Effective cleaning and decontamination of the internal air and water channels, heads and head-gears of multiple contra angle dental handpieces using an enzymatic detergent and automated washer disinfection in a dental hospital setting

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ABSTRACT

Background: Dental handpieces (DHPs) are reusable invasive medical devices that must be cleaned, decontaminated, lubricated and steam sterilized after use. DHPs have a complex internal design including narrow channels, contamination of which can compromise sterilization. DHPs are not designed for routine disassembly, making cleaning/decontamination efficacy difficult to monitor. Washer-disinfection is the preferred method of decontaminating DHPs, but few studies have investigated its direct effectiveness at reducing microbial contamination internally.

Aims: To use contra angle DHPs as a model system to investigate the effectiveness of washer-disinfection at reducing microbial contamination of internal components of multiple DHPs.

Methods: The air and water channels and heads of 10 disassembled contra angle DHPs (BienAir, Switzerland) were inoculated separately with 10^8 colony forming units (CFU) of *Pseudomanas aeruginosa*, *Staphylococcus aureus, Enterococcus hirae* or *Candida albicans* in the presence of 0.3% bovine serum albumin (BSA) (clean conditions), 3.0% BSA or 10% artificial test soil (dirty conditions). After reassembly, all 10 DHPs underwent washer-disinfection simultaneously in a Míele (Míele Ltd., Ireland) PG8528 washer-disinfector and were tested for reductions in microorganisms and protein. Additional experiments were undertaken with three lubricated DHPs inoculated with *S. aureus* and 10% test soil. All experiments were repeated in triplicate.

Findings: On average an approximate 5 log or greater reduction in microbial CFUs and a >93% reduction in protein from DHP heads and channels was consistently recorded following washer-disinfection for all DHPs under all conditions tested.

Conclusions: The internal components of multiple DHPs can be effectively cleaned and decontaminated by washer-disinfection.

Keywords: Contra angle dental handpiece, washer disinfector, decontamination, water and air channels, dental handpiece heads and gears, process challenge microorganisms, *Staphylococcus aureus*, *Enterococcus hirae, Pseudomonas aeruginosa, Candida albicans,* handpiece lubrication

Introduction

Dental handpieces (DHPs) are among the most frequently used instruments in dentistry. DHPs are reusable invasive medical devices and must be cleaned, decontaminated, lubricated and sterilized after use[1,2]. There are three basic types of DHPs including conventional or slow speed (contra angle and straight), high speed turbine and surgical. DHPs are provided with compressed air and water supplies from the dental chair unit via a flexible hose. Compressed air is used to drive the air rotor of high-speed DHPs. In conventional DHPs, the movement of dental burs is mechanically transmitted through shafts and gears and is initiated by an electric or air powered motor. In conventional DHPs compressed air is used to cool the gears. Dental unit waterlines (DUWLs) provide water to cool and irrigate tooth surfaces as the heat generated during DHP use can harm dental pulp[3,4].

The internal components of DHPs are complex and consist of narrow water and air channels, the drive rotor, and the shafts and gears in slow-speed DHPs. DHPs become contaminated externally and internally during use[5-11]. Contamination can originate from DUWLs, the compressed air supply and from the oral cavity[3,9]. DUWLs are prone to contamination with microbial biofilm from microorganisms in the supply water and from retraction of oral fluids into DHPs during use[3,4]. Smith et al. demonstrated that the internal components of DHPs are frequently contaminated with human-derived proteins[11]. The external surfaces of handpieces get contaminated with oral fluids and tissue fragments during use, all of which harbour oral microorganisms.

Current guidelines stipulate that DHPs should be decontaminated and sterilized between patients by steam sterilisation using a vacuum autoclave that has been commissioned appropriately and its various cycles validated independently[2]. The efficacy of steam sterilization can be compromised by organic material and therefore it is vital that DHPs are adequately cleaned prior to sterilization. The external surfaces of DHPs are commonly decontaminated by manually wiping with a cleaning solution followed by visual inspection[2,5,9]. The external surfaces can also be cleaned and thermally disinfected in a washer disinfector[11-13]. ISO-15883 details several methods for assessing the surface cleanliness of reusable medical devices following washer-disinfection; however, there is no specific procedure for evaluating the efficacy of washer-disinfection for the internal components of DHPs[14].

Cleaning and decontaminating the internal components of DHPs is challenging because of their complex construction and because they are not designed for routine disassembly to ensure that internal components are free of contamination[11].

Several manufacturers of devices developed to clean DHPs claim that their equipment can ensure adequate cleaning, however little independent direct evidence is available[12]. Spraying a cleaning solution into the channels and transmission components is one of the most widely used approaches to cleaning and disinfection of the internal elements of DHPs. Cleaning fluids often contain alcohols that denature proteins, which are very difficult to remove from metal surfaces[15,16]. Furthermore, the process is very difficult to validate because of the inaccessibility of the internal components of DHPs. One study demonstrated that the use of 70% alcohol to disinfect the external surface of high-speed DHPs was ineffective[16]. DHPs are not suitable for immersion in disinfectants, which can lead to metal corrosion[12].

Washer-disinfection is a reproducible process that can be validated for the external components of medical devices and is the preferred method of cleaning and decontaminating DHPs[17]. Washer disinfectors are not mandatory for dental practices in all countries[17-20]. Some studies demonstrated the effectiveness of washer disinfectors at cleaning the outside surfaces of DHPs and a few have demonstrated its efficacy at reducing organic contamination on internal components[11,21]. However, little published data is available on the direct effectiveness of washer disinfectors at significantly reducing microbial contamination from the internal components of DHPs, especially in a dental hospital setting where large numbers of DHPs must be decontaminated daily.

The purpose of this study was to use contra angle DHPs as a model system to directly investigate the effectiveness of washer-disinfection at reducing microbial bioburden of internal components of multiple DHPs deliberately contaminated with each of four challenge microorganisms in the presence/absence of organic soil in a dental hospital central decontamination unit.

Methods

Dental handpieces

BienAir Dental SA (Biel/Bienne, Switzerland) CA 1:1 L contra angle DHPs were used throughout this study and were never used for patient treatment. These DHPs consist of a head, a neck, and a sheath (Figure 1). The head houses the head gear which contains a dental bur orifice. Burs are held in place by a latch grip integrated in the head gear.

