Current Molecular Technologies for Assessing the Amount of Microbial Pathogens in Oral Plaque Biofilms

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ABSTRACT

The goal of identifying and quantifying defined oral microbial populations has gained increasing importance in clinical dentistry. Standard laboratory culture-based procedures–despite their importance for the generation of resistance profiles–are unfortunately inadequate to grow the majority of mainly anaerobic species that predominate at pathological sites, such as periodontal pockets or infected root canals. Conversely, the rapidly evolving field of nucleic-acid-based technologies is a promising approach to access the full breadth of the oral microflora. Critical to this development, however, is a proper understanding and application of the methodologies and knowledge of their limitations. In this chapter molecular tools based on real-time quantitative polymerase chain reaction (RTQ-PCR) will be described along with ways showing the computation and analysis of the datasets. RTQ-PCR allows the determination of the amount of almost any given bacterial species or the total bacterial load in oral clinical sample in a sensitive and highly reproducible way. The precise and time-efficient nature of this technique allows to run large numbers of samples and if several bacterial targets are studied in parallel to study the dynamics and potential interactions of microbial populations over space and time. This chapter will be complemented by discussing potential pitfalls that should be taken into consideration for producing proper results along with referring the reader to pertinent literature that will allow an individual deepening into the concept of molecular-based diagnosis in clinical dentistry.

INTRODUCTION

The human body (primarily the intestinal tract and the oral cavity) harbors approximately 10^{14} microbes which exceed the number of human cells by factor 10 (Bäckhed et al., 2005). In addition, the total gene pool of the human-associated microorganisms (i.e. the human microbiome of approximately 2000 different species) is at least 100 times larger than the human genome (Turnbaugh et al., 2007). Co-evolution of humans and microbes has led to mutual interdependencies with the human body benefiting from the unique metabolic capacities of the adapted microbes. Besides providing us with nutrition and vitamins and "shaping" our immune system our microflora protects us from invasion by pathogenic species through complex competitive interactions. Conversely,

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the microbes are provided with a rich buffet of glycans and a protected anoxic environment. The symbiotic relationship, however, depends on the stability of the protecting microflora. Shifts in the microbial community composition can occur quite frequently leading possibly to the development of disease or at least to the predisposition for a disease. Classical examples are oral diseases, such as periodontitis and caries but also extra-oral diseases such as inflammatory bowel disease (Crohn's disease). These chronical infectious diseases can be viewed as the result of an "ecological disaster" within the microbial community (Marsh, 2003). Knowledge about the amount and kind or microbial consortia responsible for the development of disease is becoming increasingly important with the recognition that most chronical infectious diseases are polymicrobial (Brogden et al., 2004).

In recent years, recovery of sequence-based signatures of life (using the 16S rRNA gene as target) direct from natural environments and the human body have revealed an extremely high diversity of microorganisms of which at least 90% are resistant to cultivation in the laboratory and thus with as-yet unknown functions (Amann et al., 1995). Conversely, clinical microbiology continues to rely heavily upon cultivation-based methods. The strong bias toward bacteria that are amenable to cultivation is illustrated by the fact that in a 6-year period 65% of all published microbiological research focused on just 8 bacterial genera (Hugenholtz, 2002). While there is certainly no dearth of known microbial pathogens it is even so plausible to assume that the existence of pathogens is not restricted to those being best adaptive to laboratory culture conditions. In this regard it should be noted that traditional diagnostic methods applied to syndromes of suspected infectious etiology, such as pneumonia, encephalitis, lymphocyte-predominant meningitis, pericarditis, acute diarrhea, and sepsis quite frequently fail to give a microbiological explanation. Furthermore the etiology of a large list of chronic inflammatory diseases with features of infection remains poorly understood.

Hence, nucleic acid based technologies may not only help improve daily routine diagnosis in microbiology but also to screen for novel, previously unrecognized microbial pathogens. In addition - combined with principles and concepts of microbial ecology – cultivation-independent approaches will enable to better understand the beneficial effects of the endogenous microflora as well as the consequences coming along with changes from the physiological to a pathological community. In this regard oral microbiology is a multidisciplinary research field which is equally relevant to general microbiologists as well as for dentistry. Since most oral diseases are associated with pathogenic microflora (e.g. caries, periodontitis and infected root canals with necrotic pulp tissue) knowledge of the amount and types of microorganisms involved is important for the dentist in order to choose the optimal treatment therapy. For the microbiologist the oral microflora is of interest because it can be considered as a model microbial system, with which fundamental ecological principles also valid for more complex microbial ecosystems in nature can be studied. In either case knowledge of population size and diversity of the microflora are important. In the next section the basic concept of quantifying oral microbes based on real-time quantitative PCR (RTQ-PCR) will be discussed. The following section will focus on general parameters that have to be considered when RTQ-PCR experiments are planned. The section thereafter is dedicated to a representative application along with the discussion of problems as well as possibilities to obtain reasonably interpretable results. References to pertinent literature which provides overviews of the major existing platforms, chemistries and software will be given in the text, where it is appropriate.

THE GENERAL CONCEPT OF RTQ-PCR

Background

Numerous nucleic-acid based approaches have been developed to characterize microbial

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