Glove Contamination during Endodontic Treatment Is One of the Sources of Nosocomial Endodontic *Propionibacterium acnes* Infections

Sadia Ambreen Niazi, BDS, MSc, PhD,[*†‡] *Louise Vincer, BChD,*[*] *and Francesco Mannocci, DDS, MD, PhD*[*‡]

Abstract

Introduction: The opportunistic *Propionibacterium acnes* recovered frequently from failed endodontic treatments might be the result of nosocomial endodontic infections. The study was aimed to determine if gloves worn by dentists could be one of the sources of these nosocomial infections and to investigate the *P. acnes* phylotypes involved. **Methods:** The cultivable microbiota of gloves ($n = 8$) at 4 time points (T1, immediately after wearing gloves; T2, after access cavity preparation; T3, after taking a working length/master cone radiograph; and T4, before sealing the cavity) were identified using 16S ribosomal RNA gene sequencing. *rec*A gene sequencing of *P. acnes* isolates was done. The phylogenetic relationship was determined using MEGA 6 (http://www.megasoftware.net/fixedbugs.html; Megasoftware, Tempe, AZ). Data distributions were compared using the Fisher exact test; means were compared using the Mann-Whitney *U* test in SPSSPC (version 21; IBM, Armonk, NY). **Results:** The quantitative viable counts at T4 (aerobically [2.93 ± 0.57], anaerobically [3.35 ± 0.43]) were greater ($P < .001$) than at T1 [(aerobically [0.48 ± 0.73], anaerobically [0.66 ± 0.86]) and T2 (aerobically [1.80 ± 0.54], anaerobically [2.41 ± 0.71]). Eighty cultivable bacterial taxa (5 phyla) were identified. The most prevalent ones were *P. acnes* and *Staphylococcus epidermidis* (100%). *rec*A gene sequencing ($n = 88$) revealed 2 phylogenetic lineages with type I split into type IA and type IB. Type II was prevalent on gloves. **Conclusions:** Contamination of the gloves was detected at the final stages of the treatment. *P. acnes* and *S. epidermidis* are the prevalent taxa on gloves and are opportunistic endodontic pathogens. Changing gloves frequently, after gaining access into the pulp space and also after taking the working length/master gutta-percha point radiographs, is likely to reduce the risk of root canal reinfection. *(J Endod 2016;42:1202–1211)*

Key Words

Gloves, nosocomial infection, *Propionibacterium acnes*

Significance

The microbial load of gloves increased from the beginning to the end of treatment sessions; gloves should be changed after gaining access to the pulp space and after taking an intraoperative periapical radiograph to prevent nosocomial infections of opportunistic bacteria like *Propionibacterium acnes*.

Propionibacterium acnes is an anaerobic, aerotolerant gram-positive bacillus that is a commensal bacterium on human skin (1), the oral cavity, the large intestine, the conjunctiva (2), and the external ear canal (1, 3). Numerous studies have reported *P. acnes* as an opportunistic pathogen associated with infections and inflammatory conditions (4–7). *P. acnes* infections are also linked with surgery including brain abscesses (8), osteomyelitis after lumbar puncture (9), spondylodiscitis after epidural catheterization (10), discitis after surgery (11), postoperative mediastinitis (12), endophthalmitis (13), and endocarditis (14). Recently, *P. acnes* is emerging as a well-recognized opportunistic pathogen causing various types of medical implant biofilm infection including intraocular implants (15), breast implants (16), neurosurgical shunts like ventroperitoneal and ventroatrial shunts (17), cardiovascular devices like prosthetic heart valves (18), internal fracture fixation devices, spinal hardware (19), and late prosthetic joint infections (20, 21).

Studies in dentistry have also identified *P. acnes* in parotid, periodontal, and dental infections (21, 22). *P. acnes* has been identified in studies on the endodontic microbiota (23, 24), but its importance as a pathogenic has largely been ignored because of its presence on the skin and the consequent likelihood of sample contamination. Recent studies showed that *P. acnes* recovered from primary and refractory endodontic infections is an opportunistic pathogen rather than a contaminant and might be the result of nosocomial infections occurring at the time of root canal treatment (25, 26).

P. acnes has been classified into 4 distinct evolutionary lineages by sequence analysis of a nonribosomal housekeeping gene (*recA*): type IA, IB, II, and III, which display differences in inflammatory properties, production of virulence determinants, and association with various conditions (21, 27, 28). Previous studies showed that types II and III were associated with infections of implanted prosthesis (27, 29) and are predominant phylotypes from refractory endodontic lesions (25), whereas type IA

From the Departments of *Restorative Dentistry and †Microbiology and ‡Biomaterials, Biomimetics and Biophotonics Group, King's College London Dental Institute at Guy's, King's and St Thomas' Hospital, Guy's Hospital, London, United Kingdom.

Address requests for reprints to Dr Sadia Ambreen Niazi, Department of Restorative Dentistry, King's College London Dental Institute, Floor 25, Tower Wing, Great Maze Pond, London, SE1 9RT, UK. E-mail address: sadia.niazi@kcl.ac.uk

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.05.016

and IB, which are usually recovered from skin (25, 30), were isolated from primary endodontic cases with "open" communication with the oral cavity (26).