The back of the head unit is sealed by a push button plate (Figure 1). The head gear drives the dental bur during operation and is driven by the middle gear located in the neck of the DHP (Figure 1). These DHPs are supplied with compressed air and water and they contain narrow air

and water channels and are powered by an electric motor attached to a flexible arm connected to a dental chair unit (DCU). Three pairs of small water and air outlets surround the dental bur orifice in the DHP head (Figure 1). At the Dublin Dental University Hospital (DDUH) compressed air is provided to each DCU from a central source. Water containing very low levels of microorganisms is provided to DUWLs from a central supply treated continuously with residual electrochemically-generated hypochlorous acid[22].

One of the researchers was trained to disassemble and reassemble the DHPs for microbial inoculation and recovery experiments.

Washer disinfector

A Míele (Míele Ireland Ltd., Dublin, Ireland) PG8528 washer disinfector was used throughout this study. In the DDUH the equipment is connected to a variable-speed water pump that modulates between 1-5 bar. The equipment is fitted with Míele E919 dental modules for cleaning and decontaminating DHPs (Figure 2a). Up to six modules can be accommodated in the washer, each containing adapters for 10 DHPs (Figure 2b). W&H (W&H, Bürmoos, Austria) A803 DHP adapters (Figure 2a) were used throughout the study. The enzymatic detergent Endozime® Xtreme Power (0.1% v/v) (The Ruhof Corporation, Mineola NY, USA) was used for all washer-disinfection experiments.

The parameters for each washer-disinfection cycle were as follows: (i) prewash with mains water at 22ºC for six min, (ii) cleaning with enzymatic detergent at 55ºC for eight min, (iii) rinsing with reverse osmosis purified water for five min, (iv) thermal disinfection at 92ºC for two min and (v) drying 25 min.

Challenge microorganisms

The three bacterial strains used as process challenge microorganisms are those specified in BS-EN-14561:2006[23] including *Pseudomanas aeruginosa* ATCC15442, *Staphylococcus aureus* ATCC6538 and *Enterococcus hirae* ATCC10542. The laboratory yeast strain *Candida albicans* SC5314 (ATCCMYA-2876) was also used[24]. All strains were purchased from the American Type Culture Collection and were used separately to inoculate DHP air and water channels and heads/head-gears to monitor the decontamination efficacy of washer disinfection. To prepare challenge inocula, bacterial strains were cultured on tryptone soya agar (TSA) (Oxoid Ltd./ThermoFisher Scientific., Basingstoke, UK) at 37ºC for 24 h and a single colony was inoculated into 25 ml of tryptone soya broth (Oxoid) in a 250 ml conical flask and grown at 37ºC

in a shaking incubator at 200 rpm to 10^9 colony forming units (CFU)/ml. *Candida albicans* strain SC5314 was cultured on YPD agar (MP Biomedicals, Ohio, USA) at 30°C for 24 h and a single colony was inoculated into 25 ml of YPD broth (MP Biomedicals) in a 250 ml conical flask and grown in a 30°C shaking incubator at 200 rpm to 10^9 CFU/ml.

Recovery of microorganisms from DHP channels and heads/head-gears

Before each experiment, DHPs were sterilized in a vacuum steam sterilizer at 134°C. Prior to inoculation, the small press button plate sealing the DHP head was removed, followed by removal of the head, head gear and middle gear, providing access to the openings of the air and water channels (Figure 1). The partially disassembled DHP was then positioned horizontally and 100 µl of culture inoculum supplemented with 0.3% (w/v) bovine serum albumin (BSA) (clean conditions), 3.0% (w/v) BSA (dirty conditions) or 10% artificial test soil (dirty conditions) (Edinburgh test soil, Cúram Medical, Dublin, Ireland, compliant with ISO-15883-5-2021[25]) was inoculated into both channels using an 0.3 ml insulin syringe with a 30 gauge Micro-Fine™ needle (Becton Dickson and Company, NJ, USA) and allowed to dry for 30 min. The angle in the body of the DHP ensured the head and neck were horizontal, permitting retention of the inocula in the channels. After drying, the inoculated channels were sampled by inserting sterile, tapered periopoints (02 Absorbent Points, Dentsply Sirona, Charlotte, NC). Periopoints are used for sampling periodontal pockets and are ideal for sampling narrow lumens[26]. Periopoints were placed in 1 ml phosphate buffered saline (PBS) (Oxoid) in a sterile 1.5 ml tube and vortexed for 1 min to release microorganisms. Serial dilutions were prepared in PBS and 100 µl aliquots spread in triplicate onto TSA agar for bacteria and YPD agar for *C. albicans* and incubated as described above. Following incubation, the bacterial/yeast colonies were counted and the total number of bacteria/yeasts recovered from the channels determined.

For each challenge microorganism, 100 µl of culture inoculum supplemented with 0.3%, 3.0% BSA or 10% test soil was inoculated into the head of a non-disassembled DHP placed horizontally through the dental bur orifice and allowed to dry for 30 min. The DHP head was then aseptically removed and the press button plate, the head gear and the head were placed in 5 ml of PBS in a sterile 25 ml tube and agitated for 1 min to release bacterial/yeast cells into solution (Figure 1). Serial dilutions were prepared in PBS and 100 µl aliquots plated in triplicate on TSA/YPD media and the total number of bacteria/yeasts recovered from the DHP head, head-gear and button determined.

Additional experiments were undertaken with four DHPs that were lubricated with W&H (Austria) Service Oil F1 MD-500 using an Assistina 301 plus DHP maintenance unit (W&H, Austria) according to the manufacturer's instructions prior to sterilization at 134ºC. Then the heads and channels of three DHPs were inoculated with *S. aureus* ATCC6538 in the presence of 10% test soil as described above, followed by reassembly of the DHPs and washer-disinfection. The fourth DHP was retained as a control. Following washer-disinfection, the DHPs were disassembled and the reduction in bacterial counts and protein recovered from DHP head/head-gears and channels calculated relative to the control DHP. Experiments were repeated on three separate occasions.

