MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
SUMY STATE UNIVERSITY
ACADEMIC AND RESEARCH MEDICAL INSTITUTE

Eastern Ukrainian Medical Journal

116, Kharkivska st., Sumy 40007, Ukraine
e-mail: eumj@med.sumdu.edu.ua
eumj.med.sumdu.edu.ua
ISSN: 2663-5909 (print)/2664-4231 (online)

How to cite: Dobrovolskyi D, Gevkaliuk N. Biomineralization of microstructural variations of dentinal tubules after root canal obturation using MTA-based cement with subsequent SEM analysis. *East Ukr Med J*. 2026;14(2):543-554. DOI: https://doi.org/10.21272/eumj.2026;14(2);543-554

BIOMINERALIZATION OF MICROSTRUCTURAL VARIATIONS OF DENTINAL TUBULES AFTER ROOT CANAL OBTURATION USING MTA-BASED CEMENT WITH SUBSEQUENT SEM ANALYSIS

Dmytro Dobrovolskyi
https://orcid.org/0000-0003-3993-636X
Department of Paediatric Dentistry, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

Natallia Gevkaliuk
http://orcid.org/0000-0002-7718-4616
Department of Children's Dentistry, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

ABSTRACT

Introduction. The quality of endodontic treatment of teeth with intra-root resorption process directly depends on careful instrumental, medicinal treatment and root canal filling. The use of Mineral Trioxide Aggregate (MTA) cement aggregates during root canal obturation is of concern due to its bioactive interactions with dentinal tubule phosphoapatite.

Methods. We prepared 50 curved root canals of human maxillary molars extracted according to indications and prepared them instrumentally for obturation. The intratubular biomineralization at the dentin-filling material interface was carefully analysed by scanning electron microscopy (SEM).

Results. We prepared 50 curved root canals of human maxillary molars extracted according to indications and prepared them instrumentally for obturation. The intratubular biomineralization at the dentin-filling material interface was carefully analysed by scanning electron microscopy (SEM). In the case of root canal obturation with GP and MTA sealer, increased biomineralization of dentinal tubules outside the penetrating sealant mark was confirmed by SEM observation ($p<0.05$). Mineralised structures of phosphoapatite (calcium/phosphorus ratio 1.45-1.89) were detected on the path of MTA penetration through dentinal tubules at a distance of 350-400 μm from the tubule mouth. The beginnings of crystallisation were observed along the intra- and/or intertubular dentin collagen. The depth of intratubular biomineralization was significantly increased in all pretreated dentinal tubules ($p<0.05$).

Conclusions. MTA cement and its derivatives can be used as a promising bioactive root canal filling agent to enhance biomineralization of dentinal tubules in a controlled environment.

Keywords: biomineralization, dentinal tubules, MTA cement, crystallisation activators, scanning electron microscopy.

Corresponding author: *Dmytro Dobrovolskyi, Postgraduate, Department of Paediatric Dentistry, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine*
**E-mail:* *dobrovolskyi_dv@tdmu.edu.ua*

РЕЗЮМЕ

Дмитро Добровольський
https://orcid.org/0000-0003-3993-636X
кафедра дитячої стоматології, Тернопільський національний медичний університет імені І.Я. Горбачевського МОЗ України, м. Тернопіль, Україна

Наталя Гевкалюк
http://orcid.org/0000-0002-7718-4616
кафедра дитячої стоматології, Тернопільський національний медичний університет імені І.Я. Горбачевського МОЗ України, м. Тернопіль, Україна

БІОМІНЕРАЛІЗАЦІЯ МІКРОСТРУКТУРНИХ ВАРІАЦІЙ ДЕНТИННИХ КАНАЛЬЦІВ ПІСЛЯ ОБТУРАЦІЇ КОРЕНЕВИХ КАНАЛІВ НА ОСНОВІ ЦЕМЕНТУ МТА З ПОДАЛЬШИМ СЕМ АНАЛІЗОМ

Вступ. Якість ендодонтичного лікування зубів із внутрішньо-кореневим резорбуючим процесом безпосередньо залежить від ретельної інструментальної, медикаментозної обробки та пломбування кореневого каналу. Застосування цемент на основі мінерального триоксидного заповнювача (МТА) під час обтурації кореневого каналу викликає занепокоєння через його біоактивні взаємовідносини з фосфоапатитом дентинних канальців.

Методи. В екстрагованих за показами молярах верхньої щелепи людей нами підготовлено 50 вигнутих кореневих каналів, які були інструментально підготовлені для їх обтурації. На межі дентин-пломбувальний матеріал ретельно аналізували інтратубулярну біомінералізацію за допомогою скануючої електронної мікроскопії (СЕМ).

Результати. При обтурації кореневого каналу GP і силер МТА посилена біомінералізація дентинних канальців поза межами проникаючої герметичної мітки підтверджена за допомогою SEM-спостереження ($p<0,05$). Мінералізовані структури фосфоапатиту (кальцій/фосфор співвідношення 1,45-1,89) виявлялися на шляху проникнення МТА через дентинні канальці на відстані 350-400 мкм від устя канальця. Вздовж інтра- та/або міжтрубчастого колагену дентину спостерігались зачатки кристалізації. Глибина інтратубулярної біомінералізації значно посилювалась у всіх попередньо оброблених дентинних канальцях ($p<0,05$).

Висновки. Цемент МТА та його похідні можуть бути використані як перспективний біоактивний пломбувальний засіб у кореневих каналах для посилення біомінералізації дентинних канальців у контрольованому середовищі.

Ключові слова: біомінералізація, дентинний каналець, цемент МТА, активатори кристалізаціїї, скануюча електронна мікроскопія.

