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Wylie, M., Bell, S., Nockemann, P., Bell, R., & McCoy, C. (2020). Phosphonium ionic liquid-infused poly(vinyl chloride) surfaces possessing potent antifouling properties. ACS Omega, 5(14), 7771-7781. https://doi.org/10.1021/acsomega.9b03528

Published in:
ACS Omega

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

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Phosphonium Ionic Liquid-Infused Poly(vinyl chloride) Surfaces Possessing Potent Antifouling Properties

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ABSTRACT: Microbial fouling is a costly issue, which impacts a wide range of industries, such as healthcare, food processing, and construction industries, and improved strategies to reduce the impact of fouling are urgently required. Slippery liquid-infused porous surfaces (SLIPSs) have recently been developed as a bioinspired approach to prevent antifouling. Here, we report the development of slippery, superhydrophobic surfaces by infusing roughened poly(vinyl chloride) (PVC) substrates with phosphonium ionic liquids (PILs). These surfaces were capable of reducing viable bacterial adherence by >6 log10 cfu mL−1 after 24 h under static conditions relative to control PVC. Furthermore, we report the potential of a series of asymmetric quaternary alkyl PILs with varying alkyl chain lengths (C4−C18) and counteranions to act as antimicrobial agents against both Gram +ve and Gram −ve bacteria and illustrate their potential as antimicrobial alternatives to traditional fluorinated lubricants commonly used in the synthesis of SLIPSs.

1. INTRODUCTION

Poly(vinyl chloride) (PVC) is one of the most commonly used polymers worldwide, with applications in medical device manufacture, construction, food, and electronic industries. This is due to its many advantageous properties, such as flexibility, transparency, durability, and inexpensive production. However, PVC can become colonized with microorganisms when used in some applications, such as medical devices or food processing. For example, indwelling urinary catheters and endotracheal tubes are commonly manufactured from PVC and are often required to remain in place for extended periods of time, and consequently, incidences of catheter-associated urinary tract infections and ventilator-associated pneumonia are considerable, accounting for 43 and 18.5% of all hospital-acquired urinary tract and respiratory tract infections, respectively.

To combat against bacterial colonization, several preventative methods have been employed to reduce the risk of colonization, which have included the active release of antimicrobials from device surfaces and surface modification to increase inherent antimicrobial or antiadherent properties. Unfortunately, many of these methods possess disadvantages, such as finite activity, burst release profiles, and a risk of bacterial exposure to subinhibitory concentrations of active agents, which could promote bacterial resistance.

Superhydrophobic surfaces have been widely known for their antiwetting, self-cleaning properties and are characterized by high aqueous contact angles (>150°) caused by the trapping of pockets of air between the micro- or nanoscale grooves decorating a surface with low surface energy. These properties would be extremely advantageous for antibacterial applications, potentially inhibiting bacterial surface adherence. Unfortunately, several reports have shown that these surfaces fail to prevent colonization, either through masking of the low surface energy surface with a conditioning film of proteins or dead bacteria, which is often viewed as the first step in biofilm formation, or through displacement of the trapped air pockets, allowing subsequent attachment sites for bacteria. However, the recent development of slippery liquid-infused porous surfaces (SLIPSs) by Wong et al. (2011), which rely on the infusion of a liquid into a porous surface with matching surface energy, has shown early promise in preventing biofouling by marine organisms and bacteria, such as Staphylococcus aureus and Pseudomonas aeruginosa, which are commonly associated with medical device infections. In addition, several more recent studies have demonstrated the ability of SLIPSs to resist infection and prevent thrombus formation, which is a common complication of prolonged device placement. As such, SLIPSs represent a bioinspired approach to reducing antifouling events, and the current manuscript describes the development of several phosphonium-based PILs that can be used to fabricate PVC surfaces with antifouling properties.

Received: October 22, 2019
Accepted: March 6, 2020
Published: April 1, 2020
formation in blood-contacting applications, further enhancing the potential of SLIPSs to improve medical device performance.14–16

So far, the majority of SLIPSs have been produced using fluorinated oil lubricants which are volatile and prone to evaporation if exposed to high temperatures or vacuum conditions, while those that can withstand evaporation at high temperatures are usually highly viscous at room temperature. To combat this problem, an alternative strategy has been the use of ionic liquids as a lubricating liquid.17,18 Ionic liquids provide an attractive alternative owing to their low volatility, thermal stability, and their customizable nature. Miranda et al.19 showed that the fluorinated ionic liquid, 1-ethyl-3-methyl-imidazolium bis(trifluoromethylsulfonyl)imide ([C6Mim][Tf2N]), provided similar omniphobic properties to SLIPSs infused with perfluoropolyether lubricants while demonstrating much improved stability at high temperatures under vacuum conditions and was resistant to damage from UV oxidation. Additionally, ionic liquids containing imidazolium, pyridinium, ammonium, and phosphonium cationic constituents have been investigated by Pernak et al. with many possessing potent antimicrobial activity.20

Thus, the introduction of an ionic liquid as the lubricant layer of a SLIPS platform can not only improve the stability of the slippery surface, but with careful selection of the ionic liquid, antibacterial properties can also be introduced to produce dual-functionalized antifouling surfaces. This could provide value in both preventing the adherence of bacteria on a polymer surface and eradicating approaching bacteria which could otherwise be transported with surface flow to non-SLIPS-treated sites, for example, if applied to sewage or drainage pipes.

This paper reports on the manufacture and antimicrobial assessment of SLIPS infused with phosphonium ionic liquids (PILs) aimed at producing an antifouling surface, which could show the potential to prevent biofouling of polymer surfaces. We report the manufacture of SLIPSs using a PVC substrate. A silver colloid solution was used to deposit silver nanoparticles on the PVC surface to provide a roughened substrate for lubricant infusion while also providing an underlying antifouling surface if lubricant infusion fails. PILs were synthesized and used to lubricant the porous PVC surface because of their water immiscible properties and to provide an “active” antimicrobial surface to eradicate approaching bacteria. The antimicrobial efficacy and hemotoxicity of the infused ionic liquids are reported, and the stability and antifouling properties of the synthesized PVC-SLIPSs are assessed.

