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Assessing the Penetrating Abilities of Experimental Preparation with Dental Infiltrant Features Using Optical Microscope: Preliminary Study

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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 aim of the infiltration technique is to penetrate demineralized enamel with a low viscosity resin. Icon[®] (DMG) is the first ever and so far the only dental infiltrant. Bacteriostaticity is one of the properties that should be inherent in dental infiltrants, but Icon lacks this feature.

Objectives. The aim of the preliminary study was to properly choose a dye which would allow us to assess the penetrating abilities of our own, experimental preparation with features of a dental infiltrant with bacteriostatic properties and to compare using an optical microscope the depth of infiltration of the designed experimental preparation with the infiltrant available on the market.

Material and Methods. The preparation is supposed to infiltrate decalcified human enamel and be assessed with an optical microscope. Eosin, neutral fuchsin and methylene blue were added to experimental preparation with dental infiltrant features and to Icon[®] (DMG) in order to assess the depth of penetration of the experimental solution into the decalcified layers of enamel.

Results. The experimental solution mixes well with eosin, neutral fuchsin, and methylene blue.

Conclusions. During the preliminary study, the authors concluded that the experimental solution mixes well with methylene blue, neutral fuchsin, and eosin. An addition of eosin to a preparation which infiltrates inner, demineralized enamel layers, facilitates the assessment of such a preparation with an optical microscope. A designed experimental solution with the main ingredients, i.e., 2-hydroxyethyl methacrylate (HEMA) and tetraethylene glycol dimethacrylate (TEGDMA) with a ratio of 75% to 25% penetrates the demineralized (decalcified) inner parts of the enamel and polymerizes when exposed to light. In order to assess the infiltration of the experimental solution into the demineralized enamel layers, it is required to improve the measurement techniques that utilize optical microscopy (*Adv Clin Exp Med* 2016, 25, 5, 961–969).

Key words: biomaterials, minimally invasive dentistry, dental infiltrant, experimental preparation, optical microscope.

Micro-invasive dentistry, a branch of dental medicine, has developed in recent years. Micro-invasive dentistry adopts techniques aimed at preserving hard tissue from dental caries. One of these methods, called “infiltration”, involves soaking decalcified enamel with a resin and is most effective when so-called “white spot” is clinically

observed, i.e., at the initial stage of hard-tissue demineralization.

A dental infiltrant is a substance based on polymer resins able to penetrate through the demineralized tooth surface and to close enamel canals [1]. Available literature provides a variety of names related to the infiltration technique and to the re-

quired preparation: low-viscosity resin, penetration, plastrification, therapeutic sealing, or impregnation. The technique itself is also called “non-invasive” or “ultraconservative” [1].

Infiltration allows cutting off entry routes for bacterial toxins into the subsurface areas of demineralized enamel, which, in turn, stops carious microorganisms and their toxins from entering decalcified tissues. The development of dental caries is related to bacteria present in dental plaque that, due to carbohydrate fermentation, produce organic acids. Lactic acid is one of those compounds and also one of the main factors causing enamel decalcification [2]. Organic acids decrease pH in the oral cavity, which results in the appearance of Ca^{2+} calcium and PO_4^{3-} phosphate ions and, subsequently, in enamel demineralization [2].

Moreover, the development of dental caries is related to “cariostasis”, i.e. the equilibrium between continuous processes of enamel surface demineralization and remineralization [3]. Healthy enamel never changes its color. Demineralization leads to the appearance of “white spot” lesions (*macula alba*) on the tooth surface, which result from the decreased concentration of mineral components in the subsurface layer of enamel. The “white spot” can change its color to brown, dark brown or even black.

The structure of the enamel is shaped during odontogenesis and depends on organic and inorganic components supplied to the body. Some of them, such as proteins, vitamins (A, D, C), amino acids and mineral salts (phosphorus, calcium, fluorine and iron) are vital for creating the organic base of the tooth [3]. The enamel is homogeneous, its surface is the hardest, as it contains the highest concentration of phosphate, calcium, fluorine and chlorine ions [3].

