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Comparison of two endoscope channel cleaning approaches to remove cyclic build-up biofilm

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SUMMARY

Introduction: Biofilm contributes significantly to bacterial persistence in endoscope channels. Enhanced cleaning methods capable of removing biofilm from all endoscope channels are required to decrease infection risk to patients. This head-to-head study compared cyclic build-up biofilm removal of an automated endoscope channel cleaner (AECC) with standard manual cleaning according to instructions for use (IFU) in polytetrafluorethylene channels.

Methods: Cyclic build-up biofilm was grown in 1.4-mm (representing air/water and auxiliary channels) and 3.7-mm (representing suction/ biopsy channels) inner diameter polytetrafluorethylene channels. All channels were tested for residual total organic carbon, protein, and viable bacteria. Internationally recognized ISO 15883-5:2021 alert levels were used as cleaning benchmarks for protein (3 $\mu g/cm^2$) and total organic carbon (6 $\mu g/cm^2$).

Results: The automated cleaner significantly outperformed manual cleaning for all markers assessed (protein, total organic carbon, viable bacteria) in 1.4-mm and 3.7-mm channels representing air/water/auxiliary and suction/biopsy channels, respectively. Manual cleaning failed to remove biofilm from the air/water and auxiliary channels. According to the IFU, these channels are not brushed, suggesting a potential root cause for a portion of the numerous endoscopy-associated infections reported in the literature.

Conclusion: AECC shows potential to deliver enhanced cleaning over current practice to all endoscope channels and may thereby address infection risk.

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Introduction

Flexible endoscopes are associated with the greatest number of patient infections reported in the literature compared with any other reusable medical device [1]. Studies have demonstrated repeated transmission of the same strain from contaminated endoscopes to patients over time, highlighting that the endoscope can be a long-term reservoir for infectious organisms despite reprocessing [2,3].

Biofilms have been identified in endoscope channels despite compliant cleaning and disinfection per instructions for use and clinical practice guidelines [4—6]. Primo *et al.* [4] found biofilm accumulated in new gastroscope channels within 30—60 days of clinical use and reprocessing, most frequently in air/water channels [4]. Evidence is also emerging regarding biofilm resistance to high-level disinfectants [7,8]. Recently a clinical carbapenem-resistant enterobacteriaceae (CRE) isolate from a duodenoscope-linked outbreak exhibited weak resistance against peracetic acid when in planktonic form, but strong resistance when in a biofilm form [8]. This study established a direct link between biofilm formation and high-level disinfection (HLD) tolerance in endoscopes, to nosocomial transmission of CRE [8].

Given these issues, it is clear that new cleaning methods should address biofilm removal from all channels. Biofilm removal has not historically been a requirement for cleaning validation of endoscopes. Different growth/fixation conditions yield morphologically different biofilms [9], emphasizing the importance of selecting models that are relevant to endoscopy for validation. Ribeiro *et al.*'s previously published cyclic build-up biofilm (CBB) represents the build-up of tough biofilm during clinical use and reprocessing cycles of endoscopes [10]. CBB involves a multi-day protocol of soiling, rinsing, fixation of residual soil with glutaraldehyde and drying [10]. This study compared the biofilm removal efficacy of an automated endoscope channel cleaner (AECC) with manual cleaning (MC) in polytetrafluoroethylene (PTFE) surrogates used to simulate endoscope channels using this model.

Methods

Biofilm growth

PTFE endoscope channels of 1.8 m length and 3.7 mm or 1.4 mm internal diameters (IDs) representing suction/biopsy (S/B) and air/water and auxiliary (AW/AUX) channels, respectively, were autoclaved. CBB was grown as previously described [10] with the following modifications for AW/AUX channels: 18 h bacteria-artificial test soil (ATS2015, Healthmark, Fraser, MI) circulation time; 6 h storage time; 0.3 mL/min flow rate; three fixation cycles. Sixteen replicates per condition were prepared.

Cleaning procedure

MC adhered to the endoscope (GIF/CF/PCF-190 Series, Olympus, Tokyo, Japan) and detergent (Endozime Xtreme Power, Ruhof, Mineola, NY, USA) instructions for use (IFU) for endoscope S/B and AW/AUX channels. In accordance with the IFU, brushing was not utilized in AW/AUX channels as they are inaccessible to brushes in endoscopes. AECC cleaning with the investigational device (CORIS, Nanosonics Ltd, Australia) was performed per IFU for these same channels. AECC uses a specially formulated cleaning agent and delivery mechanism to physically clean all endoscope channels. The cleaning agent is mixed with water in the device to create a saturated solution of liquid and solid. Small quantities of this mixture are then propagated through the endoscope at high velocity to clean the channels. After cleaning, the channels are flushed with water and the non-toxic, water-soluble cleaning agent is removed. An air purge then removes residual water. The endoscope is subsequently placed in an automated endoscope reprocessor for HLD or sterilization.

