

Research Article

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Attempt to assess the infiltration of enamel made with experimental preparation using a scanning electron microscope

Abstract: Introduction: The resin infiltration technique, a minimally invasive method, involves the saturation, strengthening, and stabilization of demineralized enamel by a mixture of polymer resins without the need to use rotary tools or the risk of losing healthy tooth structures.

Aim of the study:

1. To design and synthesize an experimental infiltrant with potential bacteriostatic properties.
2. To compare the depth of infiltration of the designed experimental preparation with the infiltrant available in the market using a scanning electron microscope.

Material and Methods: Composition of the experimental infiltrant was established after analysis of ^1H NMR spectra of the commercially available compounds that can penetrate pores of demineralized enamel. As the infiltrant should have bacteriostatic features by definition, an addition of 1% of monomer containing metronidazole was made. Thirty extracted human teeth were soaked in an acidic solution, which was to provide appropriate conditions for demineralization of enamel. Afterward, each tooth was divided along the coronal-root axis into two zones. One zone had experimental preparation applied to it (the test group), while the other had commercially available Icon (the control group). The teeth were dissected along the long axis and described above underwent initial observation with use of a Hitachi S-4200 scanning electron microscope.

Results: It was found that all samples contained only oxygen and carbon, regardless of the concentration of additions introduced into them. The occurrence of carbon is partially because it is a component of the preparation in

question and partially because of sputtering of the sample with it. Hydrogen is also a component of the preparation, as a result of its phase composition; however, it cannot be detected by the EDS method.

Conclusions:

1. SEM, in combination with X-ray microanalysis, does not allow one to explicitly assess the depth of penetration of infiltration preparations into enamel.
2. In order to assess the depth of penetration of infiltration preparations with use of X-ray microanalysis, it is recommended to introduce a contrast agent that is approved for use in dental materials, such as ytterbium III fluoride.

Keywords: Infiltrants; enamel; caries detection/diagnosis; minimally invasive dentistry; scanning electron microscope; preventive dentistry.

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1 Introduction

A majority of practitioners base their treatment of dental caries on an intervention at the moment when a loss becomes visible and perceptible while probing, opting for mechanical cleaning of lesions, while a small minority decides to use the infiltration method when a white spot appears. Despite the fact that it is a new technique and requires further development, the infiltration method has a number of advantages: mechanical stabilization of demineralized enamel, permanent closure of surface micropores, inhibition of lesion progression, minimization of risk of secondary caries, delayed necessity of fixing

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intervention, no risk of occurrence of dentin hypersensitivity and gingivitis, and high aesthetic values [1].

Icon, an infiltrant currently available in the market, is produced by DMG, a German company from Hamburg. Icon was introduced to the market in 2009. This compound is used for micro-invasive treatment of carious lesions for the treatment of lesions located on proximal and vestibular surfaces. The resin infiltration technique, a minimally invasive method, involves the saturation, strengthening, and stabilization of demineralized enamel by a mixture of polymer resins without the need to use rotary tools or the risk of losing healthy tooth structures. It is the first product that fills the former gap between the application of fluorine compounds and the surgical treatment of dental caries (mechanical cleaning of a loss with rotary tools). It is possible to stop lesion progression and remove an unaesthetic white spot during one dental visit [2]. The infiltration method is a very good solution for the treatment of children and teenagers in cases of both permanent and deciduous teeth. Most of all, it is painless and fast, helping small patients to overcome their fear of dental procedures, while their parents, if they take care of their children's oral cavities and follow a regular check-up schedule, can spare them pain and discomfort [3]. An ideal infiltrant material should be: hydrophobic, possess high surface tension, have a low viscosity, bacteriostatic, non-toxic, homogeneous polymerization, and be resistance to chemical and mechanical changes in the surrounding environment, and aesthetic acceptability [1].

Bacteriostatic, the ability to inhibit growth and multiplication of bacteria is an important feature, which an infiltrant should have [1]. Because Icon has no bacteriostatic property, it was decided to make an experimental infiltrant, which would feature such property.

2 Aims of the study

1. To design and synthesize an experimental infiltrant with potential bacteriostatic properties.
2. To compare the depth of infiltration of the designed experimental preparation with the infiltrant available in the market using a scanning electron microscope.

3 Materials and Methods

3.1 Composition of the experimental infiltrant

Composition of an experimental infiltrant was established after an analysis of ^1H NMR spectra of the commercially available Icon preparation (DMB company) and based on the review of available literature [4, 5]. The aim was to make a preparation, which could potentially have the features of a dental infiltrant additionally, improved with bacteriostatic properties [5]. In order to perform a quantitative analysis of the chemical composition of a mixture of compounds using NMR spectra, it is recommended to examine the spectra of each of those components to enable further comparisons. The compositions of the tested preparations were taken from their product data sheets (Icon by DMG, Excite-F by Ivoclar Vivadent, and Luxatemp Glaze&Bond by DMG) and each of those compounds was analyzed separately [5]. It was decided to choose a mixture of monomers that had the highest penetration coefficient (PC) into the pores [4,6].

Three experimental preparations were made, each with a mass of 5g and a different weight ratio of TEGDMA (Fluka) to HEMA (ACROS) monomers (100:0, 75:25, and 50:50) (tab. 1). The photo initiating system consisted of CQ (camphorquinone, Sigma-Aldrich) and DMAEMA (N,N-dimethylaminoethyl methacrylate, MERCK). Additionally, as the infiltrant should have bacteriostatic features by definition, an addition of 1% of monomer containing metronidazole (PMMA_n-MTZ) was made. This new compound was prepared at the Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology of the Silesian University of Technology in Gliwice.