Table 1. MIC and MBC for PILs against S. aureus and P. aeruginosaa

| Sample | S. aureus | P. aeruginosa |
|--------|-----------|--------------|
|        | MIC (μg mL−1) | MBC (μg mL−1) | MIC (μg mL−1) | MBC (μg mL−1) |
| trityl(tetradecyl)phosphonium docusate ([P20014][AOT]) | 500 | >1000 | >1000 |
| triptyctyl(tetradecyl)phosphonium docusate ([P20014][AOT]) | 3.91 | 7.81 | 250 | 1000 |
| triptyctadecyl(methyl)phosphonium docusate ([P3216S][AOT]) | 250 | 500 | >1000 | >1000 |
| tripentylyl(hydroxyethyl)phosphonium docusate ([P32081][AOT]) | 62.5 | 250 | >1000 | >1000 |
| triptyctadecyl(phosphonium bis(trifluoromethylsulfonyl))imid | >1000 | >1000 | >1000 |

*Results labeled >1000 μg mL−1 indicate results beyond the tested concentration range.

2. RESULTS AND DISCUSSION

In this study, several novel antimicrobial PILs were synthesized and used to provide a secondary antimicrobial mechanism to SLIPSs, which could make them more effective in preventing bacterial colonization through a dual antiadherent/antimicrobial approach. This approach has not yet been investigated and could provide further value to the already promising application of SLIPS technology to prevent biofouling. The antimicrobial activity of created SLIPS was determined, and the stability of SLIPS over time and under repeated microbial challenge was compared to that of silver-coated and control polymers. The enhanced antiadherent nature of these SLIPSs provides a method to significantly reduce microbial contamination compared to that of control polymers.

2.1. In Vitro Antibacterial Activity and Hemotoxicity of PILs. Table 1 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of each PIL against S. aureus and P. aeruginosa. All PILs, except [P666 14][Tf2N], inhibited the growth of S. aureus within the concentration range examined; however, significant differences were observed in the magnitude of activity. [P888 14][AOT] displayed an MIC which was 16–128-fold more potent than the other [AOT]-containing ionic liquids. [P666 14][Tf2N] displayed no antibacterial activity within the tested concentration range.

The antibacterial activity of the PILs was poorer against the Gram-negative bacteria, P. aeruginosa, with all ionic liquids, except [P888 14][AOT], displaying no growth inhibition, while the MIC and MBC of [P888 14][AOT] rose by 64- and 128-fold, respectively.

Research into the antimicrobial capabilities of PILs is scarce, but a link to alkyl chain hydrophobicity and antimicrobial efficacy for these liquids has been reported previously.23 This study extends this research by demonstrating that the addition of a polar side-chain moiety can affect the antibacterial activity while further supporting the importance of the cation alkyl chain length and anion identity, particularly with Gram-positive bacteria. The ionic liquids were mostly ineffective against the Gram-negative bacteria P. aeruginosa, with only [P888 14][AOT] displaying an MIC/MBC value within the examined concentration range. This can be attributed to the differences in the cell membranes of these bacteria with the long alkyl chains more able to penetrate the peptidoglycan cell wall of Gram-positive bacteria, with an alkyl chain length of 8–12 carbons optimal for bactericidal effects as has been previously reported for both quaternary ammonium salts and antimicrobial ionic liquids.24–26 Cieniecka-Rosklikiewicz et al. reported that an alkyl chain length of 8 carbons achieved effective in preventing microbial contamination compared to that of control polymers.
potential cytotoxicity as this will ultimately determine their applicability to medical or environmental applications. The hemolytic activity of PILs was evaluated against equine erythrocytes (Figure 1). The results indicate that 

\[ [P_{666}^{14}][AOT] \text{ and } [P_{181818}^{14}][AOT] \text{ are not hemolytic at concentrations up to } 25 \mu g \text{ mL}^{-1}. \] However, \([P_{888}^{14}][AOT] \text{ and } [P_{444}^{2030}][AOT] \] caused 102.3 and 99.39% hemolysis, respectively, at 25 \(\mu \)g mL\(^{-1}\) but caused <10% hemolysis at 5 \(\mu \)g mL\(^{-1}\), which was not surprising given that they were the most potent ionic liquids against \textit{S. aureus} viability, likely through a similar membrane disruption mechanism. The lack of hemolytic activity by \([P_{666}^{14}][AOT] \text{ and } [P_{181818}^{14}][AOT] \text{ compared to that by the other ionic liquids could indicate that both alkyl chain length and chain functionality play important roles in their cytotoxicity. These findings agree with previous hemolytic studies of PILs, which have reported varied hemotoxicity with changes in anion pairing and alkyl chain length.}^{26} \text{ The increased hemotoxicity of } [P_{888}^{14}][AOT] \text{ and } [P_{444}^{2030}][AOT], \text{ which exhibited the most antibacterial activity of the examined PILs, suggests that they may not be suitable for biomedical applications, although further bio-compatibility studies will be required to confirm this.} 

## 2.2. Aqueous Wettability and Stability of SLIPSs

The ability of PVC surface modification by silver coating to produce a rough substrate was confirmed by scanning electron microscopy (SEM) imaging (Figure S1). Silver-PVC presented a rough, porous surface covered in silver nanoparticles. Addition of ionic liquids to this surface caused a loss of visible surface architecture, indicating a smooth surface similar to unmodified PVC. \([P_{181818}^{14}][AOT] \text{ produced pine needle-like structures across the sample surface, and this was attributed to its waxy, semisolid nature at room temperature.}^{26} \text{ Fundamental to self-cleaning surfaces is the presence of extreme surface wettability. The infusion of PILs to the silver surface caused significant shifts toward superhydrophilic wetting (Table 2) with \([P_{444}^{2030}][AOT], [P_{666}^{14}][AOT], \text{ and } [P_{888}^{15}][AOT] \) displaying complete wetting phenomenon of 0° whereby the water droplet rapidly spreads across the surface upon contact, indicating that an extremely hydrophilic surface had been created in each case. Conversely, \([P_{666}^{14}][Tf_{2}N]^{-} \text{ reduced the hydrophilicity of silver-PVC substrates, although the surface still maintained a hydrophilic character.} \) 