Sample extraction and assays

Sterile deionized water was used to extract channels using the flush-brush-flush method (40 mL and 10 mL for 3.7 mm and 1.4 mm ID, respectively). Samples were vortexed (10 s) and sonicated (40 kHz, 5 min) twice, then vortexed for 30 s. Protein detection was performed using the bicinchoninic acid assay (Micro BCA protein assay 23235, Thermo Fisher Scientific, Waltham, MA, USA). Total organic carbon (TOC) detection was performed using the non-purgeable organic carbon method (TOC-L CPH/CPN, Shimadzu, Kyoto, Japan). *Pseudomonas aeruginosa* (ATCC15442) and *Enterococcus faecalis* (ATCC 29212) were detected by counting colony forming units (cfu) after incubation on trypticase soy agar plates (16—18 h). For S/B channels the limits of detection (LOD) and limits of quantitation (LOQ) were as follows: protein BCA assay LOQ = 0.63 $\mu g/cm^2$ and LOD = 0.19 $\mu g/cm^2$; TOC assay LOQ = 0.23 $\mu g/cm^2$ and LOD = 0.08 $\mu g/cm^2$; culture LOQ = 1.91 cfu/cm^2 and LOD = 0.19 cfu/cm^2. The limits for AW/AUX channels were as follows: protein assay LOQ = 0.42 $\mu g/cm^2$ and LOD = 0.13 $\mu g/cm^2$; TOC assay LOQ = 0.15 $\mu g/cm^2$ and LOD = 0.05 $\mu g/cm^2$; culture LOQ = 1.26 cfu/cm^2 and LOD = 0.13 cfu/cm^2.

Cleaning benchmarks

Internationally recognized ISO 15883-5:2021 alert levels were used as cleaning benchmarks for protein (3 $\mu g/cm^2$) and TOC (6 $\mu g/cm^2$).

Statistical analysis

All statistical analyses were conducted using Stata v18 (Stata Corp, College Station, TX, USA). The differences between cleaning conditions were estimated using linear regression. Statistical inference was calculated via the non-parametric bootstrap using 2000 bootstrap samples, due to non-normality of the distributions of residuals. Missing values for biofilm (Ri), caused by actual values less than LOQ or less than LOD, were imputed by assigning random values either $LOD < R_i \leq LOQ$ if below LOQ or $0 \leq R_i \leq LOD$ if below LOD. This procedure avoided artificial reduction of the within-condition variance and was repeated 1000 times and the study contrasts estimated within each resulting sample. The pooled results of the 1000 samples are reported. In effect, we utilized random processes to avoid any systematic bias in the estimated difference between cleaning conditions while also avoiding a potential artificial inflation of statistical power.

Results

In S/B channels, both cleaning methods reduced protein and TOC to below the ISO 15883-5:2021 alert levels (Figure 1). AECC significantly outperformed MC in CBB removal across all markers, and reduced protein to below the LOQ and viable *P. aeruginosa* to below the LOD. In AW/AUX channels, MC failed to clean with little impact versus positive control (Figure 2). AECC reduced both protein and *P. aeruginosa* to below the LOD and TOC to below the alert level in these channels. MC was unable to remove CBB in AW/AUX channels with residuals remaining >10 times (protein) and >3 times (TOC) the alert levels. Over 10^6 cfu/cm^2 of each organism also remained after MC.

Discussion

The AECC significantly outperformed MC in CBB removal across both simulated S/B and AW/AUX endoscope channels (Figures 1, 2).

While both cleaning methods reduced protein and TOC in S/B channels to suitable levels of cleaning according to current guidance (Figure 1), it is unclear whether organisms in residual biofilm can be successfully high-level disinfected even when residual soil is below these limits. For instance, a study from the Robert Koch Institute found a clinical CRE isolate was resistant to HLD in biofilm form [8]. Possible mechanisms for microbial resistance to disinfection include the presence and characteristics of older biofilms, genotypic diversity, production