Each of the preparation had 1% of PMMA_n-MTZ monomer added to them, then it was filtered. In order to prevent premature polymerization after the addition of 1% DMAEMA and 0.5% of camphorquinone, mixing was done in a magnetic stirrer in complete darkness and the ready

Table 1: Percentage content of experimental preparations.

Preparation 1	Preparation 2	Preparation 3
100% TEGDMA	75% TEGDMA 25% HEMA 1% DMAEMA 0.5% Camphor quinone 1% PMMA _n -MTZ	50% TEGDMA 50% HEMA

Table 2: Detailed mass composition of experimental preparations.

	Preparation 1	Preparation 2	Preparation 3
TEGDMA	5g	3.75g	2.5g
HEMA	-	1.25g	2.5g
DMAEMA	0.05g	0.05g	0.05g
Camphor quinone	0.025g	0.025g	0.025g
PMMA _n -MTZ	0.05g	0.05g	0.05g

resins were stored in dark bottles. Detailed mass composition of each preparation is listed in Table 2.

3.2 *In vitro* research methodology

Thirty extracted human molar and premolar teeth, stored in a chloramine solution, were used in this study and divided into two even groups of 15 pieces each: those which were in contact with saliva, and those which were not because their extraction was done for surgical reasons – impacted teeth (Table 3). Before the tests, chloramine solution was drained and teeth were rinsed in distilled water and then soaked in it for 24 hours.

In order to trigger initial caries, the teeth were soaked in an acidic solution, which was to provide appropriate conditions for demineralization of enamel [7]. The solution included: 3 mM of calcium chloride dihydrate (CaCl₂·2H₂O)–REACHIM, 3 mM of monopotassium phosphate (KH₂PO₄)–POCH, 50 mM of acetic acid (CH₃COOH)–CHEMPUR, 6 μM MHDP–AXB. PH of the environment was set at 5 by adding acetic acid (CH₃COOH)–CHEMPUR or potassium hydroxide (KOH)–STANLAB. Containers with the extracted teeth were placed in a heater in the temperature of 37°C in order to reflect the environment of the oral cavity. The process lasted for five weeks. Measurements and adjustments of pH were made every few days.

Table 3: Marking of teeth to constitute the base for tests
Teeth in contact with saliva (MHDP) – 15 pieces

Preparation	Marking
1	1.1.1. 1.1.2. 1.1.3. 1.1.4. 1.1.5.
2	1.2.1. 1.2.2. 1.2.3. 1.2.4. 1.2.5.
3	1.3.1. 1.3.2. 1.3.3. 1.3.4. 1.3.5.

Teeth without contact with saliva (MHDP) – 15 pieces

Preparation	Marking
1	2.1.1. 2.1.2. 2.1.3. 2.1.4. 2.1.5.
2	2.2.1. 2.2.2. 2.2.3. 2.2.4. 2.2.5.
3	2.3.1. 2.3.2. 2.3.3. 2.3.4. 2.3.5.

3.3 Used appliances

¹H NMR spectra used to establish the chemical composition of commercially available preparations were recorded with use of a UNITY/INOVA spectrometer (Varian) at a frequency of 300 MHz. A tetramethylsilane (TMS) standard and a solvent (deuterated chloroform – CDCl₃) were used.

All resin materials used in the tests were light-cured. A wireless LEDEX WL-070 lamp (Dentmate) was used for polymerization. A 5W LED diode was used as a source of blue light with a wavelength of 440 – 480 nm. The radiation power exceeded 1000 mW/cm².

Samples were examined with a Hitachi S-4200 scanning electron microscope (Institute of Material Sciences, Silesian University of Technology). This device allows magnification ranging from 20 to 500,000 times and for both qualitative and quantitative analysis of the chemical composition of a material with point, linear, and surface methods. The range of accelerating voltages ranged between 0.5 and 30 kV. The recording of images and results of an X-ray microanalysis was made with use of a Thermo Scientific software package.

3.4 Preparation application procedure

After five weeks, the teeth were removed from the solution and thoroughly dried. Afterward, each tooth was divided along the coronal-root axis into two zones by a marker line, painted with a nail lacquer. One zone had one of the three experimental preparations applied to it (the test group), while the other had commercially available Icon (the control group). The experimental preparations were applied and polymerized correctly. Both types of preparations, in the test and control groups, were applied according to the recommendations of the producer of Icon infiltrant.

3.5 Preparation of samples for microscope analysis

Having been exposed to the infiltrants, the teeth were dissected along the long axis so that each side of a dissected tooth was covered with both an experimental preparation and Icon. Subsequently, the dissected teeth were polished to a smooth surface, necessary for microscope examination.

Samples prepared in the way described above underwent initial observation with use of a Hitachi S-4200 scanning electron microscope with an accelerating

voltage of 1kV. Before the tests, the samples were sputtered with carbon. This allowed us to increase the accelerating voltage to 15kV and perform X-ray microanalysis. Observations of tooth structures were conducted with use of the secondary electron technique.

Before the analysis of the samples (polished human teeth), a test was done to establish what elements could be useful in revealing the areas where an infiltrant penetrated the tooth structures. In order to do that, one drop of each used preparation was administered to each sample plate (experimental preparation in 100%, 75%, and 25% concentration, as well as Icon). After polymerization of the plates, they were sputtered with carbon. The chemical constitution analysis was done at 15kV, using the EDS method.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

4 Results

The results of initial microscopic analysis of teeth with enamel specimens were infiltrated with experimental preparation and preparation Icon.

