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Overcoming the problem of residual microbial contamination in dental suction units left by conventional disinfection using novel single component suction handpieces in combination with automated flood disinfection

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Short Title: Improved decontamination of dental unit suction systems

KEYWORDS: Dental unit suction; decontamination; infection reservoir; disinfection; automated disinfection, dental suction handpieces; cross-infection; risk reduction

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Abstract

Objectives: Decontaminating dental chair unit (DCU) suction systems in a convenient, safe and effective manner is problematic. This study aimed to identify and quantify the extent of the problems using 25 DCUs, methodically eliminate these problems and develop an efficient approach for reliable, effective, automated disinfection.

*Method*s: DCU suction system residual contamination by environmental and human-derived bacteria was evaluated by microbiological culture following standard aspiration disinfection with a quaternary ammonium disinfectant or alternatively, a novel flooding approach to disinfection. Disinfection of multicomponent suction handpieces, assembled and disassembled, was also studied. A prototype manual and a novel automated Suction Tube Cleaning System (STCS) were developed and tested, as were novel single component suction handpieces.

Results: Standard aspiration disinfection consistently failed to decontaminate DCU suction systems effectively. Semi-confluent bacterial growth (101-500 colony forming units (CFU) per culture plate) was recovered from up to 60% of suction filter housings and from up to 19% of high and 37% of low volume suction hoses. Manual and automated flood disinfection of DCU suction systems reduced this dramatically (ranges for filter cage and high and low volume hoses of 0-22, 0-16 and 0-14 CFU/plate, respectively) ($P < 0.0001$). Multicomponent suction handpieces could not be adequately disinfected without prior removal and disassembly. Novel single component handpieces, allowed their effective disinfection *in situ* using the STCS, which virtually eliminated contamination from the entire suction system.

Conclusion: Flood disinfection of DCU suction systems and single component handpieces radically improves disinfection efficacy and considerably reduces potential cross-infection and cross-contamination risks.

Clinical Significance: DCU suction systems become heavily contaminated during use. Conventional disinfection does not adequately control this. Furthermore, multicomponent suction handpieces cannot be adequately disinfected without disassembly, which is costly in time, staff and resources. The automated STCS DCU suction disinfection system used with single component handpieces provides an effective solution.

1. Introduction

The efficient and hygienic removal of irrigants and body fluids is an important element of many modern medical procedures, for example, endotracheal secretions or blood and other fluids during and after surgery. Equipment used to provide medical suction, by its very nature, becomes contaminated by residues and microorganisms and it is not surprising that suction equipment in the hospital setting has frequently been implicated as a source of infection.[1-2] Suction is also an essential part of modern dental treatment and is used to remove fluids (e.g. saliva, blood, and irrigation water) and debris (e.g. tooth particles, dental calculus and dental amalgam) from the oral cavity during dental procedures. It is also used to minimise the release of aerosols during the preparation of tooth surfaces using high-speed dental drills and cutting instruments and during the use of ultrasonic scalers.

Suction in dentistry is usually provided by a vacuum system integrated within the dental chair unit (DCU) and is used repeatedly for successive patients with only a change of the suction tip between patients. This is standard practice in dentistry as suction has generally been considered as having a low infection risk despite studies providing evidence to the contrary.[3-6] Dental chair units are usually equipped with two types of suction device, one a high-volume and the other, a low-volume device. Each device consists of a suction hose connected to a common vacuum source at the DCU end and terminating with a removable suction handpiece at the operator's end. The high-volume device, also known as the high volume evacuator (HVE), is used to significantly reduce the release of aerosols, spray and splatter into the clinical environment during dental instrument use, whereas the low-volume device, also referred to as the saliva ejector or low volume evacuator (LVE) is used to remove excess fluids including blood and saliva from the oral cavity. Before use with each patient, a reusable or disposable sterile wide bore plastic suction tip is fitted to the HVE handpiece and a single-patient-use disposable tip is fitted to the narrower-bore LVE handpiece. These suction tips are either discarded (low volume) or cleaned and sterilised (high volume) after each patient use. Dental unit suction systems should be disinfected regularly, e.g. daily or twice daily, with a non-foaming disinfecting agent as recommended by the DCU manufacturer and it is also recommended that between patients, the suction hoses should be flushed through with clean water. In practice, the disinfection process involves aspirating a volume of disinfectant through the suction hoses, very often with the suction handpieces attached. This process is referred to as aspiration disinfection. However, HVE and LVE handpieces are usually multicomponent and may contain regulators to permit control of suction strength and are usually attached to suction hoses by means of adaptors. This creates junctions and areas that may be shielded from the aspiration disinfection process and may provide the opportunity for leakage. Ideally, suction hose handpieces would require disassembly, cleaning, disinfection, reassembly and sterilisation after each patient use to ensure proper decontamination. Dental unit manufacturers actually recommend regular disassembling, cleaning and disinfection of suction handpieces, with some recommending additional processing by daily steam sterilisation or even steam sterilisation after each patient use. The reality in busy dental clinics is that this is rarely performed. In dental hospitals and large dental clinics equipped with many DCUs, the recommended procedures would require significant staff resources and multiple sets of suction handpieces for each DCU, even if automated equipment such as washer disinfectors were used for decontamination. Furthermore, suction hose handpieces

often contain small parts and O-ring seals that can easily be lost or damaged, particularly if the fittings are dismantled regularly.

DCU suction systems have a filter component usually located in the main body or pedestal unit of the DCU to trap large particles of debris aspirated by suction hoses. Manufacturers recommend that these filters should be removed, cleaned and disinfected daily and replaced if damaged or torn. These filters and filter housings are disinfected during routine aspiration disinfection of DCU suction systems, as they are located downstream of DCU suction hoses. However, studies have shown that filter housings are prone to microbial biofilm contamination, despite regular disinfection.[6]

There are very few studies on DCU suction systems in the peer-reviewed scientific literature. One previous study reported that suction hose backflow and microbial contamination of the patient's mouth can occur with LVEs when the pressure in the mouth is less than that in the suction hose.[4,7] This could potentially occur when a patient closes their lips around the suction tip thus creating a partial vacuum. Studies have also shown that liquid can be drawn back towards the patient's mouth by gravity if the suction hose is at a level above the patient's mouth, or where the HVE is in simultaneous use.[4] To date, adverse health effects have not been reported in relation to dental suction, but parallels with medical suction suggest that infection from dental suction is possible.[8] Good practice requires that dental suction systems need to be effectively maintained and decontaminated to minimise potential risks of infection or compromising the clinical environment.

Investigations from this laboratory have identified potential infection reservoirs within DCU suction systems that are not effectively decontaminated by conventional DCU suction aspiration disinfection. Some aspects of the findings relate to multi-component suction hose handpieces and adaptors which contain areas that are shielded from disinfection during aspiration disinfection while others relate to the inadequacy of aspiration disinfection itself.

The findings of this study were used to develop a much more effective solution to DCU suction system decontamination. This consisted of a twofold approach, firstly, developing an automated disinfectant flooding system for DCU suction systems and secondly, by developing novel suction hose handpieces that can be effectively decontaminated without disassembly or removal from suction hoses during the automated disinfection cycle.

2. Materials and methods

2.1. *Chemicals and reagents*

All chemicals and reagents used were of analytical grade or molecular biology grade and unless otherwise stated were purchased from Sigma-Aldrich (Arklow, Ireland). Defibrinated horse blood was purchased from Cruinn Diagnostics Ltd. (Dublin, Ireland)

2.2. *Dental chair units*

The two DCU models used in the main part of this study were (i) a Planmeca Prostyle Compact DCU and (ii) a Planmeca Compact i DCU (Planmeca Oy, Helsinki, Finland). Both DCUs were new and were used for routine

clinical sessions at the Dublin Dental University Hospital (DDUH) for a three-month period prior to this study. Both DCUs were connected to a central vacuum source (Cattani, Parma, Italy) which services all 103 Planmeca Prostyle Compact DCUs at DDUH. Each DCU was equipped with high volume (internal diameter 15.5 mm) and low volume (internal diameter 10 mm) suction hoses (Exoflex, Kippenheim, Germany) made from polyvinyl chloride. Suction hoses were connected to the main body of the DCU and then linked by common pipework to a coarse filter housing containing removable filters. The outflow pipework from the filter housing was connected to a central waste collection vessel receiving suction waste from all DCUs at DDUH. Twenty-three 12-year old Prostyle Compact DCUs, located in three separate DDUH clinics, were used in some parts of the study. These DCUs were equipped with suction systems similar to the two new DCUs referred to above. For comparison purposes, 10 A-dec (Newberg, Oregon, USA) DCUs which were located in five separate Irish public dental clinics and which were 10 or more years old, were included in the study.

