ORIGINAL ARTICLE

CONTENTS

Morphological changes in the crystallization of mixed saliva during the treatment of dentofacial anomalies with aligners

Anton Kolomiiets[1], Monika Białoszycka[2], Alisa Pachevska[1], Iryna Simonova[3], Olena Popova[1], Nadiia Drachuk[1], Tetiana Chugu[1,]

[1]DEPARTMENT OF PEDIATRIC STOMATOLOGY, NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE
[2]SCHOOL OF MEDICINE, COLLEGIUM MEDICUM, UNIVERSITY OF WARMIA AND MAZURY, OLSZTYN, POLAND
[3]DEPARTMENT OF PHILOLOGY, NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

Aim: To investigate morphological changes in the crystallization patterns of oral fluid during orthodontic treatment with aligners.

Materials and Methods: Samples of dried saliva were analyzed from young adults aged 18–25 years of both sexes in a comparison group and in two main groups: patients treated with aligners without preventive measures and patients treated with aligners with preventive care.

Results: In the main group of young adults treated with aligners three months and six months after the initiation of treatment, disturbances in saliva crystallization were observed, which were evident from both qualitative and quantitative indicators. A study of the crystallographic pattern of a dried saliva was also conducted during the use of aligners in combination with preventive agents destruction of the clear crystal structure was lesser violations were observed. After from the initiation of treatment (three and six months after the start of treatment), a reduction in the size and number of amorphous structures was observed in the peripheral (protein) zone.

Conclusions: The use of aligners in young adults induces destructive morphological changes in the crystallization of mixed saliva. Prophylactic application of a Decasan solution demonstrates a pronounced protective effect.

KEY WORDS: crystallization, saliva, aligners, preventive care

Wiad Lek. 2026;79(5):907-917. doi: 10.36740/WLek/219790 DOI

INTRODUCTION

In recent years, saliva has become an important element of laboratory diagnostics. Researchers are continuously seeking new ways to use saliva in medical diagnostics and in monitoring treatment outcomes. The application of saliva in diagnostics is gaining increasing popularity due to the advantages it offers, not only from an economic perspective. The collection of saliva as a diagnostic material is inexpensive, non-invasive, and painless, and is also convenient for young children, older adults, and physically active individuals. Saliva can be collected at home without the involvement of medical personnel. Transportation of this material does not require special procedures, and compounds detected in saliva are generally characterized by high stability [1–4].

Saliva consists not only of secretions from the salivary glands but also of other fluid and cellular components. Water accounts for approximately 94–99.5% of its composition. The remaining fraction represents the solid component, the amount of which varies - approximately 6% under resting conditions and about 0.5% after stimulation. The main organic components of saliva include protein substances such as enzymes, serum proteins, mucins, immunoglobulins, blood group substances, kallikrein, lactoferrin, epidermal growth factor (EGF), histatin, cystatin, statherin, sialin, hormones, and vitamins A, B, C, and K. The density of saliva ranges from 1.002 to 1.012 g/mL, and its pH largely depends on the rate of secretion.

At night, when salivary secretion is slower than during the day, the pH reaches approximately 6.2–6.5; it may increase to about 8.0 due to an elevated concentration of bicarbonate ions. Salivary secretion can be stimulated by gustatory, mechanical, thermal, olfactory, visual, or psychological stimuli. Regulation of salivation occurs via the cholinergic system as well as the α- and β-fibers of the sympathetic nervous system. Basal salivary secretion averages 0.33–0.55 mL/min and varies considerably among individuals, even under standardized conditions. Following strong secretory stimulation, such as food intake, salivary flow may increase to 1.5–2.3 mL/min,

and after exposure to pharmacological agents such as pilocarpine or methacholine, it may reach up to 5.0 mL/min. The daily volume of salivary secretion depends on sleep duration, the frequency and type of meals, and the influence of emotional stimuli, averaging 1–2 liters per day. A characteristic feature of salivary secretion is its disproportionately large volume relative to the mass of salivary gland tissue and its low osmolality. Fasting saliva is hypotonic, whereas at maximal secretion it becomes isotonic with blood plasma. The volume and composition of saliva vary depending on age and sex. Relatively low salivary secretion in newborns increases with age, particularly between 3 and 5 years, reaching its first peak by the end of the first decade of life. After the age of 30, a clear tendency toward a decrease in salivary secretion is observed. Male saliva is secreted in larger volumes than female saliva and is also characterized by higher concentrations of sodium, calcium, and phosphorus. Physical exercise causes a significant increase in ion concentrations in saliva, particularly sodium [5–7].

Compounds present in saliva can be divided into two groups. The primary classification criterion is the site of formation of a given compound—either within or outside the salivary glands. Compounds synthesized in the salivary glands and classified into the first group play only a secondary role in the use of saliva as a diagnostic material. An exception within this group is secretory immunoglobulin A (sIgA), which, although lacking immunological memory, supports agglutination processes and prevents bacterial adhesion and colonization on soft tissues and teeth, acting synergistically with nonspecific defense mechanisms. Extraglandular compounds, in contrast, are formed outside the salivary glands and are transported from plasma into saliva. This transport may occur via intracellular or extracellular pathways [8–11].

Intracellular transport includes passive or specific mechanisms. Passive transport is defined as diffusion or filtration. Specific transport comprises carrier-mediated transport, energy-dependent active transport, facilitated diffusion, or pinocytosis. Extracellular transport of plasma components into saliva may occur through ultrafiltration or via disruptions of natural membranes. Compounds that enter saliva exclusively through damaged membranes include thyroxine and triiodothyronine. Thyroid hormones are examples of substances whose salivary concentrations do not reflect their systemic levels. Compounds that can be measured in saliva rather than in serum enter saliva by diffusion. Diffusion is a process dependent on three factors: molecular weight, solubility of the compound in water and/or lipids, and the degree of ionization of the compound [12–15].

Saliva not only protects the oral cavity by maintaining a buffering environment but also exhibits antibacterial and remineralizing properties, participates in taste perception, regulates water balance, and contributes to blood coagulation. Its functions also include tissue repair. Compounds that can be detected in saliva instead of serum enter saliva via diffusion [16–20].

The structures of the studied fluids are obtained through a phase transition from the liquid to the solid state by dehydration. The results of experimental studies have demonstrated that information contained in the liquid phase at the molecular level is transferred to the macroscopic level during dehydration, forming various structures that become visible to the researcher [8].

Investigations of self-organization processes in various physiological and pathological liquid media have established the primary importance of organic components present in biological fluids, even in small amounts (from 0.01 μm to 100 g/L and higher), in structure formation. The differentiation of structures into organic and inorganic components is carried out using the dehydration method, which involves applying a biological fluid onto a transparent surface in the form of droplets followed by dehydration under specific conditions. The droplet volume is determined by the ratio between the fluid's specific gravity and its surface tension forces. According to theoretical concepts, specific interacting mechanisms operate during the dehydration of biological fluids, ensuring the formation of structures within solid-phase systems and subsystems. The term facies refers to what remains of a saliva droplet after drying. A schematic representation illustrating the action of these mechanisms is provided by a sagittal-section diagram of a biological fluid droplet placed on a horizontal surface, developed by Tarasevych and Ayupova (2003) [21].