Microorganism counts in DHP channels and heads/head-gears following washer-disinfection

Each challenge microorganism preparation was inoculated separately into the heads and channels of 11 DHPs as described above. After drying, DHPs were reassembled and 10 were subjected to a washer-disinfection. The remaining inoculated DHP served as a control. Following washer-disinfection, all 11 DHPs were disassembled, sampled as described above and the log reduction in bacterial/yeast counts calculated relative to the control inoculated DHP in each case. Experiments were repeated in triplicate with all 10 DHPs for each challenge organism under clean (0.3% BSA) and two sets of dirty conditions (3.0% BSA and 10% artificial test soil).

Protein Assay

Inoculated DHP heads/rotors and channels were tested for residual protein following washer-disinfection. Tests were undertaken on samples recovered as described above. Protein was detected using the QuantiPro BCA assay kit (Sigma-Aldrich/Merck, Arklow, Ireland) according to the manufacturer's instructions. The relative reduction in protein in DHP channels and heads/head-gears from washer-disinfected DHPs was determined relative to unwashed controls.

The external surfaces of 10 DHPs were painted with 10% test soil and left to dry for 30 min followed by washer-disinfection. One additional painted DHP was retained as a control. The DHPs were visually inspected for residual test soil immediately following washer-disinfection and the surfaces were swabbed with sterile swabs soaked in 1% (w/v) sodium dodecyl sulphate (pH 11.0) and tested for protein using the QuantiPro BCA assay kit. Surfaces were also tested using the Pyromol-Test for residual protein (PEREG GmbH, Waldkraiburg, Germany) according to the manufacturer's instructions.

Results

Decontamination of DHP internal components by washer-disinfection

The internal surfaces of the head, press button plate and head-gear (all three hereafter referred to as the head) and air and water channels of contra angle DHPs were used as a model system for monitoring the efficacy of decontamination by washer-disinfection. The internal surfaces of 11 DHP heads and both channels were inoculated with one of four challenge microorganisms under clean and two sets of dirty conditions. Ten of the inoculated DHPs were then inserted into a Míele E919 dental module (Figure 2a) and subjected to washer-disinfection (see Methods). The remaining DHP in each case acted as a control to establish a baseline for recovery of microorganisms and protein in the absence of washer-disinfection. Experiments were undertaken in triplicate for each DHP under each set of conditions. Following washer-disinfection, DHPs were disassembled, and the head and channels sampled for microorganisms and residual protein. During each washer-disinfection cycle, the same DHP was consistently placed in the same position in the Míele E919 dental module (Figure 2a).

Reduction in microbial bioburden in inoculated DHP heads and channels

For each of the three challenge bacterial strains tested under clean conditions (0.3% BSA), on average an approximate 5 log or greater reduction in bacterial colony forming units (CFUs) recovered from DHP heads and channels was observed consistently for all 10 DHPs tested (Table I). Similar reductions were observed under both sets of dirty conditions. The average log reduction in *S. aureus* CFUs from DHP heads was 5.27±0.23 (3% BSA) and 5.11±0.58 (10% test soil) and from channels was 5.57±0.14 (3% BSA) and 5.59±0.16 (10% test soil). The average log reduction in *E. hirea* CFUs from DHP heads was 5.32±0.38 (3% BSA) and 5.37±0.08 (10% test soil) and from channels was 5.48±0.18 (3% BSA) and 5.58±0.13 (10% test soil). The average log reduction in *P. aeruginosa* CFUs from DHP heads was 6.07±0.05 (3% BSA) and 5.57±0.48 (10% test soil) and from channels was 5.87±0.22 (3% BSA) and 5.72±0.33 (10% test soil).

In the case of the *C. albicans* strain, on average an approximate 5 log reduction in CFUs recovered from DHP heads was recorded under clean conditions (0.3% BSA) (average 5.25±0.36) with a slightly lower log reduction recorded for DHP channels (average 4.95±0.23) (Table I). Similar results were obtained for DHP heads and channels under both sets of dirty conditions with an average log reduction in *C. albicans* CFUs from DHP heads of 5.06±0.22 (3% BSA) and 5.03±0.25 (10% test soil) and from channels of 4.93±0.09 (3% BSA) 4.97±0.43 (10% test soil).

For all four challenge microorganisms used under clean or dirty conditions, consistent log reductions in microbial count were recovered for all 10 DHPs, regardless of their position in the Míele dental module during washer-disinfection (Figure 2a). During washer-disinfection DHP1 was positioned closest to the water inlet, where the water pressure is at its highest, whereas DHP10 was furthest away (Figure 2a, Table I).

Influence of DHP position in the washer-disinfector on microbial burden reduction

Experiments were undertaken with seven DHPs inoculated with the *S. aureus* challenge microorganism and 10% test soil. Following inoculation and reassembly, one DHP was placed at position 10 (Figure 2) in each of six separate Míele E919 dental modules and subjected to washer-disinfection. The remaining DHP served as a control. Following washer disinfection, the DHPs were disassembled and tested for microorganisms and protein. All experiments were repeated three times. For each of the six DHPs, an average of >5 log reduction in *S. aureus* CFUs recovered from DHP heads and channels was observed relative to control DHPs, regardless of which of the six washer-disinfection dental modules was used (Table SII, Figure 2b).

Influence of DHP lubrication on microbial bioburden reduction

Three DHPs were lubricated using the Assistina 301 plus automated system prior to sterilization and inoculation of the heads and channels with *S. aureus* ATCC6538 in the presence of 10% test soil followed by washer-disinfection. One additional lubricated and inoculated DPH served as a control. Following washer-disinfection, the log reduction in bacterial CFUs from heads and channels was calculated relative to the control DHP. In three separate experiments, the average log reduction in bacterial CFUs was 5.96±0.1 (heads) and 5.69±0.2 (channels).

Reduction in protein in DHP heads and channels

For each of the 10 DHPs inoculated with 10% test soil in the absence of challenge microorganisms, on average a >95% reduction in protein recovered from DHP heads and channels was observed following washer-disinfection relative to unwashed inoculated control DHPs (Table SI). Similar reductions in protein levels in heads and channels were obtained with DHPs inoculated with challenge microorganisms under both sets of dirty conditions following washer-

disinfection (Table SI). No protein was detected in heads and channels inoculated under clean conditions following washer-disinfection (data not shown).