Icon® (Icon caries infiltrant; DMG; Hamburg, Germany) is the first ever and so far the only preparation which can be called a dental infiltrant, a substance based on methacrylic resins with an ability to infiltrate inside layers of demineralized (decalcified) enamel. After polymerization, Icon® creates a network which prevents cariogenic (caries-inflicting) bacteria and their metabolic by-products from entering decalcified canals of the enamel and prevents the development of dental caries. In order for a polymer resin to be used as a dental infiltrant, it needs to meet the following criteria: it has to have the ability to polymerize to a solid state, it has to display resistance to chemical and mechanical factors, no interaction with food or medicine, adequate cosmetic and aesthetic appearance on the tooth surface, and no toxicity in the environment of the oral cavity, bacteriostaticity (inhibition of bacterial growth) [1]. Bacterio-

staticity (i.e. an ability to inhibit growth and multiplication of bacteria) is one of the features which should be inherent in dental infiltrants [1]. Unfortunately, Icon lacks this feature.

There are two types of biomaterials with antibacterial properties used in dentistry: materials which release the antibacterial agent (agent-releasing materials) and materials which are permanently bound to the antibacterial agent (non-agent-releasing materials) [4]. An attempt to modify dental infiltrants can be made in two ways: if the infiltrant is supposed to release the antibacterial factor in a continuous manner, a soluble monomer should be added to the soluble resin matrix with a drug component. However, if the antibacterial agent is not supposed to be released, the drug should be immobilized in the matrix [4]. Physical and chemical properties of infiltrating resins, such as hydrophilicity, low viscosity or contact angle, and high surface tension allow for better penetration into enamel [5].

The aim of the present, preliminary study was to properly choose a dye which would allow us to assess the penetrating abilities of an experimental preparation exhibiting features of a dental infiltrant and, using an optical microscope, to compare the depth of infiltration of the designed experimental preparation with the infiltrant available on the market.

Material and Methods

The material used in the study consisted of 14 extracted human premolars and molars with preserved anatomical crowns. These retained teeth, later removed for surgical, orthodontic and prosthetic reasons, did not have any contact with the oral cavity and were not subject to constant demineralization and remineralization in that environment. Prior to processing, all teeth were carefully cleaned from soft tissues and stored in chloramine solution (NH_2Cl ; Bochemie, Katowice, Poland) with antibacterial properties. Before starting with the experiments, the teeth were washed thoroughly in distilled water (twice for $\times 5$ min), and stored in *aqua destillata* for 24 h to completely remove remnants of chloramine. The research material was randomly assigned to two groups ($n = 7$ each teeth).

Half of the research material ($n = 7$ teeth) was then moved to a container with a demineralizing solution for the period of 4 weeks [6] (Table 1).

The stable pH of solutions (5) was controlled daily and regulated by adding acetic acid (CH_3COOH ; CHEMPUR; Piekary Śląskie; Poland) or potassium hydroxide (KOH; STANLAB;

Table 1. Composition of the solution for enamel demineralization [7]

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 dihydrogenphosphate; 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

Lublin; Poland). The jars with solutions and immersed teeth were put in a heater at a temperature of 37°C to reflect the environmental conditions of the body. This process lasted 4 weeks.

Then, the teeth which were immersed in demineralizing solutions were washed twice with distilled water (30 s × 2) and dried with oil-free, compressed air (30 s).

The second half of the research material (n = 7) was removed from the chloramine solution, rinsed and dried with oil-free, compressed air (30 s). This method allowed us to prepare non-decalcified teeth for the control group.

Experimental Solution with Dental Infiltrant Features

The composition of an experimental solution with dental infiltrant features was established after the analysis of ¹H NMR spectra of the commercially available Icon infiltrant (Icon carries infiltrant; DMG; Hamburg, Germany) and based on the review of the available literature with an aim to make a preparation that could potentially have the features of a dental infiltrant additionally improved with bacteriostatic properties [8] (Table 2).

DMAEMA and CQ are factors which initialize the process of free radical polymerization. A bioactive methacrylate monomer based on PMMAAn (2-(7-methyl-1, 6-dioxo-2,5-dioxa-7-octenyl) trimellitic anhydride) with built-in MTZ (metronidazole, Acros, New Jersey, USA) was synthesized at the Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology of the Silesian University of Technology in Gliwice [9]. DMAEMA was a catalyst in that reaction. Metronidazole was supposed to be released through the reaction of hydrolysis of an ester bond created between a metronidazole particle and PMMAAn and was to retain its antibacterial features for a prolonged period [9].

