

## ANTIMICROBIAL ACTIVITY OF PHOTODYNAMIC THERAPY AGAINST MICROORGANISMS ISOLATED FROM DEEP CARIOUS LESIONS

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### ABSTRACT:

**Introduction:** Photodynamic therapy (PDT) is an innovative method, supporting routine treatment methods and accelerating the healing process. It is a method for treatment of pathogens and tumor cells with photoactive dye, in the presence of oxygen. PDT is minimally invasive and high-selective method against specific cells and is equally effective against sensitive and resistant to antibiotics microorganisms.

**Aim:** The purpose of this study was to determine the antimicrobial activity of the PDT (Fotosan-Agent High) against microorganisms isolated from deep carious lesions.

**Materials and methods:** For the purpose of this study were selected 20 patients (n=20) with primary, deep carious lesions on permanent teeth with complete root development. All prepared cavities were class I and class II, and the treatment method was indirect pulp capping. Cavities were prepared with high and low-speed handpieces and water-air cooling. Two microbiological samples were taken of each cavity:

Group 1 - Microbiological samples after cavity treatment with Aqua destillata (n=20)

Group 2 - Microbiological samples after cavity treatment with PDT (Photosensitizer Fotosan (Agent high) and irradiation with a diode laser,  $\lambda = 632$  nm, for 60 seconds) (n=20)

The samples were placed in eppendorf tube with standard transport medium and transported to the microbiological laboratory for determination of microbial number and identification of the isolated microorganisms.

The statistical analysis was made with non-parametric test of McNemar.

**Results:** In 82.35% of cases (14 of 17 cases) after treatment bacterial infection was totally eliminated. In 17.65% (3 of 17 cases) was determined reduction of bacteria count from 100 to 1000 times.

The statistical analysis with non-parametric test of McNemar presented significantly higher proportion of cases with bacterial elimination after photodynamic therapy ( $p < 0.001$ ).

**Conclusion:** After photodynamic disinfection were determined considerable elimination of microbial infection and reduction of bacterial variety and bacterial number. On the ground of these results it can be concluded, that photodynamic therapy is an effective method of treatment and removal of microorganisms from deep carious lesions and support the healing processes in the dentin and dental pulp.

**Key words:** Photodynamic therapy, antimicrobial activity, deep carious lesions, indirect pulp capping

### INTRODUCTION

The modern tissue preserving treatment such as ultra-conservative hard tissue removal, atraumatic restorative techniques and procedures for indirect pulp capping prove, that the progression of carious lesion can be stopped and damaged hard tissue can be remineralized. Stopping the progress of carious lesions and preserving the vitality and function of dental pulp is an important problem in operative dentistry, especially in tooth with incomplete root development. Removal of etiological factor, improving protective, building and trophic functions of dental pulp and thence the success of the treatment, depends on the choice of drug, its proper dosing and application, as well as on preserved regenerative possibility of the pulp, inflammation stage and presence of microorganisms. Important factors are location and defect size, patient age, hermetic seal of dental pulp against bacterial penetration and thickness of residual dentine too.

Mechanical instrumentation by cavity preparation leads to covering the cavity walls with smear layer and closing the apertures of dentinal tubules. It is possible inside to be sealed microorganisms, endotoxins and other bacterial products, that may compromise the healing process and regeneration of hard tissue and dental pulp.

Photodynamic therapy (PDT) is an innovative method, supporting routine treatment methods and accelerating the healing process. It is a method for treatment of pathogens (bacteria, fungi and viruses) and tumor cells with

photoactive dye (photosensitizer, which is activated by light with specific wavelength), in the presence of oxygen. PDT is minimally invasive and high- selective method against specific cells, because each fotosensitizer can recognize and accumulate in large quantity in them. The development of resistance after re-photoactivation is improbable, because in the microbial cell singlet oxygen and free radicals reacted with a variety of cellular structures and by different metabolic pathways. PDT is equally effective against sensitive and resistant to antibiotics microorganisms.

**AIM:**

The purpose of this study was to determine the antimicrobial activity of the PDT (Fotosan-Agent High) against microorganisms isolated from deep carious lesions.

**MATERIAL AND METHODS:**

For the purpose of this study were selected 20 patients ( n=20) with primary, deep carious lesions on permanent teeth with complete root development. The patients were without accompanying diseases. All prepared cavities were class I and class II, and the treatment method was indirect pulp capping.

