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Original article

ANTIMICROBIAL EFFECT OF A MEANS FOR ENZYME EXCAVATION (BRIX 3000) AND PHOTODYNAMIC THERAPY IN CARIOUS LESIONS OF PRIMARY TEETH – *IN VITRO* EXPERIMENT

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ABSTRACT

The selective removal of the caries dentin via enzyme methods for excavation appears to be an alternative to the conventional treatment of carious lesions in childhood. Photodynamic therapy is an effective alternative for the reduction of cariesogenic microorganisms. Brix 3000 is an enzyme-based material for excavation of carious dentin.

Aim: To study the antimicrobial effect of means for enzyme excavation (Brix 3000) and photodynamic therapy with FotoSan 630 Intro Kit to the two main cariesogenic microorganisms – *S. mutans* and *L. acidophilus*, *in vitro* experiment;

Materials and Methods: Eighty plates were prepared: group 1- 20 plates only with Brix 3000; group 2 – 20 plates only with FotoSan; group 3 – 20 plates with a combination of Brix 3000 and FotoSan; group 4 – 20 plates without an active agent.

In the agar of each plate, three 7 mm wells in diameter were made, where the Brix 3000 gel was placed, as well as discs soaked with the dye and irradiated with FotoSan and a combination of them. After 24 hours, the zone of inhibition was measured.

Results: Compared to the control group, Brix 3000 and FotoSan have a defined antimicrobial activity against *S. mutans* and *Lactobacillus spp.* By combining the two materials, their antimicrobial activity significantly increases. *S. mutans* shows greater resistance compared to *Lactobacillus spp*.

Conclusion: A combination of enzyme-based excavation and photodynamic therapy could be used successfully in the treatment of carious lesions in primary teeth.

Keywords: *S. mutans*, *Lactobacillus spp*., photodynamic therapy, Brix 3000,

INTRODUCTION

The philosophy of minimally invasive treatment is expressed in maximum preservation of hard dental structures in the treatment of carious lesions and stimulating tertiary dentinogenesis [1, 2]. An important step is the selective removal of carious dentin by enzyme-based methods, which turns out to be a reliable alternative to conventional treatment, especially to children with primary teeth [3]. During the excavation, the number and quantity of cariogenic microorganisms are reduced, decreasing the risk of unnecessary disclosure of the pulp during treatment. In the carious lesion, conditions are created for stationing the process[4].

Photodynamic therapy turns out to be an effective alternative for the reduction of cariogenic microorganisms. This technique is based on the use of a light source to activate a photosensitizer, which in the presence of oxygen, produces nascent oxygen and free radicals that irreversibly damage cellular components and change the metabolic properties of the microbial cell leading to its death [5, 6]. The most important characteristic of the interaction of biological tissue with the molecules of photosensitizing agents is in the selective attacking only the damaged microbial cells [7, 8].

Brix 3000 is enzyme-based material for chemo-mechanical excavation. Its main component is papain, which is bio-encapsulated by EBE Technology (Encapsulating Buffer Emulsion). This technology releases the enzyme, generation proteolysis to the carious dentin. When the gel meets healthy dentin, it loses its enzyme properties due to the presence of antiprotease-1-antitrypsin. In this way, Brix 3000 acts selectively, preserving the partially degraded collagen in the dentin, which can be remineralized.

Clinical removal of carious dentin, in which enzyme-based excavation with Brix 3000 and photodynamic therapy for disinfection during treatment, is a reliable alternative to the conventional treatment.

Aim

To study the antimicrobial effect of means for enzyme excavation (Brix 3000) and photodynamic therapy with FotoSan 630 Intro Kit to the two main cariesogenic microorganisms – *S. mutans* and *L. acidophilus*, *in vitro* experiment.

MATERIAL

For the experimental study of the antimicrobial efficacy of Brix 3000 and PDT with FotoSan 630 Intro Kit, 80 plates were prepared, grouped as follows:

- Group 1 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* processed with Brix 3000;
- Group 2 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* processed with FotoSan 630 Intro Kit;
- Group 3 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* treated with the combination Brix 3000 + FotoSan 630 Intro Kit;
- Group 4 – 10 plates each inoculated with *S. mutans* and *Lactobacillus spp.* without an active agent-control group

