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Removing biofilm from a endoscopic: evaluation of disinfection methods currently used*

REMOÇÃO DE BIOFILME EM CANAIS DE ENDOSCÓPIOS: AVALIAÇÃO DE MÉTODOS DE DESINFECÇÃO ATUALMENTE UTILIZADOS

REMOCIÓN DE BIOFILM DE CANALES DE LOS ENDOSCOPIOS: EVALUACIÓN DE LOS MÉTODOS DE DESINFECCIÓN QUE SE UTILIZAN ACTUALMENTE

Ana Cristina Balsamo[1], Kazuko Uchikawa Graziano[2], René Peter Schneider[3], Manoel Antunes Junior[4], Rúbia Aparecida Lacerda[5]

ABSTRACT

Laboratory experimental study that compared the effectiveness of five methods of disinfection for the removal of biofilm in gastrointestinal endoscopes. New transparent tubes of polytetrafluoroethylene (Teflon®) were used as specimens to simulate the channels of flexible endoscopes. After pre-cleaning the tubes were intentionally contaminated with Pseudomonas aeruginosa and subjected to disinfection methods. As a result, none removed 100% of these biofilms. What else physically removed biofilm was 2% glutaraldehyde in an automatic processor, probably justified by the *double clean*, since the equipment has this phase at the beginning of your cycle. The method less effective for removing plaque and other debris was the acidic electrolytic water. These results suggest that the cleaning is most striking in the removal of biofilms that disinfection of consecutive since glutaraldehyde disinfectant by machine is more efficient, it is a fastener organic waste.

DESCRIPTORS

Endoscopes
Biofilms
Disinfection
Central Supply
Hospital
Nursing

RESUMO

Estudo experimental laboratorial que comparou a ação de cinco métodos de desinfecção na remoção de biofilme em endoscópios gastrintestinais. Foram utilizados como corpos de prova tubos novos transparentes de politetrafluoretileno (Teflon®) simulando os canais flexíveis dos endoscópios. Após limpeza prévia os tubos foram contaminados intencionalmente com Pseudomonas aeruginosa para formação de biofilme e submetidos à desinfecção. Como resultado, nenhum deles removeu 100% dos biofilmes. O que mais removeu fisicamente o biofilme foi o glutaraldeído 2% em processadora automática, provavelmente justificado pela *dupla limpeza*, já que o equipamento conta com essa fase no início do seu ciclo. O método que se mostrou menos eficiente para remoção de biofilme e outros resíduos foi água eletrolítica ácida. Esses resultados sugerem que a limpeza é mais impactante na remoção de biofilmes do que a desinfecção consecutiva, uma vez que o glutaraldeído, desinfetante da máquina que se mostrou mais eficiente, é um fixador de resíduos orgânicos.

DESCRITORES

Endoscópios
Biofilmes
Desinfecção
Almoxarifado Central
Hospitalar
Enfermagem

RESUMEN

Un estudio experimental en el laboratorio en el que se comparó la acción de los cinco métodos de desinfección en la eliminación de biofilm en los endoscopios gastrointestinales. Fueron utilizados como muestras tubos nuevos transparentes de politetrafluoroetileno (Teflon®) simulando los canales de los endoscopios flexibles. Después de pre-limpieza los tubos fueron contaminadas intencionadamente con Pseudomonas aeruginosa y se sometió a métodos de desinfección. Como resultado, ningún método hay removido 100% de las biopelículas. El método que más hay removido físicamente fue 20% glutaraldehído en un procesador automático, probablemente justificado por la *doble limpio*, ya que el equipo tiene esta fase en el comienzo de su ciclo. El método es menos eficaz para eliminar la placa y la otra ruina era el agua ácida electrolítica. Estos resultados sugieren que la limpieza es más notable en la eliminación de las biopelículas que la desinfección de forma consecutiva desde desinfectante glutaraldehído de la máquina es más eficiente, es un cierre de los residuos orgánicos.

DESCRIPTORES

Endoscopios
Biofilmes
Desinfección
Central de Suministros
Hospital
Enfermería

*Taken from the dissertation "Evaluation of high-level cleaning and disinfection effectiveness for the removal of biofilm in endoscopic channels", Graduate Program in Adult Health, University of São Paulo School of Nursing, 2009.

