Review Article

Deciphering Endodontic Microbial Communities by Next-generation Sequencing

Jae M. Shin, DDS, PhD,[*†] *Ting Luo, MPH,*[†] *Kyu Han Lee, MPH,*[†] *Diogo Guerreiro, DDS, MS,*[*] *Tatiana M. Botero, DDS, MS,*[*] *Neville J. McDonald, BDS, MS,*[*] *and Alexander H. Rickard, PhD*[†]

Abstract

Introduction: Biofilms are present in more than 70% of endodontically diseased teeth. Through the advancements in the next-generation sequencing (NGS) technologies, microbiome research has granted a deeper analysis of the microbial communities living in human hosts. Here, we reviewed previous studies that used NGS to profile the microbial communities of root canals. **Methods:** A total of 12 peer-reviewed articles from PubMed were identified and critically reviewed. The study criteria were as follows: NGS platforms, sequenced bacterial hypervariable regions, teeth diagnosis with available patient information, sample characteristics, collection method, and microbial signatures. **Results:** The most common NGS platforms used were 454 pyrosequencing (Roche Diagnostic Corporation, Risch-Rotkreuz, Switzerland) and Illumina-based technology (Illumina Inc, San Diego, CA). The hypervariable regions sequenced were between the V1 and V6 regions. The patient and sample population ranged from ages 12–76 years and asymptomatic and symptomatic teeth diagnosed with pulp necrosis with or without apical periodontitis. Microbial sampling was conducted directly from the infected pulp or the extracted teeth. The most abundant phyla were Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, and Fusobacteria. The most frequently detected genera were *Prevotella*, *Fusobacterium*, *Porphyromonas*, *Parvimonas*, and *Streptococcus*. Other notable microbial signatures at different taxa levels were identified but were widely variable between studies. **Conclusions:** Technologies based on high-throughput 16S ribosomal RNA NGS can aid in deciphering the complex bacterial communities of root canal biofilms. Thus far, only a few studies have been published with relatively small sample sizes, variable sample collection protocols, and community analyses methods. Future larger clinical studies are essential with validated standardized protocols for improved understanding of the pathogenic nature of bacterial biofilm communities in root canals. *(J Endod 2018;■:1–8)*

Key Words

16S ribosomal RNA, biofilm, microbiome, next-generation sequencing, polymicrobial, root canal

Significance

NGS technology and bioinformatics tools allow open-ended community profiling of polymicrobial communities. Understanding the complex ecology of root canal microbial communities is valuable in advancing the field of endodontic microbiology and improving the clinical outcomes of endodontic treatments.

Endodontic infections involve polymicrobial communities that behave as a unit of pathogenicity (1). In the intracanal environment, microbial communities persist as surface-associated biofilms. Biofilms are defined as densely packed microbial species attached to a biotic or abiotic surface surrounded by the self-produced exopolymeric substances (2). Studies have suggested that up to 80% of microbial infections and more than 70% of endodontic infections are associated with biofilms and apical intracanal biofilms, respectively (3, 4). In endodontics, because of the recalcitrant nature of biofilms, root canal treatment is often required to treat and/or prevent apical periodontitis (5). The persistence of pathogenic biofilms in the root canal space and its surrounding environment is associated with inflammatory reactions, extreme pain, abscess formation, and cellulitis (6, 7).

The study of pathogens within intracanals has evolved from culture-based laboratory techniques to molecular strategies and now to more microbiome-based, next-generation sequencing (NGS) culture-independent strategies. Currently, large open databases are available for human microbiome samples, and studies have shown that depending on the body site, disease state, and the environmental conditions, a specific type of microbiota can be detected (8, 9). In contrast to the oral cavity, a root canal environment is unique in that it is a secluded space with highly diversified canal morphologies (10). With microbial infection (eg, dental caries), the pulp will undergo a dynamic process of experiencing pulpitis and then eventually pulpal necrosis (5). As the infection progresses, it is possible that the intracanal polymicrobial communities adapt to the changing environmental conditions by altering their microbiome structure (ie, community richness and evenness and diversity). For example, the relative abundance and prevalence of different taxa could shift, and certain populations can enter a dormant state (11). It is known in mammals and in nature that microbial communities can alter their community profiles to adapt to environmental changes and stresses for survival and maintenance (12, 13). Currently, the microbiome profiles associated with different endodontic conditions are not well characterized. Thus, to

From the *Department of Cariology, Restorative Sciences, and Endodontics, University of Michigan School of Dentistry, Ann Arbor, Michigan; and †Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan.

Address requests for reprints to Dr Jae M. Shin, Department of Cariology, Restorative Sciences, and Endodontics, University of Michigan School of Dentistry, 1011 N University, Ann Arbor, MI 48109. E-mail address: jaemshin@umich.edu

0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2018.04.003

better understand the ecologic aspects of different types of root canal polymicrobial communities, the deep-sequencing NGS approaches can be applied.

The aim of this article was to critically review the peer-reviewed articles that specifically used different NGS technologies to assess the intracanal polymicrobial communities. The reviewed parameters included

1. Sample characteristics (ie, sex, age, and anatomic and geographic location)
2. NGS platforms and the hypervariable region (HVR) sequenced
3. Sampling protocols
4. Microbial community profiles
5. A summary of the key findings

Additionally, the NGS platforms that are currently widely used and are available were further reviewed.

