Controlled Excavation and Antimicrobial Activity in the Treatment of Dentine Caries in Primary Teeth (Literature Review)

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Контролирана екскавация и антимикробна активност при лечение на дентинов кариес на временни зъби (литературен обзор)

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Summary

Modern medicine aims to minimize the intrusion in the biological integrity of the organism. For this purpose, modern methods for diagnosing a given disease in its initial stage are used, so as to apply the most sparing methods of treatment, and also, to reinforce the defense mechanisms of the organism.

The concept of minimal invasive treatment of carious lesions has been successfully applied in childhood. The micro-invasive approach is also applied with great success in the treatment of deep caries lesions in the dentine. This was made possible due to the knowledge of the regenerative and reparative capabilities of the dental pulp, and the possibility for managing of the pulp-dentine complex in the course of the treatment.

The carious dentine is the subject of excavation through various means, with classic excavation being carried out with tungsten-carbide burs.

The chemo-mechanical agents for excavation are an effective alternative to the conventional methods. In that case, the carious dentine is removed using chemical agents. They act selectively and self-limit their effect as they chemically degrade the irreversibly damaged dentine only, thus making it easy to remove with hand excavators, and at the same time does not affect dental structures having remineralization potential.

The chemo-mechanical excavating agents can be classified in two main groups:

-The first group of agents is developed on a sodium hypochlorite base. This group includes the following: GK-101, Caridex, Carisolv, etc.

-The second group of chemo-mechanical agents is enzyme based. Agents such as Papacarie, Biosolv, Brix 3000, etc are included in this group.

As an alternative method for disinfection, photodynamic therapy (PDT) also reveals good results. It achieves optimal disinfection of the residual, partially infected dentine, in cases of reversible asymptomatic pulpitis or affected dentine in deep dentine caries, which is a prerequisite for the successful outcome of the treatment. PDT uses different light sources and different photosensitizers.

Keywords*-micro-invasive approach, chemo-mechanical agents of excavation, photodynamic therapy*

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Резюме

Съвременната медицина цели минимална намеса в биологичната цялост на организма. За целта се използват съвременни средства за диагностика на дадено заболяване в неговия начален стадий, за да могат да се приложат възможно най-щадящи методи на лечение и да се подсилят защитните механизми на организма.

Концепцията за минимално инвазивно лечение на кариозните лезии успешно се прилага в детска възраст. Микроинвазивният подход се прилага и с голям успех при лечение на дълбок дентинов кариес. Това стана възможно благодарение на познанията за регенеративните и репаративни възможности на зъбната пулпа, и възможността за управление на пулпо-дентиновия комплекс в хода на лечението.

Кариозният дентин е обект на екскавация чрез различни средства, като класическата екскавация се осъществява с волфрам-карбидни борчета.

Химио–механичните средства за екскавация са ефективна алтернатива на конвенционалните. При тях кариозният дентин се отстранява с помощта на химични средства. Те действат избирателно и самоограничаващо се, защото разграждат по химичен път само необративо увреденият дентин, като по този начин той става лесно отстраним с ръчни екскаватори, а същевременно не се засягат зъбни структури, които имат потенциал за реминерализация.

Химио-механичните средства се делят на две основни групи:

Първата група са разработени на основата на натриев хипохлорид. Към тази група спадат следните средства GK-101, Caridex, Carisolv и др.

Втората група химио-механични средства са ензимно базирани. В тази група се включват следните продукти-Papacarie, Biosolv, Brix 3000.

Фотодинамичната терапия (ФДТ), като алтернатива за дезинфекция, също показва добри резултати. Чрез нея се постига оптимална дезинфекция на остатъчния, частично инфектиран дентин при обратим асимптоматичен пулпит или афектиран дентин при дълбок дентинов кариес, което е предпоставка за успешния изход от лечението. При ФДТ се използват различни източници на светлина и различни фотосенсибилизатори.

Ключови думи*-микроинвазивен подход, химио-механични средства за екскавация, фотодинамична терапия*

Micro-invasive Approach in the Treatment of Caries in Childhood

Modern medicine aims to minimize the intrusion in the biological integrity of the organism. For this purpose, modern methods for diagnosing a given disease in its initial stage are used, so as to apply the most sparing methods of treatment, and also, to reinforce the defense mechanisms of the organism [1,2,3,4].

Dental caries in childhood, being one of the most common chronic diseases, is considered to be a process which begins long before the point when clinical changes on the hard dental structures could be determined. The carious lesion is a result of this long developing process, i.e. it is a symptom of the illness [1].