The authors noted that fluid evaporation occurs unevenly across the exposed surface of the droplet. Because the hemispherical droplet has varying thickness—greater in the central region and thinner at the periphery—the evaporation of the analyzed droplet results in a non-uniform change in solute concentration. Specifically, in the peripheral region (characterized by reduced thickness), solute concentration increases more rapidly than in the central region (with maximum thickness). During these processes, osmotic and oncotic forces interact. Since osmotic forces significantly exceed oncotic forces, salts migrate toward the center of the droplet, where the concentration of dissolved substances is lower. In contrast, in the central region, proteins and other high-molecular-weight solutes release water and shift toward the droplet periphery.

As a result, the marginal amorphous zone of the dehydrated droplet is represented by structures of organic

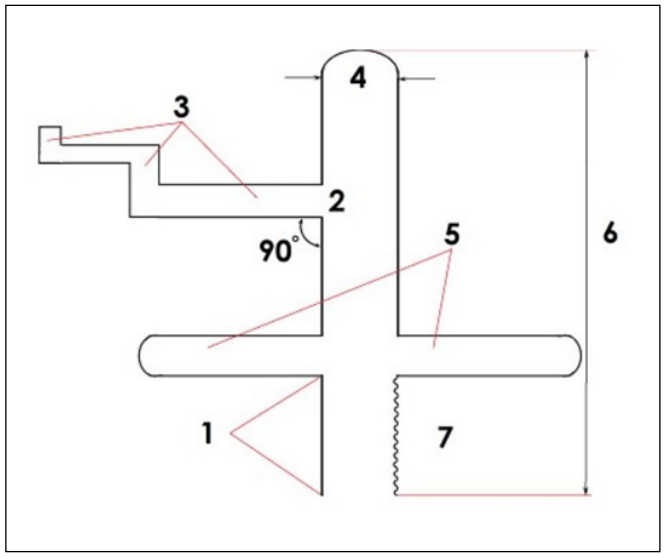

Fig. 1. Study of the crystal [21].
Marking:
Quantitative indicators:
1. Crystal length to branch point
2. Branching angle
3. Number of descendants of branches of the 1st, 2nd and other orders
4. The width of the crystal
5. Branching asymmetry
6. Crystal length
7. Number of microbranches along the length of the crystal

origin, whereas the central crystallized zone consists predominantly of salts. Thus, a highly dynamic transformation occurs, whereby unstable molecular-level structures are converted into stable macroscopic structures of the solid phase. According to the study by Annarelli et al. (2001), the central region is referred to as the zone of crystallized structures, while the peripheral region is termed the single amorphous zone [10]. The transitional zone can be observed only in studies of blood serum. The ratio between the peripheral width and the droplet diameter area is used to determine protein content in biological fluids [11]. Therefore, based on the principles of classical crystallography and early diagnosis of dental and systemic diseases, a method for crystallographic examination of one of the most accessible biological fluids—oral fluid - was proposed [12]. Oral fluid represents a highly organized and specific biological medium with unique and universal properties; it is a dynamic environment that reflects any changes occurring in the body, including pathological processes. Over recent decades, initial steps have been taken to investigate the manifestations of various diseases in the structural organization of oral fluid [13–16]. The physicochemical properties of oral fluid may be used as markers of pathological changes in the salivary glands and the oral cavity [4, 5, 8, 11, 17–21].

AIM

The aim of this study is to investigate morphological changes in the crystallization patterns of oral fluid during orthodontic treatment with aligners.

MATERIALS AND METHODS

Samples of dried saliva were analyzed from young adults aged 18–25 years of both sexes in a comparison group and in two main groups: patients treated with aligners without preventive measures and patients treated with aligners with preventive care, which consisted of using a Decasan solution in the form of rinses, applications, and irrigations twice daily (15–20 mL of solution for 30 seconds).

Oral fluid was collected using a sterile pipette in a volume of 0.2–0.3 mL from the floor of the oral cavity. Subsequently, three drops of oral fluid were applied onto microscope slides previously treated with alcohol and ether. Dehydration of the drops was carried out under standard conditions at a temperature of 22–24 °C. The micropreparations were examined using a VEGA TS 5130 MM TESCAN scanning electron microscope. Both central and peripheral zones of the oral fluid were analyzed. Fractal structures, individual crystals, and amorphous substances were evaluated. Interpretation of crystalloscopic components was performed using specialized reference tables describing the characteristics of the studied structures [21]. For quantitative assessment of microcrystals, a dedicated analysis algorithm was developed, according to which the following parameters were evaluated: length, width, and degree of curvature of the main branch; the ratio of the width of the main branch at the base and at the tip; the surface perimeter of the microcrystal; as well as the frequency and angle of dendrite deviation and the degree of symmetry of dendritic branching relative to the main axis (Fig. 1).

ETHICAL APPROVAL

The study received approval from the Institutional Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsya (protocol No. 11 of 12.01.2026), and permission to access the research was granted by University Hospital. As the study used anonymized retrospective data, informed consent was withdrawn. All procedures were in accordance with the ethical principles of the Declaration of Helsinki, which guarantees the confidentiality and anonymity of the participants.

RESULTS AND DISCUSSION

The crystallization of mixed saliva proceeded with the formation of tree-like structures. In the interpretation of