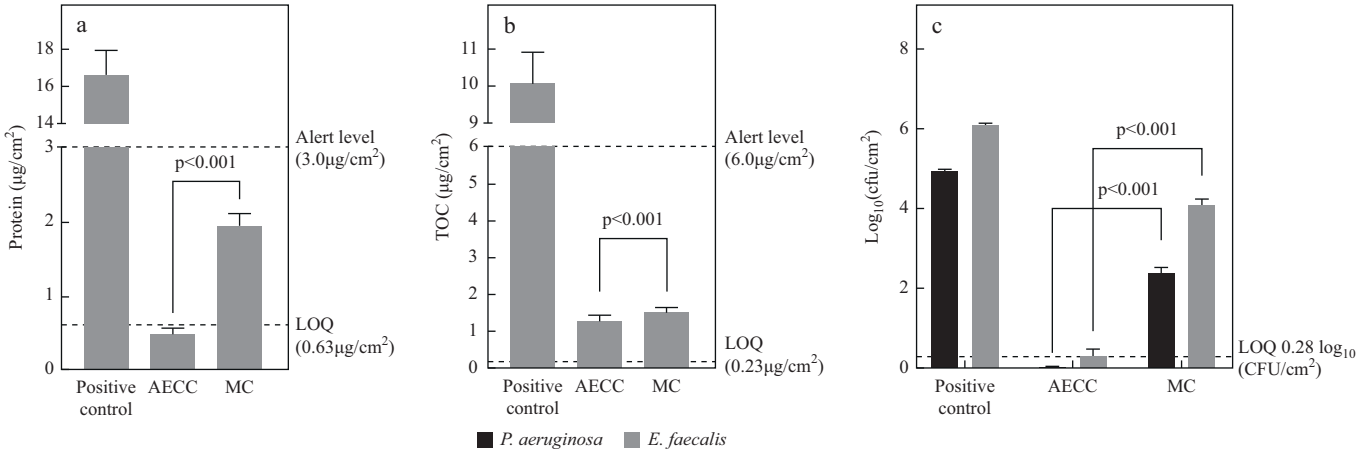

Figure 1. Cyclic build-up biofilm removal using the automated endoscope channel cleaner (AECC) or manual cleaning (MC) in simulated suction/biopsy channels (3.7 mm inner diameter). $N = 16$ per condition. (a) Protein assay. (b) Total organic carbon (TOC) assay. (c) Bacterial culture assay. cfu, colony forming units; LOQ, limit of quantitation. Alert levels per ISO 15883-5:2021: performance requirements and test method criteria for demonstrating cleaning efficacy.

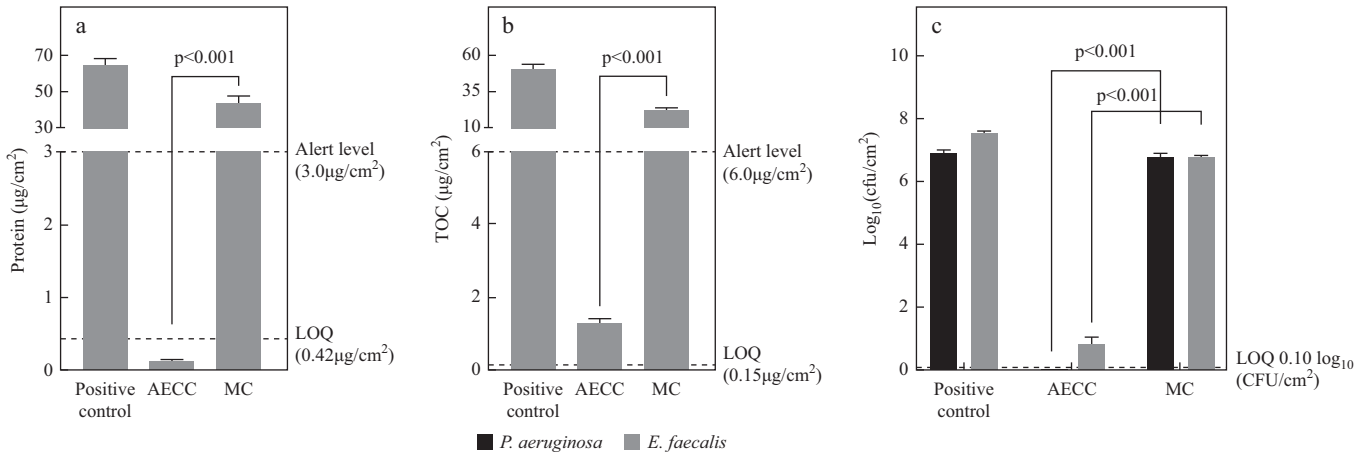

Figure 2. Cyclic build-up biofilm removal using the automated endoscope channel cleaner (AECC) or manual cleaning (MC) in simulated air/water and auxiliary channels (1.4 mm inner diameter). $N = 16$ per condition. (a) Protein assay. (b) Total organic carbon (TOC) assay. (c) Bacterial culture assay. cfu, colony forming units; LOQ, limit of quantitation. Alert levels per ISO 15883-5:2021: performance requirements and test method criteria for demonstrating cleaning efficacy.

of neutralizing enzymes and ion gradients within biofilms [6]. Age and extent of damage in working channels of clinical endoscopes may further contribute to biofilm development and inhibit effective micro-organism disinfection [4,6]. AECC significantly outperforming MC in CBB removal across all markers analysed suggests enhanced cleaning of the S/B channels is possible which could improve the effectiveness of subsequent HLD. It is important to note that these data represent optimal manual cleaning performed in laboratory conditions and do not account for errors that may occur in clinical practice settings.