The average reduction in protein from the 10 DHP heads and channels inoculated with *S. aureus* and (i) 3% BSA was 97.6±1.9% and 94.9±1.4%, respectively, and (ii) 10% test soil was 98.6±0.5% and 92.7±1.8%, respectively. The average reduction in protein from the 10 DHP heads and channels inoculated with *E. hirea* and (i) 3% BSA was 99.2±0.1% and 93.6±2.4%, respectively, and (ii) 10% test soil was 99.4±0.3% and 93.1±3.1%, respectively. The average reduction in protein from the 10 DHP heads and channels inoculated with *P. aeruginosa* and (i) 3% BSA was 98.5±0.3% and 98.6±0.6%, respectively, and (ii) 10% test soil was 97.9±1.4% and 94.2±5.1%, respectively. The average reduction in protein from the 10 DHP heads and channels inoculated with *C. albicans* and (i) 3% BSA was 98.8±0.2% and 96.3±0.3%, respectively, and (ii) 10% test soil was 99.4±0.1% and 93.2±5.4%, respectively.

For all four challenge microorganisms used under both sets of dirty conditions, consistent reductions in protein levels in heads and channels were observed for all 10 DHPs tested regardless of their position in the dental module used to retain the DHPs in the washer disinfector (Figure 2a). Similar results were obtained with the six DHPs inoculated with *S. aureus* and 10% test soil placed at position 10 in each of six separate Míele E919 dental modules (Table SII, Figure 2).

In the case of the three DHPs that were lubricated with oil and sterilized prior to inoculation with *S. aureus* and 10% test soil followed by washer-disinfection, an average of 99.69%±0.1% and 98.98%±0.3% reduction in protein was recorded for DHP heads and channels, respectively, on three separate occasions relative to inoculated but unwashed controls.

Test soil removal from the outside surfaces of DHPs by washer-disinfection

The exterior surfaces of 10 DHPs that were painted with 10% test soil and left to dry were free from visible contamination following washer-disinfection. All the DHPs were negative for residual protein using the Pyromol-Test. There was a 99.98%±0.02% reduction in protein on the DHP surfaces using the DHPs QuantiPro BCA assay relative to controls.

Discussion

Oral biomaterial and microorganisms can be retracted into DHPs during use and contaminate internal components[10,27,28]. Microbial biofilm in DUWLs provides an additional source of contamination[3,4]. The use of validated automated washer-disinfectors is currently the

gold standard for cleaning and decontaminating dental instruments. A number of studies have shown that washer-disinfection is effective at cleaning the exterior of DHPs and a few have shown its efficacy at reducing organic contamination on internal components[11,21]. However, there is very little published data on the direct efficacy of washer disinfectors at significantly reducing microbial bioburden from the internal channels and other components of DHPs, mainly due to difficulties in accessing the internal components, as DHPs are not designed to be routinely disassembled. The present study set out to address this deficit by deliberately inoculating the channels and heads of multiple contra angle DHPs four challenge microorganisms under clean and dirty conditions and monitoring the reduction in microbial bioburden and protein following washer-disinfection. The very high densities of challenge microorganisms inoculated into the DHPs was deliberately far in-excess of the levels of microorganisms contaminating the internal components of DHPs following clinical use. Sterilized DHPs were disassembled to facilitate inoculation of the internal components followed by reassembly, washer-disinfection, disassembly and sampling for microorganisms and residual protein.

An approximate five log reduction in *S. aureus*, *E. hirea* and *P. aeruginosa* CFUs recovered from DHP heads and channels was consistently observed for all 10 DHPs tested following washer-disinfection under clean and both sets of dirty conditions (Table I). On average a >93% reduction in protein was recorded for DHP heads and channels under all test conditions (Table SI). Similar reductions in microbial CFUs and protein were obtained with *C. albicans* SC5314 (Tables I and SI). Similar reductions in microbial CFUs and protein were recorded for all 10 DHPs regardless of each DHP's position in the module holding the DHPs during washer-disinfection (Figure 2, Tables I & SI). DHP10, which was furthest away from the water inlet in the washer-disinfector module, yielded similar results to DHP1 (closest to the water inlet). A series of experiments with six DHPs in which the channels and heads were inoculated with *S. aureus* and 10% test soil were undertaken with six separate dental modules, with each DHP located at position 10 (i.e., furthest away from the water inlet of each module) (Figure 2) followed by washer-disinfection. In each case, a >5 log reduction in bacterial count and a >93% reduction in protein recovered from heads and channels was consistently recorded, regardless of dental module (Table SII). Only one of the 10 adapters for DHPs was occupied in each of the six dental modules used and water freely discharged from the nine unoccupied adapters in each module during washer-disinfection.

All these findings demonstrated that the Míele PG8528 washer disinfector with the enzymatic detergent used was consistently effective at significantly reducing microbial and protein contamination of internal components and channels of multiple DHPs simultaneously. Up

to 60 individual DHPS can be decontaminated simultaneously using the Míele PG8528 washer disinfector, which is ideal for dental hospitals where large numbers of DHPs must be decontaminated daily.

The internal components of DHPs must be lubricated regularly. Winter et al.[29] postulated that the presence of lubricating oil in DHPs can be detrimental to the efficacy of steam sterilization. To determine if the presence of lubrication oil in DHPs affected the efficacy of washer-disinfection at significantly reducing microbial counts and protein levels in DHPs, three DHPs were lubricated with maintenance oil prior to sterilization followed by inoculation of both channels and heads with *S. aureus* ATCC6538 in the presence of 10% test soil. In three separate experiments with the three DHPs, a >5 log reduction in bacterial CFUs and a >98% reduction in protein was recorded for both DHP heads and channels. These findings demonstrate that oil lubrication of DHPs did not adversely affect decontamination of internal components of DHPs by washer-disinfection, at least under the conditions used. Winter et al.[31] commented that many dentists use spray cans to lubricate DHPs and that if they are used incorrectly, oil will be located throughout the internal surfaces and channels, which is a challenge for steam penetration. In the present study, lubrication was undertaken with the Assistina 301 plus automated system, which ensures correct lubrication of DHPs.

