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Microbial Challenge of Breathless™ Toothpaste with C. albicans, E. coli, S. aureus, and S. sanguis

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Abstract:
Oral conditions, such as periodontal disease are serious, not only because they can cause gum infections and the loss of teeth, but also because they have been shown to be linked to respiratory and even cardiac disease. Chlorine dioxide, in the past a relatively unknown compound, has been shown to be a powerful antimicrobial agent used in products ranging from hand creams to mouthwashes. Breathless™ is a chlorine dioxide toothpaste, based on a patented formulation. In this study, an assay was developed to determine the effectiveness of different formulations of Breathless™ against high concentrations of *C. albicans*, *E. coli*, *S. aureus*, and *S. sanguis*. Results indicated that even at the lowest concentration of chlorine dioxide tested, Breathless™ was capable of reducing the level of all of these organisms, by more than 6.4 log dilutions, after only one minute of exposure. Further assays, to determine the effects of Breathless™ against oral anaerobic organisms, are in development.

Microbial Challenge of Breathless™ Toothpaste with *C. albicans, E. coli, S. aureus* and *S. sanguis*

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ABSTRACT

Oral conditions, such as periodontal disease are serious, not only because they can cause gum infections and the loss of teeth, but also because they have been shown to be linked to respiratory and even cardiac disease. Chlorine dioxide, in the past, a relatively unknown compound, has been shown to be a powerful antimicrobial agent used in products ranging from handcreams to mouthwashes. Breathless™ is a chlorine dioxide toothpaste, based on a patented formulation. In this study, an assay was developed to determine the effectiveness of different formulations of Breathless™ against high concentrations of C. albicans, E. coli, S. aureus, and S. sanguis. Results indicated that even at the lowest concentration of chlorine dioxide tested, Breathless™ was capable of reducing the level of all of these organisms, by more than 6.4 log dilutions, after only one minute of exposure. Further assays, to determine the effects of Breathless™ against oral anaerobic organisms, are in development.

INTRODUCTION

Most people are aware that diseases of the mouth can cause serious problems in those areas that are infected. These can include chronic infections of the gums, as well as loss of teeth. Most people are unaware that these infections can cause serious problems in other areas of the body as well. Importantly, studies have shown a relationship between gum infections and heart disease, and gum infections and pneumonia among others. (1) (2)

Frontier Pharmaceutical, Inc. has developed a new toothpaste (DioxiBrite™) containing chlorine dioxide as the active ingredient. Chlorine dioxide is a well known odor neutralizer as well as a complete antimicrobial agent. It is fast acting, broad spectrum, non-irritating and non toxic. This study found that DioxiBrite™ Toothpaste was capable of killing high concentrations of microorganisms in less than one minute.

It is anticipated that in using DioxiBrite Toothpaste, potentially pathogenic microorganisms can be greatly reduced in the mouth, leading to improved oral health, and perhaps a lower rate of the serious diseases known to be related to oral infections.

(1) Beck, J.D., J. Pankow, J., H.A. Tyroler, and S. Offenbacher. 1999. Dental Infections and Atherosclerosis. Am Heart J **138:**528-533.

(2) Scannapieco, F.A. 1999. Role of Oral Bacteria in Respiratory Infection. J. Periodontol. **70:**793-802.

METHODS

CULTURING OF MICROORGANISMS: S. aureus ATCC 29213, E. coli ATCC 25922, C. albicans ATCC 10231 and S. sanguis ATCC 10556, were obtained from Remel (Lenexa, KS). Initial cultures were developed as per manufacturer's instructions. Prior to testing, S. aureus and E. coli were plated on Trypticase Soy Agar Plates, and incubated for 18-24 hours at 37°C. S. sanguis was plated on Chocolate Agar and incubated for 48 hours at 37°C. C. albicans was plated on Sabouraud Dextrose Agar and incubated for 48-72 hours at 25°C. Heavy suspensions of the microorganisms were prepared in saline. All media were obtained from Becton Dickinson (Sparks, MD).

ENUMERATION OF MICROORGANISMS IN TEST SUSPENSION: Serial ten fold dilutions of the suspensions were made. Duplicate samples of 1.0 ml of the 10^{-7} dilution and of the 10^{-8}dilution were added to sterile petri plates. Approximately 10 ml of liquid Trypticase Soy Agar at 45°C were added to the bacterial plates, and allowed to harden. Approximately 10 ml of liquid Sabouraud Dextrose Agar at 45°C were added to the C. albicans plates, and allowed to harden. Bacterial plates were incubated at 37°C for 24-48 hours. C. albicans plates were incubated at 25°C for 48-72 hours. Following incubation of the plates, colony counts were made.

CHALLENGE TESTING: DioxiBrite Toothpaste is supplied in two parts that are mixed just prior to use. In these tests 5 grams of part A and 5 grams of part B were mixed for fifteen seconds, and then incubated for 1 minute.
1.0 ml of the inoculum was then mixed with the paste and incubated for 1 minute. Following this, 90 ml of D/E Neutralizing Broth, was added to neutralize the active ingredient and dissolve the mixture. pHydrion Micro Chlorine test strips (Micro Essential Laboratory. Brooklyn, NY) were used to confirm that no residual chlorine dioxide remained after the D/E Neutralizing Broth was added. A ten-fold dilution of this D/E Neutralizing Broth diluted test sample was prepared in saline.

