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State of the Science Review

Biofilms on instruments and environmental surfaces: Do they interfere with instrument reprocessing and surface disinfection? Review of the literature

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There is a growing appreciation for the role of biofilm-embedded microbes in many different aspects of infection transmission. The format of biofilm includes traditional hydrated biofilm, build-up biofilm, and dry surface biofilm. The objectives of this article are to discuss how traditional biofilm differs from build-up biofilm and dry surface biofilm, and to review the evidence supporting infection transmission from biofilm that accumulates in reprocessed instruments and from dry biofilm that forms environmental reservoirs.

Microorganisms existing in biofilm are found in a wide variety of health care environments including infected wounds and implants, in the water taps and drains, as dry biofilm on high-touch surfaces, and also in reprocessed medical devices.[1-4] The focus of this article is on the role of biofilm found in contaminated medical devices and environmental surfaces in infection transmission.

There have been numerous reports documenting the formation of traditional biofilm in hydrated environments and how fluid flow affects the adhesion and viscoelasticity of biofilm formed.[1,2,5-7]

Generally speaking, the higher the shear stress (ie, faster fluid flow) over the biofilm attached to a surface, the stronger will be the adhesion of the biofilm to that surface and the stronger the viscoelasticity.[1,2] A number of in vitro methods have been employed to facilitate the study of microorganisms within traditional biofilm either as single species biofilm or multispecies biofilm. In nature, multispecies biofilms are the more common presentation, and they are generally more resistant to disinfectants.[5,6] However, most in vitro models evaluate mono-species biofilm as these are less variable and easier to analyze. The models for studying traditional biofilm include static models, such as reactor vessels with biofilm formed on rotating paddles submerged in fluid,[8] microtiter trays or tubes where biofilm is formed on the inner surface of the wells/tube,[5] MBEC 96-well devices where biofilm is formed on pegs immersed in fluid in the wells of the tray,[9] or beads where biofilm coats the surface.[7] In addition, there are continuous fluid flow models including lumens[10] and microscope chambers.[6] In static models, the media flow is achieved using "rocking motion" or circulation of paddles within the vessel of fluid with media refreshed on a periodic basis. In continuous fluid flow models, the fresh media is continuously perfused unidirectionally through the biofilm apparatus.

Assessment of biofilm formed under in vitro conditions has been used to characterize the stages of biofilm formation, as well as the resistance of microorganisms within biofilm to various agents that would normally kill planktonic forms of the same organisms. Akinbobola et al[11] has demonstrated that peracetic acid that is used as a high-level disinfectant (HLD) and as a liquid chemical sterilant requires higher concentrations to kill *Pseudomonas aeruginosa* in biofilm compared with the planktonic form of this organism. Furthermore, *P aeruginosa* in mature 96 and 192 hour biofilm shows <1 Log_{10} kill when exposed to 800 ppm peracetic acid after 5 minutes contact, whereas this concentration is effective in 5 minutes contact time at providing an 8 Log_{10} kill of 24-48 hour biofilm.[11] The other important aspect of traditional biofilm is that some species of bacteria can provide protection to other species of bacteria if they are embedded together in a mixed species biofilm matrix. Bridier et al[5] reported that *Bacillus subtilis*-ND isolated from an automated endoscope reprocessor produced thicker biofilm than genetic stock cultures of *B subtilis*. Furthermore, they demonstrated that 5 minutes contact with 0.35% peracetic acid could kill *B subtilis* and *Staphylococcus aureus* single species biofilm but could did not kill single species *B subtilis*-ND biofilm and could not kill *S aureus* when present as a mixed species biofilm with *B subtilis*-ND. This later phenomenon represents "bystander" protection within mixed species biofilm. Such findings using

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traditional biofilm models raise questions regarding the efficacy of the current validated manufacturer's instructions for use (MIFU) in terms of whether traditional biofilm can be removed by the existing MIFU for endoscope cleaning. It also raises questions regarding whether the current validation testing for HLDs, sterilants, and surface disinfectants is stringent enough to ensure proper decontamination of reprocessed medical devices and high-touch environmental surfaces within patient rooms.

This article focuses on evaluating the differences between traditional biofilm compared with build-up biofilm (BBF) in reprocessed medical devices, and dry surface biofilm on environmental surfaces in terms of infection transmission.