Table 2. Composition and percentage content of the experimental solution

Component	Quantity (g)	Content (%)
TEGDMA	3.75	75
HEMA	1.25	25
PMMAAn – MTZ	0.05	1*
DMAEMA*	0.05	1*
CQ*	0.025	0.5*

* 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); DMAEMA* (N,N-dimethylaminoethyl methacrylate, Merck, Darmstadt, Germany); CQ* (camphorquinone, Aldrich, St. Louis, USA)

Attempts to Change the Dye of the Experimental Solution

In order to verify if the experimental preparation penetrates demineralized layers of the enamel and to compare the depth of penetration with that achieved by the commercially-available solution (Icon), we searched for a dye agent that would allow us to make the comparison with an optical microscope.

Five experimental solutions were prepared (each of 5 g weight). Each of them contained 75% of TEGDMA, 25% of HEMA, a photo-initializing set (1% of DMAEMA and 0.5% of camphor quinone), and an addition of 0.001 g (1 mg) of the selection of dyes: Methylene blue (WarChem, Warszawa, Poland), brilliant blue (domestic), fuchsine (BDH Chemicals, Poole, England), neutral fuchsine (Michrome, London, UK) and eosin (POCh, Gliwice, Poland).

When these five preparations were ready, it was primarily checked if each of them can under-

go polymerization when exposed to an Elipar S10 polymerization lamp (3M ESPE, Sefeld, Germany).

The first selection provided the choice of three dyes: eosin, neutral fuchsine, and methylene blue for further tests due to their highest intensity of color after polymerization.

Experimental solutions with an addition of those three chosen dyes were applied to 3 non-decalcified and 3 decalcified teeth (each preparation was applied to one non-decalcified and one decalcified tooth).

Then, teeth were sectioned along their longer axis with dental diamond drills (VERDENT; Łódź; Poland), smoothed and polished (Soft-Lex; Finishing and Polishing Strips system; 3M ESPE) to obtain a smooth surface required for observation and microscopic examination. All samples were prepared by one operator, and the diamond drills and polishing Soft-Lex were renewed for each specimen. Next, the polishing surfaces were washed and dried with oil-free air-water spray (30 s) and polished to a smooth surface ready to be examined with an optical microscope. This observation led to the final selection of a dye, i.e. eosin.

After the final selection of a dye, eosin was added to the commercially-available infiltrant Icon® (DMG; Hamburg, Germany) in order to assess the depth of penetration of the experimental solution into the decalcified enamel layers.

Another 4 decalcified teeth were divided into two zones with the use of nail polish. At the same time, 4 other, non-decalcified teeth constituted the control group and were also divided into two zones. Eosin-dyed Icon® was applied to one of these zones on every tooth (on the root part marked with green nail polish).

Both dyeing preparations, i.e. commercially-available Icon® and the experimental solution, were applied to the respective zones of both non-decalcified and decalcified teeth. Both preparations were applied in accordance with the guidelines delivered with the commercially-available Icon® product. Firstly, enamel was etched for 2 min with Icon Etch (15% of HCl in a gel form), then rinsed with water for 30 s and dried with air without any water or oil traces. Subsequently, the surface of teeth was thoroughly dried with Icon-Dry® (99% ethyl alcohol) for 30 s and also dried with air without any water or oil content. Finally, the commercially-available Icon® and the experimental preparation, both dyed with eosin, were applied to non-decalcified and decalcified teeth – each one to the appropriate zones. After the first layer of the preparations was applied and 3 min lapsed, the teeth were light-cured with a polymerization lamp for 40 s. Another layer of the preparations was applied, and after 1 min. the teeth were

exposed to the second dose of polymerizing light for a period of 40 s.

After the teeth were soaked in the preparations, they were dissected (perpendicularly to the line marked with the nail polish along the long tooth) with dental diamond drills (VERDENT; Łódź; Poland), then smoothed and polished (Soft-Lex; Finishing and Polishing Strips system; 3M ESPE) to obtain the smooth surface required for observation and microscopic examination. All samples were prepared by one operator, and the diamond drills and polishing Soft-Lex were renewed for each specimen. Next, the polishing surfaces were washed and dried by oil-free air-water spray (30 s) and polished to a smooth surface. This way, a surface ready to be examined with an optical microscope was obtained.

