Persistence of dead-cell bacterial DNA in ex vivo root canals and influence of nucleases on DNA decay in vitro

Malin Brundin, DDS,[a] David Figdor, MDSc, FRACDS, DipEndo, PhD, FASM,[b] Chrissie Roth,[a] John K. Davies, BSc, PhD,[b] Göran Sundqvist, DDS, PhD,[a] and Ulf Sjögren, DDS, PhD,[a]Umeå, Sweden; and Melbourne, Australia
UMEÅ UNIVERSITY AND MONASH UNIVERSITY

Objective. The fate of DNA from bacteria that do not survive in the root canal is uncertain, yet DNA longevity may confound recovery of authentic etiologic participants in the disease process. This study assessed the recovery of PCR-detectable DNA in ex vivo human root canals and some environmental factors on the decay of microbial DNA.
Study design. Heat-killed *Enterococcus faecalis* cells were inoculated into instrumented human root canals ex vivo, and samples were taken at intervals over 2 years and analyzed by polymerase chain reaction. In an in vitro assay, heat-killed *E. faecalis* cells and extracted *E. faecalis* DNA were inoculated into various media, DNase, and culture of a DNase-producing species, *Prevotella intermedia*. Recovery of DNA was assessed by gel electrophoresis.
Results. In ex vivo human teeth, amplifiable DNA was recovered after 1 and 2 years (in 14/15 and 21/25 teeth, respectively). In vitro experiments showed that extracted DNA incubated in different media (water, 10%-50% sera, and DNase) progressively decomposed to levels below the detection limit. In corresponding assays, cell-bound DNA was more resistant to decay.
Conclusion. Amplifiable DNA is preserved after cell death, but the critical determinant is the form of DNA. Free DNA undergoes spontaneous and enzymatic decomposition, whereas cell-bound *E. faecalis* DNA persists for long periods.
(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:789-794)

Over the past decade molecular methods, particularly the polymerase chain reaction (PCR) technique, have been increasingly used to analyze the bacterial flora in the infected root canal. Compared with traditional culturing methods, the molecular approach targets specific DNA sequences, which has led to reports of a more diverse root canal flora in teeth with apical rarefaction.[1,2] Sequence-specific DNA of difficult-to-culture species, such as *Treponema denticola* and *Tannerella forsythensis* have been more frequently reported,[3-5] and previously undetected species, such as *Firmicutes* spp., *Bacteroidetes* spp., and *Tannerella forsythensis*, have been described.[2,6-9] The specificity of molecular techniques allows species to be distinguished and characterized with greater resolution than by phenotypic and biochemical analysis used in classic methods.

Supported by the Swedish Dental Association; County of Västerbotten, Public Dental Health; Australian Society of Endodontology; Australian Dental Research Foundation; and Australian Society of Endodontology, Victorian Branch.
[a]Department of Odontology/Endodontics, Faculty of Medicine, Umeå University.
[b]Department of Microbiology, Monash University.
Received for publication May 2, 2010; returned for revision Jun. 30, 2010; accepted for publication Jul. 2, 2010.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2010.07.010

The accuracy of molecular methods depends on careful, contamination-free recovery of DNA from species genuinely involved in the root canal infection. Under favorable conditions, DNA can be preserved for long periods of time, as shown in reports of microbial DNA recovery of *Yersinia pestis* from 400-year-old pulp specimens[10] and *Mycobacterium tuberculosis* in a 1,000-year-old mummy.[11] In vitro experiments have shown that DNA from dead cells of *Enterococcus faecalis* can be detected by PCR 1 year after cell death.[12]

Although all oral bacteria have the potential to invade the pulp space, the specific ecology of the root canal environment permits only a limited number of species to survive and multiply. This is reflected by dynamic changes in the composition of the root canal infection and apparent loss of some species over time.[13] What happens to cell remnants and DNA of dead microorganisms has been the subject of some speculation.[14-16] The kinetics of DNA decomposition from dead microbial cells is an important but relatively unknown process, yet there are implications for studies of endodontic infection involving PCR amplification of DNA. Should DNA from dead microbes persist, it could occasionally mean that the recovered DNA reflects the history of the infection and that some species so identified could be of minor or no importance for the disease. While it has been shown that amplifiable DNA is recoverable from dead cell cultures after 1 year in

vitro,[12] it is as yet unknown if amplifiable DNA persists and is recoverable from root canals of human teeth. Another question of interest is the influence of DNases released by microorganisms in the neighboring milieu and whether this affects DNA decomposition.

