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Efficacy of air/water syringe tip sterilization

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ABSTRACT

Background: Dental procedures involve contact between instruments and the patient's tissues, blood or saliva. This study evaluated the efficacy of the standardized sterilization of non-disposable air/water syringe tips and corrosion and contaminant build-up in these tips.
Methods: The bacterial contamination of single-use and multiple-use non-disposable air/water syringe tips after routine use and sterilization was compared to that of single-use disposable tips by microbial culturing on PCA and blood agar plates. The effect of flushing the syringe tips prior to sterilization was also measured. The amount of corrosion in single-use and multiple-use non-disposable syringes was measured by SEM and EDS analyses.
Results: Non-disposable syringe tips had significantly ($p < 0.05$) greater bacterial contamination than single-use disposable tips. There were no statistically different levels of contamination between flushed and non-flushed non-disposable syringes or between single-use and multiple-use non-disposable syringes. SEM and EDS analyses showed greater evidence of corrosion and contaminant build-up in multiple-use syringes compared to single-use non-disposable syringes.
Conclusions: Sterilization of non-disposable air/water syringes is not completely effective and rinsing, or the number of uses, does not affect the effectiveness of sterilization. There may be a lower risk of cross-infection from the use of disposable air/water syringe tips, instead of non-disposable ones.

Keywords: Air/water syringe, contamination, bacteria, corrosion.

Abbreviations and acronyms: ANOVA = analysis of variance; CDC = Centers for Disease Control and Prevention; cfu = colony-forming units; EDS = electron dispersive spectroscopy; NDS = non-disposable syringe; ONDS = old non-disposable syringe; PBS = phosphate buffered saline; PCA = plate count agar; SEM = scanning electron microscopy; UCU = urgent care unit.

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INTRODUCTION

Many dental procedures involve contact between an instrument and the patient's tissues, blood and saliva, all of which can contain potentially pathogenic microorganisms. The contact of these microorganisms with the instruments can result in adhesion and, if not removed, subsequent biofilm formation.[1] If multi-use instruments are not properly cleaned they may retain pathogens that can be transmitted to subsequent patients.

Sterilization is defined as the complete destruction of all microorganisms on an inanimate object or instrument.[2] Guidelines by the Centers for Disease Control and Prevention (CDC) state that decontamination should be carried out before sterilization.[3] Decontamination is the removal of visible contamination or bioburden and can be done by hand, instrument washer, or ultrasonic cleaner. If not properly cleaned, this bioburden can undermine the sterilization process and trapped bacteria, fungi or viruses may remain viable.

Certain medical instruments, such as mirrors, forceps, probes and clamps, are relatively easy to sterilize. However, instruments containing crevices, joints or narrow lumen are difficult to access for decontamination and can therefore harbour infectious pathogens.[4] Such medical instruments include endoscopes which have multiple internal channels, lumen and valves. Due to these complex internal structures, an endoscope can remain contaminated despite sterilization, with the most common microbiological contamination being *Salmonella*, *Pseudomonas* and *Mycobacterium* bacterial species.[5]

Handpieces used in dentistry also face this problem. They have many internal lumen and deep recesses that are not readily accessible to debridement and therefore are difficult to sterilize.[4] It has been shown that these internal areas become contaminated with oral material and are therefore potential sources for

cross-infection.[6,7] Under the CDC guidelines for infection control it is recommended that any dental device connected to the dental air/water system that enters the patient's mouth should be run to discharge water, air, or a combination for a minimum of 20–30 seconds after each patient.[3] While handpieces have retraction valves that are meant to stop infectious materials from entering the water lines between patients, dental waterlines may be contaminated with biofilms[1] and the air/water syringe may also have a biofilm refractory to sterilization.

Hypodermic needles used in dentistry are a concern due to their narrow lumen. According to the guidelines, when administering local anaesthetic, a previously unused and sterile disposable needle must be used for each patient.[3] This is due to the difficulty in effectively sterilizing the small diameter of the needle lumen. A study examining the effectiveness of sterilization on lumen at different lengths and diameters demonstrated that the longer the lumen, the less effective was sterilization of the internal surface.[8] Furthermore, the longer the lumen, the larger its diameter had to be to achieve adequate sterilization.

