxyethyl methacrylate, 2-(7-methyl-1,6-dioxo-2,5-dioxa-7-octenyl) trimellitic anhydride, metronidazole, N,N-dimethylaminoethyl methacrylate, and camphorquinone), which could potentially have the properties of a dental infiltrant with additional bacteriostatic properties [8, 9] (Table 1). The mixture was poured into dark bottles and thoroughly stirred. One gram of the commercially available Icon preparation was also transferred to a dark bottle. Two percent YbF₃ (Alfa Aesar, Ward Hill, USA) was added to both bottles (one containing 1 g of the experimental solution and the other 1 g of Icon) and then stirred with a magnetic stirrer without any access of light.

Table 1. Detailed composition of the experimental preparation

Component	Amount [g]	Percentage content [%]
TEGDMA	3.75	75
HEMA	1.25	25
PMMAAn – MTZ	0.05	1*
YbF ₃	0.02 (20 mg/1 g)	2
DMAEMA	0.05	1*
Camphorquinone (CQ)	0.025	0.5*

* as a ratio to total mass of monomers;
 TEGDMA (triethylene glycol dimethacrylate, Fluka, Buchs, Switzerland);
 HEMA (2-hydroxyethyl methacrylate, Acros, New Jersey, USA);
 PMMAAn (2-(7-methyl-1,6-dioxo-2,5-dioxa-7-octenyl) trimellitic anhydride);
 MTZ (metronidazole, Acros, New Jersey, USA);
 YbF₃ (Ytterbium trifluoride, Alfa Aesar, Ward Hill, Massachusetts, USA);
 DMAEMA (N,N-dimethylaminoethyl methacrylate, Merck, Darmstadt, Germany);
 CQ (camphorquinone, Aldrich, St. Louis, USA).

The measured grain size for ytterbium trifluoride ranged from 1.89 to 3.49 μm , which may be a hindrance for their penetration abilities into the decalcified enamel tissue as they may be too big, therefore an attempt was made to pulverize the grains with ultrasonic waves (Department of Solid State Physics, Silesian University of Technology).

Clinical Materials and Methods

The material for tests comprised 10 retained human canines, premolars and molars with preserved anatomical crowns, extracted due to surgical, orthodontic and prosthetic reasons, therefore previously not exposed to the environment of the oral cavity and thus not subjected to de- and remineralization processes. That state resulted in a homogeneous structure of tissues of those teeth. Prior to the beginning of the tests, they were stored in a chloramine solution which has antibacterial properties. Before the tests, the chloramine solution was drained and the teeth were rinsed 3 times in distilled water, then soaked in it for 24 h. Half of the research material (5 teeth) was then moved to a container with a demineralizing solution for a period of 4 weeks (Table 2).

The pH level of the solution was regulated (pH = 5) and the solution was placed in a heater for a period of 4 weeks at a temperature of 37°C. The conditions of the process were chosen in order to achieve a state as close to the environment of the

Table 2. Composition of the solution for enamel demineralization [10]

Components	Molar concentration	Quantity
CaCl ₂ × 2H ₂ O (calcium chloride dehydrate; Reachim; Moscow; Russia)	3 mM	0.441 g
KH ₂ PO ₄ (potassium dihydrogen-phosphate; POCh; Gliwice; Poland)	3 mM	0.408 g
CH ₃ COOH (acetic acid; CHEMPUR; Piekary Śląskie; Poland)	50 mM	2.88 mL
MHDP (hydroxymethylene diphosphonate; ABX; Radeberg; Germany)	6 μM	1.416×10^{-3} g

oral cavity during the beginning of the initial caries as it was possible. Every few days, the pH level was measured with a pH-meter and it was decreased with the addition of acetic acid or increased with potassium hydroxide when needed. After 4 weeks, the demineralizing solution was drained and the teeth were rinsed 3 times with distilled water, then soaked in it for 30 min, removed and dried. This method made it possible to prepare decalcified teeth with *in vitro* induced dental caries. The second group of teeth (5 pieces) was removed from the chloramine solution, rinsed 3 times with distilled water, then dried. This method made it possible to prepare non-decalcified teeth for the control group [9].

After that, each tooth was divided into 2 zones by a line drawn with a marker. The control group comprised 5 non-decalcified teeth which were also divided into 2 zones. One of the zones, marked with green nail lacquer in the root part was covered with the commercially available Icon solution (with YbF₃ added).