HOW DOES TRADITIONAL BIOFILM DIFFER FROM "ACCUMULATED" MATERIAL IN REPROCESSED MEDICAL DEVICES USED IN HEALTH CARE?

Traditional biofilm forms under continuously hydrated conditions such as water pipes, showers, taps, sinks, and others,[12,13] as outlined in Fig 1A, whereas BBF forms as an accumulation of material formed by repeated rounds of patient-exposure, cleaning and disinfection, or sterilization and dry storage as outlined in Fig 1B.[12] A major difference between the 2 types of biofilm is that each round of BBF involves exposure to chemicals (eg, HLDs or sterilants) or heat (eg, steam sterilization) that can fix any organic residuals onto the medical device surface. Thus, the multiple rounds of complete or partial fixation and complete or partial dry storage make BBF more compacted and more difficult to remove compared to traditional biofilm.[14] Azizi et al[15] demonstrated that baked on debris inside extensively patient-used Yankauer suction tips could not be eliminated despite multiple rounds of cleaning. Similarly, borescope examination of patient-used flexible endoscope channels[16,17] has revealed streaks of residual material similar to what Alfa et al[18] reported in their BBF polytetrafluorethylene channel model after a bristle brush was used for sample collection. Thaker et al[17] used a borescope to observe contact of the bristle brush used for endoscope cleaning and documented that there was no contact of the bristles on one side of the coiled endoscope channel. This incomplete contact of bristles likely contributes to the gradual accumulation of residuals in reusable medical devices over repeated uses. A traditional in vitro biofilm model for endoscope channels has been proposed,[19] however, Alfa et al[18] recently developed an in vitro model of BBF that more closely represents the "worst-case" situation for reusable flexible endoscope channels that includes partially fixed organic material and viable as well as viable but nonculturable bacteria embedded within the BBF matrix.

HOW DOES TRADITIONAL BIOFILM DIFFER FROM "DRY SURFACE" BIOFILM FOUND IN THE HEALTH CARE ENVIRONMENT?

The concept of "dry surface biofilm" was first introduced by Almatroudi et al[4] to identify that hard and soft environmental surfaces within health care facilities are not void of organisms, but rather represent a heterogenous accumulation of organisms and other material in a dry matrix. The in vitro dry surface biofilm model that they developed mimics the characteristics of surfaces within health care facilities. There are viable as well as viable but nonculturable bacteria that exist in the dry surface biofilm in a matrix of extra-cellular glycoconjugate, protein, and DNA. This dry surface in vitro model is similar to the BBF model[18] except that it uses dehydration rather than partial fixation to create the accumulated matrix. These same authors[20] subsequently used this dry surface biofilm model to show that even 20,000 ppm hypochlorite exposure for 10 minutes did not totally eradicate *S aureus*, and that the surviving viable but nonculturable organisms could recover and release viable planktonic organisms. They suggested that this dry surface biofilm model may be a useful model for more stringent assessment of the efficacy of cleaning and disinfection of health care environmental surfaces. This recommendation is further supported by Ledwoch et al,[21] who reported that the predominant organisms in dry surface biofilm from 3 hospitals evaluated were *Bacillus* spp and *Staphylococcus* spp. They question whether dry surface biofilm composed of these environmental organisms may be able to protect other organisms from the effects of surface cleaning and disinfection. Chowdhury et al[22] used the dry surface biofilm model to demonstrate that after one touch, approximately 6% of the bacteria in this dry matrix was transferred to gloved hands and then subsequently transferred to other dry surfaces. These in vitro data