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INTRODUCTION

Endoscope equipment is used in specialized services with a high demand for exams. Because of their high cost, their inventory tends to be restricted. Reuse of the equipment is approved, despite its complex structure, with long channels internally covered with polytetrafluorethylene and small luminal diameter, favoring the attachment of organic material and microorganisms and, consequently, the formation of biofilm.

Biofilms knowingly impede efficient processing and represent a challenge for material reuse. They consist of multiple layers of bacterial cells or fungi, grouped and involved in amorphous extracellular material composed of bacterial exopolysaccharides (EPS), whose function is to closely unite the cells to and between the biomaterial surfaces, constituting an extracellular matrix fundamentally composed of carbohydrates and proteins, with the presence of extracellular DNA and dead cell debris[1-2].

According to the material cleaning difficulty evaluation criteria proposed in one study[3], gastrointestinal endoscopes represent a high risk score as, besides their complex configuration, they are neither dismountable nor transparent, which hampers their internal visualization and can thus compromise the evaluation of their cleaning process. As their internal structure permits organic material accumulation and biofilm formation and direct friction with a brush is not always possible, difficulties may arise to perform the cleaning required.

In this context, endoscopes are classified as material that represents a great challenge for processing. On the other hand, they permit the entry and exit of water, as well as the use of internal cleaning artifacts, and can be immersed in detergent solution that facilitates the dissolution of dirt.

Although different specialized societies have well established gastrointestinal endoscope cleaning and disinfection recommendations, various studies discuss that the transmission of microorganisms or adverse effects in patients submitted to gastrointestinal endoscopes may be due to the formation and permanence of biofilms...

Although different specialized societies have well established gastrointestinal endoscope cleaning and disinfection recommendations, various studies discuss that the transmission of microorganisms or adverse effects in patients submitted to gastrointestinal endoscopes may be due to the formation and permanence of biofilms, making them responsible for cross-transmission of bacteria and viruses. Therefore, their authors propose the need for studies to evaluate adherence to cleaning and disinfection protocols, the elaboration of methods that permit monitoring the processing and tests to check its efficiency[1,4-8].

As biofilm formation is unavoidable in structures like endoscope channels and a causal link exists between the current causes of exogenous infections related to flexible endoscopes and bad processing quality[9-10], the aims of this study were to evaluate the effectiveness of high-level disinfection after previous brushing for biofilm removal in sample specimens that simulate flexible endoscope channels, besides comparing the methods available at health services. This research contributes by unveiling the extent to which biofilms can be eliminated from endoscope channels, using currently available resources for cleaning and high-level disinfection.

METHOD

In this comparative experimental laboratory research, the efficiency of five high-level disinfection methods for biofilm removal was tested.

In all tests, new transparent flexible tubes were used, with a length of 1m20 and an internal diameter of 2.8 mm, covered with polytetrafluorethylene (Teflon®), the same material that covers original endoscope channels. These tubes were submitted to chemical composition analysis through scanning electron microscopy, confirming their similarity with the originals.

To affirm biofilm removal differences among the five disinfection methods, probability was set at 99.98%. After professional statistic advice, a total number of 70 tubes was determined, equally distributed among the methods. From each of the 14 tubes, in turn, three surface segments of approximately 3 mm^2 were taken, representing their (previously identified) start, middle and end, totaling 210 sample segments, 42 for each processing method. The option to remove three segments increased the probability of detecting the presence of biofilm.

To obtain biofilm in the sample specimens, challenge contamination with *Pseudomonas aeruginosa* (ATCC 27853) was used, a microorganism capable of producing biofilm. Originally obtained from the culture inventory of the Microbiology Laboratory at the University of São Paulo University Hospital, this microorganism was inoculated on a MacConkey Agar plate on the day before preparing the suspension. On the date of the experiment, a suspension was prepared with $1x10^6$ colony forming units per milliliter (CFU/mL) of this microorganism in 10% TSC culture medium, using the colorimeter. To complete each lumen of the tubes, the quantity of suspension introduced was established by calculating the internal volume in relation to the tube length, totaling about eight milliliters per tube. Before contamination by this suspension, the 70 new tubes were previously submitted to manual cleaning, using water and neutral detergent, drying and sterilization in a steam autoclave.