Another factor pertinent to the cross-infection risks of multi-use instruments employed in dentistry is corrosion. Corrosion is defined as the destruction, or deterioration, of a material because of reaction with its environment.[9] Exposure to agents such as air and water can result in partial or complete dissolution, deterioration, or weakening of any solid substance.[10] Corrosion not only weakens the dental instrument, but it also creates a roughened surface that facilitates the adhesion of microorganisms and other contaminants.[11,12] If corrosion or contaminant build-up is found in areas of crevices, joints or narrow lumen in dental instruments, the chance of cross-contamination increases due to the inability to decontaminate and sterilize them correctly. Corrosion can be identified with scanning electron microscopy (SEM) and electron dispersive spectroscopy (EDS) analyses. SEM captures topographical images of the surface structure while EDS is an analytical technique used for the elemental analysis or chemical characterization of a sample.

Dental air/water syringes have the problem of both a high exposure to corrosive conditions as well as being structurally difficult to decontaminate and sterilize. Air/water syringes administer air and water to the oral cavity in order to dry tooth surfaces, as well as to remove debris for increased visibility. The passing of water and air through the metallic lumen creates a corrosive environment which is exacerbated by debridement and sterilization (especially by chemiclaves or steam autoclaves). Structurally, air/water syringes are similar to hypodermic needles. They have two long fine metallic lumen; one which administers air and the other water. There have been several studies of the bacterial contamination of, and biofilm formation on, dental water lines,[13,14] but very few studies have investigated the microbial contamination of air/water syringes. It has been shown the internal fine lumen of the air/water syringe tip are difficult to clean[4] and are contaminated after use.[15,16] Only two studies have attempted to measure the efficacy of standard sterilization on the microbial contamination of air/water syringes. One found conventional methods to be adequate,[17] the other found them to be inadequate.[18] Neither study quantified the amount of contamination on the syringes. The New Zealand Dental Association control of cross-infection in dental practice code of practice[2] states that any instrument that is incapable of being effectively sterilized for reuse must be single-use and disposable. The question addressed in this study was whether multiple-use air/water syringes retain infectious microorganisms after sterilization.

MATERIALS AND METHODS

Instruments investigated

The efficacy of the sterilization of non-disposable syringe (NDS) tips was evaluated by measuring bacterial contamination of the syringes after usage in the clinic and standard sterilization, and comparing this to the contamination detected on single-use disposable syringe (DS) tips (n = 8). Two factors that may affect the retention of viable microorganisms by re-used tips are the age of the syringe and whether or not the syringe is flushed prior to sterilization. Therefore, the bacteria associated with syringes in current clinical usage (old non-disposable syringe (ONDS) tips, n = 34) was compared with those associated with syringes that had only been used once (new non-disposable syringe (NNDS) tips, n = 34), and half of each group of syringes was flushed with water for 30 seconds with air and water after patient treatment. The required sample size was estimated using previous data on syringe tip contamination risk[16] and setting type I error at 0.05 and type II error at 0.2 (i.e. 80% power).

All syringes that were examined had been used in the mouth either as part of an examination or as part of dental treatment in the UCU (urgent care unit) at the University of Otago School of Dentistry. ONDS tips were randomly selected from existing syringe tips in the UCU while NNDS tips were new and sampled after their first use in the UCU. DS tips sterilized with ethylene oxide by the manufacturer were obtained from the Acteon Company, Bordeaux, France.

Microbiological methods

After standard sterilization procedures (a decontamination wash then steam sterilization at 135 °C for

4 minutes with a Class B cycle autoclave (AE Atheron & Sons Pty Ltd, Victoria, Australia), syringes were sampled to enumerate viable bacteria. First, the syringe tips were placed in a sterile test tube containing 12.7 mL of sterile phosphate buffered saline (PBS) solution (to completely cover the tip). The lumen of each syringe tip was then flushed with the saline solution using a sterile 6 cc endodontic syringe and a 27G 11/4 inch needle. This was to remove air and dislodge any bacteria from the tip lumen. The tips were then sonicated in an ultrasonic bath for 10 minutes to aid dislodgement of material from the syringe surfaces. After sonication, the tips were flushed again with the surrounding PBS using new sterile syringes and needles. Each test tube was then placed separately on a vortex mixer for 7 seconds to evenly distribute microorganisms within the saline solution. Immediately after vortexing, 0.2 mL samples were plated on three separate PCA (plate count agar) (which is commonly used to measure bacterial contamination of water and the environment[15]) and three separate Columbia sheep blood agar plates (which is a rich medium supporting the growth of a variety of bacterial species) using sterile laboratory techniques. The remaining solution in each test tube was then placed in a centrifuge at 3214 x *g* for 10 minutes. The supernatant was discarded and the pellet resuspended in 0.1 mL PBS and plated onto a Columbia sheep blood agar plate. All plates were incubated at 37 °C and colonies counted after 48 hours. DS tips (n = 8) were treated in the same way as the sterilized non-disposable tips. As a control for aseptic technique, tubes containing PBS but without air/water syringes were treated in a similar fashion.