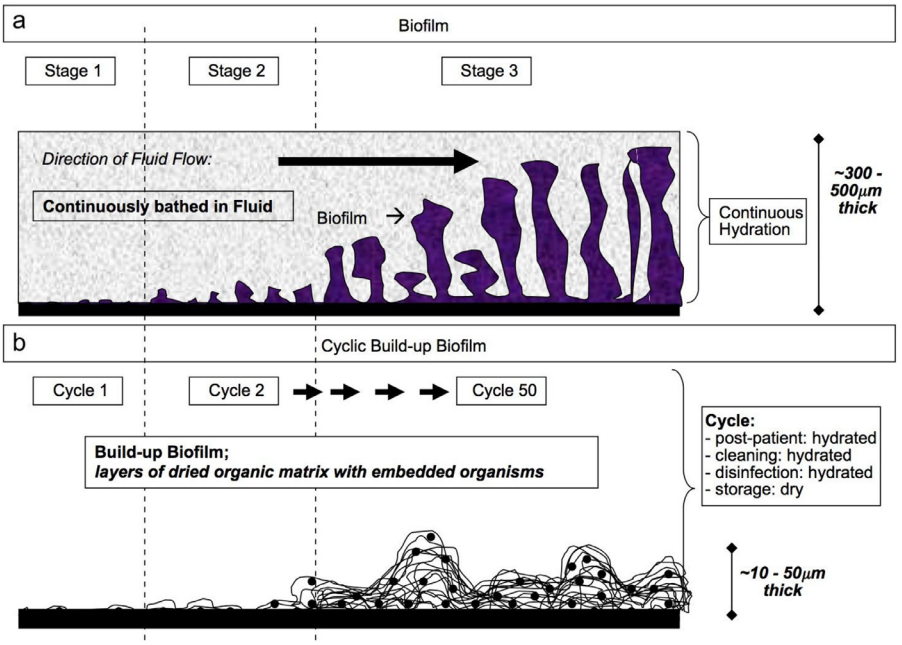

Fig 1. Comparison of traditional to cyclic build-up biofilm. Used with permission from Elsevier.[12] Traditional biofilm is formed under continuous hydration as outlined in (A) and Buildup Biofilm is formed after repeated rounds of hydration and dry conditions as outlined in (B).

suggest the dry surface biofilm in health care may act as a persistent source of pathogens.

In summary, biofilm, BBF, and dry surface biofilm all represent various types of microbial reservoirs that can be found in health care settings. Models of these environments will help us to better understand how these reservoirs may act as sources of microorganisms that might increase the risk of infection transmission in health care facilities.

EVIDENCE FOR ACCUMULATION OF ORGANIC AND MICROBIAL SURVIVAL DESPITE MIFU REPROCESSING OF MEDICAL DEVICES

The research models for biofilm and BBF suggest that disinfectant and sterilization may fail if there are organic and microbial residuals despite following the MIFU for reprocessing. So what evidence is there of biofouling of medical devices that leads to contamination despite sterilization? Deshpande et al[23] stated, "Each surgical power tool has the potential to be contaminated with proteinaceous material that aids the adsorption of bacteria to the instrument and may inhibit sterilization processes." This review clearly documented that there are contaminants detected in surgical power tools after full reprocessing. Table 1 summarizes what residuals have been found on reusable power tools,[23] and Table 2 summarizes the published reports of infection transmission from contaminated surgical devices.[24-28]

The outbreak of deep surgical site infections reported by Dancer et al[27] was related to wet packs containing instruments for orthopedic and ophthalmic surgery. This group documented that 8 of 10 visibly wet or stained surgical packs grew *Bacillus* spp and coagulase-negative *Staphylococcus* from the inner wrap as well as from the surgical instruments. Furthermore, they found the same contamination in 3 of 10 dry, intact packs. The isolates from the deep surgical site infections were also *Bacillus* spp and coagulase-negative *Staphylococcus* (although not the identical strains to the coagulase-negative *Staphylococcus* isolated from the surgical instruments). This is a case in which it was most likely that the steam sterilized instrument tray sets were contaminated poststeam sterilization due to wet packs. The risk to patients occurred because these wet packs were not identified and handled properly (ie, reprocessing staff did not deal with wet pack issues appropriately and surgery staff did not reject visibly stained instrument tray sets). However, the detection of organisms on the surgical instruments of packs not visibly stained and the deep surgical site infections that were caused shows that health care facility staff (both reprocessing and operating room staff) need to be vigilant regarding contamination after sterilization due to wet packs.