2.3. *Suction hose handpieces and adaptors*

Both of the main test DCUs were equipped with Dürr (Dürr Dental, Bietigheim-Bissingenlow, Germany) LVE handpieces and with Planmeca HVE handpieces (Figs. 1a and 1b). The LVE handpiece interfaces with an aluminium adaptor which inserts directly into the low volume suction hose while the HVE handpiece contained an adaptor which inserts directly into the high volume suction hose (Figs. 1a and 1b). The LVE handpiece had a rotary valve to regulate suction strength during use (Fig. 1a). Reusable (high volume) or disposable (low volume) aspirator tips are inserted into the suction hose handpieces for use during dental treatment (Fig. 1c). Six of the A-dec DCUs were equipped with A-dec aluminium HVE and LVE handpieces containing rotary valves for regulating suction strength and four were fitted with plastic (Cattani) HVE and LVE handpieces (Fig. 1d). All Cattani handpieces contained slide regulators for varying suction strength during use (Fig. 1d).

2.4. *Suction system coarse filters*

Suction systems in Planmeca DCUs have a coarse filter fitted on each of the high and low volume suction lines (Fig. 1e) to trap large particles of debris aspirated during use. During disinfection, aspirated disinfectant solution passes through the suction hoses, through the coarse filter and is then voided to the central suction waste collection vessel. In DDUH, these coarse filters are removed each evening and are cleaned and disinfected by immersion in Orotol Plus disinfectant solution (2%) for several minutes.

2.5. *Suction disinfectants*

Orotol Plus (Dürr Dental) was used to disinfect DCU suction systems. This product is a non-foaming agent widely used for disinfecting, deodorising and cleaning dental suction systems and contains quaternary ammonium compounds, alkaline cleaning agents, complexing agents and antifoaming agents. The product is supplied as a liquid concentrate and is diluted with water immediately before use to yield a 2% (v/v) working solution. Orotol Plus was also used to disinfect the suction systems of six of the A-dec DCUs. Green & Clean M2 (Metssys Medizintechnik GmbH, Innsbruck, Germany), containing quaternary ammonium compounds, cleaning agents and antifoaming agents, was used to disinfect the suction systems of the remaining four A-dec DCUs.

2.6. *Aspiration disinfection of DCU suction*

During this study, the routine protocol used for DCU suction system disinfection at DDUH involved twice-daily (after the morning and afternoon clinical sessions) aspiration disinfection of the suction system without removing suction hose handpieces. Between patients, suction hoses were each flushed through with one litre of clean water. Aspiration disinfection was undertaken by placing freshly prepared 2% Orotol Plus (2 litres) into an OroCup container (Dürr Dental), replacing the lid and attaching the DCU suction hoses with handpieces attached, to special adaptors present in the lid (Fig. 2a). Following activation of the DCU suction, OroCup generates an air/disinfectant mixture that is aspirated through the suction handpieces and hoses into the body of the DCU, then through the coarse filter and eventually to a central suction waste collection vessel. After disinfection, the suction hoses are removed from the OroCup and returned to a holding arm attached to the DCU (Fig. 1c). The suction systems of A-dec DCUs in public dental clinics were subjected to aspiration disinfection after each clinical session using either Orotol Plus or Green & Clean M2 disinfectant using OroCup or a similar device with the suction handpieces also in place.

2.7. *Prototype flood disinfection of DCU suction*

A novel approach for DCU suction disinfection used complete filling or flooding of the suction systems of individual DCUs with disinfectant with handpieces removed and then leaving them to disinfect for a specified period after which the disinfectant was voided to waste. This approach used a prototype system consisting of a removable disinfectant reservoir fitted to a new Planmeca Compact i DCU that was specially modified during manufacture so that the DCU suction system could be completely filled with disinfectant. The removable reservoir unit was fitted with external sockets onto which the DCU's suction hose adaptors could connect after removing the handpieces (Fig. 2b). The reservoirs were configured with air vents that permitted air displacement from the suction system during filling with disinfectant. Once the hoses were attached, the reservoir was filled with Orotol Plus disinfectant and the suction hoses filled under gravity, which took approximately two minutes. Disinfectant was then left *in situ* for three minutes before being voided to waste by activating the DCU suction. Suction hoses were then detached from the reservoir, the suction hose handpieces reattached and the DCU was then available for routine clinical use.

2.8. *Automated flood disinfection of DCU suction*

A permanently integrated and automated version of the prototype flood disinfection system called the Suction Tube Cleaning System (STCS) was developed in collaboration with Planmeca, (Fig. 2 c). This system consists of the STCS unit situated to the rear of the DCU and is fitted with ports to receive the high and low volume suction hoses. The STCS unit has a reservoir for disinfectant concentrate and a water outlet for diluting the disinfectant. Suction hose cleaning and disinfection is initiated by opening the lid of the STCS unit and inserting suction hoses into the receiving ports having the handpiece adaptors attached but handpieces removed (Fig. 2c). A predetermined volume of disinfectant concentrate (Orotol Plus) is then dispensed into the STCS disinfectant reservoir and the disinfection cycle is activated from the STCS unit by pressing a button. Water is automatically dispensed into the reservoir to dilute the disinfectant concentrate to the manufacturer's recommended working

concentration (i.e. 2%). The STCS system of each DCU is calibrated to suit the length of suction hoses used with particular DCUs, which ensures that the volume of disinfectant dispensed is adequate to completely fill the DCU suction system. Diluted disinfectant flows by gravity into the suction hoses, filter housings and associated pipework until they are completely filled or flooded with disinfectant. Air is automatically displaced from the suction system during filling. The fill process takes approximately two minutes after which the disinfectant is left *in situ* for another three minutes before an automated activation of the DCU suction evacuates it to waste. Suction hoses are then detached from the STCS unit and the hoses returned to the DCU suction hose holder arm, after which the DCU is ready for use.

2.9. *New design single component suction hose handpieces*

During this study a new design of suction hose handpiece was developed that could interface directly into the STCS receiving ports (Fig. 2d and 2e). The new handpieces were milled from polyoxymethylene (POM) and consisted of a single component without suction strength regulator valves (Fig. 2e). One end of each handpiece is tapered and ribbed on the outside to insert into suction hoses and the other is designed to hold reusable (high volume) or disposable (low volume) aspirator tips and to interface with the suction hose receiving ports of the STCS system (Fig. 2d and 2e). To facilitate effective disinfection, the inside of the new suction handpieces have a smooth bore without crevices.

2.10. *Immersion disinfection of suction hose handpieces*

One set of suction hose handpieces (one Dürr LVE handpiece and one Planmeca HVE handpiece (Fig. 1a and b)) used on the Prostyle Compact DCU were subjected to immersion disinfection weekly, over a period of ten consecutive weeks. During this period, the cleaning and disinfection protocol for the DCU suction system (including suction hose handpieces) was aspiration disinfection twice daily after the morning and afternoon clinical sessions with Orotol Plus using the OroCup system as described above (Fig. 2a). On the day of testing each week, the DCU was used for a three-hour clinical session. Following the session, the outsides of suction hose handpieces were wiped using a single-use cloth soaked in 70% (v/v) ethanol. Handpieces were then detached from their suction hoses and immersed, without disassembling, in 100 ml of Orotol Plus disinfectant for three min and agitated by using a one cm magnetic stirring bar on a laboratory magnetic stirrer. The handpieces were then removed from the disinfectant, disassembled and the components placed in 100 ml of disinfectant neutraliser for a further two min. A neutralising agent for quaternary ammonium compounds (QACs) was formulated according to the BS EN 1276:2009 standard.[9] This neutraliser consisted of 30 g/l Tween 80, 30 g/l saponin and 3 g/l lecithin. The efficacy of this neutraliser for use with Orotol Plus and Green & Clean M2 was confirmed using the dilution validation method detailed in the BS EN 1276:2009 standard document using *Pseudomonas aeruginosa* PAO1 as the reference test organism.[9] Finally the components were placed in 100 ml of sterile phosphate buffered saline (PBS) and agitated as before for three minutes. Afterwards, the components were removed and the solution centrifuged at 5400 x *g* in a Sorval RC5 Plus centrifuge (Thermo Fisher Scientific Inc., MA, USA). The supernatant was discarded and the pellet washed with fresh 5 ml of PBS, centrifuged and finally resuspended in 1 ml of PBS and dilutions plated in duplicate on PAS and CBA agar media (see section 2.11). Plates were incubated

as described below, after which time bacterial colony forming units (CFUs) were counted and the total number recovered on each type of agar medium for each handpiece was determined. A second set of experiments was performed with the same suction hose handpieces for another ten consecutive weeks as above, except that the handpieces were disassembled prior to immersion in Orotol Plus.

