ENDODONTIC MICROORGANISM SUSCEPTIBILITY BY DIRECT CONTACT TEST

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ABSTRACT

The aim of this study was to evaluate in vitro the duration of the antimicrobial effect of endodontic sealers by means of the Direct Contact Test. The sealers tested were: Endomethasone - Septodont®, Endomethasone C – Septodont®, Endion – Voco®, Diaket – ESPE®, Pulp Canal Sealer – SybronEndo®, and AH26 – Dentsply DeTrey®. The endodontopathic microorganisms (MO) confronted were: Staphylococcus aureus (Sa), Candida albicans (Ca), Enterococcus faecalis (Ef), Prevotella intermedia (Pi), Porphyromonas gingivalis (Pg) and Fusobacterium nucleatum (Fn). Test specimens of each sealer were prepared and placed on the surface of agar plates that had been inoculated with each MO, and after predetermined periods, transfers were made from the contact area between the test specimen and the cultured agar and from the area that had not been in contact with the test specimens (control). The results were read as presence/absence of microbial growth and analyzed statistically using the Kruskal-Wallis test. It was concluded that the structural features and virulence of endodontopathic microorganisms determine their response to the sealers, independently of the time during which sealers act and the mechanism by which the antiseptic reaches the microorganism, which in this case was by direct contact.

Key words: endodontic sealers, microorganisms.

SUSCEPTIBILIDAD DE MICROORGANISMOS ENDODONTOPÁTICOS POR LA PRUEBA DE CONTACTO DIRECTO

RESUMEN

El objetivo de este trabajo fue evaluar in vitro la duración del efecto antimicrobiano de los selladores endodónticos mediante la Prueba de Contacto Directo. Los selladores probados fueron: Endomethasone - Septodont®, Endomethasone C – Septodont®, Endion – Voco®, Diaket – ESPE®, Pulp Canal Sealer – SybronEndo® y AH26 – Dentsply DeTrey®. Los microorganismos endodontopáticos (MO) enfrentados fueron: Staphylococcus aureus (Sa), Candida albicans (Ca), Enterococcus faecalis (Ef), Prevotella intermedia (Pi), Porphyromonas gingivalis (Pg) y Fusobacterium nucleatum (Fn). Se prepararon las probetas con cada uno de los selladores, se colocaron sobre la superficie de placas de agar sembradas con cada MO y luego de períodos predeterminados se realizaron repiques de las zonas de contacto probeta-agar sembrado y de la zona que no estuvo en contacto con las probetas (testigo). Se realizó la lectura de los resultados: presencia/ausencia de desarrollo microbiano y se analizaron estadísticamente mediante la Prueba de Kruskal- Wallis.

Pudo concluirse que las características estructurales y la virulencia de los microorganismos endodontopáticos son determinantes de la respuesta de los mismos frente a los selladores independientemente del tiempo durante el cual estos actúen y del mecanismo por el cual el antiséptico alcance al microorganismo, en este caso por contacto directo.

Palabras clave: selladores endodónticos, microorganismos.

INTRODUCTION

The quality of endodontic treatment depends on correct diagnosis, meticulous preparation, and filling the root canal with materials having adequate physical, chemical and biological properties[1]. Proper obturation of the root canal is defined and characterized as the three-dimensional filling of any root canal, as close as possible to the cement-dentine junction. Tiny quantities of endodontic cement/sealer[1], with proven biological compatibility, are used together with the core filling to provide an adequate seal. The current core filling of choice is gutta percha[2]. Cements seal the interface between the core material of the obturation and the dentine walls of the root canal in order to achieve a hermetic, stable three-dimensional obturation[3].

The concept of hermetically sealing the root canal system has currently been discarded in favor of antimicrobial sealing, because failure is most often associated to inadequate instrumentation, antisepsis

Table 1. Microbial strains and culture mediums.