Applied processing allowed us to explicitly establish the boundaries between the basic structures of teeth, i.e. enamel and dentin.

Use of the X-ray microanalysis technique confirmed differences in the chemical constitution of enamel and dentin, which is known from the available literature. The analysis of the linear distribution of phosphorous and calcium (Figure 1) indicates that the content of those elements is higher in enamel than dentin. It is interesting that the highest content of oxygen can be found at the subsurface layer of the tooth; it was assumed that this occurrence may be related to penetration of the outside layer of enamel by an infiltrant. Such a hypothesis was based on the results of an EDS analysis of the chemical content of that preparation. It was found that all samples contained only oxygen and carbon, regardless of the concentration of additions introduced into them (Figure 1). The occurrence of carbon is partially because it is a component of the preparation in question and partially because of sputtering of the sample with it. Hydrogen is also a component of the preparation, as a result of its phase composition; however, it cannot be detected by the EDS method. Concentration of other elements, if any, is lower than the detection threshold of the device.

Unfortunately, the phenomenon in Figure 2 could not be observed in other teeth, indicating that a change in oxygen content in the tooth cross-section cannot indicate the depth of infiltration into the tooth structure. Data from the available literature pertaining to this phenomenon quote approx. 60 μm as satisfactory depth of penetration [1]. Therefore, the X-ray microanalysis scope of study was narrowed only to the subsurface area of the tooth with a width of approx. 100 μm . Example linear distribution results for chosen elements obtained from sample 2.3.3 (50:50%) are presented in Figure 3.

Fluctuations of the chemical composition visible in these photos mean simultaneous increase or decrease in

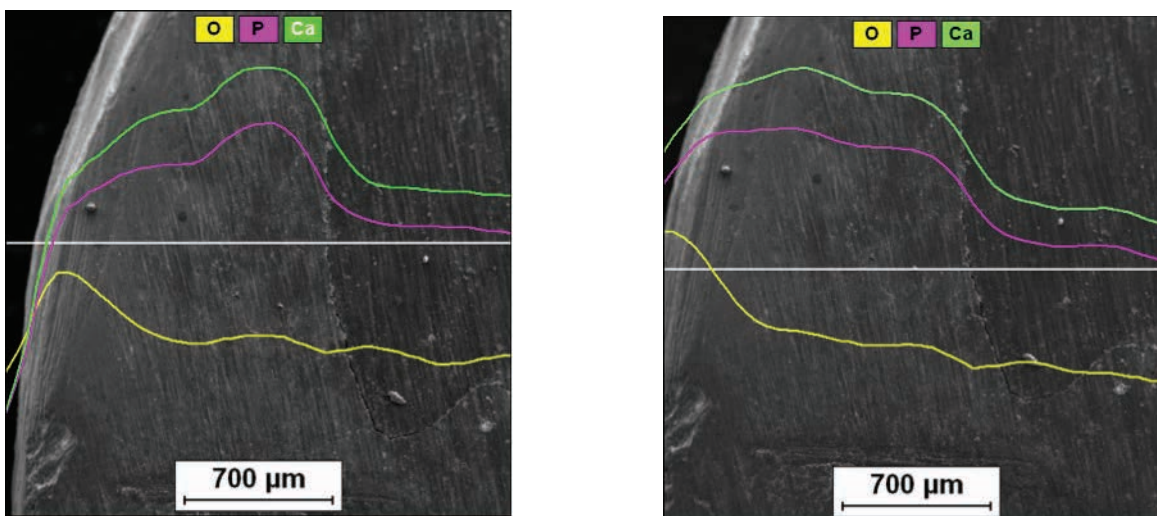


Figure 1: Change in concentration of chosen elements along the marked line.