Assessment of corrosion

The presence of corrosion or contaminant build-up in syringe lumen was evaluated by topographical and chemical analysis. A JEOL JSM-6700F field emission SEM (JEOL Ltd, Tokyo, Japan) was used to capture topographical images of the water lumen surface structure for two NNDS and two ONDS. All syringes were cut in cross-section and transverse section to gain access to the internal water lumen surface. Accelerating voltages of 5 kV and 15 kV were used with the SEM. Chemical analysis was conducted with a JEOL 2300F EDS system (Electron-Dispersive Spectroscopy) (JEOL Ltd, Tokyo, Japan). Data were acquired using an accelerating voltage of 25 kV. The areas analysed were topographical irregularities (protrusions and indentations) as identified through the SEM imaging. EDS analysis was also carried out on a dried water sample collected from a dental air/water hand-piece (without the air/water syringe attached) that was connected to the common dental water supply.

Descriptive statistics were calculated using SPSS version 20.0 (SPSS Inc, Chicago, USA). Bacterial colony numbers were firstly analysed using a two-way analysis of variance (ANOVA). Because the colony numbers were highly skewed, non-parametric Mann–Whitney test and Moses test were also used for comparing medians and ranges, respectively. The alpha value was set at $p < 0.05$.

RESULTS

ND sterilized air/water syringe tips were sampled for microbial contamination. Samples were plated on both PCA and Columbia sheep blood agar. The number of colony-forming units (cfu) on both types of agar were counted for each syringe and the total number of bacteria found on each syringe calculated. The mean level of contamination for ONDS and NNDS, flushed and non-flushed, was calculated (Table 1).

The eight control tubes which did not contain air/water syringes yielded two bacterial colonies on one agar plate (mean = 0.25 cfu/syringe). This indicated that despite aseptic technique being employed, some environmental contamination of experimental samples was experienced. The sampling of eight DS tips yielded only one bacterial colony (mean = 0.125 cfu/syringe). This is a lower level of contamination than found with the control tubes and was probably due to environmental contamination.

All four groups of NDS tips had similar ranges of contamination (Table 1). However, the ranges of bacterial contamination of the NDS tested under different conditions were significantly different from that detected with the control tubes and from that detected with the DS tips (Moses tests: $p < 0.001$). Thus, the NDS had significantly more contamination than the DS.

The NNDS did not appear to have less contamination compared to ONDS and flushing did not appear to reduce contamination (ANOVA: $F \leq 2.2$; $p \geq 0.14$). Furthermore, there was no significant interaction between the age of the syringe and the flushing

Table 1. Bacterial colony-forming units (cfu) associated with new non-disposable syringes (NNDS), old non-disposable syringes (ONDS), single-use disposable syringe tips (DS), and in syringe-free controls

Sample	Range of cfu/syringe	Mean cfu/syringe (± SD)
NNDS non-flushed (n = 17)	0 – 30.4	7.6 ± 11.5
NNDS flushed (n = 17)	0 – 24.3	4.9 ± 8.9
ONDS non-flushed (n = 17)	0 – 21.0	2.6 ± 6.8
ONDS flushed (n = 17)	0 – 21.2	3.7 ± 6.8
DS (n = 8)	0 – 1.0	0.13 ± 0.4
Syringe free control (saline) (n = 8)	0 – 2.1	0.3 ± 0.7

(ANOVA: F = 0.8; p = 0.38). When the data were grouped according to NNDS versus ONDS and non-flushed versus flushed syringes, there were no statistically significant differences between the levels of contamination (Table 2).

The lumen of NNDS and ONDS were examined with SEM and EDS to assess the amount of corrosion and contaminant build-up as this might affect the likelihood of viable microorganisms remaining after sterilization cycles. The SEM imaging showed that the water lumen surface of the ONDS had more spherical protrusions and indentations than the NNDS (Figs. 1 and 2).

SEM images from both NDS tips types showed similar underlying vertical lines which were likely due to the laminating process of the syringe construction.

Areas of the lumen of the syringe water channels that showed signs of corrosion and contaminant build-up were analysed by EDS. These areas on the NNDS yielded elements of carbon, copper, zinc and oxygen (Table 3). Areas of corrosion and contaminant build-up on the ONDS showed additional elements of sulphur, silver, tin, mercury and silicon (Table 3). EDS of areas unaffected by corrosion or contaminant build-up in both NNDS and ONDS tips indicated the air lumen to consist of copper and zinc while the water lumen consisted of copper. EDS analysis of the dried water sample gathered from a dental unit revealed high amounts of carbon, silicone, sulphur and calcium.

