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Sealing of Fissures on Masticatory Surfaces of Teeth as a Method for Caries Prophylaxis

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1. Introduction

Tooth caries, which is regarded as a social disease in many countries, affects 100% of population. For many years both high prevalence and increasing intensity of caries have inspired researchers to find effective methods of fighting the disease. The quest for caries prevention methods has led to more and more widespread sealing of fissures of lateral teeth (premolars and molars), where sealants - thanks to mechanical protection from influence of oral cavity environment, thanks to reduction of retention sites and of bacterial flora in fissures - have effectively limited the development of carious disease.

Currently known methods of caries prevention can be deemed effective to the degree justifying their widespread application. Despite this fact, dentists across the world devote most of their working time to elimination of caries's results.

In permanent dentition, tooth decay most frequently affects masticatory surfaces of molars and, as numerous studies report, very soon after their eruption 2/3 of cavities are observed just there. This is confirmed by epidemiological studies, which have been conducted in Poland since 1987 under WHO supervision (29). The studies reveal that at the end of 1970s, over 50% of permanent molars were affected by caries in children aged 6-7 years, whereas children aged 9 years had almost all first permanent molars with carious cavities or filled. In the age group 13-15 years, the percentage of teeth affected by caries reached 80%, though occlusal surfaces of teeth constitute only 12.5% of all surfaces of teeth in the oral cavity. The aforementioned epidemiological studies indicate the importance of activities towards caries prevention on masticatory surfaces.

2. Morphology and microflora of intercusp fissures in teeth

Fissures on masticatory surfaces of lateral teeth are created during development of cusps in odontogenesis, which begins in the first weeks of fetal life with formation of deciduous tooth buds, and terminates about the age of 12 years, or 15-18 years with wisdom teeth. If some disturbances occur in the process of odontogenesis, they cause anomalies within forming dentine or enamel. During early dentinogenesis, insufficient supply of whole protein, amino acids and vitamins or introduction of chemical compounds such as multi-function phosphonic acids, diuretics, cytostatics results in impairment of odontoblast function, thus inadequate base substance for developing collagen matrix is produced.

During later dentinogenesis and biosynthesis of collagen, the above mentioned factors can cause a decrease of hydrolyzing enzyme's activity, thus lowering the amount of hydroxyproline in procollagen, which in turn results in production of defective organic dentine matrix. The created dentine has reduced number of dentinal tubules, which are sinuous along their length. If the harmful factors are active during mineralization of collagen matrix, predentine is formed. Odontoblast cells produce centers of intermediate tissue between dentine and bone; so-called osteodentin is formed.

Experimental studies proved that dentine mineralization emits an inductive signal for creation of soft protein enamel matrix. If dentine mineralization is disturbed, formation of primary protein enamel matrix is delayed. Stimulation by ameloblasts results in genetically conditioned activity of odontoblasts. If ameloblasts do not develop normally, development of odontoblast cells is inhibited and their function is impaired. Due to odontoblasts' impairment, enamel is subjected to underdevelopment, such as: hypoplasias and hypomineralization. During transition from amorphous to crystal phase, inadequate supply of elements such as calcium, phosphorus, fluorine and other microelements, or introduction of substances regarded as cytotoxic, disturb enamel mineralization. Disturbance of enamel mineralization proved to be a very complex process.

In properly structured and mineralized enamel, geometrical shapes of fissures are significantly diversified. Sizes and geometrical shapes of fissures also vary considerably in each individual. Shallow and deep irregularly shaped fissures are observed, narrow at the entrance and spreading near the base. The depth of fissures varies from a few to between 10 and 20 micrometers, depending on tooth's anatomical group. It happens that the central fissure reaches enamel-dentine junction, and sometimes even ends in dentine. However its bottom is usually covered by a thin layer of enamel (27). A fissure in an erupted tooth is filled with dental plaque with microflora changing with age, and residual food particles.

Longitudinal stripping of molars allowed assessment of mean sizes of fissures in those teeth. It was observed that the central fissure in molars has an average depth of 1.1mm, and its width at fissure entrance ranges from 0.2 to 0.5mm, and the width at fissure base has a mean value of 0.1 mm. Premolars indicate a larger diversity. According to Taylor and Gwinett (81) most commonly they are funnel-shaped with a narrowing at the middle of fissure's depth; inferior premolars have more varied shapes than superior ones. Generally in inferior premolars predominant fissures are narrow at the entrance and expanding at 1/3 of length towards the base. This creates conducive conditions for development of bacterial plaque and deposition of food remains, which leads to beginning and development of carious lesions. It was also observed that enamel covering fissures, especially on first permanent molars, demonstrates a smaller fluorine content than enamel on smooth surfaces of those teeth (16,72).

A lot of interesting information has been collected on the structure and composition of dental plaque residing in hollows of masticatory surfaces of teeth. The information comes from experimental studies carried out on gnotobiotic animals and from studies on people with the use of fissure models made of Mylar foil, placed in large amalgam fillings on molars' masticatory surfaces with the use of dentures as carriers for fragments of natural teeth (third molars). Dental plaque accumulating on smooth surfaces is different from plaque deposits in fissures. Plaque in fissures on masticatory surfaces is made of Gram positive cocci bacteria, which constitute 77-89% of total bacterial flora, short rods, a small

amount of filiform microorganisms and blastomyces that are an almost regular ingredient of most plaque in intercusp fissures. Cocci form microcolonies, and bacterial plaque's matrix varies depending on the amount of culturable bacterial flora, plaque's thickness and its position in the fissure. *Streptococcus mutans* does not play any important role in early bacterial colonization of intercusp fissures. In one-day-old plaque this species can hardly be found. With time the number of these bacteria increases, but they constitute less than 10% of total streptococcus flora. Cocci are first to settle in fissures. As plaque ages, their content decreases from 62% on the first day to 46% after three days and down to 28% after eight days. After initial homing in fissures, probability of further addition of new microorganisms seems low. The structure of bacterial plaque in fissures differs from that of plaque accumulating on smooth surfaces. At fissure entrance, cocci are positioned in palisade arrangement, perpendicular to fissure's bottom, and they occur together with fusobacteria, which are considerably less numerous than in plaque on smooth surfaces. During colonization of smooth surfaces, a selection of homing microorganisms takes place. Only those microorganisms can settle which are capable of producing polysaccharides of increased viscosity. In case of fissure plaque, practically any microorganism can become its part. During early stage, the development of plaque on smooth surfaces results from bacterial cell divisions and by addition of microorganisms from saliva. Streptococci constitute almost 90% of bacteria in early plaque.

With development of methods for intercusp fissure sealing, there has been an increased interest in microflora status in carious cavities covered with sealant (75). Because of anatomical characteristics of masticatory surfaces of teeth, sealing material is introduced on bacterial plaque accumulated on the fissure's bottom. Therefore it is critical to obtain information about sealant's impact on microflora in the fissure. The sealant's impact on microflora of fissures not affected by caries has been the subject of numerous studies. Those studies were performed on implanted fragments of masticatory surfaces of teeth. Data indicate that after sealing, microflora in carious lesions becomes suppressed. However, under the sealant a presence of microorganisms capable of growing was observed, which are believed to be etiologically connected with carious processes. It was also demonstrated that sealing materials do not constitute a fully tight barrier separating fissures from oral environment, which occurs very soon after treatment.

Microbiological studies conducted by Jodkowska (32) showed that sealing of intercusp fissures not affected by caries led to a considerable reduction in the number of bacteria. The impact of this treatment on intercusp microflora varied depending on time elapsed after sealing and on the type of applied sealant. In a short time, which means 30 minutes after sealing with Nuva-Seal material, no bacteria growth was observed in 89% of examined samples. In further observation periods, after application of this material, a slow decrease of percentage of aseptic samples was observed, which reached the value of 17% after 18 months. After sealing with Concise BWSS (Brand White Sealant System) a reverse tendency was observed. Nuva-Seal material was more effective in fissure sealing than Concise (BWSS) in a short period immediately after treatment. The efficacy of sealing with this material decreased with time. On the other hand Concise (BWSS), which initially inhibited bacteria growth to a lesser degree than Nuva-Seal, proved more effective in later period of observation. After 18 months the activity inhibiting bacteria growth of both evaluated materials was similar. The most frequently isolated bacterial stains were *Streptococcus mutans* (25%), however more rarely strains classified as *Staphylococcus aureus* were obtained

(3,4%). In 71% of cases growth of only one strain was obtained, whereas in remaining cases various bacterial strains were isolated. Mixed bacterial flora was observed more frequently in material coming from teeth sealed with Nuva-Seal than from teeth sealed with Concise (BWSS).

The author also conducted microbiological studies on 30 first and second permanent molars in children whose condition of hard tissues in initial examination (visual - mirror and probe) in 16 cases suggested a suspicion of an early stage of initial caries and in 14 cases evident initial caries was diagnosed. Samples of material collected for bacteriological examination were collected after 30 minutes, two weeks, and also after two, eight, twelve and eighteen months from sealing. Samples were collected from occlusal surfaces of carious teeth covered with light-cured Nuva-Seal material and with chemically-cured Concise (BWSS). The research demonstrated that the highest reduction of culturable microorganisms in samples collected from fissures sealed with Nuva-Seal occurred 30 minutes after treatment. Then the reduction increased within the period from 2 weeks to 8 months and reached the level which was 300-fold lower compared to samples collected from control teeth. After 12 months of observation 70-fold less bacteria were cultured from sealed teeth, and after 18 months only 6.5-fold less than in samples collected from control teeth. On the other hand Concise (BWSS) material which initially inhibited bacteria growth to a lesser degree than Nuva-Seal, within the period from 2 to 8 months, indicated a 500-fold reduction of microflora compared to samples collected from control teeth. After 12 months of observation the number of culturable bacteria indicated a 100-fold reduction, and after 18 months the activity inhibiting bacteria growth of both assessed sealing materials was similar.

