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Use of Delrin Plastic in a Modified CDC Biofilm Reactor

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ABSTRACT

In this study, a modified CDC biofilm reactor was designed and the components of the reactor were primarily machined using Delrin plastic. Initially, biofilms grew well using the reactor unit. However, after approximately five runs of the reactor, bacterial growth was inhibited and biofilms no longer formed. To troubleshoot the problem, bacteria were grown in the presence of broth alone or in the presence of broth and Delrin plastic. It was determined that the reactor components containing Delrin plastic prevented growth of bacteria and the formation of biofilm. It appeared that the repeated exposure to autoclave temperature and pressure caused the Delrin plastic to decompose and leach formaldehyde into the broth, which inhibited bacterial growth. Based on these results, we propose that Delrin plastic should not be used in bacterial growth devices.

Key words: Delrin, biofilm, reactor, polymer, components, plastic

INTRODUCTION

Biofilm reactors have become essential tools in Biofilm-related research and have helped to advance our knowledge of Biofilm formation, morphology, the role of Biofilm in medicine and more (Buckingham-Meyer *et al*., 2007; Mohle *et al*., 2007; Goeres *et al*., 2009; Gilmore *et al*., 2010; Lipp *et al*., 2010; Williams and Bloebaum, 2010). Primarily, these reactors have been composed of polymeric materials and metal components. Various polymeric materials provide significant advantages in that they can be machined easily, are relatively cheap, can be autoclaved numerous times and in many instances are chemically inert.

In addition, biofilm reactors can be customized to meet specific needs of experimentation. More specifically, we modified the design of the CDC biofilm reactor (Goeres *et al*., 2005) (Biosurface Technologies, Bozeman, MT) to meet the needs of an *in vivo* model wherein the coupons from the CDC biofilm reactor (Fig. 1a) would have been too large and ultimately ineffective. The modified design of the reactor allowed for membranes made of polyetheretherketone (PEEK) to be placed into a 1 L glass vessel as they were secured in guillotine-like holders that were inserted into a slot of the reactor cap (Fig. 1b). As such, the PEEK membranes were held in the base of the reactor and exposed to shear forces of a swirling vane to promote biofilm production (Stoodley *et al*., 1999, 2001). In a future study, after biofilms have been grown on the surface of the PEEK membranes, they will be surgically placed into an animal to model biofilm-related infection. PEEK polymer membranes were used in this study because of the known biocompatibility of PEEK (Rivard *et al*., 2002), the membranes were porous and they provided a large surface area for biofilm formation.

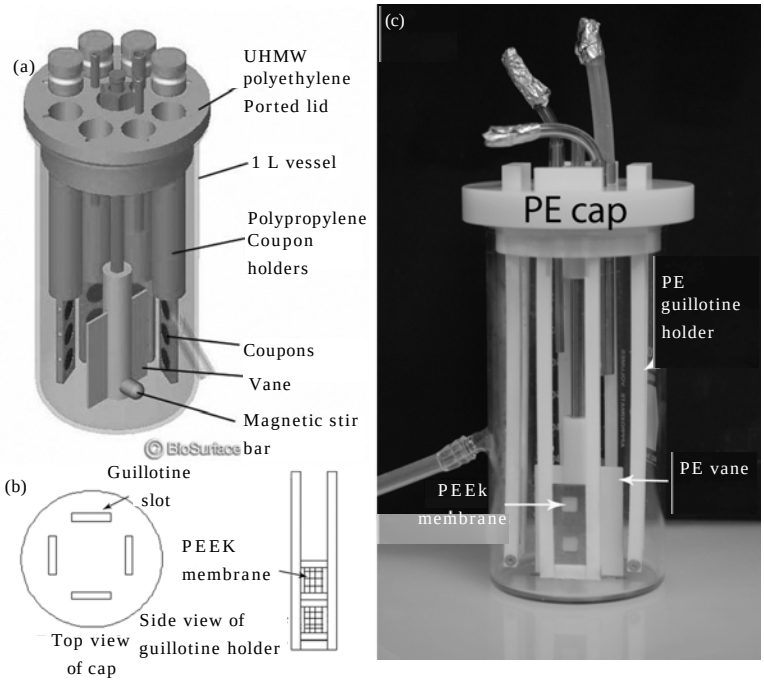

Fig. 1: (a) Schematic drawing of the original CDC biofilm reactor. (b) Schematic drawing of the cap of the modified CDC biofilm reactor and a guillotine-like holder. The holder was designed to hold PEEK membranes in the base of the reactor as they were compressed between two stainless steel plates that had a 0.64 cm^2 opening. The reactor was designed such that biofilms would form on the surface of the PEEK membranes. These membranes will be surgically inserted into an animal to model biofilm-related infection in a future study. (c) Photograph of the modified CDC biofilm reactor showing the final design of the unit. UHMWPE components are labeled with PE to show where the plastic components resided within the reactor unit. The PEEK membranes are shown being held between two stainless steel plates

The goal of this study was to determine if Delrin plastic (also known as polyoxymethylene, acetal or polyformaldehyde) was a suitable material to use for machining of the guillotine-like holders and cap of the modified reactor. As such, bacteria were grown in a broth solution within the reactor, biofilm formation on the PEEK membranes was observed and the number of bacteria on each membrane quantified.

MATERIALS AND METHODS

This study took place between January 2010 and October 2010 and was performed at the George E. Wahlen Department of Veterans Affairs Medical Center in Salt Lake City, UT. A CDC biofilm reactor was purchased from Biosurface Technologies. Delrin plastic was purchased from a local machine shop to make modified reactor components. PEEK membrane was purchased through www.smallparts.com (catalog #B000FMW8EO).