Both types of preparations, the commercially available Icon solution (with YbF₃ added) and the experimental solution, were applied to the respective parts of both decalcified and non-decalcified teeth.

The preparations were applied in accordance with the guidelines delivered with the commercially-available Icon product. After soaking the teeth with the preparations, they were dissected perpendicularly to the line marked with the nail polish, along the long tooth axis, with the use of a dental

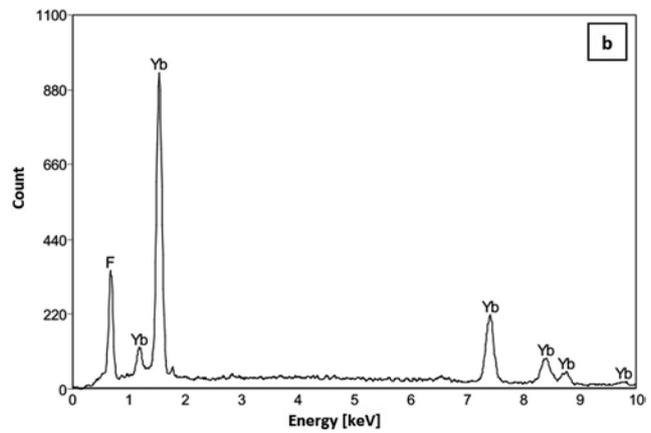
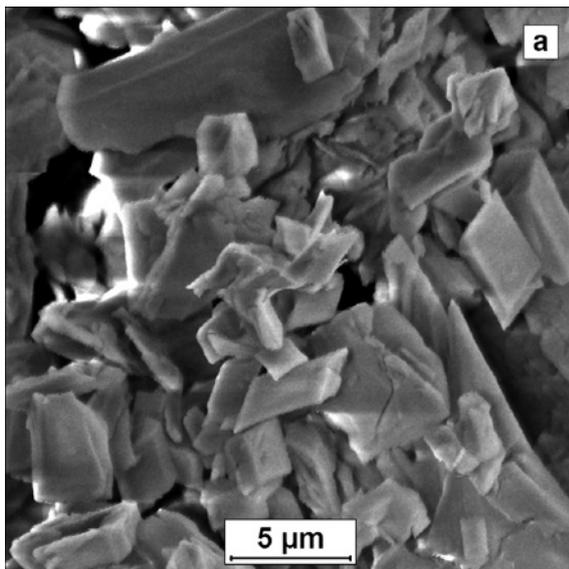


Fig. 1A. YbF₃ grains with 5 μm size before the ultrasonic wave treatment

Fig. 1B. Analysis of the chemical composition of the final material – YbF₃

diamond drill, smoothed and polished. The polished surfaces were then examined with the use of a Hitachi S-4200 electron scanning microscope.

Results

Testing Influence of Ultrasonic Waves on the Size of Ytterbium Trifluoride (YbF₃) Grains

Before applying ultrasonic waves to the original material, that is YbF₃, a test was conducted to establish its chemical composition and pictures were taken with the use of a Hitachi S-4200 electron scanning microscope equipped with a Norar Instruments EDS Voyager 3500 spectrometer (Fig. 1).

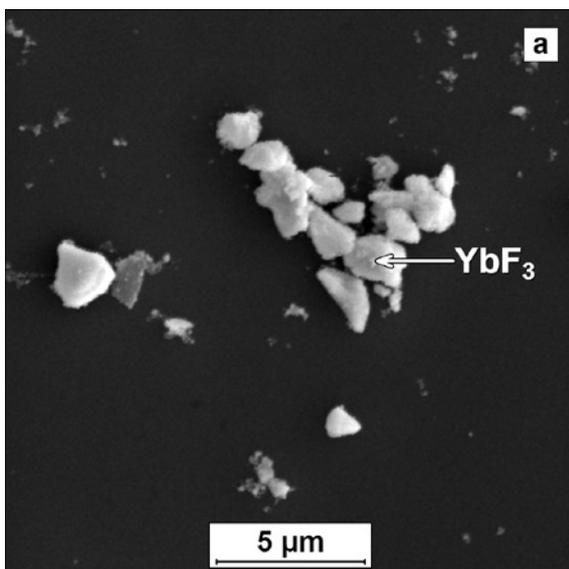


Fig. 2. YbF₃ grains after ultrasonic wave treatment

The process of the pulverization of YbF₃ was conducted with the use of a VCX750 ultrasonic processor with automatic tuning (Sonics & Materials company, Newtown, USA) at a frequency of 20 KHz and nominal power of 750 W.

