Sterilization of rotary NiTi instruments within endodontic sponges

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Abstract

Aim To determine whether the following can be sterilized by autoclaving – endodontic sponges, rotary nickel–titanium (NiTi) instruments within endodontic sponges, and rotary NiTi instruments with rubber stoppers.

Methodology Sixty-four samples of eight different endodontic sponges (n = 512) were placed into brain heart infusion broth (BHI) for 72 h. An aliquot of this was then spread onto horse blood agar and cultured aerobically and anaerobically to test sterility at purchase. Bacterial suspensions of Enterococcus faecalis, Porphyromonas gingivalis and Geobacillus stearothermophilus in BHI were used to contaminate sterile sponges and rotary NiTi instruments (with and without rubber stoppers) inserted into sponges. The various samples were autoclaved and then cultured aerobically and anaerobically. Success of sterilization was measured qualitatively as no growth. The experiment was repeated with clinically used rotary NiTi instruments (n = 512). All experiments were conducted in quadruplicate.

Results No sponges on purchase had microbial growth when anaerobically cultured but some did when aerobically cultured. All autoclaved sponges and instruments (within or without sponges, and with or without rubber stoppers) were associated with no microbial growth. All nonautoclaved positive control samples showed microbial growth.

Conclusions Autoclaving was effective in the sterilization of sponges and endodontic instruments. Endodontic sponges should be autoclaved before clinical use. For clinical efficiency and cost-effectiveness, rotary NiTi instruments can be sterilized in endodontic sponges without removal of rubber stoppers.

Keywords: autoclave, bacteria, infection, NiTi instrument contamination, rubber stoppers, sterilization.

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Introduction
Sterilization of dental instruments is a crucial part of infection control. Steam sterilization (autoclaving) involves the use of both heat and moisture maintained at a set time, temperature and pressure to eliminate microorganisms including spores (Barnett 2005). The presence of biological debris may prevent the effective penetration of steam during autoclaving (Miller & Palenik 2001). Further, biological debris with high moisture content may reduce the heat resistance of vegetative bacteria and spores, therefore facilitating the sterilization process (Van Eldik et al. 2004a).

Endodontic sponges serve as a chairside storage and mechanical cleaning aid of root canal instruments (Hubbard et al. 1975, Rutala et al. 1998, Chu et al. 1999, Linsuwanont et al. 2004, Parashos et al. 2004, Popovic et al. 2010). These studies demonstrated the importance of using nonporous, dense endodontic sponges chairside to remove biobur-
den from instruments prior to ultrasonication and sterilization.

Scouring sponges were the preferred sponges to clean rotary nickel–titanium (NiTi) instruments because their coarse top layer consists of very fine, relatively stiff fibres that enter the instrument flutes, enabling effective removal of gross debris (Parashos et al. 2004). Additionally, the nonporous scouring sponges had a greater retention of chlorhexidine gluconate solution than porous sponges (Parashos et al. 2004). Moist sponges soaked with antimicrobial solution have been shown to be more effective in mechanical cleaning of instruments when compared with dry sponges (Hubbard et al. 1975, Segall et al. 1977, Parashos et al. 2004).

Whilst it is clinically accepted that the sponges used during root canal treatment should be sterile to ensure no microbial transfer from the sponges into root canal systems, only one study has assessed the ability of various dental sponges to be successfully sterilized and concluded that steam sterilization of endodontic sponges provided the best results (Kuritani et al. 1993). Despite the common use of endodontic sponges in root canal treatment, currently no guidelines or protocols exist recommending sterilization of endodontic sponges prior to root canal procedures. Additionally, there is no literature concerning the sterilizability of rotary NiTi instruments inserted into sponges, or if the instruments can be sterilized without removal of the rubber stoppers.

Therefore, the aims of this study were (i) to determine whether endodontic sponges are sterile on purchase and whether they could be successfully sterilized, and (ii) whether rotary NiTi instruments inserted into endodontic sponges, with or without their rubber stoppers, could be sterilized.