Автор, відповідальний за листування: *Дмитро Добровольський, аспірант каф. дитячої стоматології, Тернопільський національний медичний університет імені І.Я. Горбачевського МОЗ України, м. Тернопіль, Україна*
**E-mail:* *dobrovolskyi_dv@tdmu.edu.ua*

INTRODUCTION

Endodontic treatment is a continuous, multi-stage process that aims to eliminate the source of infection and create a seal in the root canal system by filling it [1]. In theory, root canal filling material would be ideal if it were possible to fill the entire root canal system in practice. However, only a few currently published studies have confirmed the availability of such root canal obturation materials. Such materials have been studied, but the results have shown their incomplete effectiveness [2]. Mineral trioxide aggregate (MTA) has become widespread and is used in numerous treatment protocols, including root canal apex filling, perforation repair, or as an apical/coronal filling material during regenerative endodontic procedures [3]. In addition to its biological properties, MTA material also has an excellent sealing ability, which is due to the water-resistant end product of crystallisation reaction, which is resistant to moisture.

This sealing ability of MTA is largely due to its bioactive ability to form an appetite layer in contact with phosphate-containing physiological fluids, which are released in sufficiently large quantities when natural calcium is chelated from hydroxyphosphoapatite within the dentinal tubules. The release of phosphate-containing physiological fluids is explained by the corresponding reaction of sodium hypochlorite with hydrogen peroxide solution inactivation, which must be carried out to prevent formation of intra- or peritubular erosions, as previously reported [4]. These characteristics of MTA are considered important in biomineralization of dentinal tubules to enhance the retention capacity of material after root canal filling [5]. Thus, it makes it an important candidate for the choice of material for root canal filling in general.

However, MTA cannot be recommended as a routine orthograde root canal filling material, as the crystalline property and inconsistent metric characteristics of the material [6] make it difficult to use in a complex root canal system. Insufficient standardisation of the water-to-powder ratio and inadequate packaging also hinder the adaptation of MTA to the root canal wall [7]. To overcome such limitations of MTA as a root canal filling material [8], MTA was used as filling cement during the root canal filling procedure.

Recently, EndoSeal MTA, a fine powder based on pozzolanic MTA cement (Maruchi, Wonju, Korea), was introduced. Pozzolanic MTA cement is the main component of the sealant, which obtains its cement properties after a pozzolanic reaction involving calcium hydroxide and water, ensuring sufficient flow of the pre-mixed substrate through the injection tip due to the proper working consistency. Favourable mechanical characteristics such as fast setting time (about 4 minutes) and higher resistance to leaching make it more favourable than other commercially available MTAs and biological products, which have other equally positive effects. At the same time, the biocompatibility, mineralisation potential and odontogenic effect of pozzolanic MTA cement are still higher, as previously reported [9].

However, none of the studies conducted by numerous authors has yet confirmed the intratubular biomineralization ability of conventional MTA sealant when used as a material for root canal obturation. Therefore, our study was aimed at studying and evaluating the biomineralization capacity of MTA sealant under different conditions of root canal obturation.

The aim of the study was to evaluate the biomineralization capacity of dentinal tubules after root canal obturation under different conditions in teeth with simulated intra-root resorption using MTA cement-sealants and their derivatives by scanning electron microscopy.

MATERIALS AND METHODS

The study design was approved by the Bioethics Committee of I.Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Protocol No. 75 dated 01.11.2023). The procedures performed in the study involving human subjects complied with ethical standards. Patients included in the study were explained the purpose of the study and signed an informed consent.

We prepared a sample consisting of 50 roots from the number of the maxillary molars extracted for indications.

Fig. 1. Specimens prepared using the longitudinal cracks method. The untreated apical walls show pulp residues (arrows).

Method: SEM, accelerating voltage 15 kV; original magnification × 35

To detect a significant difference in the indicators of different study groups (p at the level of <0.05, <0.001), we used 50 roots of human maxillary molars with simulated intra-root resorption, the inclination angle did not exceed 30 degrees [10]. The teeth roots had fully formed apices. Teeth with cracks or root defects confirmed by microscopy (Carl Zeiss, Germany) were excluded from the study. All teeth were radiographically examined to assess the curvature of the root canals by measuring the angle between the longitudinal axis of the root and the line connecting the point starting from the root base to the long axis of the root and to its apex. The total average curvature of the root canal was 15.28° ± 7.08° (Fig.1). And the teeth roots were divided into 5 groups (10 root canals each) according to the canal curvature and obturation technique by block randomisation (Fig. 2 A).

The root canal was accessed using spherical carbide burs and Endo-Z burs. Root canal patency was achieved with a stainless steel (SS) K-file, size 10 (Dentsply Maillefer, Ballaigues, Switzerland) until the tip appeared in the apical opening. The working length was defined as 1 mm less than the measured length. After coronal flaring with Gates Glidden burs No. 2 to No. 4 (Komet, Rock Hill, SC), the root canals were processed with instruments to apical size No. 35/06 taper using the crown-down technique with ProFile rotary instruments 0.04 and 0.06 Ni-Ti (Dentsply Maillefer).