Table 2. Comparison of contamination of new non-disposable syringes (NNDS) versus old non-disposable syringes (ONDS) and non-flushed versus flushed air/water syringe tips

Syringe type	Mean cfu/ syringe (SE)	P value[#]
NNDS (n = 34)	6.3 (1.7)	0.522
ONDS (n = 34)	3.1 (1.2)	
Non-flushed (n = 34)	5.1 (1.7)	0.460
Flushed (n = 34)	4.3 (1.3)	

[#]Mann–Whitney U-test.

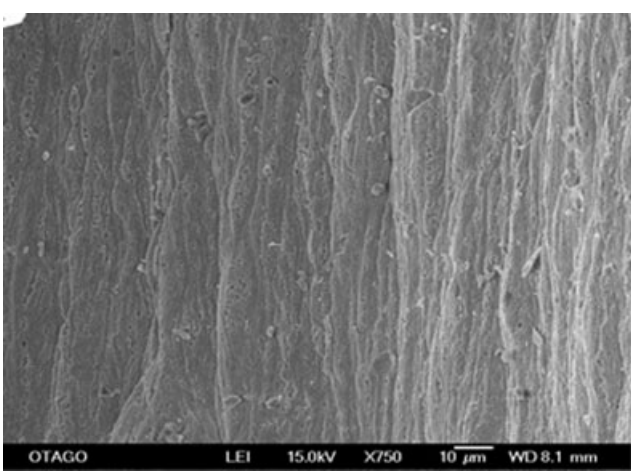

Fig. 1 SEM image showing the surface topography of the water lumen of an NNDS tip.

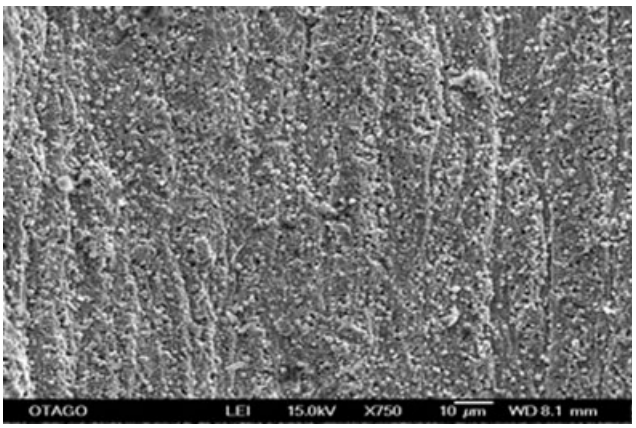

Fig. 2 SEM image showing the surface topography of the water lumen of an ONDS tip.

Table 3. Elements present in suspected areas of corrosion or contaminant build-up within the water lumens of two new non-disposable syringes (NNDS) and two old non-disposable syringes (ONDS)

Element	NNDS 1 (%)	NNDS 2 (%)	ONDS 1 (%)	ONDS 2 (%)
Carbon	32	15	14	43
Oxygen	35	26	14	14
Copper	22	43	31	23
Zinc	10	15		
Sulphur			2	2
Silicone				18
Tin			3	
Mercury			25	
Silver			11	

DISCUSSION

This study investigated whether NDS tips retain infectious microorganisms after standard sterilization procedures, and whether corrosion of the syringe lumen contributes to microorganism retention. The investigation of microbial contamination had three hypotheses. Firstly, that the standard cleaning and autoclaving processes would not be sufficient to kill all the bacteria in NDS. Secondly, that NNDS used only once in the clinic and cleaned through one cycle of sterilization would show less contamination compared to syringes placed through many patient uses and cycles of sterilization (ONDS). Thirdly, that NDS flushed after usage would show less contamination than non-flushed NDS.