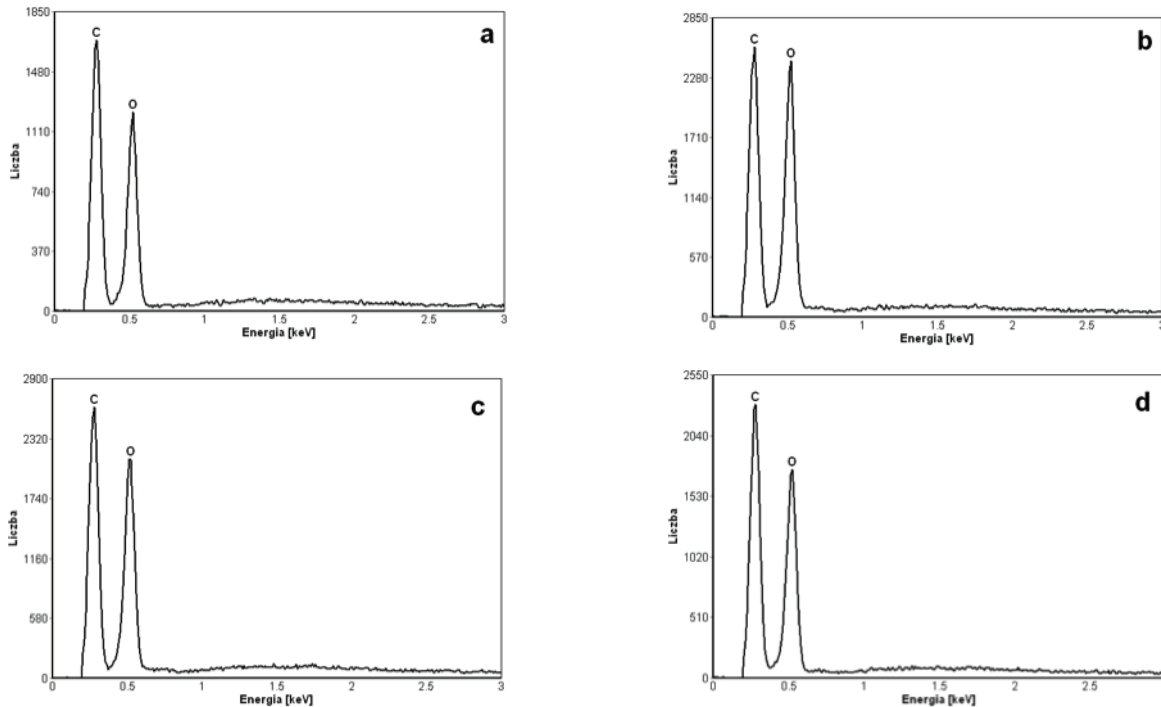


Figure 2: Results of X-ray microanalysis for a sample of ICON (a) and experimental preparation 50:50% (b), 75:25% (c), and 100% (d).

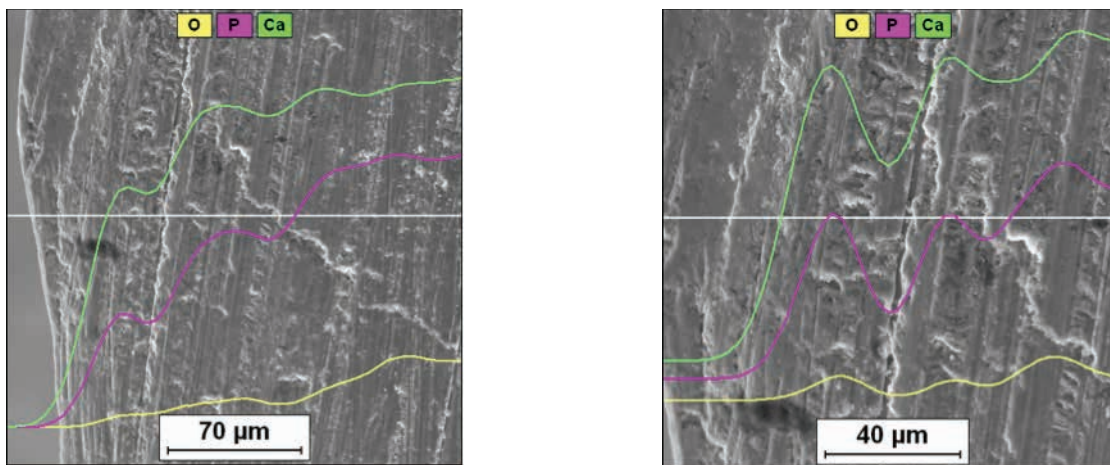


Figure 3: Change in concentration of chosen elements in sample 2.3.3 (50:50%) along the marked line.

the concentrations of all elements present in the examined samples and are an effect of the rough surface of the sample, despite the fact that it underwent polishing. Because of the rough surface, electrons are scattered and in different areas of the sample, and the number of primary electrons that induce X-ray radiation varies.

An attempt to improve effectiveness of penetration by an infiltrant through application of an etcher.

Because previous observations made with a microscope did not yield satisfactory results, it was decided to etch ready samples with three preparations: 37% orthophosphoric acid, 9.5% hydrofluoric acid, and ICON Etch

preparation (contents: hydrochloric acid, pyrogenic silicic acid, surface-active substances). The aim of the experiment was to etch tooth tissue yet leave the preparations (experimental and Icon) untouched. The etching preparations were applied on the smoothed surface within the enamel area, the cemento-enamel junction, and the root cementum (Figure 4). Such chemical preparation was meant to etch hard tooth tissues in order to make the infiltration material more visible.

Etching was applied to samples 1.1.1 (100%), 1.1.3 (100%) and 1.1.4 (100%):

- sample 1.1.1–9.5% hydrofluoric acid,

- sample 1.1.3–ICON Etch preparation,
- sample 1.1.4–37% orthophosphoric acid.

All samples were etched for 2 minutes, then rinsed with a water spray (30 sec) and dried with compressed air from a blower (30 sec). The process was repeated 10 times. Afterward, the samples were examined under the electron microscope once again.



Figure 4: Method of application of etching preparations.

The etching effect was most visible in sample 1.1.3 (100%) etched in the ICON Etch preparation. The area of enamel revealed ducts with varied orientation (Figure 5).

However, no significant differences were observed in the morphology of the enamel area, both near the tooth surface and away from it, which would allow us to establish how deep the penetration of the preparations administered to the examined teeth was. Moreover, the whole area of enamel has similar chemical composition; therefore, it cannot help in the assessment of the depth of penetration of the said preparations into the structure of dentin.

The effect of the other two reagents was even less successful. Sample 1.1.1 (100%) remained virtually unetched. The chemical composition, in points away from the tooth surface and near it, is very similar. The presence of fluorine is a distinctive feature, as it is a component of the etching reagent, suggesting accumulation of the etching products on the etched surface.

Etching of sample 1.1.4 (100%) led to accumulation of the etching products on the whole surface area of the cross-sectioned tooth.

