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Contribution of usage to endoscope working channel damage and bacterial contamination

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SUMMARY

Background: Biofilm formation has been shown to be associated with damaged areas of endoscope channels. It was hypothesized that the passage of instruments and brushes through endoscope channels during procedures and cleaning contributes to channel damage, bacterial attachment and biofilm formation.

Aim: To compare surface roughness and bacterial attachment in used and new endoscope channels *in vivo* and *in vitro*.

Methods: Surface roughness of 10 clinically used (retired) and seven new colonoscope biopsy channels was analysed by a surface profiler. For the in-vitro study, a flexible endoscope biopsy forceps was passed repeatedly through a curved 3.0-mm-diameter Teflon tube 100, 200 and 500 times. Atomic force microscopy was used to determine the degree of inner surface damage. The number of *Escherichia coli* or *Enterococcus faecium* attached to the inner surface of the new Teflon tube and the tube with 500 forceps passes in 1 h at 37°C was determined by culture.

Results: The average surface roughness of the used biopsy channels was found to be 1.5 times greater than that of the new biopsy channels (P=0.03). Surface roughness of Teflon tubes with 100, 200 and 500 forceps passes was 1.05-, 1.12- and 3.2-fold (P=0.025) greater than the roughness of the new Teflon tubes, respectively. The number of *E. coli* and *E. faecium* attached to Teflon tubes with 500 forceps passes was 2.9-fold (P=0.021) and 4.3-fold (P=0.004) higher compared with the number of *E. coli* and *E. faecium* attached to the new Teflon tubes, respectively.

Conclusion: An association was found between endoscope usage with damage to the biopsy channel and increased bacterial attachment.

Introduction

The number of endoscopy-related infections and outbreaks reported in the literature has increased rapidly over the last few years [1–4]. This increase is also reflected in the recent report by the US Food and Drug Administration, 'Infections Associated with Reprocessed Duodenoscopes' [5]. The

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increased number of reports and publications is likely due, at least in part, to improved detection of outbreaks, and many of them have been found to be associated with multi-drug-resistant micro-organisms [6–8]. Of major concern is multi-drug-resistant Klebsiella pneumoniae due to its limited treatment options and high mortality rate [8,9]. Many of these outbreaks have been associated with inadequate cleaning, particularly of the elevator mechanism of duodenoscopes [10]. However, outbreaks related to contaminated endoscopes have also been reported in cases where no lapses were identified in the reprocessing protocol [11–13], including in instances where biofilm contamination was demonstrated [14]. Positive cultures and transmission events have also been associated with endoscopes that have required critical repair despite a lack of functional defects [9]. Past studies have shown the formation of biofilm in areas of endoscope channels that were frequently associated with damage [15,16]. Pits and scratches on the surfaces of endoscope channels act as anchor points for the adhesion of biofilm and physical barriers that prevent biofilm from being removed during cleaning. Endoscope channels are made of polytetrafluoroethylene (PTFE) Teflon® which is smooth, durable and resistant to chemicals. However, Teflon tubing is not very flexible and may be damaged due to over-bending. It is proposed that the passage of instruments and brushes down endoscope channels during cleaning as well as medical procedures can also contribute to their damage.

The development of biofilm, whether by inadequate cleaning or by structural protection within damaged channel areas, adversely impacts on subsequent cleaning and disinfection. Many instrument-grade detergents have been shown to have poor efficacy for the removal of biofilm [17]. In addition, the effectiveness of disinfectants against biofilm has been shown to be reduced, as reviewed by Bridier *et al.* [18], especially if organic material is present [19]. Both *Enterococcus faecalis* and *Pseudomonas aeruginosa*, when grown as an aged, mature biofilm, survived disinfection by two commonly used endoscope disinfectants — glutaraldehyde and accelerated hydrogen peroxide [20].

Patient infections resulting from failed endoscope decontamination have also been reported in cases where guidelines and recommendations for endoscope reprocessing have been followed [12,13]. These transmission events may be related to biofilm formation in visually undetectable, damaged areas of the endoscope.

