Effect of Prion Decontamination Protocols on Nickel-Titanium Rotary Surfaces

David Sonntag, DMD, and Ove A. Peters, PD, DMD, MS, FICD†*

Abstract

Decontamination of instruments is a prerequisite for their potential reuse but may affect surface integrity. Hence, the effect of prion removal protocols on 7 brands of nickel-titanium files was investigated. Baseline debris scores were determined under magnification after staining with van Gieson's solution. After shaping root canals in vitro, rotaries were mechanically and ultrasonically cleaned followed by immersion for 24 hours in 2 M sodium hydroxide (NaOH), 6 M CH_5N_3, or 3% sodium hypochlorite (NaOCl); control files were stored dry. After sterilization, files were again stained and evaluated. Two of seven file brands demonstrated significantly higher baseline debris scores compared to final scores. Uniformly, debris could not be completely removed; there were no significant differences among groups. After immersion in NaOCl, 27.8% of instruments showed corrosion; however, no deterioration after immersion in the other solutions was found in the other groups. Regarding corrosion, no significant difference was found between brands. Based on these findings, single use of nickel-titanium rotaries appears beneficial. *(J Endod 2007;33:442–446)*

Key Words

Corrosion, decontamination, nickel titanium, prion, sodium hypochlorite

From the *Department of Operative Dentistry, Philipps-Universität, Marburg, Germany; and the †Endodontic Division, School of Dentistry, University of California, San Francisco, California.

Address requests for reprints to Dr. David Sonntag, Department of Operative Dentistry, Philipps-Universität, Marburg, Georg-Voigt-Strasse 3, 35033 Marburg, Germany. E-mail address: sonntag@staff.uni-marburg.de.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2006.12.012

Prions are proteins that have been linked to fatal neurodegenerative diseases commonly called transmissible spongiform encephalopathies. The term *prion* (PrP) was coined by Prusiner (1) in 1982, when he described a protein with a nonpathogenic isoform PrP^{C} and the infectious agent PrP^{SC} as a cause of scrapie, a veterinary disease. Similar agents may infect humans with Creutzfeld Jacob Disease (CJD), which in fact represents a group of diseases with various subgroups (2). In 1996, the CJD Surveillance Unit (Edinburgh, UK) reported a series of 10 patients with a novel form of CJD, so called variant or vCJD (3). These patients were younger than those with sporadic CJD and displayed early psychiatric and behavioral manifestations (3). The number of deaths caused by definite or probable CJD infection increased from 33 in 1990 to 108 in 2003 (4).

Several animal studies have demonstrated immunoreactivity to PrP^{SC}, not only in the trigeminal ganglion, but also in peripheral nerves (3, 5). Rutala and Weber (6) reported that prions have not yet been confirmed in peripheral nerve fibers in patients with CJD or vCJD; however, the possibility of disease transmission from that tissue cannot be ruled out (7).

Endodontic treatment may present a risk of transmission of PrP^{SC} through the intimate contact of endodontic instruments with peripheral branches of the trigeminal nerve (8). Therefore, the German Robert-Koch-Institute categorized endodontic instruments to the class of highest concern as "critical instruments class B" in 2006, because of close contact to tissue and blood (9).

The importance of prion decontamination on nickel-titanium (NiTi) instruments is controversial. In the United Kingdom, reuse of NiTi instruments is discouraged, but the Australian Endodontic Society published suggestions for instrument reuse (10, 11), whereas the American Association of Endodontists and most other governing bodies have not stated a position to date.

Reuse of NiTi rotaries requires cleaning, removal of microbial burden, and appropriate sterilization (5, 10). Unfortunately, current methods for decontaminating endodontic instruments seem unable to completely remove all biologic material (8, 12). Even brand-new instruments could not be completely cleaned prior to clinical use by ultrasonication and subsequent sterilization (13). Nevertheless, various cleaning protocols were published suggesting that reuse of NiTi instruments is feasible (10, 11, 14, 15). PrP^{SC} is highly resistant to conventional methods of chemical or thermal inactivation and to ultraviolet or ionizing radiation (16). This and a high binding affinity to metal surfaces (17) suggest specific decontamination procedures in reprocessing endodontic instruments (6). Treatments considered appropriate for decontamination include use of 1-2 M sodium hydroxide (NaOH) solution (for 24 or 1 h, respectively), 2.5 to 5% sodium hypochlorite (NaOCl) solution (for 24 or 1 h, respectively) as well as 3, 4, or 6 M CH_5N_3 (guanidine thiocyanate) solution (for 24 h, 1 h, or 15 min, respectively), followed by steam sterilization at 134°C for 18 minutes to 1 hour (7, 18). Others suggest a shorter decontamination protocol with chlorhexidine, manual cleaning, followed by 10 minutes immersion in NaOCl (1%) and 5 minutes ultrasonication before sterilization (14).