Materials and methods

Bacterial strains and growth conditions

Freeze-dried samples of Enterococcus faecalis (JKD 15036, laboratory stock from Oral Health CRC, Melbourne Dental School) and Porphyromonas gingivalis (W50, laboratory stock from Oral Health CRC, Melbourne Dental School) were revived and grown separately in brain heart infusion broth (BHI; Oxoid Pty Ltd, Heidelberg, VIC, Australia) supplemented with hemin (5 mg L⁻¹) at 37 °C under anaerobic conditions (MK3 anaerobic workstation, Don WhiteyScientific, Adelaide, Australia) with gas composition of 5% CO₂, 5% H₂ and 90% N₂ (BOC gases, Wetherill Park, NSW, Australia) for 72 h according to standard methodology carried out in this laboratory. G. stearothermophilus (ATCC 7953) was extracted from a self-contained biological indicator for steam sterilization (EZTest, Austmel Pty Ltd., Molendinar, Qld, Australia) and grown aerobically in BHI at 55 °C for 72 h.

The optical density (OD) of the bacterial cultures was determined at a wavelength of 650 nm using a Cary 50 Bio UV–visible spectrophotometer (Varian, Mulgrave, VIC, Australia). The bacterial cell number was determined by plating the serially diluted samples onto horse blood agar (HBA) plates (blood agar base No. 2, Oxoid Pty Ltd). Glycerol stocks of E. faecalis, P. gingivalis and G. stearothermophilus were prepared so that each tube contained 2 × 10⁸ bacterial cells (E. faecalis – OD 1.0; P. gingivalis – OD 1.0; and G. stearothermophilus – OD 0.85). The same batches of glycerol stocks were used throughout the experiments.

Assessing the presence of microorganisms in endodontic sponges at purchase

Sixty-four samples of eight different sponge types (Table 1) (n = 512, with four biological and four technical replicates) were placed into separate sterile containers containing 150 mL of BHI. After 72 h, 100 µL of the solution from each container was spread onto horse blood agar plates. Of these, 256 agar plates were cultured under aerobic conditions (37 °C) for 72 h. The remaining 256 HBA plates were cultured under anaerobic conditions (37 °C) for 72 h.

Initial testing of sponges

Sixty-four of each of the eight different sponge samples (Table 1) were placed into separate sealed sterilization pouches (SAFE-SEAL, Medicom, Montreal, Canada) and autoclaved (HS6610, Getinge, Murarrie, Qld, Australia) with a holding time of 3 min at 134 °C and 206 kPa. Following manual rasping with rotary NiTi instruments ten times into the sponges, the texture, colour and shape of the sponges were examined. Sponges with no change in these properties after autoclaving were considered to have the ability to withstand autoclaving and then proceeded to the next stage of the experiment. Four sponges withstood autoclaving – Endofoam PVP, Endofoam T1, Endofoam T2, and Endofoam S.
Assessing the ability of sponges to be sterilized

Sixty-four samples of the four sponge types that survived autoclaving (n = 256, with four biological and four technical replicates) were first sterilized and then contaminated with bacteria by placing them into separate sterile containers containing a bacterial suspension (*E. faecalis*, *P. gingivalis* and *G. stearothermophilus* in BHI solution). The sponges were left in the containers to allow for bacterial growth in an aerobic incubator for 72 h at 37 °C. Of the contaminated samples, 32 of each sponge type (n = 128) were autoclaved. The other 32 contaminated samples of each sponge type (n = 128) acted as a control so were not autoclaved.

After incubation, 100 µL of each solution was plated onto HBA plates; 128 agar plates were cultured aerobically and 128 plates were cultured anaerobically as described earlier. Success of sterilization in all experiments was measured qualitatively as lack of growth. All experiments were conducted in quadruplicate.

Assessing the ability of rotary NiTi instruments to be sterilized within endodontic sponges

As depicted in Fig. 1, rubber stoppers were removed from 256 sterile rotary NiTi instruments, whilst the rubber stoppers of another 256 sterile rotary NiTi instruments were left *in situ*. The instruments comprised Mtwo (n = 100) (VDW GmbH, Munich, Germany), ProTaper (n = 312) (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) and ProFile Vortex Blue (n = 100) (Dentsply Tulsa Dental Specialties).

The 512 NiTi instruments and rubber stoppers were contaminated with *E. faecalis*, *P. gingivalis* and *G. stearothermophilus* BHI bacterial suspension in an incubator. After the contamination, the rubber stoppers *in situ* were moved up and down the NiTi instruments with sterile forceps to ensure contamination between the stoppers and the instruments. The pre-sterilizing cleaning process comprised ten vigorous strokes in a scouring sponge (Endofoam PVP, Australian Dental Manufacturing, Kenmore Hills, Brisbane, QLD, Australia) soaked in 0.2% chlorhexidine solution, soaking the NiTi instruments in an enzymatic cleaning solution (Asepti Autozyme PR, Mulgrave, VIC, Australia) for 30 min, 15 min ultrasonication in an enzymatic cleaning solution and a final rinse in running tap water for 20 s (Parashos *et al.* 2004).