Tosh et al[26] reported an outbreak of knee infections with *P aeruginosa* that occurred 4-19 days after arthroscopic knee surgery. The outbreak included 7 patients who all had *P aeruginosa* isolates with identical pulsed-field gel electrophoresis patterns that matched those from the reprocessing room sink drain and from 2 suction bottles in the procedure room. These infections resulted in 3-16 days additional hospitalization, additional arthroscopic debridement, and 6 weeks of systemic antibiotic therapy. Although the arthroscopic instrument cultures were negative, the authors concluded that this outbreak was related to retained tissue within the handpiece that prevented adequate steam sterilization and resulted in contamination of the arthroscopic handpiece with *P aeruginosa*. Indeed, this study concluded that internal inspection of surgical handpieces is necessary to ensure adequacy of cleaning. Furthermore, they indicated that the Food and Drug Administration (FDA) had circulated a safety alert indicating this internal inspection should be done using a "video scope" after cleaning to ensure there was no residual fluid or tissue.[26]

The most recent infection transmission event was published in 2017,[28] and represented increased infections postcraniotomy related to reprocessing of a cavitron ultrasonic surgical aspirator. There was a variety of different bacterial pathogens grown (Table 2), but each caused serious invasive infections (ie, cerebral abscess, epidural empyema, meningitis). Sheitoyan-Pesant et al[28] concluded that the infections transmitted resulted from a change in reprocessing that was implemented in 2014 that included longer transport times before reprocessing. This prolonged transit time resulted in biological fluid that dried within the cavitron ultrasonic surgical aspirator resulting in inadequate cleaning that ultimately resulted in suboptimal steam sterilization.

In the United States, there were 51.4 million surgical procedures compared with 1.6 million endoscope procedures per year in 2010.[29] Although the infection transmission rates from surgical instruments are lower than for reusable flexible endoscopes,[29] it still represents a substantial number of infections that could be prevented.

Table 1
Surgical power tool contamination after patient-use, cleaning, and disinfection

Power Tool	Specialty	Contaminants after Decontamination
Rotary	Dentistry	Bacteria including *Staphylococcus aureus*
		Hepatitis B DNA
		Hepatitis C DNA
	Orthopedic	Protein
		DNA
		Pseudomonas
Ultrasonic	Ophthalmology, neurosurgery, dentistry	Blood protein bacteria fungi
		Eye lens tissue
		Viruses
Laser	Dermatology	Cellular debris herpes simplex virus HIV viral DNA
		Bacteria
Robotic	Surgery	Protein

Information extracted from reference.[23]

EVIDENCE FOR ACCUMULATION OF ORGANIC MATERIAL AND MICROBIAL SURVIVAL DESPITE MIFU REPROCESSING OF FLEXIBLE ENDOSCOPES

The recent outbreaks of multidrug resistant organisms related to contaminated endoscopes has focused attention on the reprocessing of such devices. In a recent review, Grein and Murthy[30] indicated that gastrointestinal endoscopy is one of the most commonly performed medical procedures in the world, and that in the United States there are approximately 20 million endoscopy procedures per year. Exogenous infection due to contaminated endoscopes are the most common cause of device-related nosocomial infection in the United States.[30] They indicated there have been 25 multidrug resistant outbreaks from 2010-2015 related to contaminated duodenoscopes. The recent review by McCafferty et al[31] indicated that 7 of 18 outbreaks of infections related to contaminated endoscopes occurred despite no apparent breaches in the MIFU, but the authors indicate that biofilm may still have contributed to the persistent contamination. They cautioned that inadequate cleaning, endoscope defects, and inadequate drying during storage may all contribute to unrecognized biofilm formation.[31] Furthermore, Johani et al[32] reported that 47% of air-water and instrument channels from patient-used colonoscopes and gastroscopes were culture positive.

Using quantitative polymerase chain reaction and next generation sequencing they reported that the average bacterial load in air-water and instruments channels was 1×10^3 colony-forming units (CFU)/cm and up to 6.6×10^2 CFU/cm, respectively.[32]

A key question is whether the existing validated MIFU for endoscope reprocessing can eliminate traditional biofilm. Alfa et al[14] used the polytetrafluorethylene biofilm model to demonstrate that after 5 repeated rounds of overnight biofilm formation followed by full MIFU reprocessing (including brushing and manual pump-assisted cleaning and automated endoscope reprocessor using liquid chemical

Table 2
Summary of clinical infections on surgical instruments related to disinfection of sterilization failure

