Strength of evidence in current endodontic microbial research

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The comments of Dr. Jose Siqueira(1) to my letter to the editor(2) are very much welcome as they help to discuss certain issues in contemporary endodontic microbiology.

It is appreciated that there is a remarkable consensus of opinion on several points, namely: (1) PCR(3) is a very sensitive technology, (2) molecular genetic approaches are of great scientific value, (3) culture techniques remain important in microbiology, (4) PCR can be applied to identify as-yet-uncultivable species, (5) such species of microbes may in the future become cultivable, (6) molecular genetic methods detect dead microbes, (7) molecular techniques have been successfully applied to recover many-hundred-year-old DNA, (8) contamination of microbial samples is a very serious concern and (9) methods used in research should be appropriate and in steps with change.

However, there are important issues of contention too. Dr. Siqueira has dealt at length on the advantages of the molecular genetic methods - a further point on which there is full agreement. But understanding the importance and application of the technology is not sufficient on its own. Research that utilizes this technology, or any technology, must be performed with a methodological rigor and scientific standards that allows safe and justifiable conclusions.

Let me give an example. Controls for microbial contamination were shown to be essential for culture studies in order to get authentic results. The procedures for taking microbial samples from infected root canals have been well established by Möller. (4) It is essential that the tooth and surrounding area be thoroughly cleaned and disinfected before preparation of the access cavity. After gaining access and before reaching the pulp chamber, a second cleaning, disinfection and inactivation are essential. This must be followed by a control for contamination of samples to check that no contaminating species remain at the surface before entry to the pulp chamber. That a second decontamination step is essential was shown in a classic work more than 35 years ago in which bacteria were found to thrive under restorations. (5) These methods are well established for culture studies, yet most molecular studies contain none or inadequate molecular contamination procedures and controls.

To my knowledge, only one study has applied these steps: a first and second cleaning, disinfection and oligonucleotide decontamination followed by sampling for contamination control. (6) They found that use of pumice, hydrogen peroxide and iodine resulted in contaminating DNA recovered from 45% of investigated teeth. Furthermore, cleaning by pumice, hydrogen peroxide and NaOCl, yielded 13% of samples that were positive for contaminating DNA. Thus, the study (6) showed the importance of a second round of cleaning, greater effectiveness of NaOCl as against iodine and the challenge of achieving a contamination-free surface before entry to the pulp chamber. The point was confirmed again in a more recent study (7) in which the initial cleaning and decontamination procedures were done using pumice, hydrogen peroxide and NaOCl but was not followed by the second round of cleaning procedures. The result was that contamination controls showed (7) contaminating “… bacterial DNA in all … samples”.

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These findings illustrate an essential step for valid isolation of microbial species from the root canal, namely accurate recovery without contamination. More than 40 years ago, this lesson was learnt and applied for culture work\(^3\), yet studies using highly sensitive molecular methods appear to have ignored this valuable lesson of the past. In brief: while research should keep pace with change\(^8\), the lessons learnt previously and from other scientific approaches must not be discarded when it comes to applying molecular tools for the study of the endodontic microbial flora.

Dr. Siqueira commented\(^1\) on various aspects on the application of molecular genetic methods in endodontic microbiology. It is appreciated that he concurs with the serious problem of contamination and the inadequacy of certain terminology to convey the idea of “as-yet-culture-difficult”\(^13\) microbes. He also recognized that (1) the DNA based methods can identify dead microbes from the root canal, and (2) how-long DNA from dead bacteria can survive in the infected root canal is currently unknown. Nevertheless, in the latter context he believes that the detection of hundreds of year old ancient DNA\(^9\)-\(^12\) in animal and human remains by molecular genetic methods by independent researchers is not a matter of concern! In the absence of evidence it is difficult to agree with such opinions. The fact that independent researchers using molecular genetic methods have repeatedly detected ancient microbial DNA, particularly the 400-year-old *Yersinia pestis* DNA in human dental pulp,\(^10\) serves to illustrate that DNA from dead microbes can survive for hundreds of years in pulp space under certain circumstances. Those ‘circumstances’ are also currently unknown. It is, therefore, safe to say that there is currently an absence of convincing hard evidence to assume that all DNA from dead microbes would be quickly fragmented in necrotic root canals to a level below detection limits.

Another issue is the identification of microbial species in infected root canals.\(^13\) There is no doubt about the usefulness of molecular tools for the precise taxonomical identification of the microbes. But the issue is about the validity of the ‘newly identified’ organisms as the authentic residents of infected root canals to be considered as the etiological agents of pulpal and apical disease. Whilst it is true that some species that are culture difficult or not-yet-culturatable have been detected by molecular approaches, most of the ‘new’ species are merely re-classified or split off from other genera. It may be pointed out that in principle any metabolically active microorganism living in sufficient numbers in an infected root canal can participate in inflammation of the periradicular tissues. That means all viable microbes in root canal are ‘candidate pathogens’ for endodontic disease. Therefore, it is important to show that the microbial DNA detected by molecular methods really originates from viable root canal microbes.

My statement that “…it has become fashionable to negate great achievements of the past”\(^2\) was not intended to suggest that some say microbial culturing has become irrelevant or outdated. Rather, it is the attitude of some researchers to avoid meeting the basic tenets that are required to relate a pathogen to a disease and provide robust evidence for their conclusions. It is obvious that a mere detection of already known microbes or ‘discovering’ new microbes, cultured or “as-yet-culture-difficult”\(^14\) at disease sites using any technology is the most preliminary and relatively simple of the stringent requirements needed today to implicate the organisms in question to causation of diseases. That is the issue raised by me in that context and it remains unaddressed. Therefore, the onus is on
the researchers, particularly those using DNA-based methods in endodontic microbiology, to provide proof for causation of pulpal and periapical disease in the contemporary sense.

In summary, there are currently several issues that are debatable in endodontic microbiology. They are debatable because questions remain unanswered due to lack of sound scientific data. Therefore, the endodontic community is rightly concerned about the reliability, authenticity and validity of the information obtained from such studies.

References