Limitations

The study was limited to contra angle DHPs. The internal design of turbine DHPs is different, as they lack gears and usually have less bearings. Because of the higher rotational speeds of turbine DHPs, there are greater opportunities for suck-back via the bur orifice when the devices are stopped resulting in internal contamination. Nonetheless, the internal architecture of the contra angle DHPs used here is complex, and all were consistently decontaminated by washer-disinfection.

Conclusions

In a dental hospital setting multiple DHPs can simultaneously be effectively decontaminated internally and externally by washer-disinfection using an enzymatic detergent.

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Conflict of interest statement

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Figure Legends

Figure 1. Photograph showing an example of the contra angle dental handpiece (DHP) model used in this study and some of its internal components. (a) Frontal view of a DHP showing the angled main body and the head, neck and sheath. The head contains an opening into

which a dental bur is fitted. The bur is driven by internal gears powered by an electric motor. (b) View of a DHP with the head removed showing the openings of the narrow-compressed air and water channels at the top of the image. (c) Image showing components of a disassembled DHP including the head with the bur opening and water and air outlets at the 1 o'clock (marked with a white arrow), five o'clock and 8 o'clock positions (top left), the press button plate that closes the back of the DHP head (top centre), the DHP head gear (top right) into which a dental bur fits and the middle gear shaft that powers the head gear (bottom).

Figure 2. Photographs showing Míele PG8528 washer disinfector E919 dental modules. (a) The image shows a Míele E919 dental module equipped with 10 W&H (Bürmoos, Austria) DHP adapters with contra angle DHPs *in situ*. The white arrow shows the dental module water inlet, and the smaller black arrows show the direction of flow of water within the module. The DHPs are numbered 1-10 and show the relative positions of the 10 DHPs subjected to washer-disinfection throughout this study (Tables I and SI). During washer-disinfection, water/cleaning solution is injected under pressures up into the internal lumens and channels of each DHP via the adapter as well as onto the outsides of each DHP by the washer-disinfector spray arms. (b) The Míele PG8528 washer-disinfector can accommodate up to six E919 dental modules, each of which contains adapters for up to 10 DHPs. The dental module in the foreground is fitted with W&H DHP adapters. The other five modules are fitted with other DHP adapters that were not used in this study.

Table I: Reduction in the density of four challenge microorganisms recovered from internal components of 10 contra angle dental handpieces (DHPs) under clean and dirty conditions following washer disinfection relative to inoculated DHPs not subjected to washer-disinfection

			Log_{10} reduction in bacterial count[a] (+/- standard deviation)										
Challenge microorganism	**Conditions[b]**	**DHP[c] site**	**DHP1**	**DHP2**	**DHP3**	**DHP4**	**DHP5**	**DHP6**	**DHP7**	**DHP8**	**DHP9**	**DHP10**	**Overall average**
***Staphylococcus aureus* ATCC 6538**	Clean (0.3% BSA)	Head	5.06 (0.98)	5.40 (0.41)	5.33 (0.53)	5.46 (0.31)	5.57 (0.17)	5.48 (0.28)	5.43 (0.36)	5.22 (0.56)	5.36 (0.47)	5.46 (0.31)	**5.38 (0.43)**
		Channels	5.50 (0.05)	5.50 (0.05)	5.44 (0.90)	5.50 (0.05)	5.50 (0.05)	5.50 (0.05)	5.50 (0.05)	5.50 (0.05)	5.39 (0.24)	5.33 (0.34)	**5.46 (0.09)**