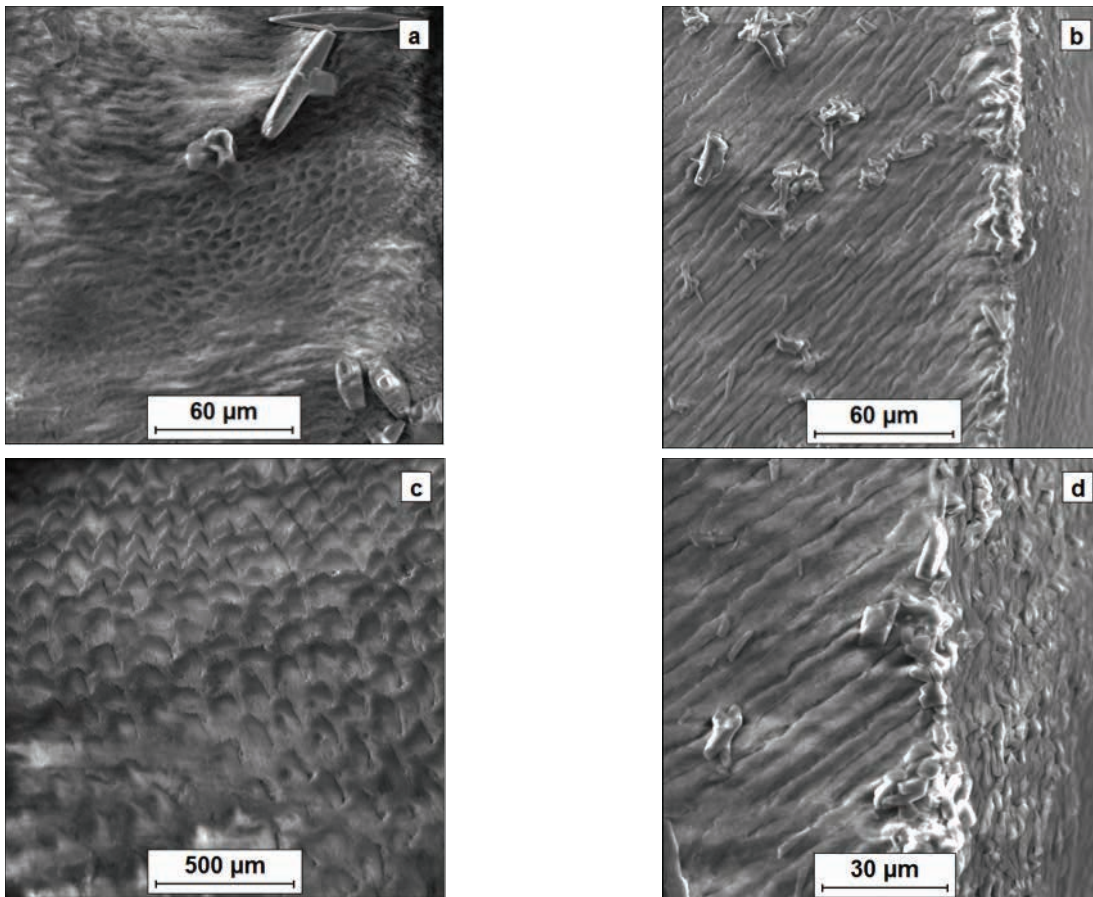


Figure 5: View of surface of cross-section of enamel in tooth 1.1.3 (100%) after etching.

Because the expected result was not achieved, the etching process was repeated with its time extended to 1 hour. When compared to the unetched state, it was found that the polished surface, which was subjected to the etching reagent, was significantly cracked (Figure 6). A chemical analysis of the composition of that layer was performed using the EDS method, which revealed the presence of fluorine (Figure 7). Fluorine is a component of the etching reagent; therefore, one can assume that structures present at the polished surface are etching products strongly bound to tooth tissues. An attempt to remove those products resulted in failure.

Etched samples were analyzed again for changes in concentrations of chosen elements along the marked lines. One group of measurements was made similarly to the unetched samples, i.e. along the line perpendicular to the tooth surface, while another group of measurements was collected along the line very near the tooth surface, parallel to its surface. It was assumed that ducts etched before the application of preparations are the main entry

points for the infiltrant to get into the structure of enamel and it would be easier to verify that assumption if the chemical composition analysis was conducted in a direction perpendicular to those ducts.

Results obtained during that analysis are presented in Figure 8. The layout of obtained lines of change in the concentration of calcium, phosphorus, and oxygen does not allow us to establish how deep the infiltrant penetrated the inside of the tooth.

The tests conducted so far show that assessment of the depth of penetration of experimental preparations into enamel is impossible with use of scanning electron microscopy and X-ray microanalysis techniques. Application of etching reagents to tooth tissues on the surface of ready samples did not reveal that area.