For biofilm formation, a system (Figure 1) was set up with the following materials: two-liter glass flasks with lid, polyvinyl chloride (PVC) and silicon extensions, clamps and an 0.2 micrometer filter to filter air from the system and prevent contamination of the culture medium. In the lid of the flasks, eight perforations were made to introduce eight PVC extensions, fixed with Araldite® rapide epoxy glue. This set was sterilized in a steam autoclave at 134° C during five minutes. The solution with the TSB culture medium was prepared in the glass flasks and then autoclaved at 121° C during 15 minutes. On the day of the experiment, about eight milliliters of *Pseudomonas aeruginosa* inoculum – 10^6 CFU/mL – were injected in each of the tubes of the sample specimens and their extremities were attached in circular shape and incubated at 37° Celsius for one hour. This procedure allowed the microorganism to interact with the surface of the sample specimens[11].

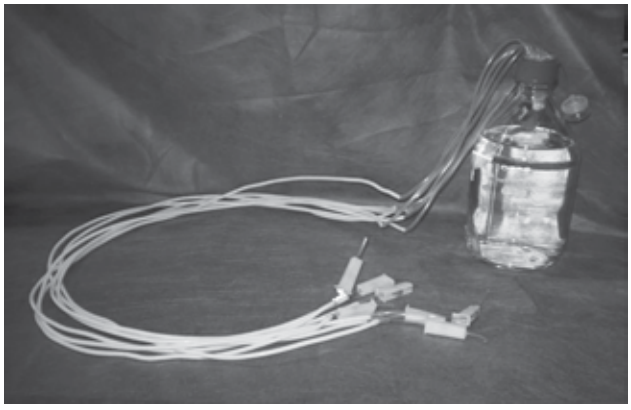
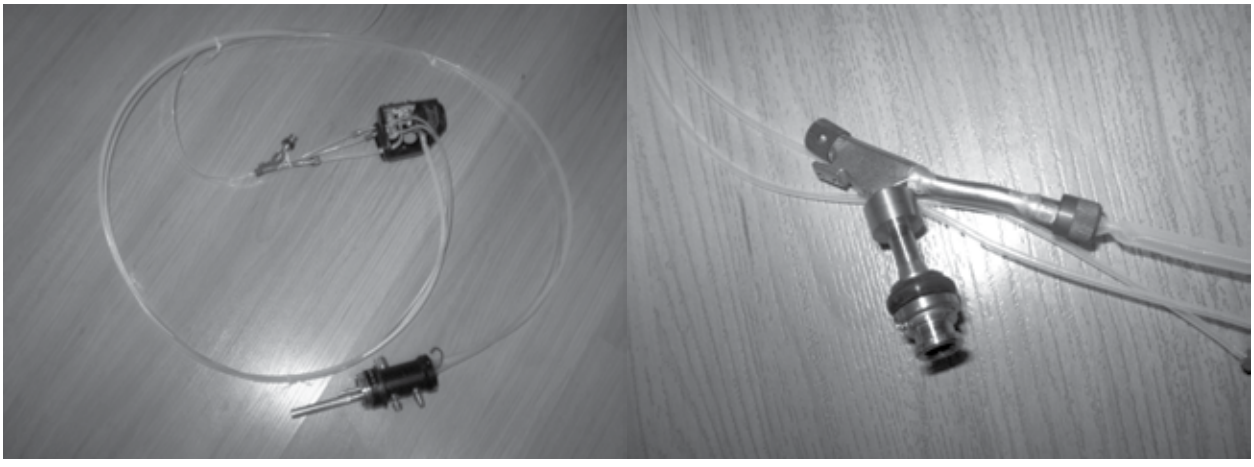


Figure 1 – Experimental model for biofilm formation on sample specimens

At the end of this period, the lid with the PVC extensions was screwed onto the mouths of the flask containing the culture medium, their extremities were attached to the tubes previously contaminated with the *Pseudomonas aeruginosa* suspension and the culture medium ran into the distal end of the system, permitting a slow and constant flow, controlled by the clamps, for a random six-hour period to promote biofilm formation. The choice of this period was established to simulate the endoscope usage time in a hospital context, which functions in six-hour shifts, although the biofilm was obtained after one hour in the laboratory result. Then, the clamps were closed, the Teflon® tube removed from the system, the clamp on the extremity it was connected to was identified with the letter F to indicate the end (*final* in Portuguese) of the tube and the specimens were forwarded to the Endoscopy Service for the cleaning and disinfection phases. Positive and negative controls were performed for each disinfection method. In the positive control, contaminated sample specimens were not submitted to cleaning and disinfection to prove that uniform biofilm formation occurred across the extent of the lumens. In the negative control, a new and clean tube was submitted to the steam autoclave sterilization process. Both controls took place at the same time as the experiments.