2.11. *Microbiogical sampling of DCU suction system*

Following disinfection of DCU suction systems, various suction system sites were sampled for residual microbial contamination using Copan Venturi Transystem sterile cotton swabs (Brescia, Italy) pre-moistened with filter sterilised neutralising agent. The suction system sites selected for sampling included (i) the interior of each suction hose immediately adjacent to where suction handpieces are attached, (ii) both suction hose handpiece adaptors (Fig. 1a & b), (iii) filter cage housing (Fig. 1e), (iv) the internal components of the high volume suction handpiece (Fig. 1b) and (v) the internal components of the low volume suction handpiece (Fig. 1a). Suction hoses were swabbed thoroughly to a depth of 12 cm. Prior to sampling, the coarse filter housing was opened, the filter removed and the internal area swabbed. Surfaces were swabbed thoroughly using both back and forth and perpendicular strokes. Each swab was then placed in a tube of sterile hydrated sodium alginate supplied with the swab, labelled, packaged and immediately transferred to the microbiology laboratory for culture. Swabs were lawned on culture media within 2 h of sampling using a zigzag pattern with swab rotation. Samples were cultured on four different culture media to maximise the potential recovery of contaminating bacterial species. Swabs were plated consecutively on (i) *Pseudomonas aeruginosa* selective agar (PAS) consisting of Pseudomonas agar base (Oxoid Ltd., Basingstoke, UK) supplemented with cetrimide (200 µg/ml) and sodium nalidixate (15 µg/ml) (Oxoid Pseudomonas CN Selective Supplement), (ii) *Pseudomonas* selective agar (PA) consisting of Pseudomonas agar base (Oxoid) supplemented with cetrimide (10 µg/ml), fusidic acid (10 µg/ml), and cephaloridine (50 µg/ml) (Oxoid Pseudomonas CFC Selective Supplement), Columbia blood agar (CBA) (Lip Diagnostic Services, Galway, Ireland) and R2A agar (Lip Diagnostic Services). PAS and PA agar plates were incubated at 30°C for 48 h, CBA plates were incubated at 37°C for 48 h and R2A agar plates were incubated at 20°C for up to ten days. PA and PAS media select for *Pseudomonas* and related species, R2A selects for aerobic heterotrophic environmental bacterial species and CBA was used to culture human derived bacterial species. Following incubation, plates were examined and colonies counted using a Flash and Go™ automatic colony counter (IUL Instruments Ltd., Barcelona, Spain). Plates yielding semi-confluent growth harboured between 101-500 CFU/plate. Plates with confluent bacterial growth were determined to contain between >501 CFU/plate. To obtain a quantitative estimate of CFUs present on swabs yielding confluent growth on plating on all four media, the heads of 50 such swabs were cut off and vortexed separately in 10 ml phosphate buffered saline (PBS). These samples were then serially diluted in PBS and plated. Selected colonies of each type present were stored in Protect bacterial preservers (Technical Service Consultants Ltd., Lancashire, UK) at -80°C.

2.12. *Identification of bacterial isolates*

Definitive identification of isolates recovered on all media was undertaken by determining the DNA sequence of a segment of the small ribosomal subunit rRNA gene and by comparing the sequences with consensus sequences

for individual bacterial species in the EMBL/GenBank nucleotide sequence databases using the BLAST family of computer programmes (http://www.ncbi.nlm.nih.gov/BLAST/)[10]. Genomic DNA from bacterial isolates was prepared using the Qiagen DNeasy kit system (Qiagen, Crawley, UK) according to the manufacturer's instructions as described previously.[11] A variable segment of the 16S rDNA gene of each bacterial isolate was amplified by PCR as described previously, using approximately 30 ng of purified genomic DNA as template with the universal primers 533F (5'-AGAGTTTGATC/TA/CTGGCTCAG-3') and 142R (5'-CGGC/TTACCTTGTTACGA-3'). These primers amplify a region of approximately 950 bp to 1.5 kb of the 16S rDNA gene of all bacterial species. Amplified PCR products were purified using the Sigma GenElute PCR clean up kit according to the manufacturer's instructions and then sequenced commercially by Source Bioscience (Waterford, Ireland).

2.13. *Statistical analysis*

All statistical analyses were performed using GraphPad Prism v.5 (GraphPad Software, San Diego, USA). Statistical significance was determined using an unpaired, two-tailed Student's t-test with 95% confidence interval (C.I.).

3. Results

3.1. *Sampling of DCU suction sites prior to disinfection*

During this study DDUH was equipped with 103 Planmeca Prostyle Compact DCUs. To ascertain baseline levels of suction system contamination at the outset of this study, suction system sites including suction hoses, suction handpieces and coarse filter housings were sampled directly in all DCUs after patient treatment sessions but prior to disinfection. The majority of these samples yielded either semi-confluent (101-500 CFU/plate) or confluent (> 501 CFU/plate) growth on a variety of nutrient or selective media including PAS, PA, R2A and CBA. The CFU range in confluent samples (determined by serially diluting bacteria recovered from 50 individual swab samples vortexed in PBS) for all media was 1098 - 6.14 x 10^6 CFU/swab (mean average 1.03 x 10^6 ± 1.52 x 10^6 CFU/swab). Some LVE suction handpieces and their corresponding suction hoses and coarse filter housings contained visible saliva and blood generated during non-surgical dental treatment.

3.2. *Residual contamination of suction system following aspiration disinfection*

One Planmeca Prostyle Compact and one Planmeca Compact i DCU were used for the main part of this study. Both DCUs were used daily for dental treatment and disinfected using aspiration disinfection with Orotol Plus. Immediately after disinfection, the following sites were sampled with swabs dipped in neutralising agent; the coarse filter housings (Fig. 1e), the internal surfaces of both low and high volume suction hoses adjacent to their suction handpieces, the interior of the suction handpieces and the suction hose handpiece adaptors (Fig. 1a and 1b). The Compact i and Prostyle DCUs were sampled once weekly for 27 and 25 weeks respectively. Results of sampling were combined for both DCUs as very similar data were obtained from each DCU separately.

The majority of samples from the filter housings and high and low volume suction hoses (n=52 for each site) showed bioburden still remaining post-aspiration disinfection (Table 1). The coarse filter housing samples yielded bacterial growth in 94-97% of cases of which 40-60% were semi-confluent (101-500 CFU/plate) depending on the culture medium. Similarly, 73-100% of low volume suction hose samples were contaminated and up to 37% of samples yielded semi-confluent growth (Table 1). In contrast, high volume suction hose samples yielded a lower level of bacterial contamination (56-65%) with only 2-19% of samples yielding semi-confluent growth (Table 1, upper panel).

Preliminary experiments revealed that suction hose handpiece adaptors in DDUH were primarily contaminated with *P. aeruginosa* and related species and thus samples from these sites were cultured on PA and PAS culture media. It was noted that suction hose handpiece adaptors (Fig. 1a and 1b) still yielded heavy contamination following aspiration disinfection. The majority of both low volume (33/52, 63.5%) and high volume (38/52, 73%) handpiece adaptors yielded semi-confluent growth (101-500 CFU/plate), with the remaining samples yielding between 89-418 CFU/plate. Both HVE and LVE handpieces (Fig. 1a and 1b) were also found to remain contaminated post-aspiration disinfection 7/52 (6-13.5%) and 12/52 (15-23%) of samples from HVE and LVE handpieces respectively, yielding semi-confluent growth on CBA, R2A, PAS and PA culture media.

Additional neutralised swab samples were taken from 23 randomly chosen 12 year-old Planmeca Prostyle Compact DCUs from three separate DDUH clinics immediately after Orotol Plus aspiration disinfection with handpieces attached to suction hoses. Fourteen of these DCUs were equipped with Dürr LVE handpieces, identical to those used on the two new DCUs (Fig. 1a), and nine were equipped with Cattani LVE handpieces (Fig. 1d). Swab samples were taken weekly for five consecutive weeks from filter housings and LVE handpiece adaptors and for three consecutive weeks for the LVE handpieces and cultured on CBA. The vast majority (101/115, 87%) of filter housing samples remained contaminated, of which 44/115 (38%) yielded semi-confluent growth. Similarly, the vast majority (103/115, 98%) of LVE handpiece adaptors also remained contaminated with 94/115 (82%) yielding semi-confluent growth. All 14 Dürr and nine Cattani LVE handpieces remained contaminated, yielding average bacterial counts of 548 (±267) CFU/plate (range 134-1000 CFU/plate) and 339 (±167) CFU/plate (range 110-500 CFU/plate), respectively. In parallel with these experiments, neutralised swab samples were taken from 20 additional 12 year-old Planmeca Prostyle Compact DCUs from the same three clinics immediately after Orotol Plus aspiration disinfection but with suction handpieces removed in order to determine whether the presence of suction handpieces affected the disinfection efficacy of distal suction system sites. Swab samples were taken weekly for five consecutive weeks for filter housings and from both suction hoses adjacent to where the handpieces attach and cultured on CBA. Again the vast majority (90/100, 90%) of filter housing samples were contaminated of which 40/100 (40%) yielded semi-confluent growth. All low volume hose samples were contaminated of which 42/100 (42%) yielded semi-confluent growth. The majority (62/100, 62%) of high volume hoses samples were contaminated, however, only 3/100 (3%) yielded semi-confluent growth. These two sets of experiments with 12-year-old DCUs confirmed the results obtained in long-term studies with the two new test DCUs (Table 1), but also showed that the presence or absence of handpieces during aspiration disinfection resulted in similar levels of residual contamination at distal sites.

