Vol. 104 No. 4 October 2007

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology

ENDODONTOLOGY

Editor: Larz S. W. Spångberg

Abusing technology? Culture-difficult microbes and microbial remnants

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The advent of molecular genetic methods has revolutionized life sciences as a whole. It has its share of impact on microbiology in general and on oral and endodontic microbiology in particular. The current knowledge in endodontic microbiology is based on the application of several methods, such as conventional histology, correlative light and electron microscopy, scanning confocal laser microscopy, microbial culturing, and biochemical and molecular techniques. All these methods have different degrees of limitations with respect to sensitivity, specificity, and etiologic relevance. Among the DNA-based methods, the polymerase chain reaction (PCR)[1] has enabled detection of microbes by amplification of their DNA, when PCR has been targeted at 16S rRNA gene sequences for taxonomic identification. These genetic methods have not only confirmed the microbial species that have been previously detected by culture methods[2,3] but also facilitated the identification of "as-yet-culture-difficult"[3] endodontic organisms. The sophisticated PCR technology accurately replicates and amplifies the DNA, but a valid scientific procedure remains essential,[4] without which the resulting data has only very limited etiologic relevance. Apart from the possible contamination of samples, the molecular technique does not differentiate between viable and nonviable organisms, but can pick up a minuscule amount of microbial DNA that is amplified during PCR,[1] resulting in an exponential accumulation of several million copies of the original DNA fragments.

It has been estimated that more than 500 different species of microorganisms live in a healthy human mouth. This estimate is expected to rise by another 200 species with judicious application of molecular methods. All of them have equal opportunity, at least theoretically, to invade and establish in the exposed pulp space. But, for each pulp-diseased tooth, only a very limited number of microbial species has been found to do so. Even with highly sensitive and specific molecular methods, the number of identified intracanal species remains low. This disparity between the potential and actual number of species in infected canals is due to the selective environment of the root canal. A large majority of the microbial species die off, possibly leaving certain cellular remnants, including the DNA. Vital pulp may contain host nucleases that can degrade free microbial DNA. But the necrotic root canal, with many pockets of dry areas, is a favorable place for persistence of bacterial remnants that include DNA. This is particularly so with root canal–treated and obturated teeth. There is no evidence yet that all free microbial DNA, particularly those located in the drier parts of necrotic root canals, are degraded by nucleases released from microbes living and/or dying in the root canal. How long DNA from dead microorganisms may persist in the root canal is unknown.[5,6] It may be pointed out that application of PCR methods enabled researchers to detect and amplify DNA fragments of *Mycobacterium tuberculosis* from hundreds-of-years-old human remains[7-9] and from that of a 17,000-year old extinct bison.[10] Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp helped to diagnose ancient septicemia and deaths in humans.[11] These reports on the

Received for publication May 31, 2007; accepted for publication Jun 1, 2007.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2007.06.003

detection of ancient DNA cannot be dismissed as instances of contamination. The data derived from the molecular technique, therefore, require very careful interpretation in the light of the technique's many advantages and numerous limitations. Although the DNA-based molecular method is superior for microbial sensitivity and specificity, it is prone to false positive results owing to detection of dead organisms and/or contaminants.

Refinements of the molecular methods, such as the application of real-time PCR, using larger primers, and targeting ribosomal RNA or messenger RNA may mitigate some of the "high sensitivity" and "nonviability" problems of molecular techniques. Likewise, improvements in culture techniques may help to grow organisms that are hitherto labeled as uncultivable. Let us not forget that many anaerobes and more fastidious spirochetes that are routinely grown today were once considered as uncultivable. What is uncultivable today may become cultivable tomorrow. Further, the term "uncultivable" does not mean that the organisms in question would not grow in a human body. If an organism cannot grow in or on a host, it is a very unlikely candidate to be the cause of a disease. Conversely speaking, an organism that grows in the relatively stable and reproducible environment of a mammalian body (in comparison to the extremes of open nature) should become amenable to the ingenuity of researchers to grow them in laboratory conditions.

It is about 5 years now since the 2 papers that changed the equation in endodontic microbiology were written.[2,3] The time has come to compare by independent researchers the taxonomic data obtained by application of refined molecular methods in conjunction with appropriate culture studies with those of early nonquantitative PCR-based assays, to arrive at the realistic data on root canal microflora. Understandably, it will take a certain time for such objective synthesis to happen in endodontic microbiology. Lately, it has become fashionable to negate great achievements of the past. Sound understandings on the etiology of microbial diseases are under threat of erosion or even reversal by statements such as: "In the event DNA from dead cells is detected, the results by no means lack significance with regard to participation in disease causation."[6] It must be stated with all emphasis that a mere observation of the presence of certain microbial remnants is not sufficient to implicate the organisms in question as etiologic agents of diseases. The current data on "uncultivable microbes" of necrotic root canals are at best only evidence that these organisms, by virtue of their own efforts and/or otherwise, "visited" pulp spaces of the teeth under investigation. Therefore, a great responsibility rests with contemporary researchers to provide convincing evidence that the "as-yet-culture-difficult" organisms reported using the molecular genetic methods are viable root canal microbes that cause pulpal and periapical disease.

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