Author [Ref]	Surgical Device	Disinfection/Sterilization	Pathogen [Infection]	Issue
Zaluski, et al[24]	Phacoemulsifier [eye surgery]	Steam	*Pseudomonas aeruginosa* [endophthalmitis]	Contamination of internal lines
Gillespie, et al[25]	Needle guide for transrectal biopsy	High-level disinfectant with OPA [overnight soak]*	*P aeruginosa* [septicemia]	Encrusted channel contamination
Tosh, et al[26]	Arthroscopic handpieces	Steam sterilization	*P aeruginosa* [knee infections]	Tissue retained inside handpieces after cleaning
Dancer, et al[27]	Orthopedic and ophthalmologic surgical instruments	Steam: wet-packs and intact packs	*Bacillus* spp, coagulase-negative staphylococci [deep skin and soft tissue infections]	Instruments in intact wrapped packs contaminated
Sheitoyan-Pesant, et al[28]	Ultrasonic surgical aspirator used in craniotomy surgery	Steam	*Propionibacterium acnes*, *Staphylococcus capitis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus faecalis*, [brain abscess, epidural empyema, meningitis]†	Inadequate cleaning due to process change

NOTE. Data extracted from the references listed in column one.[24-28]
OPA, ortho-phthalaldehyde.
*Data shows that even extended overnight soaking does not eliminate bacteria if there is a lot of organic residuals within the needle guide (ie, build-up biofilm).
†Days to infection postcraniotomy ranged from 3-107 days.

sterilization with peracetic acid), there were viable bacteria detected when a nonenzymatic detergent was used (regardless of the type of channel cleaning brush or pull-through device used). The high level of surviving bacteria (>2 Log_{10}/cm^2) correlated with residual clumps of biofilm observed on scanning electron microscopy when bristle brushes were used for cleaning (Fig 2). Thaker et al[17] reported that using a borescope they were able to see that bristle brushes do not reliably contact the inner channel surface when passing through a coiled region of the endoscope. This likely explains why residual biofilm may remain in the endoscope channel despite following the MIFU for reprocessing. It also supports the Alfa et al[14] data showing residual clumps on scanning electron microscopy analysis despite 3 "up-down" passes of the bristle brush. These clumps of residual biofilm are very resistant to the liquid chemical HLD/sterilization process, and can result in contaminated endoscope channels after full MIFU reprocessing.[32-34]

In the United States, the FDA released a series of "supplemental measures" for sites offering duodenoscopy procedures.[35] These recommendations included performing HLD 2 consecutive times, performing HLD followed by liquid chemical sterilization, or performing HLD followed by ethylene oxide sterilization and culture. The recently published clinical studies have provided data to confirm that none of these supplemental recommendations was any more effective than a single round of HLD.[36-39] The recent survey by Thaker et al[40] indicated that these supplemental recommendations have been implemented to varying degrees across the United States. They reported that duodenoscope culture, HLD followed by ethylene oxide sterilization, HLD followed by liquid chemical sterilization, and HLD performed twice has been implemented in 53.5%, 12%, 34.5%, and 63.1% of sites respectively. Furthermore, the Centers for Disease Control and Prevention[41] position on these supplemental measures is, "Because there is currently insufficient evidence. . .these methods have not been included as essential elements of a reprocessing program." As such, it would seem that the FDA supplemental measures (other than culture) are no longer recognized as effective in eliminating contamination of duodenoscopes after patient-use and full reprocessing.