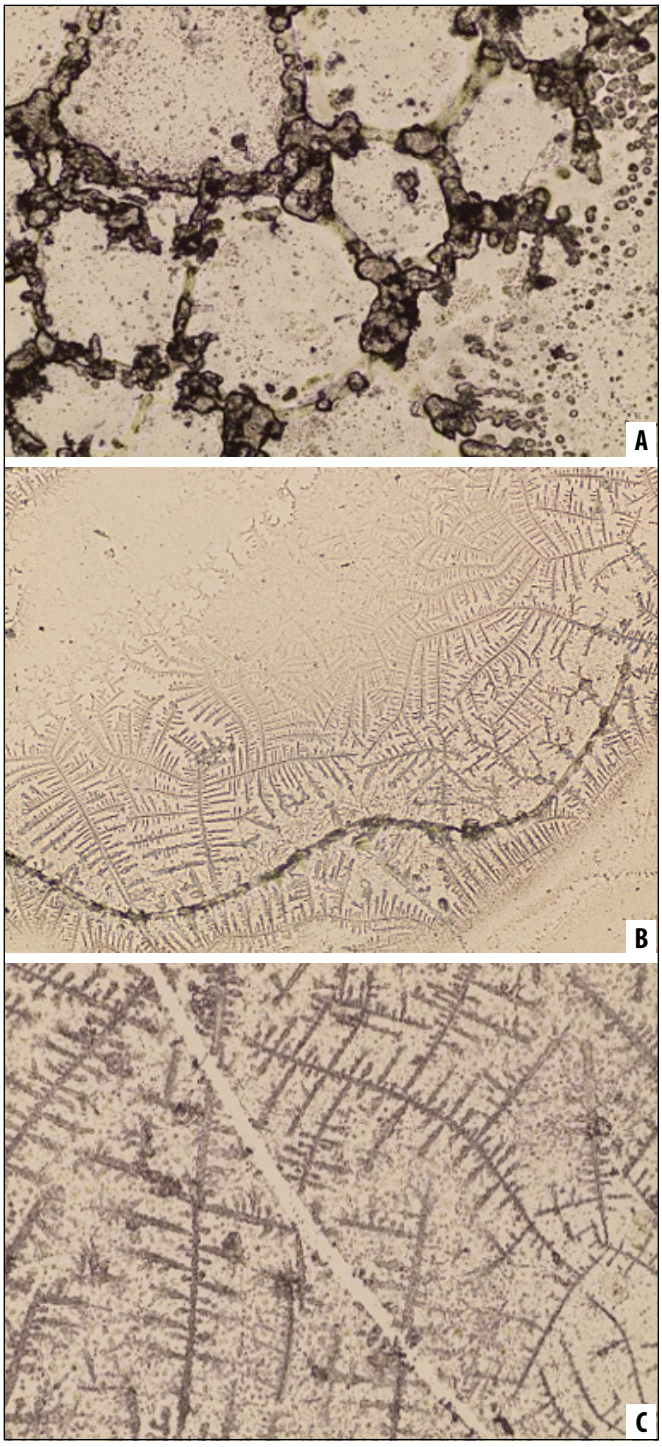

Fig. 2. Characteristics of saliva crystallization in healthy individuals. **A** - Peripheral (protein) zone. Magnification ×100. **B** - Transitional zone. Magnification ×100. **C** - Central (saline) zone. Magnification ×40
Source: Own materials

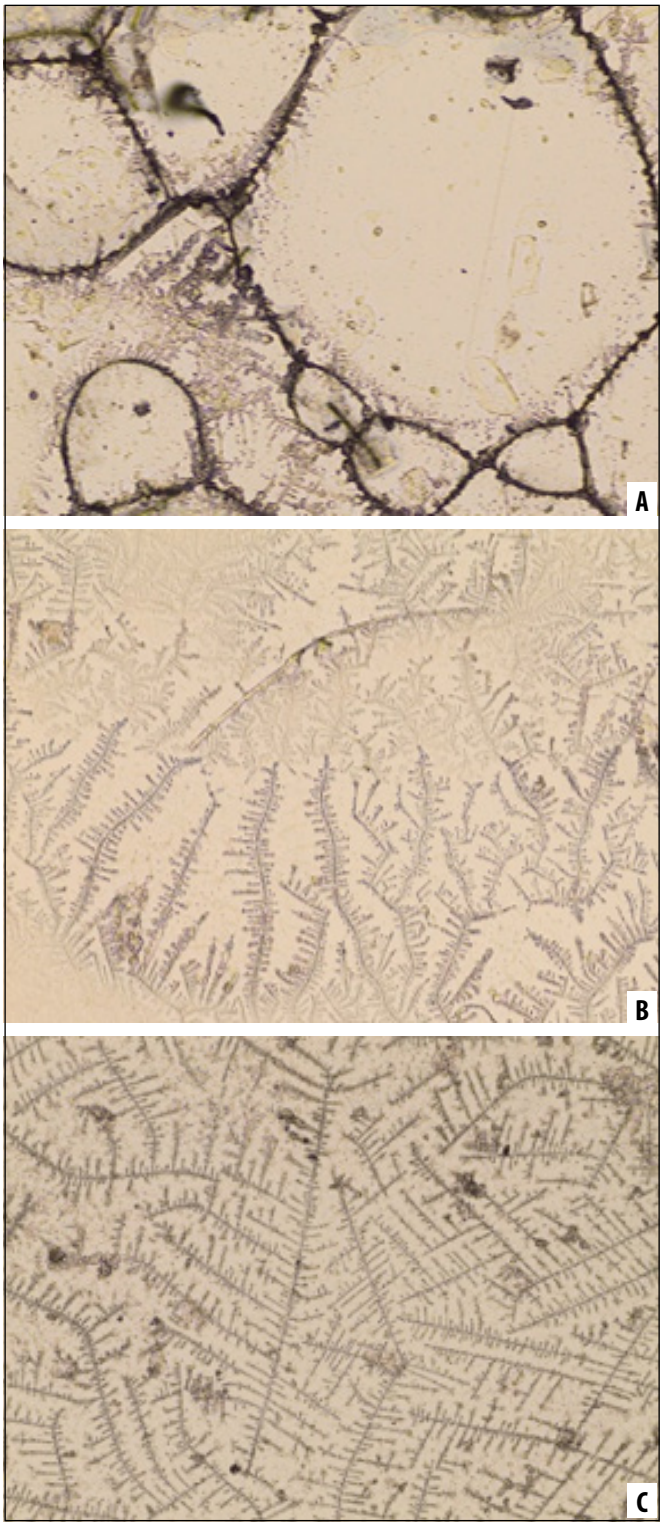

Fig. 3. Characteristics of mixed saliva crystallization at the beginning of treatment of dentofacial anomalies (DFA) with aligners. A - Peripheral (protein) zone. Magnification ×100; B –Transitional zone. Magnification ×100; C -Central (saline) zone. Magnification ×100
Source: Own materials

crystallograms, quantitative characteristics were taken into account, including crystal length up to the branching point, branching angle, the number of first-, second-, and higher-order generations, crystal width, total crystal length, and the number of microprojections along the crystal length. Qualitative features included uneven thickness within a single structure, asymmetry of branching, curvature of the main crystal trunk, crystals with indistinct contours, flat crystals, destructive-type changes, trunks without branching, and cruciform crystals.

