

SUPPLEMENTARY INFORMATION

Use of a Cellulase from *Trichoderma reesei* as an Adjuvant for *Enterococcus faecalis* Biofilm Disruption in Combination with Antibiotics as an Alternative Treatment in Secondary Endodontic Infection

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Table S1. Antimicrobial susceptibility profile of *E. faecalis* strains by the Kirby-Bauer method (inhibition zone)^a

| Antibiotic | CLSI Reference (mm) | | | <i>E. faecalis</i> strains | | | | | | | | | | | | | | | | | |
|----------------------------------|---------------------|-------|-----|----------------------------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | R | I | S | E.F M1 | E.F M2 | E.F M3 | E.F M4 | E.F M5 | E.F M6 | E.F M7 | E.F M8 | E.F M9 | E.F M10 | E.F M11 | E.F M12 | E.F M13 | E.F M14 | E.F M15 | E.F M16 | E.F M17 | E.F M18 |
| Amoxicillin/Clavulanate 20/10 µg | ≤16 | | ≥17 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| Clindamycin 2 µg | ≤14 | 15-20 | ≥21 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| Cephalothin 30 µg | ≤14 | 15-17 | ≥18 | R | I | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| Vancomycin 30 µg | ≤14 | 15-16 | ≥17 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |

S= Sensitive, I= Intermedius, R= Resistant.

a=According to CLSI (2021)

Table S2. Extended antimicrobial susceptibility profile of selected *E. faecalis* strain (Dilution test, MIC)^a

| Antibiotic | CLSI Reference ($\mu\text{g/mL}$) | | | Susceptibility profile of M4 |
|------------------------------|-------------------------------------|-------------|------------|------------------------------|
| | Sensitive | Intermedium | Resistant | |
| Ampicillin | ≤ 8 | | ≥ 16 | S |
| High level Gentamicin | ≤ 16 | 15-16 | ≥ 8 | S |
| Ciprofloxacin | ≤ 1 | 2 | ≥ 4 | S |
| Levofloxacin | ≤ 2 | 4 | ≥ 8 | S |
| Erythromycin | ≤ 0.5 | 1-4 | ≥ 8 | R |
| Linezolid | ≤ 2 | 4 | ≥ 8 | S |
| Vancomycin | ≤ 4 | 8-16 | ≥ 32 | S |
| Doxycycline | ≤ 4 | 8 | ≥ 16 | R |
| Tetracycline | ≤ 4 | 8 | ≥ 16 | R |
| Nitrofurantoin | ≤ 32 | 64 | ≥ 128 | S |

S= Sensitive, I= Intermedius, R= Resistant.

^a=According to CLSI (2021)

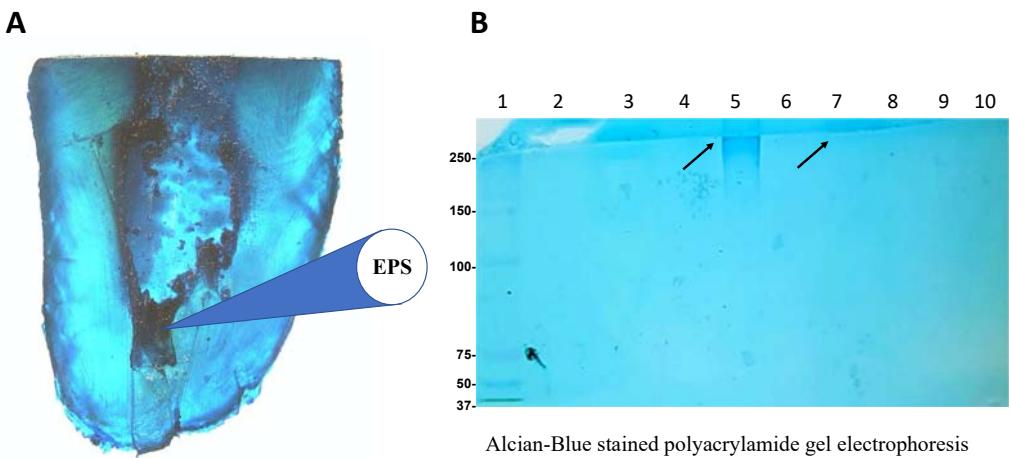


Figure S1. Biofilm staining using Alcian Blue. **(A)** Periapical stained biofilm of *E. faecalis* shown in an infected tooth. **(B)** Biofilm produced by the clinical isolate of *E. faecalis* and separated in SDS-PAGE (10%). Lanes: 1, Pre-stained protein marker; 3, Hydrolase alone; 5, Untreated biofilm; 7 Treated biofilm with the hydrolase. Equal amounts of the biofilm and hydrolase were loaded. Black arrows point to the band of the untreated (Lane 5) and treated biofilm (Lane 7).