The bioburden within used gastrointestinal endoscopes, as estimated using a brush or flush technique, can be as high as 9.4 $\log_{10}$ organisms per device [21]. However, using this technique, the time taken for bacteria to attach to an undamaged endoscope channel cannot be determined. When compared with surgical interventions, endoscopy procedures are less time-consuming. Alfa *et al.* [21] found that the maximum and average procedure times were 70 and 32 min, respectively, when duodenoscopes were used, and 55 and 25 min, respectively, when colonoscopes were used. The objectives of the present study were: (i) to determine the extent of channel surface damage in clinically used endoscopes obtained from Australia; (ii) to determine how quickly bacteria can attach to new endoscope channel using Teflon tubing as a model; and (iii) to develop an in-vitro assay to model the effect of repeated clinical use on endoscope surface integrity and its effect on bacterial attachment.

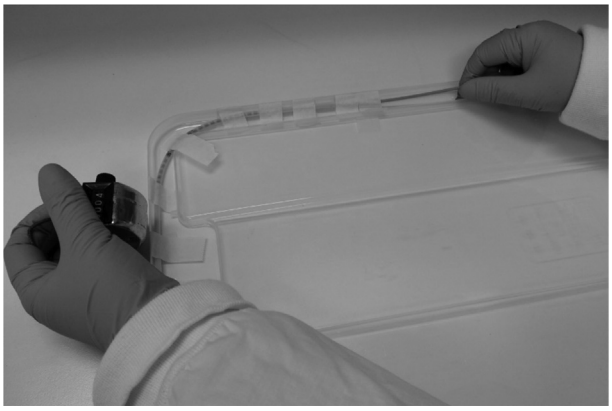

Figure 1. Flexible endoscope biopsy forceps passing through a new Teflon tube.

Methods

Presence of channel damage in clinically used colonoscopes

Clinically used colonoscope biopsy channels (N=10) from a variety of brands were received from endoscope repair services in Australia and subjected to surface profile analysis by a contact profilometer. For comparison of results, seven new endoscope biopsy channels were also assessed.

A 2-cm section was obtained from each channel and soaked in 5 M sodium hydroxide overnight at room temperature to remove all biological material. A test piece of tubing was subjected to this treatment previously, and scanning electron microscopy showed that sodium hydroxide removed all biological soil and did not otherwise affect the tubing surface. Each section was then rinsed in distilled water, cut longitudinally and dried using filtered nitrogen gas. The samples were subsequently processed in an Alpha-Step 500 Surface Profiler (Tencor, Mountain View, CA, USA), which uses a stylus to scan the surface profile longitudinally and calculates the arithmetic average deviation of the channel profile from the central line, or average roughness (in nm).

The surface profile was measured in two sequential channel areas that were selected at random and scanned by stylus. The scan length was 200 μm at a speed of 40 μm/s. For each sample, the average roughness of the two channel areas was calculated.

Development of an in-vitro assay to model channel surface damage

It was assumed that damage to the endoscope channel surface can occur frequently due to the passage of instruments, such as biopsy forceps, so an in-vitro experiment was performed to simulate clinical use to determine approximately how many times an instrument needed to be passed to cause some damage. The central portion of a 60-cm piece of PTFE Teflon tubing was bent at an angle of between 90° to 120°, and a pair of flexible endoscope biopsy forceps with a diameter of 2.8 mm was passed repeatedly through the Teflon tubing as shown in Figure 1. The number of passages of the biopsy forceps was fixed at 50, 100, 200, 500 and 1000 times. One passage

was pushing the forceps into the tube and pulling the forceps out, to simulate a biopsy being taken.

A 2-cm section of tubing was removed from the bent area (30 cm from the end of the tubing) for atomic force microscopy (AFM) analysis. Images of 10x10 μm (512x512 pixels) were acquired by Bruker MultiMode 8 in air in scanasyst mode with a scanasyst air probe (tip radius 2 nm, spring constant 0.4 N/m). Five random images were taken of each sample for posterior second order plane fit to assess the roughness of each sample. Average roughness was calculated using nanoscope analysis software.

Bacterial attachment to Teflon tubing

Culture conditions in flow system

Bacterial attachment to Teflon tubing was determined using a modification of an in-vitro flow system developed to form reproducible biofilm on Teflon tubing for efficacy testing of detergents against biofilm [17].