Stringent conditions, which are mandatory for the reprocessing of nondisposable endodontic instruments used with a recognizable risk of vCJD/CJD, are potentially hazardous to NiTi files. The corrosive nature of chlorine may preclude its use for disinfection of NiTi files (6, 19). Manufacturers generally recommend avoiding acidic ($pH < 6$) or alkaline ($pH > 8$) solutions during reprocessing in order to avoid instrument damage (20). Most manufacturers recommend discarding instruments af-

ter a specified number of uses or whenever visible deformation is observed. The "one-patient" single use of NiTi rotaries is not strictly recommended.

Berutti et al. (21) found that NiTi files, immersed for 5 minutes in NaOCl, were significantly less resistant to cyclic fatigue compared to files that had not been in contact with NaOCl. On the other hand, several studies have demonstrated that NiTi rotaries may be reused without intracanal failure (22, 23), even after contact with NaOCl.

Considering the significant economical and medical impact of reuse of endodontic instruments, more information on the effects of decontamination methods seems to be needed. Hence, the aim of this study was to determine whether currently recommended prion decontamination protocols are adequate to clean NiTi rotaries without damaging the instrument surface.

Materials and Methods

Instrument Selection and Staining

Batches of unused NiTi rotary instruments were used for this study (Alpha File, Brasseler, Lemgo, Germany; FlexMaster, VDW, Munich, Germany; K3, NT Company, Chattanooga, TN, USA; MTwo, VDW; ProFile, Dentsply Maillefer, Ballaigues, Switzerland; ProTaper, Dentsply Maillefer; RaCE, FKG, La Chaux-de-Fonds, Switzerland). All had tip size 30 and 0.04 taper except ProTaper Finishing File 3. All instruments were stained immediately after removal from their packages by immersion in van Gieson's solution (1.2% picric acid, 1% acid aqueous fuchsine) for 3 minutes to disclose organic material (12). Care was taken to grasp the instruments at the shank, and powder-free gloves were used while handling the instruments to avoid contamination. After baseline evaluation, rubber stoppers were removed and 20 instruments of each type were randomly assigned to three experimental and one control group (see below). Subsequently, instruments were dried with an air-supply device. Instruments were stored in covered Petri dishes except during the cleaning and scoring procedures to minimize exposure to dust and exogenous debris.

Scoring System

The working part of each instrument was examined at 50× magnification consistently at four consecutive levels, beginning from file tip, using a stereomicroscope (Stemi DV4, Zeiss, Wetzlar, Germany). Photographs were taken with a digital camera (Nikon 4500, Tokyo, Japan). At each level, the files were sequentially examined on four sectors by rotating them by 90°, aided by a custom-made holder (Feinmechanische Werkstätten, Philipps-University, Marburg, Germany).

Deposits on the instrument surface were classified as stained or unstained by van Gieson's solution (Fig. 1). Stained debris was recorded using the criteria listed in Table 1. Only one category of debris was assigned to each site examined. If stained and unstained debris was found concurrently, only the stained, organic debris was recorded because of its potentially higher infectious load.