The concept of minimal invasive treatment of caries lesions is successfully applied in childhood. This is the modern treatment approach which aims to achieve optimal preservation of the hard dental structures and stimulate the regenerative and healing capabilities of the organism in the carious lesion area [5,6,7].

The micro-invasive approach is also applied with great success in the treatment of deep caries lesions in the dentine. This was made possible due to the knowledge of the regenerative and reparative capabilities of the dental pulp, and the possibility for managing of the pulp-dentine complex in the course of the treatment [6,8].

The alternative techniques used for the microinvasive cavity preparation are air abrasion, zone abrasion, polymer burs, round burs from zirconium dioxide ceramic, hand-tool excavation, chemo-mechanical excavation, enzyme excavation, ultrasonic excavation and laser excavation [1,2,3].

Each of these methods has its positive and negative sides, specific indications for application, specific work protocols and different impact mechanisms on the excavated dentine. What they have in common is their sparing, selective excavation and a possibility for maintaining the reversibly damaged dentine [8].

Micro-invasive Excavation

The carious dentine is excavated through various means with classic excavation being carried out with tungsten-carbide burs. The use of conventional burs at low speed is still the most widespread method for caries excavation. From a modern point of view, their drawback is the lack of selective removal of the irreversibly infected dentine only and preservation of the partially demineralized one [9,10,11].

The conventional low speed burs caries excavation is not in line with the principles of micro-invasive treatment. These burs do not operate selectively and remove healthy dental structures. Polymer and ceramic burs are also unfit for this purpose due to their high cost and single use. They also cause certain discomfort due to the vibrations they produce. There are several factors behind the pain and discomfort they cause, namely: dentine sensitivity; mechanical stimulation - the pressure they put on the tooth; the loud noise of the turbine; thermal stimulation - increase in temperature of the cutting tip of the bur [12].

The ultrasound can also be used in the micro-invasive treatment of caries. The advantage of the ultrasound, compared to conventional methods, is the reduction of vibrations, noise and mechanical trauma during operation, and the sparing excavation [1].

Another method for excavation is air abrasion, in which particles of aluminum oxide are used and directed by compressed air. The particles enter the carious lesion at high speed and remove small parts of the irreversibly damaged dental structure. Unfortunately, air abrasion does not meet the practical expectations with regards to micro-invasive cavity preparation. The indications of this method remained highly limited [13,14,15,16].

Er:YAG lasers are widely applicable in pediatric dentistry for cavity preparation. They operate at a wavelength of 2940 nm since it has the best water absorption capability. Er:YAG lasers are suitable for micro-invasive cavity preparation as it has been proven that they have a selective effect on the completely decomposed dentine only, they lack the drawbacks of noise and vibrations found in rotary tools, and simultaneously reduce bacterial colonization [1].

Additional Antimicrobial Effect in Micro-invasive Excavation

The principle of micro-invasive excavation requires the selective removal of the highly infected dentine only. The preservation of affected dentine in deep carious lesion or partially infected dentine in closed asymptomatic pulpitis requires additional antimicrobial measures, which along with the use of calcium-hydroxide liners during interim obtruation, would provide the necessary antimicrobial effect for limiting any residual microbial flora and arresting the carious process, as well as stimulating tertiary dentinogenesis and the healing process in the pulp [17].

The ozone therapy is an antimicrobial method applied in the treatment of caries after the micro-invasive cavity preparation has been carried out. The ozone has a bactericidal effect as it destroys the microbial cellular membrane. The purine acid produced by the microorganisms, related to the carious disease, is oxidized by the ozone down to an acetate and carbon dioxide. The ozone has several mechanisms of action-antimicrobial, anti-inflammatory, anesthetic, detoxifying and can activate the metabolism of carbohydrates, lipids and proteins [18]. One of the apparatuses used in dental medicine is HealOzone, Curozone Inc., Canada. The ozone is brought to a silicone attachment of varying shapes depending on the different teeth and is held to the tooth for at least 10 seconds. The ozone treatment lasts from two to three minutes. The nozzle has a vacuum system through which microorganisms are removed not only from the main cavity, but also from all hidden niches [1]. The ozone therapy can be applied in the following cases-in the treatment of periodontitis, disinfection of root channels, teeth whitening, desensitization of sensitive teeth, wound healing, etc [18].