	Dirty (3% BSA)	Head	5.73 (0.07)	5.51 (0.02)	5.73 (0.07)	4.79 (0.85)	4.45 (0.77)	5.24 (0.16)	5.68 (0.16)	4.75 (1.27)	5.59 (0.16)	5.22 (0.52)	**5.27 (0.23)**
		Channels	5.61 (0.23)	5.33 (0.47)	5.79 (0.07)	4.94 (1.42)	5.66 (0.28)	5.67 (0.27)	5.79 (0.07)	5.48 (0.50)	5.79 (0.07)	5.70 (0.15)	**5.57 (0.14)**
	Dirty (10% Test soil)	Head	5.53 (0.23)	4.82 (0.89)	5.14 (0.56)	4.82 (0.89)	5.16 (0.59)	4.86 (0.79)	5.14 (0.75)	5.17 (0.55)	5.39 (0.53)	5.13 (0.59)	**5.11 (0.58)**
		Channels	5.62 (0.23)	5.71 (0.20)	5.61 (0.38)	5.24 (0.86)	5.77 (0.10)	5.71 (0.10)	5.22 (0.76)	5.82 (0.00)	5.49 (0.59)	5.77 (0.10)	**5.59 (0.16)**
***Enterococcus hirea* ATCC 10542**	Clean (0.3% BSA)	Head	5.06 (0.67)	5.12 (0.76)	5.58 (0.09)	5.04 (0.90)	5.52 (0.23)	5.63 (0.15)	5.63 (0.15)	5.49 (0.13)	5.58 (0.09)	4.94 (0.69)	**5.36 (0.28)**
		Channels	5.82 (0.00)	5.82 (0.00)	5.51 (0.41)	5.77 (0.10)	5.67 (0.27)	5.71 (0.19)	5.77 (0.10)	5.77 (0.10)	5.51 (0.14)	5.70 (0.22)	**5.70 (0.07)**
	Dirty (3% BSA)	Head	5.57 (0.17)	5.52 (0.08)	5.57 (0.17)	5.52 (0.23)	5.57 (0.17)	4.47 (1.24)	4.98 (1.12)	5.43 (0.36)	5.57 (0.17)	4.96 (0.57)	**5.32 (0.38)**
		Channels	5.57 (0.21)	5.57 (0.21)	5.62 (0.21)	5.62 (0.21)	5.62 (0.21)	5.49 (0.50)	5.62 (0.21)	5.02 (0.21)	5.06 (0.98)	5.62 (0.21)	**5.48 (0.18)**
	Dirty (10% Test soil)	Head	5.38 (0.67)	5.77 (0.00)	5.28 (0.85)	5.65 (0.21)	4.77 (0.77)	5.32 (0.40)	4.92 (0.88)	5.32 (0.40)	5.55 (0.39)	5.77 (0.00)	**5.37 (0.08)**
		Channels	5.75 (0.41)	5.69 (0.31)	5.45 (0.10)	5.65 (0.25)	5.45 (0.10)	5.52 (0.03)	5.75 (0.41)	5.36 (0.25)	5.63 (0.22)	5.55 (0.08)	**5.58 (0.13)**
***Pseudomonas aeruginosa* ATCC 15442**	Clean (0.3% BSA)	Head	5.71 (0.27)	5.38 (0.83)	5.83 (0.10)	5.73 (0.19)	5.83 (0.10)	5.74 (0.22)	5.19 (1.03)	5.78 (0.17)	5.78 (0.17)	5.78 (0.17)	**5.67 (0.24)**
		Channels	5.96 (0.23)	5.96 (0.23)	5.96 (0.23)	5.82 (0.40)	5.72 (0.40)	5.52 (0.53)	5.96 (0.23)	5.96 (0.23)	5.96 (0.23)	5.89 (0.29)	**5.87 (0.18)**
	Dirty (3% BSA)	Head	5.91 (0.32)	6.12 (0.09)	6.12 (0.09)	6.17 (0.00)	6.06 (0.09)	6.12 (0.09)	6.12 (0.09)	6.17 (0.00)	5.84 (0.08)	6.12 (0.09)	**6.07 (0.05)**
		Channels	5.77 (0.06)	5.86 (0.18)	5.91 (0.27)	5.91 (0.27)	5.91 (0.27)	5.91 (0.27)	5.79 (0.37)	5.91 (0.27)	5.91 (0.27)	5.75 (0.07)	**5.87 (0.22)**
	Dirty (10% Test soil)	Head	5.49 (0.71)	5.10 (1.15)	5.09 (1.03)	5.85 (0.14)	5.85 (0.29)	5.85 (0.23)	5.90 (0.23)	5.90 (0.23)	5.54 (0.47)	5.10 (1.52)	**5.57 (0.48)**
		Channels	5.90 (0.29)	5.90 (0.13)	5.90 (0.13)	5.34 (1.21)	5.96 (0.23)	5.54 (0.70)	5.50 (0.92)	5.91 (0.29)	5.45 (0.24)	5.81 (0.15)	**5.72 (0.33)**
***Candida albicans* ATCC MYA-2876**	Clean (0.3% BSA)	Head	5.17 (0.32)	5.26 (0.40)	5.26 (0.40)	5.26 (0.39)	5.26 (0.39)	5.26 (0.36)	5.26 (0.36)	5.26 (0.37)	5.26 (0.37)	5.26 (0.37)	**5.25 (0.36)**
		Channels[c]	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	4.95 (0.23)	**4.95 (0.23)**
	Dirty (3% BSA)	Head	4.65 (0.84)	4.81 (0.61)	5.21 (0.44)	4.61 (0.90)	5.21 (0.44)	5.21 (0.44)	5.21 (0.44)	5.21 (0.44)	5.21 (0.44)	5.21 (0.44)	**5.06 (0.22)**
		Channels[d]	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	4.93 (0.09)	**4.93 (0.09)**
	Dirty (10% Test soil)	Head	4.98 (0.61)	5.27 (0.17)	5.27 (0.17)	4.92 (0.22)	4.97 (0.34)	5.27 (0.17)	5.27 (0.17)	4.66 (1.15)	5.16 (0.310	4.55 (1.35)	**5.03 (0.25)**
		Channels	4.73 (0.85)	4.81 (0.71)	5.09 (0.23)	4.91 (0.53)	5.09 (0.23)	5.03 (0.33)	5.09 (0.23)	5.09 (0.23)	4.80 (0.74)	5.09 (0.23)	**4.97 (0.43)**