All contaminated sample specimens were previously submitted to manual cleaning after immersion in Rioquímica® enzymatic detergent (containing proteases, amylases and lipases) and then distributed among the five different disinfection methods: 1) basic 2% glutaraldehyde solution (Cidex® – Johnson&Johnson) in manual method; 2) basic 2% glutaraldehyde solution (Cidex® – Johnson&Johnson) in automated method, using the Lifemed® Endolav® endoscope cleaner/disinfector; 3) 0.09%-0.15% active peracetic acid (Anios® Anioxyde 1000) in manual method; 4) 35% peracetic acid in automated method, using the *Steris® System* sterilizer, whose active principle is 35% peracetic acid itself (sterilizing concentrate *STERIS®* 20); 5) acidic electrolytic water produced *in situ* in the Cleantop® processor. The cleaning and disinfection methods followed the recommendations in the Endoscope Processing Manual of the Brazilian Gastrointestinal Endoscopy Nursing Society[12].

In the manual disinfection methods, the tubes were immersed in the test-disinfectant after cleaning and their lumens were filled with the help of a 10-ml sterilized syringe. Contact times with the disinfectant were established according to the product manufacturers' recommendations. After removing the solution, the tubes were rinsed under running tap water using a water pistol, the lumens were dried with a compressed air pistol and the external surface was dried with a clean cloth.



Figures 2 and 3 - Endoscope prototype

For the automated methods, prototypes (Figure 1) were created which permitted the adequate fitting of the test tubes into the machine connectors, guaranteeing contact between the disinfectant and the internal and external surfaces of each tube.

At the end of the processing phase (cleaning and disinfection), the three segments were removed from each of the 70 tubes, which were prepared with platinum in high vacuum and stored in a desiccator until they were subject to scanning electron microscopy (Brand FEI, model Quanta 600 – FEG) analysis to detect the presence or not of residual biofilms. The likelihood ratio test[8] was used to compare the segments from the start, middle and end of the tubes for each disinfection method. Significance was set at 5% and statistical power at 95%.

RESULTS

Table 1 demonstrates the results for the presence of biofilm attached to the sample segments after each tested disinfection method.

Table 1 – Frequency distribution of presence of biofilm in different sample segments after the application of the tested disinfection methods

	PRESENCE OF BIOFILM IN SAMPLE SEGMENTS					
a) 0.09% to 0.15% peracetic acid with manual process	**Presence**	**%**	**Absence**	**%**	**Total**	**%**
Start	10	71.43	4	28.57	14	100
Middle	12	85.71	2	14.29	14	100
End	10	71.43	4	28.57	14	100
Total	32	76.19	10	23.81	42	100
b) Ácido peracético com processo automatizado (sistema Steris®)	**Presence**	**%**	**Absence**	**%**	**Total**	**%**
Start	5	35.71	9	64.29	14	100
Middle	2	14.28	12	85.72	14	100
End	3	21.42	11	78.58	14	100
Total	10	23.80	32	76.20	42	100
c) Glutaraldeído 2% com processo manual	**Presence**	**%**	**Absence**	**%**	**Total**	**%**
Start	14	100	-	-	14	100
Middle	11	78.57	3	21.42	14	100%
End	5	35.71	9	64.28	14	100%
Total	30	71.42	12	28.58	42	100%
d) Glutaraldeído 2% com processo automatizado	**Presence**	**%**	**Absence**	**%**	**Total**	**%**
Start	1	7.14	13	92.86	14	100
Middle	1	7.14	13	92.86	14	100
End	-	-	14	-	14	100
Total	2	4.76	40	95.24	42	100
e) Água eletrolítica ácida com processo automatizado	**Presence**	**%**	**Absence**	**%**	**Total**	**%**
Start	11	78.57	3	21.43	14	100
Middle	4	28.57	10	71.43	14	100
End	10	71.43	4	28.57	14	100
Total	25	59.52	17	40.48	42	100

Based on Table 1, it is verified that none of the processing methods was able to completely remove the biofilms. The result of the automated method using 2% glutaraldehyde can be considered satisfactory though, removing almost all inoculum, followed by the automated process using peracetic acid (Steris® system), totaling ten sample segments contaminated with biofilm. In the other methods, the biofilm that remained was practically equivalent, totaling between 25 and 32 sample segments.