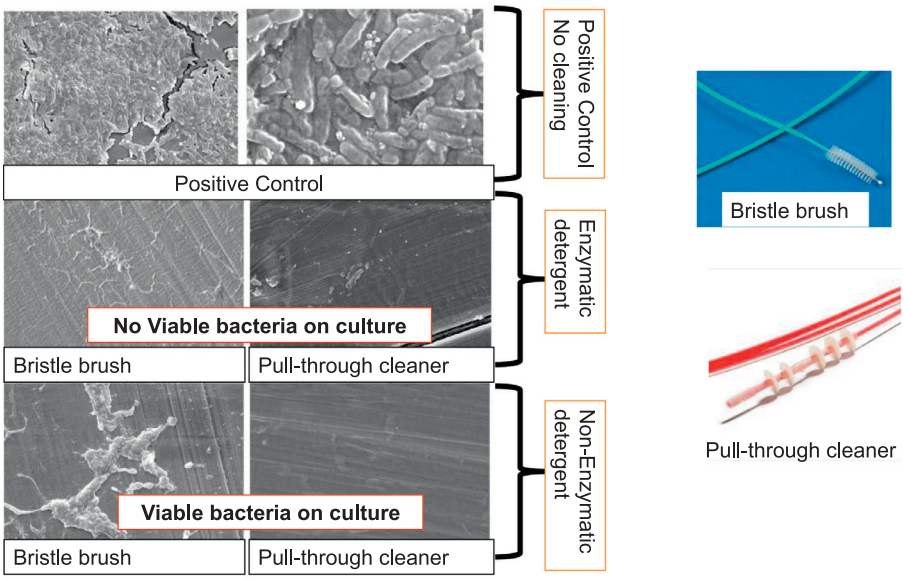

Fig 2. Manufacturer's instructions for use inadequate to eliminate traditional biofilm. The positive control shows the confluent build-up biofilm with channels. After 5 repeated rounds of manufacturer's instructions for use reprocessing with enzymatic detergent and bristle brush or pull-through cleaner, there were no detectable viable bacteria. When a nonenzymatic detergent was used with the same types of channel cleaners, viable bacteria were detected (highest level with bristle brush cleaning). Scanning electron microscopy image from Alfa (2018). Images of bristle brush and pull-through cleaner from manufacturer's websites. Used with permission from Elsevier.[18]

This issue of contaminated endoscopes continues to persist as the recent clinical study by Rauwers et al[42] used sample collection that involves neutralization and concentration of the sample (ie, optimal culture protocol) and reported that 15% of 150 duodenoscopes tested were contaminated after full reprocessing and storage (CFUs ranged from 1 to >100 CFU). The contamination detected included yeast and a range of *Enterobacteriaceae* as well as *P aeruginosa*, *Enterococcus* spp, and *S aureus*. They concluded that the current duodenoscope reprocessing and process control procedures are not adequate. The recent study by Johani et al[32] showing that fully reprocessed patient-used gastroscopes and colonoscopes contained up to 10^2 to 10^3 CFU/cm in gastroscope and colonoscope channels supports the inadequacy of current process control procedures.

The recent published data from Ofstead et al[16] has shown that there are debris that accumulates within the suction channel of flexible endoscopes, and that moisture can be found in 49%-95% of channels in a wide range of endoscopes after overnight storage, despite having had an alcohol flush and forced air drying in an automated endoscope reprocessor.[43] Analysis of the fluid within endoscope channels using Fourier transform infrared spectroscopy indicated that it contained simethicone residuals.[44] Simethicone is used to disperse bubbles on the mucosal surface during endoscopic procedures to enhance ability to detect polyps and other abnormalities. Simethicone consists of silica as well as silicon oil that are both insoluble in water or alcohol. Accumulation of simethicone residuals have been reported to occlude the air-water channel, and that removal was not possible using routine detergent solutions or alcohol flushes.[45] Recent analysis by Barakat et al[46] has shown that despite reprocessing, simethicone remains in the endoscope channel regardless of whether a low, medium, or high concentration was used and regardless of using 2 repeated automated endoscope reprocessor cycles. This same group subsequently documented that forced air drying using an automated unit was significantly better at reducing fluid residuals in endoscope working channels compared to manual forced air drying, but it could not eliminate all fluid residuals.[47] Olympus have recommended against the use of simethicone in gastrointestinal endoscopy because their current MIFU may not be able to eliminate it from the endoscope channels.[48] Olympus further instructed that if sites determine that the benefit of using simethicone outweighs the risk or potential reprocessing difficulties then they should consider administering it orally (eg, as part of bowel prep) or through the biopsy port rather than the water bottle and that it should be used in the lowest effective concentration.

In summary, the formation of biofilm or BBF within endoscope channels is related to the efficacy of cleaning, adequate drying for storage, and the impact of simethicone residuals on preventing adequate drying, thereby potentiating the risk of contamination of fully reprocessed endoscopes.