The results for the \([AOT]^{-}\)-containing ionic liquids were unexpected considering the hydrophobic nature of both the phosphonium cations and [AOT]. However, it is important to consider that [AOT] is a known anionic surfactant, which lowers surface tension between water and a hydrophobic surface because of the presence of amphiphilic components within its structure. Therefore, it is possible that upon contact with the [AOT] ionic liquids, [AOT] molecules quickly arrange themselves at the water–lubricant interface, projecting the more hydrophilic regions of [AOT] outward toward the water droplet to facilitate hydrogen bonding, allowing rapid spreading across the SLIPS, while the hydrophobic phosphonium cation portion of the ion pair may provide a hydrophobic barrier to further water encroachment toward the PVC surface, as presented in Figure 2. Venkatesan et al. have recently reported on the molecular interactions of PILs at the ionic liquid/aqueous interface using molecular dynamic modeling.\(^{27} \text{ Venkatesan demonstrated that initial interaction begins with a simultaneous diffusion of anions into the aqueous phase and water molecules into the ionic liquid, which quickly reaches an equilibrium, resulting in the formation of multiple planar interfaces, and causes the ionic liquid phase to become positively charged. This shift in charge leads to reorientation of the phosphonium cations toward the ionic liquid/aqueous interface, as presented in Figure 2, while the hydrophobic alkyl chains of the tetraalkylyphosphonium cation prevent its diffusion into the aqueous layer.} \text{ Srinivasa Rao et al. have also reported several imidazolium-AOT ionic liquids to be water miscible.}^{28} \text{ [P}_{181818}^{14}][AOT]-SLIPS possessed the highest water contact angle of the docusate ionic liquid surfaces with an angle of 24.49°, which may be caused by the considerable hydrophobic nature of the octadecyl chains or through the semisolid/waxy nature of the surface of \([P_{181818}^{14}][AOT]-SLIPS, \text{ which produced a nonuniform needle-like surface roughness (Figure 2). Previous research of self-cleaning surfaces has shown that surfaces exhibiting extreme contact angles of either superhydrophobic (＞150°) or superhydrophilic (＜5°) nature can be successful at preventing bacterial attachment.}^{29} \text{ Within the developing field of SLIPS, the majority of initial research was based on infused fluorinated oils, resulting in most antibacterial SLIPS exhibiting hydrophobic properties.}^{12,17} \text{ However, more recently, several hydrophobic SLIPSs have been reported, although they have been designed toward water harvesting and anti-icing applications rather than bacterial anti fouling.}^{30,32} \text{ Changing the counterion from [AOT] \textsuperscript{−} to [Tf}_{2}N\textsuperscript{−} caused a significant increase in contact angle compared to silver-PVC. This increase can be attributed to the high fluorine content of the [Tf}_{2}N\textsuperscript{−} anion making the ionic liquid more hydrophobic relative to the [AOT] \textsuperscript{−} ionic liquids, and this result follows the same trend as surface wettability results for hydrophobic dialkylimidazolium ionic liquids coupled with [Tf}_{2}N\textsuperscript{−} and }

### Table 2. Static Contact Angle Measurements of Modified PVC Samples and PVC Control

| sample                  | contact angle (deg) |
|-------------------------|---------------------|
| PVC                     | 75.34 ± 0.37        |
| silver-PVC              | 49.68 ± 5.36        |
| \([P_{666}^{14}][AOT]-silver-SLIPS\) | 0 ± 0               |
| \([P_{888}^{14}][AOT]-silver-SLIPS\) | 0 ± 0               |
| \([P_{181818}^{14}][AOT]-silver-SLIPS\) | 24.49 ± 2.34        |
| \([P_{444}^{2030}][Tf_{2}N]-silver-SLIPS\) | 0 ± 0               |
| \([P_{666}^{14}][Tf_{2}N]-silver-SLIPS\) | 70.17 ± 4.79        |

Contact angle measurements represent mean ± SD (n = 5).

![Figure 1. Hemolytic activity of PILs against equine erythrocytes. Each value is the mean of four replicates and error bars ± SD. PBS and 16 mM cetylpyridinium bromide were used as a negative (0% hemolysis) and positive (100%) control, respectively.](https://pubs.acs.org/doi/10.1021/acsomega.9b03528)
coated on various surfaces with water contact angles ranging between 56 and 75°.

A recent study on the potential of SLIPS in preventing marine biofouling has shown promising results; however, its success was limited because of the loss of lubricants during immersion in artificial seawater over a 7 day period. Recently, Shillingford et al. reported that non-fluorinated surfaces suffer from the displacement of their fluorinated oil lubricant layer, leading to increased contact angle hysteresis compared to surfaces that are fluorosilanized, which may compromise their antifouling properties.

The release of ionic liquids from SLIPSs in this study was examined in phosphate-buffered saline (PBS) over a 14 day period and is shown in Figure 3.

The release of ionic liquids from SLIPSs in this study was examined in phosphate-buffered saline (PBS) over a 14 day period and is shown in Figure 3.

Table 3. Zone of Inhibition Measurements for SLIPS Samples, after 24 h Incubation, against S. aureus and P. aeruginosa (n = 3)

| sample            | S. aureus growth under sample surface? | zone of inhibition (mm) | P. aeruginosa growth under sample surface? | zone of inhibition (mm) |
|-------------------|---------------------------------------|-------------------------|------------------------------------------|-------------------------|
| PVC               | yes                                   | 0.0 ± 0.0               | yes                                      | 0.0 ± 0.0               |
| silver-PVC        | no                                    | 0.5 ± 0.5               | no                                       | 1.3 ± 0.6               |
| [P_{666 14}] [AOT] | no                                    | 4.0 ± 0.0               | no                                       | 0.7 ± 0.3               |
| [P_{888 14}] [AOT] | no                                    | 4.0 ± 0.6               | no                                       | 1.2 ± 0.8               |
| [P_{181818 1}] [AOT] | no                                    | 2.0 ± 0.0               | no                                       | 0.0 ± 0.0               |
| [P_{444 2OH}] [AOT] | no                                    | 5.5 ± 0.5               | no                                       | 1.0 ± 0.0               |
| [P_{666 14}] [Tf2N] | no                                    | 0.3 ± 0.3               | no                                       | 1.0 ± 0.0               |

Based on the stability data in this study, no significant change in surface wetting behavior was observed for all PIL-SLIPS after 14 days, which confirmed that a layer of ionic liquid was still present. This was further supported by the sustained antimicrobial efficacy of [P_{888 14}] [AOT]-SLIPS after repeated bacterial challenges (Section 2.3). However, stability was performed under static conditions, and so further dynamic testing is required to fully assess their stability for flow applications.