As for the presence of biofilm in the segments (start x middle x end), a statistically significant difference was found among them in the manual disinfection method using 2% glutaraldehyde and in the automated method using acidic electrolytic water, indicating that results were not uniform in the same sampling unit.

In the manual method with glutaraldehyde, this difference was obtained between the initial and final portions ($p < 0.001$), showing that the biofilm remained attached in 35.71% of the sample segment surfaces. On the other hand, no statistically significant difference ($p = 0.067$) was found between the initial and middle portions but, when comparing the middle and final segments, significant differences were found ($p = 0.039$). No biofilm or debris was found in only 11 of the analyzed segments (11/42). Only one sample unit contained a fragment of the EPS layer. No attached bacteria were found without the EPS (Table 2). The presence of biofilm or isolated bacteria without EPS or EPS without the presence of bacteria, identified through electron microscopy, indicated inefficient cleaning of the channels.

In the automated method with acidic electrolytic water, a significant difference was found between the initial and middle segments (p = 0.039). The other pairs showed no significant mutual differences. Using electron microscopy, however, it was verified that segments without biofilm were not clean. Some segments contained countless isolated or grouped bacteria, while others only revealed the EPS layer without the presence of bacteria (Table 2). The presence of biofilm or isolated bacteria *beyond* the biofilm, or EPS without the presence of bacteria, observed through electron microscopy, indicated inefficient cleaning of the channels.

In the automated method using 2% glutaraldehyde, no significant difference was observed when comparing all segments: start x middle (p = 0.999; start x end, p = 0.466; middle x end, p = 0.466). Although this method removed the biofilm from most sample segment surfaces, only 19.04% (8/42) of these contained no debris – bacteria or EPS (Table 2).

Table 2 – Frequency distribution of presence of biofilm and other debris after manual cleaning and disinfection with different methods

METHOD	PERACETIC ACID				GLUTARALDEHYDE				ACIDIC ELECTROLYTIC WATER	
	Manual [0.09% to 0.15%]		Steris® System		Manual [2%]		Automated [2%]			
	n	%	n	%	n	%	n	%	n	%
No debris	2	4.762	28	66.67	11	26.19	8	19.05	-	-
Biofilm	32	76.19	10	23.81	30	71.43	2	4.762	25	59.52
EPS only	2	4.762	4	9.524	1	2.381	30	71.43	3	7.14
Bacteria only	6	14.29	-	-	-	-	2	4.762	9	21.43
EPS/bacteria on extremities	-	-	-	-	-	-	-	-	5	11.90
Total	**42**	**100**	**42**	**100**	**42**	**100**	**42**	**100**	**42**	**100**

As for the peracetic acid, neither the manual nor automated methods presented significant differences between the segment positions (respectively, 0.15% start x middle, p = 0.802; start x end, p = 0.999; middle x end, p = 0.802; and start x middle, p = 0.487; start x end, p = 0.222; middle x end, p = 0.487). In the automated method, the presence of attached bacteria without the exopolysaccharide (EPS) layer or of EPS without the bacteria was considered as debris instead of biofilm. When using this processor, a proportion of 28 clean segments in 42 sample units (66.66%) was obtained (Table 2).

Table 2 summarizes the results obtained for the presence of biofilm and debris in the different sample specimen segments. In many samples, the biofilm remained attached when using 0.09% to 0.15% peracetic acid, and in less samples when using automated disinfection with 2% glutaraldehyde. The presence of the EPS layer at one end of the sampling unit but only bacterial cells at the other end was observed in the same segment only in the method using acidic electrolytic water.