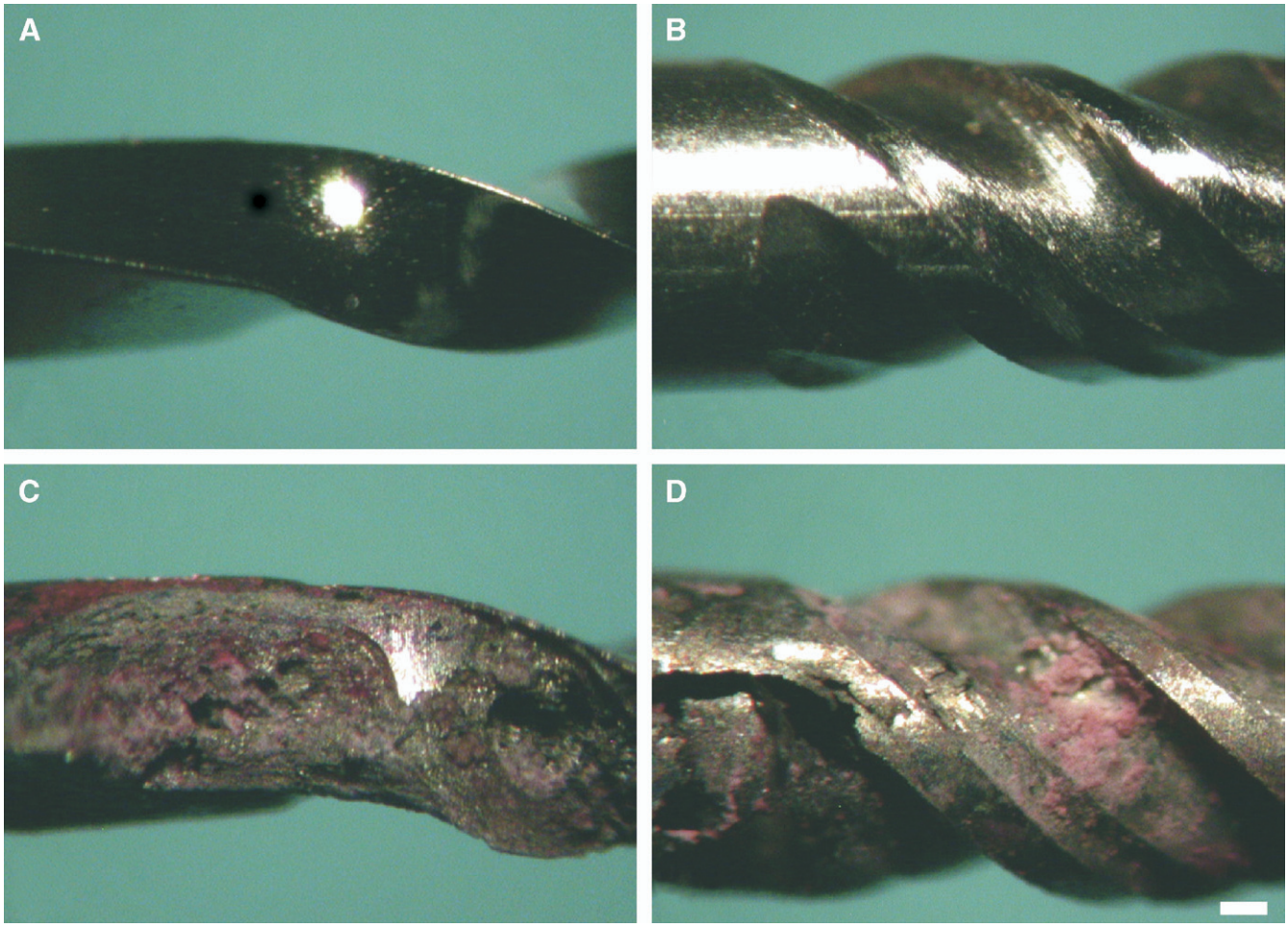

Figure 1. Rotary files before (*A*, *B*) and after (*C*, *D*) reprocessing, stained with van Gieson's solution, original magnification 50×, white bar indicates 100 μm. *A* and *C* show the same RaCE instrument, *B* and *D* the same K3 instrument. Note concurrence of localized stain and significant corrosion after immersion in NaOCl solution.

TABLE 1. Scores for stained and unstained debris after immersion of rotary instruments in van Gieson's solution

Debris categories
1 Clean
2 Particulate matter stained yellow, orange, or red
3 Unstained particulate matter
Extent of stained debris
1 Slight: Few scattered particles spaced apart
2 Moderate: Numerous particles with areas of continuous coverage
3 Heavy: Packed with debris

Evaluation was done at 50× magnification.