Each challenge microorganism was inoculated separately into the head and air and water channels of 11 DHPs. Ten of these DHPs were processed by washer-disinfection. For each washer-disinfector cycle, one inoculated DHP was left untreated as a control. Following washer-disinfection, all 11 DHPs were tested for recovery of microorganisms using periopoints as described in the Methods and the log reduction in bacterial/yeast counts calculated relative to the untreated control inoculated DHP in each case. The results shown are the average of three separate experiments for each DHP with each challenge microorganism. Abbreviations: BSA, bovine serum albumin; DHP, dental handpiece.

[a]Average reduction in bacterial count from three separate experiments.

[b]The artificial test soil (Edinburgh test soil, Cúram Medical, Dublin, Ireland) used was compliant with ISO-15883-5-2021(25)

[c]Bacterial recovery data shown for channels represent the average recovery data from both air and water channels for each DHP tested with each challenge microorganism. Bacteria recovered from heads includes organisms recovered from the DHP head, press button plate, head gear and middle gear of each DHP.

[d]No viable *Candida* cells recovered by culture following washer-disinfection, thus all of the readings for the 10 DHPs are identical.

Figure 1

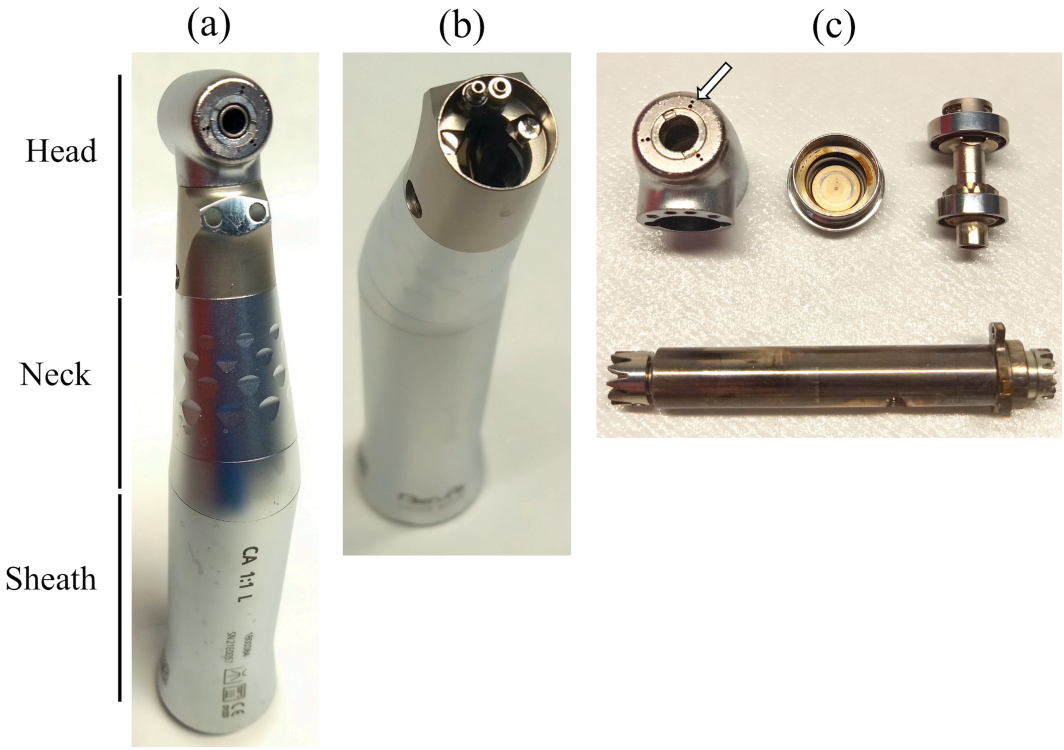

Figure 2

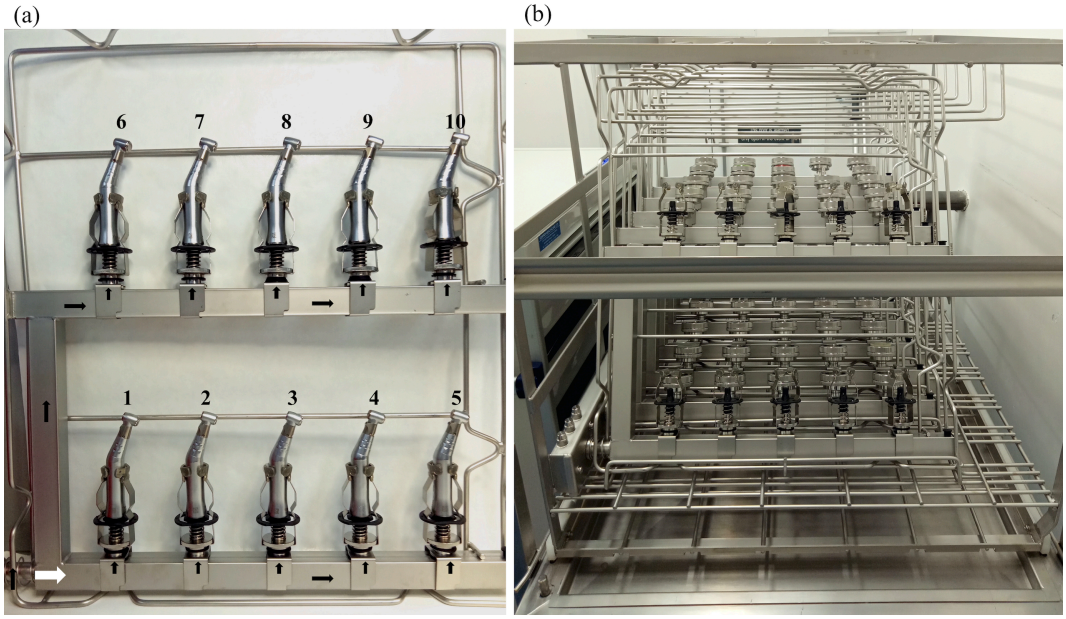

Table SI: Reduction in protein recovered from the internal components of 10 contra angle dental handpieces (DHPs) under two different sets of dirty conditions following washer-disinfection relative to inoculated DHPs not subjected to washer-disinfection