The bacterial inoculum was prepared by removing a single, fresh colony of *Escherichia coli* (Strain K12) from a horse blood agar plate, emulsifying it in 100 mL of tryptic soy broth (TSB) and incubating it at 37°C for 7 h. The absorbance of the resulting culture was diluted to give a reading of 0.3 at a wavelength of 620 nm, which correlates with a concentration of approximately 10^8 bacterial cells/mL. One millilitre of this bacterial culture was added to 99 mL of TSB to create the inoculum for the flow system. The Teflon tubing was connected to the growth media and a peristaltic pump using sterile gloves, and the media was circulated at 75 mL/h for the required time. The media and the Teflon tubing were both kept in a water bath at 37°C during the experiment (Figure 2). At specified time points, the tubing was disconnected from the flow system, the external surface of the detached tubing was wiped serially with Matrix (Whiteley Corporation, North Sydney, Australia) — a marketed biofilm remover, 70% ethanol and sterile water — and cut aseptically into 5-cm sections. Each 5-cm piece of tubing was rinsed in 10 mL of phosphate-buffered saline (PBS) three times to remove detached bacteria, cut aseptically into five 1-cm pieces, sonicated in 5 mL of PBS in an ultrasonic bath (Soniclean, Adelaide, Australia) for 10 min with a sweeping frequency of 42–47 kH at 20°C and vortexed for 1 min, followed by serial 10-fold dilution, standard plate culture and colony-forming unit (cfu) count.

Variability of bacterial attachment and growth along the Teflon tubing

The Teflon tubing was attached to the apparatus and the media was circulated for 16 h as describe above. The external surface of the detached tubing was wiped clean, the tube was cut aseptically into 20 5-cm pieces, and the cfu count of each piece was determined as described above. The experiment was repeated an additional two times. Within-experiment coefficient of variation was calculated by dividing the standard deviation (SD) by the mean (100 x SD/mean) to give a dimensionless measure of variance.

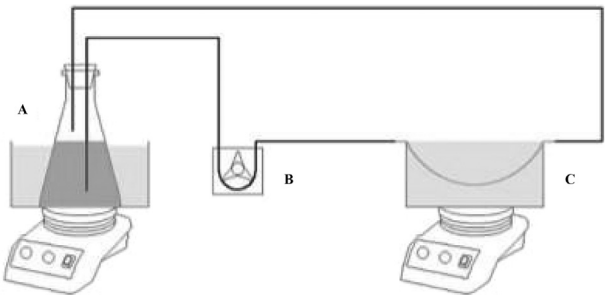

Figure 2. Schematic of flow system apparatus used for bacterial attachment assay to Teflon tubing. A, bacterial inoculum in growth media; B, peristaltic pump; C, Teflon tubing.

Bacterial attachment over time to new Teflon tubing

The aim of this experiment was to determine a time point where bacterial attachment was consistent but bacterial numbers were low. At set time points of 30 min and 1, 2, 4 and 6 h, the pump was stopped and a pre-marked 25-cm length of tubing was removed for analysis. The remaining tubing was re-attached aseptically before the pump was restarted. The 25-cm length of tubing was cut aseptically into five 5-cm pieces in order to obtain technical replicates for each time point.

Bacterial attachment to damaged Teflon tubing

Biopsy forceps were passed in and out of Teflon tubing 500 times (N=7) as described above. The damaged tubing was trimmed to a 7-cm length (including the bent section) and new Teflon tubing was also cut into a 7-cm length (N=7). Both new and damaged Teflon tubing pieces were connected to each other in the flow system, and *E. coli* (Strain K12) was circulated for 1 h as detailed above. One centimetre from each end of the Teflon tubing pieces was trimmed (connection sites) leaving the middle 5-cm length, and the cfu count was determined. The experiment was repeated using another seven pieces of new and damaged tubing and *Enterococcus faecium* (American Type Culture Collection 35667) as described above.

Statistical analysis

Student's t-test was used to test the null hypothesis that the surface roughness of biopsy channels increases following clinical use. To test for differences in bacterial attachment to unused and scratched Teflon tubing, Student's t-test was used for normally distributed data and Mann–Whitney Rank Sum Test was used for non-normal data.

Results

Presence of channel damage in clinically used colonoscopes

The inner surface of clinically used colonoscope biopsy channels were significantly rougher (54.7±16.9 nm) compared with new colonoscope biopsy channels (35.8±7.44 nm) (P=0.03) (Figure 3). This indicates that used endoscopes have more deviations in their surface profile compared with a central line, demonstrating that the surface profile of endoscope channels changes with use of the device.