2.3. In Vitro Antibacterial Activity of SLIPSs. Table 3 shows the inhibitory zones produced by SLIPS samples against S. aureus and P. aeruginosa. All SLIPS samples inhibited the growth of S. aureus after 24 h incubation and followed a trend similar to that shown in Table 1. [P_{888 14}] [AOT] and [P_{444 2OH}] [AOT] displayed the largest inhibition zones, although at 4 and 5.5 mm, respectively, these were relatively small zones and indicated minimal transfer of ionic liquid from the sample. Parallel examination with P. aeruginosa showed reduced zones for the majority of surfaces, except silver-PVC control and [P_{666 14}] [Tf2N] which each displayed an almost 3-fold increase.

The ionic liquids used in this study possessed progressively longer alkyl chains attached to a phosphonium cation with hydrophobicity increasing from [P_{444 2OH}] [AOT] < [P_{666 14}] [AOT] < [P_{888 14}] [AOT] < [P_{181818 1}] [AOT]. With [P_{888 14}] [AOT] possessing the most potent activity, this suggests that the differing alkyl chain lengths play an important role in determining the antibacterial efficacy. This corresponds with the previous literature on ionic liquids using different cations such as imidazolium ionic liquids.

Disk diffusion assays were conducted on SLIPSs to identify if the ionic liquid leached from the surface when exposed to aqueous media. As can be seen from Table 3, the addition of a silver coat to PVC produced an antimicrobial surface with inhibitory zones of 0.5 and 1 mm for S. aureus and P. aeruginosa, respectively, and may be attributed to limited dissolution of Ag+ ions into the ionic layer and diffusion into
the aqueous medium and agrees with the previous literature. The addition of ionic liquids to the surface caused an increase in the inhibitory zone against \textit{S. aureus}, relative to silver-PVC, except those infused with \([\text{P}_{666}\text{14}]\text{[Tf}_2\text{N}]\). This was likely facilitated by improved dissolution and diffusion of the ionic liquids because of the presence of a docusate counterion. Alternatively, it is possible that the docusate anion possesses inherent antibacterial properties, while the \([\text{Tf}_2\text{N}]^-\) anion does not; however, Kruszewska et al. have previously reported an MIC of 3000 μg mL$^{-1}$ for sodium docusate, which is considerably higher than the docusate counterion. Similarly, the underlying silver substrate surface.

\[ [\text{P}_{666}\text{14}]\text{[AOT]}\text{-SLIPS} \]

Produced the largest inhibitory zone against \textit{S. aureus}; this was likely due to its smaller molecular size and the presence of a hydroxyl functional group, which improved the diffusion of the ionic liquid into the aqueous medium. The remaining \([\text{AOT}^-]\)-ionic liquids followed the inverse trend of decreasing inhibitory zone with increasing alkyl chain length with \([\text{P}_{181818}\text{1}]\text{[AOT]}\) possessing the smallest zone of 2 mm. However, \([\text{P}_{666}\text{14}]\text{[Tf}_2\text{N}]\) had the smallest inhibitory zone against \textit{S. aureus} of <0.5 mm which was smaller than uninfused silver-PVC samples. This result agrees with the MIC results obtained in Table 1, which showed \([\text{P}_{666}\text{14}]\text{[Tf}_2\text{N}]\) to possess no antimicrobial activity against either bacteria, while the decrease in zone size compared to silver-PVC suggested that the liquid ionic coat reduces the ability of Ag$^+$ ions to diffuse into the surrounding media. It is likely that \([\text{P}_{666}\text{14}]\text{[Tf}_2\text{N}]\) was unable to dissolve in the aqueous media because of the significant hydrophobicity imparted by both the long-chain cation and the high fluorine content of the anion. The lack of dissolution would reduce the ability of the ionic liquid to come in contact with bacteria and prevent a true MIC from being determined and has similarly been reported by Pernak et al. when assessing the antimicrobial activity of choline-based \([\text{Tf}_2\text{N}]^-\) ionic liquids.

Zones of inhibition for \textit{P. aeruginosa} followed a similar trend to those measured for \textit{S. aureus} with silver-PVC and all SLIPSs displaying inhibition. However, the size of inhibitory zones was smaller for all samples except silver-PVC and \([\text{P}_{666}\text{14}]\text{[Tf}_2\text{N}]\)-SLIPS. This decrease was in agreement with the decrease in antibacterial efficacy of these ionic liquids against \textit{P. aeruginosa}, as shown in Table 1. In addition, silver-PVC samples possessed the largest inhibitory zone, which suggests that the addition of a PIL layer reduces the dissolution and diffusion of Ag$^+$ ions into the surrounding media and prevents aequous contact with the underlying silver substrate surface.

In addition to inhibition assays, SLIPSs were immersed in inoculated media for up to 7 days. All SLIPS samples reduced viable bacterial adherence by at least 4 log$_{10}$ reductions after 24 h, when challenged with a 1 × 10$^7$ cfu mL$^{-1}$ inoculum density of \textit{S. aureus} and \textit{P. aeruginosa}, as shown in Table 4.

Coating of PVC with silver caused complete inhibition of viable \textit{S. aureus} adherence at 24 h and achieved a statistically significant 4 log$_{10}$ reduction in \textit{P. aeruginosa} adherence at 24 h. Metallic silver coatings do not possess inherent antiadherent properties, and so, it is envisioned that these results occurred because of the silver surface generating silver ions upon contact with the aqueous media, which subsequently killed \textit{S. aureus} and \textit{P. aeruginosa} cells upon approach or settlement on the silver surface. A recent study by Xu et al. demonstrated the ability of a polystyrene polymer coated with a silver nanosheet to provide a 1 log$_{10}$ and 2 log$_{10}$ reduction in \textit{S. aureus} and \textit{P. aeruginosa} adherence, which agrees with the results seen with the silver-coated PVC substrate. Silver ions have also been shown to eradicate as much as 99.999% of a 10$^7$ cfu mL$^{-1}$ \textit{P. aeruginosa} inoculum within 6 h at concentrations as low as 0.08 mg L$^{-1}$. SLIPSs produced with a silver-PVC substrate displayed remarkable ability to prevent viable bacterial attachment, with all variants achieving complete inhibition of viable adherence (below the limit of detection of <50 cfu mL$^{-1}$) of \textit{S. aureus} at both 4 and 24 h, representing 4.5 and 5.5 log$_{10}$ reductions, respectively, with similar results observed against \textit{P. aeruginosa} at 4 and 24 h.