			Percentage reduction in protein[a] (+/- standard deviation)										
Conditions[b]	**Challenge microorganisms**	**DHP site[c]**	**DHP1**	**DHP2**	**DHP3**	**DHP4**	**DHP5**	**DHP6**	**DHP7**	**DHP8**	**DHP9**	**DHP10**	**Overall average**
Dirty (3% BSA)	*Staphylococcus aureus* ATCC 6538	Head	98.1 (1.5)	97.3 (2.3)	97.8 (2.3)	98.1 (1.8)	98.2 (1.4)	98.2 (1.6)	98.3 (1.2)	98.5 (1.1)	98.1 (1.7)	97.3 (3.9)	**97.6 (1.9)**
		Channels	95.1 (1.1)	94.6 (1.4)	94.8 (1.4)	95.2 (1.8)	95.9 (0.3)	94.6 (0.9)	95.1 (1.2)	95.1 (1.9)	94.1 (2.8)	94.5 (2.1)	**94.9 (1.4)**
	Enterococcus hirea ATCC 10542	Head	99.3 (0.3)	98.8 (0.6)	99.2 (0.2)	99.1 (0.6)	99.3 (0.5)	99.5 (0.2)	99.7 (0.3)	99.6 (0.3)	98.6 (0.8)	99.3 (0.4)	**99.2 (0.1)**
		Channels	92.6 (2.6)	93.5 (0.8)	92.9 (1.6)	92.5 (1.0)	95.1 (6.3)	93.6 (1.2)	94.6 (1.0)	92.7 (2.1)	95.5 (5.3)	93.1 (2.7)	**93.6 (2.4)**
	Pseudomonas aeruginosa ATCC 15442	Head	98.7 (0.4)	98.5 (0.6)	98.1 (1.1)	98.6 (0.6)	98.7 (0.3)	98.2 (0.5)	98.4 (0.7)	98.1 (0.9)	98.7 (1.2)	98.8 (1.1)	**98.5 (0.3)**
		Channels	98.4 (1.0)	98.9 (0.6)	98.6 (0.7)	98.8 (0.5)	98.5 (0.6)	98.3 (0.9)	98.7 (0.4)	98.8 (0.6)	98.4 (0.8)	98.8 (0.3)	**98.6 (0.6)**
	Candida albicans ATCC MYA-2876	Head	98.9 (0.7)	98.3 (0.3)	98.9 (0.5)	98.9 (0.3)	98.7 (0.1)	98.1 (0.4)	98.4 (0.3)	98.9 (0.5)	99.1 (0.6)	99.2 (0.5)	**98.8 (0.2)**
		Channels	96.9 (0.2)	95.9 (0.5)	96.5 (0.2)	96.2 (0.3)	96.2 (0.4)	96.3 (0.5)	96.1 (0.7)	95.9 (1.2)	96.4 (0.4)	96.5 (0.5)	**96.3 (0.3)**
Dirty (10% Test soil)	*Staphylococcus aureus* ATCC 6538	Head	98.7 (0.5)	98.3 (0.7)	98.7 (0.5)	98.7 (0.8)	98.5 (0.2)	98.6 (0.8)	98.5 (0.2)	98.7 (0.3)	98.4 (0.8)	98.5 (0.5)	**98.6 (0.5)**
		Channels	92.8 (0.4)	92.5 (1.4)	92.7 (2.0)	92.8 (1.9)	92.8 (2.6)	92.9 (2.1)	93.1 (1.6)	92.5 (2.3)	92.5 (2.3)	92.6 (1.9)	**92.7 (1.8)**
	Enterococcus hirea ATCC 10542	Head	99.6 (0.3)	99.2 (0.4)	99.3 (0.1)	99.5 (0.3)	99.1 (0.2)	98.9 (0.6)	99.6 (0.2)	99.6 (0.3)	99.3 (0.4)	99.4 (0.2)	**99.4 (0.3)**
		Channels	92.3 (2.7)	92.5 (3.9)	92.8 (3.8)	94.7 (1.9)	93.2 (2.4)	93.3 (4.1)	94.1 (2.2)	93.6 (3.2)	92.3 (4.6)	92.4 (4.3)	**93.1 (3.1)**
	Pseudomonas aeruginosa ATCC 15442	Head	97.6 (0.9)	98.5 (0.8)	98.1 (1.6)	97.8 (2.3)	97.9 (1.9)	97.7 (1.2)	98.2 (1.7)	97.9 (1.8)	98.1 (1.5)	97.4 (1.2)	**97.9 (1.4)**
		Channels	93.3 (4.7)	95.1 (4.2)	93.9 (6.3)	95.5 (3.7)	95.2 (4.5)	94.1 (6.2)	94.3 (6.2)	94.4 (6.2)	93.5 (4.9)	92.5 (4.9)	**94.2 (5.1)**
	Candida albicans ATCC MYA-2876	Head	99.4 (0.2)	99.4 (0.2)	99.6 (0.4)	99.1 (0.3)	99.6 (0.3)	99.5 (0.3)	99.2 (0.2)	99.5 (0.3)	99.2 (0.2)	99.5 (0.2)	**99.4 (0.1)**
		Channels	95.1 (3.1)	92.5 (5.9)	93.1 (5.9)	93.4 (4.8)	92.9 (5.6)	92.8 (6.1)	92.8 (5.6)	92.9 (5.2)	93.1 (5.8)	93.2 (5.8)	**93.2 (5.4)**
Dirty (10% Test soil)	No challenge microorganisms	Head	97.9 (1.5)	97.6 (1.4)	93.9 (6.3)	96.8 (3.6)	98.6 (0.9)	96.2 (5.5)	98.4 (1.6)	97.9 (1.7)	96.4 (3.0)	95.9 (5.0)	**96.9 (2.7)**
		Channels	95.3 (5.5)	95.2 (6.0)	95.2 (5.7)	94.9 (6.4)	94.6 (6.4)	95.3 (5.3)	94.9 (5.9)	94.9 (6.2)	95.1 (5.9)	95.1 (5.8)	**95.1 (5.9)**

Each challenge microorganism was inoculated separately into the head and air and water channels of 11 DHPs. Ten of these DHPs were processed by washer-disinfection. For each washer-disinfector cycle, one inoculated DHP was left untreated as a control. Following washer-disinfection, all 11 DHPs were tested for recovery of protein as described in the Methods and the percentage reduction in protein calculated relative to the untreated control inoculated DHP in each case. The results shown are the average of three separate experiments for each DHP under each set of conditions.

[a]Mean average result from three separate experiments.

[b]The artificial test soil (Edinburgh test soil, Cúram Medical, Dublin, Ireland) used was compliant with ISO-15883-5-2021.

[c]Protein levels shown for channels represent the average recovery data from both air and water channels for each DHP tested under each set of conditions. Protein recovered from heads includes protein recovered from the DHP head, press button plate, head gear and middle gear of each DHP under each set of conditions (see Figure 1). Abbreviations: BSA, bovine serum albumin; DHP, dental handpiece.

Reference: International Organization for Standardization (ISO). BS EN ISO 15883-5. 2021. Washer-disinfectors — Part 5: Performance requirements and test method criteria for demonstrating cleaning efficacy. London: British Standards Institute.

Table SII: Reduction in the protein recovered and density of *Staphylococcus aureus* ATCC 6538 under dirty conditions recovered from internal components of six contra angle dental handpieces (DHPs) in each of six different washer-disinfector dental modules following washer disinfection relative to inoculated DHPs not subjected to washer-disinfection

DHP site		**Log Reduction in Bacterial Count[a] (+/- Standard deviation)**	**Percentage protein reduction[a] (+/- Standard deviation)**
Module 1 Position 10	Head	5.86 (0.1)	99.63 (0.2)
	Channels	5.37 (1.5)	94.42 (5.7)
Module 2 Position 10	Head	5.36 (0.7)	99.76 (0.2)
	Channels	5.33 (0.8)	94.10 (6.5)
Module 3 Position 10	Head	5.53 (0.8)	99.56 (0.3)
	Channels	5.65 (0.3)	95.12 (5.4)
Module 4 Position 10	Head	5.37 (1.1)	99.64 (0.2)
	Channels	5.00 (1.4)	93.83 (7.5)
Module 5 Position 10	Head	5.34 (0.9)	99.64 (0.3)
	Channels	5.11 (1.0)	94.10 (6.8)
Module 6 Position 10	Head	5.16 (1.4)	99.62 (0.3)
	Channels	5.07 (0.9)	95.03 (5.1)

[a]Bacterial recovery and protein data shown for channels represent the average recovery data from both air and water channels for each DHP tested with each challenge microorganism in three separate experiments. Bacteria recovery data from heads includes bacteria recovered from the DHP head, press button plate and head gear of each DHP (see Figure 1).