The chemo-mechanical agents for excavation are an effective alternative to the conventional methods. In that case, the carious dentine is removed using chemical agents. They act selectively and self-limit their effect as they chemically degrade the irreversibly damaged dentine only, thus making it easy to remove with hand excavators, and at the same time not affecting the remineralization potential of dental structures.

Chemo-mechanical Excavation-Micro-invasive Approach

The chemo-mechanical excavating agents can be classified in two main groups:

-The first group of agents is developed on a sodium hypochlorite base. They destroy the hydrogen bonds in the completely decomposed collagen of the carious dentine only and preserve the partially denatured dentine, thus facilitating selective excavation using hand instruments. This group includes the following: GK-101, Caridex, Carisolv, etc [19].

-The second group of chemo-mechanical agents is enzyme-based. Agents such as Papacarie, Biosolv, Brix 3000, etc are included in this group [20,21].

These agents act atraumatically and they are used with hand excavators. They also have selective, bacteriostatic and bactericidal effect, with their active substance softening and removing the irreversibly denatured collagen painlessly and preserving the partially decomposed collagen in the dentine, which can be restored and remineralized [20,21].

As early as 1975 *Habib et al* presented a method for the removal of carious tissues with a 5% sodium hypochlorite solution, with the sodium hypochlorite used as a non-specific proteolytic agent for removing of the carious dentine. Numerous attempts have been made to improve this methodology since then. The use of sodium hypochlorite only is toxic to healthy tissues nearby. For this purpose, a solution that adds sodium hydroxide, sodium chloride and glycine, to the sodium hypochlorite was created. This modified formula containing N-monochloroglycine is called GK-101 [22].

GK-101 is more effective than using sodium hypochlorite only, but with it the removal of the carious tissues is slower. GK-101 operates in the following manner: it chlorinates the free amino groups and, most likely the amino groups of the peptide protein bonds in the dentine thus forming chlorine-protein compounds [23].

The first chemical agent created which selectively removes the carious dentine is sodium hypochlorite, buffered by a mixture of amino acids (amino butyric acid), sodium chloride and sodium hydroxide. The purpose of the added mixture of amino acids is to dampen the aggressiveness of the sodium hypochlorite on the healthy dentine and improve its effectiveness on the denatured collagen in the carious dentine. Thus, the carious dentine could more easily be removed with a manual, spoon-shaped excavator. This caries removal system is called Caridex (National Patent Medical Products: New Brunswick, NJ, USA) and it has not proven itself particularly successful in the clinical practice [24,25].

What followed is the improvement of Caridex with the creation of a new system for the removal of carious dentine. This was Carisolv

(Carisolv, Mediteam Dental Sävedalem, Sweden), a gel which also contains sodium hypochlorite and various amino acids-glutamic acid, leucine and lysine. The gel is applied onto the carious lesion after which the carious dentine is removed with purpose built and patented manual excavators. The method is proven to be effective in removing the carious dentine and is well received by the patients.

Sodium hypochlorite alkalizes the environment and reacts with the amino acids resulting in the formation of mono- and dichloramine. The chloramine interacts with the collagen and dissolves the torn fibrils. Carisolv stays in the cavity for 30 seconds after which the carious mass is excavated. The partially denatured dentine remains unaffected. The remaining preserved fibrils are stabilized into bundles and become nuclei for internal mineralization of the dentine, which is stimulated in the course of the treatment by the underlying odontoblasts [1].

Through the use of confocal microscopy it is proven that Carisolv is effective in excavating the carious lesion. It has been observed that the microorganisms in the dentine tubules and especially those in the enamel-dentine junction survive, which is an indication of the gel's weak effectiveness in those areas. This is due to the difficulty excavating in the area of the enamel-dentine junction with hand instruments. On the other hand, however, the study reveals that these microorganisms are far less active, most likely due to the bactericidal activity of Carisolv [22,23].

Carisolv is a material which has found its way into the medical practice, especially in the treatment of primary teeth in childhood due to the fact that it is atraumatic and selectiveness, when combined with mechanical excavation and hand excavators.

Enzyme Agents for Selective Excavation

The enzyme method of caries excavation is similar to the chemo-mechanical one. The difference in enzyme-based excavation is in the different mechanism for selective activity, which is more sparing than the one of hypochlorite. It is considered that enzyme excavation can more precisely remove the denatured collagen that has lost the integrity of its triple strand structure [24].