To our knowledge, this study provides the first investigation of CBB removal in 1.4-mm channels representing the AW/AUX channels of gastrointestinal endoscopes (Figure 2). While AECC met cleaning guidance in AW/AUX channels, the high levels of residuals remaining after MC suggest HLD is unlikely to provide an adequate margin of safety, particularly as HLD may be compromised against organisms in biofilms [7,8]. The data show standard MC practice is inadequate at removing CBB from narrow endoscope channels which cannot be brushed, providing probable root cause for a portion of the numerous endoscopy-associated infections reported in the literature [4—8]. Primo *et al*. [4] found that the majority of AW endoscope channels had extensive biofilm accumulation after only 30—60 days of patient-use and reprocessing cycles [4], and Johani *et al*. [5] found AW endoscope channels had a greater build-up of biofilm compared with working channels [5]. The complexity of AW channels including their varying diameter, attachment to valve seats and other structures, sharp bends and various joins further adds to the difficulty of achieving cleaning.

A limitation of the present study was that MC was conducted in the laboratory with perfect IFU adherence. This does not reflect the case in reprocessing facilities where technicians are often under time pressure. Simulated endoscope channels were used to assess cleaning performance. Growing biofilm in isolated channels allows for proper extraction of residual cleaning markers. The inability to effectively sample narrow AW channels within endoscopes without destructive testing, is a known issue for biofilm recovery and limits biofilm detection clinically [5].

The present findings highlight the importance of using stringent, relevant biofilm models for evaluating cleaning efficacy for flexible endoscopes. ISO15883-5:2021 lists an example biofilm model that can be optionally used for flexible endoscope cleaning validation. This standard model involves continuously hydrated PTFE tubes inoculated with a single species (*P. aeruginosa*) in growth medium circulated for three to four days. The CBB model is a more stringent model (fixed, multi-species biofilm grown in an organic test soil), developed to represent a challenge closer to that found in clinical practice [10]. While not all scopes are exposed to glutaraldehyde fixation in clinical use, glutaraldehyde serves to create a worst-case laboratory model in five days, whereas clinical biofilms can form over much longer periods. Continuously hydrated biofilms such as the ISO15883 biofilm are morphologically different to fixed build-up biofilms such as CBB, which yield compact layers of dried organic matrix with embedded organisms [9,10]. Use of the CBB model in the present study revealed a clear need for enhanced cleaning in channels that cannot be brushed. Selection of a relevant biofilm model in validation studies is of paramount importance for evaluation of new cleaning approaches [10].

The AECC tested here cleans via the mechanical action of the cleaning agent passing through the lumens at high velocity. Mechanical action that provides adequate friction was previously shown to be critical for CBB cleaning over other parameters such as chemical action, temperature and contact time [10]. Flush-only methods resulted in higher residual bacteria regardless of detergent used [10]. This is consistent with our findings that liquid detergent flushing alone cannot remove biofilm. The ability to deliver physical cleaning of biofilm from all channels, regardless of diameter, should be considered a key indicator of cleaning success. Automation of cleaning can also eliminate the human factors associated with MC of endoscope channels.

In summary, effective cleaning of endoscope channels is critical to ensure that HLD or sterilization are effective and that endoscopes are safe for re-use. This study showed that MC with strict IFU adherence failed to remove CBB in 1.4-mm channels but removed biofilm to below alert levels in 3.7-mm channels. Methods that deliver physical cleaning of biofilm across all channels are needed. The AECC tested in this study demonstrated significantly enhanced CBB removal in simulated S/B channels, as well as simulated AW/AUX channels which are unable to be brushed. While further clinical studies are needed, AECC shows potential to deliver enhanced cleaning over current practice to all endoscope channels and thereby address infection risk.

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Author contributions

Conceptualization: L.M., J.B. Methodology: all authors. Formal analysis: L.M., V.S., M.P.J. Investigation: V.S. Writing — original draft: L.M. Writing — review and editing: all authors. Supervision: J.B.

Conflict of interest statement

L.M., V.S., L.Y.T. and J.B. are employees of and hold stock in Nanosonics Ltd. M.P.J., K.V. and M.A. have received consulting fees from Nanosonics Ltd.

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