

# In Vitro Antimicrobial Effects of 3 Root Canal Sealers on *Actinomyces Radicidentis*

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**Objective:** To determine the *in vitro* antimicrobial effects of 3 endodontic sealers—AH Plus®, Sealapex®, and Tubli-Seal®—on *Actinomyces radicidentis*, a bacterial species commonly found in root canals.

**Methods:** Prior to the experimental procedures, bacterial identification tests, such as Gram staining, catalase, and API 20A, were performed, and the bacteria were identified as *A. radicidentis*. The agar diffusion susceptibility test was performed to determine the areas of bacterial growth inhibition and, consequently, the microbial resistance of the 3 sealers against *A. radicidentis*. Chlorhexidine was used as a positive control, and saline solution was used as a negative control.

**Results:** Tubli-Seal cement had an average diameter of inhibition zones in the 3 panels of 22.73 mm, that of AH Plus was 17.13 mm, and that of Sealapex, 11.99 mm. A one-way ANOVA test showed that there were significant differences between the 3 cements ( $p < 0.05$ ).

**Conclusion:** Tubli-Seal® showed the highest levels of antimicrobial activity, which was followed by AH Plus® with the next highest levels and, finally, Sealapex® with the lowest levels of antimicrobial activity. [P R Health Sci J 2014;33:71-73]

*Key words:* Antimicrobial, Endodontic sealers, *Actinomyces radicidentis*

Since the discovery of microorganisms in root canals (1), several researchers have characterized these microorganisms and their role in the development of pulpal necrosis, periapical lesions, and chronic periapical abscess; therefore, the control and elimination of these microorganisms is vital for endodontic success (2).

The isolation and cultivation of several species of bacteria from infected root canals is now possible owing to technological advances in the transportation and collection of microorganisms (3). Microbiological studies have shown that a large number of Gram-negative bacteria play a role in the etiology of a majority of root canal infections. It has been found that root canal infections normally occur because of the presence of anaerobic bacteria (4), which play a vital role in the inflammation process by producing enzymes and endotoxins, chemotactically inhibiting neutrophils and phagocytosis, allowing the migration of lysosomal enzymes, participating in the immune response, interfering with the antibiotic sensibility, and thus, facilitating the maintenance of painful periapical lesions (5). Previous studies have shown the diverse range of microorganisms present in apical lesions and have linked these microorganisms to endodontic failure (6). *Actinomyces radicidentis* has also been identified in root canals as another bacterial species associated with apical lesions and endodontic failure (7). The presence of bacteria in root canals and in the periapical region is the main cause of endodontic failure, and therefore, eradication of such microorganisms from the root canal is an important goal of endodontic treatment (8). To avoid bacterial growth, endodontic filling materials should have an associated antibacterial effect.

The aim of this study is to determine the *in vitro* antimicrobial effect of 3 endodontic sealers on *A. radicidentis*, a bacterial species found in root canals.

## Material and Methods

*A. radicidentis* (Culture Collection; University of Goteborg, Sweden 36733 T) was grown and maintained in brain–heart infusion (BHI) broth (Scharlau Chemie, S.A., Barcelona, Spain) with 5% CO<sub>2</sub> (GENbag, bioMérieux, Marcy-l'Étoile, France) at 37°C for 48 h. Gram staining catalase, and API 20A (bioMérieux, Marcy-l'Étoile, France) tests were performed to confirm the identity of the bacterial species.

Three commonly used sealers were tested: AH Plus® (DENTSPLY DeTrey GmbH, Konstanz, Germany), an epoxy resin–based material; Tubli-Seal® (Kerr Italia S.r.l, Salerno,

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Italy), a zinc oxide–eugenol sealer; and Sealapex® (Kerr Italia S.r.l, Salerno, Italy), a calcium hydroxide–calcium silicate complex sealer. These 3 sealers were prepared according to the manufacturer instructions.