The batch comprised of 0.01 g of YbF₃ and 15 mL of C₂H₅OH. The components were placed in a polyurethane syringe (with 20 mL volume), then it was subjected to pulse ultrasonic waves (5 s on, 1 s off) in a solid type tip with an inner diameter of 13 mm for a period of 6 h with the power set to 80% (power density: 113 W/cm²). As a result of the ultrasonic wave treatment of YbF₃, the grains obtained in that way had a diameter of less than 1 μm (Fig. 2).

However, the microscopic imaging of the results of the treatment shows that the grains tend to aggregate (merge) and form larger particles. This is very unfavorable in the case of a dental infiltrant.

Analysis of Penetration of Decalcified Enamel by YbF₃

The tested regions of the tooth were covered with Icon (containing YbF₃) or the experimental preparation. In order to establish if the cross-section contained YbF₃ grains, an X-ray microanalysis of several dozens of particles which were similar in size and shape to YbF₃ grains after ultrasonic wave treatment was made. However, in most cases, their chemical composition was the same as the composition of the tooth. The above-mentioned tests included the subsurface area of the enamel of teeth soaked with both compounds: Icon (with YbF₃) and the experimental solution. Not even a single YbF₃ particle could be found in the part of the tooth covered with the first of the mentioned compounds. Howev-

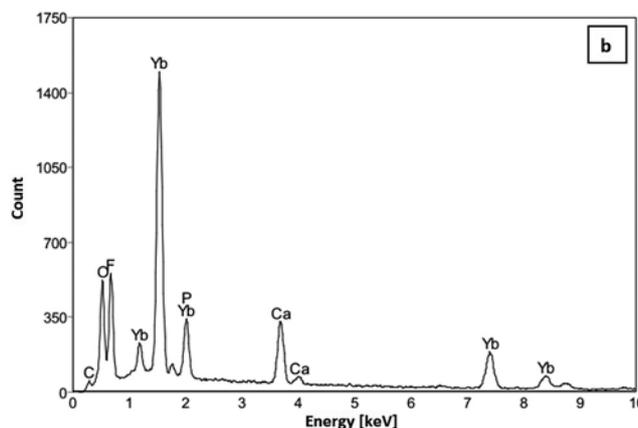
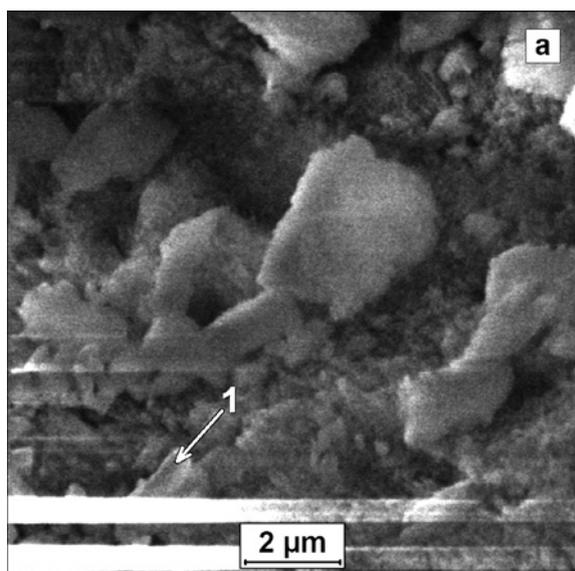


Fig. 3A. Image of enamel in the area in contact with the experimental compound with a revealed YbF₃ particle (marked with number 1)

Fig. 3B. X-ray spectrum graph for that particle

er, examination of the area in contact with the second solution revealed 2 such particles (Fig. 3).

It should be noted that it is very difficult to find YbF₃ particles. To support this, the particle marked in Fig. 3A with number 2 is morphologically similar to number 1, however its content is similar to that of the tooth. It is also worth stressing that YbF₃ particles were found at a significant distance from the surface of the tooth, however no presence of them was found in the subsurface layer of enamel (where both preparations should be infiltrating). Therefore it is possible that the location of those particles is not a result of the penetration of tooth structures, as their comparatively large sizes make it very improbable or even impossible, but rather due to contamination of the material during its preparation for the tests.

