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A new buildup biofilm model that mimics accumulation of material in flexible endoscope channels

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ABSTRACT

The objective of this study was to develop a new build up biofilm (BBF) model that was based on repeated exposure to test soil containing *Enterococcus faecalis* and *Pseudomonas aeruginosa* and repeated rounds of fixation to mimic the accumulation of patient material in endoscope channels during reprocessing. The new BBF model is a novel adaptation of the minimum biofilm effective concentration (MBEC) 96-well model where biofilm is formed on plastic pegs. The new MBEC-BBF model was developed over eight days and included four rounds of partial fixation using glutaraldehyde. There was 6.14 Log_{10} cfu/cm^2 of *E. faecalis* and 7.71 Log_{10} cfu/cm^2 of *P. aeruginosa* in the final BBF. Four detergents (two enzymatic and two non-enzymatic) were tested alone or in combination with orthophthalaldehyde, glutaraldehyde or accelerated hydrogen peroxide to determine if BBF could be either removed or the bacteria within the BBF killed. None of the detergents alone could remove the biofilm or reduce the bacterial level in the BBF as determined by viable count and scanning electron microscopy. The combination of detergents and disinfectants tested provided a 3 to 5 Log_{10} reduction in viable bacteria but no combination could provide the expected 6 Log_{10} reduction. Our data indicated that once formed BBF was extremely difficult to eliminate. Future research using the BBF model may help develop new cleaning and disinfection methods that can prevent or eliminate BBF within endoscope channels.

1. Introduction

Flexible endoscopes are used for therapeutic procedures as well as diagnostic purposes including; visualization and sample collection (PHAC, 2010; AAMI, 2015). Endoscopic clinical techniques are continuing to expand and the number of endoscopic procedures per year has greatly increased (Kovaleva and Buss, 2012). Overall, the rate of infections attributed to transfer of infectious agents from contaminated endoscopes is low but recent publications have demonstrated that these rates may be higher than previously thought as there is poor documentation and reporting of such events (Ofstead et al., 2010). In the past few years there has been world-wide documentation of outbreaks associated with antibiotic-resistant organisms due to contaminated flexible endoscopes (Aumeran et al., 2012; Carbonne et al., 2010), with recent USA outbreaks attributed to transmission of carbapenem-resistant Enterobacteriaceae (CRE) arising from endoscopic retrograde cholangiopancreatography (ERCP) procedures (Wonderf et al., 2015; Ubhayawardana et al., 2013; Epstein et al., 2014). It has been difficult to understand the documented survival of microorganisms in flexible endoscopes despite repeated rounds of reprocessing using manufacturer validated cleaning and disinfection methods which should preclude biofilm development.

Accurate reprocessing of flexible endoscopes includes meticulous cleaning, followed by high level disinfection (HLD) or sterilization with further drying before storage (Pineau et al., 2008). Most contemporary flexible endoscopes cannot be heat-sterilized and have narrow internal channels that are difficult to clean and disinfect (Kovaleva et al., 2010; Rutala and Weber, 2013; SGNA, 2013; Fushimi et al., 2013). Organic material binds and inactivates many chemical disinfectants and some disinfectants such as glutaraldehyde and alcohol fix protein, thereby creating a physical barrier of denatured protein that can shield microorganisms from subsequent HLD (GESA, 2011).

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Biofilm development is an important issue in environmental niches, such as, water pipes, and treatment plants, but it is also an important issue in medical implants and a variety of other infectious processes (Marion et al., 2006; Costerton, 1999; Costerton et al., 1999; Dolan and Costerton, 2001; Olson et al., 2002; Zhong et al., 2009; Fang et al., 2010; Kovaleva et al., 2010; Bridier et al., 2012; Roberts, 2013; Gökçen et al., 2014; Ren et al., 2013). In flexible endoscopes, accumulation of organic matter and microorganisms is likely the result of multiple cleaning and disinfection cycles over the life of the instruments (Alfa and Howie, 2009a, 2009b; Pajkos et al., 2004). The cyclic buildup of organisms and organic material on devices that have been reprocessed multiple times is different from the development of traditional biofilm that forms in a constantly hydrated environment (Zhong et al., 2009). Many years ago Pajkos et al. (2004) warned that even small breakdowns in the reprocessing protocol could result in a buildup of material within the endoscope and encourage biofilm development. Because of this narrow "margin of safety" and the recognition of build-up biofilm within endoscope channels, research efforts have focussed on developing methods to evaluate effective ways to eliminate biofilm that may form in flexible endscope channels.

The minimum biofilm eradication concentration (MBEC) model was developed by Ceri et al. (1999) to facilitate testing antibiotic susceptibility of bacteria in biofilm as this was thought to be more representative of therapeutic outcomes in clinical infections where the infecting organisms had formed biofilm. The MBEC enables the formation of biofilm on pegs in a 96-well format and thus allows testing of a wide range of different agents or antibiotics using a very small "footprint" (Ceri et al., 1999; Alfa and Howie, 2009a, 2009b; LaPlante and Mermel, 2009; Kovaleva et al., 2010; Bridier et al., 2011; Coraça-Hubér et al., 2012; Takei et al., 2013; Abbanat et al., 2014; Parker et al., 2014; Laverty et al., 2015). Recently, the MBEC method for testing antibiotic susceptibility of organisms within biofilm has been validated (Parker et al., 2014).