A possible explanation for the remarkable efficacy of these SLIPSs can be attributed to the surface hydrophilicity displayed by each SLIPS. Several studies have shown that surfaces with superhydrophilic or markedly hydrophilic surfaces reduced bacterial adherence. All \([\text{AOT}^-]\)-ionic liquid SLIPSs achieved extremely low or superhydrophilic contact angles with water. It is possible that these surfaces facilitate the formation of a layer of water molecules on top of the lubricant layer in a process known as “water layer theory”. When this occurs, there is a reduced interaction between the bacterial cell surface and solid surface and inevitably reduces cell adhesion.

In the case of the SLIPS discussed in this study, it is possible this same mechanism occurs but on top of the lubricant layer, providing a further barrier to bacterial adhesion and may also provide a platform through which bacteria are brought into contact with the ionic liquid front, enabling contact kill mechanisms to dominate. \([\text{P}_{666}\text{14}]\text{[Tf}_2\text{N}]\)-SLIPSs were unable to provide complete prevention of detectable adherence for both bacteria at 24 h, and it is noteworthy that \([\text{P}_{666}\text{14}]\text{[Tf}_2\text{N}]\) produced SLIPSs with a water contact angle of 70.17°, which,

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**Table 4. Number of Viable Bacteria of \textit{S. aureus} and \textit{P. aeruginosa} Adhered to the Surface of PVC Control and SLIPS Samples, Incubated at 37 °C, after 4 and 24 h ($n = 5$)**

| Sample               | \textit{S. aureus} | \textit{P. aeruginosa} |
|----------------------|---------------------|-----------------------|
| PVC                  | 4.10 ± 0.31         | 4.93 ± 0.09           |
| silver-PVC           | 2.82 ± 0.21         | 4.93 ± 0.09           |
| silver-[P$\text{666}\text{14}$][AOT]-SLIPS | n.d                 | 1.39 ± 1.28           |
| silver-[P$\text{666}\text{14}$][Tf$_2$N]-SLIPS | n.d                 | n.d                   |
| silver-[P$\text{181818}\text{1}$][AOT]-SLIPS | n.d                 | n.d                   |
| silver-[P$\text{666}\text{14}$][Tf$_2$N]-SLIPS | n.d                 | n.d                   |
| silver-[P$\text{666}\text{14}$][P$_\text{666}\text{14}$][AOT]-SLIPS | n.d                 | n.d                   |

*“A result of “n.d” denotes a bacterial adherence below the limit of detection of 50 cfu mL$^{-1}$.\footnote{A result of “n.d” denotes a bacterial adherence below the limit of detection of 50 cfu mL$^{-1}$}*

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https://dx.doi.org/10.1021/acsomega.9b03528
ACS Omega 2020, 5, 7771–7781
although still hydrophilic, was significantly higher than all other ionic liquid-infused SLIPSs. Surfaces with moderate hydrophilicity or moderate hydrophobicity have been linked to enhanced bacterial attachment; Duo et al. found that the Gram-positive bacteria *Micromonospora purpurea* adhered optimally on surfaces with contact angles between 54 and 130°.\(^{45}\) This is supported by previous studies on ionic liquids by Pernak et al. who found that imidazolium ionic liquids coupled with a \([\text{TF}_2\text{N}]\) anion displayed no antimicrobial activity against several bacteria, including *S. aureus* and *Escherichia coli*. Pernak attributed this to the poor water solubility of \([\text{TF}_2\text{N}]\)-ionic liquids in aqueous media,\(^{46}\) and this is also experienced with \([\text{P}_{666\,14}][\text{TF}_2\text{N}]\) which has a reported aqueous solubility of 0.26 mM.\(^{47}\)

When SLIPS samples were exposed to three repeated bacterial challenges (10⁶ cfu mL⁻¹) over a period of 7 days, the antiadherent efficacy of the SLIPS coatings was lost with no significant reductions detected except for \([\text{P}_{888\,14}][\text{AOT}]\)-SLIPS (Figure 4). \([\text{P}_{888\,14}][\text{AOT}]\)-SLIPS displayed remarkable antibacterial activity, with a >6 log₁₀ reduction in viable bacterial adherence maintained after 7 days. This result, coupled with the low inhibition zone diameters observed in Table 3, indicates that \([\text{P}_{888\,14}][\text{AOT}]\) infusion is maintained on the silver substrate and can provide an effective antibacterial surface for up to 7 days.

The failure of the majority of SLIPS surfaces to prevent colonization may have been caused by isolated areas of the rough PVC surface becoming exposed to bacterial cells as shear forces on the surface may have led to a loss of ionic liquid, which would reduce the lubricant layer thickness providing bacterial cells with anchoring sites on the underlying silver-PVC surface, as shown in Figure 5. Further cell adhesion and lubricant durability studies will be conducted to fully elucidate the mechanism behind the differing efficacies of the SLIPSs during prolonged bacterial exposure.

### 3. CONCLUSIONS

There is an urgent need for improved and novel strategies to address biofouling which has a significant financial and practical consequence on the marine, medical, food, and construction industries. This study has described the development of a novel ionic liquid-infused SLIPS that combines the antiadherent properties of early SLIPS development, with the addition of an antimicrobial component to enhance their potential application in antifouling applications. These ionic liquid-infused surfaces reduced viable bacterial adherence by up to 4 log₁₀ reductions after 24 h compared to the control PVC polymer. Furthermore, this performance was maintained in one SLIPS variant upon repeated bacterial challenge over a 7 day period. Further work to assess the biocompatibility of the PILs and the efficacy of the synthesized SLIPS in dynamic flow conditions will be conducted to further assess their potential as an antifouling surface for the prevention of microbial fouling.

This work highlights the potential use of ionic liquids as a multifunctional lubricant to manufacture SLIPS with additional properties to their inherent antiadhesive nature, in this instance with respect to providing fouling resistance for at least 7 days.