The research conducted on the test batch proves that secondary electron (SE) imaging is not very useful in the case of an analysis of YbF₃ particles. Therefore the technique of backscattered electron (BSE) imaging was used to observe and record the structures of the second polished cross-section of a decalcified tooth. The amount of those electrons depends mainly on the atomic numbers of the elements in each region of the sample: the higher the number, the stronger the signal. Due to that fact, images of the structure are brighter in those places where the atomic number of components is higher. Therefore, this method is particularly useful for searching for single, tiny particles with a significantly different chemical composition than its surrounding, like in the case of our samples. The tooth protection preparations used in the tests contained YbF₃ additions which were virtually impossible to trace with the use of the EDS method. During the tests, it was also found that the sizes of those particles were a hindrance to their pene-

tration into decalcified enamel structures, however their presence was found as particles morphologically similar to those which were the result of the pulverization process. As they were similar in shape to the particles of the Icon compound and the experimental solution, their differentiation in images obtained with the use of the secondary electron (SE) technique was very difficult and more like trial and error. They are much easier to distinguish in BSE images because they occur as brighter spots than the surrounding structure of the tooth and the analyzed preparations (Fig. 4).

However, in this case, the area with different morphology was located in the upper part of the tooth. It also had a slightly different structure than the area previously described. The common feature was a significant presence of ytterbium trifluoride particles.

The examples shown above suggest that the backscattered electron technique gives much better results than the secondary electron method as this makes it possible to localize even very small YbF₃ particles. Nevertheless, it does not help to establish the mechanism which governs the appearance of ytterbium trifluoride particles in the subsurface area of enamel and even deeper. The appearance of those particles in the deeper parts of enamel suggests that they moved there during the preparation of samples, that is during the polishing of the cross-sectioned teeth.

Analysis of Penetration of Non-decalcified Enamel by YbF₃

The tested regions of the tooth were covered with Icon and the experimental preparation. Simi-

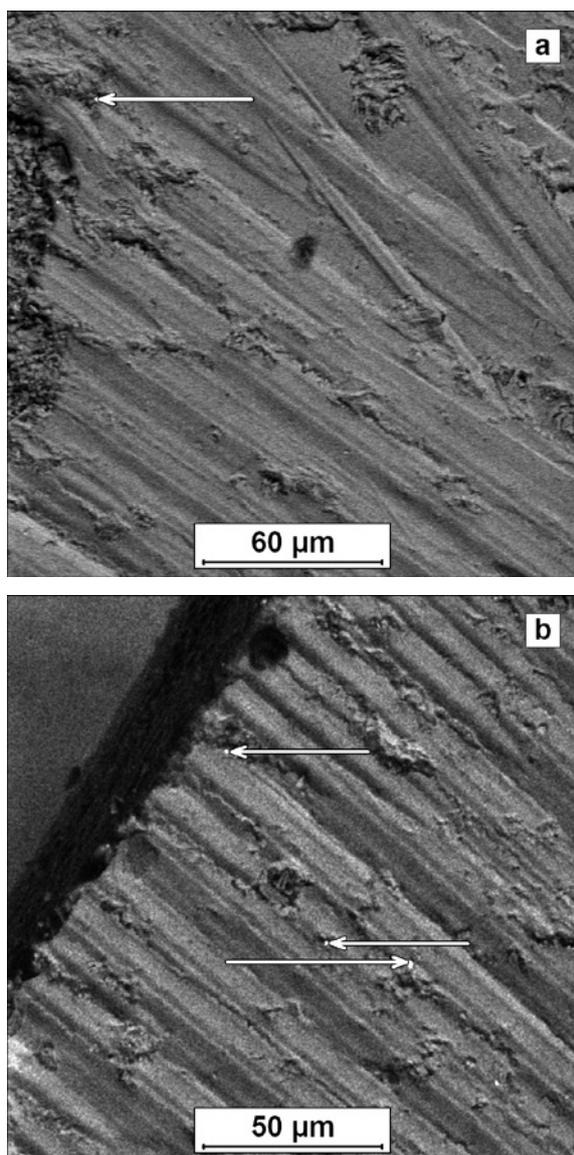


Fig. 4 A, B. YbF₃ particles (some marked with arrows) found in tooth enamel in the area covered with the experimental preparation

