RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

RSC Advances

RSCPublishing

COMMUNICATION

Plasma polymerization of 1,1,1-trichloroethane yields coating with robust antibacterial surface properties

Cite this: DOI: 10.1039/x0xx00000x

Thomas D. Michl[a,b], Bryan R. Coad[b], Michael Doran[c], Amanda Hüsler[a], Jules D. P. Valentin[b], Krasimir Vasilev[b], Hans J. Griesser[b]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Novel, highly chlorinated surface coatings were produced via a one-step plasma polymerization (pp) of 1,1,1-trichloroethane (TCE), exhibiting excellent antimicrobial properties against the vigorously biofilm-forming bacterium *Staphylococcus epidermidis*.

Biofouling with its implications causes reoccurring substantial financial loss.[1] Bacteria are a major contributor to biofouling, this urges the need for permanent antibacterial coatings for a variety of applications, such as water filtration, bioreactor membranes, food packaging and biomedical devices. [2, 3] Because biofouling is such a serious problem, the approaches to combat its formation are manifold, such as using biocidal coating containing organo-tin or quarternary amine compounds.[3] However, these coatings bear certain drawbacks. For example, the well-known antibacterial quaternary ammonium compounds usually require a wet-chemical activation step using toxic and carcinogenic alkylation reagents in the presence of solvents or the use of expensive and exotic starting materials.[4-6]

An all-embracing solution however, has not been found so far. Colonization of a surface by bacteria is a stepwise process starting with planktonic bacteria attaching to a surface upon which they start multiplying and secreting a thick extracellular matrix forming a biofilm. Bacteria imbedded within this biofilm are much more resistant to outside xenobiotics which is why it is of importance to kill them at the surface in their planktonic before attachment.[7] Highly desirable in the design of antibacterial surfaces is the goal of generating robust, yet financially affordable coatings that withstand bacterial colonization over long periods of time. Due to its scalability, speed of process and lack of solvent usage, plasma polymerization is used to generate thin functional coatings on an industrial scale. These process advantages have led to the research of antimicrobial coatings generated via plasma polymerization.[8] However, all reported plasma polymerized coatings so far involve several production steps and often include the use of solvents, nullifying the advantage of being solvent free.

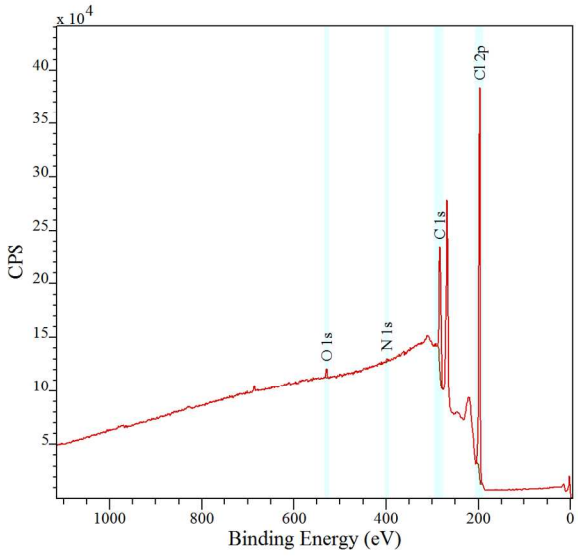

Figure 1: XPS survey spectra of dry 1,1,1-TCEpp

Various chlorinated hydrocarbons, such as dichlorobenzene, are being used for sanitary purposes which gave rise to the inspiration to utilize them in our study as a starting material.[9] 1,1,1-trichloroethane was selected due to its low toxicity when compared to other halogenated solvents. Plasma polymerization of chlorinated monomers has been reported previously.[10, 11] However, to the best of our knowledge their potential short and long-term antibacterial properties were never reported. The aim of this study was to investigate the chlorinated monomer TCE as a promising candidate to generate antibacterial plasma polymer coatings in one step, which represents a potentially considerable improvement in comparison to multi-step approaches."

We report the use of TCE as a volatile precursor to generate surface coatings via reduced pressure radio frequency plasma polymerization in one step yielding surfaces which are highly bactericidal to the vigorously biofilm producing *Staphylococcus epidermidis (S.epi)*. Furthermore, those surfaces exhibit persistent antimicrobial properties even after soaking or washing with water.

	% C	% N	% O	% Cl	% Si
Dry	54.6	0.7	1.3	43.2	-
Wet	56.9	1.2	9.3	31.9	0.5
Washed	55.4	2.4	15.2	27.0	-

Table 1: XPS data obtained

The plasma polymerization and deposition onto polyethylene terephthalate (PET) coverslips was carried out in a custom-built reactor as reported before.[12, 13] In short, PET coverslips were first treated with air plasma to clean the surface. Subsequently, TCE vapours were introduced into the chamber; followed by its plasma deposition at a fixed monomer flow rate and power input. Afterwards, the plasma polymerized TCE (TCEpp) surfaces were either stored in air (Dry), covered with 1 ml of deionized water (Wet) or washed 20 times × 1 ml with deionized water over the course of 1 month (Washed). The surfaces were characterized using X-ray photoelectron spectroscopy (XPS), time of flight secondary mass spectroscopy (ToF-Sims) and ellipsometry. All of which are methods commonly used to characterize plasma polymer films.[14, 15] Bacterial testing was carried out using the clinically isolated *S.epi* strain (ATCC 35984), in either a 24 h test utilizing Safranin or a time lapse experiment using the live-dead BacLight™ stain. All spectra and detailed testing protocol are provided in the supporting information.