All NDS tips had levels of contamination significantly higher than background sterility controls and

more contamination than DS tips. This indicates that some bacteria which enter NDS are able to survive the sterilization process of a class B cycle autoclave and therefore supports the hypothesis that the standard cleaning and autoclaving processes are not sufficient to kill all the bacteria in all NDS tips. It should be noted, however, that of the 68 NDS investigated no bacterial contamination could be detected on 40 (59%). The detection of bacteria on sterilized NDS confirms an earlier report in which 50% of sterilized air/water syringes were found to contain viable bacteria.[16] This previous study, however, only examined 16 ONDS and was only semi-quantitative – it did not measure the number of viable bacteria on the syringes.[16]

The effect of undertaking the recommended flushing procedure at the end of the clinical session on contamination of the syringe was measured. Flushing involved spraying air and water simultaneously through the air/water syringe tip for 30 seconds prior to cleaning and sterilization. There was no statistically significant difference between contamination levels of flushed and non-flushed air/water syringes. Despite these results flushing of air/water syringes is still recommended as, unless the precautionary measure of using DS tips is employed, it will help dislodge potentially infectious foreign material from the lumens of the NDS tips. A possible source of the microbial contamination of the syringes is the dental water lines. A previous study of the water from the water lines of 12 dental chairs from two clinics at the University of Otago School of Dentistry showed that the mean bacterial concentration was ~300 cfu/ml (Cannon RD, unpublished results). Thus, the level of bacterial contamination of air/water syringes observed in this study (4.7 cfu/syringe) is small compared to the number of bacteria coming through the dental water lines. It should be noted that this study focused on the wide range of bacteria that grow on PCA and blood agar. It remains to be determined whether fungi and viruses on air/water syringes also survive routine sterilization processes.

The effect of the number of sterilization cycles on the bacterial contamination of NDS was also investigated. NNDS tips that had been used once on a patient and exposed to one cycle of the sterilization processes were compared to syringes (ONDS) that had been in use in the dental school for several years with many uses and sterilization cycles (it was not possible to determine the number of sterilization cycles for these syringes). Again there was no statistically significant difference between the contamination levels of NDS tips used once and those used many times. However, differences between NNDS and ONDS were found through SEM and EDS analyses.

The SEM results showed ONDS to have a much more irregular water lumen surface compared to NNDS. This indicates either corrosive breakdown (and subsequent pitting of the metallic surface) or contaminant build-up within the lumen, or both. This is supported by research that has shown that the pitting of metallic surfaces is a typical feature of corrosion[19] and that contaminant build-up on metallic surfaces often presents as spherical masses.

The EDS analysis showed that both NNDS tips and ONDS tips contained the expected metallic elements copper and zinc. Copper is very resistant to corrosion. However, it has been shown that upon exposure to humidity, the rate of corrosion increases with increasing zinc content.[20] As both the clinical use and the sterilization processes expose air/water syringes to high levels of humidity, corrosion is likely.

In addition to these expected elements, other elements were found in areas of corrosion or contaminant build-up within the ONDS tips. These included high levels of silicone and sulphur. An EDS of water gathered directly from a dental unit revealed high amounts of carbon, silicone, sulphur and calcium. These findings suggest there was retention of water components within the syringe lumen. Retention of water is typical of long structures with small lumen diameters due to capillary action. The retention of liquid within metallic devices, or stagnation, leads to a reaction with the metal and results in corrosion.[19] The ONDS also contained the elements mercury, silver and tin. These were not found in the water EDS but are common elements in dental amalgam, which is used in the dental school clinics. This demonstrates how materials from the mouth can contaminate syringe tips through capillary action. Thus, there is the potential for blood cells and blood-borne viruses to be drawn into the syringe lumen and beyond. Most importantly, it demonstrates that cross-infection is indeed possible. The retention of materials such as amalgam in the ONDS not only potentially increases the health risk of mercury exposure, but also poses a problem for 'non-mercury' dental practitioners. There is a risk in joint or large practices and hospitals where some practioners use amalgam and some do not, that practitioners who only do non-amalgam work may still expose patients to mercury by employing ONDS tips used by other practitioners. The increased surface roughness and additional elements found in the ONDSs suggests that prolonged use of NDS tips can lead to corrosion and contaminant build-up. Areas of corrosion and contaminant build-up are of concern as they create a roughened surface that facilitates the adhesion of bacteria. Although the sterilized ONDS did not show significantly greater contamination than the NNDS, if the sterilization process failed, biofilms associated with the older syringes may pose a greater contamination risk than newer syringes.

In conclusion, the results from the bacterial growth analyses suggest that there is a low but extant risk of cross-infection from the use of NDS tips as even the class B cycle autoclave was unable to sterilize the narrow lumen of these devices. Although the research did not demonstrate contamination by particular pathogenic organisms, it did demonstrate that this is indeed possible as bacterial contamination was detected. To reduce the risk of infection from the air/water syringe tips, DS could be used. Negligible contamination was detected on sterile DS tips provided by the manufacturer. Thus, there is a possible benefit from the use of DS but attention should also be given to the quality of the water provided by the dental water lines.

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