larly to the case of decalcified teeth, the backscattered electron (BSE) technique was initially used to find the presence of YbF₃ particles on the cross-sectioned and polished surface. Then, the chemical composition of chosen particles was assessed with the use of the EDS method. The presence of ytterbium proves them to be YbF₃ particles. The procedure used in the test made it possible to establish the presence of numerous ytterbium trifluoride particles on the cross-sectioned surfaces. The highest occurrence was found in the subsurface area, irrespectively of the preparation used to cover the surface of the tooth. Selected images of the particles found are shown in Fig. 6 A, while the X-ray spectra of them are shown in Fig. 6 B.

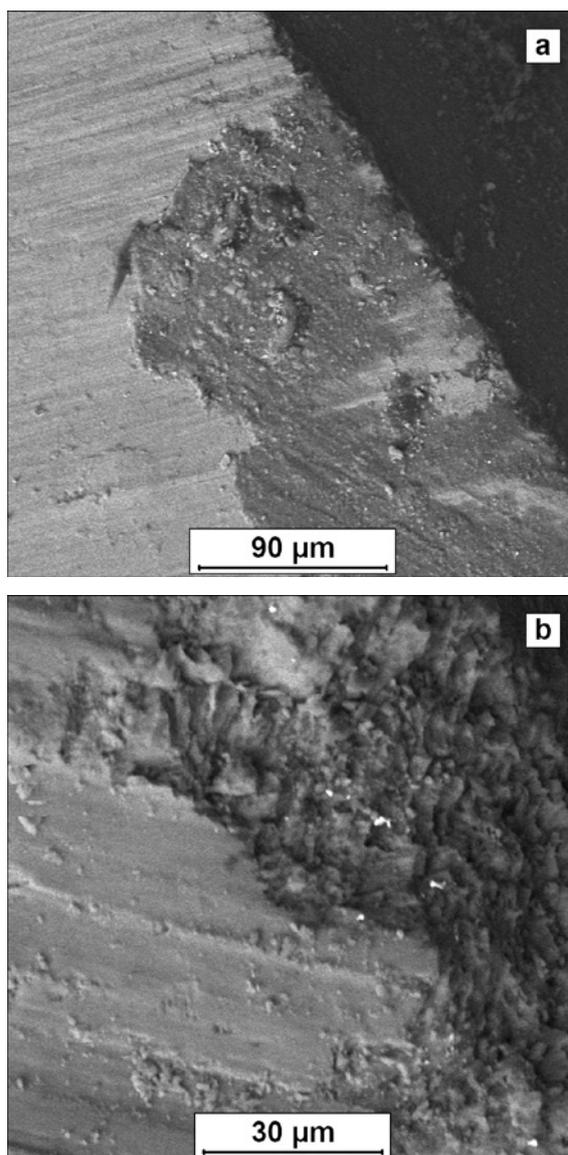


Fig. 5 A, B. Images of the cross-sectioned surface area covered with Icon preparation

Discussion

In most cases, the initial caries occurs in anatomical pits and fissures on the surfaces of lateral teeth and in occlusal contact points which are difficult to reach in both prevention and treatment. That is why pit and fissure sealants became popular. When a new material, that is a dental infiltrant, came onto the market, researchers started comparing it to the sealants. Paris et al. conducted a comparative study to assess the penetration of the infiltrant and a sealant in the case of carious lesions in pits and fissures [11]. The penetration ratio in the case of teeth qualified as ICDAS code 2 type and soaked with the infiltrant was higher than in the case of teeth to which the sealant was applied. As far as the value of the penetration index for the teeth qualified as ICDAS codes 0 and 1 is concerned, no