The machinist with whom we consulted had previous experience working only with Delrin plastic. He suggested that we make the guillotine-like holders out of Delrin since it was easy to machine, cheap and could be autoclaved.

The guillotine-like holders were machined to a length of 20 cm with a groove of 3 mm into which stainless steel plates could be inserted (Fig. 1b). Stainless steel screws were used to hold the guillotine-like holders together. A cap was also machined having four slots into which the holders could be inserted (Fig. 1b).

Following machining of the Delrin plastic parts for the modified reactor, the reactor was assembled such that eight PEEK membranes were lowered into the reactor as they were held in the guillotine-like holders (four holders held two membranes each for a total of eight PEEK membranes within the modified reactor unit). As such, the membranes could be exposed to shear forces of a swirling paddle in the base of the reactor (Fig. 1b). After rinsing the reactor thoroughly with distilled water, it was autoclaved prior to use.

To grow the biofilms, the reactor was filled with 500 mL of brain heart infusion broth (modified) and aseptically inoculated with ~1.5×10^8 cells of methicillin-resistant *Staphylococcus aureus*. The paddle was swirled at 130 rpm and the unit was incubated on a hot plate set at 34°C for 24 h. A flow of 10% brain heart infusion broth (modified) was flowed through the reactor for an additional 24 h.

RESULTS

For approximately five runs of the modified reactor, biofilms developed on the surface of the PEEK membranes with an average of ~3×10^9 cells per membrane. However, as we used the modified reactors over approximately five runs, we began to notice that there was more than a 3 log reduction in the number of cells within our biofilms. After another three to five runs of the reactor, bacterial growth within the reactor was inhibited completely, with no growth in the broth or on the membranes of the reactor system.

To troubleshoot the problem, we grew bacteria in 500 mL of broth alone within the biofilm reactor. The ability of bacteria to grow in the broth while in the presence of PEEK, stainless steel or Delrin plastic was also tested. More specifically, bacteria were grown in 10 mL of broth that contained a 1 cm^2 section of PEEK membrane material, in 10 mL of broth that contained 1 cm^2 of PEEK membrane and a stainless steel plate and in 10 mL of broth and a stainless steel plate alone. Finally, we grew bacteria in the modified reactor that had only the Delrin plastic reactor components in place. Notably, each of the broth samples was inoculated with ~1.5×10^8 bacterial cells.

Results indicated that bacterial growth was overtly evident in all of the broth samples with the exception of the broth that had been exposed to the Delrin plastic. These results further indicated that the Delrin plastic contained or eluted some toxic compound that had been inhibiting bacterial growth and thus biofilm formation.

Based on these findings, we made an updated version of the modified biofilm reactor using Ultra High Molecular Weight Polyethylene (UHMWPE) in place of those parts that were made of Delrin plastic (Fig. 1c). Following more than 10 runs of this autoclaved reactor, we have had no problems with bacterial growth or biofilm formation on the surface of the PEEK membranes.

DISCUSSION

Although there is a paucity of data in the literature with respect to the use of Delrin plastic in bacterial growth devices, LaIuppa *et al.* (1997) showed that its use in an *ex vivo* cell expansion system had an adverse effect on the proliferation of hematopoietic progenitor cells

(LaIuppa *et al.*, 1997). Use of Delrin plastic in biomaterials should be limited as it may release toxic formaldehyde into human patients (Kusy and Whitley, 2005). Ohlin and Linder suggested the same (Ohlin and Linder, 1993).

However, in contrast to these reports, Penick *et al.* (2005) were unable to confirm the observation of LaIuppa *et al.* (1997). as they found no inhibition of cellular proliferation in an *in vitro* bioreactor system that contained components made of polyoxymethylene that was autoclaved even up to 20 times, yet they recognized that they used a different cell type and material handling may have contributed to the difference (Penick *et al.*, 2005). Further, in direct response to the suggestion of Kusy and Whitley (2005), Zilberman (2005), argued that there have been no reports of complications due to toxicity with the use of Delrin plastic in biomaterials (particularly dental crowns and bridges), suggesting that it is safe to use clinically (Zilberman, 2005). Clinical data have corroborated those suggestions (Brown and Mayor, 1978; MacAfee and Quinn, 1992).

The results of this investigation had a similar outcome as that of LaIuppa *et al.* (1997). More specifically, results strongly suggested that when Delrin plastic that had been autoclaved numerous times was exposed to the broth in which bacteria were inoculated, bacterial growth was inhibited. From the literature cited above and the material safety data sheet for Delrin plastic, wherein it is stated that if Delrin is exposed to high pressure and high temperature systems numerous times, which in our case was autoclaving, it can begin to decompose, there is strong evidence to suggest that the Delrin plastic in our system began to decompose and released formaldehyde into the broth, ultimately preventing bacterial growth in our modified biofilm reactor. Furthermore, there has been no inhibition of bacterial growth within the reactor unit after the Delrin plastic components were replaced with UHMWPE components.

The difference in cellular viability and proliferation that have been seen by investigators in *in vitro* and *ex vivo* systems may be due to (1) differences in surface area of Delrin that comes in contact with liquid, (2) the machining process of various components and/or (3) the manufacturing process that the Delrin plastic underwent prior to purchase. These possibilities remain to be determined, but may help explain the disparity in results.

In conclusion, the goal of this study was achieved. We determined that in this specific application, i.e., for our modified biofilm reactor system, Delrin plastic was not a suitable material to support growth of bacteria in broth and prevented the formation of biofilm on the surface of PEEK membranes. It is our suggestion that since there is the possibility that Delrin plastic may prevent bacterial growth, that this material not be used to manufacture bacterial growth devices, but that alternate materials such as UHMWPE be used.

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