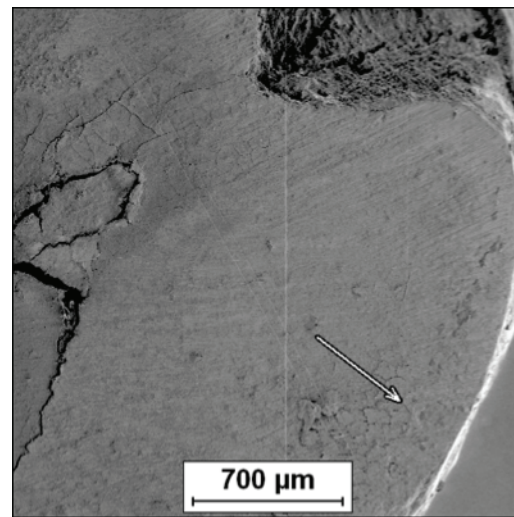
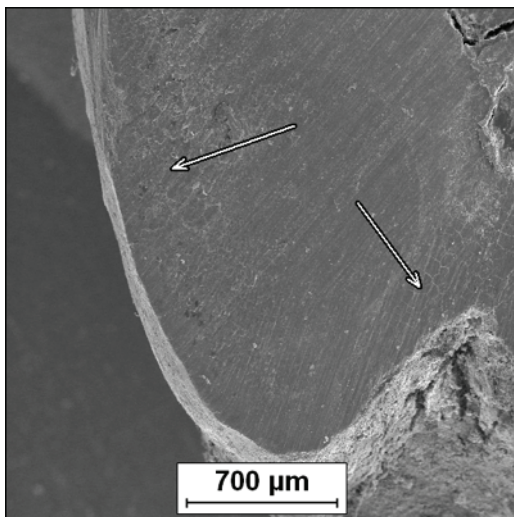


Figure 6: Polished surface of tooth 2.3.3 (50:50%) after etching the subsurface layer with 9.5% hydrofluoric acid.

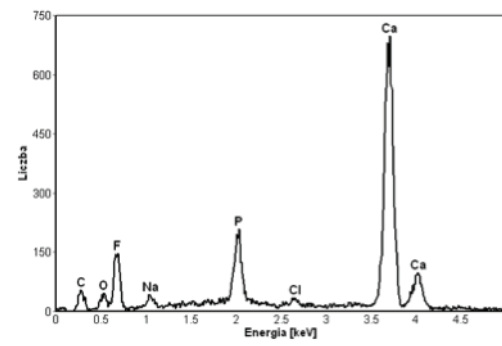
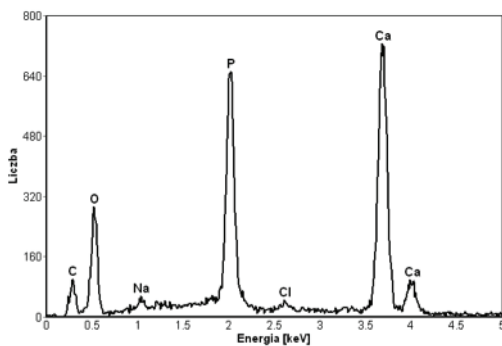


Figure 7: Results of X-ray microanalysis of the polished surface of a tooth before (left side) and after etching it (right side) with 9.5% hydrofluoric acid.

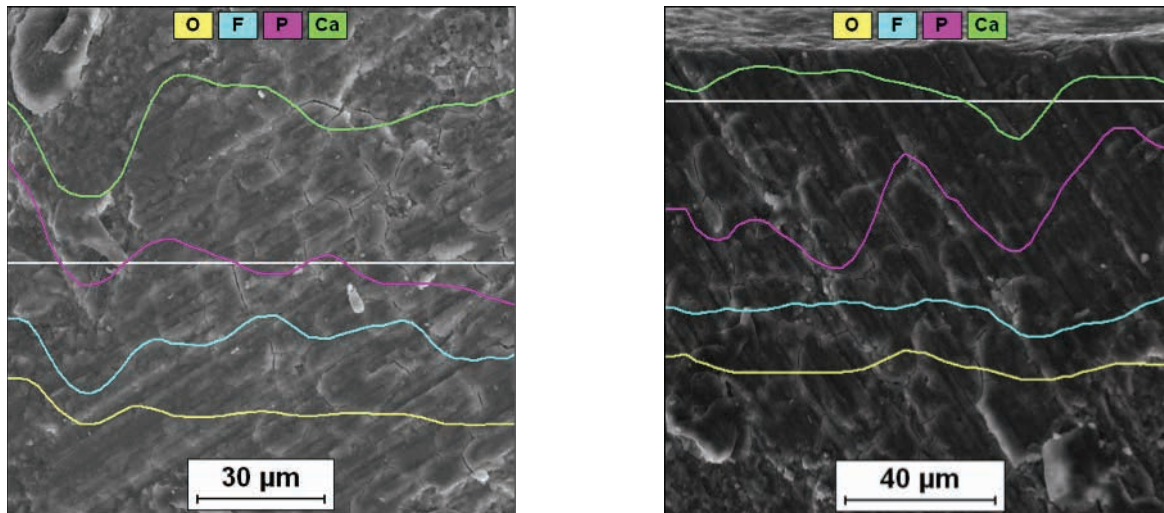


Figure 8: Changes of concentration of selected elements in sample 2.3.3 (50:50%) after etching along a line perpendicular (left side) and parallel (right side) to the tooth surface.

5 Discussion

Paris et al. took 33 experimental preparations with varied composition and attempted to assess their usefulness for infiltration of enamel damage [4]. Those preparations were mixtures of 2 monomers from bis-GMA, UDMA, TEGDMA, and HEMA groups in various mass proportions (100:0, 75:25, 50:50, 25:75, 0:100) with ethanol (0%, 10%, or 20%). All parameters required to assess the penetration coefficient (PC) were examined. PCs for those created preparations ranged from 0.2 to 474.9 cm/s. Those experimental preparations that contained bis-GMA and UDMA had high viscosity, which resulted in a low PC. Addition of ethanol decreased viscosity, surface tension, and contact angle of the mixtures. The highest PC value was found in the preparations, which contained TEGDMA, HEMA, and 20% ethanol (470 cm/s). However, a significant addition of HEMA monomer and ethanol decreases the efficiency of treatment, as its polymerization process is more difficult. Therefore, mixtures with high TEGDMA content and a small amount of HEMA and ethanol have a high potential for future applications [4].