3.3. *Residual contamination of suction system following flood disinfection*

The second part of this study investigated disinfecting DCU suction systems by completely filling or flooding the suction system with Orotol Plus disinfectant. Initially this involved using a new Planmeca Compact i DCU with a special disinfectant reservoir fitted during manufacture (Fig. 2b). Suction hoses with suction handpieces removed from their adaptors could interface with sockets on the side of the reservoir (Fig. 2b). Disinfectant added to the reservoir filled the DCU suction hoses/system under gravity, was left *in situ* for three minutes and then voided to waste by suction system activation. A second approach to flood disinfection was undertaken using a novel suction disinfection system which was integrated into the new Planmeca Prostyle DCU during manufacture (Fig. 2c). The system referred to as STCS, is fitted to the rear of the DCU pedestal and possesses ports into which suction hoses are inserted after first removing the suction handpieces.

Neutralised swab samples were taken weekly for 20 consecutive weeks following flood disinfection of the suction systems for both the Compact i DCU fitted with the prototype disinfectant reservoir and from the Prostyle DCU equipped with the integrated STCS. The samples were plated on the same four media used for the aspiration disinfection study. Results of sampling were combined for both DCUs (n=40 for each site) as very similar data were obtained with each DCU separately (Table 1, lower panel). There was an extremely significant reduction ($P < 0.0001$) in the level of residual contamination observed on all agar media for all sample sites using flood disinfection compared to aspiration disinfection (Table 1). Residual contamination of the coarse filter housing, high and low volume hose samples following flood disinfection ranged from 3-15%, 0-8% and 6-25%, respectively, depending on the culture medium used, however, the levels of residual contamination were low (filter cage range 0-22 CFU/plate, high volume hose range 0-16 CFU/plate and low volume hose 0-14 CFU/plate) (Table 1).

3.4. *Residual contamination of suction hose handpieces following disinfection*

Flood disinfection using the prototype reservoir and STCS method did not permit disinfection of suction hose handpieces due to the necessity for their removal in the process. For this reason, separate experiments were performed with suction handpieces (one Dürr LVE handpiece and one Planmeca HVE handpiece (Fig. 1a and 1b)) fitted to the new Prostyle Compact DCU. Handpieces were subjected to immersion disinfection weekly for ten consecutive weeks. During this period, the DCU's suction system and handpieces were subjected to aspiration disinfection with Orotol Plus at the end of the morning and afternoon clinical sessions. Immediately following aspiration disinfection, the handpieces were detached and without disassembly, were immersed in Orotol Plus for three minutes. Following disinfection, the handpieces were disassembled, soaked in neutralising agent and sampled for residual microorganisms by plating onto CBA, R2A and PAS agar media. This experiment was repeated for another ten weeks but with the handpieces being disassembled prior to immersion in Orotol Plus.

All samples (n=20) from the non-disassembled handpieces showed residual bacterial contamination. The average counts from the HVE handpiece on CBA, R2A and PAS media were 489(±145), 484(±150) and 400(±116) CFU/sample, respectively. The LVE handpiece yielded higher average counts of 1722(±516),

1819(±532), and 1556(±439) CFU/sample, respectively. No residual bacterial contamination was detected in any of the samples (n=20) from the disassembled suction hose handpieces after disinfection.

3.5. *Contamination of DCU suction in public clinics*

The suction systems of 10 A-dec DCUs located in five Irish public dental clinics were also investigated for residual contamination following aspiration disinfection where the suction handpieces were still attached to the suction hoses. The suction systems of six of the DCUs were routinely disinfected with Orotol Plus whilst the remaining four used Green & Clean M2, a similar DCU suction disinfectant. The vast majority (19/20, 95%) of coarse filter housing samples in these DCUs exhibited residual bioburden with the majority (15/20 75%) yielding confluent growth (>501 CFU/plate) on CBA and R2A culture media. Confluent growth was also obtained from the majority of low volume suction hose samples 18/20 (90%). In contrast, lower levels of residual bacterial contamination were found in high volume suction hose samples as only 7/20 (35%) yielded confluent growth and 4/20 (20%) yielded semi-confluent growth. The majority of the remaining samples (8/20, 40%) yielded no residual contamination.

The majority, 15/20 (75%) of LVE handpieces were heavily contaminated, yielding confluent growth (>501 CFU/plate). In contrast, 7/20 (35%) of HVE handpieces yielded confluent growth and 3/20 (15%) yielding semi-confluent growth. HVE handpiece suction hose adaptors were also contaminated as 11/20 (55%) yielded semi-confluent growth. However, 6/20 (30%) of these adaptors yielded no growth at all. LVE handpiece suction hose adaptors yielded the highest level of contamination with 19/20 (95%) yielding confluent growth.

Swab samples from HVE and LVE handpieces of each of the 10 A-dec DCUs that yielded confluent growth on CBA and R2A agar were vortexed in 10 ml of PBS, serially diluted and plated on CBA agar. The average bacterial density recovered from the 10 samples was $4.2 \times 10^8 \pm 5.2 \times 10^8$ CFU/swab (range 6×10^4-1.45×10^9 CFU/swab).

3.6. *Novel design suction handpieces*

Due to the considerable residual contamination problems detected with suction hose handpieces and of the difficulties associated with effective disinfection without disassembling, new single component handpieces were designed without suction strength regulators and with a smooth interior bore (Fig. 2e). The handpieces were designed to accept suction aspirator tips and also to interface with the suction hose ports of the STCS disinfection unit (Figs. 2c and 2d). A series of experiments were performed with two separate one-year-old Planmeca Compact i DCUs equipped with the STCS system and the new design suction handpieces. The two DCUs were used routinely for clinical sessions at DDUH and were disinfected twice daily with the new design suction handpieces attached to suction hoses after morning and afternoon clinical sessions using Orotol Plus and the STCS system. For ease of analysis the results obtained with both DCUs were combined. None of the samples taken from low volume hoses (n=60), high volume suction hoses (n=60), low volume handpieces (n=60), or high volume handpieces (n=60) yielded any bacterial growth on CBA agar. Similar results were obtained on R2A agar, apart from one low volume hose sample that yielded < 10 CFU/plate. The vast majority (55-56/60, 91.7-93.3%) of filter cage samples yielded no growth on CBA or R2A agars, respectively. Two of the remaining samples (3.3%)

yielded < 10 CFU/plate on both media, two samples yielded between 10-100 CFU/plate on CBA, one of which yielded the same density range on R2A. The remaining sample yielded between 101-500 CFU/plate on both media.

3.7. *Other suction handpiece issues*

Orotol Plus at its working concentration (2%) was found to have an adverse affect on the integrity of aluminium components of the Planmeca HVE handpiece (Fig. 1b) and on the LVE handpiece hose adaptor (Fig. 1a). Following prolonged use, it was observed that the aluminium components of HVE handpieces and LVE handpiece adaptors of all 103 DCUS with which DDUH is equipped, became corroded and pitted following twice-daily aspiration disinfection with Orotol Plus (Fig. 3a-3c). Immersion of aluminium components from new Planmeca HVE handpieces and new LVE handpiece hose adaptors in Orotol Plus for several hours resulted in visible corrosion. This corrosion became more evident with longer immersion times (e.g. 24 h). These problems were resolved by replacing the aluminium components of HVE handpieces with plastic parts (Fig. 3d) and by replacing aluminium LVE handpiece hose adaptors with adaptors made of polyoxymethylene (POM) (Fig. 3d). However, following routine aspiration disinfection with Orotol Plus (data not shown), replacing these components did not cause a noticeable reduction in residual contamination of the handpieces or adaptors fitted to DCUs used for clinical sessions.