The purpose of the present study was to assess the influence of different factors on the decay of post–cell death *E. faecalis* DNA. Specifically, the aims were to: 1) assess recovery over time of PCR-detectable DNA in root canals of ex vivo teeth that were instrumented and inoculated with dead *E. faecalis* cells; and 2) assess in vitro the influence of microbial nucleases on the fate of microbial DNA.

MATERIALS AND METHODS

Ex vivo experiments

Preparation of bacteria. Overnight cultures of *E. faecalis* (strain JH2-2) were harvested, washed, and resuspended in distilled filtered sterile water. The cells were heat killed by incubation at 60°C for 36-72 hours and resuspended in ultrapure water (Sigma, St. Louis, MO, USA). This method has been previously shown to kill bacteria, leaving cell walls intact.[17] Heat-killed cells were confirmed to be dead by plating aliquots from each batch. Aliquots of the suspension (0.1 mL) were stored at −20°C until use.

Selection of teeth. Forty-four permanent intact teeth with mature root apices and no cracks were selected from collections of anonymous extracted teeth stored in 50% glycerine/50% ethyl alcohol. All teeth were soaked in 2.5% NaOCl for 10 minutes and then rinsed in sterile saline solution for 15 minutes. After drying, the apices were covered with 2 layers of nail varnish. The teeth were embedded to the cementoenamel junction in plaster, radiographed, numbered, and stored at 25°C in 100% humidity.

For experiments, the teeth were randomly divided into 3 groups: 2 groups of 13 teeth each and 1 group of 18 teeth. Three teeth in each group were used for control samples.

Root canal instrumentation and inoculation. All procedures were performed with a strict aseptic technique to ensure accurate sampling from root canals. After cleaning the tooth surface with pumice, teeth were isolated with rubber dam, taking great care to have a tight seal between rubber dam and tooth. Teeth were carefully cleaned with H_2O_2 (30%, 1 minute) followed by NaOCl (2.5%, 2 minutes), and the operating field was further scrubbed with 5% iodine tincture. The cervical area was sealed with glue (Cervitec; Ivoclar Vivadent, Schaan, Liechtenstein) at the rubber dam–tooth interface. Disinfection was completed with a final iodine tincture wash. Before entering the pulp, a surface control sample was obtained by scrubbing the access surface with a foam pellet (Disposable Mini-Sponge Applicator; 3M Espe, St. Paul, MN, USA) soaked in 5% thiosulfate solution. The pellet was transfered to 5 mL Fluid Thioglucolate Medium (FTM)[18] and incubated at 37°C for 7 days. Using the same procedure, a second sample was taken with the pellet in 1 mL Tris-EDTA (TE; Sigma) and immediately frozen at −20°C.

A conventional access cavity was prepared, and root canals were instrumented up to a size #40 Hedström file to just short of the apical foramen. Between instruments, canals were irrigated with copious sterile saline solution. After cleaning and shaping, samples were obtained by filling the canals with sterile saline solution, creating dentin shavings by filing the walls, and absorbing the canal contents onto paper points. Alternate points were transfered to 5 mL FTM or 1 mL TE buffer and incubated or stored as described above. Root canals were then inoculated with 10 μL thawed heat-killed *E. faecalis* cells (10^{10} cfu/mL). Control teeth were inoculated with 10 μL distilled filtered sterile water. One or two disposable minisponge applicators were inserted into the coronal root canal, and access cavities were sealed with ≥4-mm-deep zinc oxide–eugenol cement.