Strain	Culture media
Staphylococcus aureus (Sa)	BHI agar[1]
Enterococcus faecalis (Ef)	Mitis Salivarius agar[2]
Candida albicans (Ca)	Saboureaux agar[3]
Prevotella intermedia (Pi) *Porphyromonas gingivalis (Pg)* *Fusobacterium nucleatum (Fn)*	Blood agar with vitamin K and hemin[4]

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[2]Lab. Disco. Detroit, Michigan. EEUU.
[3]Lab. Britania SA. Argentina.
[4]Summanen P, Baron EJ, Citron DM. Wadsworth Anaerobic Bacteriology Manual. Publishing Company. California. USA. 5th Edition. 1993.

or obturation, particularly in inaccessible canals of multi-rooted teeth and unsealed lateral canals[4-6]. From a microbiological standpoint, the endodontic sealer must be able to modify environmental conditions by acting on the pH, and ideally it should contain a substantive antiseptic with microbicidal, or at least microbiostatic effect, to prevent microbial proliferation in the canal and periapical spaces. The most commonly used *in vitro* technique for evaluating the antimicrobial activity of endodontic sealers is the Agar Diffusion Test (ADT)[7-10], which consists of comparing the diameters of the inhibition halos obtained by contact of the sealers with cultures on agar plates. The ADT requires careful standardization of inoculum density, preparation and storage of the culture medium. In turn, the diffusion process will depend upon the size and number of test specimens per plate, the distance between them, the adequate contact between them and the agar and the incubation time and temperature needed. In addition, this technique has certain limitations, such as its semi-quantitative nature, the impossibility of measuring the activity of the soluble components continuously, and does not distinguish between microbicidal and microbiostatic effects.

Another type of methodology is the Direct Contact Test (DCT), which evaluates microbial viability resulting form the direct contact between the microorganisms and the sealer for a controlled time, independently of the solubility and diffusion of its antimicrobial components. It thus provides significant advantages, including reproducibility, simultaneous evaluation of several test specimens per plate and continuous measurement of microbial growth. Some materials show major inhibition by ADT because there are diffusible components in their makeup, while others do not because they cannot diffuse in agar. It is therefore important to run both tests in order to evaluate the antimicrobial activity of an endodontic sealer[11-14].

The antimicrobial effectiveness of an endodontic sealer must be measured as a function of time[15,16]. Some papers only study initial inhibition[8,11,13], with some of them saying that maximum effectiveness is recorded up to the time the material sets, and others saying that it declines over the hours immediately following. Nevertheless, a prior study enabled us to prove that the effect lasts over time[7].

The aim of this study was to assess the antimicrobial effect of six endodontic sealers against facultative aerobic and anaerobic strains involved in endodontic infections, using the Direct Contact Test.

MATERIALS AND METHODS

The following sealer cements were tested: **Endomethasone** - Septodont®, **Endomethasone C** – Septodont®, **Endion** – Voco®, **Diaket** – ESPE®, **Pulp Canal Sealer** – SybronEndo®, and **AH26** – Dentsply DeTrey®; the facultative aerobic microorganism strains were *Enterococcus faecalis, Staphylococcus aureus, Candida albicans,* and the anaerobic microorganism strains were *Porphyromonas gingivalis (ATCC 33277), Prevotella intermedia (ATCC 25611)* and *Fusobacterium nucleatum (ATCC 25586).*

Sealers were prepared according to the manufacturers' instructions, and a tuberculin syringe used to inject them into sterile glass cylinders 1 cm in diameter placed in the sterile agar (Table 1), and kept at 37°C for 24 hours until the sealers set. Then the test specimens were removed from the agar and stored at room temperature for 1, 7, 17, 28 days. Following each of these periods, the test specimens were placed on agar plates inoculated with an 0.25 suspension of Mc Farland Scale 1 for each microorganism. Plates were incubated at 37°C under the conditions of time and atmosphere required by each microorganism (aerobic and facultative strains were incubated for 24 hours in aerobiosis and microaerobiosis respectively, and anaerobic strains were incubated for 4 days in an anaerobic jar).

After the first 6 hours of contact, each test specimen was lifted, and a calibrated inoculating loop was used to take a sample from the area that the test specimens had been touching and streak it onto surface of fresh sterile agar plates, which were then incubated. The test specimens were immediately

Table 2: Results for 6, 24 and 72 hours contact between sealers and *Enterococcus faecalis*.