### 4. EXPERIMENTAL SECTION

#### 4.1. Materials.

Unplasticized PVC of 0.2 mm thickness (1.4 g/cm³) was purchased from Goodfellow Cambridge Ltd. (Huntingdon, UK). Silver nitrate (AgNO₃) (>99.0%), ammonium hydroxide (NH₄OH) (28–30% NH₃ basis), α- (+)-glucose, and sodium hydroxide (NaOH) (>97%) were purchased from Sigma-Aldrich (Gillingham, Poole, UK). Ethanol (>98%) was supplied by JT Baker (Teugseweg, The Netherlands). In all cases, reagents were used as received, while polymer substrates were washed with ethanol and deionized water before use to remove any surface contaminants.

Trihexyl(tetradecyl)phosphonium docusate (\([\text{P}_{666\,14}][\text{AOT}]\)), trioctyl(tetradecyl)phosphonium docusate (\([\text{P}_{888\,14}][\text{AOT}]\)), and trioxadecyl(methylphosphonium docusate (\([\text{P}_{353535\,14}][\text{AOT}]\)) were synthesized using the following materials—trihexyltetradecylyphosphonium chloride, trioctyltetradecylyphosphonium chloride, and docusate sodium (all obtained from Sigma-Aldrich, Poole, UK), trioctadecylyphosphine (Cytec, Canada), and iodomethane (99%) (Alfa Aesar, Heysham, UK). Toluen, pentane, chloroform, and diethyl ether were used as solvents (Sigma-Aldrich, Poole, UK). Trihexyl(tetradecyl)phosphonium bis(trifluoromethylsulfonylimide (\([\text{P}_{666\,14}][\text{TF}_2\text{N}]\)) and tributyl(hydroxyethyl)-phosphonium docusate (\([\text{P}_{444\,20\,14}][\text{AOT}]\)) were kindly provided by Queen’s University Ionic Liquid Laboratory.

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**Figure 4.** Number of viable bacteria adhered to the surface of silver-PVC and silver-PVC-SLIPSs compared to PVC control, incubated at 37 °C for 7 days, when repeatedly challenged with 10⁶ cfu mL⁻¹ of *S. aureus* and *P. aeruginosa*. Error bars represent ±SD.

**Figure 5.** Illustration of incomplete coverage of the roughened PVC surface with ionic liquid, allowing the attachment and colonization by bacteria.
Table 5. Quaternary PIL Structures

| Ionic Liquid                                      | Cation | Anion |
|--------------------------------------------------|--------|-------|
| Trihexyl(tetradecyl) phosphonium docusate         | ![Image](https://example.com/image1.png) | ![Image](https://example.com/image2.png) |
| [P666 14][AOT]                                  |        |       |
| Trioctyl(tetradecyl) phosphonium docusate         | ![Image](https://example.com/image3.png) | ![Image](https://example.com/image4.png) |
| [P888 14][AOT]                                  |        |       |
| Trioctadecyl(methyl) phosphonium docusate         | ![Image](https://example.com/image5.png) | ![Image](https://example.com/image6.png) |
| [P1818 13][AOT]                                 |        |       |
| Trihexyl(tetradecyl)phosphonium bis(trifluoromethylsulfonylimide) | ![Image](https://example.com/image7.png) | ![Image](https://example.com/image8.png) |
| [P666 14][TF2N]                                 |        |       |
| Tributyl(hydroxyethyl)phosphonium m docusate     | ![Image](https://example.com/image9.png) | ![Image](https://example.com/image10.png) |
| [P444 30][AOT]                                  |        |       |

(QUILL), Queen’s University Belfast, UK. Structures of the ionic liquids are shown in (Table 5).

*S. aureus* (ATCC 6538) and *P. aeruginosa* (PA01) were stored on Microbank cryopreservative beads (Pro-Lab Diagnostics, Cheshire, UK) at −80 °C and used to subculture in a Mueller–Hinton broth (MHB) at 37 °C when required. All microbiological supplies including MHB, quarter-strength Ringer’s solution (QSRS), PBS, and tryptic soy broth were obtained from Oxoid Ltd. (Basingstoke, UK).

### 4.2. Methods

#### 4.2.1. Synthesis and Characterization of Quaternary Phosphonium Docusate Ionic Liquids

4.2.1.1. [P666 14][AOT] and [P888 14][AOT]. Trihexyl(tetradecyl)-phosphonium docusate ([P666 14][AOT]) and trioctyl-(tetradecyl)phosphonium docusate ([P888 14][AOT]) were synthesized using trihexyl(tetradecyl)phosphonium chloride and trioctyl(tetradecyl)phosphonium chloride, respectively, using a previously described method. Sodium docusate (11.104 g, 25 mmol) was placed in a 250 mL round-bottom flask and dissolved with stirring in 100 mL of chloroform. The trialkyltetradecyl halide salt ([P666 14][Cl] = 6.58 g, 12.67 mmol, [P888 14][Cl] = 7.62 g, 12.63 mmol) was then dissolved and added to the sodium docusate using three 20 mL aliquots of chloroform. The reaction vessel was then fitted with a reflux condenser and left to stir at 65 °C for 24 h and then allowed to cool to room temperature. The mixture was transferred to a separating funnel, and 100 mL of distilled water was added and shaken vigorously. The mixture was left to separate for 24 h, and the lower aqueous phase was decanted off and washed twice more using the same procedure. The solution was placed on a rotary evaporator at 50 °C and 140 rpm to remove chloroform solvent and then placed under high vacuum overnight. Finally, the solution was dissolved in 10 mL of ether, sonicated at 37 kHz for 15 min, and filtered to remove any remaining NaCl. The filtrate was vacuum evaporated for 2 h to remove ether and placed under high vacuum overnight to yield the dry product. A total yield of 10.38 g (93.66%) and
was then filled with a reflux condenser and left to stir at 40 °C for 24 h. Toluene was evaporated under vacuum at 50 °C using a rotary evaporator at 140 rpm for 3 h. The product solution was left to dry overnight under high vacuum. The crude product was dissolved in 5 mL of pentane and sonicated at 37 kHz for 15 min. The solution was then filtered with 5 mL of aliquots of pentane. The residue was dried under high vacuum overnight. Ion exchange with sodium docusate was then performed as described previously for $[P_{666}\,14][\text{AOT}]$ and $[P_{888\,18}][\text{AOT}]$ to give a total yield of 1.139 g (37.98%).