An analysis of isolation of individual bacteria strains and the number of isolated microorganisms in various periods of observation after sealing with both assessed materials indicated that Concise (BWSS) material had a more favorable effect on microflora of carious lesions than Nuva-Seal material. After application of Concise (BWSS) a less frequent development of bacteria etiologically associated with caries, belonging to *Streptococcus mutans* (*S. mutans*) species was observed. Microorganisms belonging to this species occurred solely in material collected from fissures sealed only during certain periods of observation. Numbers of *S. mutans* in material collected after sealing with Concise (BWSS) material were - in various periods of observation - generally much lower than numbers observed after application of Nuva-Seal material. After use of Nuva-Seal material, a less frequent development of mixed bacterial flora was observed. Clinical observations of carious teeth sealed with both assessed materials, which were performed during 18-month period, indicated that only in three cases, where sealant was damaged, slightly deepened carious cavities were observed under sealant. Studies by other researchers confirm the results obtained by the author and conform with the above results, while deepening of carious cavities, if it occurs, seems small (39). This is due mainly to the presence of a mechanical barrier which blocks an inflow of new microorganisms and of substrate for bacterial transformations. Moreover, acid etching of sealed teeth and regenerative capability of dentine may to some degree inhibit further progression of existing carious lesions in fissures (1). Study by Heller *et al.*, who assessed occurrence of caries in previously healthy teeth and in teeth with initial caries, where fissures were sealed in some patients of each group, observed after five years that among previously healthy teeth caries developed in 13% of not sealed teeth, whereas in case of sealed teeth the percentage was smaller and equaled 8% (28). After five years, in the group with previously diagnosed caries the condition of 52% of

not sealed teeth and of 11% of sealed teeth deteriorated. According to the authors, sealing of teeth previously affected by caries was fully justified because through application of sealants - instead of conventional preparation and filling of cavities - one of the prophylactic methods against development of carious centers can be applied. Further research may bring more data on this issue.

Healthy enamel, especially its surface layer, however well mineralized during tooth eruption, undergoes continual chemical and physical changes throughout organism's life. Those changes may be favorable, yielding better mineralized, more mature enamel, or they may be unfavorable, especially when enamel is covered by bacterial plaque. Fermentable carbohydrates diffuse into dental plaque where they are metabolized and transformed into organic acids with low pH (lactic, formic, pyruvic acids) and with high pH (butyric, propionic, acetic acids). These acids may be buffered by various systems or they can diffuse into dental plaque or into oral fluid. However, they can also penetrate through the plaque-enamel surface and partly demineralize crystals on and immediately under the surface of enamel. The external surface of enamel is in the state of dynamic balance between demineralization and remineralization, which periodically follow each other.

Fissures in lateral teeth are particularly predisposed for caries development. Most typically caries starts in enamel of fissures. We know that it develops due to disturbances between demineralization and remineralization phases of enamel covered with bacterial plaque. Organic acids produced by bacteria penetrate through the plaque-enamel contact surface in a dissociated or non-dissociated form and diffuse in the liquid phase among enamel crystals or inside crystals. Continual elution of calcium and phosphorus ions, decrease of pH value, ion concentration and other factors have impact on diffusion, on amount of acids penetrating into enamel and on loss of majority of minerals from hydroxyapatites. Progression of carious processes in enamel of fissures depends on consecutive attacks of acids and further destruction of enamel's crystal structure. Early carious lesions are characterized by varied advancement of demineralization in the surface layer and inside the tissue, where the highest loss of non-organic compounds and degradation of prismatic structure is observed in the so-called subsurface layer. With a growing loss of non-organic components of enamel, its porosity increases. Demineralization of enamel in fissures starts at fissure entrance. The main demineralization center spreads sideways from isthmus of fissure along central fissure. Outside layer of enamel with early carious lesions observed on microphotographs indicated a considerably higher degree of mineralization compared to underlying areas of enamel and dentine. Conducted research confirms that in the beginning stage of carious lesion's development the degree of demineralization of fissures' enamel depends on topography and depth of fissures. The deeper and more diversified in shape the fissure, the higher the observable degree of demineralization. The degree of demineralization decreases towards fissure base. In wide fissures the degree of demineralization was smaller. The period of the first three years after tooth eruption is regarded to be of the highest susceptibility for caries development on masticatory surfaces. In dentine, demineralization period depends on the depth and width of fissures. At the entrance of all fissures, both narrow and wide, demineralization area was the largest. At fissure base the area of dentine was preserved and did not indicate symptoms of demineralization, whereas in deep fissures demineralization at fissure base was less pronounced. Narrow fissures indicated a smaller loss of non-organic compounds. In the narrowest fissures demineralization was deepest but in majority of fissures it was limited to

the upper part, not reaching the fissure base. The described associations between dentine fissure morphology and range of tissue demineralization in conditions of experimental exposure to caries-inducing factor continued for eight weeks. However, the loss of minerals and dentine tissues was higher on the seventh day of the experiment compared to the loss observed eight weeks after the experiment (45).

3. Development of sealing methods and applied materials

Available methods for caries prophylaxis by means of fluorine compounds, limited consumption of carbohydrates and systematic removal of dental plaque - which effectively inhibit caries on smooth surfaces of teeth (from 35% to over 90%) - do not significantly impact the occurrence of caries on masticatory surfaces of teeth. Since 1965 the search for effective methods of prophylaxis against caries on occlusal surfaces of teeth has been considering individual anatomical characteristics of this surface and has been leading towards isolating of fissures in premolars and molars from the impact of oral environment (quot. after 37). The method which evoked special interest among clinicians consists in fissure and pit sealing on masticatory surfaces, i.e. in mechanical isolation of clinically healthy tissues from oral environment.

Attempts to control caries of masticatory surfaces of lateral teeth have a history of over one hundred years. The oldest concept was a removal of not lesioned fissures during preparation of carious cavities on masticatory surfaces. It was a so-called prophylactic dilatation, invented by Webb and strongly propagated by Black (quot. after 72). Nevertheless, as early as 116 years ago it was observed that prophylactic activities at the time of occurrence of carious events are frequently belated or insufficient. In 1895 Wilson performed prophylactic procedure of filling fissures of permanent molars with cement. Thus he realized Hunter's idea from 1778, who observed that fissure blocking may inhibit development of caries. In 1917 Hove introduced silver impregnation applying ammonia solution of silver nitrate, and in 1950 Ast applied zinc chloride (quot. after 78). Removal of healthy enamel (odontotomy, eradication, prophylactic dilatation) was especially controversial because most applied methods yield unsatisfactory caries reduction and masticatory surfaces turned brown-black.

Willingness to use less invasive methods led to search for new materials. The development of organic chemistry, especially polymers, greatly contributed to progress in prophylaxis of occlusal surfaces of teeth. In the course of performed studies, several chemical compounds were singled out which proved useful for fissure sealing. Generally they belonged to four groups (cyanoacrylates, polyurethanes, epoxy resins and bis-GMA resins).

Cyanoacrylates, which were first adhesive materials used for this procedure, did not gain clinical approval, because they clung to tooth surfaces for a very short time and frequently caused dentine complications. Polyurethanes, which demonstrated poor adhesion to enamel, were applied in caries prophylaxis mainly thanks to content of fluorine compounds. However the two groups of chemical compounds did not act up to expectations.

Only the introduction of epoxy resins by Schröder in 1953 and later of bis-GMA resins by Bowen in 1963 raised new hopes for clinicians. The new materials allow fissure isolation without disturbing healthy enamel. Apart from many advantages, such as: mechanical endurance, chemical resistance, relatively good adhesion, low contractility during

polymerization, had one major disadvantage - lack of solid bonding to tooth's hard tissue, resulting in short-term retention of material on sealed teeth.

Only Buonocore in 1965, followed by Gwinett, Sharp and Silverstone demonstrated that permanent bonding of materials with enamel can be obtained after its etching. When etched enamel became a permanent element of clinical procedures with adhesive materials, adhesion and stability of bonding of compound materials with hard tissues of teeth increased on the principle of micromechanical retention. Sealing based on application of bis-GMA resins was introduced less than 50 years ago. Due to matrix type, content of filler particles, presence (or absence) of fluorine ions and efficacy of prophylactic sealant, they were divided into four generations.

Studies on first-generation sealants (sealants polymerized with UV light, started in late 60s) demonstrated diversified prophylactic efficacy of lateral tooth sealing. Their effectiveness depended on the number and type of sealed teeth (first molars, second molars and premolars), applied methodology of procedure, methodology of assessment and the fact that procedures were generally performed by doctors. Representatives of this generation were: Nuva-Seal, Alpha-Seal, Espe 717, Saga-Sealant, Lee-System. After 10-year-long observation, retention of material was 68.0% - 180 sealed teeth in children aged 7-8 years (31). Other authors after five years of observation obtained retention from 19.3% to 63.0% (70,72) and corresponding caries reduction from 57.9% to 43.0% (64).