Alfa and Howie (2009a, 2009b) developed a buildup biofilm (BBF) adaptation of the MBEC biofilm model where they used repeated cycles of exposure to high levels of organic material and microorganisms, followed by HLD, rinsing and drying that more closely replicated the repeated cycles used for endoscope reprocessing. This was a useful BBF model but it was time-consuming as it was based on 30 days of repeated cycles. Although Costa Luciano et al. (2016) reported on the impact of detergents and high level disinfectants using the hydroxyapatite MBEC model this evaluation was for traditional biofilm (i.e. no repeated rounds of HLD). There is still need for a model that represents build-up that accumulates after repeated cycles of endoscope reprocessing but that takes a short time to form.

The primary objective of our study was to modify the Alfa and Howie (2009a, 2009b) MBEC model to create a new buildup biofilm model (MBEC-BBF) that takes only a few days to create (similar to the Costa Luciano et al. (2016) model) but that still mimics the accumulation of material in flexible endoscope channels after repeated rounds of reprocessing. Our second objective was to use this MBEC-BBF model to investigate the effectiveness of various detergents and HLDs to remove or inactivate the BBF-associated microorganisms.

2. Methods

2.1. MBEC build-up biofilm (BBF) model

2.1.1. Formation of BBF

Pseudomonas aeruginosa (ATCC 15442) and *Enterococcus faecalis* (ATCC 29212) were used for buildup biofilm formation in MBEC™ Biofilm Inoculators (Innovotech®, Edmonton, Alberta, Canada). These bacteria were selected as they represent organisms that can cause biofilm-associated infections in humans as well as contamination of flexible endoscopes.

The bacteria were stored as stock cultures in skim milk at −20 °C. Prior to experiments, the bacteria were subcultured twice on blood agar (BA) consisting of Tryptic Soy Agar (TSA) with 5% sheep blood (Oxoid, Toronto, Canada) incubated at 35 °C aerobically for 24 h. The inoculum used to create BBF consisted of Artificial Test Soil (ATS) (Artificial Test Soil: US patent 6,447,990) containing approximately 10^8 cfu/mL of both *P. aeruginosa* and *E. faecalis*.

Buildup biofilm was formed in the MBEC model over an eight-day time-frame and included four rounds of sub-optimal HLD. Briefly: each well of the MBEC tray was inoculated with 150 μL/well of ATS with microorganisms and biofilm allowed to form at RT for 2 days on a rocking table (8 full "back and forth" rocks per minute). On days 3, 4 and 5 the pegs were then rinsed (200 μL/well of sterile tap water three times at 30 s), disinfected (1:50 dilution of glutaraldehyde – see Table 1) using 175 μL/well for 2 min (i.e. sub-optimal HLD because the concentration and time were less than the manufacturer's recommendations), and rinsed (200 μL/well of sterile tap water five times at 60 s). The MBEC pegs were then exposed to ATS with microorganisms for 2 h at RT and then rinsed (200 μL/well of sterile tap water three times at 30 s) The MBEC pegs were then immersed in 150 μL of sterile tap water overnight at RT. On day 5 after the treatment previously described, the pegs were exposed to 150 μL/well of ATS with microorganisms and placed on a rocking table at room temperature (RT) for 2 days (over the weekend). On day 8 (Monday) the MBEC pegs were then rinsed, exposed to 1:50 dilution of glutaraldehyde and rinsed as previously described. The final BBF was now fully developed and used for subsequent testing.

Table 1
Description of detergents and disinfectants evaluated.

Detergent name	Type of detergent	Company	Use-DILUITION	Exposure time and temperature
D1	Prolystica Enzymatic 2× concentrate enzymatic	Steris - Mississauga, ON, Canada	4 mL/1 L	5 min 30 °C
D2	Prolystica Neutral 2× concentrate non-enzymatic	Steris -Mississauga, ON, Canada	4 mL/1 L	5 min 30 °C
D3	Neodisher® alkaline non-enzymatic	Dr. Weigert - Hamburg, Germany	30 mL/1 L	10 min 30 °C
D4	Endozime® Bio-Clean – enzymatic detergent	Ruhof - Mineola, New York, USA	8 mL/1 L	2 min 30 °C
Disinfectant name	**Type of disinfectant**	**Company**	**Re-use (days)**	**Time of exposure and temperature**
GLUT	Metricide™ glutaraldehyde 2.6%	Metrex – Sybron, Oakville, ON	14 days	20 min Room temperature
OPA	Ortho-phthalaldehyde 0.55%	Cidex® - Gargrave, N. Yorkshire, UK	14 days	10 min 20 °C
AHP	Accelerated hydrogen peroxide 2%	Revital-Ox Resert®, Mississaga, ON	14 days	5 min Room temperature