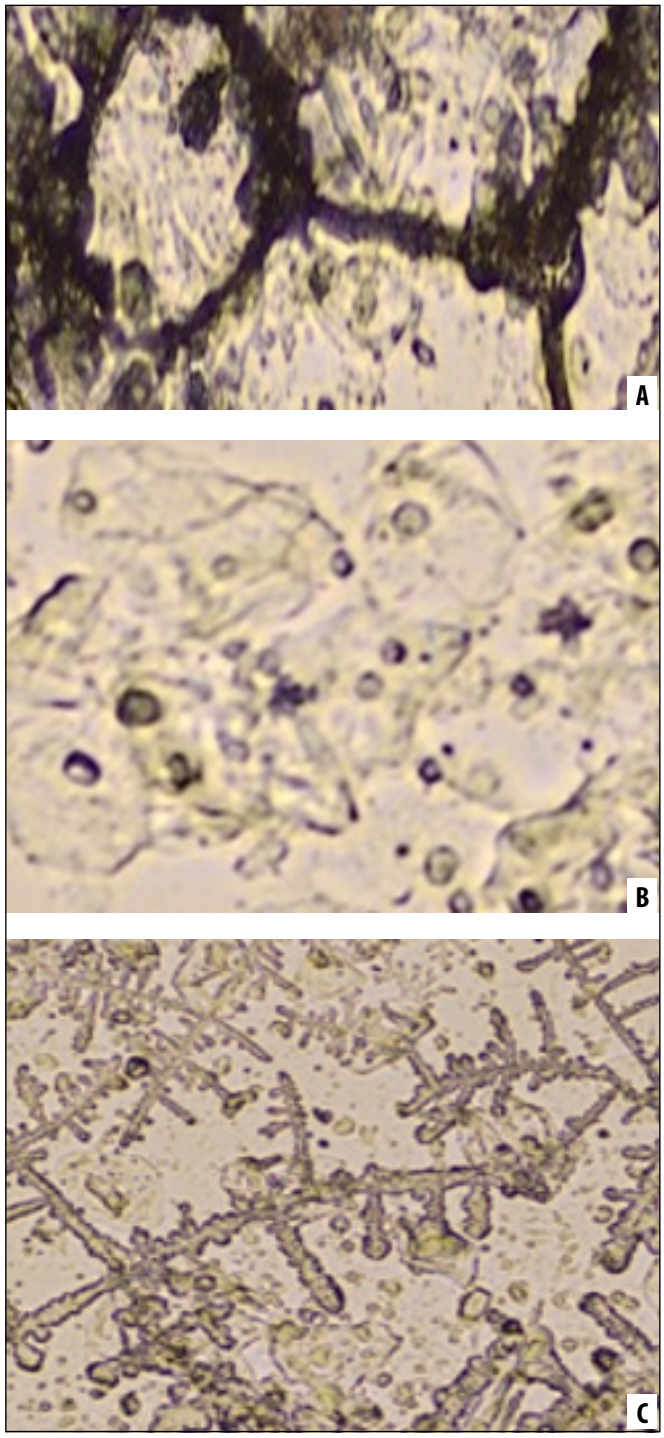

Fig. 4. Another type of crystallization at the beginning of treatment of DFA with aligners. A - Peripheral (protein) zone. Magnification ×100. B - Transitional zone. Magnification ×100. C - Central (saline) zone. Magnification ×100
Source: Own materials

The analysis of the crystallographic pattern demonstrated that, in the comparison group, crystals of uneven thickness predominated, along with structures exhibiting asymmetric branching. There were few crystallization trunks without branching and with curved main trunks. Minimal values were observed for such features as flat crystals, split crystals, unbranched trunks, and cruciform crystals.

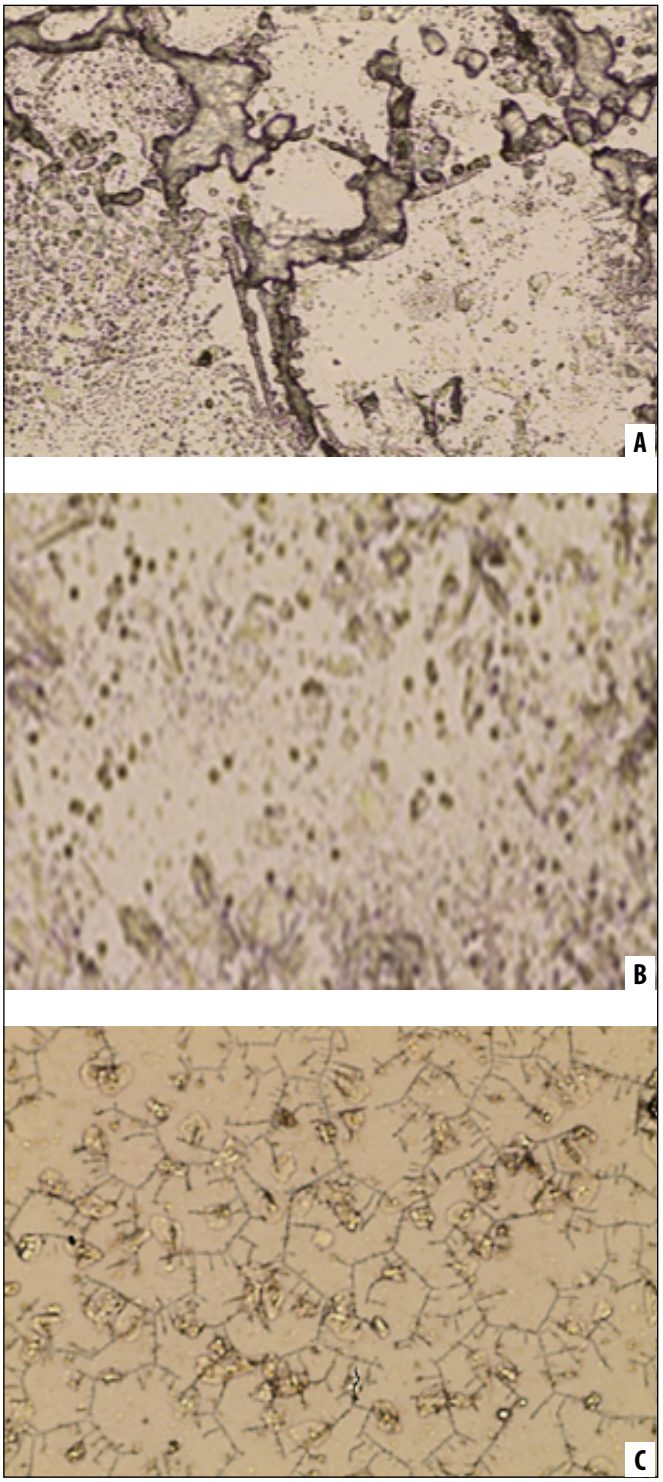

Fig. 5. Characteristics of mixed saliva crystallization during treatment of DFA with aligners after 3 months. **A** - Peripheral (protein) zone. Magnification ×100; **B** -Transitional zone. Magnification ×100; C - Central (saline) zone. Magnification ×40
Source: Own materials

In practically young adults, crystallization of saliva in the central (saline) zone was characteristically tree-like (fern-like). Large elongated crystalloprismatic structures were identified, which were properly fused with one another. In some areas, these structures were intercon-