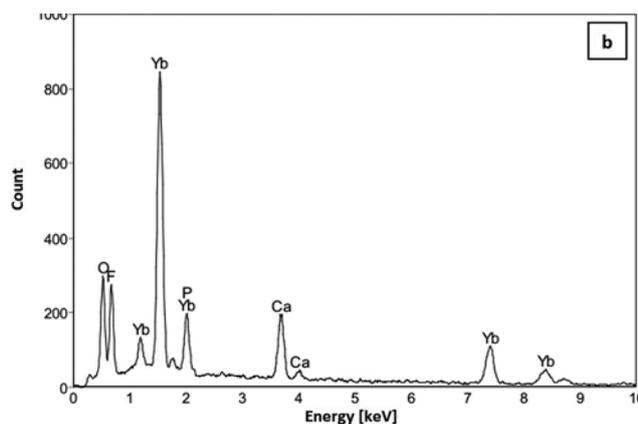
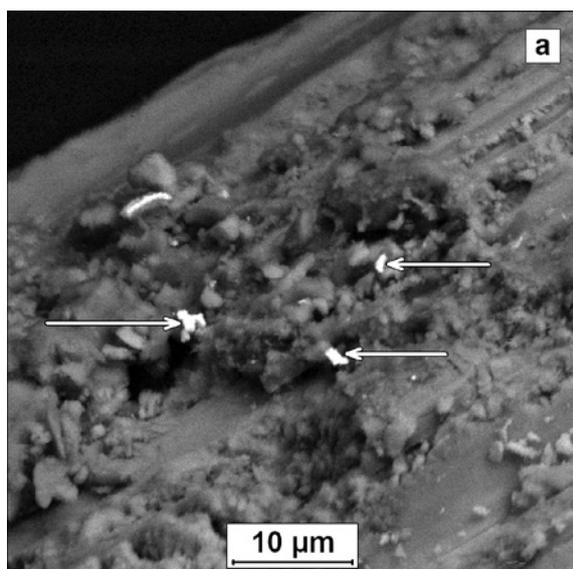


Fig. 6A. Images of cross-sectioned teeth with the revealed YbF₃ particles

Fig. 6 B. X-ray spectra obtained for those particles

significant differences were found. The results obtained during that study show that a pit and fissure sealant applied after etching with H₃PO₄ only infiltrates near the surface area of demineralized enamel, while the infiltration improves after etching with the use of HCl. Paris et al. reported that the infiltration of pits and fissures conducted with use of a resin is more efficient in the inhibition of carious lesions than a sealant [11].

That was the main reason for the initiative undertaken as part of our own research, to improve the material known as the dental infiltrant. Icon is the only commercially available preparation for the infiltration of decalcified enamel. However, the preparation does not meet all the requirements of infiltration materials as it does not have any ability to inhibit or stop the growth and multiplication of bacteria. The experimental solution, which was designed for our own research, potentially has this feature when metronidazole was introduced as a part of its composition. The mechanism of action of metronidazole is based on infiltration of the microorganism cell through passive diffusion and then, as a result of the reduction process, the creation of free radicals which damage its DNA and lead to its death [6]. Ferredoxin plays an important role in the process of the reduction of metronidazole. It is a peptide which transports electrons and it can be found only in anaerobic microorganisms. This is probably why metronidazole is only active against bacteria with anaerobic metabolism [6].

The main mechanism of action of a dental infiltrant is based on the penetration of the decalcified enamel and mechanical blockage of canals in order to prevent the penetration of the deeper tooth tissues by bacterial toxins and then microorganisms. Previous studies mainly focused on the

improvement of the penetration coefficient (PC) values [12]. Paris et al. conducted tests on 60 extracted primary molars showing active non-cavitated proximal lesions using 4 experimental preparations with different penetration coefficients as infiltrants (PC 63, PC 185, PC 204 and PC 391). Ethanol was used as an additive. On the basis of those *in vitro* tests, it was found that the decalcification of enamel showing as initial (proximal) lesions can be thoroughly soaked with low-viscosity resins. Prolongation of the exposure time to 5 min, application of a preparation with higher PC or an addition of ethanol did not result in deeper penetration of the applied experimental solution into the tissues [12].

In our current research, we used ytterbium trifluoride (YbF₃) as one of the components of the experimental dental infiltrant. It was added to the experimental preparation as a result of previously conducted research, in order to facilitate the assessment of the penetration abilities into the demineralized parts of enamel when examined with the use of a secondary electron (SE). Due to the fact that ytterbium is a heavy element, it is easy to detect in X-ray microanalysis, which makes it possible to assess the penetration abilities of the preparation. It also gives a highly visible contrast during electron microscopy. The combination of electron scanning microscopy and X-ray microanalysis does not make it possible to establish unequivocally the depth of infiltration of a preparation into enamel if those compounds do not have a contrast agent as one of their ingredients. What is more, the ingredient has to be accepted as a dental material, such as ytterbium trifluoride. Moreover, fluorine strengthens enamel and takes part in the process of remineralization. That is why we decided to add ytterbium trifluoride as a component

of the experimental solution to potentially protect against the development of dental caries.