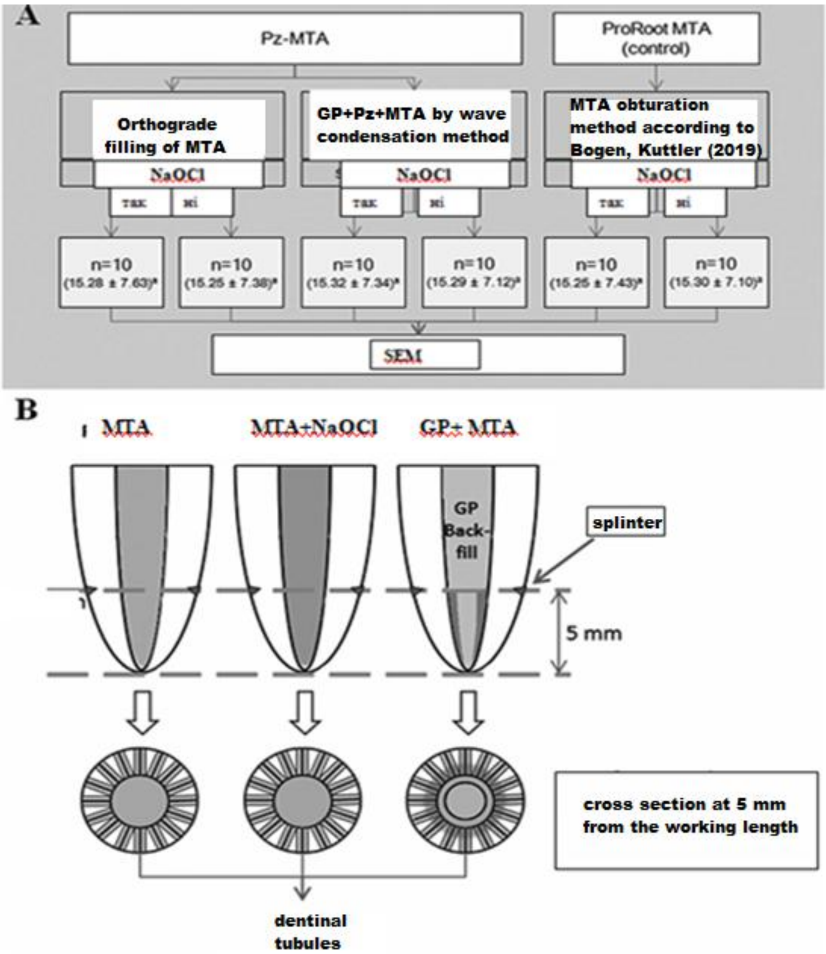


Fig. 2. A: Flow chart with a diagram of the experimental groups of teeth distribution. The number in parentheses shows the mean and standard deviation of the canal curvature of each root group, and their identical upper case indices, which show no significant difference in the mean canal curvature values (p>0.05). B: Schematic diagram of root sample preparation for SEM method evaluation.

Notes: MTA – mineral trioxide aggregate (filler); MTA - mineral trioxide aggregate (filler) based on pozzolana; GP - gutta-percha; NaOCl - sodium hypochlorite; SEM - scanning electron microscopy

The simulation of intra-root resorption was reproduced by preparing the inner part of the root canal with a thin carbide endodontic bur under the control of an electron microscope (Karl Kaps, Germany). The root canals were treated with 2 ml of 5.25% sodium hypochlorite (NaOCl) solution and immersed in a 17% ethylenediaminetetraacetic acid (EDTA) solution at pH 7.2 for 1 min before final rinsing with 2.5 ml of 5.25% NaOCl solution. The latter was inactivated with H_2O_2 solution. All irrigants were activated using ultrasonic devices (P5 Newtron1 XS; Satelec, Acteon Group, Mérignac, France).

Instrumented root canals were rinsed well with sterile distilled water and dried with sterile paper pins. For the root canal obturation procedure, 10 canals were obturated with gutta-percha according to the size and taper of the canal and MTA sealer (EndoSeal MTA, bioceramic sealer) using the continuous condensation wave (CW) technique. For a control comparison, another 10 canals were obturated exclusively with MTA ProRoot. The pre-mixed MTA sealing cement was released using an injection syringe and tip system. Canals (n=10) filled with ProRoot MTA according to the obturation technique proposed by Bogen G, Kuttler (2019) were also used as a positive control. ProRoot MTA was mixed with distilled water according to the manufacturer's instructions and applied gradually using a Lee block, cold gliders, and an S-Condenser. The SS K-file, 1 or 2 sizes smaller than the main apical file (MAF), was used to seal the apex (3-4 mm), and increasing K-files in an ascending gradation were gradually used for further compaction. Finally, the coronal part of the tooth was tamped with a manual plugger to complete the root canal obturation. For pretreatment with buffered phosphate-buffered saline (PBS), an additional 10 canals were selected for each experimental group. They were immersed in sterile PBS solution for 1 minute and dried using sterile paper canal filling pins. After obturation, the teeth were stored at 4°C in a 2.4% glutaraldehyde solution. For the SEM study, the preparations were prepared by the conventional method and viewed in a scanning electron microscope SEM JEOL-25 -TA220 (Japan).

Biomineralization of the dentinal tubules of the samples was assessed after 12 weeks in a scanning electron microscope to characterise the microstructural variations of the dentinal tubules. Each tooth was mounted in an acrylic block and each mesiobuccal or distobuccal root was separated from the teeth using a low-speed diamond disc tip under water cooling (Isomet Low Speed Saw; Buehler, Lake Bluff, IL). Separated roots were split horizontally for cross-sectional analysis at 5 mm above the level from the apex (see Fig. 2B). The root segments were quickly rinsed in distilled water and sputtered with chemically pure aluminium for SEM observation with an accelerating voltage of 15 kV. At the interface of the main root canal and obturation material, the depth of its penetration into the dentinal tubules was recorded and the character of intratubular mineralisation was assessed.

Statistical processing of the results was performed using one-way analysis of variance and Tukey post hoc test with SPSS software (SPSS Inc., Chicago, IL) to assess differences between the experimental groups. For each study group, intratubular biomineralization was investigated using a two-sample t-test. The value at the level was set at $p<0.05$.

RESULTS

SEM analysis of root canal specimens obturated with GP (gutta-percha) using MTA (bioceramic) sealer showed direct penetration of MTA into the apical part of the root canal as a whole (Fig. 3) and into the dentinal tubules with subsequent formation of apatite crystals, which were found in the form of densely packed conglomerates in the dentinal tubules (Fig. 4).

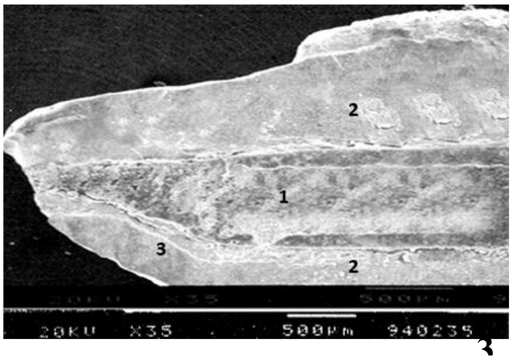


Fig. 3. General view of an MTA-cemented root canal. Designations: 1 - MTA; 2 - dentin; 3 - line of instrumented root canal expansion.

Method: SEM; accelerating voltage 15 kV; original magnification × 35.