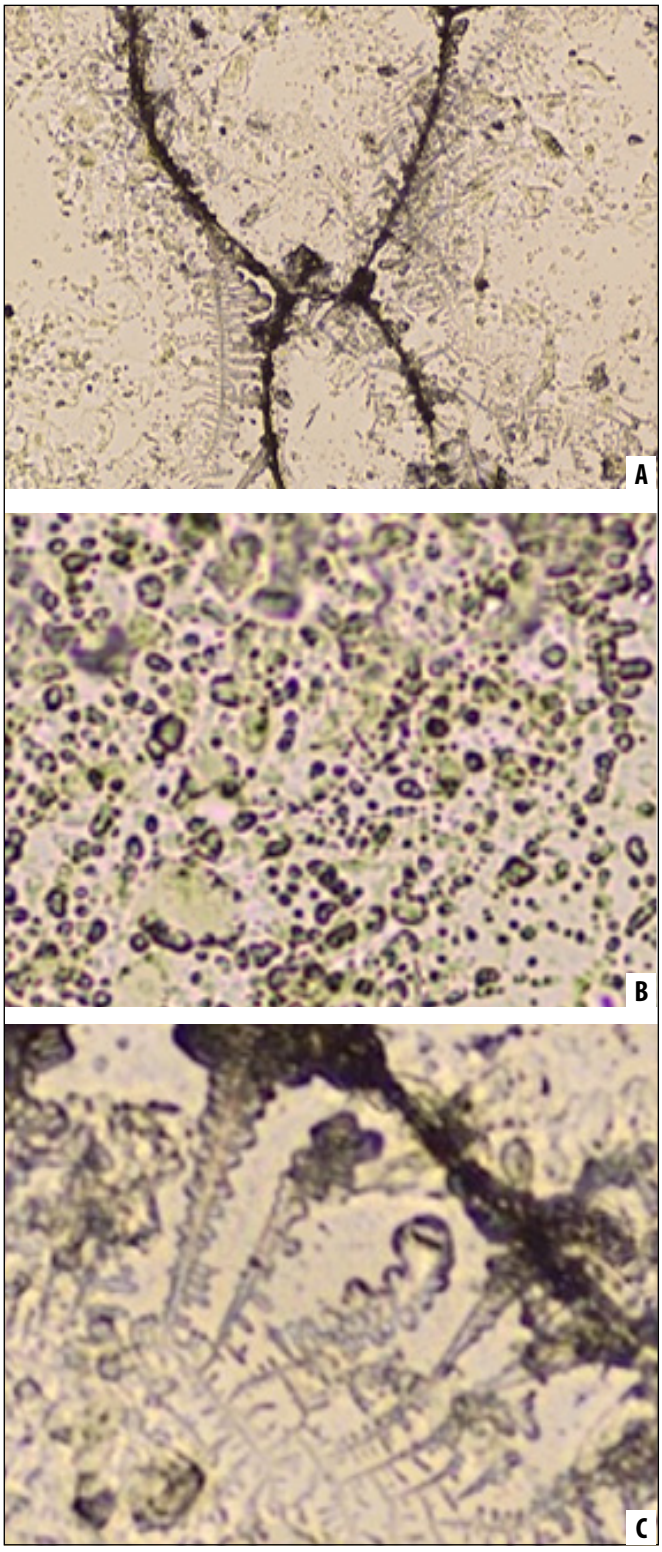

Fig. 6. The second type of crystallization in 25% of patients in group during treatment of DFA with aligners after 3 months. **A** - Peripheral (protein) zone. Magnification ×100; **B** -Transitional zone. Magnification ×100; **C-** Central (saline) zone. Magnification ×100
Source: Own materials

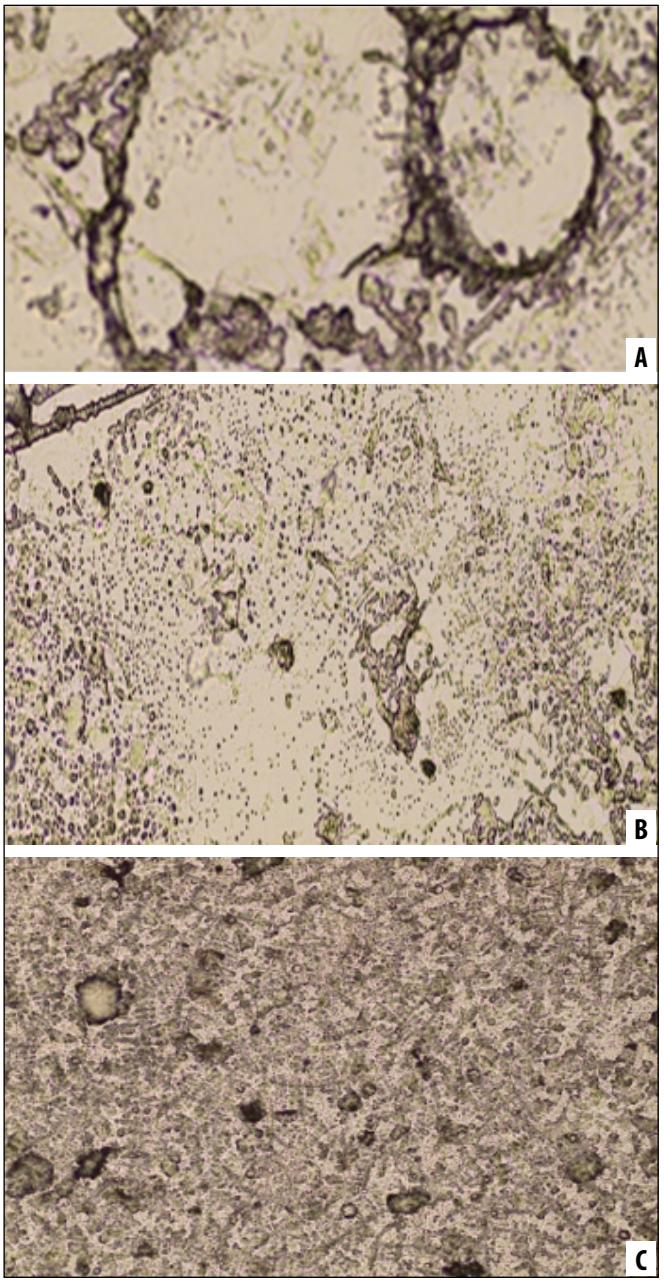

Fig. 7. Characteristics of mixed saliva crystallization during treatment of DFA with aligners 6 months after the beginning of therapy. A - Peripheral (protein) zone. Magnification ×200; B -Transitional zone. Magnification ×100. C - Central (saline) zone. Magnification ×200
Source: Own materials

nected; in the center, individual star-shaped crystals, as well as structures resembling twigs or branches, were observed. In the peripheral zone, amorphous spherical and ellipsoidal formations were noted. The transitional zone was relatively narrow (Fig. 2A-C).

The study of saliva crystallization patterns in the group of healthy young adults showed that, overall, the obtained data are consistent with the results reported by other researchers [27].

