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Using an efficient biofilm detaching agent: an essential step for the improvement of endoscope reprocessing protocols

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Summary Biofilms develop inside endoscope channels even when valid endoscope reprocessing protocols are applied. The use of an efficient biocide is not sufficient if the channels are not cleaned thoroughly prior to disinfection. This study compared new anti-biofilm combinations of detachment promoting agents with a cleaning product in current use. Tests were performed using Teflon tubing and a contamination device that reproduces conditions that are prevalent during endoscopy. Products were subjected to static + brushing or dynamic treatments, and their ability to remove a preformed biofilm was assessed. The residual biofilm after treatment was assessed and compared with untreated controls. The percentage of surface covered by biofilm was measured after staining with crystal violet. Culturable bacteria levels were determined by plating the bacteria scraped from the tubing surface and counting the colony-forming units (CFU). Further tests were performed on actual endoscopes that had been contaminated artificially. Biofilm removal was confirmed by scanning electron microscopy. This study showed that the new anti-biofilm products prevented the build-up of biofilm and removed a mature biofilm (approximately 10^8 CFU/cm^2), whereas protocols based on detergent-disinfectants containing quaternary ammonium compounds showed low efficacy as these protocols and products fixed the biofilm on the endoscope surfaces. The new procedure and agents represent a new approach to biofilm control

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that may improve the efficacy of endoscope reprocessing, and reduce the risk of transmitting infections.

Introduction

During endoscopy, the environment provides optimal conditions for the growth of biofilms. Most studies related to endoscope contamination have reported the presence of biofilms on the inner surface of endoscope channels.[1–4] Biofilms represent not only a reservoir of pathogenic bacteria that can detach, resume their planktonic state, and contaminate the patient, but also a source of endotoxins that may enter the circulation of the patient through ruptured mucosae and cause systemic disorders.[5] These observations suggest that biofilm removal is of clinical relevance in endoscopy.

With regard to potential action on biofilms, peroxyacetic acid is considered to be a superior disinfectant compared with glutaraldehyde.[3] However, although peroxyacetic acid is known to be a very good biocide, previous studies performed on haemodialysis systems have shown that it has low efficacy for biofilm removal.[6] This has been confirmed in situ following experimental contamination of actual dialysis machines, suggesting that biofilm removal should be attempted with cleaning agents rather than disinfectants.[7]

The anti-biofilm efficacy of a cleaning agent should be related to its ability to detach biofilm from a surface. A new anti-biofilm procedure, the Pronetron® procedure, has been developed recently for the disinfection of haemodialysis machines.[7,8] This procedure provides complete detachment of biofilm due to sequential and synergetic action of two detachment promoting agents:[9] a complex enzymatic mixture (Pronetron α) and a specific alkaline detergent solution (Pronetron β). The Pronetron agents can be used for preventive maintenance (preventing the development of a biofilm) based on the use of a detergent solution alone, and corrective maintenance (removal of an existing biofilm) based on enzymatic treatment followed immediately by treatment with an enriched detergent solution. The objective of the present study was to assess the efficacy of the Pronetron procedure for biofilm removal during endoscope reprocessing. Tests were performed in vitro using plain Teflon tubing and artificially contaminated endoscopes.

Materials and methods

Artificial contamination

Contamination device

A continuously stirred tank reactor was developed as a contamination device to reproduce the conditions prevalent during endoscopy. This included a beaker filled with a contaminating medium comprising 1:10 diluted brain heart infusion (BHI; Difco, le Pont de Claix, France) supplemented with human serum (0.1%) (Sigma, Saint Quentin Fallavier, France). The beaker was stirred gently to mimic movements within a human body.

Test pieces

Several test pieces were prepared from translucent Teflon tubing (Masterflex® tubing, 4 mm ID, Fisher Bioblock Scientific, Illirch, France). One end of each test piece was immersed in the contaminating medium to enable the medium to rise up the channel via capillary action. The other end of each test piece was run through the cap of the beaker and covered with a 0.45-μm filter (Millipore, Saint Quentin en Yvelines, France) that filtered the air and prevented external contamination of the medium. The system was kept in a water bath at 37 °C.