The studies show the possibility of removing the carious dentine with particular enzymes. In 1989 Goldberg and Kale successfully removed softened carious dentine, using bacterial Achromobacter collagenase, which does not affect the dentine layers below the carious lesion. Moreover, in a recent study the pronase enzyme was also used as a non-specific proteolytic enzyme, originating from Streptomyces greseus, for the removal of carious dentine [25].

A gel, containing pepsin in phosphoric acid and a sodium bio-phosphate buffer (SFC-III, V 3M ESPE, Seefeld, Germany) was created. The phosphoric acid dissolves the non-organic component of the carious dentine, while the pepsin having reached the organic mass of the carious dentine dissolves the denatured collagen only. SFC-III must be used with plastic excavators. A study with a micro-CT scan of extracted teeth, processed with SFC-V proves that the gel displays similar activity to those of Carisolv [23,24,26].

Chemo-mechanical agents, based on the effects of enzymes include several products. These are Papacarie, Papacarie Duo, Brix 3000.

Enzyme chemo-mechanical removal of carious dentine with a papain product from papaya was introduced in 2003 by Bussadori, who developed PapacarieTM (a word which means "caries consuming" in Portuguese). Papain is extracted from the leaves and fruits of the Carica Papaya tree, which could be found in tropical regions such as Brazil, India, South Africa and Hawaii [24].

The Papacarie gel is comprised of papain, chloramine, toluidine blue, salts, preservatives, coagulants, stabilizers and deionized water. Papacarie contains small amounts of chloramine, with the main effect of the gel owed to the presence of the papain enzyme, while the chloramines are added to improve the removal of the denatured dentine structures [26,27].

The mechanism of action of chemo-mechanical enzyme-based agents has not yet been fully confirmed. *Bussaduri et al* report that the selective enzyme-based removal of the infected dentine is based on the fact that the carious dentine lacks the anti-protease alpha-1-antitrypsin, which inhibits the proteolysis of the collagen, which in turn allows the papain to be activated. When the collagen is only partially denatured and still has this anti-protease, this leads to the papain inactivity and its proteolytic activity arrest. Thus, selective enzyme proteolytic activity can be put into effect in excavating carious dentine [24,27].

Papacarie action is exhibited in the separation of polypeptide chains and hydrolysis of cross-linked collagen bonds. Oxygen is released immediately after their dissolving, which explains the appearance of the bubbles on the surface during clinical work [27].

An *in vitro* study, using fibroblast culture at different concentrations, demonstrated the lack of Papacarie cytotoxicity [26].

Presently, a new product called Brix 3000 is also being produced. It is also a gel, comprised of papain 3000 U/mg 10%. The papain is bio-capsulated using the incredible E.B.E technology (encapsulating buffer emulsion) which has an immobilizing and stabilizing effect and increases enzyme activity. This leads to a greater proteolytic effect, with the aim to remove the collagen in the decomposed tissue, greater antibacterial and anti-fungal activity, as well as a greater antiseptic effect on the tissues [23,25].

Comparative Studies on Various Methods of Chemo-mechanical Excavation

A number of studies compare the various means for selective excavation of carious dentine, pertaining to the duration of the manipulation, the psychological effect on the patients, particularly if they are children, the morphological characteristics of the residual dentine, the bonds between the dentine surfaces excavated by different means with the adhesion of the obturation material, the degree of micro-invasiveness, the degree of antimicrobial activity in regard to the residual microflora in the carious lesion, etc [22,23].

Reddy et al compare the antimicrobial activity of Carisolv and Papacarie toward Lactobacillus spp. The study includes 40 primary molars of 20 children between the ages of four and eight. The inclusion criteria are: a deep carious lesion; a lack of night and spontaneous pain; a lack of pain during percussion. The first group was excavated with Carisolv, and the second - with Papacarie. Microbiological samples were taken from all of the teeth before and after excavation. The results show that there is no statistically significant difference in the reduction of the microorganisms between the two agents [24].

El-Tekeya et al have conducted a comparative study on the antimicrobial activity of Carisolv and Papacarie. They examine 45 primary molars of children between the ages four to eight, by dividing them into three groups of 15 molars. Group one was excavated with Carisolv, group two- with Papacarie and group three was manually excavated with the use of an excavator only. A microbial sample was taken before and after the excavation of the carious dentine in each of the groups. The study shows that Papacarie is more effective in reducing cariogenic microorganisms compared to Carisolv and manual excavation [25].