As the available literature shows, researchers who actively looked for better parameters of infiltrants focused only on their physical and chemical properties, ignoring their antibacterial effect. None of those preparations had any antibacterial components [4]. This inspired us to include that factor in our research. It is very difficult to build any preparation having antibacterial properties into a mixture of resin monomer. Previous experience of cooperation between the Department of Conservative Dentistry, the Department of Endodontics and Organic Chemistry, Bioorganic and Biotechnology, Silesian University of

Technology resulted in a successful attempt to connect metronidazole and PMMA [8]. Inclusion of MTZ in the composition of resins did not disturb the polymerization process of the experimental infiltrant. Metronidazole is highly active against anaerobic bacteria and protozoa, but only partially effective against aerobic and facultative anaerobic bacteria (*Streptococcus* spp., *Lactobacillus*) that are mainly responsible for carious demineralization. In spite of this, it was decided to continue research on new monomer having antibacterial properties and use it in the field of micro-invasive dentistry, strictly speaking, the broadening of research on decalcified tooth tissue infiltration. The results present very early stage of the study of a new preparation, which due to its attributes is possible that in the future will be able to be used in the treatment (prevention) of demineralization in dental cementum (in the course of periodontal diseases, where the anaerobic microorganisms take main part in the etiology). Metronidazole is commonly used in dentistry, especially in treatment of periodontal diseases and acute necrotizing ulcerative gingivitis. A new bioactive methacrylate monomer based on PMMA (2-(7-methyl-1,6-dioxo-2,5-dioxo-7-octenyl) trimellitic anhydride, the synthesis given in with built-in metronidazole (PMMA-MTZ) was synthesized at the Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology in Gliwice [8]. DMAEMA was a catalyst in that reaction. The obtained monomer has two isomers (*meta* and *para*). It was assumed that the synthesized resin could be successfully incorporated in binding systems used in dentistry, acting as a double agent: an antibacterial agent and an adhesive monomer. MTZ could be released through the reaction of hydrolysis of an ester bond created between a MTZ molecule and PMMA and

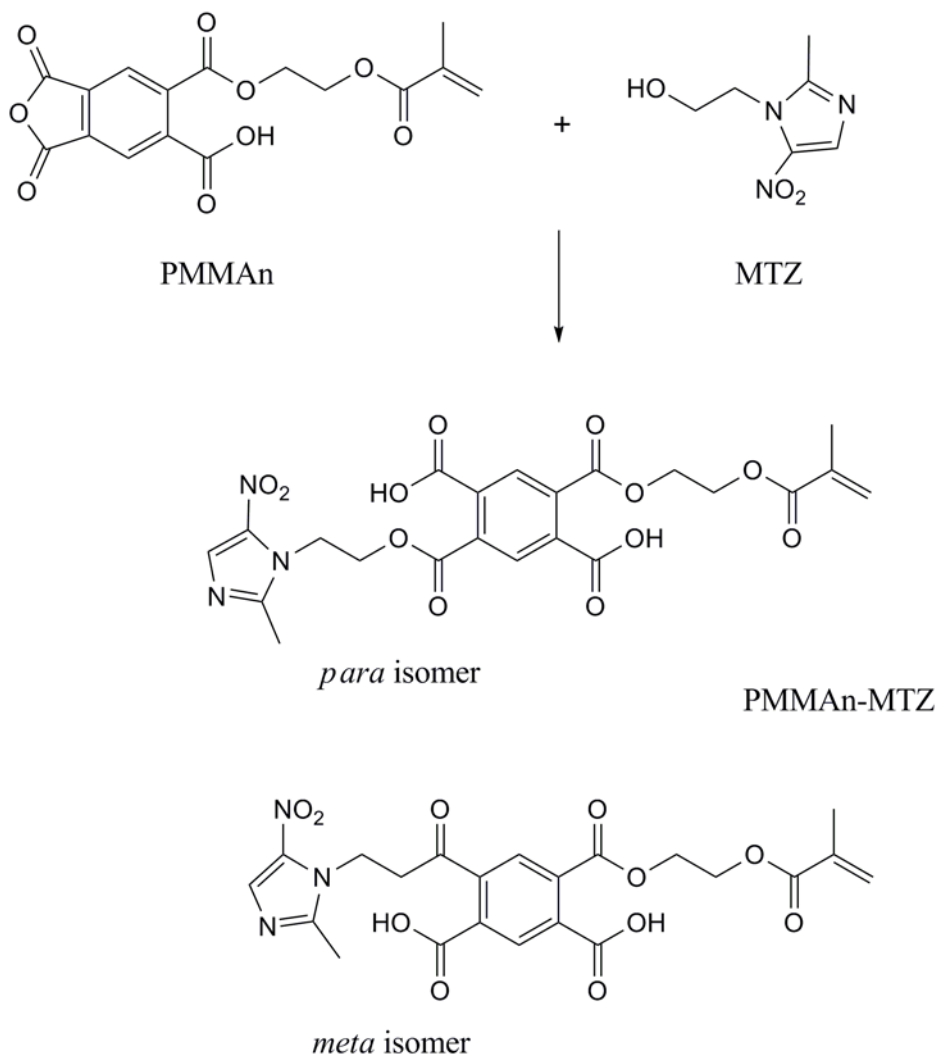


Figure 9: PMMAAn-MTZ monomer synthesis reaction.

could retain its antibacterial features for a prolonged period. Figure 9 shows the monomer synthesis reaction.