The study demonstrated that, in the main group of young adults treated with aligners, at the initial stage of treatment the crystallographic pattern was most frequently (68%) characterized by a distinct arrangement of large elongated prismatic crystals radiating from the center of the droplet

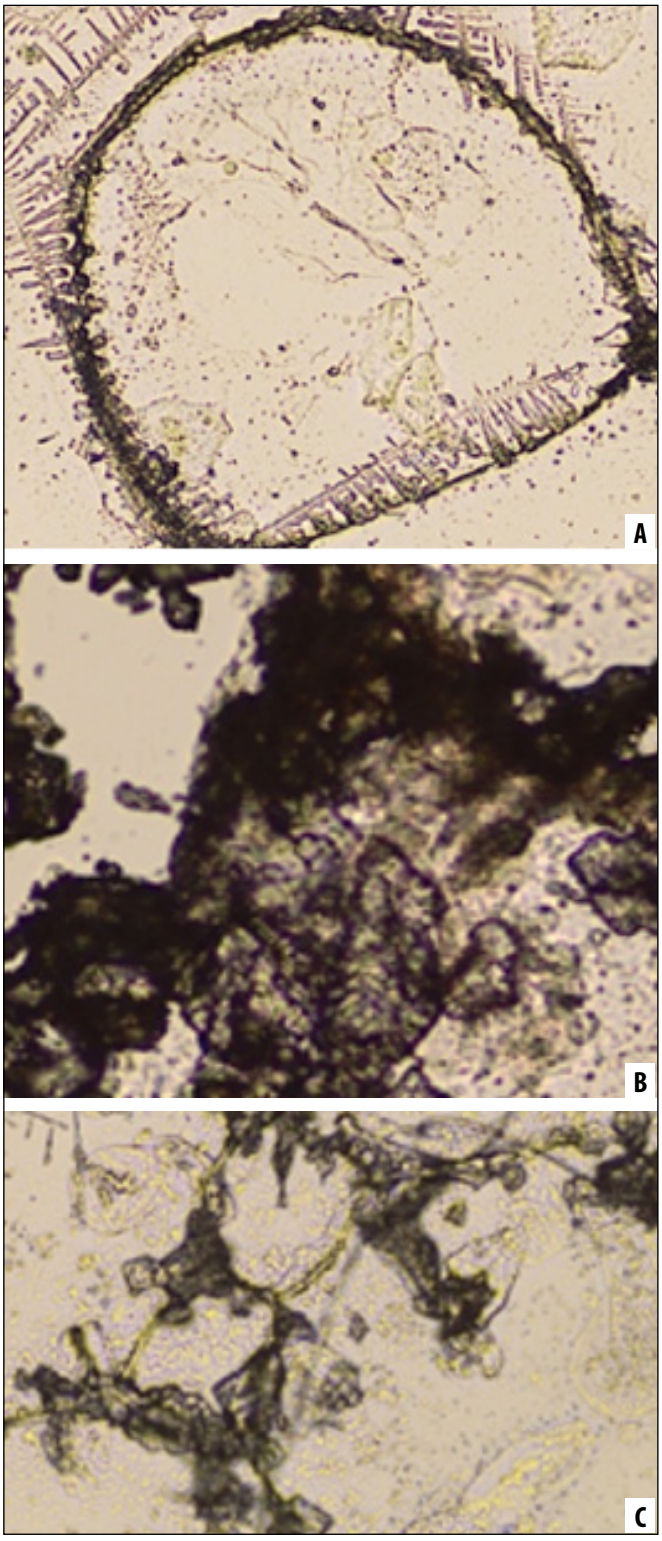

Fig. 8. Disruption of crystallization of mixed saliva according to the "coral-branch" type during treatment of DFA with aligners 6 months after the beginning of therapy. A - Peripheral (protein) zone. Magnification ×200; B -Transitional (saline) zone. Magnification ×200. C - Central (saline) zone. Magnification ×200
Source: Own materials

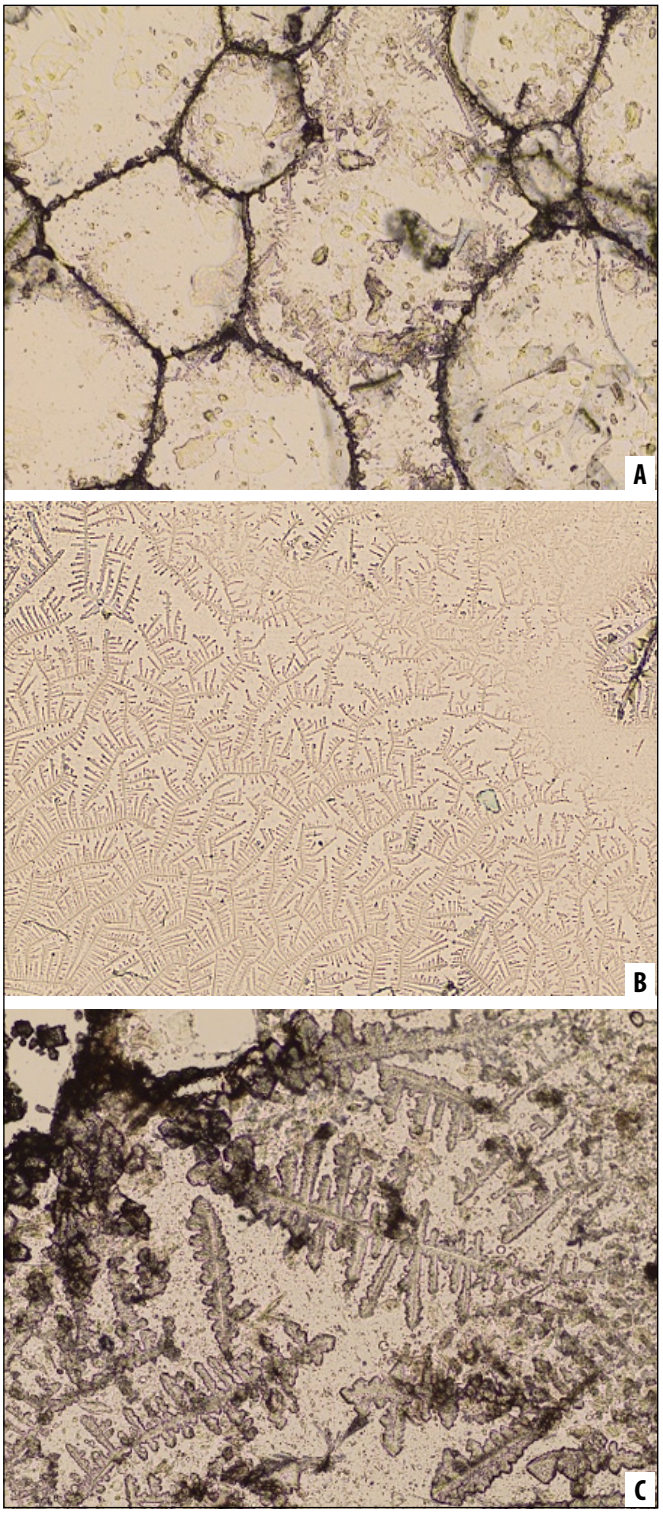

Fig. 9. Characteristics of mixed saliva crystallization during treatment of DFA with aligners at the beginning of therapy combined with the use of preventive agents. **A** - Peripheral (protein) zone. Magnification ×200; **B** -Transitional (saline) zone. Magnification ×100; **C** - Central (saline) zone. Magnification ×200
Source: Own materials

and merging with one another to form so-called horsetail- or fern-like structures. The radial processes of the "fern leaves" extended through the transitional zone toward the peripheral zone. In the peripheral zone, these processes were arranged uniformly in the form of cracks. The transitional zone was wide and contained a certain number of crystals (Fig. 3A-C).

