A MODEL OF STANDARTIZATION IN CONTAMINATION OF ROOT CANALS SYSTEM FOR LABORATORIAL STUDIES WITH LASER SCANNING CONFOCAL MICROSCOPY ANALYSIS: *EX VIVO* STUDY

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Enterococcus faecalis is the main bacteria responsible for failures in the endodontic treatment for the fact that they are able to invade the dentinal tubules. Given the due importance of the bacterial quantity as well as its level of penetration within the dentinal tubules for effectiveness analysis of antimicrobial substances in a tooth, this research aimed to determine the minimum incubation period for the bacterial growth and penetration within the dentinal tubules in order to develop a standardized model of contamination for laboratorial research, since there is no one available and scrutinized in the literature. Sixteen single-rooted bovine teeth had their crowns cut, their root canals instrumented, were then sealed, waterproofed and placed in cryogenic tubes for autoclaving. In order to verify the sterilization process, two randomly chosen teeth were sectioned longitudinally submitted to fixing with glutaraldehyde and gold metallisation and were observed in scanning electronic microscopy for analysis. From the fourteen remaining teeth, two were put in BHI culture for 24 hours with subsequent analysis of the turvity of the medium, totalizing twelve remaining teeth and 100µl of a suspension containing approximately 1.96 x 10^8UFC/ml of *E. faecalis* (138 UFPEDA - Federal University of Pernambuco, Department of Antibiotics) was inoculated into the root canal and each cryogenic tube was filled with BHI culture. According to the incubation time, the teeth were randomly divided into four groups (n = 3): G1 - 72 hours; G2 - 07 days; G3 - 14 days; G4 - 21 days. Every seven days the groups were subjected to catalase test, bile esculin, plating on blood agar and Gram staining to certify the exclusive presence of *Enterococcus faecalis*. After the incubation time for each group, the contaminated culture medium went through serial dilutions with subsequent solid plating for the counting of colony-forming units. The specimens were cut longitudinally, stained with LIVE / DEAD® BacLight™ Bacterial Viability Kit and were analyzed by Laser Scanning Confocal Microscope evaluating the bacterial viability and penetration. The results were analyzed using the Kruskal-Wallis test with a 5% level of significance. This work concluded that the bacterial growth and the penetration was present in the first 72 hours, dispensing longer observational periods for the accomplishment of scientific works in this area.

Keywords: Enterococcus faecalis, incubation, dentinal tubule, confocal

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