Second-generation sealants (chemically polymerized, late 60s and early 70s) indicated a higher prophylactic efficacy compared to first-generation sealants (22). Their better effectiveness was due to introduced modifications, which resulted in improved physical-chemical properties (added filler particles) and improved retention to hard tissues of teeth, for example: Concise BWSS from 3M Kerr (PFS) Delton, Concise EBS (Enamel Bond System). Light-cured sealants, which also belong to the second generation, demonstrated lower microleakage (19-20%) compared to chemically cured sealants (50-67%). After 10 years 50% total retention of second-generation sealants was obtained, also a higher percentage of partial retention and of caries reduction (from 40 to 63%) compared to first-generation sealants (70,72). They were represented by light-polymerized Concise (BWSS), Prismashield resins.

Studies on third-generation sealants (polymerized with visible light - the first half of the 80s) demonstrated efficacy which was comparable with second-generation sealants as far as material retention and caries reduction are concerned. After five year of observation it was about 77% (65,66,67). This generation is represented by Concise LVC, Helioseal.

The fourth generation includes sealants with fluorine (late 80s). They are based on bis-GMA resin, urethane dimethacrylate, aliphatic methacrylates. Fluorine is released from fluosilicate glass (Helioseal F) or added in the form of sodium fluoride (Fluoroshield, Fissurit F, Ultraseal XT). The presence of fluorine in sealing material decreases caries risk on sealed surface, even when microcracks occur on sealant's surface. Lasting low concentration of this element in the oral cavity is especially significant. Fluorine level in dental plaque is from 6 ppm to 300 ppm, whereas in saliva it is from 0.001 ppm to 9.4 ppm. Sealants with fluorine such as: Helioseal F and Ultraseal XT were characterized by low viscosity, which enabled easy penetration of material into deep, narrow fissures. Moreover, they do not feature phase separation (i.e. sedimentation of ingredients), which makes them different from other

fluorine sealants. Another advantage of Helioseal F and Ultraseal XT sealants is lack of air bubbles in material, which constitutes a common problem with sealing materials. Laboratory tests demonstrated that sealants with fluorine release twice as much fluorine ions within nine days compared to the number of fluorine ions released from glass-ionomer cements (68). In own experimental studies, the number of fluoride anions released from five sealants was determined by direct potentiometry method with the use of fluoride ion selective electrode. In order to obtain constant pH of contact solution and standard solutions, TISAB buffer was used, which eliminated any influence of alien ions. The potentiometer showed the potential of a given solution, while the amount of fluorine was calculated on the basis of calibration curve obtained for standard solutions. The study demonstrated that the value of fluorine ion emission level varied depending on the type of sealing material, type of fluorine compound in sealant, time of testing (short-term from 0.5 to 7 days and longitudinal during 15 study periods from 7 to 371 days). During the whole study period, the highest level of released fluorine ions was observed in Ultraseal XT (22.83 mgF/mm^2 after 12 months and 28.10mgF/mm^2 after 13 months of observation), and a much lower level in Fissurit F (from 13.04mgF/mm^2 after 12 months of observation and 9.30 mgF/mm^2 after 13 months of observation). The lowest level of released fluorine ions was observed for the three remaining sealants: Pentraseal, Ionoseal, Helioseal (4.40, 3.90, 3.40mgF/mm^2 respectively) and after13 months of observation for Helioseal, Pentraseal i Ionoseal (5.8, 4.0, 3.5mgF/mm^2 respectively). It was also observed that release of fluoride ions from sealants containing various fluorine compounds is a long-term process, though amounts of released fluoride ions are small (33).

Clinical studies comparing retention of sealants with fluorine to retention of sealants without fluorine, after one-year observation indicated a higher retention of sealants with fluorine. After three years of observation retention of materials with fluorine was similar. The obtained effect of reduced caries by application of sealants with fluorine was 83.9%, whereas with sealants without fluorine the result was 70% (30). The net gain index (which indicates the actual number of teeth saved from caries thanks to sealing) for 100 teeth sealed by application of sealants with fluorine was 30 teeth saved from caries, while only 15 teeth were saved by application of sealants without fluorine.

Another group of sealants which became a permanent part of caries prophylaxis are glass-ionomer cements (GICs). Thanks to their hydrolytic properties they are capable of forming solid and long-lasting bonds with hard tissues of teeth, non-etched enamel and dentine, and are characterized by similar release of active fluoride ions to surrounding fissures. However, it should not be forgotten that glass-ionomer cements used for sealing of lateral teeth are susceptible to impact of big masticatory forces, thus their application should be limited because of their low resistance to wear. Clinical studies showed low retention index for GICs used as sealants in observation periods from six months to seven years (73). Although a high percentage of sealant loss was observed , it was not directly related to development of caries (25,57,74,76). Examples of such sealants were: ASPA, Fuji III, Ketac Cem, Ketac Bond, Chemfil Superior.

At the beginning of the 90s the quality of glass-ionomer cements was improved by addition of bis-GMA and HEMA (2-hydroxyethyl methacrylate) resins. HEMA monomer was combined with a copolymer based on chains of itaconic acid, having - through amide groups - metacrylan groups polymerizing when exposed to light, which led to development

of resin-modified glass-ionomer cements (RMGICs) and polyacid-modified resins which were tested as sealants. In *in vitro* studies, resin-modified glass-ionomer cements demonstrated the same or better adhesive properties and release of fluorine ions. Moreover, the addition of resins made glass-ionomer cements stronger and less brittle. As far as protection against secondary caries is concerned, no significant differences were observed between materials. A low retention index was obtained for Ketac GIC and Vitremer RMGIC after five years (57). The total retention index was less than 2% (1.6%) for both materials. The authors explained low retention with long-term use and insufficient resistance to abrasion of both materials. In another research Forss and Halme noticed 10% total retention after seven-year-long observation of conventional glass-ionomer cement (26). There have been numerous published reports comparing the efficacy of GIC sealants with efficacy of materials based on bis-GMA resins. They covered observation periods from one month to four years. However, due to various research methods, diversification of age groups, anatomical characteristics of observed teeth and number of repeated sealant applications, the results can not be compared, therefore their value is limited. Nevertheless the studies demonstrated decidedly worse retention among glass-ionomer cements compared to retention of sealants based on bis-GMA resins (25,62). Low retention of GICs was due to the fact that before application of sealant, tooth enamel was not subjected to any preparatory procedures aimed at improving retention, but only - according to instruction - teeth were cleaned of dental plaque, rinsed and dried. Probably the main cause of GIC loss may be its inadequate adhesion to enamel. Such explanation seems to confirm results of *in vivo* and *in vitro* studies on the impact of various preparatory procedures on GIC's adhesion to tissues of teeth. They indicate that adhesion can be improved by proper conditioning of enamel before application of cement. 30-second etching with 50% citric acid before application of cement or 10-second etching with 10% polyacrylic acid resulted in 26% retention after two years and 10% after seven years. The reasons for such unsatisfactory results are poor adhesion of cement to enamel, lack of procedures improving adhesion and brittleness of GICs. An improvement of cement's physical properties suggested by the authors, e.g. liquidity of cement which was created as a result of perfecting Fuji III, was not confirmed in two- and four-year studies reporting 4% retention, whereas with application of a comparable sealant based on bis-GMA resins - Delton - the values obtained after two and four years were respectively 79% and 61%. The discussed issue is GIS's ability to release fluorine, which enables anticarietic activity on neighboring tissues and facilitates remineralization of existing early carious centers in enamel. Observation of carious process after six and twelve months of observation in several cases in both study groups prevents formulation of an opinion on anticarietic activity of glass-ionomer cements. Mejer and Mjör after six and twelve months of study observed considerable loss of cement on 66% of masticatory surfaces sealed with Fuji III, however they did not observe carious lesions, whereas in case of sealing with bis-GMA resins (Delton and Concise), whose total retention after five years was as high as 90%, caries was present on 5% of masticatory surfaces (48). This suggests that even a small amount of cement which remains in fissures is enough for long-lasting caries prevention. *In vitro* studies demonstrated that fissures sealed with GIC are more resistant to demineralization than unsealed fissures, even if presence of sealant is not confirmed clinically, authors associate the anticarietic effect it with possible residue of cement remains at the fissures' bottom and continual release of fluorine by glass-ionomer cements. Some attention should also be paid to antibacterial activity of glass-ionomer cements against *S. mutans* and *S. sorbinus*. Suppression of colony growth is related to the

ability to release fluorine ions. Therefore the choice of GIC as fissure sealant must result from other reasons than good retention or cariostatic property. Certainly it can be an alternative e.g. in the absence of proper humidity control, with partial tooth eruption or with mentally or physically handicapped patients. Apart from that, glass-ionomer cements have another advantage of simple application technique. Increased efficacy of sealing can be obtained by reapplication of sealant during routine visits, however this will increase cost of treatment. That is why only certain clinical situations can constitute an indication for such treatment, for example difficult children or those with a high caries risk (23).

It was observed that resin-modified cements demonstrate much stronger bonding forces with dentine (almost threefold) compared to conventional glass-ionomer cements, but considerably weaker than sealants based on bis-GMA resins. Retention of glass-ionomer cements after 6 months equaled 93%, after 24 months from 82.5% to 86%, and after four-year observation only 35% (50,51,86). Baseggio *et al.* assessed the efficacy of Vitremer modified glass-ionomer cement compared to Fluoishield conventional sealant based on bis-GMA resins containing fluorine. During three-year studies on sealants in second molars in 320 patients aged 2-16 years, retention of sealant modified with glass-ionomer cement was 5.10%, whereas retention of sealant based on bis-GMA resin equaled 91.08%, and total loss of retention was observed in 6.37% and 7.65% of sealed teeth respectively. Caries on masticatory surfaces was observed in 20.06% of teeth sealed with materials modified with glass-ionomer cement and in 8.91% of teeth sealed with materials based on bis-GMA resin (7).