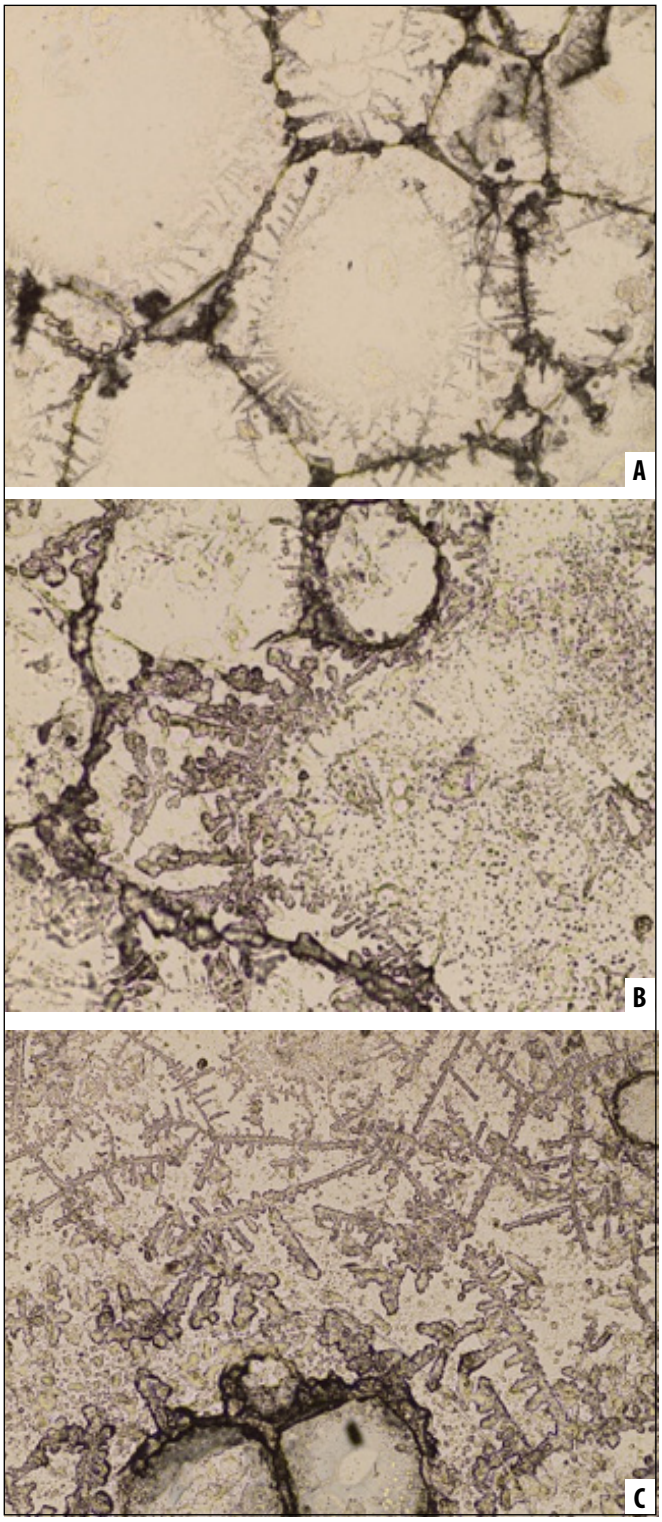

Fig. 10. Characteristics of crystal formation in mixed saliva during the treatment of DFA with aligners and the use of preventive measures after 3 months. **A** - Peripheral (protein) zone. Magnification ×200; **B** –Transitional (saline) zone. Magnification ×100; **C** - Central (saline) zone. Magnification ×200
Source: Own materials

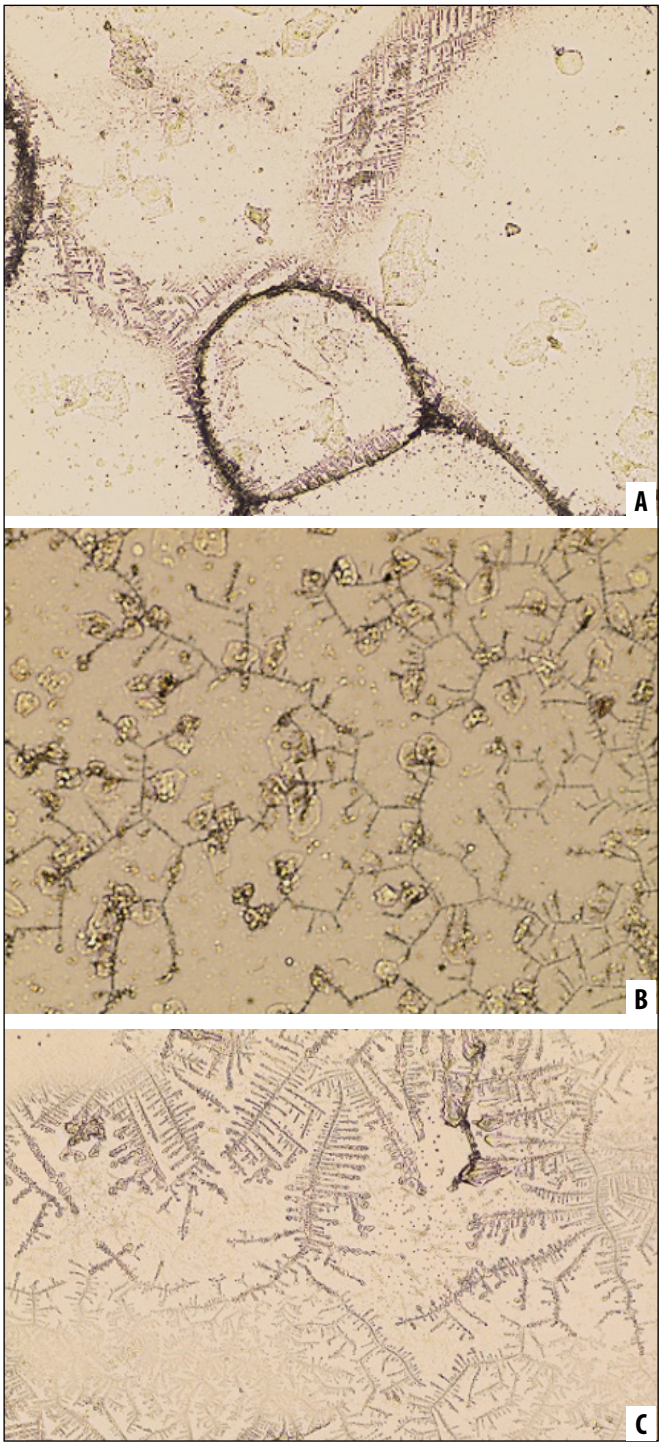

Fig. 11. Characteristics of crystallization of mixed saliva during the treatment of DFA with aligners and the use of preventive agents after 6 months. **A** - Peripheral (protein) zone. Magnification ×200; **B –**Transitional (saline) zone. Magnification ×100; **C** - Central (saline) zone. Magnification ×200
Source: Own materials