4.2.2. Nuclear Magnetic Resonance Spectroscopy of Quaternary PILs. PILs were analyzed by proton, carbon-13, and phosphorous-31 nuclear magnetic resonance ($^1$H NMR, $^{13}$C NMR, and $^{31}$P NMR, respectively) spectroscopy. PILs were dissolved in deuterated chloroform (CDCl$_3$) for analysis. Results from $^1$H, $^{13}$C, and $^{31}$P NMR analysis are detailed in the following for each PIL.

4.2.2.2.1. Tributyl(tetradecyl)phosphonium Docusate $[P_{666\,14}][\text{AOT}]$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta_H$ 4.08–3.84 (m, 5H), 3.18–2.98 (m, 2H), 2.16 (br, 8H), 1.53 (m, 1H), 1.40 (m, 17H), 1.16 (m, 48H), and 0.78 (m, 24H).

4.2.2.2.2. Trioctyl(tetrade cyclyl)phosphonium Docusate $[P_{888\,18}][\text{AOT}]$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta_H$ 4.12–3.93 (m, 5H), 3.30–3.09 (m, 1H), 2.31 (br, 8H), 1.62 (br, 1H), 1.51 (m, 17H), 1.24 (m, 60H), 0.96–0.86 (m, 24H).

4.2.2.2.3. Trioc tylex(tetradecyl)phosphonium Docusate $[P_{666\,14}][\text{AOT}]$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta_H$ 4.22–3.92 (m, 5H), 3.23–3.06 (m, 2H), 2.21 (br, 6H), 1.92–1.88 (d, 3H), 1.61 (br, 1H), 1.45 (m, 17H), 1.22 (m, 96H), 0.85–0.83 (m, 21H).

4.2.2.2.4. Tributyl(hydroxyethyl)phosphonium Docusate $[P_{644\,20\,14}][\text{AOT}]$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta_H$ 4.16–3.90 (m, 7H), 3.11 (d, $J = 3.81$ Hz, 2H), 2.59 (m, 1H), 2.25 (br, 8H), 1.53 (br, 8H), 1.27 (m, 22H). 0.99–0.84 (m, 21H).

$^{13}$C NMR (400 MHz, CDCl$_3$): $\delta_C$ 171.47, 169.05, 67.90 (d, $J = 7.51$ Hz), 61.76, 55.33 (d, $J = 7.23$ Hz), 38.63 (d, $J = 2.27$ Hz), 38.50 (d, $J = 7.63$ Hz), 33.92, 30.26 (d, $J = 3.26$ Hz), 30.05 (d, $J = 6.76$ Hz), 28.90, 28.86, 27.65, 27.00, 24.32, 24.18, 23.95 (d, $J = 15.59$ Hz), 23.72, 23.67, 23.61, 23.40, 22.96 (d, $J = 2.10$ Hz), 22.51, 19.56, 19.09, 14.06 (d, $J = 3.46$ Hz), 13.62, 13.49, 13.44, 10.93, 10.89, 10.85, 10.77.

$^{31}$P NMR (400 MHz, CDCl$_3$): $\delta_P$ 33.16 (s).

4.2.2.2.5. Trihexyl(tetrade cyclyl)phosphonium Bis(trifluoromethanesulfonyl)imide $[P_{666\,14}[\text{TF$_2$N}]]$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta_H$ 2.13–2.06 (m, 8H), 1.48–1.21 (m, 48H), 0.91–0.86 (m, 12H).

$^{13}$C NMR (400 MHz, CDCl$_3$): $\delta_C$ 121.51, 118.31, 31.91, 30.87, 30.57 (d, $J = 14.33$ Hz), 30.20 (d, $J = 14.21$ Hz), 29.64 (t, $J = 3.34$ Hz), 29.47, 29.34, 29.24, 28.79, 22.67, 22.26, 21.50 (d, $J = 4.62$ Hz), 18.73 (d, $J = 47.50$ Hz), 14.09, 13.82.

$^{31}$P NMR (400 MHz, CDCl$_3$): $\delta_P$ 32.93 (s).

4.2.3. In Vitro Antibacterial Activity of PILs. S. aureus (ATCC 6538) and P. aeruginosa (PAO1) were maintained on cryopreservative beads (Microbank) at −80 °C and cultivated in 100 mL of MHB at 37 °C when needed. Overnight broth was centrifuged (3000 rpm, 12 min) and resuspended in PBS to achieve a final inoculum density of approximately 1 $\times$ 10$^8$ cfu mL$^{-1}$.

The microbiological performance of the ionic liquids was assessed using MHB inoculated with S. aureus or P. aeruginosa according to a standardized broth dilution method.$^{25}$ A stock solution of 0.2% w/v of ionic liquid was freshly prepared in sterile MHB by stirring at 37 °C until dissolved and then filtered using a sterile 0.22 μm membrane. Serial twofold dilutions were prepared to provide a concentration range of 7.6294 $\times$ 10$^{-10}$ to 0.1% w/v. Inoculum was diluted in MHB to produce a final inoculum density of 1 $\times$ 10$^8$ cfu mL$^{-1}$, verified by viable count. The inoculum (100 μL) was added to an equal volume of ionic liquid and incubated for 24 h (37 °C, 100 rpm). Positive (100 μL of MHB/100 μL of inoculum) and negative controls (200 μL of MHB) were included in each assay. A minimum of five replicates of each dilution and controls were included.

Determination of the MIC was taken as the lowest concentration that produced no visible growth after 24 h incubation. MBC was then established by spreading 20 μL of suspension from wells exhibiting no visible growth onto Mueller–Hinton agar (MHA) plates, which were incubated for 24 h in a static incubator at 37 °C and then examined for 99.9% killing, taken as the lowest concentration that prevented no growth on MHA plates after 24 h.