It was observed, following several years of use in DDUH, that many Dürr LVE handpiece valves used to regulate suction strength leaked (Fig. 1a and Fig. 3e). Disassembly of the valves from 50 Dürr LVE handpieces revealed the presence of extensive microbial biofilm determined both by microscopy and culture and in many cases the O-ring seals were either absent or had perished (Fig. 3f). Blood was evident in several cases. These findings indicate that Dürr LVE handpieces can leak fluids aspirated during use if the O-ring seals are damaged or absent.

3.8. *Blood splatter*

Removal of LVE handpieces for flood disinfection identified an issue with blood splatter. It was discovered that if blood was present inside a suction hose handpiece adaptor (Fig. 3g), when the handpiece was detached from the hose adaptor, blood could be splattered onto the person removing the handpiece. This effect was verified in the laboratory by pipetting 250 µl of horse blood into the handpiece attached to the adaptor, vortexing, then pulling them apart inside a cone of filter paper (Fig. 3h). It was found that in 90% (n=10) of cases blood splatter was generated.

3.9. *Bacterial species recovered from suction*

Twenty-three bacterial species recovered from DCU suction systems were identified by 16S rDNA sequencing (Table 2). These included several Gram-positive species commonly isolated from humans including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus, Staphylococcus warneri*, *Streptococcus mitis*, *Streptococcus pneumoniae* and *Streptococcus salivarius*. *Staphylococcus aureus* is a major human pathogen, whereas the other staphylococci are common skin commensals. *Staphylococcus epidermidis*,

Streptococcus mitis and *Streptococcus salivarius* are commonly present in the oral cavity. Several environmental Gram-negative bacterial species were also identified including a number of potentially pathogenic species including *Pseudomonas aeruginosa*, *Serratia marcescens* and *Stenotrophomonas maltophilia.* Other Gram-negative environmental species identified included species commonly found in dental unit waterlines such as *Comamonas acidovorans*, *Novosphingobium subartica* and a range of *Sphingomonas* species (Table 2). The common oral yeast species *Candida albicans* was also identified from several suction site samples. Environmental bacterial species were the predominant bacterial species recovered from DCU suction sites.

Pseudomonas aeruginosa was most frequently recovered from coarse filter housings, LVE handpiece adaptors and from the suction strength regulator valves of Dürr LVE handpieces. *Pseudomonas fluorescens* and *Pseudomonas putida* were most frequently recovered from coarse filter housings and suction hoses. Staphylococcal species were most frequently recovered from suction hoses, suction handpieces and suction hose handpiece adaptors. Oral streptococci were recovered from throughout the suction system.

Oral streptococci and Gram-negative environmental bacterial species were the predominant bacterial species recovered from suction system sites in the public dental clinic A-dec DCUs.

4. Discussion

The moist conditions that exist within dental suction systems are conducive to the growth and proliferation of microbial biofilms.[6] Dental unit suction systems by their very nature become contaminated with oral and dental waterline-derived microorganisms and therefore have to be cleaned and decontaminated regularly.[3] There is a scarcity of studies in the scientific literature on the infectious potential of dental suction, and of the few published studies, most focus on the possibility of cross-contamination of patients due to backflow and pressure changes in the low volume suction.[3,4,7] The complexity of DCU suction systems can mean that disinfection is not straightforward. Dental practitioners are advised to follow manufacturer's instructions for suction system decontamination. In general terms, this involves the aspiration of a recommended cleaning/disinfecting solution through the suction system via the suction hoses on a daily or twice daily basis. The objective is to clean/disinfect the suction hoses and internal suction system components including the coarse filter housing and suction system pipework. Suction hose handpieces also form part of the DCU suction system, but their effective decontamination is more challenging. Previous studies from this laboratory using the phenolic DCU suction system disinfectant Puli-Jet[6] and more recent preliminary studies with the quaternary ammonium-containing disinfectant Orotol Plus revealed that conventional aspiration disinfection of DCU suction systems left significant residual microbial bioburden and therefore the purpose of this study was to develop more effective disinfection approaches.

The first part of this study investigated the efficacy of the aspiration disinfection method on two new Planmeca DCU suction systems. After being used routinely for patient treatment, several suction system sites were swabbed for residual microbial contamination, weekly for 25-27 weeks (n=52 samples per site) following aspiration disinfection with Orotol Plus. Swabs were cultured on a variety of nutrient and selective agar media to maximise the recovery of environmental and human-derived bacterial species used as marker organisms for microbial contamination. Results demonstrated that conventional aspiration disinfection left significant residual

contamination throughout the DCU suction system (Table 1). The results revealed that a majority of samples from coarse filter housings (up to 60%) and low volume suction hoses (up to 37%) yielded semi-confluent growth (101-500 CFU/plate) depending on the culture media used (Table 1). Semi confluent growth was also found in 38% of coarse filter housing samples taken from 23 additional 12 year-old Planmeca DCUs. Furthermore, residual contamination of DCU suction following aspiration disinfection was not confined to Planmeca DCUs as post-disinfection sampling (n=20) of 10 A-dec DCUs in five public clinics revealed similar considerable residual contamination. Samples from the coarse filter housing and low volume suction yielded confluent bacterial growth (average density of $4.2 \times 10^8 \pm 5.2 \times 10^8$ CFU/swab) in 75% and 90% of cases, respectively. Similar results were reported in a previous study where twice daily aspiration disinfection of suction systems from Planmeca DCUs using the phenolic disinfectant Puli-Jet left considerable residual microbial contamination in suction hoses and filter housings.[6] The study found that 78.4% (29/37) of suction hoses sampled after disinfection yielded confluent growth (≥1000 CFU/swab) of *Pseudomonas* species.

The underlying reason(s) behind failure to minimise microbial contamination in DCU suction systems is probably related to the disinfection process itself. Aspiration disinfection generates an air/disinfectant mixture that is sucked through the suction system with the intent of coating the internal surfaces but does not completely fill the system. This could result in lack of contact by the disinfectant or inadequate contact time and therefore it is probable that particular areas are not adequately disinfected, especially areas harbouring considerable bioburden.

In an attempt to improve the efficacy of disinfection of DCU suction systems experiments were undertaken with a new Planmeca Compact i DCU that was equipped with a removable disinfectant reservoir that permitted complete filling or flooding of the suction system with Orotol Plus disinfectant (Fig. 2b). The DCU was used for routine dental treatment and disinfected twice daily by flood disinfection. When the sampling protocol used in the aspiration disinfection part of the study was applied to the flooding disinfection study, an extremely significant and consistent reduction ($P < 0.0001$) in levels of contamination at all sites tested (Table 1, lower panel) was recorded. These findings indicated that completely filling the DCU suction system with Orotol Plus provides superior disinfection relative to aspiration disinfection, probably due to increased contact time, contact area and disinfectant availability throughout the suction system.

Based on these results an integrated system for automated flood disinfection of DCU suction systems was developed in collaboration with Planmeca. The system is called the Suction Tube Cleaning System (STCS) and permits the DCU suction system to be filled with disinfectant automatically (Fig. 2c). An additional series of experiments was undertaken with a new Planmeca Prostyle DCU equipped with STCS that was used for routine dental treatment and disinfected twice daily by flood disinfection. Samples were taken post-disinfection for 20 consecutive weeks from suction system sites and cultured. Again, there was an extremely significant and consistent reduction ($P < 0.0001$) in levels of contamination at all sites tested (Table 1, lower panel). The development of STCS not only significantly improved DCU suction system decontamination but also simplified and automated the process. The set up time for STCS decontamination takes about 30 seconds per DCU.

There is no consensus between manufacturers regarding the cleaning and decontamination of DCU suction handpieces. In general, it is recommended that suction handpieces should be cleaned/disinfected separately from the rest of the suction system, either following each patient use or more usually on a daily basis. The DCU manufacturer Planmeca recommends daily autoclaving of suction handpieces and dismantling them for cleaning on a weekly basis,[12] whereas A-dec recommends that suction handpieces should be dismantled and cleaned/disinfected daily but steam sterilisation is optional.[13] The dental suction handpiece manufacturer Dürr recommends that suction handpieces should be dismantled and cleaned/disinfected after each use followed by steam sterilisation.[14] Cleaning of handpieces involves detachment from the suction hose, dismantling into individual components, immersing in disinfectant or thermally disinfecting using a washer disinfector, greasing of O-rings, reassembling and then steam sterilisation. Some manufacturers further specify that handpiece lumens should be cleaned daily with a brush using a recommended cleaning agent. To accommodate these recommendations suction handpiece cleaning/decontamination requires very considerable resources in terms of staff time and expertise, use of cleaning and sterilisation equipment and replacement handpieces, especially in dental hospitals equipped with many DCUs. Handpieces also have to be reassembled correctly following cleaning/decontamination and there is considerable potential for human error in this regard. Incorrectly assembled handpieces may not function correctly and may leak aspirated fluids/aerosols during use. Furthermore, because suction handpieces are multicomponent, small parts can easily be damaged or lost during reprocessing. In the authors' experience many dentists are unaware of manufacturers recommendations for decontamination of suction handpieces by dismantling and the necessity for further processing as described above. In reality, for logistical reasons and due to time constraints in busy dental clinics, dental suction handpieces are seldom disassembled. Suction handpieces are generally left attached to their hoses and suction disinfectant is aspirated through them during routine DCU suction system disinfection. This was the situation in DDUH prior to this study. After residual contamination of handpieces was identified as a problem in DDUH, as an interim measure all DCU suction handpieces were removed, dismantled, disinfected, cleaned and reassembled every evening, a process that took one staff member fours hours to complete at a cost of approximately €16,000 per annum.