Compomers, also called composite materials modified with polyfunctional acids, contain bis-GMA monomers, deionized glass which constitutes mechanical filler with 42-67% of volume and particle size 0.7-5µm, and monomers containing COOH acidic functional groups. Thanks to glass content they are capable of releasing fluorine contained in glass, but in a much lesser quantity than GICs or resin-modified GICs (63). A two-year assessment of sealants based on bis-GMA resins (Fissurit F, Fissurit FX, compomer Dyract Seal and ormocer Admira Seal) demonstrated that retention of the compomer (Dyract Seal) was much lower compared to the other sealants. However, no significant differences were observed in marginal tightness of assessed sealants and in caries presence on surfaces of sealed teeth (88). In research comparing two materials - one compomer and one based on bis-GMA resin - special emphasis was placed on advantages of compomers, which are easy to use, chemically and physically durable, and they release fluorine. No difference was observed between applied sealant and its retention and between the degree of caries reduction and material retention (quot. after 24). In another study in which GIC-based material with zinc and fluorine was used (Jonosit Seal DMG), after six years of observation in 77.5% of cases retention on occlusal surfaces of teeth was confirmed. Presence of caries was closely related to retention of material. In case of total retention of sealant, caries reduction was 99.6%, whereas with total loss of material after five years caries reduction was 69% (34). Other authors, such as Aranda *et al.* and deLuca-Froga *et al.*, who assessed activity and preventive performance of resin-modified glass-ionomer cements (RMGICs) and composite materials modified with polyfunctional acids, observed that retention index changed after one-year observation, rising from 20% to 95.9% (4,18). An important factor which must be considered in case of glass-ionomer cements used as sealants is the fact that even after clinical loss of material, a small amount of sealant remains at the fissure's bottom with fluorine still being released, thus protecting masticatory surface. Higher grow of caries was observed in the

control group compared to the group with applied sealant. Anticarietic protective effect of glass-ionomer cements remained even after their loss. Masticatory surface was protected in the period most susceptible to caries, i.e. during the first year after tooth eruption (82). In this period there is no contact of opposing teeth and children have difficulties with appropriate hygienic procedures. According to Forss and Halme, after this period the risk of caries is lower and consequences of sealant loss would be less significant (26). Therefore it was assumed that children from the study group would have a higher caries development than children from the control group, because the latter were older. However the research yielded opposite results. It should be stressed that glass-ionomer cements are recommended for sealing erupting teeth due to difficulties with proper isolation of teeth during application, while glass-ionomer cements are less sensitive to humidity, and because these teeth are susceptible to caries (62).

In a research where retention and efficacy of fissure sealing with glass-ionomer cements was assessed after reapplication of material, caries reduction on masticatory surfaces three years after sealing was 66.5% compared to untreated control group (42). This suggests that lost material should be supplemented in order to ensure the best protection of occlusal surface. The obtained results were better compared to sealing without supplementation of lost material (82). Repetitive application requires time an results in higher cost of treatment. Therefore such prophylactic program may be directed rather at patients from high risk groups, though it may also be applied for the whole population (55). With tooth eruption, all permanent molars should be sealed, which will prevent caries development and consequently will lead to a decrease in the value of the DMF score. However, costs of promoting such a program will be higher, because surfaces which probably would never be carious, would also be sealed (41). In case of sealing only teeth with high caries risk, occurrence of caries would decrease, as well as cost of treatment, which constitutes an important factor in public health protection (9). Thus it is necessary to establish which teeth indicate a higher caries risk and to choose appropriate material.

4. Aim and requirements for sealing materials

The aim of sealant application is:

- mechanical closure of anatomical fissures, crevices and pits on masticatory surfaces of lateral teeth, upper distal-lingual fissures, buccal surfaces of inferior teeth and blind pits in incisors,
- sealing of intercusp fissures from penetration of oral fluids, microorganisms and their substrates which start carious process,
- obtaining mechanical microretention of sealing materials,
- obtaining clinical efficacy in caries reduction on occlusal surfaces.

Requirements for sealing materials:

- appropriate period of activity, fast and easy application,
- fast bonding of material,
- fast curing,
- proper viscosity of material allowing deeper penetration into narrow fissures,
- good and long-lasting adhesion to enamel walls,
- thermal and mechanical properties similar to characteristics of hard tissues of teeth,

- low sorption and solubility in oral environment,
- resistance to abrasion,
- good tolerance by patient (must not disturb occlusion),
- topical and general non-toxicity of material,
- flexible equivalence with tooth tissues
- long-term anticarietic activity.

Appropriate period of activity, fast and easy application of sealants, fast bonding and curing should be within time limits accepted by the patient. Long curing may cause anxiety in a child, leading to moisturizing of treatment area, to outflow of material beyond the sealed site and thus to disturbance of proper polymerization (10). It occurs especially with little children, where sealed teeth are not yet fully erupted and covered with a fold of mucous membrane. Therefore chemically cured resins, which are cured from 45 to 90 seconds after initial mixing of material until completion of curing are not recommended. Materials polymerized with halogen light should be used, as they have a more favorable, unlimited activity time and are characterized by fast curing up to 20-40 seconds, depending on intensity of lamp-emitted light.

Constitution of materials for sealing fissures is generally similar to constitution of composite materials used to fill cavities in hard tissues of teeth. The difference is that fissure sealants have more liquid consistency than fillers, which allows penetration into etched fissures. Sealants based on bis-GMA formula contain amine accelerators and an initiator - benzoyl peroxide. Light-cured sealing materials require a lamp emitting wavelengths of 340-400 nanometers to initiate polymerization. Contemporary sealants are made of one ingredient which does not need mixing. It consists of three parts of viscous bis-GMA monomer, which is diluted with one part of MMA monomer (methyl methacrylate) in order to obtain material of relatively low viscosity. The activator is 2% methylbenzyl ether, which in the presence of 1-2% benzoyl peroxide and light initiates polymerization. Research on appropriate selection of monomer and diluent is very important and provides information about obtained physical properties of sealants. Replacement of methacrylate monomer with other monomers leads to changes of viscosity, impacts extension or shortening of curing time, and alters its hydrophilic character. Efficacy of sealing depends on resin's ability to penetrate fissures before its curing, i.e. on the ability to create a mechanical barrier for carious process. In order to produce an adequately strong bonding and secure proper retention, sealant has to flow over the surface of etched enamel and penetrate microfissures on etched surface. However, it is believed that resin's penetrative ability depends on etching pattern and enamel's moisture, surface tension of material, its viscosity and penetration index. Modifications in application of various monomers resulted in change of cuing time.

Studies on sealants' tightness confirmed the occurrence of microleakages for all assessed sealing materials (e.g. Epoxylite 9075, Nuva Seal, ESPE 717, Concise Enamel Bond, Kerr Fissure, Sealant Delton). Microleakage was observed on the sealant-enamel interface, and in case of glass-ionomer cements, dye penetrated through the whole surface of material. Nuva-Seal seemed the most resistant to dye penetration, though after two weeks some number of cases where dye penetration on the sealant-enamel interface occurred could be observed. In most studied cases ESPE 717 and Epoxylite 9075 materials demonstrated microleakage on the sealant-enamel interface and through the material. The occurrence of microleakage was related to hydrophobic structure of materials and their porosity, which constitutes a better

barrier for dye penetration. The barrier resulting from material's hydrophobic properties is not stable and with time it deteriorates, which results in microleakage.

ASPA and Poly F glass-ionomer cements contain polyacrylic matrix, which is hydrophilic thanks to carboxyl groups. These materials are permeable to water and dye solutions. 24 hours after their application it was observed that aqueous dye solutions penetrated through the sealant-enamel interface and through the material. Another study on Concise, Delton, Kerr, Nuva-Seal sealants with the use of aqueous dye solutions with $Ca45$ and $S35$ radioactive isotopes did not show dye penetration when enamel was etched and sealant permanently and tightly adhered to enamel. Also extension of enamel curing time up to 30 seconds resulted in 32% decrease of microleakage occurrence compared to teeth etched for a shorter time.

Studies determining microhardness of sealants demonstrated varied degree of this parameter from initial 7.51±0.62 for Concise EB and 4.78±0.52 for ASPA cement, up to values increasing in time after three months: 16.49±0.29 for Concise EB and 55.28±1.31 for ASPA cement. Compared to sealants based on bis-GMA formula, ASPA cements after three months demonstrated higher stability and a higher flexibility factor. With increased amount of sealant, rigidity of material also increases along with susceptibility to deformation, that is Young's modulus. The best materials are those with Young's modulus below 10 GPa.

Solubility and disintegration of bis-GMA type sealing materials in oral environment is low and has no clinical significance. Compared to bis-GMA resins, glass-ionomer cements undergo dissolution and disintegration to a much higher degree. Water absorption of sealing materials is low, which is beneficial as it contributes to closure of marginal fissure created by polymerization contraction.