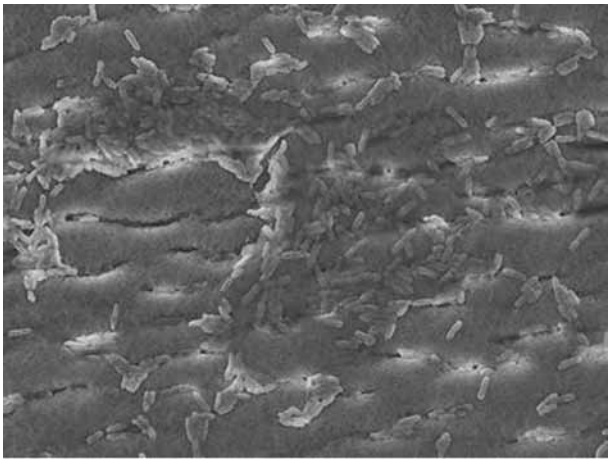


Figure 4 - Biofilm after disinfection using 0.09% to 0.15% peracetic acid

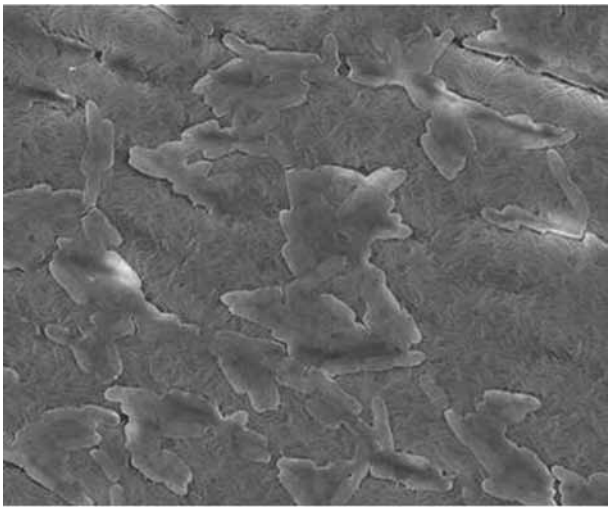


Figure 5 - Biofilm after automated disinfection using 2% glutaraldehyde

Figures 4 to 10 illustrate the presence of biofilm, EPS and bacteria after the disinfection methods.