Development of an in-vitro assay to model channel surface damage

Average roughness of damaged Teflon tubing, as assessed by AFM, is illustrated in Figure 4. Surface roughness values of Teflon tubing with 100, 200, 500 and 1000 forceps passages were 1.05, 1.12, 3.2 and 2.05 times greater than the values for

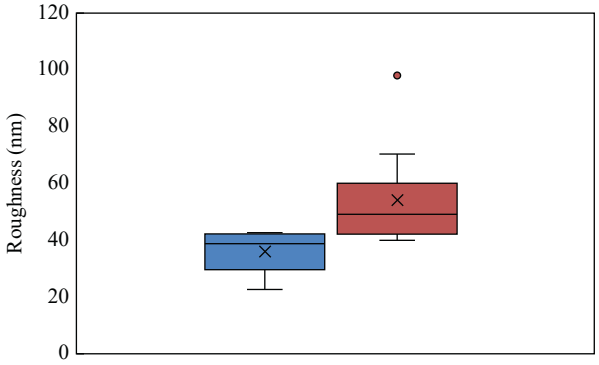

Figure 3. Comparison of roughness values of clinically used (red bar) and new (blue bar) endoscope biopsy channels tested by surface profiling (P=0.03).

new Teflon tubing, respectively (P=0.025), with the highest surface roughness generated by 500 passages.

Bacterial attachment to Teflon tubing

Variability of bacterial attachment and growth along Teflon tubing

Twenty 5-cm segments were available for cfu determination from three flow system runs. For Runs 1 and 2, all segments were within 2 SD of the run mean. Only one of 19 segments from Run 3 was greater than 2 SD from the mean value for Run 3. The within-run coefficient of variation was very low (Table I).

Bacterial attachment over time to new Teflon tubing

Attachment of bacteria to new Teflon tubing was evident by 30 min (the shortest time point tested). By 2 h, the number of attached bacteria had increased 10-fold, and by 4 h, the number of bacteria had increased 100-fold (Figure 5).

Table I
Mean number of colony-forming units per centimetre of tubing, standard deviation (SD) and coefficient of variation (CV) following 16 h of bacterial attachment

Run	Segments	Mean $\log_{10}$/cm	SD	CV	Segments >2 SD
1	20	5.69	0.34	6.0	0
2	19	6.21	0.33	5.4	0
3	19	6.03	0.22	3.7	1

Segments >2 SD, number of segments more than 2 SD from the mean value for each run.

Bacterial attachment to damaged Teflon tubing

The number of *E. coli* and *E. faecium* attached to Teflon tubes with 500 biopsy forceps passes was 2.9-fold (P=0.021) and 4.3-fold (P=0.004) higher than the number of bacteria attached to the new Teflon tubing, respectively (see Figure 6).

Discussion

Endoscope reprocessing can be affected by various factors. Problems with automatic endoscope reprocessors (AERs) and failure to adhere to recommendations and guidelines provided by professional societies are most frequently cited as the cause of endoscope reprocessing failure [7,22,23]. Additionally, improper maintenance of endoscopes and AERs can compromise endoscope reprocessing, which can lead to patient infections and outbreaks [9,13]. However, infection outbreaks linked to endoscopy have occurred even in cases where guidelines were followed strictly and no endoscope reprocessing errors were identified [12,24]. Continued endoscope contamination has also occurred following repeated decontamination by high-level disinfectants [24].

Given the complex design of gastrointestinal endoscopes, they can be damaged easily. Scanning electron microscopy has

