UNIVERSIDADE FEDERAL DO CEARÁ
CURSO DE ODONTOLOGIA
DO *CAMPUS* DE SOBRAL

REPORT

Sobral, 03.12.2018.

From: Prof. Dr. Bruno Carvalho de Vasconcelos

To: Angelus Dental Products Company

Subject: Report submission.

Dear,

Saluting them, in view of the conclusion of the requested experiments related to the analysis of the antimicrobial capacity against the biofilm of E. faecalis of the intracanal medication material called BIO-C TEMP; I hereby pass on the results of the evaluations to the company.

Initially, it is necessary to emphasize that this in vitro methodology used, previously approved by the company and showed in the Annex at the end of this Report, does not fully reflect the condition to which the material will be exposed when used clinically. However, regardless of this aspect, this methodology allows to perform comparisons with control groups and / or with other materials available on the market for the same purpose.

According to the results expressed in Table 1, it can be highlighted that the BIO-C TEMP showed antimicrobial activity against both strains evaluated, showing counts always lower than those presented by the control groups. However, although it is possible to numerically determine this activity, the statistical test was not able to determine a statistically significant difference between the groups. Controversially, it is understood that differences greater than 3 Logs reflect significant differences, so it is possible to state that the BIO-C TEMP intracanal medication was effective against #29212 strain in direct contact condition.

Table 1. Count of CFU/mL of the BIO-C TEMP intracanal medication paste against E. *faecalis* strains under different conditions.

	Direct contact		Indirect contact	
Group	*ATCC 29212*	*ATCC 4083*	*ATCC 29212*	*ATCC 4083*
BIO-C TEMP	1,14E+08	1,23E+07	1,22E+08	1,47E+08
Control	1,58E+12	8,30E+09	6,43E+09	7,40E+08

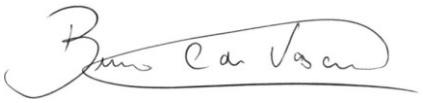
Statistical analysis performed with the Kruskal-Wallis and Dunn test with $P < 0.05$.

In view of the above, we believe that the tested intracanal medication has potential for clinical use, however, it is suggested that more tests be carried out as a way of looking for alternatives to increase its antimicrobial activity; also, it is suggested to perform tests on teeth with both culture methods and confocal laser scanning microscopy.

UNIVERSIDADE FEDERAL DO CEARÁ
CURSO DE ODONTOLOGIA
DO *CAMPUS* DE SOBRAL

This being for the moment, we are available for any clarifications and development of new studies.

Kind regards,



Prof. Dr. Bruno Carvalho de Vasconcelos
Professor Adjunto IV

Bruno Carvalho de Vasconcelos, DDS, MSc, PhD
Email: bcv@ufc.br

ANNEX

(Methodology taken from the previously approved Project)

Antimicrobial activity against biofilms formed membrane

Biofilm formation

The E. faecalis biofilms formation will be induced in a pore cellulose nitrate membranes of 0.22 μm in size and 13 mm in diameter. The membranes will be placed on the surface of TSB blood agar plates. Then, 1 mL aliquots of the bacterial suspension will be applied to the membrane surfaces and the plates will be stored for 48 hours at 37º C.

Direct and indirect contact test

For the material-biofilm direct contact test, 40 μL of the intracanal medications will be placed on the biofilm formed in the membranes. For indirect contact, sterile membranes will be placed over the membrane containing biofilm, and the material will be applied over the sterile membrane.

In the indirect test the same technique will be used, with the difference that a nitrocellulose membrane will be interposed between the biofilm and the sealer. Two membranes will be used for each material, with three repetitions on different occasions. The contact time for both tests will be 30 minutes at 37º C. After the contact period, the material will be removed from the membrane surface in direct contact. In indirect contact, the cellulose membrane with the material will be discarded. For the control group, membranes with biofilm formed without any contact with the materials will be used.

The membranes will be transferred to a flask containing 2 mL of sterile phosphate-buffered saline (PBS) and slightly agitated to remove loosely adhered cells. Then, they will be transferred to another flask containing 2 mL of PBS and agitated by sonication for 1 minute (30 sec + 15 sec + 15 sec) intercalated in an ice bath. A serial dilution of 1:10, 1: 100, 1: 1000, 1: 10000, 1: 1000000, 1: 1000000 and 1: 100000000 will be performed and 100 μL aliquots of each dilution will be plated on TSB blood agar. Colony forming units (CFU) will be counted after incubation for 24 hours at 37º C.