Sealants' resistance to abrasion depends on type of material, content, size and arrangement of filler's particles, as well as on anatomical conditions of sealed teeth. It is assumed that abrasion of sealants based on bis-GMA formula occurs due to impairment of silane bonding agent. Abrasion is a two-phase process: the first phase is characterized by abrasion of polymer and exposure of filler particles, whereas in the second phase they are torn out of the material. Abrasion of sealant on masticatory surface begins on fissure periphery in sites with the thinnest layer of material. Defects on fissure periphery are retention sites, where dental plaque accumulates. The rate of material abrasion also depends on the method of bonding basic ingredients and on the way of material application. Air bubbles trapped in material are sites with decreased resistance to abrasion. The degree of abrasion also depends on activity of separate tooth groups during mastication and on anatomy of tooth and its position in the arch. Sealant abrasion is observed in distal part of superior molars' masticatory surface twice as frequently as in medial parts of those teeth. Moreover, it has been proved that the highest abrasion occurs soon after sealant placement and it decreases with time. The lowest sealant loss was observed in inferior first premolars compared to the other premolar teeth. After 30 months mean value of material volume loss for premolars was 0.43±0.24mm², whereas mean depth of material loss was 221.8±115.1µm. In *in vitro* studies, abrasion, penetration and deformation four most commonly used bis-GMA resins sealants was assessed. It was proved, that materials were abraded to a different degree. The observed level of material wear increased as follows: Delton-21.5; Kerr-22.3; Nuva Seal-23.9; Carbimet -3.,0 (*10^{-4} mm²/mm). The assessed sealants differed considerably in degree of penetration and formability, where the lowest values were observed for Kerr, and the

highest values were observed for Nuva Seal, which was conditioned by material composition. For example, Kerr had 40% of quartz filler particles by volume, which increased its resistance to abrasion and decreased the risk of damaging the surface layer of resin susceptible to abrasion. Due to the necessity of avoiding mastication impairment, sealing material should be present only in anatomical fissures and crevices. Therefore its excess should be removed from occlusal surface.

Every substance of foreign origin which is introduced into oral environment may have an adverse impact and induce certain biological or health effects. Almost all organic ingredients can be washed out from polymerized sealing material by organic solvents, e.g. methanol, ethanol or water. Released formaldehyde and metacrylic acid are particularly dangerous, the latter creating an inhibitory oxygen layer in the surface layer of sealant after polymerization and it can release into oral environment for a long time (up to 115 days after polymerization). Admittedly it does not cause toxic effect, but it may contribute to a local allergic reaction. During monomer particle disintegration, alcohol may be formed (e.g. from bis-GMA resin - bivalent alcohol). Further metabolism of alcohol occurs in digestive tract, leads to release of bisphenol A (BPA), which is a constituent of many compounds, such as bis-GMA, bis-EMA (bisphenol ethoxylate dimethacrylate), TEGDMA (triethylene glycol dimethacrylate), applied in sealing materials. Bisphenol A was detected in saliva of patients subjected to prophylactic fissure sealing. The substance belongs to the group of chemical compounds called xenoestrogens, which - joining with estrogen receptors - imitate the activity of natural hormones, thus they may impact human health. In a study by Olea *et al.* (56) on the presence of bisphenol A, it was detected at the level of 90-931µg/ml in saliva samples collected from patients one hour after sealing. The study also demonstrated that sealing material exposed to 100°C temperature for 30 minutes in buffers with pH 1 and pH 13, released bisphenol A in concentrations causing increased proliferation of MCF-7 cells up to one hour after application, which indicates induction of para estrogenic activity. In other studies authors assessing seven different sealants *in vitro* did not confirm the presence of bisphenol A in any of the assessed materials. However, determinable amounts of TEGDMA were detected in all studied sealants. It can also be supposed that with proper proceeding, bisphenol A will not be released if substances reacting with each other have a sufficient degree of purity. Only in products containing bis-GMA, small amounts of bisphenol A are released, and it occurs only directly after material curing. In most studies bisphenol A was detected in saliva only directly after application of sealant, and this was the case especially with "older" generation of Delton sealant, which still contained bis-DMA (bisphenol A dimethacrylate) in concentrations not affecting the organism. Approximate calculations of bisphenol A released from sealants showed that its level is lower than 1.5%, and such concentrations are too small to cause carcinogenic effect. A study assessing the presence and concentration of BPA (bisphenol A) in blood serum and saliva in 30 individuals aged 18-40 years before sealing and one, three and twenty-four hours after sealing (Delton Pit&Fssure Sealant Dentsplay) indicated the presence of bisphenol A in all examined patients before sealing at the level from 0.07 to 6.00 ng/ml. The level of bisphenol A three hours after sealing had the highest value and after twenty-for hours it returned to the same value as before sealing. The highest recorded concentrations of bisphenol A after a single application and after four subsequent applications were 3.98mg/ml and 9.08mg/ml respectively. Bisphenol A was not observed in blood serum at any stage of the study (89). Exposures to BPA from other sources than resins contributed to a change of BPA level in saliva. What is

more, the amount of sealant's dose used in the study did not impact BPA concentration in blood serum. Further research is necessary to establish the impact of BPA from sealants on estrogen balance.

Chemical compounds contained in sealing materials may have a destructive influence on genetic material in DNA and RNA; it was proved that as many as 14 components of composite materials indicate genotoxicity. On the other hand it was not demonstrated if materials based on bis-GMA and UDMA (urethane dimethacrylate) indicate mutagenicity. It is known that TEGDMA (triethylene glycol dimethacrylate) used as componomer in nontoxic concentrations has mutagenic properties, it impairs proper cell structure, e.g. through interactivity with cell membrane, and its functionality, e.g. decreasing cell's glutathione level, which is responsible for cell structure protection and detoxication. Apart from that it changes expression level of many genes which are important for proliferation processes, control of cell cycle and cell death as well as for DNA replication and repair.

Dentine bonding systems, used to increase adhesion of sealant to hard tissues of teeth, including glutaraldehyde, proved mutagenic and genotoxic in some *in vivo* studies (89).

Data from available studies indicate that concentrations necessary to evoke mutagenic reaction in *in vivo* conditions are much higher than those which evoke a reaction in a patient. Data on mutagenic impact of materials show that their components act mainly in *in vitro* conditions. Such situation occurs especially in case of dentine bonding systems and light-activated glass-ionomer cements. It was proved that resin-modified glass-ionomer cements (Vitrebond) evoked genotypic reactions. The cause was 2-phenylindole chloride which had cytotoxic effect on cell cultures.

Therefore all materials introduced into oral cavity in certain conditions may harmfully impact the organism through releasing specific doses of chemical compounds.

4.1 Technique of sealing procedure

4.1.1 Tooth selection

Sealing procedure should be performed not later than four months after eruption of first permanent molars, at the age of 5-6 years, but practically even after 10 years of age - premolars at the age of 8-12 years, especially if caries is observed in deciduous teeth and first permanent molars, second permanent molars at the age of 11-13 years, and the procedure can be performed up to the 15th year of age. In a population with a high caries risk, also deciduous molars and permanent premolars should be sealed.

Sealant should be applied in teeth with narrow and deep fissures and in teeth with developmental abnormalities on masticatory surfaces. Sealing depends mainly on meticulousness during clinical procedure, i.e. isolation of teeth from moist oral environment, thorough cleaning of occlusal surfaces, etching, rinsing, drying and application of sealant.

4.1.2 Isolation of teeth to be sealed

This is a very important stage of the procedure. Maintaining the area dry is critical for successful sealing procedure. Usage of dental dam, saliva ejector and alternatively lignin rolls is recommended.

Difficulties with proper procedure occur during sealing of molars in maxilla (neighboring parotid gland) and in fissures of freshly erupted molars in maxilla and mandible, when a fold of mucous membrane covers tooth crown which is not fully erupted.

4.1.3 Cleaning of masticatory surface

Masticatory surfaces should be carefully cleaned of bacterial plaque with the use of a hard rotating brush with paste containing a small amount of pumice but no fluorine compounds. Many published reports assess various of cleaning masticatory surfaces before procedure and techniques of applying sealant. One of suggestions is to use a brush dampened with hydrogen peroxide, without pumice. Research demonstrated that after cleaning with pumice, its particles are forced into fissures, which may impair resin's penetration into fissures. Botti et al. assessed cleaning of occlusal surfaces from dental plaque with the use of a synthetic fiber brush and PROPHYflex 3 device (11). The results suggest that the assessed device was the most effective in removing dental plaque compared to cleaning by synthetic fibers. Preparation of masticatory surface by air abrasion was conducted with Air Flow sandblaster at 4.5-7 bars of pressure and at 90° angle from a distance of 1 mm. The abrasive material is aluminum trioxide. The high value of kinetic energy of particles ejected as a stream enables effective preparation of hard tissues. It is recommended to use grains below 50 microns and to abrade intermittently (35,54). Sound systems are based on pneumatic scaler working within the range 6000-6500Hz, vibration amplitude 60-1000 microns. The correct pressure should not exceed 1.5N. Diamond drill bits with 25 and 40 micron coating.

Laser etching with Er Cr:YSGG, 2W and 40Hz laser (8) and erbium laser Er:YAG 7W, Er:YAG (5.5W) (49,70,71). Er:YAG laser demonstrates a 15-fold higher water absorption than CO_2 laser and 20,000-fold higher than Nd:YAG laser. Activity time is thousandths of a second with a minimal energy dose. The energetic level for enamel ablation is 3.3J/cm^3 and for dentine ablation it is 2.8J/cm^3. Carisolv system and sodium hypochlorite demonstrated not only effective cleaning of fissures but also antibacterial properties (49,87). Fissure surface preparation with compressed air was assessed by Kramer N *et al.* (43), whereas Mosemi *et al.* studied the impact of masticatory surface preparation with Er Cr YSGG laser and air abrasion, as well as with 37% phosphoric acid on bonding strength on the wall with INSTRON servohydraulic machine. Samples were divided into three groups. In group A, masticatory surface was etched with acid only, in group B air abrasion and acid were applied, and in group C initial etching with laser and phosphoric acid was performed (53). The results indicate that bond strength was highest in the group where air abrasion and acid etching were applied, compared to the group with acid only etching or initial preparation by means of a laser and phosphoric acid etching (43).