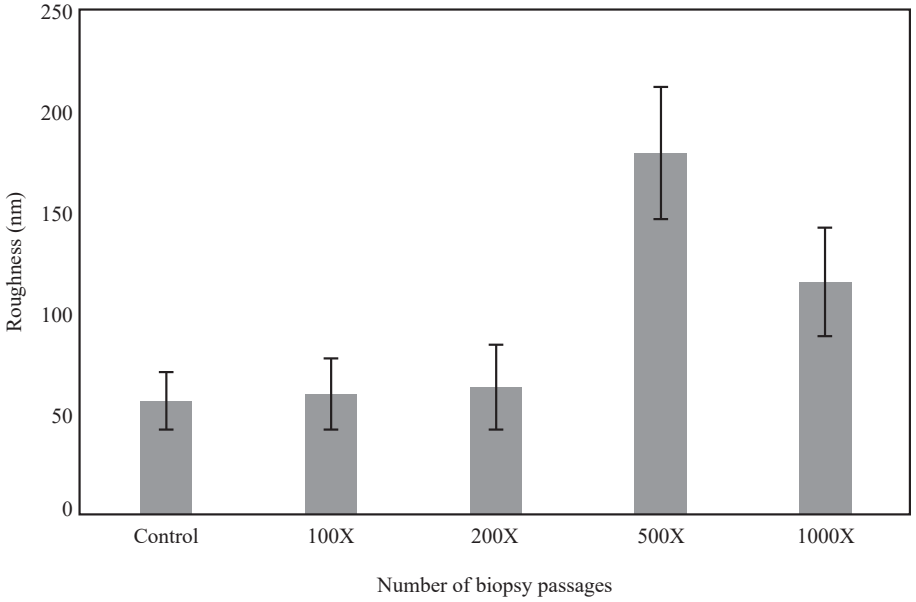

Figure 4. Comparison of roughness values of Teflon tubing for different numbers of biopsy forceps passages, as analysed by atomic force microscopy.

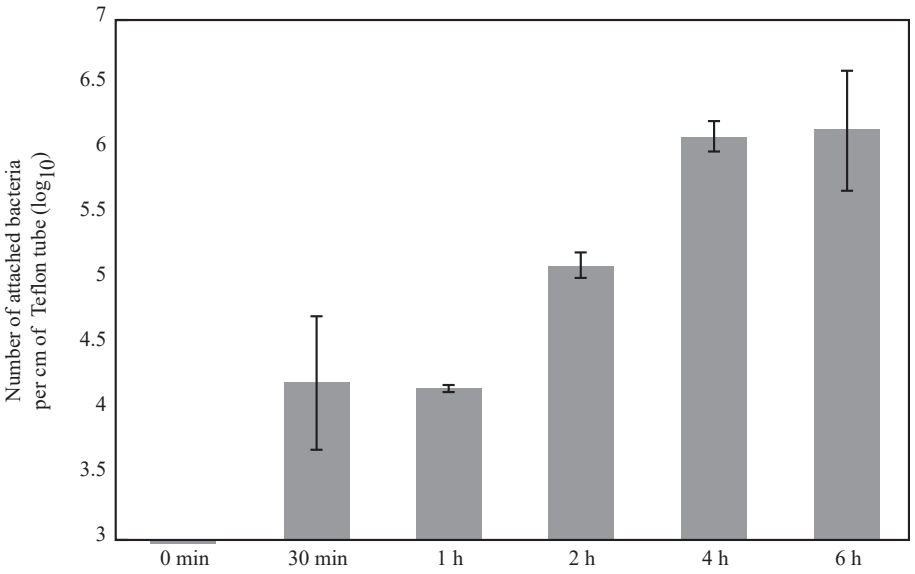

Figure 5. Attachment of viable bacteria to new Teflon tubing generated by an in-vitro model of flow contamination.

visually confirmed endoscope channel damage and its association with the presence of biofilm-containing bacteria of various morphologies [16]. Previous studies have shown that damage to endoscopes can result in patient infections [9,13,15,25]. Although based on a small number of colonoscopes, this study found that the internal channels of clinically used endoscopes were significantly rougher than new endoscope channels, demonstrating that the longitudinal surface profile of endoscope channels is shaped by routine use. A recent evaluation of gastrointestinal endoscope channels using borescopes over a 2-month period not only proved that there was channel damage, but also that the irregularities within the channels changed over time [26].

Increased roughness associated with channel damage provides a good habitat for bacteria and patient soil to attach. Soil and bacteria are partially protected within dips and crevices, which decreases the efficacy of cleaning and facilitates the growth of biofilm. Rough surfaces have been shown to retain more bacteria in the presence of test soil containing blood [27]. Therefore, the presence of damage on endoscope surfaces may contribute to bacterial adherence. Bisset *et al.* found a significant relationship between the number of times an endoscope had been used and the frequency of isolating organisms from it [22], and Hervé and Keevil found evidence of large amounts of protein and abraded biofilm in decommissioned clinical endoscopes [28]. A limitation of this study was the assumption that bacteria attached equally along the length of the channel over short periods of time. While this was found to be true following 16 h of culture, this was not the case for shorter time periods. The authors attempted to minimize any differences by circulating the media through the tubing five times over a 2-min period. This ensured that the bacteria were able to attach along the whole length of the tube virtually simultaneously. The authors chose to study attachment for 1 h as this is more reflective of the time that colonoscopes are used clinically, and initial experiments showed that sufficient bacteria attached within this time frame for meaningful statistical analysis. The bacterial number attached to the tubing at 1 h was $\log_{10}4.16$ and had an SD of only 0.03.