Contamination procedure

The evening before each test, the test pieces were filled with 0.1% human serum and kept at 37 °C overnight so that serum components could attach to the channel surface, increasing the adherence potential for the bacteria. At the same time, the medium was inoculated with approximately 10^6 colony-forming units (CFU)/mL of a bacterial culture containing 25% *Pseudomonas aeruginosa* PAO1, 25% *Staphylococcus epidermidis* ATCC 49134, 25% *Enterobacter cloacae* ATCC 10699, and 25% *Klebsiella pneumoniae* ATCC 700831. After incubation for 12 h at 37 °C, the contamination level in the medium had stabilized at approximately 10^9 CFU/mL. The serum was drained from the test pieces, which were rinsed with sterile water and immersed in the contaminating medium. During the test, fresh medium was added continuously using a peristaltic pump that delivered a sterile 1:10

dilution of BHI at 10 mL/min. Simultaneously, the same volume of used medium was removed. Thus, the medium was renewed completely every 45 min.

Contamination of actual endoscopes

For artificial contamination of actual endoscopes, the endoscopes were equipped with a new operating channel. The inlet and outlet of the operating channel were connected to the contamination device described above. The contaminating medium was run through the channel by the peristaltic pump (flow rate 100 mL/min). The evening before each test, the operating channel was filled with 0.1% human serum, incubated overnight at 37 °C, then emptied and rinsed with sterile water.

Treatments

Screening of various products using translucent Teflon tubing

The first experiment involved testing five products with Teflon tubing in order to determine whether the presence of quaternary ammonium in the composition of the products helped to improve the anti-biofilm efficacy. Different formulations of detergents included in the Pronetron procedure were tested and compared with a currently used detergent-disinfectant (Table I). Each product was tested according to French guidelines: two static or dynamic cleaning steps (of 10 min and 5 min contact time), separated by rinsing with tap water.[10] Products containing surfactants intended for manual cleaning were used in static + brushing treatments. Products that did not contain surfactants intended for use in automatic washer-disinfectors were used in dynamic treatments.

The contamination device was run for 4 h until the whole surface of the tubing was covered with a homogenous biofilm made of microcolonies and isolated adhering cells. Test pieces were selected at random to undergo a specific treatment or to be left untreated as controls. Treatments were performed as follows.

- For static + brushing treatments, the test pieces or the complete endoscope were immersed in the test product, and the inner surface of the channels was brushed for 1 min using a specific channel brush (Olympus, Rungis, France). The test pieces were left static in the solution for a specific time.
- For dynamic treatments, which mimic the running of automatic washer-disinfectors, the test pieces were connected to silicone tubing (4 mm ID, Masterflex, Fisher Bioblock Scientific, Illirch, France) set up as a recycling loop, and the test product was circulated through the test piece by a peristaltic pump at 200 mL/min.

All of the test pieces were rinsed with tap water after treatment. At the end of this experiment,

Table I Screening of translucent Teflon tubing. Composition of test products, conditions of use and quantitative results

Test product	Use	Composition	Microscopic observation of treated surfaces	Surface coverage (%)	Culturable bacteria (CFU/cm^2)
No treatment (control)			Homogenous biofilm	47 ± 5	$1.3 \times 10^5 \pm 5 \times 10^3$
Pronetron β1	Static 0.5%	Phosphates, hydrates, minerals, surfactants IV ammoniums[a]	Large amounts of residues	22 ± 2	5 ± 1
Pronetron β2	Dynamic 0.5%	Phosphates, hydrates, minerals IV ammoniums	Large amounts of residues	25 ± 5	2 ± 0.5
Pronetron β3	Static 0.5%	Phosphates, hydrates, minerals, surfactants	No biofilm	<0.05	<0.1
Pronetron β4	Dynamic 0.5%	Phosphates, hydrates, minerals	No biofilm	0.05	<0.1
Current product	Static 0.4%	Surfactants, minerals, sequestering agents IV ammonium + biguanides	Large amounts of residues	27 ± 5	1 ± 0.1

CFU, colony-forming units.
[a] Quaternary ammonium compounds.

the two most efficient products (one for manual cleaning and one for automatic cleaning) were selected for testing on actual endoscopes.