Due to the fact that ytterbium trifluoride grains are too big, an attempt was made to pulverize them with the use of ultrasonic wave treatment. That treatment uses ultrasonic waves with a frequency range between 20 and 100 kHz in order to cause cavitation and change the physical and chemical properties of the material, particularly to disintegrate the cell structures and pulverize the particles, just like in the case of the current study [13]. The cavitation effect is a result of an explosion of gas bubbles which appear as a result of sudden changes in the pressure and temperature [13]. The main factors which cause pulverization of particles are mechanical forces which appear while the cavitation bubbles collide and the wave which is a result of their implosion [13].

The development of various branches of medicine, including dentistry, has enabled the use of nanoparticles in dental materials to improve their properties. Nanoparticles are particles with dimensions of 0.1 μm (100 nm) and less. They are used in dentistry to improve the mechanical properties of materials based on composite resins. Filtek Supreme Universal Restorative nanocomposite is an example of such dental material. It is used to reconstruct anterior and lateral teeth [14]. There have already been attempts to use YbF₃ in dentistry. Prentice et al. conducted research on the effect of ytterbium trifluoride and barium sulfate (VI) nanoparticles on the reactivity and strength of a glass-ionomer cement. Commercially available glass-ionomer powder was mixed in various proportions with nanoparticles of ytterbium trifluoride and barium sulphate (VI). The results obtained by that team show that an addition of ytterbium trifluoride nanoparticles shortens the procedure and hardening times, decreases the strength of the cement (an addition of 1 or 2% w/w of ytterbium trifluoride significantly influences the strength of the cement), yet increases its hardness. Moreover, ytterbium trifluoride accelerates the hardening reaction of the glass-ionomer cement [14]. As one of the features of ytterbium trifluoride is releasing F-fluoride ions which remineralize enamel, it is widely used in dental mate-

rials in the form of nanoparticles with sizes in the 10^{-9} m range, which improves the penetration of anatomical enamel fissures, as well as increases the adaptation ratio and resistance to wear [15]. These studies inspired us to use ytterbium trifluoride in our own research as an addition to the experimental solution.

Our results, obtained with the use of electron scanning microscopy, did not confirm the penetration into enamel either in the case of commercially-available Icon or in our own experimental preparation, as ytterbium trifluoride YbF₃ was not found in the subsurface layer of enamel. The reason was probably the size of the ytterbium trifluoride YbF₃ particles which were added to both preparations: commercially-available Icon and our own experimental solution. In comparison with the diameter of decalcified enamel pores, their sizes were too big (YbF₃ particles tend to aggregate and form bigger structures), which made infiltration of both preparations very difficult or even impossible. However, the presence of their particles was still found, which may suggest that the occurrence of those particles was not the result of infiltration of the preparations into enamel but the outcome of a mechanical displacement of the solutions which occurred during the preparation of the samples, more precisely speaking during their polishing.

In the case of the one examined tooth, the presence of YbF₃ grains was found both on the surface of the teeth and deeper inside, therefore it is difficult to establish the infiltration mechanism which worked in that case. The use of YbF₃ nanoparticles seems reasonable as, in such cases, all particles will have similar sizes and that can prevent blockage of decalcified enamel canals by bigger ytterbium trifluoride YbF₃ grains.

The authors conclude that the backscattered electron technique gives much better results than the secondary electron method as it makes it possible to localize even very small particles of a contrast agent. In order to prevent a blockage of the decalcified enamel tissue by ytterbium trifluoride (YbF₃) grains, a nanoparticle form of that compound should be used (that is particles with sizes in the range of 10^{-9} m).

Acknowledgments. We would like to thank Professor Mirosław Gibas from the Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology of the Silesian University of Technology in Gliwice, Professor Janusz Szala from the Institute of Material Sciences, Silesian University of Technology and Professor Marian Nowak from Institute of Physics, Silesian University of Technology for their help in the preparation and conducting of the laboratory part of this research. We would like to thank Tomasz Szklarski, PhD, for preparing the images.