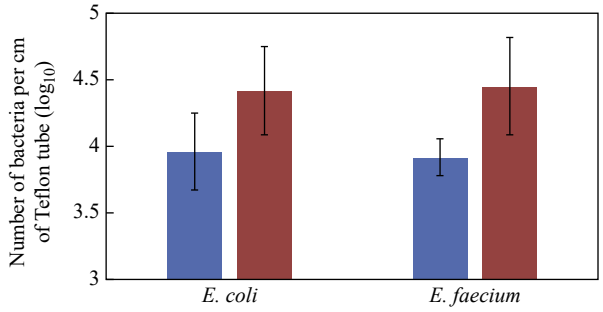

Figure 6. Viable bacteria attached to Teflon tubes generated by an in-vitro model of flow contamination. Blue bars, control; red bars, scratched; *E. coli*, *Escherichia coli*; *E. faecium*, *Enterococcus faecium*.

In this study, surface roughness was evaluated using two different methods —surface profiler and AFM. Unfortunately, neither method proved to be ideal. AFM analysis offers visualization of defects at a microbiological scale, but only allows for inspection of very small surface areas [29,30]. To overcome this issue, the surface roughness of five areas selected at random was examined. The magnitude of damage caused by the passage of biopsy forceps through the Teflon tubing exceeded the measurement capability of the nanometre scale. Better approaches should be used to assess surface roughness over larger areas of endoscope channels in order to reduce sampling error. Despite this, the authors were still able to demonstrate that the passage of biopsy forceps through Teflon tubing 500 times increases surface roughness and therefore contributes to endoscope channel damage.

In clinical practice, monitoring endoscope damage can be challenging given the complex structure of the instrument. Current research methods involve the use of destructive processes to assess the internal surface of endoscopes. Leak tests and visual inspection using borescopes are frequently recommended for detection of endoscope defects [31,32]. Repeated positive microbial cultures from a single endoscope can also

suggest endoscope channel damage. However, leak tests and microbial cultures are not reliable methods for determining endoscope damage [33]. In addition, it is not known if the bacterial recovery rate from damaged channels is the same as that from undamaged channels using clinical endoscope sampling techniques or sonication.

Although endoscopes are typically only in contact with patients for a short period of time during endoscopy procedures, gastrointestinal endoscopes are routinely contaminated with high microbial loads due to the large population of bacteria resident in the gastrointestinal tract. This study used an in-vitro model to demonstrate that large numbers of bacteria attach to Teflon tubing within 30 min of exposure. This finding suggests that bacteria not only contaminate the internal channels of endoscopes, but also adhere to their surfaces during endoscopy procedures. Therefore, additional recommendations regarding prevention of bacterial attachment to endoscope surfaces should be adopted. As bacterial attachment can be facilitated by deposition of organic material within medical devices, minimizing the time between clinical use of flexible endoscopes and instrument reprocessing is fundamental for prevention of bacterial adherence.

The relationship between internal damage and endoscope contamination should be studied further in order to improve professional guidelines and ensure patient safety. The search for an appropriate method to assess endoscope channel damage continues, given that this study demonstrated that: (i) viable bacteria attached to new Teflon tubing within 30 min of surface contact; (ii) more bacteria attached to damaged endoscopes than new endoscopes; and (iii) recommendations on endoscope maintenance and repair are still empirical in guidelines.

In conclusion, this study confirmed the association between endoscope usage and increased surface roughness secondary to physical damage, as well as increased bacterial attachment. The increased roughness of the interior surface of used endoscope channels provides a favourable habitat for bacteria and patient soil to attach, making cleaning and decontamination more difficult, and facilitating biofilm growth. The tubing subjected to 500 passages (in and out) of biopsy forceps posed a significantly greater risk of bacterial contamination than tubing subjected to 200 passages. This study suggests the need to design tools that do not damage endoscopes, and regular inspection of endoscope damage to prevent biofilm contamination.

Conflict of interest statement
None declared.

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