TABLE 2. Baseline level of contamination, expressed as numbers of sectors found in each category

File	Clean	Stained debris			Unstained debris
		Slight	Moderate	Heavy	
Alpha File	37	0	0	0	43
FlexMaster	21	13	0	0	46
K3	0	20	57	0	3
MTwo	42	8	0	0	30
ProFile	13	8	0	0	59
ProTaper	34	15	0	0	31
RaCE	19	28	0	0	33

Instrument Contamination and Decontamination

After baseline determination, the instruments were used to shape canals of extracted teeth that had been collected in accordance with the guidelines of the Internal Review Board of the Philipps-University and stored immediately after extraction in 0.12% thymol solution. Teeth were decoronated with a diamond disk to ensure straight-line access to the canals. Root canals were prepared in a crown-down technique for approximately 10 seconds to contaminate the working part of the rotary. All files were visually checked for complete coverage with debris. After completion of the contamination process, the instruments were immersed directly in distilled water to avoid drying of dentin debris.

Published PrP^{SC} decontamination protocols (18, 24) were consulted and partially modified to accommodate NiTi rotary files. After immersion in distilled water for 1 hour, each file was brushed with a nylon toothbrush (Oral B Advantage Plus, South Boston, MA) for 20 strokes. During brushing, files were mounted in a stand and then rinsed with tap water. Immediately thereafter, files were placed in a LavEndo file stand (VDW) into an ultrasonic bath with distilled water (model 1510, Branson, Danbury, CT) for 10 minutes. Then, files were rinsed under running water for 10 to 15 seconds, air dried, and placed for 24 hours at room temperature in one of four Petri dishes.

The following solutions were used to decontaminate instruments ($n = 35$, 5 instruments of each file type in each group):

- Group 1: NaOH, sodium hydroxide (2 M, pH 13.8, Fisher Chemicals, Fair Lawn, NJ)
- Group 2: NaOCl, sodium hypochlorite (3%, pH 12.3, Clorox, Oakland, CA)
- Group 3: CH_5N_3, guanidine thiocyanate (GdnSCN; 6 M, pH 6.0, Merck, Darmstadt, Germany)
- Group 4: Control, air-dried (after immersion in distilled water)

After immersion, the files were rinsed under running water for 15 seconds, air-dried, pooled, and placed in an airtight box. All 140 files from the control and experimental groups were examined at 50× magnification for debris as detailed above.

In addition, files were evaluated for signs of corrosion, which was identified visually by the presence of localized areas of surface pitting with or without corrosion products deposited onto the surface. More extensive corrosion reactions were identified by the presence of large and/or deep pits. The presence of corrosion products was determined through precipitates in the decontaminating solution or deposits on the metal instrument surface.

Finally, instruments were inserted into dry foam sponges before steam sterilization at 134°C (237°F) for 18 minutes. Subsequently, instruments were immersed in van Gieson's solution, rinsed with distilled water, and checked again for debris as described above.

Statistical Analysis

The SPSS 12.0 package (SPSS Inc., Chicago, IL) was used for statistical evaluation. Frequencies of presence of contamination and corrosion (nominally or ordinally scaled values) were tabulated and χ^2 tests were used for comparisons at $p < 0.05$. If a significant difference was registered with the χ^2 test, the individual p-values were specified using standardized residues.

Results

Organic Debris

At baseline, organic debris was present on the surfaces of six instrument brands (Fig. 1). Only gold-colored, titanium nitride–coated Alpha File instruments showed no visible yellow-, orange-, or red-stained debris. Presence of stained debris ranged from 10% (MTwo, ProTaper) to 96.3% (K3) on the assessed segments (Table 2).

After canal instrumentation, decontamination, and sterilization, RaCE and K3 instruments that had highest debris scores at baseline demonstrated a significant reduction of organic debris ($p < 0.01$). No differences in the presence of debris were found for any of the other instruments (Table 3).

Immersion in any of the decontamination solutions tested resulted in no significant differences ($p = 0.54$, χ^2 test); the control group (dry storage) showed similar results to the experimental groups.