4.1.4 Etching

After protecting a site from access of saliva, after cleaning and drying, etching procedure can be started. In order to obtain enamel etch, acid solution has to be spread on intercusp fissure surface for 15 seconds. The recommended concentration of phosphoric acid is 37%. The introduction of gel etches allowed to limit the impact of acid used to prepare enamel surface before application of sealant and to decrease the risk of uncontrollable contact of etch with dentine. Gels are applied only once, whereas liquid solutions of acids require multiple application. Etches in the gel form penetrate fissure enamel to the same degree as

liquid etches. Acid penetration is hampered by impurities left over after mechanical cleaning of fissures.

It is advisable to repetitively moisten enamel with acid solution during etching, in order to unblock dentine microtubules by removal of calcium salts precipitated during chemical reaction, which limit the effect of etching. After introduction of one-stage etching (of enamel and dentine), dentists have been using self-etching systems based on non-washable acid monomers (low-concentration acids, 10% phosphoric or maleinic acid, 2.5% nitric acid or their mixtures including extra ingredients such as metal ions, glycerin) (2), which eliminate the rinsing phase thus shortening application time and greatly limit the risk of infection of the operative site by saliva and the risk of a mistake during application, which makes the procedure insusceptible to operator's manual "skills".

A soft brush is recommended for etch application, whereas cotton wool balls are not advisable as they contain bubbles of trapped air hampering even spreading of etching solution. Acid solution leads to creation of microfissures in enamel, whose presence enables sealant's penetration deep into tissues. Microscopically, enamel surface becomes rough and appears matt, in electron microscope view - pits of various shapes are visible which were created by a loss of some enamel prisms. The pits are 50-52μm deep.

4.1.5 Rinsing

After etching, the etched surfaces of teeth are rinsed with high pressure water for 10-20 seconds in order to remove acid and residue after the chemical reaction of etching. If etching was performed with an acid in gel form, rinsing time should be doubled.

4.1.6 Drying of etched surface of teeth

After rinsing the tooth's surface should be dried with compressed air for about 15 seconds and isolated from saliva by dental dam which constitutes a simple and effective method of protecting operative field against infection by microorganisms from saliva. This is necessary because any water remaining after rinsing as well as organic impurities from saliva left on the etched surface, impair sealant's penetration into microfissures, thus decreasing the strength of bonding between sealant and enamel. In case of contact with saliva, etching should be repeated for 15 seconds with subsequent rinsing and drying of the tooth. It should be checked with special attention that the compressed air is not polluted by water or oil.

4.2 Application of sealant

Sealant is applied on tooth's surface with delicate brushes made of camelhair, by means of special applicators with a plastic ball or with a cannula resembling a syringe needle. The time required for sealant application and its quantity depend on material type. Chemically cured sealants have a limited curing time and have to be mixed and placed before hardening commences. Otherwise they become too dense and do not reach the required depth in microfissures created by etching. Light-cured sealants have unlimited bonding time, which allows placing of resin when its viscosity is lowest. Too little sealant may cause early material loss, because it cannot cover the whole etched surface, which results in low retention. Too much sealant may impair occlusion or cause unsatisfactory retention due to

break-off or abrasion of material. If a loss or inaccuracy in covering of surface is observed, excess material should be removed with a coarse and fine coated diamond drill with subsequent polishing until smooth (58,60). Surveys among children and their parents on acceptance of colored sealants (red, pink, yellow, white, opalescent) indicated that a great majority of individuals prefer - for aesthetic reasons - white and opalescent sealants.

4.3 Factors impacting effective sealing

Efficacy of sealing procedure is determined by: retention of sealant and obtained caries reduction. Factors impacting retention of sealing materials are:

- condition of tooth surface before sealing,
- morphology of fissures,
- degree of tooth eruption (and probably the time of tooth exposure to oral environment before sealing),
- anatomical features characteristic for an anatomical group,
- individual susceptibility to caries,
- type of sealing material,
- precision of sealing procedure.

Varied morphology of fissures in lateral teeth makes diagnostics of caries in fissures by means of dental mirror and probe ineffective (about 25% probability of caries detection). Fissure penetration with a sharp dental probe causes iatrogenic damage of enamel surface, often affected by demineralization processes. As literature reports, a less invasive method is diagnosing with the use of (5,59) electrical conductivity measurement (ECM), quantitative light-induced fluorescence (QLF) (5) and DIAGNOdent (58,68) in order to assess sensitivity and specificity of caries detection on occlusal surfaces (5,59). ECM demonstrates high sensitivity of between 93% and 96%. However specificity of this method is relatively low - less than 80% (71-77%). Specificity at the average level of about 75% means that 25% of healthy tooth surfaces is recognized and diagnosed as diseased and qualified for treatment, including invasive therapy. The ECM method enables long-term observations of carious lesions.

The FOTI method (fiber-optic-transillumination) serves to diagnose carious lesions, primarily on masticatory surfaces. The sensitivity of the test does not exceed 57%. In laser diagnostics the phenomenon of QLF and DIAGNOdent device are used. The sensitivity of the laser method enables detection of tiniest fluorescence changes related to demineralization of hard tissues. Research indicates the advantage of laser measurement compared to bitewing radiogram in examining occlusal surface lesions. The probability of caries detection in fissures by means of electric conductivity measurement is 93%, whereas the accuracy of X-ray diagnostics (bitewing radiogram) is 75% (46). Sensitivity of microbiological tests (determination of *Streptococus mutans* count) is 67% (40,84). Comparison of clinical test results with radiological results indicated that during clinical study 15% of teeth did not demonstrate any carious centers in fissures which were detectable by means of radiological examination. During repeated clinical tests performed by the same team on individuals aged 14, 17 and 20 years, no carious centers were observed, whereas during radiological examinations carious centers in enamel and dentine were observed respectively in 26.0%, 37.5% and 50.0% of examined teeth. Application of the

radioluminescence method showed carious centers in 32.4% of sealed teeth which during previous clinical tests were deemed healthy (85). In 58% of teeth caries was observed in fissures, which included enamel and dentine (19).

The impact of fissure morphology on sealant's retention is associated with conditions of mechanical retention of material. Sealant is retained better in deep fissures than in shallow ones, and also better in fissures with initial caries, where fissure surface is uneven, than in fissures free from caries.

The degree of tooth eruption and tissues' exposure time to oral environment under sealant have a significant impact on the prophylactic effect of sealing (6). Healthy enamel during tooth eruption is relatively well mineralized, however the process of forming the non-organic composition of the tissue is not yet finished and is subjected to constant physical and chemical changes. Physical changes are various types of abrasion, microcracks and fractures of enamel which occur when crystals of enamel surface layer become fully etched by acids from consumed food. Chemical changes include enamel maturing, which results from continuous dynamic exchange of ions between plaque and fluid in oral cavity on one hand and enamel surface on the other one. Study results indicate that anatomical group of teeth, their position in the dental arch (superior, inferior) may also impact prophylactic efficacy of tooth sealing. It has been proved that sealing of inferior teeth yields better results than sealing of superior ones. Higher efficacy of sealing of inferior teeth is caused by better access of the operator to masticatory surfaces being sealed, better penetration of viscous sealant into fissures due to pressure gradient. An analysis of available literature and own studies indicates that the lowest results are obtained in case of sealing superior second premolars. It is connected - among other things - with small occlusal surface, short fissures and proximity of the parotid gland. A comparison of sealant retention on premolars and molars shows that sealing materials demonstrate better retention on molars. Caries reduction in premolars is also lower compared to reduction on molars after sealing procedure. Lower caries reduction in premolars may be associated with later eruption of those teeth and their generally lower susceptibility to caries compared to molars (36).

Type of sealing material has been deemed a particularly important factor which may impact the effect of sealing procedure to a greater degree than other factors. In caries prophylaxis of lateral teeth various methods and materials are used, e.g. fluoride varnish, resins based on bis-GMA formula (reinforced or not by microfiller particles) enriched or not by fluoride ions (44) and conventional glass-ionomer cements (chemically cured) (77,38), reinforced by silver filings (cements) and HEMA resin-modified cements (7), resins modified with polyacid (7), semi-fluid materials (13,14), ormocers (12,15). Sealing materials are characterized by liquid consistence which allows their inflow into fissures, however it is believed that sealant bonds with etched enamel on cuspal slopes and not at fissure bases (3).

Results of studies on fluoride varnishes showed that despite their efficacy in suppression of caries on flat surfaces of teeth, they are less effective in suppression of caries on masticatory surfaces. This is due to the fact that compared to bis-GMA resins and glass-ionomer cements, fluoride varnishes are retained at the most for a few days only, therefore their prophylactic effect depends on the number of fluoride ions released while the varnish remains on the tooth. Fluoride varnishes reduce caries from 50 to 70% depending on frequency of application. In case of three applications during one year, caries reduction on masticatory surfaces is from 50 to 56%, whereas with three yearly applications during three

years the value increases to 70% (61). It seems that the long-term prophylactic effect of sealing procedure depends not only on the above mentioned factors - with reference to sealed masticatory surface and whole dentition - but also other factors, such as: type of fluoride compounds and frequency of its application, level of oral infection in a given individual, number of microorganisms from *Streptococcus mutans* group in saliva and person's dietary and hygienic habits. All mentioned factors may also be subjected to change in a certain period of time as a result of carious process activity modifications in a given individual. Matalon *et al.* (47) assessed antibacterial properties of sealing materials which contained fluorine. Four sealants were used: Helioseal F, Ultraseal XT, Dyract Seal and GC Fuji Triage. Samples of materials were "aged" for 30 days and rinsed daily with 0.05% solution of NaF for 14 days. After the last rinsing, a culture of *Streptococcus mutans* was added (about 1 x 10^6), which was placed on the surface of each sample and cultured for one hour at 37°C. Results indicated that GC Fuji Triage and Dyract Seal had antibacterial properties for 24 hours after the last exposure to fluorine. GC Fuji Triage maintained strong bactericidal activity up to 48 hours after fluorine rinse. After 72 hours none of the materials demonstrated antibacterial properties. The 30-day aging process resulted in total elimination of their antibacterial properties, but two-week daily rinsing with 0.05% solution of NaF restored antibacterial capability for glass-ionomer cement and compomer (47).