The polished surfaces were then examined with the use of ME 1000 optical microscope (DELTA Optical) equipped with PHAMIAS 2003 v. 1.3B software, which is a standard measurement method using software with an optical microscope.

Results

Dye Choice

After the first selection, i.e. polymerization trials for five dyes: methylene blue, brilliant blue, fuchsine, neutral fuchsine and eosin, three of dyes were chosen (neutral fuchsine, methylene blue and eosin) due to their highest color intensity. After the application of the experimental solutions dyed with three chosen dyes to both non-decalcified and decalcified teeth, images from the optical microscope were obtained in order to check if the experimental solution penetrates the inner layers of demineralized enamel (in the case of decalcified teeth), which are presented in Fig. 1–5.

Figure 1A presents the enamel surface of a non-decalcified tooth which was covered with an experimental solution dyed with methylene blue. No traces were observed of the dye penetrating into the non-decalcified layers of enamel (thus no penetration of the experimental solution). The spot marked with an arrow is a groove filled with the dyed experimental solution, not showing any in-depth penetration.

Figure 1B presents the enamel surface of a decalcified tooth soaked with an experimental solution, also dyed with methylene blue. The spots marked with arrows show the degree of penetration of the dye, thus indicating also the penetration of the experimental solution.

Figure 2A presents the enamel surface of a non-decalcified tooth which was covered with an

Fig. 1. (A) – non-decalcified tooth enamel surface covered with methylene-blue-dyed experimental solution; (B) – decalcified tooth enamel surface covered with methylene-blue-dyed experimental solution (×40 magnification)

Fig. 2. (A)– non-decalcified tooth enamel surface covered with neutral-fuchsine-dyed experimental solution; (B) – decalcified tooth enamel surface covered with neutral-fuchsine-dyed experimental solution (×40 magnification)

experimental solution dyed with neutral fuchsine. No traces were observed of the dye penetrating into the non-decalcified layers of enamel (thus no penetration of the experimental solution). The spot marked with an arrow, similarly to the non-decalcified tooth covered with the methylene-blue-dyed experimental solution, is a groove filled with the experimental solution dyed with neutral fuchsine and does not show any in-depth penetration.

Figure 2B presents the enamel surface of a decalcified tooth soaked with an experimental solution, also dyed with neutral fuchsine. The spots marked with arrows show the degree of penetration.

Figure 3A presents the enamel surface of a non-decalcified tooth with an experimental solution dyed with eosin. No traces of the dyed agent penetrating (thus no penetration of the experimental solution) into the non-decalcified layers of enamel were observed.

On the other hand, Fig. 3B presents the enamel surface of a decalcified tooth similarly covered

with an experimental solution with an eosin addition. The spot marked with an arrow shows the degree of penetration.

On the basis of the images presented above, it was found that eosin-dyed solutions have better visibility for a researcher who uses an optical microscope. Therefore, before commencing any further research to assess the depth of penetration of experimental solutions into the decalcified layers of enamel, it was decided to dye both the experimental solution and the commercially-available Icon[®] preparation with eosin.

As a result of applying the commercially-available Icon[®] and the experimental solution, both dyed with eosin, to non-decalcified and calcified teeth, the following images (Fig. 4 and 5) from an optical microscope were obtained.

Figure 4A presents the enamel surface of a non-decalcified tooth which was covered with the commercially-available Icon[®] preparation dyed with eosin. No traces of the dye penetrating into the non-decalcified layers of enamel (thus no penetration

Fig. 3. (A) – non-decalcified tooth enamel surface covered with eosin-dyed experimental solution; (B) – decalcified tooth enamel surface covered with eosin-dyed experimental solution ($\times 40$ magnification)

Fig. 4. (A) – non-decalcified tooth enamel surface with eosin-dyed commercially-available Icon preparation applied to it; (B) – non-decalcified tooth enamel surface with the eosin-dyed experimental preparation applied to it ($\times 40$ magnification)

of the Icon[®] preparation) were observed, which is correct, as the preparation should only penetrate demineralized (decalcified) parts of enamel. The spot marked with an arrow is not a sign of penetration but a groove which was filled with the preparation during its application to the enamel surface.