TABLE 3. Presence of clean surfaces before and after reprocessing

Before reprocessing	After reprocessing						
	Alpha File (66, 82.5%)	FlexMaster (56, 72.7%)	K3 (46, 57.5%)	MTwo (61, 76.3%)	ProFile (62, 77.5%)	ProTaper (68, 85%)	RaCE (61, 76.3%)
Alpha File (37, 35.9%)	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
FlexMaster (21, 26.3%)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
K3 (0, 0%)			$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
MTwo (42, 52.5%)				N.S.	N.S.	N.S.	N.S.
ProFile (13, 16.3%)					$p < 0.001$	$p < 0.001$	$p < 0.001$
ProTaper (34, 42.5%)						$p < 0.001$	$p < 0.05$
RaCE (19, 23.8%)							$p < 0.001$

Significant differences are shown; numbers and percentages in parentheses indicate clean surfaces of NiTi instruments. N.S., nonsignificant.

Corrosion

Instruments of group 2 that had been in contact with 3% NaOCl showed corrosion in 27.8% of assessed sectors (Fig. 1*B*, *D*). The other decontamination solutions and dry storage did not cause corrosion; this difference was significant ($p < 0.001$). Frequently, corrosion was surrounded by light red-stained organic debris (Fig. 1).

Comparing instrument brands, K3 demonstrated corrosion in 25% of the sectors evaluated; Alpha File, MTwo, and RaCe were corroded in 20%; ProFile as well as ProTaper showed signs of deterioration in 15%; and FlexMaster in 10%. The χ^2 test showed no significant difference ($p = 0.849$) among the groups because of the number of files examined.

Discussion

Decontamination of surgical instrument surfaces is an important task prior to reuse. Although contamination after use is expected, most root canal instruments evaluated in this study had organic debris on their surfaces also before use.

Several methods have been used in the past to assess NiTi instrument surfaces, for instance, scanning electron microscopy (SEM) and photoelectron spectroscopy. In this study, examination was realized at 50× magnification using a stereomicroscope. Dissecting microscopes with magnification in the range of 15× to 45× were used previously by others (12, 14). Using a stereo- or dissecting microscope is advantageous because organic debris can be detected easily after staining, for instance, with van Gieson's solution. This dual color dye was used in prior studies to demonstrate organic debris (12, 14) and consists of picric acid and acid fuchsine. The resulting pattern in denser structures (e.g., erythrocytes and muscle tissue) is a yellow stain, whereas more loosely composed tissues (e.g., collagen and reticulin) stain red (25). Remaining debris on the nickel-titanium surface could lead to crevice corrosion if the instruments are left in chloride-containing solutions (26).

Our findings on remaining debris are supported by other studies (5). Stained and unstained debris was found on new, unused files with a dissection microscope after immersion in staining solution (10). SEM studies revealed significant differences among unused rotary files (13, 15), similar to results in this study. In some cases, even epithelial cells have been found on new unused files (27, 28). Hence, the need for cleaning and sterilization prior to use seems obvious.

Prions have been shown to possess a high binding affinity to and tenacity on metal surfaces (17). However, debris adhering to clinically used rotary instruments may consist of various bacterial and host proteins. Hence, prior to using prion preparations, e.g., 263 k scrapie brain homogenate (24), it should be determined whether current decontamination protocols remove adhering debris.

In spite of the consistent finding that debris remains on used instruments, interpretations of the results vary. Although Australian research groups support the reuse of instruments after effective cleaning (11, 12, 14, 19), European and North American authors suggest single use of instruments (5, 8, 21, 29). Moreover, the pertaining Australian/New Zealand Standard AS/NZS 4187:2003 stipulates that instruments should be "clean to the naked eye (macroscopic) and free from any protein residues" (14). However, the safest and most unambiguous method to ensure that there is no risk of residual infectivity on contaminated instruments and other material is to discard them after use (5, 18).

An equally important problem may be surface deterioration after cleaning processes. In this study, immersion in NaOCl resulted in signs of corrosion in more than a quarter of all instrument sectors; no corrosion was found in the other solutions or the control group. There were no significant differences between file brands.