In the first part of this study, over a period of 25-27 weeks, the efficacy of aspiration disinfection of two new Planmeca DCU suction systems was investigated where the suction handpieces were left attached during disinfection. Swab sampling of handpiece adaptors (components that link handpieces to suction hoses, see Figs. 1a and 1b) and LVE and HVE handpieces showed that 63.5% and 73% of samples (n=52 in each case) from LVE and HVE handpiece adaptors and 15-23% and 6-13.5% of samples from LVE and HVE handpieces (n=52 in each case), respectively, yielded semi-confluent growth. Similar findings were obtained with additional samples taken once weekly from LVE handpiece adaptors and LVE handpieces from 23 12 year-old Planmeca DCUs over a period of 3-5 weeks. Sampling of handpieces and their respective adaptors of 10 A-dec DCUs located in five public clinics showed that the post-disinfection contamination was not specific to DCU manufacturer or location, as confluent growth was recovered in 75% of LVE handpieces and 95% of LVE handpiece adaptors. To confirm the findings that aspiration disinfection cannot adequately disinfect suction handpieces, a pair of handpieces from

the new Planmeca Prostyle Compact DCU used daily for routine dental treatment were removed once weekly following aspiration disinfection and immersed in Orotol Plus for three minutes, then disassembled and the components immersed in neutralising agent after which time viable microorganisms were recovered by vortexing the components in PBS. All samples from the handpieces showed residual contamination with average bacterial counts ranging from 400 to 1819 CFU/sample, depending on the culture medium used. In contrast, a similar set of experiments using handpieces disassembled prior to immersion in Orotol Plus yielded no bacterial growth when sampled. These findings confirm that multicomponent dental suction handpieces need to be dismantled for effective decontamination as they contain areas shielded from disinfection when assembled. Finally, it was observed that removal of LVE handpieces from their hose adaptors generated blood splatter if blood was present in the suction handpiece/adaptor junction (Fig. 3g and 3h). This phenomenon was reproducible in the laboratory and highlights a potential risk of infection with blood-borne viruses to dental healthcare staff as blood splashes to mucus membranes have previously been shown to transmit viral infection to other healthcare personnel.[15]

Another issue arising from failure to disassemble Dürr LVE handpieces prior to cleaning/disinfection was also identified. Many such handpieces were found to leak aspirated fluids around the suction strength regulator valve, a phenomenon that was associated with damaged or absent O-ring seals following prolonged use (Fig. 3f). This highlights the fact that the hygienic integrity of these handpieces requires regular inspection and maintenance of components to prevent contamination to the exterior contact surfaces of handpieces by leaked microorganisms or aspirated oral fluids.

In order to simplify suction handpiece decontamination, new single component, smooth bore HVE and LVE handpieces were developed (Fig. 2e). These novel handpieces lack valves to regulate suction strength and do not require handpiece adaptors as they interface directly with suction hoses. The handpieces can also couple directly with the STCS suction disinfection system as they are designed to be disinfected without removal from the suction hoses (Figs. 2c to 2e). A series of experiments was then undertaken using two one-year-old Planmeca Compact i DCUs equipped with STCS and these new design suction handpieces in order to determine the efficacy of handpiece disinfection. The two DCUs were used routinely for dental treatment and were disinfected twice daily-using STCS and Orotol Plus. Thirty separate samples were taken from several suction system sites from each DCU immediately following disinfection and cultured on CBA and R2A media. None of the samples (n=60 in each case) from low or high volume hoses or the associated suction handpieces yielded any bacterial growth. Similarly, 91.7-93.3% of the associated coarse filter housing samples yielded no bacterial growth. These findings demonstrated that the new suction handpieces can be disinfected effectively using the STCS system. Furthermore, the combination of flood disinfection and the new handpieces provides a simple and automated approach to DCU suction system decontamination that does not require extensive reprocessing of suction handpieces. This new system facilitates the simultaneous effective disinfection of all parts of DCU suction, a process that is automated and takes about five minutes to complete. Since completion of this study, the DDUH has installed 113 new Planmeca DCUs, all of which are equipped with the STCS and the new LVE and HVE suction handpieces. The STCS system and handpieces are provided as an option with new Planmeca DCUs at a cost of approximately

€1,460. This cost is more than offset in suction system decontamination efficacy and in staff time saved by not having to remove and dismantle suction handpieces for reprocessing. The absence of vacuum regulation on the new design handpieces has not been an issue in the delivery of dental care at DDUH. In cases where vacuum regulation maybe desirable for the provision of dental care to some special needs or paediatric patients, mini Yankauer LVE suction tips containing a thumb port to regulate suction can be used.

Corrosion of aluminium LVE handpiece adaptors and aluminium components of HVE handpieces associated with the use of Orotol Plus disinfection of DCU suction systems was observed in DDUH. Untreated aluminium generally has very good corrosion resistance, primarily due to the formation of a thin oxide layer, which prevents further oxidation. This oxide layer is stable within the pH range of 4-9, however, Orotol Plus at working concentration has a pH of 10-11. The prevalence of *P. aeruginosa* in LVE handpieces and associated adaptors may also have contributed to the corrosion as it is known to have biocorrosive effects on aluminium alloys.[16] The combination of Orotol Plus-induced corrosion of aluminium components and the prevalence of *P. aerugoinosa* may have had an additive effect as increased surface roughness leads to increased bacterial biofilm attachment and subsequent biocorrosion. A previous study from this laboratory reported an association between *P. aeruginosa* from DCU suction systems and corrosion of steel DCU components.[6] There is an important design obligation on DCU manufacturers to ensure compatibility of materials with the suction cleaning/disinfection agents that they recommend for use with their DCUs. In the present study, replacing aluminium suction adaptor and handpiece components with plastic alternatives resolved the issue of Orotol Plus-associated corrosion.

The bacterial species identified from post-disinfection suction system samples were a mixture of environmental and human-derived organisms (Table 2). Many of the Gram-negative aerobic heterotrophic environmental species such as *Pseudomonas* and *Sphingomonas* species, amongst others, probably originated in the dental unit waterlines as they have frequently been recovered from DCU water.[10,17-20] DCU suction is used to remove DCU waterline water from the oral cavity during dental treatment and to reduce aerosols generated by dental handpieces and ultrasonic scalers. Some of these species may be of concern in the dental treatment of immunocompromised patients such as those with cystic fibrosis.[8] The majority of human-derived bacterial species identified were Gram-positive cocci including staphylococcal and streptococcal species. Barbeau and colleagues found a similar array of bacterial species in DCU suction systems including Gram-negative species such as *P. aeruginosa, P. fluorescens* and *Sphingomonas paucimobilis* and Gram-positive species including *S. aureus* and *S. epidermidis*.[3] Barbeau *et al*. also reported a virtual absence of oral streptococci, whereas in the present study we found α-hemolytic streptococci in a number of samples, particularly from low volume suction. Gram-negative bacteria such as *P. aeruginosa* were most frequently recovered from the suction system of DCUs from DDUH, whereas Gram-positive bacteria such as oral streptococci were most frequently isolated from DCUs in the five dental public clinics. Merchant and Molinari also reported the recovery of Gram-negative bacilli and staphylococci from DCU suction hoses and coarse filter housings.[5] The staphylococcal species recovered in the present study from DCU suction sites included *S. aureus*, *S. epidermidis*, *S. haemolyticus* and *S. warneri* and probably originated from the oral cavities of dental patients. These species are common skin commensals and *S.*

aureus, *S. epidermidis* frequently colonise the nose, from where they can be trafficked into the oral cavity.[21] *Staphylococcus aureus* is a significant and versatile human pathogen responsible for numerous healthcare-associated and community-associated infections worldwide. It can cause a wide variety of infections related to its ability to express an extensive range of virulence factors, toxins and antimicrobial agent resistance determinants, many of which are encoded by mobile genetic elements.[22-24]