Before cavity preparation the mouth was rinsed with an antiseptic solution , the teeth were cleaned, polished and isolated. Cavities were prepared with high and low-speed handpieces and water- air cooling. The preparation of the pulp wall was completed finally with new, sterile, round steel burs. Two microbiological samples were taken of each cavity:

Group 1 - Microbiological samples after cavity treatment with Aqua destillata (n=20)

Group 2 - Microbiological samples after cavity treatment with PDT (Photosensitizer Fotosan (Agent high) and irradiation with a diode laser,  $\lambda = 632 \text{ nm}$ , for 60 seconds)

(n=20)

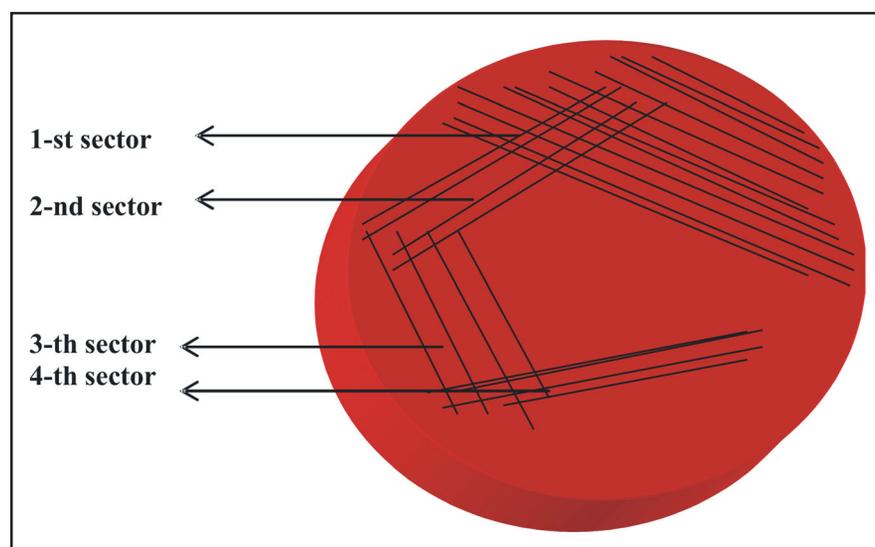
The samples were placed in eppendorf tube with standard transport medium (Stuart medium) and transported to the microbiological laboratory for determination of microbial number and identification of the isolated microorganisms.

In our study there were used different culture media: blood agar (BA) with 5% sheep blood , MacConkey agar (MC), Saburou (S) Trypticase soy broth (T) and elective - selective medium - chocolate agar supplemented with vancomycin for fastidious Gram -negative bacteria (SCA), which was developed by a team at the Department of Microbiology, Medical University of Sofia. The culture media, as well as tests for species identification REMEL RAPID ID, Crystal system, were manufactured by Oxoid and Becton Dickinson (BD) BBL. After overnight incubation initial screening was carried out and pure cultures were isolated. If suppressed microbial growth was found, cultivation was extended to 48 hours. Biochemical tests and examination of microscopic morphology were performed with 24 or 48 hour microbial culture.

The culture conditions were consistent with the supposed etiology for obtaining an optimal result [1, 2, 4]. Cultures of BA and SCA were incubated necessarily in the presence of 5 % to 10 % CO<sub>2</sub> and high humidity, and all other media in the presence of atmospheric O<sub>2</sub> in 36°C for 24-48 h.

The quantitative method was used to inoculate the secretions of all media et following scheme: 1 sector 30-40 strokes, following culturing from 1 sector (third terminal ) to sector 2, 4 strokes from 2 to sector 3, 4 strokes from 3 to sector 4, and 4 strokes also (Fig. 1). After cultivation grewed colonies in different sectors were counted up and were compared with standard scale to bacterial count (number of colony forming units) determination.

The statistical analysis was made with non- parametric test of\* McNemar.



**Fig. 1.** Quantitative method of strokes

## RESULTS:

In Group 1 absence of bacterial growth was found in 3 cultures and from 17 cultures were isolated various microbial species. There were found 14 different microbial species (9 of them were Gr + microorganisms and 5 - Gr- microorganisms). All isolated bacteria were aerobic or facultative anaerobic. In 10 cultures was isolated one microbial species, in 6 cultures- two microbial species, and in 1 culture- three

different microbial species. (Tab. 1.)

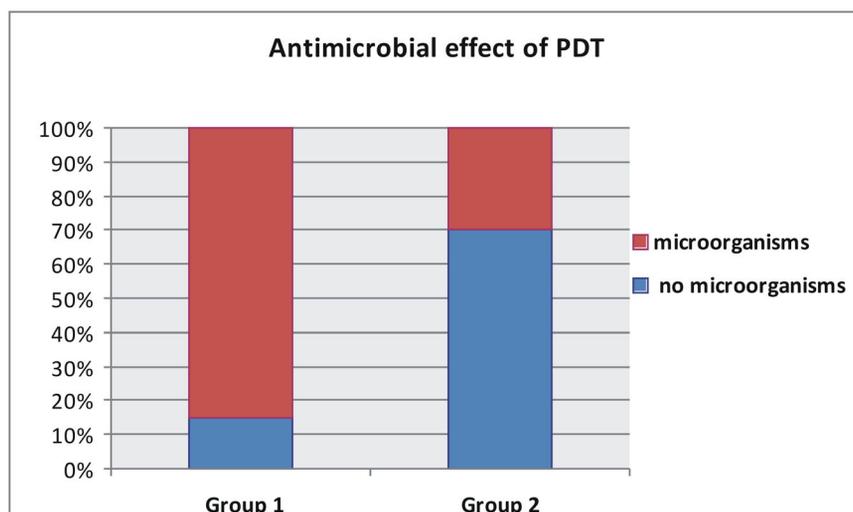
In Group 2 absence of bacterial growth was found in 17 cultures and from 3 cultures were isolated various microbial species. There were found 4 different microbial species (3 of them were Gr + microorganisms and 1- Gr-microorganism). All isolated bacteria were aerobic or facultative anaerobic. In 2 cultures was isolated one microbial species, and in 1 culture- two microbial species. (Tab. 1.)

