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Bacteria of asymptomatic periapical endodontic lesions identified by anaerobic cultivation and genetic methods

Aim The purpose of the present study was to recover, identify and visualize bacteria in asymptomatic periapical endodontic lesions.

Methodology Microbiological samples were taken from mucosa, surgically exposed bone and periapical lesions in 30 patients. The samples were cultured anaerobically and biochemical/enzymatic profiling completed with the API bioMerieux system. In 34 patients the samples from the periapical lesions were examined using the checkerboard DNA–DNA hybridization technique with 40 whole genomic bacterial probes. Twenty periapical lesions were fixed and examined by means of the fluorescence *in situ* hybridization (FISH) technique using a probe specific for the domain *Bacteria* and species-specific probes in combination with epifluorescence and confocal laser scanning microscope.

Results Twenty-one of the 30 lesions were positive for bacterial growth, and between 1 and 10 species, mostly anaerobes, were recovered from the lesions. With the checkerboard method bacterial DNA was identified in all 34 lesions, and the number of species was higher than after culture (between 11 and 39) with anaerobes dominating. With the FISH technique, bacteria were observed in 50% of the lesions. A variety of different morphotypes, cocci, rods, spiral- and spindle-shaped bacteria, were seen to coaggregate forming micro-colonies, or the bacteria were spread out among cells and fibres in the tissue. Hybridization with probe for *Porphyromonas gingivalis* gave a positive signal in one lesion. The confocal laser scanning microscope allowed three-dimensional reconstruction, exact localization and observation of the spatial distribution of the microorganisms.

Conclusions Microbial invasion of periapical endodontic lesions may take place in asymptomatic teeth.