Methods

A meta-analysis review approach was avoided because the application of NGS for endodontic microbiome research is still nascent. Hence, we conducted an extensive literature search using PubMed to identify peer-reviewed original research articles that used NGS for profiling human root canal microbial communities. Studies were included if the articles used NGS methodologies for intracanal microbial samples. For studies that used both NGS and non-NGS microbial identification methods (ie, culture-based approaches, targeted and broad-based polymerase chain reaction, microarray technology, and so on), only the NGS findings were included for this review.

The PubMed key search terms included [endodontic infection], [root canal], [root canal microbial communities], [16S rRNA], [next-generation sequencing], [pyrosequencing], [biofilm], and [microbiome]. The search was conducted with individual key words and in combinations using 2 or more key words together in a single search. For article selection, there were no time line restrictions (all articles until August 31, 2017). To further increase the depth of our literature search, the reference lists of the selected articles were carefully screened to find relevant studies to our article. Based on our search strategies, a total of 12 articles were identified, selected, and reviewed after removing duplicate articles. The literature search was conducted independently by 2 investigators (J.M.S. and T.L.).

Results

Sample Characteristics

The sample characteristics are listed in Table 1. Six out of 12 studies did not assign a specific sex (ie, male or female) for their sample population. The age of the sample population ranged from 12–76 years old, which was obtained from 8 studies (Table 1). The geographic location of the studies included Brazil (4), the United States (2), the Netherlands (1), Sudan (1), South Korea (1), Estonia (1), Greece (1), and Turkey (1) (Table 1). The total sample size ranged from 7 to 48 samples, with a mean of 19 samples (Table 1). The teeth sampled included incisors, premolars, and molars (Table 1). For multirooted teeth, the number of canals and specific morphology information were not provided. The diagnosis of teeth included symptomatic and asymptomatic pulp necrosis with apical periodontitis (Table 1). Studies that included external canal microbial samples (eg, periapical soft tissue) and/or abscess samples were excluded for this study.

NGS Platforms and HVRs Sequenced for the Detection of Root Canal Microorganisms

The NGS platforms used in the reviewed studies included 454 GS-FLX (Roche Diagnostic Corporation) and Illumina MiSeq and HiSeq (Illumina, Inc, San Diego, CA) (Table 1). The Roche 454 GS-FLX can sequence read lengths of 700 to 800 bases and is capable of generating approximately 700,000 reads per run (26). The Illumina MiSeq and HiSeq reads per run are in the 20 to 30 millions and up to 3 billion, respectively (Table 2). However, compared with the 454 platform, the base pair read length is significantly shorter at 100 to 250 (Table 2).

The HVRs sequenced included V1 to V2, V1 to V3, V3 to V4, V4, and V5 to V6 (Table 1). Studies that used Roche 454 platforms sequenced HVRs V1 to V2 (14, 17, 19, 23), V1 to V3 (16, 20), V4 (15, 25), and V5 to V6 (18). Studies that used Illumina MiSeq sequenced V3 to V4 (22) and V4 (24), and studies that used HiSeq sequenced the V6 region (21).

Root Canal Sampling Protocols

To identify the microbial communities present in the infected root canal environment, investigators used either direct *in vivo* pulp sampling or extracted pulp sampling protocols (Table 1). For the *in vivo* sampling, the protocols began with rubber dam isolation followed by coronal access preparation with sterile dental burs, disinfection of the pulp chamber, inactivation of the disinfectant, hand filing to the apex or to the working length with sterile files, and sterile files and paper points for bacterial collection. The collected microbial samples were then transferred to a transport medium (ie, Tris-EDTA buffer) for DNA extraction.

The alternative approach for sampling was sampling from extracted teeth. After tooth extraction, the outer surface of the tooth was wiped off repeatedly with a disinfectant for exterior sterilization. The teeth were then frozen for cryogenic pulverization. Once the teeth were sectioned under liquid nitrogen and ground, the powdered samples were refrozen or processed immediately for DNA extraction.

For multicanaled teeth (ie, premolars and molars), the samples collected from separate canals were pooled into one and analyzed as a single sample. The most common types of disinfectants used during sample collection were hydrogen peroxide (3%–30%), sodium hypochlorite (0.5%–5.25%), and iodine. When sodium hypochlorite was used, sodium thiosulfate was used as the inactivating solution.

The Root Canal Microbial Community Profiles

For processing and analysis of 16S ribosomal RNA sequencing data, Quantitative Insights Into Microbial Ecology (QIIME) and mothur were the most commonly used bioinformatics pipelines; both produce reliable and comparable results (33, 34). The number of operational taxonomic units (OTUs) detected ranged from 179 to 916 (mean = 471 from 11/12 studies; Vengerfeldt et al's study (21) was excluded because the authors presented the detected OTUs as a range) (Table 3). The most widely accepted way for OTU clustering was based on a 97% 16S ribosomal RNA sequence similarity threshold. For bacterial taxa annotation, the reference databases used were RDP, SILVA, Greengenes, Human Oral Microbiome Database, and Core (35–39). The number of phyla and genera detected ranged from 5 to 24 (mean = 12) and 35 to 317 (mean 136), respectively (Table 3). The most common phyla detected based on abundance and prevalence in root canals included Bacteroidetes, Firmicutes, Actinobacteria, Fusobacteria, and Proteobacteria (Table 3 and Fig. 1). Phyla detected in low abundance (<5%) included Spirochaetes; Synergistetes; TM7; and some rare phyla such as Tenericutes, Deinococcus-Thermus, Chloroflexi, Cyanobacteria, OD1, SR1, and Acidobacteria. The most common genera detected were *Prevotella*, *Tannerella*, *Parvimonas*,