Chowdhry et al studied 90 primary molars of children aged from six to nine comparing the effectiveness of three different methods of excavation, the time necessary to carry out the procedure, as well as the attitude of the children towards the treatment. They divided the patients into three different groups based on the method of excavation-conventional excavation, chemo-mechanical excavation with Carisolv and excavation with Papacarie. The most effective in terms of caries excavation was the conventional method, with no statistically significant differences between the other two methods. All three groups demonstrated no statistically significant differences in relation to the quantity of microorganisms before and after the excavation. In relation to the duration, the treatment in the first group with conventional excavation was the shortest, in comparison to the other two. There is no statistically significant difference in the duration between groups two and three. All children had a positive attitude toward the chemo-mechanical excavation [26]. These results match those of *Banerjee et al, Maragakis et al, Yazici et al, Peters et al* [27,28,29,30].

Although Carisolv and Papacarie take slightly longer than the conventional method of excavation, these methods can effectively remove carious dentine and are positively received by children.

Photodynamic Therapy and its Application in the Treatment of Caries with Minimal Intervention

In essence, photodynamic therapy works by forming free radicals or active oxygen with a rapid rate of decay. These radicals cause an instant death of the cell where the photosensitizer is located. Because they decay and deactivate extremely fast, they pose no danger to the neighboring cells [31].

The most important function in the interaction of biological tissues with the molecules of the photosensitizing agents is in the selective targeting of the damaged cell or structures of the organism only (e.g. infected dentine) [32].

The PDT consists of a photosensitive molecule that absorbs an adequate wavelength light and light source. This light-excited molecule, the photosensitizer (PS), can induce two reactions that may happen simultaneously (Type I and II reactions) [33]. In Type I reactions, the excited triplet PS reacts with biomolecules such as nucleic acids, lipids and proteins by transferring an electric charge that produces radicals and radical ions. These radicals react with the molecular oxygen to form reactive oxygen species (ROS) hydrogen peroxide, hydroxyl radicals and superoxide anion. In Type II reaction, being in an excited triplet state the photosensitizer transfers energy to the oxygen in the fundamental triplet state (a process called triplet-triplet annihilation), forming a singlet oxygen [34,35,36].

Scherer et al have recently proposed two new reactions, Type III and Type IV. In these reactions, the cytotoxic effect occurs even in the case of lack of oxygen in the structure within the cells. Type III PSs are antioxidant carrier sensitizer that are able to decrease the radical concentrations in the target cells and generate a singlet oxygen. In Type IV reaction, the photosensitizer cannot bind to the target molecule. After light irradiation, a process of photoisomerization may occur during this type of reaction. This process may lead to intracellular remodeling that facilitates PS binding to the cellular target [37].

The effectiveness of photodynamic therapy depends on a number of factors such as the amount of light used in the target cell (source type) as well as the duration of the light exposure. There are various light sources that can be used for photodynamic therapy. Such sources are a halogen light, light diode (LED), laser diode, helium-neon laser. Laser radiation in the red visible and near infrared region is able to pass through demineralized dentine [38].

Besides the various light sources, a number of photosensitizers can also be used. Such are: methylene blue, toluidine blue, curcumin, azul-cyanine, rose bengal. According to *Nagata et al* in order for photosensitizers to be effective they must exhibit the following characteristics: to interact with the cellular wall and membrane of the cariogenic microorganisms; to penetrate the bacterial cell and perform their internal function; to secrete enough singlet oxygen when irradiated with light [39].

The most widely used photosensitizer is methylene blue. This molecule belongs to the phenothiazine and is soluble in water and alcohol. The photosensitizer efficiency in PDT is related to its intense absorption in the UV-visible region, whose maximum absorption wavelength is 664nm, within the spectral region of 600 to 1000nm (phototherapeutic window). It allows for a deep penetration of light in the biological tissues and expressive quantum yield for singlet oxygen formation [40,41]. Methylene blue has characteristics that promote good interaction with microorganisms, such as: positive molecular charge and low molecular mass. Methylene blue has an action in both Gram-positive and Gram-negative microorganisms. Gram-positive microorganisms are more efficiently inactivated due to the fact that the transport of positively charged molecules into the cell is facilitated. These bacteria have teichoic acids that give a negative charge to the outer surface. This makes the particular photosensitizer suitable for inactivation of cariogenic microorganisms [42].