Figure 4B also presents the enamel surface of a non-decalcified tooth which was covered with the experimental preparation dyed with eosin. Similarly to Fig. 5A, no traces of the experimental preparation penetrating into the non-decalcified layers of enamel were observed, which again is correct, as the preparation should only penetrate demineralized (decalcified) parts of enamel. The spots marked with arrows are grooves filled with the preparation during its application to the enamel surface.

Figure 5A presents the enamel surface of a decalcified tooth which was covered with the commercially-available Icon[®] preparation dyed with eosin. Traces of the dye (eosin) penetrating

into the decalcified layers of enamel (thus penetration of the Icon[®] preparation) are visible, which is favorable, as the preparation should only penetrate demineralized (decalcified) parts of the enamel. The spots marked with arrows show penetration areas where the commercially-available Icon[®] preparation infiltrated enamel.

Figure 5B presents the enamel surface of a decalcified tooth which was covered with the experimental preparation dyed with eosin. Traces of the dye (eosin) penetrating into the decalcified layers of enamel (thus penetration of the experimental preparation) are visible, which proves that the experimental solution has one of the features of a dental infiltrant, i.e. it penetrates the decalcified inner layers of enamel.

The average penetration depth of both commercially-available Icon[®] and our own experimental preparation with use of an optical microscope equipped with PHAMIAS 2003 v.1.3 B software. Measured values are presented in Table 3.

Fig. 5. (A) – decalcified tooth enamel surface with eosin-dyed commercially-available Icon preparation applied to it; (B) – decalcified tooth enamel surface with the eosin-dyed experimental preparation ($\times 40$ magnification)

Table 3. Penetration depth of commercially-available Icon[®] and our own experimental preparation assessed with use of an optical microscope

Type of preparation	Penetration depth [μm]
Commercially-available Icon [®]	6.33
Experimental preparation	2.40

Discussion

The phenomenon of infiltration in medicine is defined as penetration of a liquid into the pores or grooves of a solid substance. This notion appears in cases of treatment conducted using low-viscosity resins with high penetration abilities in relation to subsurface carious lesions. Resin infiltration, as one of micro-invasive techniques of carious lesion treatment, allows for the reduction or even cessation of the carious process at the stage when a white spot appears [1]. Due to the higher porosity in decalcified enamel, an increase of water and choline is observed. Moreover, the occurrence of micropores and intercrystal spaces opens a route for the diffusion of acids which, in turn, dissolve minerals. On the basis of the above-described phenomena, an infiltration technique, which uses a low-viscosity resin, was designed. Micropores, which appeared as a result of demineralization, are filled with a low-viscosity resin, which blocks access into the inner layers of hard tooth tissues for toxins. The procedure improves the mechanical properties of damaged enamel. Moreover, organic and inorganic substances present in enamel and the resin jointly create an acid-resistant barrier which further improves the effectiveness of this technique in the fight against dental caries [1].

Kielbassa A.M. et al. noticed that the surface tension and contact angle have an effect on the penetrating abilities of resins with a high penetration coefficient into the inner layers of enamel. Moreover, resins with lower viscosity penetrate in greater volume and might create a layer of protection for tooth tissues. Penetration of $60\ \mu\text{m}$ into enamel is sufficient enough to prevent further demineralization [1].

Dental infiltrants have a low contact angle. Tangent angle is the angle between the line tangent to a liquid (resin) and the surface (enamel). It is related to surface energy and surface tension. The low contact angle indicates hydrophilic properties, which are very desirable in case of dental infiltrants. Infiltrating compounds with low viscosity have limited inner resistance and minimal friction forces within inner layers of the substance (resin), so they move simultaneously [1, 10].

Icon[®] preparation, due to its content, is prone to discoloration. Rey N. et al. conducted a study to assess possibilities of dyeing Icon[®] preparations in comparison to 4 other materials with adhesive abilities: Clearfil SE Bond, Heliobond Syntac Classic, Optibond FL and Scotchbond Universal Adhesive [11]. They obtained results which indicate that Icon[®] has the highest degree of dye when compared to other adhesive materials [11]. Icon[®] preparation consists mostly of TEGDMA, which features higher water absorption than Bis-GMA, one of the ingredients of Clearfil, Heliobond and Optibond [11].