Postinoculation sampling. Inoculated and control teeth were incubated (25°C; 100% humidity) and subsequently sampled at 3, 6, 12, and 24 months. At the respective interval, teeth were polished with pumice and 2 mm of the temporary filling removed. Rubber dam, disinfection, and surface control samples were performed as above. After removal of the remaining temporary filling, the canals were filled with sterile saline solution, dentin shavings created by filing the walls, and the canal contents absorbed onto paper points and transfered to FTM and TE, respectively, as described above. Samples for PCR were frozen (−20°C) until processing. Teeth that had been sampled at 6 and 12 months were resealed with zinc oxide–eugenol, the canals were left empty, and the teeth were resampled at 24 months as above.

Detection of E. faecalis DNA by 16S rRNA gene PCR. Tubes were thawed (37°C; 10 minutes) in heat blocks, vortexed for 1 minute, and the contents transfered to Eppendorf tubes and centrifuged (16,100*g*, 2 minutes). DNA from *E. faecalis* was extracted from cell pellets according to the manufacturer's protocol (Bacterial Genomic DNA kit; Gene Elute, Sigma). Briefly, pellets were resuspended thoroughly in 200 μL lysosyme solution (45 mg/mL) with added mutanolysin (250 U/mL) and incubated at 37°C for 30 minutes. RNase A (20 μL) was added at room temperature (2 minutes), then proteinase K (20 μL) and Lysis Solution C (200 μL) incubated at 55°C (10 minutes). Ethanol

(200 μL, 99.5%) was added to the lysate and mixed thoroughly. The tube contents were transfered into binding columns, centrifuged (9000*g*, 1 minute), and washed with the manufacturer's recommended solutions. After addition of elution solution, columns were incubated at room temperature for 5 minutes. Eluates (200 μL) were transfered to sterile DNA- and RNA-free tubes (Sarstedt, Numbrecht, Germany) and frozen (−20°C) until use.

Enterococcus faecalis–specific oligonucleotide primers[19] were used that produce a 138-bp amplicon. PCR amplifications were prepared in a 25 μL final reaction volume: 10 μL total DNA template, 6 pmol each primer, 100 μmol/L dNTPs (Qiagen, Hilden, Germany), 2.5 μL 10× PCR buffer (Qiagen; $MgCl_2$ was added to a final concentration of 2.5 mmol/L Mg^{2+}), 1.7 U HotStarTaq DNA Polymerase (Qiagen), and Ultrapure water (Sigma) to make up the final reaction volume.

PCR amplification was performed in a DNA thermocycler (Mastercycler gradient; Eppendorf, Hamburg, Germany). Initial denaturation was uniform at 95°C (15 minutes), followed by 35 cycles of 94°C (30 seconds), 68°C (35 seconds), and 72°C (35 seconds), and a final step of 72° C for 5 minutes. Ultrapure water (Sigma) replaced the bacterial DNA template in the negative control. *E. faecalis* (strain JH2-2) was used as positive control.

Amplification products were analyzed by gel electrophoresis (1.5% agarose in 0.5% TBE buffer; Gibco, Invitrogen, Grand Island, NY, USA), stained with 1 μg/mL ethidium bromide (Sigma), and photographed in ultraviolet light. A 100 bp DNA ladder (Invitrogen, Fredrick, MD, USA) was used as a molecular weight marker.

In vitro experiments

Spontaneous decay of DNA. DNA from *E. faecalis* was extracted as described above. Sera from 4 healthy human adults were pooled (PHS) and inactivated at 56°C for 30 minutes. Decay of both extracted and cell-bound DNA was assessed in various media.

Extracted DNA was inoculated into Ultrapure water (Sigma), PHS (10% and 50% diluted in Ultrapure water) and Elution Solution (Sigma) for up to 21 days (1,000 ng DNA, 100 μL). At test intervals, 20 μL was withdrawn from each tube and stored frozen (−20°C) for later analysis by gel electrophoresis.

Heat-killed cell-bound DNA was inoculated into the same media as above (3,200 ng DNA, 160 μL). Samples (10 μL) with cell-bound DNA were isolated and precipitated with ethanol before analysis. The DNA pellet was washed with 70% ethanol and resuspended in 10 μL Ultrapure water (Sigma). After precipitation, DNA products were analyzed by gel electrophoresis.