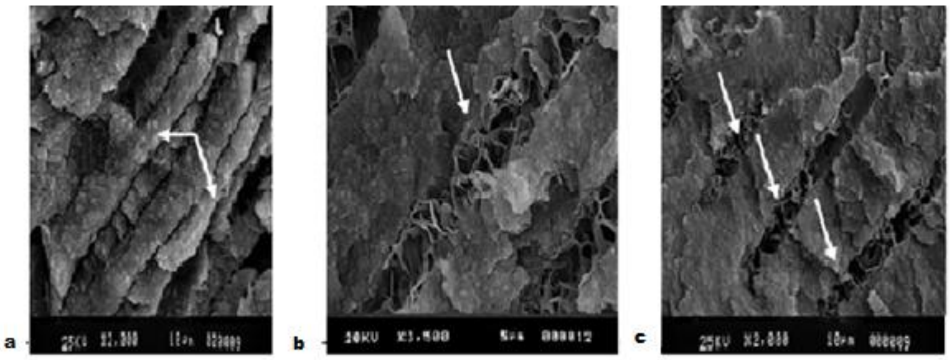


Fig. 4. SEM image of gutta-percha with MTA: a - MTA sealant cement penetrated into dentinal tubules (arrows) at the level of the dentinal tubule mouth in the form of a 'finger tongue'; b - distant areas of biomineralized dentinal tubules (arrows) beyond the penetration of MTA cement at a distance of 50-100 µm, densely packed and organised apatite nanoprecipitates in the dentinal tubules are visible; c - consistently biomineralized dentinal tubules (arrows) at a distance of 100-150 µm from their mouths.

Method: SEM; accelerating voltage 20 kV; original magnification × 2000; scale bar = 10 µm

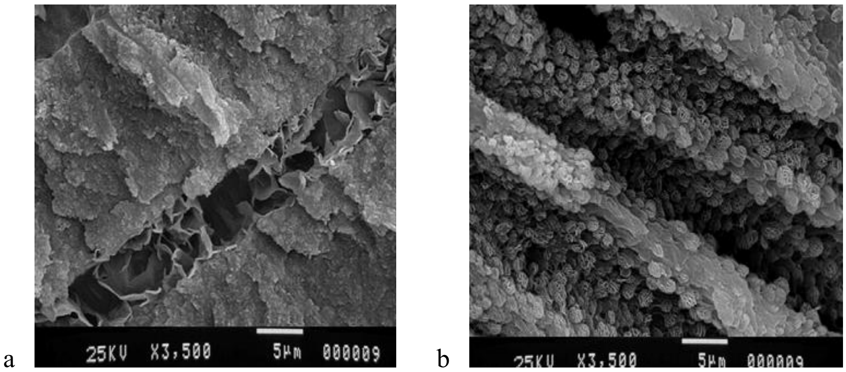


Fig. 5. General view of petal (a) and spherical (b) biomineralization in dentinal tubules during MTA root canal sealing

On the other hand, the adaptation and close localisation of material to the main canals and intratubular biomineralization near the openings of the dentinal tubules were observed when obturation with MTA alone. The mineralised structure formed at the material-dentin interface was confirmed at the level of the dentinal tubule lumen, although there was insufficient material at the loci of direct MTA penetration into the tubules. The patterns of apatite crystallisation at the entrance to the dentinal tubules (mouth) in the complex of both GP materials with MTA filling cement were similar and were observed in the samples shown in (Fig. 5.a, b) and MTA samples (Fig. 6, a, b).

However, crystallisation of apatite is altered along the dentinal tubule: agglomerated MTA precipitates rarely completely occlude dentinal tubules (Fig. 7, a, b).

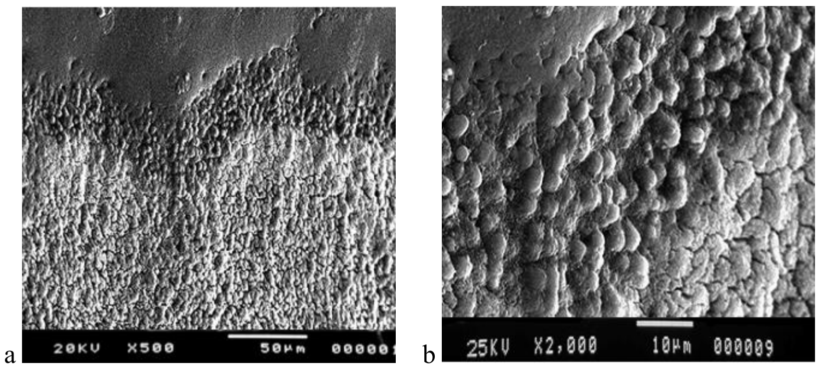


Fig. 6. Conformational changes of apatite crystals on the surface of MTA particles caused by intratubular biomineralization. Crystallisation of dentinal tubule material in the form of microspheres

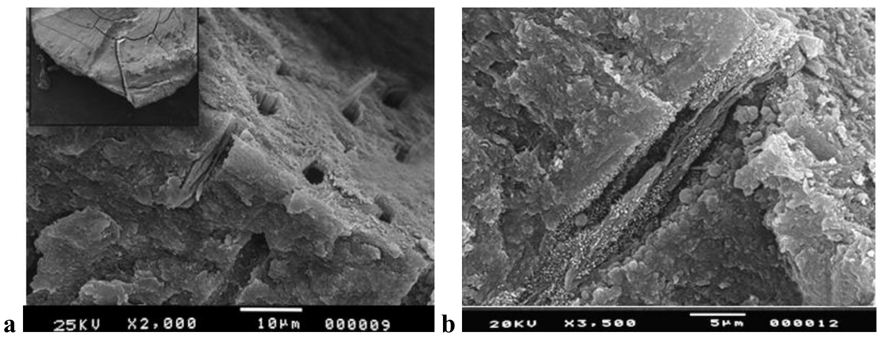


Fig. 7. General view of intratubular biomineralization stimulated by MTA sealant: a - area of a horizontally split specimen encased in gutta-percha and MTA cement, showing the boundary at a distance of 350-400 µm from the dentinal tubule mouth; b - pre-crystallisation phase, when crystallisation aggregates similar to collagen fibrils are observed

At the same time, continuous and consistent crystallisation along the tubular lumen is observed when filling with a group of sealing materials (GP + MTA) (Fig. 8, a, b).