Preventive maintenance efficacy on actual endoscopes

A second experiment was performed on actual endoscopes that had been contaminated artificially in order to assess the efficacy of selected products for preventing biofilm growth when used regularly between endoscopy sessions. Three endoscopes were used for this study; two to test the efficacy of two previously selected products and one (untreated) as a control. Every hour, the endoscopes were removed from the contamination device, submitted to either dynamic or static + brushing treatment, rinsed and then reset in the contamination device. The treatments were performed according to French guidelines and at the recommended concentration. The experiment was stopped after 8 h of contamination, allowing the development of a mature, thick biofilm on the surface of the untreated control.

Corrective maintenance efficacy on actual endoscopes

A third experiment was performed to study the efficacy of the Pronetron procedure in the removal of an existing mature, thick biofilm. To do so, the control (untreated) endoscope from the second experiment was subjected to the following.

- A 0.5% solution of Pronetron α (multi-enzymatic mixture containing amylases, lipases and proteases) was run through the operating channel for 20 min, followed by rinsing with tap water.
- The endoscope was immersed in a 2.5% solution of Pronetron β*Plus* (enriched formulation of Pronetron β3 containing a low level of bleach) for 20 min, the operating channel was brushed manually, followed by rinsing with tap water and sterile water.

Analysis

Treated and untreated pieces of tubing or actual endoscope channels were cut into three segments that were analysed according to methods developed previously for the study of haemodialysis biofilms.[11]

Direct staining

Translucent pieces of tubing were stained with a 0.25% crystal violet solution (Labo-Moderne, Paris, France) for 5 min, cut lengthwise and observed with an optical microscope (Leica DMLB, Solms, Germany) connected to a couple charged device camera (Sony, Clichy, France). Six pictures were taken per piece of tubing and surface coverage was determined using Scion Image software.

Scanning electron microscopy

The internal surface of operating channels from actual endoscopes was observed by scanning electron microscopy (Hitachi S800, Tokyo, Japan). The biofilm was fixed with a 4% glutaraldehyde solution (Sigma, Saint Quentin Fallavier, France) in a 0.2 M cacodylate buffer (Merck, Darmstadt, Germany), dehydrated in alcohol and metallized with gold.

Culturable bacteria counting

The inner surface of 2-cm-long channels was scrapped using a tooth microbrush (Crinex, Montrouge, France). Subsequently, the microbrush was immersed in 1 mL of a sterile phosphate-buffered saline solution (Oxoid, Basingstoke, UK), and the bacterial aggregates were dissociated by sonication for 3 min and vortexed for 1 min. The resulting suspension was plated in triplicate on tryptic soy agar plates (Difco, le Pont de Claix, France), incubated for 48 h at 37 °C, and then the CFUs were counted.

Results

Screening of various products using translucent Teflon tubing

Products that were detergent-disinfectants and which contained quaternary ammonium compounds (Pronetron β1, β2, currently used detergent-disinfectant) had a low efficacy for biofilm removal. Conversely, Pronetron β3 and β4, which only had detergent properties, ensured complete biofilm removal (surface coverage below detection limit) in static + brushing and dynamic treatment(s), respectively.

Table I shows the means and coefficients of variation ($N = 3$) for surface coverage and number of culturable bacteria for the five treatments. Representative images of the tubing surfaces are shown in Figure 1.

Pronetron β3 and β4 were then selected for the second experiment.