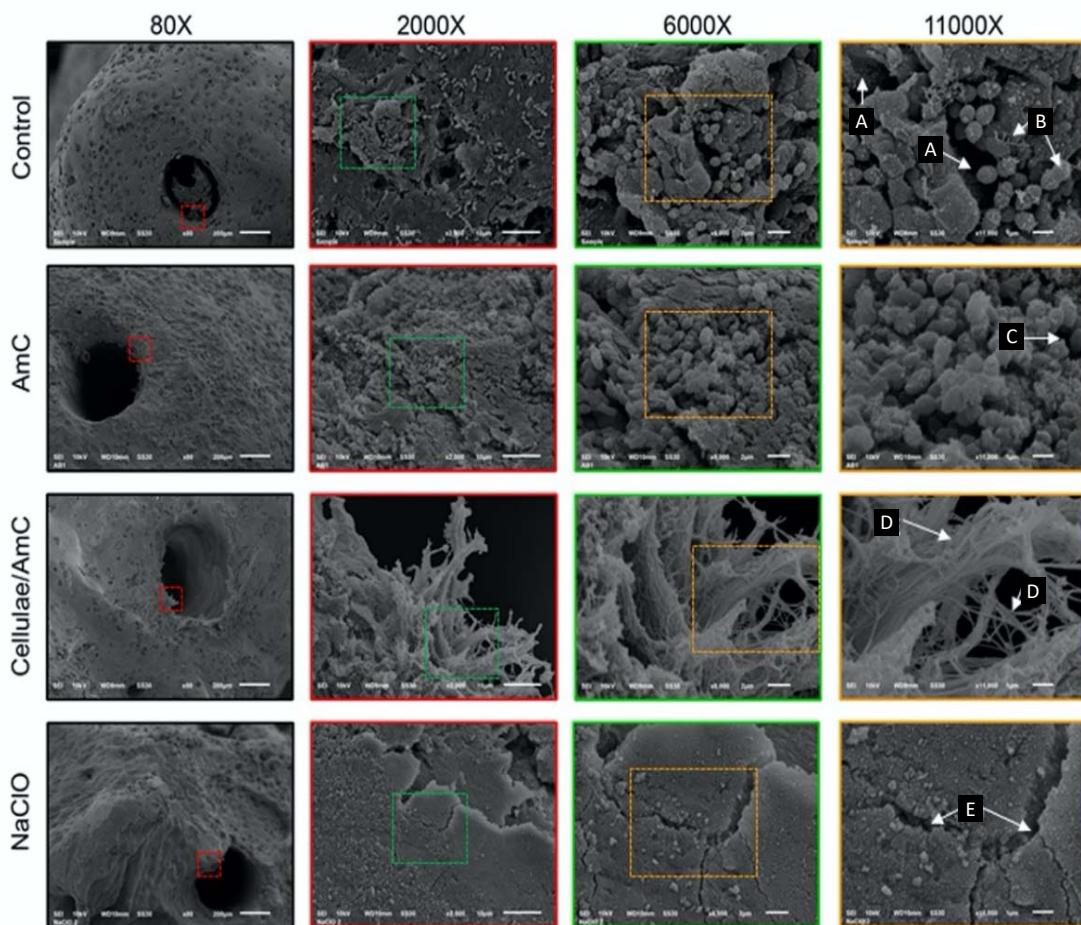


Figure S2. Periapical biofilms after irrigation. The arrangement of images shows the biofilms by treatment group in rows. The increasing order from left to right can be observed in the magnifications of each group. The color boxes show the amplification zones in the micrograph on the right. The last column shows the greatest magnification, with signs in the structure. AmC, Ampicillin and clavulanic acid. A) and C) Possible communication pores, B) EPS excreted by the bacteria, D) Possible fibers resulting from the hydrolysis of EPS polymers by CEL, E) Possible microfractures in dentin.