Sealer	6 hours Time (days)				24 hours Time (days)				72 hours Time (days)			
	1	7	17	28	1	7	17	28	1	7	17	28
Endomethasone	1	1	1	1	0	1	1	1	0	0	1	1
Endomethasone C	1	1	1	1	1	1	1	1	1	1	1	1
Endion	1	1	1	1	1	1	1	1	1	1	1	1
Diaket	1	1	1	1	1	1	1	1	0	0	1	1
Pulp Canal Sealer	1	1	1	1	0	1	1	1	0	0	1	1
AH26	1	1	1	1	1	0	0	0	1	0	0	0

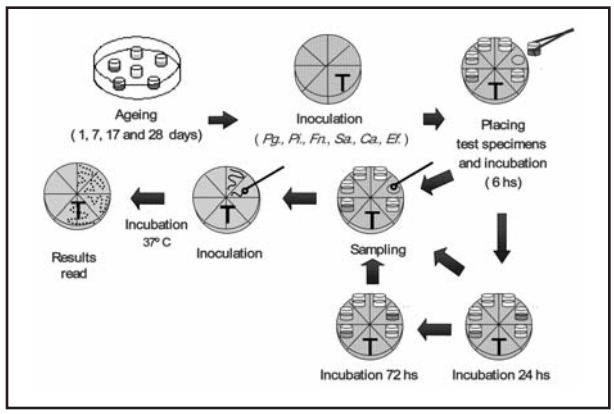

Fig. 1: Methodology. Pg: Porphyromonas gingivalis, Pi: Prevotella intermedia, Fn: Fusobacterium nucleatum, Sa: Staphylococcus aureus, Ca: Candida albicans, Ef: Enterococcus faecalis T: Control.

replaced onto the surface of the contact plate agar using sterile tongs, and incubation continued up to 24 and 72 hours. An area of the inoculated agar that had not been in contact with the test specimens was used as a control. This procedure was carried out again under the same conditions, at 24 and 72 hours contact sub-times, for the ageing time periods established: 1, 7, 17 and 28 days (Fig. 1).

The results were assessed considering presence or absence of microbial growth and statistical analysis was performed using the Kruskal-Wallis Test.

RESULTS

For the group of **aerobic and facultative microorganisms** it was found that all sealers were bactericidal against *Staphylococcus aureus* after 24 hours for all experimental times and sub-times. *Candida albicans* was sensitive to all sealers for all times and sub-times. The results obtained with *Enterococcus faecalis* (*Ef*) where somewhat uneven, showing it to be resistant to the six sealers tested at the 6-hour sub-time. At 24 hours contact it was sensitive only to Endometasone and Pulp Canal Sealer, with 1 day of test specimen ageing. AH26 was bactericidal against it as from 7 days aging. At 72 hours contact for test specimens aged for 17 and 28 days, *Ef* developed in the presence of all the sealers tested except for AH26 (Table 2).

For the group of **anaerobic microorganisms** it was found that *Prevotella intermedia* developed only in contact with Endion and at most of the experimental sub-times and times. After 6 hours in contact with the test specimens aged for 1 day, *Porphyromonas gingivalis (Pg)* colony forming units (CFU) were found with Endomethasone C, Endion and AH26; but growth was inhibited at the rest of the sub-times and times evaluated. All the sealers tested, for all sub-times and times, were bactericidal against *Fusobacterium nucleatum.*

Statistical evaluation using the Kruskal-Wallis Test of all the values obtained for **aerobic and facultative microorganisms** showed a significant effect for the microorganism factor ($p \leq 0.05$) and not for the sealer factor ($p > 0.05$) (Table 3).

For **anaerobic microorganisms** no significant effect was found for the microorganism and sealer factors ($p > 0.05$) (Table 4).

Table 3: Kruskal-Wallis test for facultative aerobic microorganisms.

Aerobic/facultative microorganisms	
Sealer	$p > 0.05$
Microorganism	$p \leq 0.05$
Time	$p > 0.05$

Table 4: Kruskal-Wallis test for anaerobic microorganisms.

Aerobic microorganisms	
Sealer	$p > 0.05$
Microorganism	$p > 0.05$
Time	$p > 0.05$

No significant difference was found for the time factor ($p>0.05$) (Tables 3 and 4).