**Tab. 1.** Microbial isolates from deep carious lesions

Sample No	Group 1	Group 2
1.	<i>Staphylococcus aureus</i> 10 000 cfu/ml	No growth
2.	No growth	No growth
3.	<i>Streptococcus mitis</i> 100 000 cfu/ml	No growth
4.	<i>Staphylococcus aureus</i> 1 000 cfu/ml <i>Actinomyces newii anitratus</i> 10 000 cfu/m	No growth
5.	<i>Streptococcus anginosus</i> 10 000 000 cfu/ml	<i>Streptococcus anginosus</i> 10 000 cfu/ml
6.	<i>Enterobacter cloacae</i> 10 000 000 cfu/ml <i>Streptococcus mitis</i> 100 000 cfu/ml <i>Enterococcus faecalis</i> 10 000 cfu/ml	<i>Enterobacter cloacae</i> 10 000 cfu/ml <i>Streptococcus mitis</i> 1 000 cfu/ml
7.	<i>Streptococcus mutans</i> 10 000 cfu/ml	No growth
8.	<i>Streptococcus intermedius</i> 10 000 cfu/ml	No growth
9.	<i>Bacillus brevis</i> 100 000 cfu/ml <i>Streptococcus mitis</i> 10 000 cfu/ml	<i>Bacillus brevis</i> 100 cfu/ml
10.	<i>Streptococcus mutans</i> 100 000 cfu/ml	No growth
11.	<i>Enterobacter cloacae</i> 10 000 cfu/ml	No growth
12.	<i>Streptococcus gordonii</i> 100 000 cfu/ml <i>Neisseria subflava</i> 10 000 cfu/ml	No growth
13.	<i>Streptococcus mitis</i> 100 000 cfu/ml <i>Haemophilus parainfluenzae</i> 10 000 cfu/ml	No growth
14.	No growth	No growth
15.	<i>Streptococcus mutans</i> 100 000 cfu/ml	No growth
16.	<i>Corynebacterium xerosis</i> 10 000 cfu/ml <i>Streptococcus mitis</i> 100 000 cfu/ml	No growth
17.	<i>Staphylococcus aureus</i> 10 000 cfu/ml	No growth
18.	<i>Streptococcus mitis</i> 100 000 cfu/ml	No growth
19.	<i>Streptococcus intermedius</i> 10 000 cfu/ml <i>Neisseria polysacharea</i> 10 000 cfu/ml	No growth
20.	No growth	No growth

In 82.35% of cases (14 of 17 cases) after treatment bacterial infection was totally eliminated. In 17.65% (3 of 17 cases) was determined reduction of bacteria count from 100 to 1000 times.

The statistical analysis with non- parametric test of McNemar presented significantly higher proportion of cases with bacterial elimination after photodynamic therapy (  $p < 0.001$ ). (Fig.2)



**Fig.2.** Effect of photodynamic therapy against microorganisms, isolated from deep carious lesions

### DISCUSSION:

Isolation and identification of sensitive Gram -negative pathogens, may be hindered by the presence of other microbial species participated in inflammation or saprophytes. These problems can be removed by means of selective media, containing antibiotic supplement, which inhibit the growth of Gram-positive concomitant microorganisms such as Chocolate agar with vancomycin, which was used in our study.

In Group 1 were isolated various microbial species from 85 % of cultures. There were found 14 different microbial species- *Streptococcus*(5), *Staphylococcus*(1), *Actinomyces*(1), *Enterococcus*(1), *Corynebacterium*(1), *Enterobacter*(1), *Haemophilus*(1), *Neisseria*(2), *Bacillus*(1). Gr + microorganisms were more than Gr- microorganisms.

In Group 2 were isolated microbial species from 15 % of cultures. There were found 4 different microbial species- *Streptococcus* (2), *Enterobacter*(1), *Bacillus*(1).

Gr + microorganisms were more than Gr- microorganisms.

ganisms.

In both groups isolated microorganisms were predominantly aerobic or facultative anaerobic Gr+ bacteria. In Group 2 there were found lower microbial number and lower variety of microbial species. In 82.35% of cases after treatment bacterial infection was totally eliminated. *Streptococcus*, *Enterobacter* and *Bacillus* were relative steady to PDT, but there was found reduction of bacteria count from 100 to 1000 times.

### CONCLUSION:

After photodynamic disinfection were determined considerable elimination of microbial infection and reduction of bacterial variety and bacterial number. On the ground of these results it can be concluded, that photodynamic therapy is an effective method of treatment and removal of microorganisms from deep carious lesions and support the healing processes in the dentin and dental pulp.

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