In the group of sealants (GP with MTA), various nanostructures of precipitates, mainly petal flakes, were found in a stratified or organised spherical form, or in the form of a mixed structure (Fig. 9, a, b).

It is interesting to note that the mineralised structural apatite observed in dentinal tubules was confirmed at a distance of 350-400 μm from the tubule mouth, and crystallisation nuclei were observed along intra- and/or intertubular collagen fibres (Fig. 10).

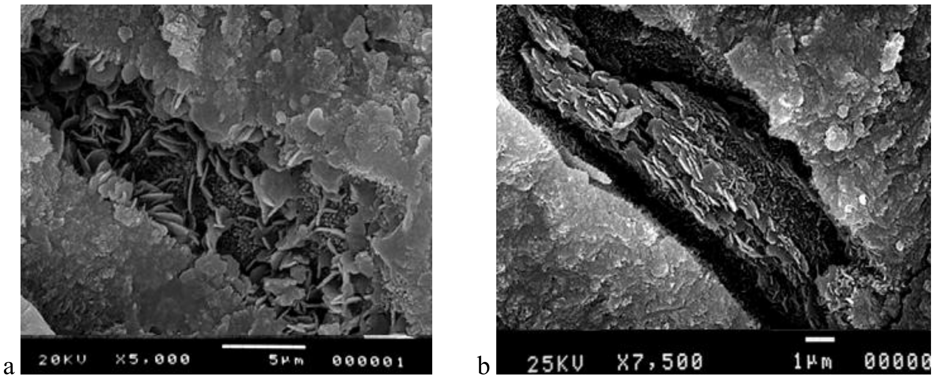


Fig. 8. Various configurational changes of apatite crystals on the surface of MTA particles caused by intratubular biomineralization: a - mixed flakes; b - organised and aggregated crystallisation plates, assembled into aggregates that stick together into one continuous elongated aggregate that can be used for dentinal tubule filling

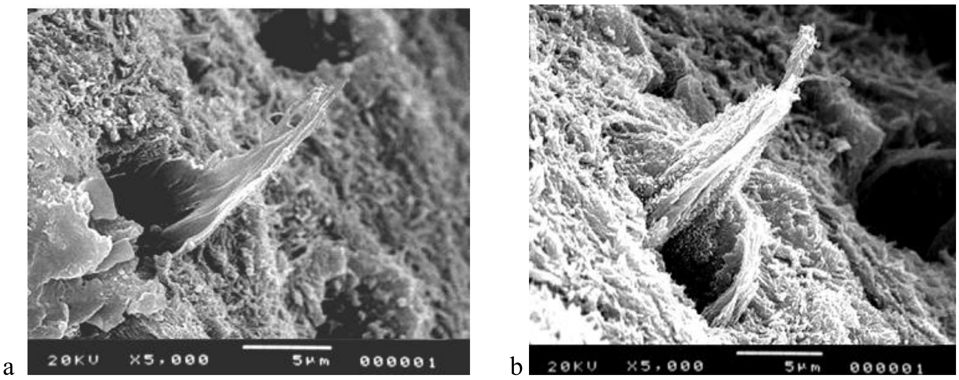


Fig. 9. Various configurational changes of apatite crystals on the surface of MTA particles caused by intratubular biomineralization: a - sequential intratubular biomineralization in a lamellar form; b - longitudinal agglomeration of precipitates in the form of collagen-like fibrils. *Method: SEM; accelerating voltage 20 kV; original magnification × 5 000; scale bar = 5 µm*

It is noteworthy that although the obturation material adheres closely to the canal wall (Fig. 11), from time to time there is a depressurisation of the dentinal tubule openings, as previously reported by other authors [11]. In our study, it was found that the preparations from the root canal wall, which was previously filled with MTA, after acid dentin etching, clearly show the initial sections of dentinal tubules filled with this MTA (Fig. 10, inset). 'Dentin columns' on the MTA surface, which is adjacent to the root canal wall and serves as a replica of dentinal tubules filled with MTA sealant to a tubular length of ≈300 µm from their mouths. The 'dentinal columns' on the MTA surface look like palisades near the root canal wall, corresponding to the 'finger-like tongues' shown in Figure 4a, and are well amenable to morphometry, indicating the quality of cleaning of this wall before filling with MTA sealant.

A completely different SEM picture is found in the teeth of patients of groups 2 and 3:

1) in 85% of the samples, the sealant does not fill the root canal to the apex (Fig. 12, a);

2) there is no close contact between the sealant and the root canal wall (Fig. 12, b);

3) the structure of the sealant itself has microdefects and cracks (Fig. 12, c).

The filling material in patient of groups 2, 3 and 4 has poor adhesion to the root canal walls, forming a gap of ≈ 20-30 μm, and there are pronounced defects in the material itself (Fig. 13, a, b) and (Fig.14).

The obtained result indicates the need to improve the methodology of root canal filling, since the free spaces between the adhesive and hybrid layers may be a locus for the development of microflora, which in case of depressurisation of the apical root canal opening can cause secondary infection of dentin with all the consequences that arise in this case.

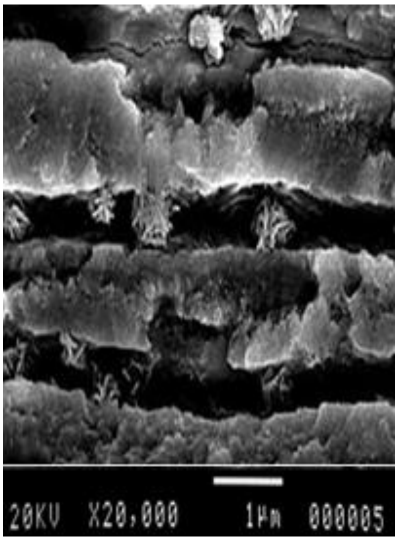


Fig. 10. General view of crystallisation nuclei along the dentinal tubules (arrows) of the group 5 patients after sealing the root canal with MTA at a distance of 350-400 μm from their mouth

In the samples of group 5, root canals sealed with GP gutta-percha with MTA sealer showed direct penetration of the sealant and biomineralization to a significantly greater depth of the dentinal tubule than in other groups. Pretreatment with NaOCl and EDTA significantly contributed to the depth of biomineralization in the tubules ($p<0.05$). Root canals obturated exclusively with MTA without pretreatment with NaOCl showed a minimal depth of biomineralization without penetration of materials into the dentinal tubule.