The location of the pitting caused by corrosion appears to be random (21). Interestingly, we found concurrence of staining with van Gieson's solution in the same areas that showed corrosion. This phenomenon may result from small gaps between debris and the NiTi surface, which leads possibly to crevice corrosion. Crevice corrosion was demonstrated for implants and orthodontic wires (30, 31). Darabara et al. (32) concluded that NiTi may be not susceptible to pitting or crevice corrosion in NaOCl solution. However, others reported corrosion of NiTi files subjected to NaOCl (5, 19).

To our knowledge, no staining of NiTi files that were subject to NaOCl immersion has been performed. Hence, the observed concurrence of stained debris and corrosion need to be verified and a potential causative mechanism evaluated.

It should be emphasized that, contrary to manufacturers' cleaning guidelines, alkaline solutions such as NaOH and NaOCl were used in this study to substantiate cleaning recommendations of the World Health Organization (WHO) pertaining to prion contamination. Immersion in NaOCl led to pitting corrosion with deep defects, whereas no surface damages could be detected after immersion in NaOH. Particularly with regard to corrosion, GdnSCN (CH_5N_3, pH 6.3) appears to be suitable for disinfection of NiTi instruments. Conflicting evidence was reported for torque and fatigue resistance after immersion in NaOCl (19, 33), and no information is currently available regarding potential effects of NaOH and GdnSCN in NiTi instrument performance.

Prion decontamination with GdnSCN has been recommended (34); however, the WHO classified the solution as an insufficient disinfectant (18). Hence, in principle, the only applicable disinfectant appears to be NaOH, in spite of its basic pH. Further studies of torque resistance and cyclic fatigue are required before the use of NaOH solution could be recommended. Furthermore, 1 N NaOH solution readily reacts with CO_2 in air to form carbonates that then neutralize NaOH and reduce its disinfecting properties. The solution should be prepared fresh for each use from solid pellets or stock solution (18); however, working with this hazardous solution may not be practical.

Finally, as demonstrated by the control group that was stored dry in this study, regardless of the solution selected, instruments for reuse should be kept moist between the time of exposure to infectious materials and subsequent decontamination and cleaning (18).

In conclusion, despite meticulous cleaning under laboratory conditions, all solutions used were not able to completely clean rotary instruments of organic debris. Significant corrosion was observed with NaOCl immersion. Single use of rotary instruments is therefore recommended to prevent the transmission of infectious diseases and to reduce the hazard of corrosion.

Acknowledgments

Rotary files used in the present study were kindly supplied by the above-mentioned manufacturers of NiTi instruments. The authors thank Carl Zeiss, Wetzlar, Germany, for the generous loan of a Stemi DV microscope.

References

1. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982;9:136–44.
2. Azarpazhooh A, Leake JL. Prions in dentistry—what are they, should we be concerned, and what can we do? Tex Dent J 2006;123:421–8.
3. Smith AJ, Bagg J, Ironside JW, Will RG, Scully C. Prions and the oral cavity. J Dent Res 2003;82:769–75.
4. The National CJD Surveillance Unit. URL: http://www.cjd.ed.ac.uk, last accessed 10/19/2006.
5. Aasim SA, Mellor AC, Qualtrough AJ. The effect of presoaking and time in the ultrasonic cleaner on the cleanliness of sterilized endodontic files. Int Endod J 2006;39:143–9.