Increased efficacy of sealing of masticatory surfaces can be obtained by improving sealant's retention, elimination of marginal leakage, which prevent caries development under sealant (14,15,33,52,83). Original laboratory research on improving efficacy of sealing by modification of etching time (23, 30, 60 seconds) and application of intermediate system of dentine bonding indicated, that bonding systems applied on enamel etched with acid fill spaces created by partly dissolved enamel and penetrate deeper. After polymerization a hybrid layer is created. Application of bonding systems (Concise LCWS and Helioseal) showed that depending on etching time and type of sealing material with or without bonding system (Schotbond MP i Dyract) it was observed that with extended etching time the bonding strength of Concise LCWS on enamel wall without bonding system decreased, whereas the bonding strength of Heliosal increased. After application of bonding systems appropriate for a given sealing material, with extended etching time, strength of bonding on enamel wall decreased for Concise LCWS sealant with Schotbond MP intermediate bonding system and increased for Helioseal with Syntac intermediate system. In case of etched dentine covered directly with sealant, bonding strength on enamel's wall was insignificantly different, whereas with the use of intermediate systems (appropriate for a given sealing material) an increased bonding strength on the wall was observed. In case of Concise LCWS with Schotbond MP - 2.7-fold, in case of Heliomolar with Syntac > threefold (33).

Studies on morphology of Concise LCWS and Helioseal jonts with hard tissues of teeth indicated occurrence of two types of joints: gapless (adhesion on all surface), and with microfissure between tooth tissues and sealing material. The width of microfissures varied depending on sealing material - in case of Helioseal, microfissures were 30-90μm wide with dominating width of 30μm, whereas in case of Concise LCWS from 10-50μm with dominating width of 25μm. Moreover, micropores with diameter of up to 8μm were observed in Helioseal material, whereas in Concise LCWS material - vertical and horizontal microcracks of sealant extending to dentine were observed. After application of intermediate bonding systems and sealants, gapless joints were observed, however few had microfissures, which were from 1-50μm wide.
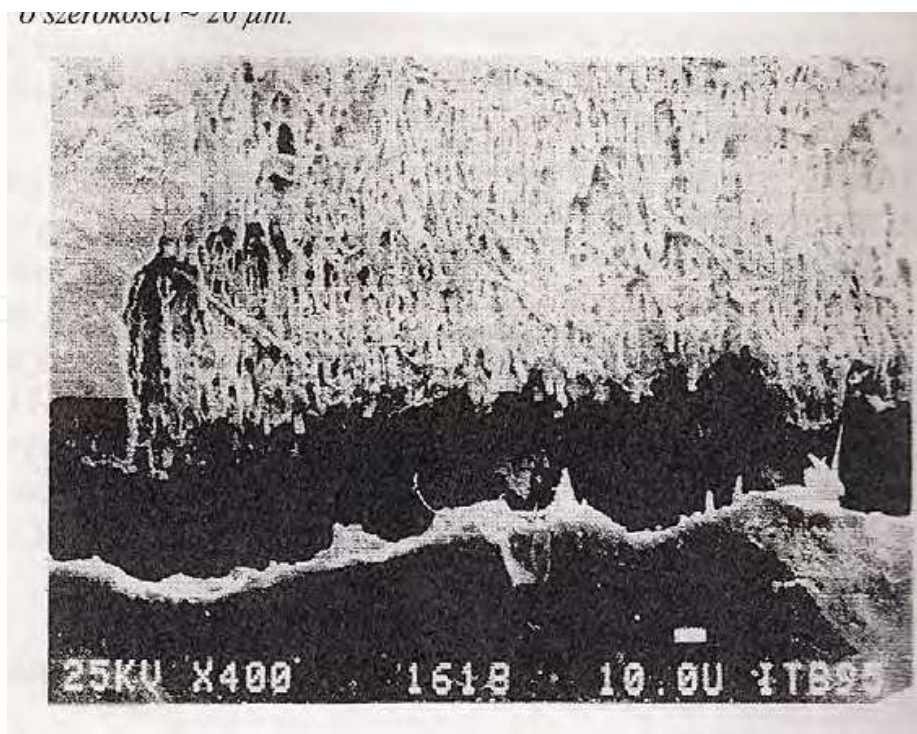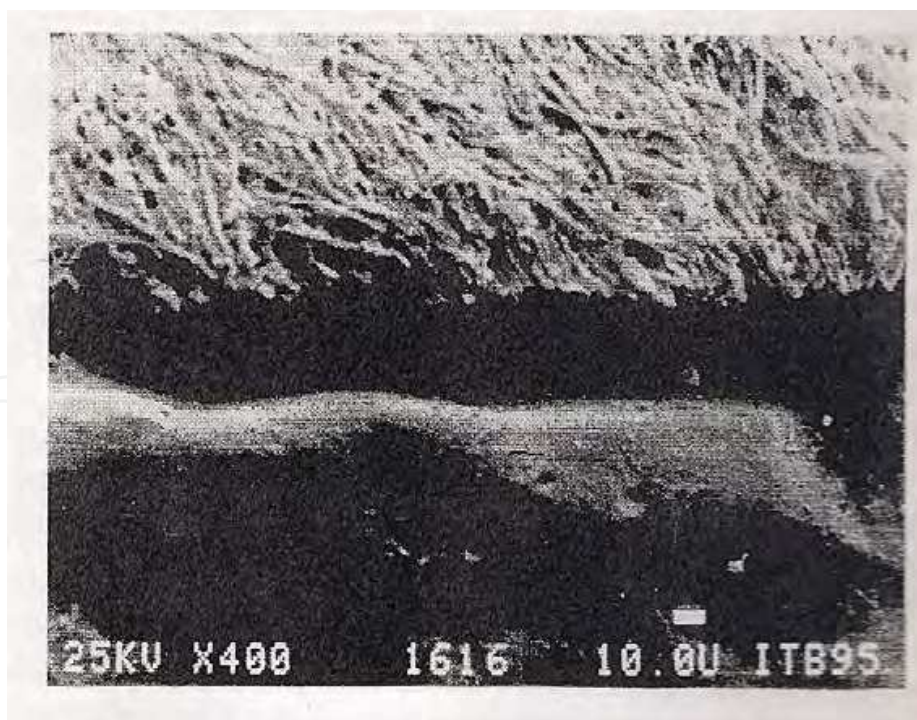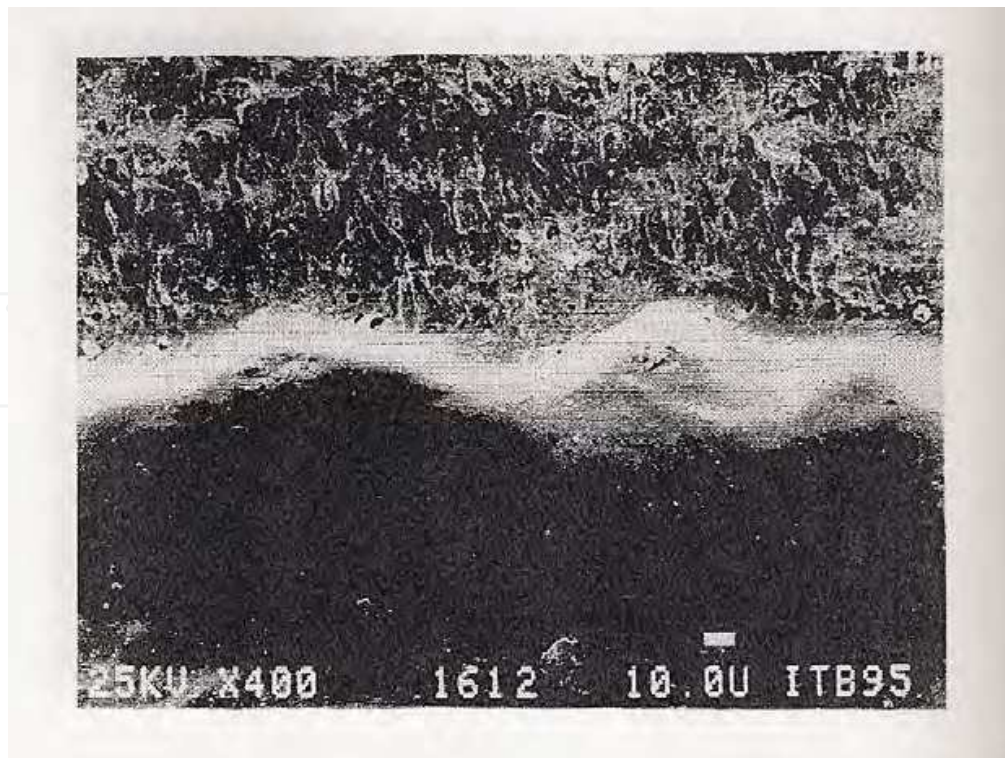
Fig. 1. The SEM microphotograph of the section of etched dentin coated directly with H sealer (horizontal level section). Visible (from the top) microstructure of the dentin and the fissure between the dentin and the sealer ~ 20 μm broad.
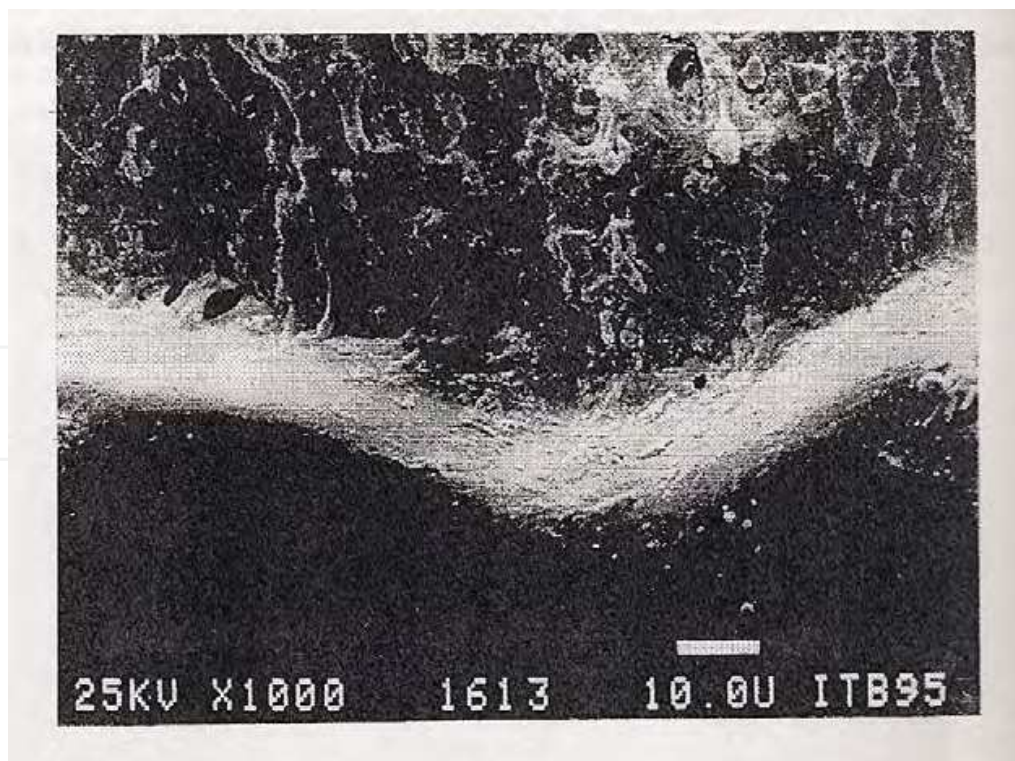