Steiner-Oliveira et al conducted an *in vivo* study by comparing the effects of various light sources and various photosensitizers. They examined 32 children who had at least one primary molar with a deep carious lesion, reaching the second half of the dentine. The examined teeth were included into three groups: (1) group one (control)- disinfection of the residual dentine at the bottom of the cavity with 2% chlorhexidine; (2) group two- with a LED light source and ortho-toluidine blue applied for 60 seconds, after this period the photosensitizer was activated with a LED source with a wavelength of 630nm, 100mW power, energy 9 J; and (3) group three- with photosensitizer-methylene blue 0.01% for five minutes, activated by a low frequency laser in the red spectrum with a wavelength of 660nm, 100mW power, energy 9 J. A microbiological sample was taken from the dentine before and after the treatment. The results show no statistically significant difference in the photosensitizers used or in the light sources. In all of the groups in the study a significant reduction in the number of microorganisms was observed [43].

Lara Alves et al studied 20 children with active carious lesions of primary molars. Their aim was to study the quantities of *S. mutans* before the excavation of the carious dentine, after the excavation of the carious dentine and after FDT. In both groups the infected dentine was removed down to affected dentine with conventional method. A methylene blue photosensitizer was used and activated by a laser with a wavelength of 660nm, red spectrum, 100mW power, energy 640 J/cm^2. The results show a statistically significant reduction in the quantity of *S. mutans* after the selective removal of the infected dentine and after the use of FDT. The reduction of *S. mutans* is 76.4% after the excavation of the carious dentine and 92.6% after the use of FDT. The experiment demonstrates that even if the infected dentine, containing the greatest quantity of microorganisms, they can still be found in the affected dentine after the excavation [38].

FDT can be considered as a good alternative for the elimination of the remaining microorganisms, increasing the chances of a successful treatment.

Neves et al examined 19 children with active carious lesions. The inclusion criteria were 9: radiographically, the depth of the carious lesion reaches the second half of the dentin; lack of spontaneous or night pain; no fistula in the gingival area; no indications of periapical changes. The preparation of the cavity was made using a conventional method. The first microbiological sample was taken from the residual dentine on the bottom of the cavity. The second microbiological sample was taken again from the bottom of the cavity, after photodynamic therapy. Methylene blue was used as a photosensitizer, placing it in the cavity for five minutes, before being activated. The results show no statistically significant difference in the quantity of microorganisms before and after the use of FDT [42].

The difference in the results of the different authors could be owed to a difference in the dosimetry that was used. Another factor may be the limited penetration of the photosensitizer in the carious dentine. It has been shown that the penetration of toluidine blue and methylene blue in healthy dentine are, respectively 130 and 190µm, and this penetration is even smaller in carious dentine 56.2µm below the surface [44]. Lethal photosensitization probably occurs predominantly in the outer layers of the carious tissue. This fact could occur due to the inability of the photosensitizer to spread into the inner layers or the inability of the light to be totally transmitted [44].

The concentration of the dyes may also affect the FDT. It is known that high concentrations of dyes can induce the phenomenon of self-quenching; reducing the amount of the light that actually reaches the bacteria and induces the generation of reactive oxygen species. Wavelength is a crucial factor in the light's ability to penetrate into the structures. Sources with a greater wavelength allow the light to penetrate deeper into the tissues. This is why red light sources are preferred in photodynamic therapy [36].

Comparing the effectiveness of toluidine blue and methylene blue, both photosensitizers exhibit a reduction of the microorganisms, when combined with red light. Regardless, the effectiveness of photodynamic therapy may vary according to the type of microorganisms. Nevertheless, the efficiency of the therapy can vary according to the genus of the bacteria. Gram-negative bacteria were shown to be more resistant to the therapy due to the greater complexity of their cell membranes. The main microorganisms participating in the cariogenic process are *S. mutans* and *Lactobacillus spp*. which are Gram-positive, but there may be great variations and changes in the environment of the lesion as the front of the carious lesion progresses into the dentine [45].

Chemo-mechanical methods for excavation of carious dentine show great results in the context of the micro-invasive approach. They achieve a controlled removal of the fully damaged dentine only, and only the dentine that allows internal remineralization is preserved. These treatment methods are extremely suitable for non-cooperative children, as the unpleasant sensation caused by conventional methods of preparation can be avoided, as well as the unnecessary removal of hard dental tissues.

As an alternative method for disinfection, photodynamic therapy also reveals good results. It achieves optimal disinfection of the residual, partially infected dentine, in cases of reversible asymptomatic pulpitis or affected dentine in deep dentine caries, which is a prerequisite for the successful outcome of the treatment.

Chemo-mechanical methods and photodynamic therapy can be successfully used in childhood.

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