Preventive maintenance efficacy on actual endoscopes

The artificial contamination system allowed the development of a mature biofilm (9.6×10^7

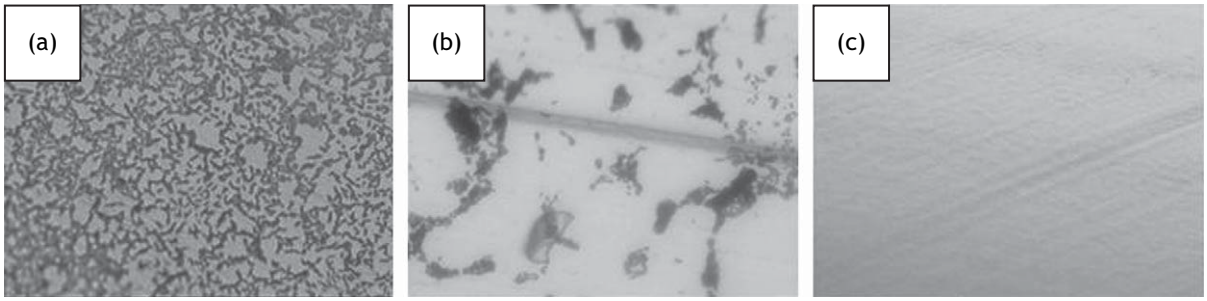

Figure 1 Screening on translucent Teflon tubing. Observations with an optical microscope (original magnification × 40) after staining with crystal violet. (a) Homogenous biofilm made of microcolonies and isolated adherent cells (untreated control). (b) Residual biofilm after treatment with detergent solutions containing quaternary ammonium compounds. (c) Residual biofilm after treatment with detergent solutions free of quaternary ammonium compounds.

CFU/cm^2) within 8 h on the inner surface of the operating channel of the non-treated control endoscope. Conversely, no biofilm developed within the same period in the operating channels of the endoscopes treated with Pronetron β3 and β4. Quantitative results and corresponding pictures are given in Table II and Figure 2.

Corrective maintenance efficacy on actual endoscopes

The control endoscope treated once according to the corrective Pronetron procedure showed complete removal of biofilm on the inner surface of its operating channel. Quantitative results are given in Table II.

Discussion

This study highlighted the high efficacy of detachment promoting agents for biofilm removal from contaminated operating channels of endoscopes, and supports application of the Pronetron procedure during the cleaning steps recommended by the French guidelines prior to disinfection.[10]

Most authors relate the poor quality of disinfection of endoscopes to inadequate compliance with reprocessing protocols.[1,3,5] This may well be true; due to the increase in endoscopy practice, time devoted to reprocessing needs to be as short as possible and reprocessing procedures are not always followed thoroughly. However, another explanation could be related to the low efficacy of the cleaning products, as suggested by Pajkos *et al.* who studied the inner surface of 25 endoscope channels and found biofilms on 17, despite documented evidence of excellent compliance with reprocessing protocols.[12] Pajkos *et al.* concluded that 'more efficacious cleaning agents are required to remove biofilm from the endoscope surface'. The present experiments support this point of view and demonstrated that, although bacterial adherence may be very low during an endoscopy session and usually below detection and quantification limits, bacterial accumulation can

Table II Experiments on actual endoscopes. Efficacy of the Pronetron procedure for preventive and corrective maintenance

Treatment	Procedure	Observation with scanning electron microscopy	Culturable bacteria (CFU/cm^2)
No treatment (control)	–	Mature, thick biofilm	9.6×10^7
Preventive treatment	Pronetron β3, 0.5%, static treatment for 15 min + brushing	No biofilm	<0.1
Preventive treatment	Pronetron β4, 0.5%, dynamic treatment for 15 min (200 mL/min)	No biofilm	<0.1
Corrective treatment	Pronetron α, 0.5%, static treatment for 20 min then Pronetron β*Plus*, 2.5%, static treatment for 20 min + brushing	No biofilm	0.1

CFU, colony-forming units.