To date, few studies have focused on infections in patients and/or dental healthcare staff resulting directly from exposure to contaminated DCU suction systems and none has reported exemplary cases. A number of studies however, have highlighted the potential for cross-contamination between patients due to backflow/pressure changes in low volume suction.[3-5] It is very possible that DCU suction-associated infections have gone undetected or unreported because of failure to associate exposure with the development of specific infections after dental treatment. The present study demonstrates that high levels (sometimes > 1 x 10^6 CFU) of significant human pathogens including *S. aureus* and *P. aeruginosa* can remain in DCU suction systems despite following recommended disinfection protocols. Residual contamination was also evident in multicomponent suction handpieces following disinfection. Some of these were subject to leakage due to disinfectant corrosion or component degeneration following long-term use. Such leaking suction handpieces can contaminate the gloved hands of the dentist or dental nurse and potentially result in cross-contamination of instruments, equipment and surfaces in the patient treatment zone, or directly introduce high levels of pathogenic bacteria into the oral cavity of the patient being treated. Contaminated equipment and surfaces in healthcare environments are well-recognised sources of healthcare-associated infection.[25] There is an onus in healthcare to reduce the risk of infection from all sources including dental suction. Studies on dental suction are rare and the present study focuses attention on a neglected area of infection prevention and control in dentistry. The combined use of automated flood disinfection and single component handpieces provides a demonstrably effective solution to minimise potential infection risks from dental suction by eliminating contamination sources.

5. Conclusions

The results of this study demonstrate the effective failure of conventional aspiration disinfection in DCU suction systems. This leaves significant residual microbial and other contamination in suction hoses and coarse filter housings, probably due to insufficient disinfectant contact time and availability. This problem was resolved by development of the automated DCU suction cleaning/disinfection STCS system that enables DCU suction systems to be completely filled with disinfectant and retained for a specified period, followed by automated evacuation to waste. This study also showed that conventional multicomponent dental suction hose handpieces cannot be effectively cleaned/decontaminated without first detaching them from their suction hoses followed by disassembly to expose areas that are shielded from disinfectant action. Routine disassembly of suction handpieces followed by cleaning/disinfection requires considerable resources in staff time and replacement handpieces, especially in dental hospitals equipped with many DCUs. In essence, suction handpieces and DCU suction systems have to be cleaned/disinfected separately. The development of novel, single-component, smooth bore

handpieces allows effective cleaning/decontamination of DCU suction systems, hoses and suction handpieces using the STCS system, all without the need to remove handpieces from suction hoses. These developments provide a simple, rapid and automated solution to provide effective DCU suction system decontamination and eliminate potentially substantial infection reservoirs from the clinical workspace.

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Figure legends

Fig. 1- Components of the suction system of the DCUs used in this study. (**a)** A Dürr LVE handpiece disassembled (right) and assembled (left). 1 indicates the aluminium adaptor that inserts into the LVE suction hose. **(b)** A Planmeca HVE handpiece disassembled (right) and assembled (left). 1 indicates the adaptor that inserts into the HVE suction hose. **(c)** Suction handpieces attached to suction hoses on a DCU. Key: 1, HVE suction hose; 2, LVE suction hose; 3 reusable HVE suction tip; 4, single-use LVE suction tip; 5, adaptor fitted to LVE suction hose into which the LVE handpiece is attached. **(d)** A Cattani LVE handpiece disassembled (right) and assembled (left). This handpiece uses the same type of adaptor shown in panel (a) to insert into the LVE suction hose. **(e)** View of the suction system coarse filters located within a DCU. The HVE and LVE systems each has its own coarse filter to trap large particles of debris aspirated during suction use. Some DCU models have only one coarse filter. Filters are removed from the DCU each evening and cleaned and disinfected by immersion in Orotol Plus solution. Disinfectant aspirated through the suction hoses passes through the coarse filter housings (*) and filters (yellow components) and is then voided to waste.

Fig. 2 – Conventional aspiration disinfection and flood disinfection of DCU suction. **(a)** Aspiration disinfection. Disinfectant is placed in the OroCup container and suction hoses with handpieces are attached to receivers in the lid. Suction is activated from the DCU and the air/disinfectant mixture generated is aspirated through the hoses and attached suction handpieces. **(b)** An experimental reservoir used for flood disinfection of DCU suction. Suction hoses without handpieces are inserted into

767 ports on the reservoir, which is then filled manually with Orotol Plus disinfectant, which then flows into the hoses and the
768 internal components of the DCU suction system until completely filled. Following three minutes, the DCU suction is
769 activated and disinfectant is voided to waste. **(c and d)** Novel automated DCU suction flood disinfection system (STCS)
770 fitted to a Planmeca Compact DCU. STCS consists of a suction hose attachment unit fitted to the rear of the DCU.
771 Disinfectant concentrate is placed in the reservoir **[1],** suction hoses with the new single component LVE and HVE
772 handpieces are inserted into the ports **[2]** and the disinfection cycle activated by pressing a button **[3]**. A water outlet **[4]**
773 dispenses a predetermined volume of water to dilute the disinfectant concentrate, which then completely fills the DCU
774 suction system. Following three minutes the DCU suction activates automatically voiding the disinfectant to waste. **(e)** New
775 single component HVE (left) and LVE (right) suction handpieces developed during this study.

776 **Fig. 3 –** Corrosion, leakage and biofilm issues associated with DCU suction handpieces and adaptors. **(a)** The aluminium
777 LVE handpiece adaptor on the right shows corrosion following prolonged use with Orotol Plus. **(b)** Corroded LVE handpiece
778 adaptor showing extensive pitting. **(c)** The aluminium component of the Planmeca HVE handpiece on the right shows
779 corrosion following prolonged use with Orotol Plus. **(d)** New design Planmeca HVE handpiece and hose adaptors that resist
780 corrosion by Orotol Plus. The handpiece on the left has a new plastic central component and interfaces with the HVE hose
781 using a new design adaptor made of polyoxymethylene (POM). The Dürr LVE handpiece shown on the right interfaces with
782 the LVE hose using a new design adaptor made of POM. **(e)** Detail of Dürr LVE handpiece showing blood leaking around the
783 suction strength regulator following clinical use. **(f)** Suction strength regulator valves removed from Dürr LVE handpieces
784 used at DDUH. The discolouration on both valves is due to microbial biofilm. The arrow points to a groove missing an O-
785 ring. **(g)** A suction hose handpiece adaptor after removal of the handpiece following clinical use showing heavy blood
786 contamination. **(h)** Blood splattered onto filter paper following detachment of the LVE handpiece from the suction hose
787 adaptor harbouring blood shown in panel (g).

788

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Table 1- Comparative bacterial contamination of dental unit suction system sites following aspiration disinfection[a] and flood disinfection[b] with Orotol Plus

Suction site (n=52 per site)		**Coarse filter housing**				**High volume suction hose**				**Low volume suction hose**			
Aspiration disinfection		**Bacterial density in CFU/plate following disinfection with Orotol Plus**											
	Culture medium	CBA	R2A	PAS	PA	CBA	R2A	PAS	PA	CBA	R2A	PAS	PA
	CFU/plate												
	<10	2%	2%	0%	6%	22%	10%	23%	12%	4%	35%	4%	0%
	10-100	30%	27%	50%	30%	38%	36%	33%	46%	50%	38%	53%	60%
	101-500	58%	60%	40%	56%	0%	19%	0%	2%	37%	0%	25%	33%
	501 - 1000	4%	8%	2%	2%	0%	0%	0%	0%	8%	0%	6%	6%
	>1000	2%	0%	2%	2%	0%	0%	0%	0%	0%	0%	2%	0%
	CFU Range[c]	0 - >1000	0-500	0 - >1000	0 - >1000	0-108	0-144	0-88	0-84	0-10	0-39	0 - >1000	0-300
	% sites contaminated	96%	97%	94%	96%	60%	65%	56%	60%	98%	73%	100%	99%
Suction site (n=40 per site)													
Flood disinfection	**CFU/plate**												
	<10	13%	10%	3%	5%	0%	0%	0%	8%	17%	3%	25%	20%
	10-100	2%	2%	0%	3%	3%	0%	0%	0%	3%	3%	0%	3%
	101-500	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	501 - 1000	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	>1000	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	CFU Range[c]	0-13	0-22	0-10	0-2	0-14	0-22	0	0-4	0-10	0-14	0-8	0-12
	% sites contaminated	15%	12%	3%	8%	3%	0%	0%	8%	20%	6%	25%	23%

[a] The suction systems of a Prostyle Compact and a Compact i DCU were subjected to aspiration disinfection with Orotol Plus after morning and afternoon clinical sessions with suction handpieces attached to the suction hoses (Fig. 2a). Immediately afterwards swabs soaked in disinfectant neutraliser were used to sample the sites indicated once weekly for 27 and 25 weeks, respectively, and cultured on CBA, R2A, PAS and PA agar media.