Decay of DNA by DNase I. DNase I (Invitrogen, Carlsbad, CA, USA) was diluted to 8 concentrations (0.5, 0.3, 0.13, 0.06, 0.03, 0.02, 0.01, and 0.004 U). Extracted DNA (4 μL, 78 ng) and cell-bound DNA, respectively, were incubated in different concentrations of DNase I (4 μL) and 1 μL DNase buffer. Samples were incubated at 37°C for 30 minutes. The reaction was stopped by adding EDTA (1 μL, 25 mmol/L) and incubation at 65°C for 10 minutes. For control samples, 78 ng DNA was incubated with 1 μL DNase buffer and Ultrapure water. Samples containing cell-bound DNA were isolated and precipitated as above. DNA products were analyzed by gel electrophoresis.

Coincubation experiments. A DNase-producing strain of *Prevotella intermedia* (ATCC 25611) was grown on blood agar or Fastidious Anaerobe Agar (FAA; Laboratory M, Bury, U.K.) with 10 mg/L vitamin K (37°C, 4 days) in an anaerobic box (10% H_2 and 5% CO_2 in N_2). Cells were harvested from plates and resuspended in phosphate-buffered saline solution.

Aliquots (6 μL) of a *P. intermedia* suspension (OD 2.3) were placed in Eppendorf tubes, to which either extracted DNA (3 μL, total 21 ng DNA) or heat killed *E. faecalis* cells were added and the tubes incubated at 37°C. At intervals, the tubes containing extracted DNA were centrifuged (13,000*g*, 1 minute) and the supernatant removed and stored frozen (−20°C) until further analysis by gel electrophoresis.

In parallel, experiments with cell-bound DNA were performed. The products were kept frozen for later analysis. Tubes were thawed (37°C, 10 minutes) in heat blocks, DNA was extracted as described previously, PCR amplification with *E. faecalis*–specific oligonucleotide primers was performed (35 cycles), and amplification products were analyzed by gel electrophoresis. As a control, cell-bound and extracted DNA was added to a non–DNase-producing strain of *Streptococcus mutans* (NCTC 10449). The samples were analyzed as above.

All in vitro experiments were repeated 2-4 times.

RESULTS

Ex vivo

The results of the ex vivo study are presented in Table I. One year after inoculation, the DNA was recoverable in 14 of 15 teeth. After 2 years, amplifiable *E. faecalis* DNA was detected in 21 of 25 teeth. Taken together, the results demonstrate that amplifiable DNA is recoverable from root canals 2 years after cell death.

Extensive controls were undertaken throughout the study to check for contamination. Samples for PCR and culture were taken from the surface of all teeth before

Table I. Recovery of amplifiable *Enterococcus faecalis* DNA from ex vivo human teeth

Postinoculation time (mo)	*Experimental teeth**	*Control teeth**
3	9/9	0/3
6	10/10	0/3
12	14/15	0/3
24†	21/25	0/5‡

*Number of teeth with positive polymerase chain reaction/total number of teeth.
†Teeth sampled at 6 and 12 months were resampled at 24 months.
‡One control not done for technical reasons.

entering the pulp space, and no bacterial growth or PCR-detectable *E. faecalis* DNA was recovered in any case. Of samples taken after canal instrumentation, there were no recoverable viable bacteria and no amplifiable *E. faecalis* DNA.

After 3-12 months' incubation, contamination control samples from all tooth surfaces before reentry were negative for bacterial growth by cultivation, but there was PCR-detectable *E. faecalis* DNA at the surface of 1 of 44 teeth. This tooth belonged to the 3-month group and was excluded from these results. Surface control samples taken at 24 months were negative for both culturing and PCR.

In vitro

Extracted *E. faecalis* DNA incubated with sera (10% and 50%) decomposed within 24 hours. Extracted DNA decomposed to below the detection limit in 7-13 days in water, but was recoverable for >21 days in elution solution (Table II).

DNase I enzymatically degrades DNA, as shown by complete decomposition of 78 ng DNA by DNase I in <30 minutes. The DNase activity in *P. intermedia* culture resulted in degradation of extracted DNA to a nondetectable level by 3 hours. In comparison, there was no degradation over 3 hours when extracted DNA was incubated in an *S. mutans* culture.