DISCUSSION

The main task of endodontists remains the search for the optimal material for filling root canals. However, the technological progress of obturation methods has not yielded statistically proven significant results on the predominant effect of a particular method on the treatment outcome. Radiological examination of depulpated teeth of patients may reveal densely filled root canals, but they do not support a biologically sealed root canal system from the surrounding periapical tissue. To date, microcomputed tomography has been proposed, which can evaluate the ability of sealing materials and the effectiveness of root canal filling, while not providing sufficient information about the filling of the root canal system. In this regard, confirmation of mineralization in the dentinal tubules can provide secondary evidence that indicates the possibility of filling the root canal system.

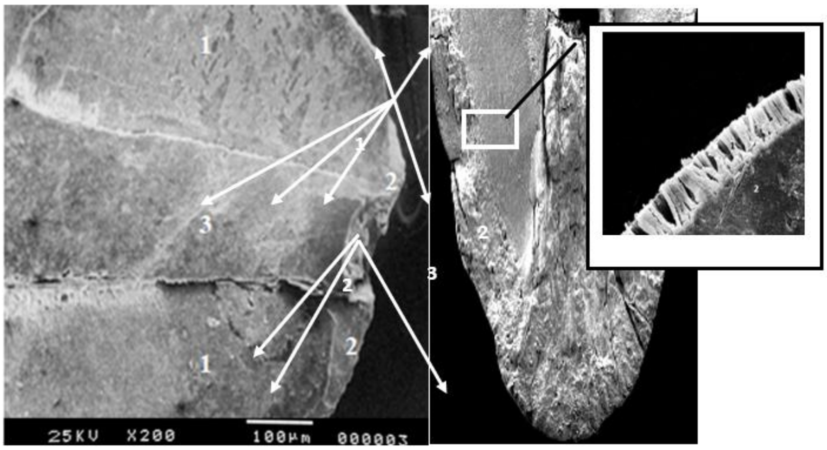
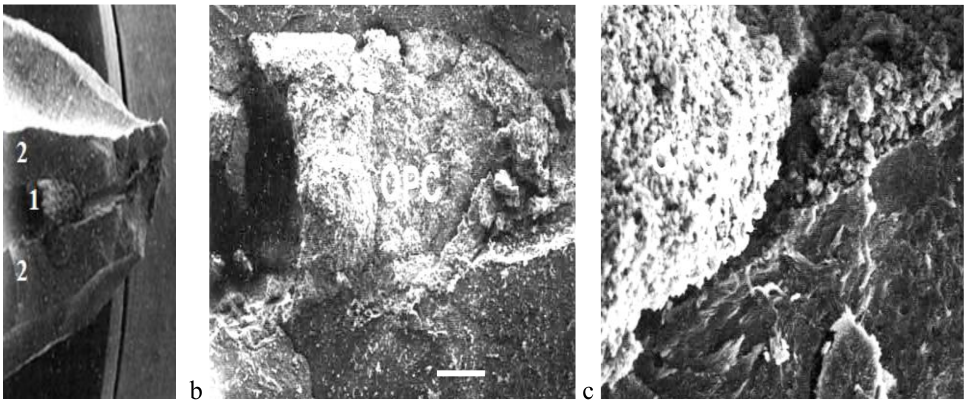


Fig. 11. General view of the apical part of the root canal filled with MTA cement. Abbreviations: 1 - dentin; 2 - cement; 3 - MTA; notation in the photo inset: 1 - dentin columns; 2 - MTA

Therefore, our study was conducted to extrapolate and comparatively evaluate the biomineralization capacity of the currently widely available MTA cement with enhanced clinical convenience. In our study, MTA cement showed deeper intratubular biomineralization. This provides convincing evidence in favour of MTA (bioceramics) as a bioactive root canal sealant when combined with hot GP filling and condensation by vertical pressure. In addition, premedication with NaOCl followed by EDTA before final intratubular obturation promotes mineralization of both MTA and sealer MTA (110-150%) and ProRoot

MTA during canal obturation (130-190%). Although the intratubular depth of MTA mineralization was rather limited compared to the studies of other authors (Reyes-Carmona), in which NaOCl treatment was performed, the strategic importance of enhancing the biomineralization capacity is the inclusion of phosphate anion as the nucleus of the initial condensation of the material on the root canal wall. This, in our opinion, is the basis of the morphological substrate of high biocompatibility of this filling material, unlike others that are often used in endodontic practice.



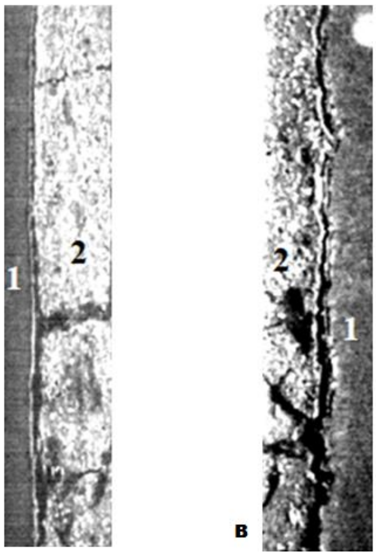
a b c

Fig. 12. General view of the sealant in the apical part of the root canal, which is not completed to the apex (a, b) and has cracks in its structure. Designations: 1 - gutta-percha; 2 - dentin

Pretreatment with phosphate anions obtained by sequential impregnation with PBS could enhance the formation of such crystallization and biomineralization foci, which is known as the polymer-induced liquid precursor (PILP) of this process [12]. The phosphate anions in PBS are considered to be an intra-channel environment that becomes uniform or more labile in the process of contact with PILP, enhancing the formation of a prenucleation cluster and its further growth during crystallization on collagen fibres exposed after removal of the lubricated layer by EDTA pretreatment. Such treatment can also affect the crystallization process after penetration with amorphous precursors. However, there are no reports on the clinical use and biomineralization capacity of EDTA as a final treatment solution before root canal obturation. In view of this, the results of our SEM analysis are the first confirmed report of biomineralized apatite that penetrates 350-400 µm deep into dentinal tubules with a variety of crystal nanostructures such as petals (phlox) and flakes, in stratified or organized spherical forms.