[b] The suction systems of the same DCUs used for aspiration disinfection were subjected to flood disinfection with Orotol Plus twice daily with suction handpieces detached from the suction hoses in a separate set of experiments for 20 consecutive weeks. The Compact i DCU had its suction system flood disinfected using a prototype disinfectant reservoir (see section 2.7 and Fig. 2b) and the Prostyle Compact's suction system was flood disinfected using the integrated and automated Planmeca STCS system (see section 2.8 and Figs. 2c and 2d). Immediately afterwards swabs soaked in disinfectant neutraliser were used to sample the sites indicated once weekly for 20 weeks and cultured on CBA, R2A, PAS and PA agar media.

[c]The CFU range shown was determined from colony counts on plates inoculated directly from swab samples. For samples yielding confluent growth (i.e. >501 CFU per plate), the quantitative bacterial density range determined by serial dilution of bacteria recovered from swab**s** by vortexing in PBS for all media was 1098 - 6.14 x 10^6 CFU/swab (mean average 1.03 x 10^6 ± 1.52 x 10^6 CFU/swab).

Table 2- Bacterial species isolated from dental unit suction systems during the study

Bacterial species*	**Gram-stain**
Aeromonas salmonicida	Negative
Acidovorax temperans	Negative
Comamonas acidovorans	Negative
Novosphingobium subarctica	Negative
Pseudomonas aeruginosa	Negative
Pseudomonas fluorescens	Negative
Pseudomonas putida	Negative
Serratia marcescens	Negative
Sphingomonas aerolata	Negative
Sphingomonas paucimobilis	Negative
Sphingomonas trueperi	Negative
Stenotrophomonas maltophilia	Negative
Arthrobacter agilis	Positive
Kocuria palustris	Positive
Micrococcus luteus	Positive
Rhodococcus fascians	Positive
Staphylococcus aureus	Positive
Staphylococcus epidermidis	Positive
Staphylococcus haemolyticus	Positive
Staphylococcus warneri	Positive
Streptococcus pneumoniae	Positive
Streptococcus salivarius	Positive
Streptococcus mitis	Positive

*Identified by DNA sequence analysis of the variable region of 16S rDNA gene. [11,17,19]

Figure 1

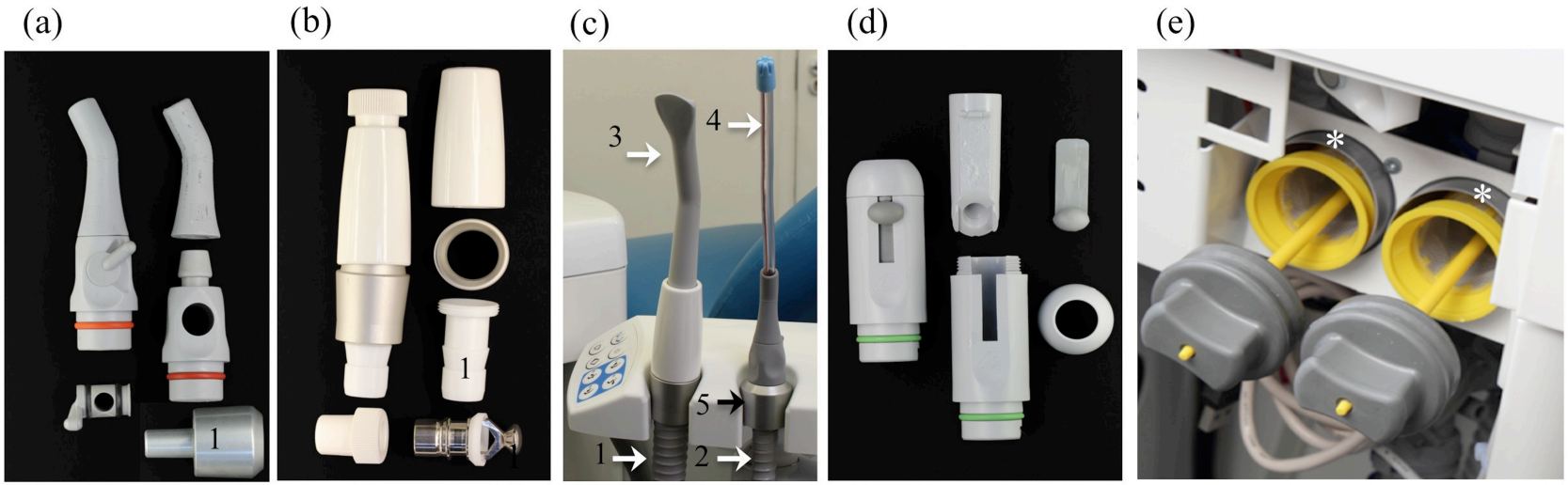

Figure 2

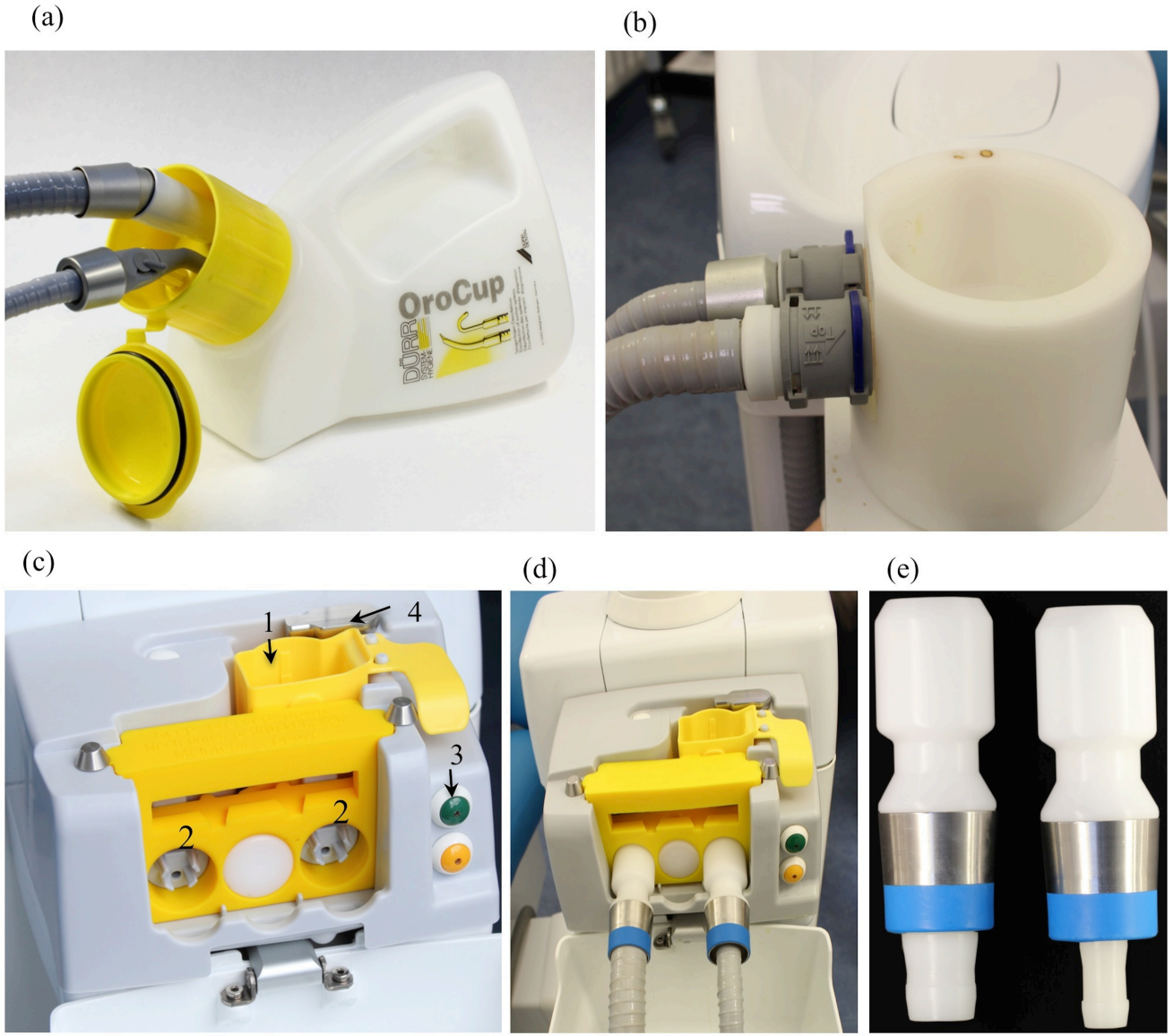

Figure 3