DISCUSSION

This study showed the resistance of *Enterococcus faecalis* to the sealers that were tested, except for AH 26, which contains hexamethylentetramine (HMT) as a catalyzing agent for the setting or hardening reaction, during which formaldehyde, which is a powerful antimicrobial agent, is released.

The HMT chemical reaction releases formaldehyde very slowly, which could explain why AH 26 has bactericidal effect on *Enterococcus faecalis* only at contact sub-times 24 and 72 hours for the test specimens aged for 7, 17 and 28 days. Similar studies[11] using the direct contact test found that AH 26 had a bacteriostatic effect on *Enterococcus faecalis* until 6 contact hours and that there was an increase in bacterial growth towards the end of the study (18 hours).

The main antimicrobial agents in Diaket, which is a vinyl resin, are zinc oxide and chlorophenols. It was found in this study that the test specimens aged for 1 and 7 days had a bactericidal effect on *Enterococcus faecalis* only after 72 hours contact. This might be due to the slow release of chlorine and regeneration of phenol[17] from Diaket after it sets.

Other sealers that were also effective against *Enterococcus faecalis* were Endomethasone and Pulp Canal Sealer, whose formulas contain zinc oxide - eugenol and iodine. Endomethasone also contains paraformaldehyde. The results of both were similar at the 24- and 72-hour contact sub-times, and there was inhibition by the Endomethasone test specimens that had been aged for 1 day and the Pulp Canal Sealer aged for 1 and 7 days. The loss of effectiveness over time might be due to the sublimation of iodine plus the strong action of the zinc oxide eugenol, particularly the latter. Pizzo et al. obtained the same results with these sealers at 24 hours contact with 1- and 7-day samples[18].

Gomes et al. found that there was no significant difference between Endomethasone and Endomethasone C when tested against *Enterococcus faecalis* using methodology similar to ours with 18 hours contact with test specimens aged for 24 and 48 hours and 7 days[13]. In contrast, we found that Endomethasone C showed no inhibitory activity at any of the times and sub-times evaluated, possibly due to the formaldehyde content in Endomethasone. Considering the result of Gomes et al.[13] and the fact that in our study Endomethasone and Pulp Canal Sealer were equally effective, it may be inferred that the latter would be the sealer of choice, following the worldwide trend of replacing toxic substances for more biocompatible materials.

Candida albicans and *Staphylococcus aureus* were the most sensitive microorganisms to most of the sealers tested.

On analyzing the results obtained for the group of anaerobic microorganisms, we noticed, on the whole, that they were highly susceptible to the sealers tested, except for Endion against *Prevotella intermedia* and *Porphyromonas gingivalis*. Endion is a glass ionomer cement whose antimicrobial agent is the fluoride ion. It is a halogen compound that can inhibit the enzyme enolase in anaerobic glycolysis and block the reaction. *Prevotella intermedia* is a moderately saccharolytic bacteria[19], so Endion might have a certain bacteriostatic effect on it, at the expense of fluoride release.

On the other hand, environmental pH becomes acidified in presence of Endion as a result of the release of polyalkenoic acid during the setting reaction[20]. This pH condition is harmful to most microorganisms; nevertheless, previous studies[21,22] have shown that black pigmented bacteria such as *Prevotella intermedia* and *Prevotella nigrescens* can survive at low pH values. It may therefore be inferred that the development of *Prevotella intermedia* in the presence of Endion even 28 days after setting is also due to its resistance to acid environments.

Fusobacterium nucleatum showed no growth at any contact time during the periods evaluated. This behavior might be due to the fact that *Fusobacterium nucleatum* is more pathogenic in mixed culture than in pure culture[23, 24] due to its nutritional dependence[25,26]. These factors may cause its marked susceptibility to all the sealers tested and all the time periods set.

CONCLUSIONS

It was concluded that under the conditions of this study:

- The effectiveness of the sealers tested does not depend on the anaerobic microorganisms confronted ($p>0.05$), but on sealer formulation.
- The susceptibility of aerobic/facultative microorganisms to the sealers tested ($p\leq0.05$), might be

due to their structural characteristics, virulence factors or the ecological conditioning factors in the environment.

- Contact time between sealer and microorganisms is not determining regarding antimicrobial effectiveness.

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