Although the obturation material adheres closely to the root canal wall, from time to time there is a defect in the fit and partial depressurization of the mouth of the dentinal tubules, as previously reported by other authors (Bird).

Both gutta-percha and MTA could not penetrate the dentinal tubules regardless of their different particle sizes. Rather, they showed mineralized structures similar to crystallization “nuclei” connecting the material interface with dentin further from the level of the dentinal tubule mouth. These structures should represent surface flocculated crystals that could have formed on the material and that were grown from a crystallization precursor.

a


B

Fig. 13. General view of the contact between the sealant and the root canal wall in group 2 (a) and group 3 (b). Designations: 1 - dentin; 2 - sealant

In fact, the results of our study showed that such petal-like structures (phlox) were the result of the biomineralization potential of MTA cement, which is able to enhance the interaction with phosphate-containing compounds of dentinal tubules after calcium chelation in their walls. However, the “nuclei” of crystallization found in the dentinal tubules of compacted GP with MTA are the result of material penetration due to vertical pressure condensation transmitted through the thermoplasticized gutta-percha and are clearly different from such "nuclear structures" in other groups. In addition, the small particle size of MTA could contribute to this induction of more stable crystallization precursors for efficient ion diffusion, in contrast to higher molecular weight particles in cements with other structures.

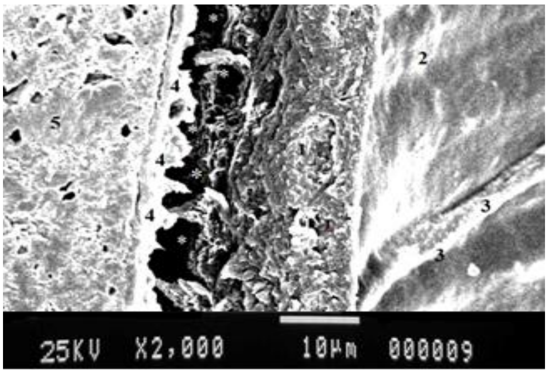


Fig. 14. SEM image of the area of contact with the root canal wall (1), with a homogeneous dentin structure (2) and dentinal tubule profile (3) after root canal treatment, where the adhesive layer (4) on the side of the filling material and the hybrid layer on the side of the root canal wall, (5) filling material are clearly visible. However, free spaces between the adhesive and hybrid layers are clearly visible (*)

The average particle size of white ProRoot MTA is 10 microns, while all particles after polymerization have a size of 20-50 microns. The resulting paste with such particle aggregates becomes promising for orthograde root canal filling. On the contrary, finely dispersed MTA pulverized cement with an average particle size of 1.5 μm becomes thixotropic when the material is released through the needle tip and further compressed by vertically directed pressure. It then seeps or grinds into the dentinal tubules to form sealed plugs and apatite crystallization "nuclei" for subsequent intratubular biomineralization.

Such stable precursors can induce expansion of crystallization along the dentinal tubules with secondary crystal nucleation among individual disordered phase nanoparticles on the crystallization "nuclei", which ensures consistent biomineralization as deeper sections of the dentinal tubules are compacted. In fact, the root canals treated with MTA showed a slight blockage of some areas by agglomerates at a limited depth, where MTA cement-induced crystals were also visible. They were of a different shape than those caused by gutta-percha, within the elemental composition of Ca/P ratio similar to hydroxyapatite, which is confirmed by the results of X-ray energy dispersive microanalysis on the SEM console "EDAR" [13].

Among the various apatite structures, particles with a grain size of less than 100 nm, in at least one direction, have a higher surface area of activity as an ultrafine structure, which leads to enhanced bioactivity than coarser crystals [14]. We can confirm the direct formation of mineralization foci in groups of GP and MTA sealants, extended beyond a depth of more than 300 μm. At the same time, MTA cement as a sealant demonstrated a favourable biomineralization pattern for filling the root canal system, while inside the canals filled with MTA sealant alone, such fine structures were not enough.

An in-depth study on the correlation between crystallography and trace element composition of cement-induced biomineralization by MTA-sealing of dentinal tubules requires further investigation. In the present study, the use of MTA cement as a sealant in combination with a well-fit gutta-percha cone and vertical pressure sealing resulted in consistent biomineralization of dentinal tubules. Preconditioning with NaOCl + H_2O_2 before root canal obturation triggers the PILP process and leads to increased biomineralization of the material and its distribution into the dentinal tubules beyond the penetrated MTA ('finger tongue') in different tubules. Thus, MTA-cement as a root canal sealant provides new opportunities for biohermetic root canal filling.

CONCLUSIONS

1. Depending on the type of root canal wall pretreatment, different types of sealants have their own specific characteristics of contact with the root canal wall, which is manifested in the delicate relationship with the mouths of the dentinal tubules, the depth of penetration of the sealant into them, the conditions of biomineralization with the appearance of crystallization "nuclei" along the dentinal tubules, as well as the nature of contact with the root canal wall.

2. The depth of MTA penetration into the dentinal tubules is confirmed morphometrically and is ≈ 300 μm, which significantly increases the retention capacity of the filling material and increases the prognostic reliability of endodontic treatment.

3. After performing various techniques of obturation of the root canals of previously extracted teeth according to indications, the biomineralization ability of dentinal tubules in teeth with simulated intra-root resorption using Mineral Trioxide Aggregate (MTA) cement - sealants and their derivatives showed the best result in both penetration into the dentinal tubules and their sealing.