References

- [1] **Skucha-Nowak M, Tanasiewicz M, Gibas M, Twardawa H:** Barriers securing the patient's own tissues against influence of the oral cavity environment than can be used in low invasive dentistry. *Pol J Environ Stud* 2012, 21, 25–29.
- [2] **Subramaniam P, Girish Babu KL, Lakhota D:** Evaluation of penetration depth of a commercially available resin infiltrate into artificially created enamel lesions: An *in vitro* study. *J Conserv Dent* 2014, 17, 146–149.
- [3] **Kmieć Z:** Powstawanie i właściwości szkliwa. In: *Histologia i cytofizjologia zęba i jamy ustnej*. Eds: Stoces I, Sokołowska-Trelka A. Wydawnictwo Elsevier Urban & Partner, wyd. I, Wrocław, Poland, 2007, 34–52.
- [4] **Skucha-Nowak M, Gibas M, Tanasiewicz M, Twardawa H, Szklarski T:** Natural and controlled demineralization for study purposes in minimally invasive dentistry. *Adv Clin Exp Med* 2015, 24, 891–898.
- [5] **Kielbassa AM, Müller A, Gerhard CR:** Closing the gap between oral hygiene and minimally invasive infiltration technique of incipient (proximal) enamel lesions. *Quintessence Int* 2009, 40, 663–681.
- [6] **Bronk M, Samet A, Dąbrowski K:** Metronidazol–lek, który nie utracił pierwotnej aktywności przeciwbakteryjnej. *Zakażenia* 2010, 10, 35–38 [in Polish].
- [7] **Kasten M:** Repetytorium z leków przeciwdrobnoustrojowych cz. VIII – klindamycyna, metronidazol i chloramfenikol. *Mayo Clin Proc* 1999, 74, 825–833.
- [8] **Skucha-Nowak M, Tanasiewicz M, Gibas M, Twardawa H:** Analysis of the composition of preparations used as a barrier to protect tissues of the patient against the influence of the environment in the oral cavity. *Pol J Environ Stud* 2013, 22, 53–57.
- [9] **Skucha-Nowak M:** Attempt to assess the infiltration of enamel made with experimental preparation using a scanning electron microscope. *Open Med. Cent Eur J Med* 2015, 10, 238–248.
- [10] **Yang F, Mueller J, Kielbassa AM:** Surface substance loss of subsurface bovine enamel lesions after different steps of the resinous infiltration technique: A 3D topography analysis. *Odontology* 2012, 100, 172–180.
- [11] **Paris S, Lausch J, Selje T, Dorfer CE, Meyer-Luckel H:** Comparison of sealant and infiltrant penetration into pit and fissure caries lesions *in vitro*. *J Dent* 2014, 42, 432–438.
- [12] **Paris S, Soviero VM, Chatzidakis AJ, Meyer-Lueckel H:** Penetration of experimental infiltrants with different penetration coefficients and ethanol addition into natural caries lesions in primary molars. *Caries Res* 2012, 46, 113–117.
- [13] **Nowak M, Kotyczka-Morańska M, Szperlich P, Bober Ł, Jesionek M, Kępińska M, Stróż D, Kusz J, Szala J, Moskal G, Rzychoń T, Młyńczak J, Kopczyński K:** Using of sonochemically prepared components for vapor phase growing of SbI₃•3S₈. *Ultrasonics Sonochem* 2010, 17, 892–901.
- [14] **Prentice LH, Tyas MJ, Burrow MF:** The effect of ytterbium fluoride and barium sulphate nanoparticles on the reactivity and strength of a glass-ionomer cement. *Dental Mater* 2006, 22, 746–751.
- [15] **Kupka T, Tanasiewicz M, Gibas M, Dąbrowska A:** Porównawcze badania laboratoryjne eksperymentalnego uszczelnacza dołków i bruzd zębowych SPBS (Smart Polymer – based Bio-Sealant). *Czas Stomatol* 2006, 59, 191–196 [in Polish].

Address for correspondence:

Małgorzata Skucha-Nowak
Department of Conservative Dentistry with Endodontics
Medical University of Silesia in Katowice
Plac Akademicki 17
41-902 Bytom
Poland
Tel.: +48 32 282 79 42
E-mail: mskucha-nowak@sum.edu.pl

Conflict of interest: None declared

Received: 19.03.2016

Revised: 25.05.2016

Accepted: 21.06.2016