During endodontic treatment, maintaining sterility as much as possible is crucial. Microorganisms can contaminate the root canal if there is insufficient cross infection control leading to root canal failure (25, 31–33). To reduce contamination from the patient to the practitioner and vice versa, universal precautions focused on barrier use have been developed (34). The Decontamination: Health Technical Memorandum 01-05: Decontamination in primary care dental practices 2013 version (https://www.gov.uk/government/publications/decontamination-in-primary-care-dental-practices) promotes the use of gloves, masks, protective eyewear, and gowns. The infection control regimens suggested by the European Society of Endodontology 2006 (35) and endodontic textbooks (36, 37) focus on hand washing techniques, wearing of personal protective equipment to be changed between patients, and the use of barrier techniques within the surgery. Clean nonsterile boxed gloves are one of the key factors involved in cross infection control during endodontic treatment. Studies have shown that gloves can get contaminated before (38, 39) and also after use in clinical dentistry, thus being a potential source for microbial contamination of the operative field (34, 40, 41). Contamination of gloves by the patient's saliva or skin or by bacteria present in the surgery can cause the inoculation of these bacteria into the root canal during treatment (41). So far, little information is available regarding microbial contamination of gloves during different stages of endodontic treatment and their role in causing nosocomial endodontic infections. The objective of this study was 2-fold: first to determine the potential source of nosocomial endodontic *P. acnes* infections by investigating the microbiota of the pair of gloves worn by the dentists at 4 different time points during endodontic treatment sessions and second to investigate the phylotypes of *P. acnes* if isolated among the microbiota present on the gloves.

Materials and Methods

Gloves Sampling

Glove samples were collected from 8 postgraduate endodontic students performing endodontic treatment on patients in the Endodontic Department of the Dental Institute at Guys' and St Thomas' Hospital, King's College, London, UK. The clinical operative surfaces were disinfected before treatment using Clinell Universal Wipes (GAMA Healthcare Ltd, London, UK). The surfaces were separated into clean and dirty zones and cling film barriers applied to the X-ray equipment including the collimator and exposure button, the operating microscope, the dental unit, and the dental chair. Barrier sleeves (Henry Schein, Melville, NY) were placed over the 3-in-1 syringe and handpieces. The gloves used in this study were commercially available Sempercare Nitrile Gloves (Sempermed, Semperit Industrial Products, Birmingham, UK) packaged in boxes of 100 units. The postgraduate dentists were instructed to wear a single pair of gloves for the entire treatment session of a single patient. The dentist used Cutan Hand Wash (Deb Group, Derbyshire, UK) following the 6-step hand washing technique recommended by Guy's and St Thomas' National Health Service foundation trust (http://www.guysandstthomas.nhs.uk/patients-and-visitors/infection/washing-your-hands.aspx#na). The soap was lathered and rubbed on the palms, backs of hands, between fingers, fingertips, thumbs, wrists, and nails for 15 seconds up to 1 minute. Hands were rinsed under clean running water, and taps were turned off without touching them directly. Hands were dried with clean disposable paper towels. The procedure was complied with the World Health Organization guidelines on hand hygiene in health care 2009 (http://apps.who.int/iris/bitstream/10665/44102/1/9789241597906_eng.pdf). Gloves were worn immediately after drying the hands.

The samples were collected using sterile cotton swabs rubbed all around gloves worn on both hands at 4 time points during the treatment session: T1, immediately after wearing the gloves at the beginning of the treatment before touching anything with the gloved hands; T2, after access cavity preparation; T3, after taking a working length/master cone radiograph; and T4, before sealing of the cavity before rubber dam removal. The swabs were suspended into 1 mL PRAS medium (Oxyrase, Mansfield, OH) and immediately transported on ice to the laboratory.

Sterile swabs were also collected as the negative control from the unhandled gloves taken immediately out from the glove boxes with the help of sterile tweezers. The swabs were plated onto fastidious anaerobe agar (FAA) supplemented with 5% (v/vP) horse blood (LabM, Lancashire, UK) and incubated anaerobically (in the MACS-MG-1000-anaerobic workstation; Don Whitley Scientific Ltd, West Yorkshire, UK) for 7 days and aerobically for 3 days at 37°C.

Microbial Analysis of Samples

Each sample (T1–T4) was dispersed by vortexing with glass beads, serially diluted in fastidious anaerobe broth (Lab M), and plated onto nonselective media; duplicate plates of FAA were supplemented with 5% (v/v) horse blood (Lab M). The FAA plates were incubated anaerobically for 7 days and aerobically for 3 days at 37°C. After incubation, colonies were counted, and a predetermined number of colonies (anaerobically: n = 20/time point, aerobically: n = 20/time point, maximum 160/glove) were randomly selected for Gram staining and molecular identification. The protocol used for selecting and picking the colonies was consistent with the one described in previous studies (25, 26).

Identification of Isolates

All randomly selected isolates were subcultured on FAA plates and grown for 24 hours. Bacterial genomic DNA was extracted by boiling

TABLE 1. Quantitative Viable Counts of the Gloves at 4 Time Points during Endodontic Treatment

Microbial counts as log10 per sample (mean ± SE)							
T1		T2		T3		T4	
Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
0.48 (0.73)	0.66 (0.86)	1.80 (0.54)*	2.41 (0.71)*	2.44 (0.41)†‡	2.86 (0.68)†	2.93 (0.57)§‖	3.35 (0.43)§

SE, standard error; T1, immediately after wearing the gloves at the beginning of the treatment; T2, after access cavity preparation; T3, after taking a working length/master cone radiograph; T4, before sealing of the cavity before rubber dam removal.

*Values at T2 significantly greater than values at T1 ($P < .001$).

†Values at T3 significantly greater than T1 ($P < .01$).

‡Values at T3 significantly greater than values at T2 ($P < .05$).

§Values at T4 significantly greater than values at T1 and T2 ($P < .001$).

‖Values at T4 significantly greater than values at T3 ($P < .05$).