A diffusion susceptibility test was performed to evaluate the antibacterial activity (9) of the sealers in 1 Petri dish of 15 x 150mm containing brain–heart infusion (BHI Agar) (Scharlau Chemie, S.A., Barcelona, Spain). Three equidistant wells of 4mm depth and 4mm diameter were punched in the agar plate with the help of a Pasteur pipette (Normax, Marinha Grande, Portugal). A direct colony suspension of *A. radidentis* was prepared in sterile saline; the turbidity was adjusted to a 0.5 McFarland scale (Densimat, bioMérieux, Lyon, France), and the agar plate was plated with 150 µl of the bacterial suspension with the help of a sterile micro-pipette (Gilson, Inc., Middleton, WI, USA).

The sealers were mixed with a sterile spatula on a sterile glass slab according to the manufacturer instructions and, immediately after manipulation, each of the 3 wells was filled with a different sealer. This procedure was performed in triplicate. After the pre-diffusion of the test materials for 2 h at room temperature, all the plates were incubated at 37°C in an anaerobic cabinet (AnaeroJar [AG0025], Oxoid Ltd, Hampshire, England) and supplied with 5% CO<sub>2</sub> for 48 h. Positive and negative controls consisted of a plate with 2 equidistant paper disks saturated with 10 µl of test solution for 1 min, one with 2% chlorhexidine (CHX-Plus, Vista Dental Products, Racine, WI, USA) as a positive control and the other with a saline solution (Paracélsia, Industria Farmacéutica, S.A., Porto, Portugal) as a negative control; the 2 disks were incubated for the same period under identical conditions. This procedure was performed in triplicate, as well.

After this, pictures were taken of each of the Petri dishes, together with a millimeter ruler. The images were taken (perpendicular to each of the Petri dishes) with a digital Nikon camera (Nikon D90, Nikon Corp., Japan), using a focal length of 20 cm. The images were transferred to an image-processing software (Macnification, Orbicule, Inc., Leuven, Belgium), where, with the image of the millimeter ruler present in each, the photographs were calibrated. Next, the distance from the center of each inhibition zone to a sharp edge was measured. Three lines were measured for each inhibition zone; the average of these 3 values was multiplied by 2 to obtain the diameter of each inhibition zone.

Data are presented as the mean ± standard deviation. Differences among groups were analyzed using one-way analysis of variance (ANOVA) for multiple comparisons and a Bonferroni t-test using SPSS statistical software. A p-value of less than 0.05 was considered significant. All experiments were performed at least 3 times with at least 5 replications per experiment.

## Results

In the Petri dishes used for positive and negative controls, the disc impregnated with saline, used as negative control, showed

no inhibition zone and the disc impregnated with chlorhexidine, used as positive control, showed an inhibition zone.

The descriptive statistics for each cement are shown in Table 1.

All of the cements showed inhibition halos. In all three panels, the average diameter of the inhibition zone of Tubli-Seal was 22.73 mm, that of AH Plus (also in 3 panels) was 17.13 mm, and that of Sealaplex (same conditions) was 11.99 mm.

The one-way ANOVA showed significant differences between the 3 cements ( $p < 0.05$ ). The Bonferroni test (Table 2) verified that there were significant differences between AHPlus and Sealapex ( $p = 0.03$ ), AHPlus and Tubli-Seal ( $p = 0.02$ ), and Sealapex and Tubli-Seal ( $p < 0.01$ ).

The 95% confidence interval shows that Sealapex has the smallest inhibition zones diameter and Tubli-Seal had the largest inhibition zone diameter.

## Discussion

Clinical implications and microbiology of bacterial persistence after treatment procedures are an important issue in endodontics because bacteria have been shown to play a major role in the persistence or development of apical periodontitis lesions after root canal treatment (10).

Most of the studies that investigated the microbiota present in the filled root canals of teeth associated with post-treatment apical periodontitis have demonstrated the occurrence of *Actinomyces* species in 3 to 24% of the teeth (4,11).

The agar diffusion test has been widely used to evaluate the antibacterial activity of dental materials. This method permits a direct comparison between materials and also indicates which sealers are likely to have the highest antimicrobial activity within the root canal system. Because of the obvious limitations of *in vitro* studies, clinical inferences should be drawn with strict caution (12).