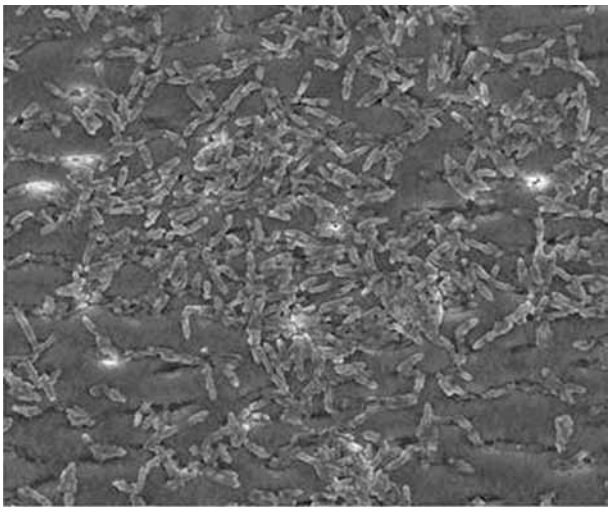


Figure 6 - Biofilm after manual disinfection using 2% glutaraldehyde

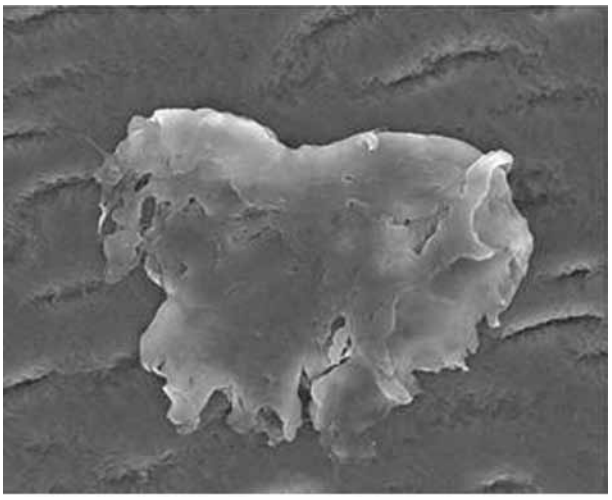


Figure 9 – EPS fragment after disinfection using acidic electrolytic water

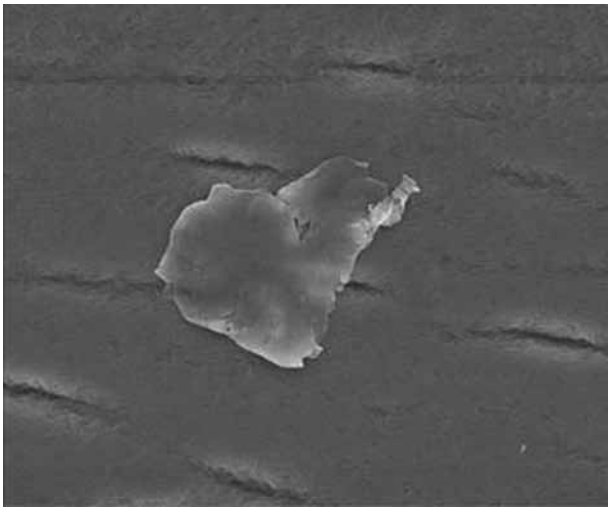


Figure 7 - EPS fragment after manual disinfection using 2% glutaraldehyde

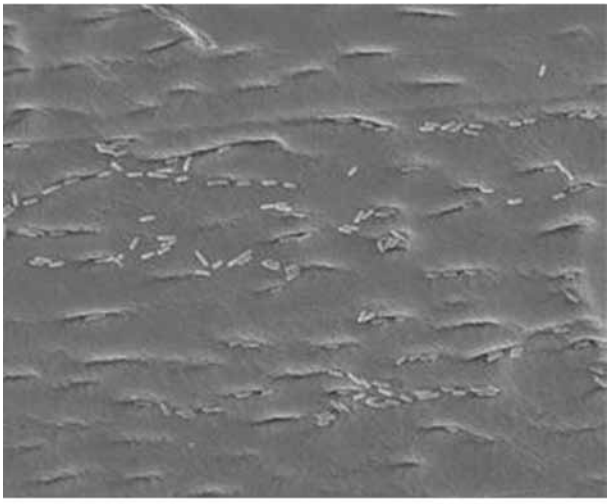


Figure 10 – Bacterial cells after disinfection using acidic electrolytic water

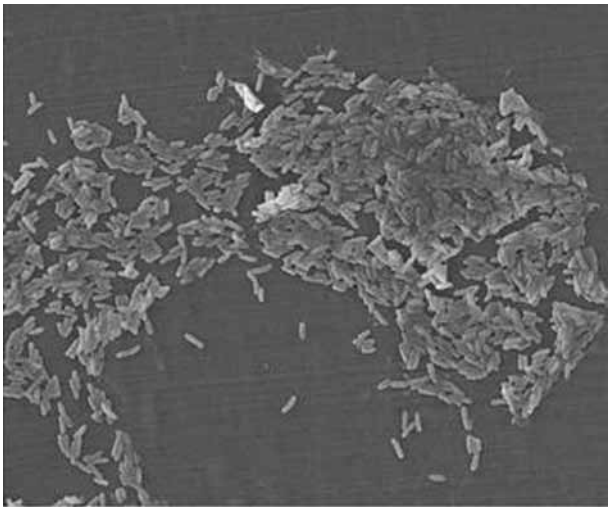


Figure 8 - Biofilm after disinfection using acidic electrolytic water

DISCUSSION

This study showed that processing through the methods commonly used in Brazil was unable to remove all biofilm attached to the lumen surfaces of the test specimens that simulated the flexible endoscopic channels. The results enhanced the understanding that the processing of this care equipment still represents a challenge to institutions, researchers and official health entities. The phases of this process need to be reconsidered and discussed, including the use of manual cleaning artifacts, efficacy of cleaning agents, microbial activity of disinfectants and, mainly, the ability of this whole apparatus to remove biofilm.

A research showed that the number of times the endoscope was contaminated was directly proportional to the number of occasions on which the equipment was used, highlighting the presence of *Pseudomonas aeruginosa*,

in gastroscopes as well as colonoscopes[4]. The authors conclude that these findings reflect the microorganism's capacity to survive the cleaning and disinfection process.

At two Brazilian hospital, it was also demonstrated that *Pseudomonas aeruginosa* was the microorganism most isolated from ready-to-use endoscope samples and highlighted the biofilm formation ability of this microorganism[14].

Authors consider that, although difficult, the removal of biofilm can be achieved through mechanic cleaning and brushing, but some disinfectants are capable of removing the biofilm while others are not[1,15]. In our research, differences were found in the disinfectants' effect on the biofilms. While some removed the biofilm from most of the sample segments, others only removed the exopolysaccharide (EPS) layer, while bacterial cells remained, and vice-versa.