These NiTi instruments were then inserted into sterile endodontic sponges and were placed into separate sterile plastic containers containing BHI solution and underwent autoclaving, followed by aerobic incubation at 37 °C for 72 h. Each sponge held different numbers of instruments to simulate possible clinical combinations, that is five sponges, each containing 1–8 instruments, and four sponges, each containing 9 or 10 instruments (n = 256).

NiTi instruments were removed from the sponges after autoclaving and directly inserted into HBA

### Table 1 Details of the endodontic sponges tested

<table>
<thead>
<tr>
<th>Sponges</th>
<th>Colour and shape</th>
<th>Size</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endofoam C</td>
<td>Light blue, low density; round.</td>
<td>Diameter: 5.5 cm Thickness: 0.5 cm</td>
<td>Australian Dental Manufacturing, Kenmore Hills, Brisbane, QLD, Australia</td>
</tr>
<tr>
<td>Endofoam PVP</td>
<td>Pink high density, green scouring and white low density; cubic</td>
<td>Diameter: 4 cm Width: 4 cm Thickness: 4 cm</td>
<td>Australian Dental Manufacturing, Kenmore Hills, Brisbane, QLD, Australia</td>
</tr>
<tr>
<td>Endofoam T1</td>
<td>Grey; triangular</td>
<td>Diameter: 5.5 cm Thickness: 1.6 cm</td>
<td>SybronEndo, Orange, CA, USA</td>
</tr>
<tr>
<td>Endofoam T2</td>
<td>Green; triangular</td>
<td>Diameter: 5.5 cm Thickness: 1.6 cm</td>
<td>SybronEndo, Orange, CA, USA</td>
</tr>
<tr>
<td>Endofoam S</td>
<td>Grey; square without adhesive</td>
<td>Diameter: 4.6 cm Width: 4.6 cm Thickness: 2.9 cm</td>
<td>VDW GmbH, Munich, Germany</td>
</tr>
<tr>
<td>Endofoam SA</td>
<td>Grey; square with adhesive</td>
<td>Diameter: 4.6 cm Width: 4.6 cm Thickness: 2.9 cm</td>
<td>VDW GmbH, Munich, Germany</td>
</tr>
<tr>
<td>Endo Ring foam</td>
<td>Yellow; triangular</td>
<td>Diameter: 5.3 cm Thickness: 1.5 cm</td>
<td>VDW GmbH, Munich, Germany</td>
</tr>
<tr>
<td>Interim Stand foam disc</td>
<td>Blue; round</td>
<td>Diameter: 5.5 cm Thickness: 1.7 cm</td>
<td>VDW GmbH, Munich, Germany</td>
</tr>
</tbody>
</table>
using sterile forceps. For NiTi instruments with rubber stoppers in situ, rubber stoppers were first removed from the instruments and both inserted into HBA plates using sterile forceps to test for bacterial growth.

Assessing the ability of clinically used rotary NiTi instruments to be sterilized within endodontic sponges

This experiment followed exactly the same protocols previously explained (Fig. 1) except that clinically
contaminated rotary NiTi instruments were tested. These instruments comprised Mtwo \( (n = 272) \) (VDW GmbH), ProTaper \( (n = 212) \) (Dentsply Tulsa Dental Specialties) and ProFile Vortex Blue \( (n = 28) \) (Dentsply Tulsa Dental Specialties). These instruments were obtained from three specialist endodontic practices and had been pre-processed by them.

**Results**

**Assessing the presence of microorganisms in the sponges at purchase**

Only Endofoam PVP sponges and Interim Stand foam discs (Table 1) exhibited microbial growth when cultured aerobically, but not anaerobically. The creamy white colour and smooth surfaces of these colonies suggested they may be *C. albicans*.

**Assessing the ability of endodontic sponges to be sterilized**

None of the autoclaved endodontic sponges \( (n = 128) \) had microbial growth when cultured under aerobic and anaerobic conditions, whilst all the positive control (nonautoclaved) endodontic sponges \( (n = 128) \) had positive microbial growth.