The measurement of the inhibition zones (done in some studies with agar diffusion test) (12) is carried out using a millimeter ruler directly on the top of a Petri dish—measuring the diameter of the inhibition zone of each material used—probably because the inhibition zone is uniformly circular. The zones of inhibition obtained in our study were irregular, so we decided to do the measurement as described in Materials and Methods to compensate for these irregularities (9).

The results of the agar diffusion test showed that all 3 sealers exhibited antibacterial activity against *A. radidentis*. The antibacterial properties of Tubli-Seal® can be explained by the presence of the compound eugenol (13). It has been shown that zinc oxide–eugenol-based sealers are very effective antimicrobial agents (14). The antimicrobial effect of epoxy resin–based sealers, such as AH Plus®, might be related to the release of formaldehyde during the polymerisation process (15). Kaplan et al. showed the antimicrobial activity of AH Plus® against *A. israelii* (14).

Sealapex®, a calcium hydroxide–based sealer, shows antimicrobial activity in the dentine and periapical zone owing

**Table 1.** Descriptive analysis of the value obtained in the inhibition zone of each cement.

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Limit	Upper Limit		
AH Plus	3	17.12	0.83	15.05	19.19	16.54	18.08
Sealapex	3	11.99	1.10	9.25	14.73	10.94	13.14
Tubli-Seal	3	22.72	1.25	19.61	25.83	21.36	23.82

**Table 2.** Bonferroni post-hoc test: Multiple comparisons

(I) Cement	(J) Cement	Mean Difference (I-J)	95% Confidence Interval	
			Lower Limit	Upper Limit
Tubli-Seal	AH Plus	5.60 (*)	2.70	8.49
Tubli-Seal	Sealapex	10.73 (*)	7.84	13.62
AH Plus	Sealapex	5.13 (*)	2.24	8.02

A mean difference of <0.05 is considered significant. Multiple comparison test showed significant mean differences in diameter of inhibition zones by cement

to calcium hydroxide the alkaline pH activity. The antimicrobial mechanism of calcium hydroxide is based on the liberation of hydroxyl ions and the inhibition of enzymes in the bacterial cytoplasmic membrane, consequently changing the chemical composition of the cellular components and modifying the nutritional support, thus producing a cytotoxic effect (16).

The results of the agar diffusion test do not depend only on the toxicity of the material for the particular microorganism, but are also highly influenced by the diffusibility of the material across the medium (17); this explains the difference in results obtained using different sealers, which vary according to their diffusibility across the medium.

In conclusion, our study showed that all 3 sealers exerted an *in vitro* antimicrobial effect against *A. radidentis*, and that, of the three, Tubli-Seal® showed the highest antimicrobial effect against these bacteria.

## Resumen

**Objetivo:** Determinación *in vitro* del efecto antimicrobiano de tres selladores de conductos—AH Plus®, Sealapex® y Tubli-Seal®—contra *Actinomyces radidentis*, una especie bacteriana regularmente encontrada en los conductos radiculares. **Métodos:** Antes de iniciar el proceso, se realizaron las pruebas de identificación bacteriana Gram coloración, catalasa y API 20A. La bacteria fue identificada como *A. radidentis*. Para determinar las áreas de crecimiento de inhibición bacteriana se realizó la prueba de susceptibilidad mediante difusión en agar, y, en consecuencia, la resistencia microbiana de los tres selladores frente a *A. radidentis*. Se utilizó clorhexidina en el control positivo así como solución salina en el control negativo. **Resultados:** El cemento Tubli-Seal® presentó una media en el diámetro de la zona de inhibición de las tres placas de 22.73 mm, AH Plus® de 17.13 mm y Sealapex® de 11.99 mm. El test de ANOVA indicó diferencias significativas entre los tres cementos

( $p < 0.05$ ). **Conclusión:** Tubli-Seal® mostró mayor actividad antimicrobiana seguida de AH Plus® y finalmente Sealapex® mostró menor actividad antimicrobiana.

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