Fig. 2. The SEM microphotograph of the section of etched dentin coated directly with H sealer (horizontal level section). Visible (from the top) microstructure of the dentin and the fissure between the dentin and the sealer (30 - 90 μm broad). Clearly seen micro-pores in the sealer reaching the breadth of 8 μm.

Fig. 3. The SEM microphotograph of the section of etched dentin coated directly with Sy and H sealer (horizontal level section). Visible (from the top) microstructure of the dentin and the fitting hybrid layer and sealer.

Fig. 4. The SEM microphotograph of the section of etched dentin coated by indirect bonding system Sy and H sealer. Visible magnified fragment of middle part of photography fig. 3.

In vitro and *in vivo* studies on application of X-ray microanalysis for assessment of penetration of selected elements included in sealing materials penetrating into dentine indicated that independent of type of conditions - *in vitro* or *in vivo* - an increased value of elements included in sealants and intermediate materials bonding to dentine was observed. Depth of penetration and concentration of elements in dentine depended on the method used for preparation of tissues. The highest concentration of elements on much wider and deeper surface was observed in *in vivo* samples, i.e. when tissue was etched, covered with intermediate material and then sealed, which shows that sealant infiltrated into sealed tissues, improving adhesion and tightness of the joint (33).

One of the most decisive directions of research is improvement of adhesion of sealing materials to tooth's hard surface, because in that way a microleakage of microorganisms or their substrates can be eliminated. The main disadvantage of contemporary adhesive materials is their limited stability *in vitro*. The most frequently quoted reason for clinical failure is loss of retention and marginal tightness, thus in order to extend clinical period of sealant use, one should focus on improvement of bonding stability of those biomaterials with tooth's tissues. The effectiveness of direct bonding of modern sealants is quite good, independent of their type, however during clinical trials bonding efficacy of some materials decreases dramatically, whereas others demonstrate higher stability (20). Results of clinical studies depend not only on patients and used materials but also on external factors, such as operator's skills, type of light source, isolation method, type of instruments used for finishing of sealing material. Factors connected with the patient, such as age, oral hygiene, have a higher impact than any properties of sealing material. Also occlusal tension, eccentric stress is one of the main reasons for sealant loss. Additionally, apart from occlusal load, temperature changes in oral cavity may have a negative impact on tooth/material interface, which is associated with repeated contractions/expansions of filling material resulting from higher expansion factors of thermal contraction than those for tooth's tissues.

Tooth/sealing material interface may also be destroyed by its exposure to water and human bacterial enzymes present in saliva. Penetration of water into hybrid layer and subsequent release of resin components leads to ineffective polymerization *in situ* and to their degradation. Hydrolysis of remaining hydroxyapatites and collagen fibers insufficiently covered with resin additionally impairs long-term efficacy of bonding. Interfacial junction sealing material/tooth is subjected to both chemical and mechanical degradation. Chemical degradation is hydrolysis and plasticization of resin components, also associated with water penetration. Hydrolysis may disrupt a conventional bond, join different units of collagen fibers as well as resin polymers. The process can be aided by bacterial enzymes and by dentine itself. Then in turn catabolites are released together with residual monomers which may weaken the bonding mechanics and promote more water penetration. Water may also decrease fiction forces between polymer chains, which is called resin plasticization. Repetitive mechanical loads can also damage bonding cohesion. During each mastication cycle, the interfacial junction is strained. At some sites, concentration of tension may exceed the bond's resistance to disruption, which results in formation of cracks that spread and lead to damage of material cohesion. A bond is more resistant to cracking in adhesive systems with microfiller particles compared to systems without microfiller particles (21,80).

All resin-based adhesive materials while shrinking induce tensions on interfacial junction, which may lead to crack formation and consequently to microleakage. It was proved that a microleakage may appear between the hybrid layer and dentine even when no crack is observed (2,79).

5. Summary

Taking into consideration a high prevalence and intensification of caries of lateral teeth in children, apart from other methods of caries prophylaxis, such as contact fluorization, which protects flat and contact surfaces of teeth - it is advisable to apply fissure sealants of lateral teeth, i.e. molars and premolars, starting with freshly erupted permanent first molars through subsequently erupting posterior teeth. In order to decrease caries prevalence in our population, it is necessary to conduct awareness-forming oral hygiene training, to create motivation for proper tooth brushing, to advise a diet aimed at reduction of carbohydrate consumption and elimination of additional meals.

Results of numerous laboratory tests indicated that extension of enamel etching time significantly increased the stability of bonds with sealing material. Application of intermediate adhesive systems on enamel increased the strength of enamel bonding with sealing materials only slightly, whereas their application on dentine significantly improved bonding stability (about threefold) compared to dentine coated with sealant alone. An assessment of morphology of dentine joints where dentine was coated with bonding agents and sealant demonstrated occurrence of gapless joints, whereas when sealant was applied directly on dentine, joints with microcracks were formed. Elements included in sealing materials and adhesive systems penetrate into dentine deep and wide, improving retention and tightness of bonding with hard tissues of teeth.

Sealants with fluorine release it into hard tissues of teeth. The level of emission depends on type of fluorine compound and its concentration in sealant. On the basis of literature analysis and own longitudinal clinical studies as well as laboratory tests I confirm the advisability of sealing procedure, expanding it not only on permanent first molars, but including also second molars and - in children requiring special care and having a high caries risk - premolars. Laboratory tests confirmed that that adhesion and tightness of joints between sealants and tooth's hard tissues can be increased, and microleakage can be minimized.

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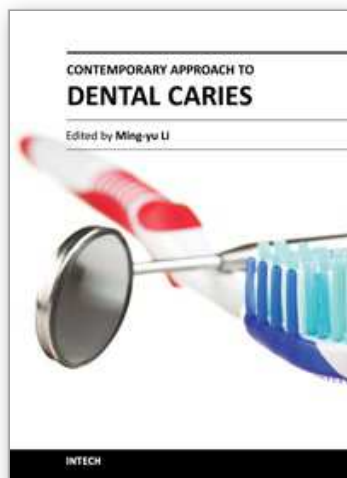
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Edited by Dr. Ming-Yu Li

With an update of the recent progress in etiology, pathogenesis, diagnosis, and treatment of caries, it may be said that the final defeat of dental caries is becoming possible soon. Based on the research in this area in recent decades, "Contemporary Approach to Dental Caries" contained the caries in general, the diagnosis of caries, caries control and prevention, the medical treatment of caries, dental caries in children and others such as secondary caries. This book provides the reader with a guide of progress on the study of dental caries. The book will appeal to dental students, educators, hygienists, therapists and dentists who wish to update their knowledge. It will make you feel reading is profitable and useful for your practice.

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