EVIDENCE OF THE ROLE OF DRY SURFACE BIOFILM IN TRANSMISSION OF MICROBES CAUSING HOSPITAL-ACQUIRED INFECTIONS

The presence of dry surface biofilm (Fig 3) formed on high-touch health care surfaces raises the question regarding its role in transmission of pathogens that ultimately cause hospital-acquired infections.[4] It also raises questions regarding the efficacy of removal and inactivation of microbes within dry surface biofilm by currently used cleaning-disinfection methods routinely used in health care. Ledwoch et al[21] investigated 3 different hospitals and reported that 95% of surfaces harbored dry surface biofilm. They also used DNA analysis to show that *Bacillus* spp and *Staphylococcus* spp were the predominant organisms found within dry surface biofilm from all 3 health care facilities. They found there was a high proportion of the *S aureus* detected in dry surface biofilm that were methicillin-resistant *S aureus*, but they indicated that the clinical significance of these findings is unknown. The study by Johani et al[32] demonstrated using an in vitro model that gloved hands touching the dry surface biofilm could transmit the organisms from this matrix to subsequently touched fomites.

Although more clinical studies are needed, the data to date indicate that dry surface biofilm likely plays a role as an environmental reservoir for hospital-acquired infections.

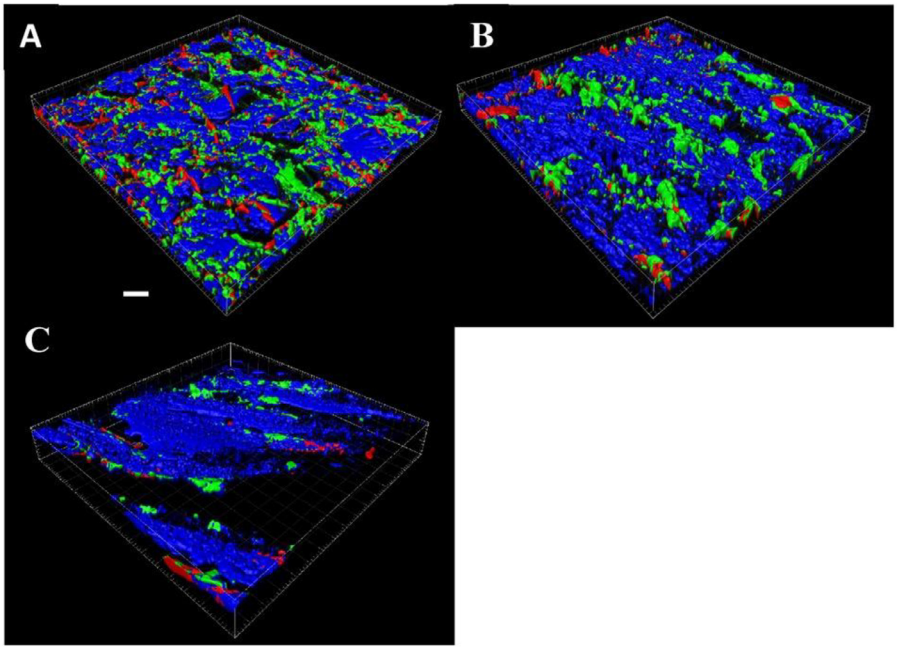

Fig 3. Dry surface biofilm. The distribution of protein (blue), bacterial DNA (red), and glycoconjugate (green) in dry surface biofilm. Dry surface biofilm at 12 days (A) and 18 days (B) compared to clinical dry surface biofilm on a glove box (C). Used with permission from Elsevier.[4]

CONCLUSIONS

The data reviewed from the published literature confirmed that there is evidence of infection arising from contaminated reusable surgical instruments and flexible endoscopes. These are exogenous infections arising from contaminants on reusable medical devices that are introduced into the patient during the clinical procedures (ie, laparoscopic surgery, rigid or flexible endoscopy). There is strong evidence that exogenous infection transmission has occurred for both flexible and rigid endoscopes due to biofilm or BBF formation within narrow channels. Although the evidence for exogenous infection transmission from sterilized surgical instruments is not as conclusive as for high level disinfected endoscopes, it still highlights the role that biofilm or retained secretions and tissue may play if cleaning is not effective. The role of dry surface biofilm in exogenous hospital-acquired infection transmission related to environmental surfaces requires further study. In all situations of biofilm, BBF, and dry surface biofilm there is a need for appropriate testing methods to more stringently assess the efficacy of manufacturer's reprocessing instructions and efficacy of environmental disinfection, as well as medical device high-level disinfection and sterilization methods.

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