In some patients from the aligner group, isolated cruciform crystals with a smaller number of dendritic formations were observed in the central (saline) zone of the specimen. In such cases, the peripheral zone was narrowed and exhibited both radial cracks and multidirectional fine cracks. Very rarely (in 8% of patients), no saliva crystallization was observed in the central zone; instead, a large number of amorphous structures with scattered

crystal fragments and dendritic formations were present. In these cases, the peripheral (protein) zone was narrow, appearing as a strip with numerous chaotically arranged cracks and crystal-like formations (Fig. 4A-C).

Three months after the initiation of treatment, disturbances in saliva crystallization were observed, which were evident from both qualitative and quantitative indicators. In the center of the facies, crystallization still had a fern-leaf appearance; however, the leaves were smaller in size. At the periphery, cracks as well as crystal-like and amorphous formations were again observed (Fig. 5A-C).

Only 25% of patients exhibited saliva crystallization in the form of an amorphous pattern with isolated scattered 'fragments.' These 'fragments', radiating from the center of the facies, merged with one another in the peripheral zone, which was narrow and appeared as a strip (Fig. 6A-C).

Six months after the initiation of treatment, examination of dried facies revealed that, in the majority of patients (65%), there was crystal disintegration of the saliva, splitting of the crystal apex, and absence of microprocesses (Fig. 7A-C).

In 20% of patients, branching was completely absent; the processes were long and deformed, as were their apices. When branching was observed, it was limited only to the crystal apex in the form of a spike.

Occasionally, a so-called "coral branch," that is, unilateral branching, was observed. The crystallization pattern was so severely disturbed that it made quantitative assessment of saliva crystallization impossible (Fig. 8A-C).

Thus, it was established that the use of aligners leads to disturbances in mixed saliva crystallization. The peak of these disturbances occurs at the sixth month of orthodontic treatment.

A study of the crystallographic pattern of a dried saliva droplet (facies) was also conducted during the use of aligners in combination with preventive agents.

At the beginning of treatment, a distinct pattern of the dried saliva droplet was observed, with medium-length prismatic crystals extending from the center to the periphery; isolated fern-like structures were noted in the transitional zone.

The peripheral (protein) zone contained a large number of amorphous structures (Fig. 9A-C).

Three months after the start of treatment, destruction of the clear crystal structure was observed in the central (saline) zone of the facies. In the center of the droplet, individual star-shaped crystals were identified; throughout the entire area of the droplet, isometrically arranged crystalline structures of star-shaped, rounded, and irregular forms were observed (Fig. 10A-C).

After 6 months from the initiation of treatment, a reduction in the size and number of amorphous structures was observed in the peripheral (protein) zone; an expanded transitional zone with a small number of crystal-like structures; and isolated fern-like crystals in the central (salt) zone (Fig. 11A-C).

Analysis of the quantitative parameters of salivary crystallization in patients treated with aligners for DFA demonstrated that, after 3 months from the start of treatment, the crystal length decreased on average by 1.5 times, and after 6 months by 3 times in both groups. A progressive decrease in the number of branching offspring was noted (from an average of 5 at baseline to 3 at month 3 in the aligner group; and from an average of 12 at baseline to 7 at month 3 in the group receiving preventive measures; at month 6 of treatment, the values decreased to 1 and **2** branches, respectively). Crystal width decreased at months 3 and 6 of aligner treatment by 3 times, from (0.09 ± 0.003) to (0.03 ± 0.007) mm. In contrast, treatment with orthodontic appliances combined with preventive measures resulted in a 2 times reduction in crystal width at month 3, from (0.08 ± 0.002) to (0.04 ± 0.006) mm, followed by an increase at month 6 to a value 4 times higher than baseline - up to (0.32 ± 0.009) mm ($p \leq 0.05$).

At the 3rd month of aligner treatment, the crystal length decreased on average by 45% - from (0.125 ± 0.037) to (0.067 ± 0.002) mm and correspondingly from (0.40 ± 0.016) to (0.21 ± 0.001) mm. By the 6th month of treatment, this parameter was, on average, 4 times lower in both treatment groups - (0.031 ± 0.003) mm and (0.10 ± 0.001) mm, respectively ($p \leq 0.05$). The number of microbranches of the main crystal trunk at the 3rd month of treatment decreased 2.7 times in the aligner-only group and 2 times in the aligner plus prophylaxis group, while at the 6th month it decreased 4 times in both groups compared with baseline values. The branching angle, which increased 2 times at the 3rd month of treatment in the aligner-only group - from (85 ± 0.1) to (170 ± 2.1) degrees - showed almost no change (only a 5% increase) in the aligner plus prophylaxis group - from (90 ± 1.8) to (95 ± 1.8) degrees. At the 6th month, this parameter was respectively 30% and 10% lower - (60 ± 3.2) and (77 ± 2.4) degrees - compared with baseline values ($p \leq 0.05$).

CONCLUSIONS

The use of aligners for the treatment of dental anomalies in young adults aged 18–25 years induces destructive morphological changes in the crystallization of mixed saliva at 3 and 6 months after the initiation of treatment. Prophylactic application of a Decasan solution demonstrates a pronounced protective effect.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Monika Białoszycka
School of Medicine, Collegium Medicum,
University of Warmia and Mazury
Olsztyn, Poland
e-mail: monika.margaretka@gmail.com

ORCID AND CONTRIBUTIONSHIP
Anton Kolomiiets: 0009-0007-6454-2721 A C D E
Monika Białoszycka: 0009-0005-9371-0398 A E F
Alisa Pachevska: 0000-0002-6041-3814 B C D
Iryna Simonova: 0000-0002-3501-1813 B D E
Olena Popova: 0000-0002-7793-021X E
Nadiia Drachuk: 0009-0002-2715-0650 E
Tetiana Chugu: 0000-0001-9605-6196 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

RECEIVED: 14.01.2026
ACCEPTED: 19.03.2026