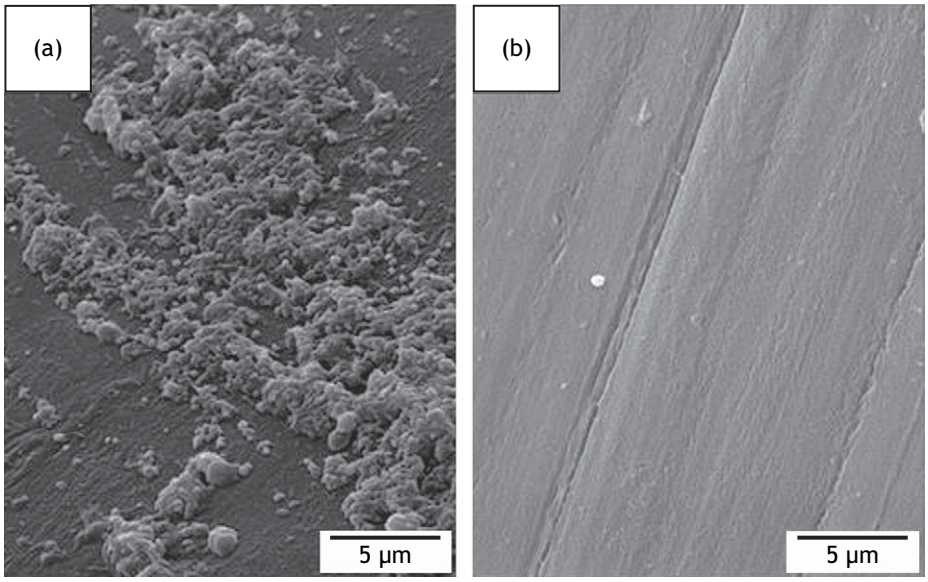

Figure 2 Experiments on actual endoscopes after artificial contamination. Observation of the inner surface of the operating channels with scanning electron microscopy. (a) Control. (b) Internal surface of the channel after treatment with the Pronetron procedure.

lead to the development of a mature biofilm in time. Biofilm development inside endoscope channels can create a vicious circle because biofilms decrease the efficacy of reprocessing protocols, which become less and less able to prevent biofilm growth. According to Pajkos *et al.*, cleaning solutions currently used for endoscope reprocessing are unable to remove deposits and adherent bacteria completely.[12] This was confirmed by the present results. Many complex solutions combining detergent, enzymatic and disinfectant properties have been developed in the hope that a '3-in-1' solution would lead to a higher efficacy and reduce the reprocessing time. In agreement with Vickery *et al.*, the present authors believe that some inadequate combinations may have antagonistic effects.[2] The Pronetron procedure includes enzymes and detergents, but the two types of product are never mixed together. Other experiments showed that a mixture of Pronetron α and β had low efficacy (unpublished data). The same results were found in the present study with a mixture of a detergent and a disinfectant, such as quaternary ammonium compounds. These chemicals are known to be good disinfectants, but they also seem to be good fixing agents. The authors suggest that they should only be used for applications in which thorough cleaning is not necessary. An adequate endoscope reprocessing protocol should include products that have a specific action and which are used in a definite order. For example, an efficient curative maintenance protocol for removing a biofilm that is already present inside an endoscope channel may include enzymatic predigestion, followed by removal of the biofilm with a highly efficient cleaning agent, followed by disinfection with peroxyacetic acid. An efficient preventive maintenance protocol for reducing biofilm build-up inside endoscope channels may only include the efficient cleaning agent followed by the disinfectant. A two-step regular preventive cleaning protocol with enzymes followed by detergent might be conceivable, but the price and the handling conditions would not be acceptable. These protocols could be an answer to Nelson, who concluded that new initiatives to enhance endoscope reprocessing are increasingly important to maintain public confidence.[3]

Another problem related to endoscope reprocessing has been reported by some authors who found biofilms growing inside automated washer-disinfectors.[1,4,13] This was first described in 1992, but remains a problem today.[14] The use of an anti-biofilm agent inside washer-disinfectors could reduce biofilm build-up inside both endoscope channels and hydraulic pathways of endoscope reprocessors.

This study should alleviate the concerns of authors who have noted the lack of studies evaluating the performance of cleaning agents, and it proposes a new approach to biofilm removal by the use of combinations of detachment promoting agents. If included in endoscope reprocessing protocols, these products may improve the efficacy of disinfectants and reduce the risk of transmitting infections.

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