Cell-bound DNA was more resistant to decay in all media over the test period (Table II). In summary, extracted *E. faecalis* DNA was more susceptible to decay than cell-bound DNA.

DISCUSSION

Longevity and durability of microbial DNA is of great relevance to diverse disciplines from paleomicrobiology to endodontic research. In the former, DNA longevity has been essential for addressing questions about disease history in century-old specimens,[10,20,21] although in studies of the root canal microbiota DNA longevity may confound recovery of authentic etiologic participants in the disease process. Using a PCR assay, the present study sought to clarify some factors affecting DNA longevity and recoverability. After inoculation of heat-killed *E. faecalis* cells in human teeth ex vivo, amplifiable DNA was recovered after 1 and 2 years (in 14/15 and 21/25 teeth, respectively). In experiments with nuclease-producing bacteria, coincubation of cell-bound and free DNA showed that longevity depended on the relationship of DNA to heat-killed cells, i.e., free DNA decayed rapidly whereas cell-bound DNA was more resistant to decay regardless of the medium and presence of nucleases.

Breakdown of DNA occurs due to spontaneous decomposition or enzymatic fragmentation of the DNA strand. Spontaneous decomposition occurs by hydrolysis, oxidation, and methylation, and the rate of the decay is dependent on temperature, humidity, pH, and oxygen tension.[22,23] It has been previously shown in an in vitro study that DNA from dead *E. faecalis* cells is PCR detectable 1 year after cell death.[12] By inoculating heat-killed *E. faecalis* cells into human teeth ex vivo, the present study went a step closer to real-world conditions in the human root canal, revealing that amplifiable DNA is recoverable 1 and 2 years after cell death. In vitro, there is an ~1,000-fold decay over 1 year,[12] and it is reasonable to think that a similar reduction in quantifiable DNA occurred in human teeth ex vivo. Yet after 2 years, the amount of DNA was above the amplifiable detection limit, possibly owing to stabilization of the DNA molecule which is known to bind to minerals[24] and adsorb to hydroxyapatite in dentin and bone.[22]

The infected root canal is a dynamic environment in which some species fade and others flourish depending on environmental and nutritional factors over time.[13,25] As the available nutrition changes from glycoproteins to peptides and amino acids,[26,27] the microbial mix matures and thriving proteolytic bacteria dominate the infection. Many of these species produce DNases that conceivably influence DNA decomposition of the declining saccharolytic species. Microbial nucleases are known to contribute to DNA degradation in nonsterile environments.[28] Therefore, we investigated the effect of nuclease-producing species on DNA decay. A species common to root canal infections, *P. intermedia*,[29-31] was selected for its potent nuclease capability.[32] When extracted DNA from *E. faecalis* was incubated in a *P. intermedia* culture, there was rapid DNA decomposition to below the detection limit (Table II), which is consistent with a study showing DNA decomposition within 6 hours in a growing culture of DNase-producing bacteria.[33] The accelerated rate of DNA decay is likely due to the presence of microbial nucleases. This was explored further by incubation of extracted DNA with DNase I, which is known to have

Table II. *Enterococcus faecalis* DNA decay in various media assessed by gel electrophoresis of isolated DNA product

	DNase I	*Sera (10%)*	*Sera (50%)*	*Water*	*Elution solution*	*Prevotella intermedia*
Extracted DNA	<30 min	<24 h	<24 h	7-13 d	>21 d	<3 h
Cell-bound DNA	No decay in 30 min	>21 d	>21 d	>21 d	>21 d	>7 d*

*Assessed by polymerase chain reaction (PCR).

the capability to split the DNA molecule.[34] Under these conditions, DNA was destroyed within minutes when exposed to DNase I (Table II).