**Assessing the ability of rotary NiTi instruments to be sterilized within endodontic sponges**

None of the autoclaved endodontic instruments \( (n = 128) \) when inserted into endodontic sponges, with or without rubber stoppers, had any microbial growth aerobically or anaerobically, nor did the rubber stoppers. Similarly, those instruments not within the sponges with or without rubber stoppers \( (n = 128) \) also had no microbial growth, nor did the rubber stoppers. All positive control samples, instruments and rubber stoppers \( (n = 256) \), showed microbial growth.

**Assessing the ability of clinically used rotary NiTi instruments to be sterilized within endodontic sponges**

Prior to autoclaving, 79% of the 256 clinically used rotary NiTi instruments tested positive for microorganisms when cultured aerobically and 69% anaerobically. None of the autoclaved endodontic instruments \( (n = 128) \) when inserted into endodontic sponges with or without rubber stoppers had microbial growth when aerobically and anaerobically cultured, nor did the rubber stoppers. Similarly, those instruments not within the endodontic sponges \( (n = 128) \), and the rubber stoppers, also had no microbial growth.

**Discussion**

*G. stearothermophilus*, *P. gingivalis* and *E. faecalis* were used for the bacterial contamination in this study. *G. stearothermophilus* is a rod-shaped, Gram-positive thermophile bacterium capable of growth in the high temperature range of 30 °C–75 °C (Nicholson *et al.* 2000, Nazina *et al.* 2001) and is commonly used as a challenge organism in sterilization validation studies (Munro *et al.* 1999, Lemieux *et al.* 2006, ISO 2009). *P. gingivalis* is a Gram-negative anaerobe commonly associated with periodontal disease (Lamont & Jenkins 1998) and frequently isolated from root canals of infected teeth with periapical abscesses (Jacinto *et al.* 2006). *E. faecalis* is a Gram-positive facultative anaerobic bacterium frequently found in the gastrointestinal tract and root filled teeth (Molander *et al.* 1998, Roças *et al.* 2004, Stuart *et al.* 2007). It can survive and grow in a wide range of temperatures (10 °C–45 °C) and can withstand temperatures up to 60 °C for 30 min (Tendolkar *et al.* 2003). It is also reported that *E. faecalis* can remain viable on inanimate surfaces for up to 4 months (Kramer *et al.* 2006). Therefore, these microorganisms represented clinically and scientifically relevant contaminants in the various experiments of this study.

Many studies have found endodontic instruments to be nonsterile and have debris at purchase despite claims made about their sterility (Zmener & Spielberg 1995, Martins *et al.* 2002, Parashos *et al.* 2004, Van Eldik *et al.* 2004b). The use of nonsterile instruments in the oral cavity may increase risk of pathogen transmission (Segall *et al.* 1977, Zmener & Spielberg 1995). Infection control guidelines stipulate that instruments must be autoclaved before their first use, which is a vital step to achieve sterility of dental instruments (AS/NZS 2003, Hancock *et al.* 2013). However, there is no single measurement recognized to test the sterility of an item (Dunn 2002). Unfortunately, there is limited research available to recommend any guidelines or protocols on which to base sterilization procedures (Miller 2000). It is important that any guidelines or protocols are based on scientifically obtained and consistent clinically relevant data (Rutala *et al.* 1998). The cleaning and sterilization recommendations made by...
various groups may not be practical, justifiable or achievable (Rutala et al. 1998).

Packaged endodontic instruments can be successfully sterilized by autoclaving (Van Eldik et al. 2004a). These instruments may then need to be introduced into an endodontic sponge chairside. To date, there have been only two studies examining the ability of endodontic instruments to be sterilized within sponges. Those studies tested stainless steel hand files inserted into synthetic sponges and found that they were able to be successfully sterilized by autoclaving (Boyd et al. 1994, Velez et al. 1998); however, dry heat sterilization was less successful despite the fact that the integrity of the sponges was not compromised (Velez et al. 1998).