4.2.4. Hemolysis Assay of AOT-Containing PILs. To assess the potential of phosphonium-AOT ionic liquids to illicit a hemolytic effect, ionic liquids were assessed for the ability to induce hemolysis of equine erythrocytes according to a previous method.$^{51}$ Briefly, 10 mL of fresh defibrinated equine blood was rinsed three times with an equal volume of PBS by centrifugation at 900g for 15 min. Erythrocytes were resuspended in PBS to a concentration of 4% (v/v). Erythrocyte suspension (100 μL) was added to each well of a 96-well microtiter plate and exposed to varying concentrations of ionic liquid for 1 h at 37 °C in an orbital incubator. Exposed solutions were subsequently centrifuged at 1000g for 15 min, and 100 μL was transferred to a fresh 96-well microtiter plate and spectrophotometrically read at 414 nm to determine hemoglobin release. Hexadecyltrimethylammonium bromide (8 mM) was used as a positive control (100%)
hemolysis) and PBS as a negative control (0% hemolysis). Samples were run in quadruplicate, and percentage hemolysis was calculated as follows

\[
\% \text{ haemolysis} = \frac{(\text{ionic liquid absorbance at 414 nm} - \text{PBS absorbance at 414 nm})}{(\text{CTAB absorbance at 414 nm} - \text{PBS absorbance at 414 nm})} \times 100
\]

4.2.5. Manufacture of a Porous Polymer Substrate and SLIPS. Silver coating of substrates was achieved through utilization of the well-documented Tollen’s reagent. Tollen’s reagent has been previously used with success to produce a silver coat on substrates such as polymers and glass, mainly for spectroscopic analysis sample preparation.\(^{52,53}\)

Tollen’s reagent was produced as follows: the wells of a 24-well microtiter plate were filled with 2.20 mL of 0.15 M AgNO₃. Concentrated NH₄OH was then added dropwise with stirring until a yellow-brown precipitate formed. Further dropwise addition of concentrated NH₄OH continued until the precipitate just dissolved, with the solution becoming clear. NaOH (0.56 mL, 1 M) was added to each well producing a black solution, which upon further dropwise addition of concentrated NH₄OH and stirring returned to a clear solution. PVC samples (10 mm × 10 mm), previously etched in ethanol for 1 min, were placed into each well, and 0.23 mL of 0.15 M glucose was added to reduce the ionic silver to metallic silver, which was accompanied by a color change from colorless to yellow to brown/black with a gray/silvery shine signalling the deposition of silver in the solution. Samples were left in this solution for 30 min and then removed and immediately washed with deionized water to remove excess silver hydroxide deposits. Samples were then washed a second time with deionized water and left to air-dry before use.

Samples were transformed into SLIPS by the addition of synthesized PILs. Ionic liquid was diluted to produce a 20% v/v ionic liquid in ethanol stock solution. Stock solution (50 μL/cm²) was infused onto the sample surface and left for 1 h to allow ethanol evaporation. This was repeated on the reverse side of the sample. Samples were then washed with deionized water and left to air-dry before use.

4.2.6. SEM of SLIPSs. Samples were characterized using a Hitachi High Technologies TM3030 scanning electron microscope (Berkshire, UK) using adhesive carbon disks to attach samples to the aluminum sample stub. Each sample was analyzed at 800X magnification using an accelerating voltage of 15 kV. Images were then captured using the TM3030 software.

4.2.7. Determination of Static Water Contact Angle of SLIPSs. The static contact angle was determined using a modified First Ten Angstroms (Virginia, USA) FTA1000 B-Class contact angle analyzer and images captured via a coupled video camera and FTA32 software. Static contact angle was calculated using a 4 μL droplet of deionized water as a wetting agent, and measurements were conducted at room temperature.

4.2.8. Stability of SLIPS in Aqueous Media. The stability of SLIPS was assessed by immersing samples in 10 mL of PBS and incubating at 37 °C. Samples were removed at 0, 24, 48, and 168 h, allowed to dry in an air oven, and cooled to room temperature, and then static water contact angle measurements were taken as described previously.

4.2.9. In Vitro Antibacterial Activity of PIL-Infused SLIPS. The antibacterial activity of SLIPS materials was assessed on MHA inoculated with P. aeruginosa or S. aureus according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion method.\(^{54}\) MHA plates were inoculated by dipping a sterile cotton swab in 10⁴ cfu mL⁻¹ inoculum and swabbing the surface of the plate in a side-to-side motion from top to bottom. This was repeated twice more with the plate rotated 60° each time to ensure complete coverage of the agar surface. Plates were allowed to dry for 10 min, and then 10 × 10 mm samples of each SLIPS were placed on the surface of the inoculated MHA plates and incubated at 37 °C for 24 h. Nonmodified PVC was used as a positive growth control. The width of the inhibition zone with no bacterial growth, excluding sample width, was measured.

In addition, the antibacterial activity of SLIPS materials was assessed in immersed conditions. SLIPS samples (10 × 10 mm) were placed in individual wells of a 24-well microtiter plate and immersed in 1 mL of a 1 × 10⁶ cfu mL⁻¹ inoculum and incubated for 4 and 24 h. Samples were aseptically removed and rinsed three times in QSRS and sonicated for 10 min. Serial dilutions were made in PBS and plated on MHA plates and incubated for 24 h at 37 °C. Microbial growth was assessed using the standard Miles and Misra technique.\(^{55}\)

Repeated exposure to bacterial inoculum was also conducted to assess the durability of the antibacterial properties of each SLIPS. SLIPS samples were challenged with a 10⁶ cfu mL⁻¹ inoculum several times over a 7 day period. Experiments were set up as described above, and inoculated media were removed and replaced with fresh 10⁶ cfu mL⁻¹ inoculum on day 2, day 4, and day 6. SLIPS samples were removed on day 7 and processed as described above for 4 and 24 h assays.

4.2.10. Statistical Analysis. Statistical analysis was performed using GraphPad Prism 6 statistical software. Statistical significance in static contact angle measurements was determined using a one-way analysis of variance with post-hoc comparisons evaluated using Dunnett’s multiple comparisons test. The effect of surface modifications on viable bacterial adherence was evaluated using the Kruskal–Wallis test, with post-hoc comparisons carried out with Dunn’s test. In all cases, p < 0.05 denoted significance and n = 5.
Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03528

Notes
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This study was supported by funding from the Department for Employment and Learning, Northern Ireland, and the Engineering and Physical Sciences Research Council (EPSRC Reference: EP/R043345/1).

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