The PMMAAn-MTZ monomer could potentially be a bacteriostatic additive to resins used in the infiltration technique, which we attempted to do. Inclusion of MTZ as a component of the experimental infiltrant did not disrupt polymerization of the obtained compound. The quality of work with the experimental infiltrant did not differ from the quality of work achieved with the commercially available Icon preparation.

Biomaterials with antibacterial properties are used in dentistry. They can be divided into two main groups [9]:

- materials which release the antibacterial agent (*agent-releasing materials*),
- materials, which are permanently bound to the antibacterial agent (*non-agent-releasing materials*).

These agents are used mostly in composites and binding systems. In the case of composite materials, the

task of an antibacterial agent is to protect the compound against dental plaque sticking to it and to prevent bacteria from penetrating the filling. In the case of binding systems, antibacterial agents prevent the occurrence of secondary caries due to flaws in tightness and penetration of the filling by bacteria. Dental infiltrants are polymer resins, which can be modified in two ways. If there is a need for the antibacterial agent to be released continuously, the resin matrix requires the addition of a soluble monomer with the agent built in. On the other hand, if the agent is not required to be released, it should be immobilized in the matrix. An addition of a soluble antibacterial agent to the matrix is quite an easy task. Chlorhexidine is commonly used for that purpose and works well as an inhibitor of development for streptococci in the oral cavity. However, the addition of such an agent has a negative effect on the properties of the material. It was found that composite materials containing an antibacterial agent

are less resistant to stress and stretching and difficult to harden. Moreover, release of the agent from the matrix increases porosity of the surface. The composites with immobilized antibacterial agents have better mechanical properties, but they react only to those bacteria, which are in direct contact with them [9].

The choice of the experimental base, i.e. human teeth extracted for orthodontic and prosthetic reasons as well as impacted teeth, was not accidental, as that kind of material is difficult to obtain. It is much easier to obtain bovine teeth for experiments and most researchers use them [7, 10-12]. Moreover, they are more homogeneous as far as their chemical composition is concerned and are not often exposed to the effects of anticaries therapies (for example with fluoride), unlike human teeth extracted from the oral cavity. Additionally, bovine teeth have a significantly larger surface, which makes the work of the researchers easier.

As reported by literature, it is suggested to avoid bovine teeth for studies of infiltrant materials, as the enamel tissue of bovine teeth has higher porosity and facilitates diffusion of the infiltrant into the demineralized area [13]. This very feature excludes such hard tissue from a potential range of choices for infiltrant studies. Human tooth enamel is a very difficult and unrewarding tissue to study in the field of minimally invasive dentistry; however, it is the only tissue, which can guarantee a reliable result in such studies. According to *Buzalaf et al.* and *Mellberg*, bovine teeth can be considered an acceptable alternative for human teeth if used for research in cariology [13, 14].

As aforementioned, the choice of human teeth for research was not accidental. We decided to compose two tooth groups:

1. Teeth which were exposed to saliva (for example, through extraction due to orthodontic and prosthetic reasons),
2. Teeth which did not have any contact with saliva, as their extraction was conducted due to surgical reasons – compacted teeth.

Teeth, which were exposed to saliva, can feature enamel tissue with non-homogeneous chemical composition. This is partly due to genetic influence but mostly a result of environmental factors such as diet, exposure to fluorine and its compounds, patient's age, and taken medicine, which can create huge differences in response to an acidic environment within one tooth. However, saliva-exposed teeth undergo the process of infiltration under *in vivo* conditions.

Compacted teeth are definitely less frequently used as a base for research, as they are difficult to obtain. The

enamel surface of those teeth are more porous and the process of demineralization advances much faster than in the case of teeth with mature enamel, which are in direct contact with the environment of the oral cavity [13]. Because of that, we decided to include compacted teeth in our test base, as their enamel reflects the development base for white spot carious lesions in young patients.

On the basis of the available literature, one can find that decalcified enamel of human teeth was most frequently assessed with use of a confocal microscope rather than a scanning electron microscope (SEM) [11, 15, 16].

Few researchers based their observations on electron microscope imaging material. The results of our own research, when compared to other studies, confirm that the efficiency of that methodology, without any changes in the process of preparation of the assessed material, is very low. *Gomez S. et al.* assessed the depth of penetration of a binding system into the enamel tissue of human teeth with SEM imaging. In order to depict the depth of penetration of a sealer, they etched previously readied preparations (decalcified human teeth covered with a sealer) for 24 hours. However, the results they obtained were not statistically significant [17] and, therefore, comparable with our findings.

On the basis of previous research, in order to assess the depth of penetration of an infiltrant preparation with use of X-ray microanalysis, it appears that a contrast agent is required; however, it has to be approved to be used in dental materials, for example ytterbium III fluoride.

6 Conclusions

1. SEM, in combination with X-ray microanalysis, does not allow one to explicitly assess the depth of penetration of infiltration preparations into enamel.
2. In order to assess the depth of penetration of infiltration preparations with use of X-ray microanalysis, it is recommended to introduce a contrast agent that is approved for use in dental materials, such as ytterbium III fluoride.

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Conflict of interest statement: Authors state no conflict of interest

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