PROSPECTS FOR FUTURE RESEARCH

Prospects for future research consist in the studied effect and quality of root canal cleaning with intra-root resorption.

ETHICAL CONSIDERATIONS

The study design was approved by the Bioethics Committee of I.Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Protocol No. 75 dated 01.11.2023). The procedures involving human subjects complied with ethical standards. The patients taking part in the study signed an informed consent.

AUTHOR CONTRIBUTIONS

Dmytro Dobrovolskyi [A,B,C,D]

Natallia Gevkaliuk [A,D,E,F]

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

FUNDING

None.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

1. Siqueira JF. Strategies to Treat Infected Root Canals. Journal of the California Dental Association [Internet]. 2001 Dec 1;29(12):825–35. Available from: http://dx.doi.org/10.1080/19424396.2001.12223244
2. Zmener O, Pameijer CH, Serrano SA, Vidueira M, Macchi RL. Significance of Moist Root Canal Dentin with the Use of Methacrylate-based Endodontic Sealers: An In Vitro Coronal Dye Leakage Study. Journal of Endodontics [Internet]. 2008 Jan;34(1):76–9. Available from: http://dx.doi.org/10.1016/j.joen.2007.10.012
3. Torabinejad M, Parirokh M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview – part II: other clinical applications and complications. International Endodontic Journal [Internet]. 2017 Oct 11;51(3):284–317. Available from: http://dx.doi.org/10.1111/iej.12843
4. Tay FR, Pashley DH, Rueggeberg FA, Loushine RJ, Weller RN. Calcium Phosphate Phase Transformation Produced by the Interaction of the Portland Cement Component of White Mineral Trioxide Aggregate with a Phosphate-containing Fluid. Journal of Endodontics [Internet]. 2007 Nov;33(11):1347–51. Available from: http://dx.doi.org/10.1016/j.joen.2007.07.008
5. Revankar V, Prathap M, Shetty KhK, Shahul A, Sahana K. Effect of biomineralization ability on push-out strength of proroot mineral trioxide aggregate, mineral trioxide aggregate branco, and calcium phosphate cement on dentin: An In vitro evaluation. Journal of Pharmacy And Bioallied Sciences [Internet]. 2017;9(5):121. Available from: http://dx.doi.org/10.4103/jpbs.jpbs_120_17
6. Gandolfi MG, Siboni F, Primus CM, Prati C. Ion Release, Porosity, Solubility, and Bioactivity of MTA Plus Tricalcium Silicate. Journal of Endodontics [Internet]. 2014 Oct;40(10):1632-7. Available from: http://dx.doi.org/10.1016/j.joen.2014.03.025
7. Moazami, Fariborz et al. "Evaluation the Solubility and the Porosity of the Nano Fast Cement Comparing to the Mineral Trioxide Aggregate: An *in vitro* Study." *Journal of dentistry (Shiraz, Iran)* vol. 24,1 (2023): 28-33. https://doi.org/10.30476/DENTJODS.2021.92684.1668
8. Haji TH, Selivany BJ, Suliman AA. Sealing ability in vitro study and biocompatibility in vivo animal study of different bioceramic based sealers. Clinical and Experimental Dental Research [Internet]. 2022 Nov 17;8(6):1582–90. Available from: http://dx.doi.org/10.1002/cre2.652
9. Abuarqoub D, Aslam N, Jafar H, Abu Harfil Z, Awidi A. Biocompatibility of Biodentine™ ® with Periodontal Ligament Stem Cells: In Vitro Study. Dentistry Journal [Internet]. 2020 Feb 8;8(1):17. Available from: http://dx.doi.org/10.3390/dj8010017
10. Tavares KIMC, Pinto JC, Santos-Junior AO, Duarte MAH, Guerreiro-Tanomaru JM, Tanomaru-Filho M. Effect of Additional Apical Preparation on Retreatment of Curved Root Canals Filled with Different Sealers. European Journal of Dentistry [Internet]. 2022 Sep 8;17(03):636–41. Available from: http://dx.doi.org/10.1055/s-0042-1750693

11. Bird DC, Komabayashi T, Guo L, Opperman LA, Spears R. In Vitro Evaluation of Dentinal Tubule Penetration and Biomineralization Ability of a New Root-end Filling Material. Journal of Endodontics [Internet]. 2012 Aug;38(8):1093–6. Available from: http://dx.doi.org/10.1016/j.joen.2012.04.017
12. Yao S, Jin B, Liu Z, Shao C, Zhao R, Wang X, et al. Biomineralization: From Material Tactics to Biological Strategy. Advanced Materials [Internet]. 2017 Feb 23;29(14). Available from: http://dx.doi.org/10.1002/adma.201605903
13. Gevkaliuk NO, Sydliaruk NI, Martyts YM, Pynda MY, Krupei VY, Mykhailiuk VM. Differentiation of the structure of dentinal tubules and dentinal canaliculi in human teeth of different ages . Regul. Mech. Biosyst. [Internet]. 2024May27 [cited 2025Jun.26];15(3):394-0. Available from: https://medicine.dp.ua/index.php/med/article/view/1004
14. Yoo YJ, Lee Y, Yoo J, Perinpanayagam H, Yoo C, Kang H, et al. Intratubular Biomineralization in a Root Canal Filled with Calcium-Enriched Material over 8 Years. Materials [Internet]. 2017 Dec 5;10(12):1388. Available from: http://dx.doi.org/10.3390/ma10121388

INFORMATION ABOUT THE AUTHORS

Dmytro Dobrovolskyi

Postgraduate, Department of Paediatric Dentistry, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine.

https://orcid.org/0000-0003-3993-636X

*E-mail: dobrovolskyi_dv@tdmu.edu.ua

Natallia Gevkaliuk

Doctor of Medical Sciences, Professor, Department of Children's Dentistry, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine.

http://orcid.org/0000-0002-7718-4616

*E-mail: gevkalyuk@tdmu.edu.ua

Received: 07.08.2025

Accepted for publication: 08.11.2025

Published: 23.06.2026