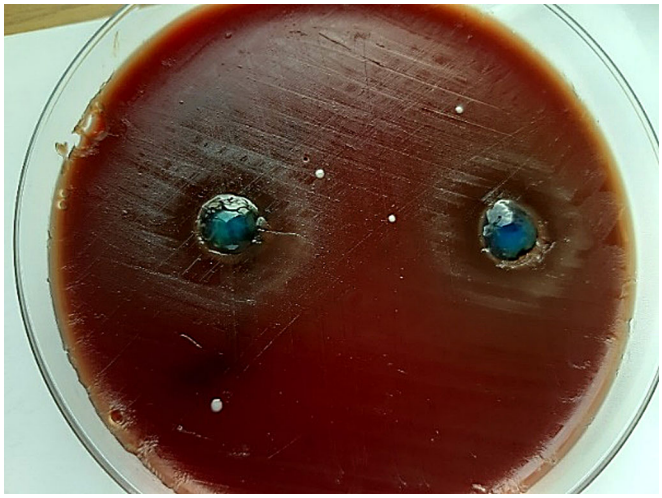
MICROBIOLOGICAL METHOD:

Lyophilized strains of *S. mutans* and *Lactobacillus spp.* come to life after culturing in broth and repeated subculturing of blood agar and CO_2 atmosphere. After obtaining a pure 24-hour culture, it was standardized according to McFarlan 0.5 Mueller-Hinton agar plates (EUCAST 2020) were inoculated with 5% horse blood and 20 mg/I NAD using a sterile tampon.

In the agar of each plate were made three wells with a diameter of 7mm., where the Brix 3000 gel was applied, as well as discs soaked with the dye and irradiated with FotoSan (photodynamic therapy) and the combination of them.

The formulation thus prepared was incubated for 24-48 hours in a thermostat at 36°C in a CO_2 atmosphere. Inhibition zones were reported, including a well with a diameter of 7mm. and the zone of actual inhibition. The average of the zones of inhibited growth was calculated in each group.

Image 1. Zone of inhibition obtained by the combined action of Brix 3000 and FotoSan



The SPSS (version 19, SPSS Inc., USA) was used for the statistical processing of the results. A 95% confidence interval ($p<0.05$) was chosen as a level of significance at which the null hypothesis is rejected.

RESULTS

1. Antimicrobial activity of Brix 3000, FotoSan and their combination to *S. mutans*

The next table represents the values of reported zones of *S. mutans* inhibition growth in millimeters.

Table 1. Antimicrobial activity of Brix 3000, PDT with FotoSan and their combination to *S. mutans*

Average arithmetic values of the zone of inhibition, in mm			
Group	N	Mean± SD	Ind T-test
Group 1 – Brix 3000	10	9.60 ± 0.699	$t_{1,2} = 4.837$ $p_{1,2} < 0.05$
Group 2 – FotoSan	10	8.30 ± 0.483	$t_{1,3} = -20.813$ $p_{1,3} < 0.05$
Group 3 – Brix 3000+FotoSan	10	17.20 ± 0.919	$t_{1,4} = 11.759$ $p_{1,4} < 0.05$
Group 4 – control group	10	7.00 ± 0.00	$t_{2,3} = -27.110$ $p_{2,3} < 0.05$ $t_{2,4} = 8.510$ $p_{2,4} < 0.05$ $t_{3,4} = 35.101$ $p_{3,4} < 0.05$

The average value of the antimicrobial activity of Brix 3000 only against *S. mutans* is 9.60 mm. The antimicrobial activity of Brix 3000 is double when compared to that of FotoSan, in separate usage. The best antimicrobial effect could be observed in ingroup 3, which includes a combination between Brix 3000 and FotoSan ($p<0.05$).

2. Researching the antimicrobial activity of Brix 3000, FotoSan and their combination to *Lactobacillus spp.*

Table 2 presents the antimicrobial activity of Brix 3000, FotoSan and their combination to *Lactobacillus spp.*

Table 2. Antimicrobial activity of Brix 3000, FotoSan and their combination to *Lactobacillus spp.*

Average arithmetic values of the zone of inhibition, in mm			
Group	N	Mean± SD	Ind T-test
Group 1 – Brix 3000	10	11.20 ± 0.789	$t_{1,2} = 8.547$ $p_{1,2} < 0.05$
Group 2 – FotoSan	10	8.70 ± 0.483	$t_{1,3} = -20.889$ $p_{1,3} < 0.05$
Group 3 – Brix 3000+FotoSan	10	19.20 ± 0.919	$t_{1,4} = 16.837$ $p_{1,4} < 0.05$
Group 4 – control group	10	7.00 ± 0.00	$t_{2,3} = -31.983$ $p_{2,3} < 0.05$ $t_{2,4} = 11.129$ $p_{2,4} < 0.05$ $t_{3,4} = 41.983$ $p_{3,4} < 0.05$

Used separately, Brix 3000 demonstrates a higher antimicrobial activity compared to FotoSan ($p<0.05$). The two materials combined have higher antimicrobial activity in comparison to their individual use.