The complex architecture of root canal instruments (Segall et al. 1977, Zmener & Spielberg 1995, Marsicovetere et al. 1996, Johnson et al. 1997, Linsuwanont et al. 2004) makes their cleaning and sterilization difficult. Parashos et al. (2004) developed a cleaning protocol for rotary NiTi endodontic instruments, which involved both mechanical and chemical cleaning procedures (Parashos et al. 2004, Popovic et al. 2010) and that completely removed bioburden at a microscopic level on all surfaces of the instruments. Although there is evidence that endodontic instruments can be sterilized in the presence of biological debris (Miller & Sheldrake 1991, Boyd et al. 1994), the cleaning of these endodontic instruments to remove bioburden eliminates the majority of the microorganisms (Chu et al. 1999). In this context, whilst beyond the scope of this paper, it is relevant to mention that some authors have recommended single use of rotary NiTi instruments due to a perceived difficulty in reprocessing (Aasim et al. 2006, Sonntag & Peters 2007). However, other authors have contrary views, evidence and recommendations (Saunders 2005, Parashos & Messer 2007, AAE/CAE 2011). In any case, the current study presents evidence applicable to initial processing and reprocessing of rotary NiTi root canal instruments.

The use of a sponge during the reprocessing of NiTi instruments will clean all the surfaces of the instruments simultaneously (Parashos et al. 2004). It also assists in preventing needle stick injury when compared with dry wiping of NiTi instruments with a scouring pad or gauze (Miller 2002). Hence, this present paper focused on the cleaning and sterilizability of rotary NiTi instruments when inserted into single-use endodontic sponges. To this end, this study has proved that rotary NiTi instruments can be completely and reliably free of microbial growth including spores after autoclaving.

This study also revealed that NiTi instruments of different complex designs showed equal effectiveness of sterilization. Importantly, the study showed that both newly purchased NiTi instruments and used instruments can be sterilized within endodontic sponges. Different numbers of the NiTi instruments within sponges, varying from one to ten per sponge, were tested in acknowledgement of the different systems in place in different dental practices. Ten instruments were the maximum tested because of the size limitation of the sponges. The results showed that the sponges and the NiTi instruments can be sterilized regardless of the number of instruments within the sponges.

It is important to note that there was microbial growth in two of the sponge types under aerobic conditions at purchase. This growth was likely C. albicans based on their classic fungal colony morphology of shape, form, colour and margin (Deacon 1997). In the microbiology laboratory in which these experiments were performed, the manufacturer-packaged sponges were removed from their packages using sterile instruments and strict aseptic protocols. Contamination may have come from several sources including pre-existing fungal spores within the sponges at purchase or environmental contamination during storage and preparation. In the laboratory, all experimental procedures were performed under a class II biosafety cabinet, personal protective equipment (masks, gloves, goggles and gown) was worn at all times, and all handling forces were sterilized prior to use. Hence, the chance of laboratory contamination was negligible.

Fungal spores can spread through air, water or skin contact and can survive an extended period of time without nutrients (Deacon 1997). They will reactivate when they encounter nutrients, such as those provided on the HBA plate used in this experiment. Fungi, in particular C. albicans, have been found to be associated with both primary root canal infections and failed root canal treatments (Gomes et al. 2010), which raises the concern that the use of nonsterile endodontic sponges may lead to contamination of the root canal system.

In this study, all sponges were opened from their bulk packaging and tested simultaneously, but in a clinical scenario, it is unlikely they would all be used immediately. Rather, the open package could potentially become contaminated by microorganisms present in the environment. Therefore, it becomes important to sterilize endodontic sponges before use.
and store them appropriately to reduce the risk of contamination. Ideally, this would be in individual autoclave packages that would be opened chairside immediately prior to use, as is anecdotally already undertaken in some private dental practices. Transmission of new pathogens from endodontic sponges into root canal systems can be effectively prevented by sterilization procedures (Woods et al. 1996, Barnett 2005), and this study demonstrated that steam sterilization procedures are effective for sponges and endodontic instruments.

Lastly, the recommendation to remove rubber stoppers from rotary NiTi instruments prior to steam sterilization (ADA 2012) has been shown in this study not to be necessary, at least demonstrated by the three bacterial species tested. Importantly, the protocol in this study was designed to ensure that microbial contamination between the rubber stoppers and instruments was confirmed, yet, upon autoclaving, no microorganisms survived. Hence, rotary NiTi instruments can be sterilized and handled without removal of rubber stoppers which, again, avoids sharp injuries.

Conclusions

Some endodontic sponges are not sterile on purchase but can be sterilized by steam sterilization.

Rotary NiTi endodontic instruments can be sterilized effectively with or without removal of rubber stoppers when inserted into endodontic sponges. All endodontic instruments including sponges should be autoclaved before their clinical use to achieve an acceptable sterility assurance level.

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References


