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Gene expression of adhesion factors and biofilm formation by Fusobacterium nucleatum strains isolated from root canals

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Aim To investigate several adhesion factors of *F. nucleatum*, one of the main species found in root canals, and correlate it with its structural behaviour in biofilms, by means of real time PCR and confocal microscope (CLSM) volumetry.

Methodology Four clinical isolates of *F. nucleatum* were collected from patients, isolated and cultivated in anaerobic chambers, identified and stored. ATCC strain and isolates were cultivated in planktonic and biofilm forms and their RNA purification and transcription to cDNA done. Real-time PCRs of genes FomA (adhesion to other Gram-negative species) and RadD (adhesion to Gram-positive) were performed. Seven-day biofilms produced from the strains were also evaluated by CLSM, comparing their total volume by the software BioimageL v2-1. The percentage of life and dead bacteria was also measured, by means of emitted fluorescence from the Life &Dead dye at the biofilms. Kruskal-Wallis test followed by Dunn were performed for statistical analysis.

Results The expressions of both adhesion genes were similar between each other. When in a planktonic form, the ATCC strain expressed these genes more than isolates. When in a biofilm form, the strain number 12 expressed the genes more than strains 13 and 20. The most voluminous biofilm was produced by the ATCC strain.

Conclusions The gene expressions varied when the strain was a clinical isolate, compared to the ATCC strain. When cultivated in a planktonic form, the ATCC produced more transcripts and when in a biofilm form, strain number 12 expressed more genes when compared to other strains. The ATCC strain produced the biggest one-species biofilm. The supposition is that *F. nucleatum* needs more interactions with other species *in vivo* and it is probably why the isolates required associations and produced smaller biofilms when in a pure culture.

Acknowledgements Funding source: FAPESP (São Paulo State Foundation) number 2010/20186-3.