3. Comparative analysis between the antimicrobial activity of *S. mutans* and *Lactobacillus spp.*

Table 3. Comparison of the antimicrobial activity of Brix 3000, FotoSan and their combination to *S. mutants* and *Lactobacillus spp.*

Group	S. Mutans		Lactobacillus sp.		Paired S-test
	N	Mean± SD	N	Mean± SD	
Group 1 – Brix 3000	10	9.60 ± 0.699	10	11.20 ± 0.789	t=-9.798 p=0.000
Group 2 – FotoSan	10	8.30 ± 0.483	10	8.70 ± 0.483	t=-2.449 p=0.037
Group 3 – Brix 3000+FotoSan	10	17.20 ± 0.919	10	19.20 ± 0.919	t=-13.416 p=0.000
Group 4 – control group	10	7.00 ± 0.00	10	7.00 ± 0.00	

It could be observed from the table that the antimicrobial activity of Brix 3000, used separately, is higher than *Lactobacillus spp.* when compared to *S. mutans.* FotoSan demonstrates a relatively similar antimicrobial activity to both microorganisms. The stronger antimicrobial effect of the combination of both materials used simultaneously is to *Lactobacillus spp.*

DISCUSSION

Our results revealed that both Brix 3000 and FotoSan possess an expressed activity with regards to the two main studied microorganisms, *S. mutans* and *Lactobacillus spp*. The papain, which is the main component of Brix 3000, is an end protein with bacteriostatic, bactericidal and anti-inflammatory activity. [9,10]. The papain could inhibit the growth of the bacteria since it could degrade the peptide bonds in microorganisms to dipeptides and amino acids. The enzyme papain is included in the sulfhydryl proteases group. This shows that it has a sulfhydrylene residue in its active place, which affects the bacterial cell wall and the cytoplasmic membrane [11].

The essence of photodynamic therapy is the creation of free radicals or of the active oxygen with a short life span. These radicals cause the immediate death of the cell, in which the photosensitizer lies. They have a very short life span and could be deactivated very rapidly, and thus they do not endanger the neighboring cells [11].

Brix 3000 has a higher antimicrobial activity to *S. mutans* and *Lactobacillus spp* in comparison to the photodynamic therapy as an individual activity. When the two materials are combined, the activity is increased. This proves a potentiated antimicrobial effect of the combination between Brix 3000 and photodynamic therapy with FotoSan to both microorganisms - *S. mutans* and *Lactobacillus spp.*

A number of studies prove the antimicrobial activity of the papain, which is the main component of Brix 3000. Goyal et al. compare the conventional method of excavation with the enzyme one by studying 25 children aged between 5 and 9 years. The microbiological samples are taken before and after excavation of the carious dentine. The results obtained reveal a significant reduction in the number and amount of *S. mutans* and *Lactobacillus spp.* [12]. Similar results are also achieved by Singh et al. by discovering a reduction in the amounts of *S. Mutans* and *Lactobacillus spp.* [13].

Various authors conduct *in vitro* studies of the antimicrobial effect of the photodynamic therapy against *S. mutans*and*Lactobacillusspp*by researching different sources of light and different photosensitizers. *Araujo et al.* demonstrate a high antimicrobial effect of the photodynamic therapy to *S. Mutans* and *Lactobacillus spp.* by using curcumin as a photosensitizer and blue light with wavelength 450 nm [14]. In their research, *Melo et al.*use a diode laser and toluidine blue and prove the high antimicrobial effect of the photodynamic therapy [15]. Their results concur with ours. The same results are achieved by *Ricatto et al.*in their work, although they use methylene blue as

photosensitizer [16].

The chemio-mechanical technique of excavation of the carious dentine with Brix 3000 in primary teeth demonstrates excellent results in the context of the minimally invasive method. Through the technique, a controlled excavation of the completely damaged dentine only, and only the dentine, which could stimulate a tertiary dentinogenesis and internal remineralization, is reserved.

The potentiated antimicrobial effect, proven by us, of the means of enzyme excavation and follow-up procedure of photodynamic therapy with FotoSan, represents a good reason to recommend the studied means in selective excavation to soft, partially infected dentine in the course of treatment of deep dentine caries or asymptomatic closed pulpitis. The method is sparing and achieves dentine reserving for internal remineralization and stimulation of the tertiary dentinogenesis as well as a healing process on the part of the dental pulp.

CONCLUSIONS:

Brix 3000 for enzyme excavation has a two times higher antimicrobial activity when compared to FotoSan, used separately, to *S. mutans* and *Lactobacillus spp*;

By combining Brix 3000 with photodynamic therapy with FotoSan, their antimicrobial activity is significantly increased;

S. mutans is more resilient to Brix 3000 and the photodynamic therapy with FotoSan in comparison to *Lactobacillus spp.*

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Abbreviations:
PDT - photodynamic therapy

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