On the basis of the results mentioned above, we decided to use dye-absorbing abilities of Icon[®] in our own research and to qualify it into the control group. Both Icon[®] and our own experimental preparation contain mainly TEGDMA. As proved by Sideridou et al., resins which contain mainly TEGDMA have the highest penetra-

tion value, which is very desirable in the case of treatment methods which utilize infiltration with a resin [12]. TEGDMA has the highest degree of water absorption, which, in turn, leads to dyeing the resin and explains why Icon[®] changes its color the most after being kept in dyed agents. In other words, this ability of Icon[®] makes it a transport dye with water inside the resin and changes its color [12]. Our own research confirms this ability of preparations which contain high amounts of TEGDMA in the case of decalcified enamel.

The aim of this study was to assess the penetrating abilities of an experimental solution which features properties of a dental infiltrant into the inner layers of decalcified human enamel with the use of an optical microscope. We examined images of polished teeth soaked with dyed preparations: Commercially-available Icon[®] and the experimental compound was examined under an optical microscope (Fig. 5A, 5B) and we attempted to design a preparation which could potentially have favorable properties of a dental infiltrant. Like commercially-available Icon[®], the designed experimental solution meets an essential requirement of a dental infiltrant, i.e. the ability to penetrate the demineralized (decalcified) parts of enamel, which is a phenomenon favorable in relation to treatment methods which utilize the resin infiltration technique. Moreover, it demonstrates polymerization abilities which turn it into a solid state material.

We were also able to assess the average penetration depth of both commercially-available Icon[®] and our own experimental preparation with the use of an optical microscope equipped with PHAMIAS 2003 v. 1.3B software. Measured values are presented in Table 3. Microscope observations conducted by Subramaniam et al. demonstrate that the depth of penetration of Icon[®] preparation into extracted human teeth ranges between 6.06 and 3.32 μm [13]. The obtained results prove that there is a high variety of penetration by infiltrating resin. This may possibly be due to the fact that their research material comprised of premolar teeth extracted for orthodontic reasons, which were thus exposed to the environment of the oral cavity and, therefore, subject to de- and remineralization processes, which, in turn, resulted in varying concentrations of both main and trace compounds, making the structure non-homogeneous. The research of demineralization process at the stage of a white spot is very difficult to conduct as the structure of the enamel constitutes the so-called low-porosity barrier. Removal and perforation of the subsurface layer of enamel is the main factor which decides about successful infiltration of the demineralized lesion [7].

The choice of the material which is to be used in the research is of essential importance. Therefore, based on our own experience, we chose to use retained human teeth as the research material. Retained teeth have no contact with the environment of the oral cavity and thus they are not subject to the de- and re-mineralization processes. On the other hand, the obtained results of infiltration depth for both preparations are still very small as the depth of canals etched in enamel with the use of Icon-Etch amounts to 30–40 μm , while a dental infiltrant should seal them thoroughly, i.e. block access of caries-inflicting bacteria and by-products of their metabolism [14]. Moreover, the available literature shows that the penetration of 60 μm into the enamel is sufficient enough to prevent further demineralization [1]. The results obtained during our own research are lower but similar to those described by Subramaniam et al. [13]. Our study is based on own tests conducted on the human enamel material. Most other researchers use bovine teeth in their research on infiltration because this kind of material is easier to obtain. However, it features higher porosity in comparison to human teeth, which makes it easier for the infiltrants to penetrate inner layers of enamel to a significant degree [15–17].

It should also be noted that the difference in penetration depth values between both preparations is very significant: The depth of Icon[®] penetration is almost 3 times higher than in the case of the experimental solution, whereas the difference of the penetration coefficients value is small. As presented in Fig. 5A and 5B, it is very difficult to draw boundaries of penetration for both preparations; therefore, the quoted depth values of penetration for both Icon[®] and the experimental solution are approximate.

During the preliminary study, the authors concluded that the experimental solution mixes well with the methylene blue, neutral fuchsine and eosin. An addition of eosin into a preparation which infiltrates the inner, demineralized layers of human enamel facilitates the assessment of such preparation with an optical microscope. A designed experimental solution with the main ingredients, i.e. 2-hydroxyethyl methacrylate (HEMA) and tetraethylene glycol dimethacrylate (TEGDMA), and a ratio of 75% to 25% penetrates the demineralized (decalcified) inner parts of enamel and polymerizes when exposed to light. In order to assess the infiltration of the experimental solution into the demineralized layers of tooth enamel, it is required to improve the measurement techniques which utilize optical microscopy.

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