ENUMERATION OF MICROORGANISMS IN CHALLENGE SAMPLE: Five 2-ml aliquots of the neutralized test sample were added to sterile petri plates. Duplicate 1 ml samples of the neutralized diluted test sample, and of the ten-fold saline dilution, were added to sterile petri plates. Approximately 10 ml of liquid Trypticase Soy Agar at 45°C were added to the bacterial plates, and allowed to harden. Approximately 10 ml of liquid Sabouraud Dextrose Agar at 45°C were added to the C. albicans plates, and allowed to harden. Bacterial plates were incubated at 37°C for 24-48 hours. C. albicans plates were incubated at 25°C for 48-72 hours. Following incubation of the plates, colony counts were made.

CONTROL STUDIES: Control studies were run, in which a 10 ml sample of saline was challenged, instead of 10 grams of the toothpaste. The tests were run as described above, except that test samples were diluted 1 x 10^{-5} to 1 x 10^{-7} to obtain plates that could be counted.

Results

- DioxiBrite caused a reduction of greater than 8.38 logs of microorganism when tested with S. sanguis. (Table 1)
- DioxiBrite caused a reduction of greater than 8.04 logs of microorganism when tested with E. coli. (Table 2)
- DioxiBrite caused a reduction of 6.44 logs of microorganism when tested with S. aureus. (Table 3)
- DioxiBrite caused a reduction of 7.73 logs of microorganism when tested with C. albicans. (Table 4)

Table # 1

Test Organism: **S. sanguis ATCC 10556**

Test Sample Product)	Challenge Inoculum / (Log#cfu/ml Product)	Recovered (Log#cfu/ml	Log Reduction
DioxiBrite™ Toothpaste	2.4 x 10^{8} / **8.38** (1)	0 (3)	>8.38
Control (Saline)	2.4 x 10^{8}	7.2 x 10^{7} / **7.85** (2)	

Table # 2

Test Organism: **E. coli ATCC 25922**

Test Sample Product)	Challenge Inoculum / (Log#cfu/ml Product)	Recovered (Log#cfu/ml	Log Reduction
DioxiBrite™ Toothpaste	1.1 x 10^{8} / **8.04** (1)	0 (3)	>8.04
Control (Saline)	1.1 x 10^{8}	5.9 x 10^{7} / **7.77** (2)	

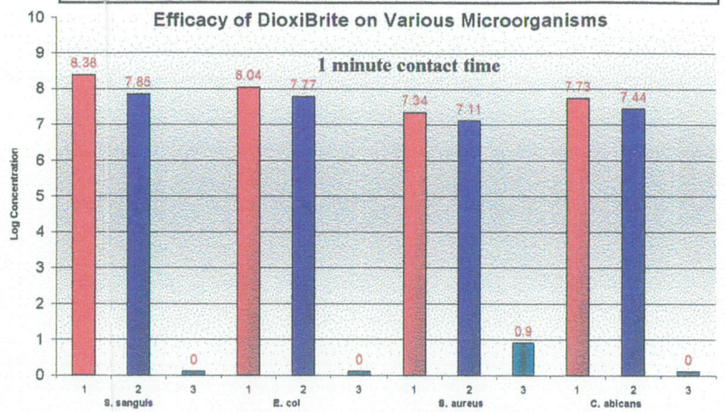
Table # 3

Test Organism: **S. aureus ATCC 29213**

Test Sample Product)	Challenge Inoculum / (Log#cfu/ml Product)	Recovered (Log#cfu/ml	Log Reduction
DioxiBrite ™ Toothpaste	2.2 x 10^{7} / **7.34** (1)	**0.90** (3)	6.44
Control (Saline)	2.2 x 10^{7}	1.3 x 10^{7} / **7.11** (2)	

Test Organism: **C. albicans ATCC 10231** Table # 4

Test Sample Product)	Challenge Inoculum / (Log#cfu/ml Product)	Recovered (Log#cfu/ml	Log Reduction
DioxiBrite™ Toothpaste	5.4 x 10^{7} / **7.73** (1)	0 (3)	>7.73
Control (Saline)	5.4 x 10^{7}	2.8 x 10^{7} / **7.44** (2)	



KEY: 1=Challenge Inoculum 2=Recovered Control 3= Recovered Test

DISCUSSION

This was a pilot study designed to evaluate the effectiveness of chlorine dioxide as an antimicrobial agent in toothpaste. Results indicated that DioxiBrite Toothpaste killed all of the organisms in three of the four challenges. In the fourth case the number of organisms were reduced to less than one millionth of the original concentration.

Designing an assay to determine the effectiveness of the toothpaste was difficult and several obstacles had to be overcome. The most important of these was that the viscosity of the paste made it difficult to mix the organisms both quickly and effectively. While these processes were optimized, it is likely that in those samples in which some organisms survived the challenge, this may have been the cause. The data also indicate that the D/E Neutralization Broth also had an inhibitory effect on growth, which probably accounted for not retrieving 100% of the organisms in the control samples.
The results generated by this study have an immediate and practical application. Based upon this work, Frontier Pharmaceutical, Inc. is evaluating DioxiBrite Toothpaste for efficacy against plaque and gingivitis.

The results described above were obtained with organisms that can survive in air. Westbury Diagnostics, Inc. is currently developing an assay that will allow the testing of DioxiBrite Toothpaste with anaerobic microorganisms, as well.