In contrast, when heat-killed *E. faecalis* cells were incubated in a *P. intermedia* culture or with DNase I, amplifiable DNA remained present throughout the observation period. These results demonstrate the importance of DNA form to its longevity in the environment. DNA in heat-killed *E. faecalis* cells possesses a much greater ability to resist decomposition than free DNA. It is possible that the results with *E. faecalis*, which has a tough cell wall, may not be transferable to other species, yet under favorable conditions longevity of microbial DNA has been described for various species.[10,11]

In vivo, the necrotic root canal likely contains small amounts of serum that perfuses from the inflamed periapical tissue. While it sustains the microbial flora, it may also influence DNA decomposition. Human serum contains a DNase concentration of 3-23.9 ng/mL.[35,36] Therefore, we assessed recovery of amplifiable DNA in various media and found that incubation of extracted DNA in serum resulted in rapid decomposition compared with incubation in water (no detectable DNA by gel electrophoresis at 24 hours and 7-13 days, respectively). It should be noted that the serum in these experiments had been heat treated, which might inactivate the DNase but could also affect DNase inhibitors in sera which are heat labile.[37] Although DNA decomposition occurred with heat-treated serum, it is reasonable to assume that untreated serum would have the same effect, but confirmation and a mechanism awaits clarification by further experiments.

The present study shows that in vitro there is the potential for preservation of amplifiable DNA after cell death and that a critical determinant for DNA longevity is the form of DNA, i.e., whether the cells die with intact cell walls or lyse and release their DNA into the local milieu. If the DNA is free in the environment, it is likely to undergo spontaneous and enzymatic decomposition, however cell-bound DNA may persist for long periods. Exactly how bacteria die in the root canal and what is the fate of cell constituents remain unanswered, yet it is likely that both forms occur in infections in vivo.

REFERENCES

1. Siqueira JF, Jr., Rôças IN, Souto R, de Uzeda M, Colombo AP. Checkerboard DNA-DNA hybridization analysis of endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:744-8.
2. Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG. Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res 2002;81:761-6.
3. Gonçalves RB, Mouton C. Molecular detection of *Bacteroides forsythus* in infected root canals. J Endod 1999;25:336-40.
4. Siqueira JF, Jr., Rôças IN, Favieri A, Santos KR. Detection of *Treponema denticola* in endodontic infections by 16S rRNA gene-directed polymerase chain reaction. Oral Microbiol Immunol 2000;15:335-7.
5. Baumgartner JC, Khemaleelakul SU, Xia T. Identification of spirochetes (treponemes) in endodontic infections. J Endod 2003;29:794-7.
6. Conrads G, Gharbia SE, Gulabivala K, Lampert F, Shah HN. The use of a 16S rDNA directed PCR for the detection of endodontopathogenic bacteria. J Endod 1997;23:433-8.
7. Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie D, Coldero L, et al. Molecular identification of microorganisms from endodontic infections. J Clin Microbiol 2001;39:3282-9.
8. Sakamoto M, Rôças IN, Siqueira JF, Jr., Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. Oral Microbiol Immunol 2006;21:112-22.
9. Siqueira JF, Jr., Rôças IN, Paiva SS, Magalhães KM, Guimarães-Pinto T. Cultivable bacteria in infected root canals as identified by 16S rRNA gene sequencing. Oral Microbiol Immunol 2007;22:266-71.
10. Drancourt M, Aboudharam G, Signoli M, Dutour O, Raoult D. Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. Proc Natl Acad Sci U S A 1998;95:12637-40.
11. Salo WL, Aufderheide AC, Buikstra J, Holcomb TA. Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. Proc Natl Acad Sci U S A 1994;91:2091-4.
12. Young G, Turner S, Davies JK, Sundqvist G, Figdor D. Bacterial DNA persists for extended periods after cell death. J Endod 2007;33:1417-20.
13. Fabricius L, Dahlén G, Öhman AE, Möller ÅJR. Predominant indigenous oral bacteria isolated from infected root canals after varied times of closure. Scand J Dent Res 1982;90:134-44.
14. Nair PNR. Abusing technology? Culture-difficult microbes and microbial remnants. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:569-70.
15. Siqueira JF, Jr.On the issue of uncultivated bacteria and dead cell detection by molecular methods: reply to Dr. Nair's commentary. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:5-8.
16. Nair PNR. Strength of evidence in current endodontic microbial research. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:8-10.