Cleaning agents are more effective to remove biofilm than disinfectants, as the forms are able to detach the biofilm from the surface[15]. In this research, the best processing result involved two cleaning phases using enzymatic detergent.

In one study, the authors elaborated a system to produce biofilm in 16 hours, using *Escherichia coli* bacteria, in Teflon® tubes, used for endoscope channels[16]. The goal of these authors was to check the efficacy of detergents recommended for endoscope cleaning to remove biofilm, three of which contained enzymes and one did not. The cleaning procedures did not use a brush to scrub the surfaces, but merely immersion during eight minutes. Differences among the products were found, as the non-enzymatic detergent removed more biofilm. In the topic *Letters to the editor*, it was published that the non-enzymatic detergent, called *Matrix®,* tested in that research, contains a significant quantity of quaternary ammonium compound (not cited by the research author), resulting in the removal of the biofilm from the Teflon® tubes. The same author affirms that the biofilm was reduced because the detergent was associated with the disinfectant[17].

In our research, although the internal surfaces of the sample tubes were scrubbed with a brush, the results showed that the cleaning process was insufficient to remove the biofilm. It should be reminded that the presence of dry organic material makes cleaning more difficult and enhances the creation of biofilms. These, in turn, make it difficult for the chemical agent to penetrate and, thus, microbial death does not occur. In places without a nursing team to perform equipment cleaning and disinfection immediately after use, like at night or during emergency care, the endoscope awaits processing by the regular team, which only happens on the next workday. Hence, the endoscope surface is exposed to organic material for a long time, sufficient for biofilm to form.

Endoscope processors offer advantages in comparison with manual processing: they automatize and standardize important processing phases, reduce the probability of omitting a phase and minimize the team's exposure to chemical products. The processor reduces the possibility of human errors and generally involves devices to be connected to water filter systems, which avoid the equipment's contamination by opportunistic microorganisms found in reservoir water[18]. The same author describes that previous manual cleaning with a brush can be more effective than automated cleaning and that adding this phase would increase total processing time by 40 to 60 minutes. Also, it is reported that automatic processors neither use 70% alcohol for the final flush nor monitor the concentration of the chemical product automatically, and that machine compartments should be frequently evaluated to avoid any accumulated dirt and biofilm formation in its internal circuits.

In our study, the best biofilm removal result was obtained in the automated processing method that includes another cleaning phase with enzymatic detergent. These findings allow us to infer that the difference obtained is more related to the accomplishment of another cleaning phase than to the chemical product used, in this case 2% glutaraldehyde, which is an organic waste fastener. One important issue is the fact that endoscope processors come with their own devices that fit into the holes of the endoscope, guaranteeing contact between the cleaning agent and chemical product in the internal equipment channels through a direct, pressurized flow without return. The automatic processor with 2% glutaraldehyde most removed biofilm, but maintained a high percentage of segments with EPS layers (more than 60%). Manual processing with 0.09% to 0.15% peracetic acid and 2% glutaraldehyde were the methods that most retained biofilm.

Peracetic acid has been recommended for alternative high-level disinfection instead of aldehyde derivatives because of its low toxicity and biodegradability, although its antimicrobial effectiveness is similar[19]. In a previous publication, however, the same author demonstrated, with other collaborators, that some peracetic acidic formulae fixed biofilm while other did not[20]. They found that stabilized, i.e. ready-to-use peracetic acid did not fix the biofilm and concluded that, when choosing a disinfectant product for high-level disinfection, not only the germicide's bacterial activity should be considered, but also its ability not to fix biofilm.

CONCLUSION

In conclusion, none of the disinfection methods tested totally removed the biofilm; the most efficient was the use of 2% glutaraldehyde in automated equipment, and the least effective was acidic electrolytic water in automated equipment. As the manual application of 2% glutaraldehyde did not obtain a similar response to the automated

method, and also due to the fact that this product fastens debris, the present study results suggest that cleaning is more effective to remove biofilm than consecutive disinfection; this is justified by the fact that the automatic processor with this product includes a cleaning phase at the start of its cycle. This research alerts to the capacity of microorganisms to form biofilms within one hour after contamination, reinforcing the need to clean the endoscope soon after its use, so as to avoid environments that favor their development. As the microorganisms present in rinsing water are capable of forming biofilm, we suggest using bacterial filters for the endoscopes' rinsing water, as well as testing other disinfectants available in the market in disinfecting washers.

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