6. Rutala WA, Weber DJ. Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. Clin Infect Dis 2001;32:1348–56.
7. Bebermeyer RD, Powell JF, Hobdell MH, Durban EM. Dental practice implications of prion diseases. Quintessence Int 2003;34:38–44.
8. Smith A, Dickson M, Aitken J, Bagg J. Contaminated dental instruments. J Hosp Infect 2002;51:233–5.
9. Robert-Koch-Institut. URL: www.rki.de, last accessed 10/12/2006.
10. Parashos P, Linsuwanont P, Messer HH. Effective cleaning protocols for rotary nickel-titanium files. Aust Endod J 2003;29:23–4.
11. Van Eldik DA, Zilm PS, Rogers AH, Marin PD. Microbiological evaluation of endodontic files after cleaning and steam sterilization procedures. Aust Dent J 2004;49:122–7.
12. Linsuwanont P, Parashos P, Messer HH. Cleaning of rotary nickel-titanium endodontic instruments. Int Endod J 2004;37:19–28.
13. Parirokh M, Asgary S, Eghbal MJ. An energy-dispersive X-ray analysis and SEM study of debris remaining on endodontic instruments after ultrasonic cleaning and autoclave sterilization. Aust Endod J 2005;31:53–8.
14. Parashos P, Linsuwanont P, Messer HH. A cleaning protocol for rotary nickel-titanium endodontic instruments. Aust Dent J 2004;49:20–7.
15. Van Eldik DA, Zilm PS, Rogers AH, Marin PD. A SEM evaluation of debris removal from endodontic files after cleaning and steam sterilization procedures. Aust Dent J 2004;49:128–35.
16. Taylor DM. Inactivation of transmissible degenerative encephalopathy agents: a review. Vet J 2000;159:10–7.
17. Flechsig E, Shmerling D, Hegyi I, et al. Prion protein devoid of the octapeptide repeat region restores susceptibility to scrapie in PrP knockout mice. Neuron 2000;27:399–408.
18. World Health Organization. WHO Infection control guidelines for transmissible spongiform encephalopathies. Geneva, Switzerland: WHO, 1999. URL: www.who.int/csr/resources/publications/bse/WHO_CDS_CSR_APH_2000_3/en, last accessed 10/19/2006.
19. O'Hoy PY, Messer HH, Palamara JE. The effect of cleaning procedures on fracture properties and corrosion of NiTi files. Int Endod J 2003;36:724–32.
20. Dentsply Maillefer. Direction for use of ProTaper. Ballaigues, Switzerland, 2006.
21. Berutti E, Angelini E, Rigolone M, et al. Influence of sodium hypochlorite on fracture properties and corrosion of ProTaper Rotary instruments. Int Endod J 2006;39:693–9.
22. Yared GM, Bou Dagher FE, Machtou P. Cyclic fatigue of ProFile rotary instruments after clinical use. Int Endod J 2000;33:204–7.
23. Svec TA, Powers JM. The deterioration of rotary nickel-titanium files under controlled conditions. J Endod 2002;28:105–7.
24. Lemmer K, Mielke M, Pauli G, Beekes M. Decontamination of surgical instruments from prion proteins: in vitro studies on the detachment, destabilization and degradation of PrPSc bound to steel surfaces. J Gen Virol 2004;85:3805–16.
25. Young B. Wheather's functional histology: a text and colour atlas, 4th ed. Philadelphia, PA: Elsevier, 2000.
26. Lucas LC, Lemons JE. Biodegradation of restorative metallic systems. Adv Dent Res 1992;6:32–7.
27. Marsicovetere ES, Clement DJ, del Rio CE. Morphometric video analysis of the engine-driven nickel-titanium Lightspeed instrument system. J Endod 1996;22:231–5.
28. Miller CH. Applied research still needed on infection control procedures. Am J Dent 2000;13:285–6.
29. Arens FC, Hoen MM, Steiman HR, Dietz GC Jr. Evaluation of single-use rotary nickel-titanium instruments. J Endod 2003;29:664–6.
30. Eliades T, Eliades G, Athanasiou AE, Bradley TG. Surface characterization of retrieved NiTi orthodontic archwires. Eur J Orthod 2000;22:317–26.
31. Guindy JS, Schiel H, Schmidli F, Wirz J. Corrosion at the marginal gap of implant-supported suprastructures and implant failure. Int J Oral Maxillofac Implants 2004;19:826–31.
32. Darabara M, Bourithis L, Zinelis S, Papadimitriou GD. Susceptibility to localized corrosion of stainless steel and NiTi endodontic instruments in irrigating solutions. Int Endod J 2004;37:705–10.
33. de Castro Martins R, Bahia MG, Buono VT, Horizonte B. The effect of sodium hypochlorite on the surface characteristics and fatigue resistance of ProFile nickel-titanium instruments. Oral Surg Oral Med Oral Path Oral Radiol Endod 2006;102:99–105.
34. Van Everbroeck B, Pals P, Martin JJ, Cras P. Antigen retrieval in prion protein immunohistochemistry. J Histochem Cytochem 1999;47:1465–70.