17. Kort R, Keijser BJ, Caspers MPM, Schuren FH, Montijn R. Transcriptional activity around bacterial cell death reveals molecular biomarkers for cell viability. BMC Genomics 2008;9:590.
18. Carlsson J, Sundqvist G. Evaluation of methods of transport and cultivation of bacterial specimens from infected dental root canals. Oral Surg Oral Med Oral Pathol 1980;49:451-4.
19. Sedgley CM, Nagel AC, Shelburne CE, Clewell DB, Appelbe O, Molander A. Quantitative real-time PCR detection of oral *Enterococcus faecalis* in humans. Arch Oral Biol 2005;50:575-83.
20. Pötsch L, Meyer U, Rothschild S, Schneider PM, Rittner C. Application of DNA techniques for identification using human dental pulp as a source of DNA. Int J Legal Med 1992;105: 139-43.
21. Drancourt M, Raoult D. Palaeomicrobiology: current issues and perspectives. Nat Rev Microbiol 2005;3:23-35.
22. Lindahl T. Instability and decay of the primary structure of DNA. Nature 1993;362:709-15.
23. Höss M, Jaruga P, Zastawny TH, Dizdaroglu M, Pääbo S. DNA damage and DNA sequence retrieval from ancient tissues. Nucleic Acids Res 1996;24:1304-7.
24. Aardema BW, Lorenz MG, Krumbein WE. Protection of sediment-adsorbed transforming DNA against enzymatic inactivation. Appl Environ Microbiol 1983;46:417-20.
25. ter Steeg PF, van der Hoeven JS. Development of periodontal microflora on human serum. Microb Ecol Health Dis 1989;2:1-10.
26. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol 1994;78:522-30.
27. ter Steeg PF, van der Hoeven JS, de Jong MH, van Munster PJ, Jansen MJ. Enrichment of subgingival microflora on human serum leading to accumulation of *Bacteroides* species, *Peptostreptococci* and *Fusobacteria*. Antonie Van Leeuwenhoek 1987;53:261-71.
28. Josephson KL, Gerba CP, Pepper IL. Polymerase chain reaction detection of nonviable bacterial pathogens. Appl Environ Microbiol 1993;59:3513-5.
29. Sundqvist G, Johansson E, Sjögren U. Prevalence of black-pigmented *Bacteroides* species in root canal infections. J Endod 1989;15:13-9.
30. Baumgartner JC, Watkins BJ, Bae KS, Xia T. Association of black-pigmented bacteria with endodontic infections. J Endod 1999;25:413-5.
31. Tomazinho LF, Avila-Campos MJ. Detection of *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia*, and *Prevotella nigrescens* in chronic endodontic infection. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:285-8.
32. Leduc A, Grenier D, Mayrand D. Outer membrane–associated deoxyribonuclease activity of *Porphyromonas gingivalis*. Anaerobe 1995;1:129-34.
33. Gerceker D, Karasartova D, Elyürek E, Barkar S, Kiyan M, Özsan TM, et al. A new, simple, rapid test for detection of DNase activity of microorganisms: DNase Tube test. J Gen Appl Microbiol 2009;55:291-4.
34. Nagata S. DNA degradation in development and programmed cell death. Annu Rev Immunol 2005;23:853-75.
35. Aitken ML, Burke W, McDonald G, Shak S, Montgomery AB, Smith A. Recombinant human DNase inhalation in normal subjects and patients with cystic fibrosis. A phase 1 study. JAMA 1992;267:1947-51.
36. Miyauchi K, Ogawa M, Murata A, Nakano I, Funakoshi A, Mori T. Serum deoxyribonuclease I determined by a radioimmunoassay and an enzymatic assay in malignant diseases. Clin Chim Acta 1989;184:115-9.
37. Macanovic M, Lachmann PJ. Measurement of deoxyribonuclease I (DNase) in the serum and urine of systemic lupus erythematosus (SLE)–prone NZB/NZW mice by a new radial enzyme diffusion assay. Clin Exp Immunol 1997;108:220-6.

Reprint requests:

Dr. Malin Brundin
Department of Odontology/Endodontics
Faculty of Medicine
Umeå University
Umeå, 90187
Sweden
malin.brundin@odont.umu.se