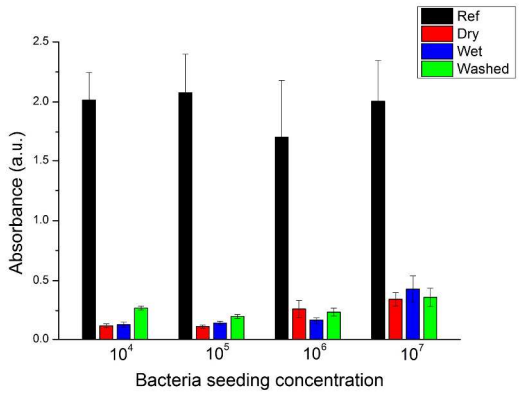

Figure 2: Bacterial testing results after 24 h

As can be seen in Table 1 and Figure 1, TCEpp stored in air exhibits a high chlorine content of more than 43 %. This is somewhat lower compared to the chlorine content of TCE itself; which suggests fission of carbon-chlorine bonds of TCE during the polymerization and the elimination to some degree. The very low concentrations of nitrogen and oxygen are most likely a result of post plasma polymerization aging, as has been observed in other plasma polymers.[16] The ToF-Sims spectra in negative mode (see supporting information) of TCEpp stored in air is dominated by peaks at 34.97 / 36.97 and 69.95 / 71.95 amu; corresponding to Cl^- and Cl_2^-. In positive mode two characteristic peaks can be seen at 82.94 and 84.94 amu; indicating that the molecule fragments contain chlorine. Many more fragments in pairs 2 amu apart and with a "less than whole" amu can be found throughout the spectra, all pointing to a multitude of fragments containing at least one chlorine. On the other hand, "more than whole" fragments such as 91.05 and 105.07 amu can be found. These are most likely alkyl chain fragments coinciding with the aforementioned carbon-chlorine fission and chlorine elimination. The measured thickness via ellipsometry (supporting information) was 169.4 ± 0.2 nm after 2 minutes of total polymerization time. This indicates a fast pp growth rate compared to other monomers such as n-heptylamine.[17]

The bacterial testing results, as seen in Figure 2, indicate a considerable reduction of bacteria and biofilm after 24 h when compared to the reference for all bacteria seeding concentration. Only at very high seeding concentrations did the TCEpp show decreased efficacy; however, such high seeding concentrations are less realistic in real life situations such as wound healing.[18] The time lapse experiment, presented in Figure 3, further solidifies the antibacterial properties of TCEpp. Here, mostly dead bacteria (orange/red) could be seen at all time intervals and their overall number hardly increased even after 6 h of incubation. This is especially the case when compared to the reference on which the bacteria showed vigorous proliferation.

Cytotoxic measurements have shown the toxic effect of the synthesized TCEpp surfaces towards certain mammalian cell lines (see supporting information and Figure S1). These results reflect the limitations of the chlorinated plasma polymers and would probably preclude their use in implantable materials. Regardless of these limitations, the authors envision numerous other applications such as air-filters and disposable surgical gowns, just to name a few.

A desirable aspect for antibacterial surfaces is their longevity and resistance to external factors such as washing. For this

purpose TCEpp coated coverslips were either immersed in deionized water for at least 24h or washed with distilled water twenty times over the course of one month prior to bacterial testing. TCEpp that came into contact with water showed in comparison to its dry stored counterpart a decrease in chlorine content, as seen in the XPS results in Table 1. Wetting led to a decrease of the overall chlorine content to 31.9% and to 27% in the case of repeated washing. Interestingly, the carbon percentage seems to undergo only small changes whereas the biggest difference is the decrease of chlorine, which is counterbalanced mostly by an increasing oxygen content. The slight increase of nitrogen and moderate one in case of oxygen for the wet and washed TCEpp was most likely due to post polymerization aging in water.[19, 20] It appears that the plasma polymer network contains chlorinated, non-crosslinked fragments, which can leach into solution as implied by the loss of chlorine seen in XPS. The loss of chlorine could suggest a release mechanism of bactericidal chlorinated oligomers into solution and is subject of further research. Minute traces of silicon were observed; most likely in form of silicone contaminations; however, at such low concentration they were very unlikely able to influence the surface chemistry. Altogether, the XPS confirms the presence of a chlorinated plasma polymer even after contact with water. The ToF-Sims spectra in negative mode of the wetted or washed surfaces did not change compared to the dry TCEpp with Cl^- and Cl_2^- still being the dominant peaks. Conversely though, in positive mode, the intensity of lower molecular "more than whole" peaks increased. More interestingly however, these small chemical differences did not impair its antibacterial activity; as can be seen in the almost identical bacteria reduction on surface in Figure 2.

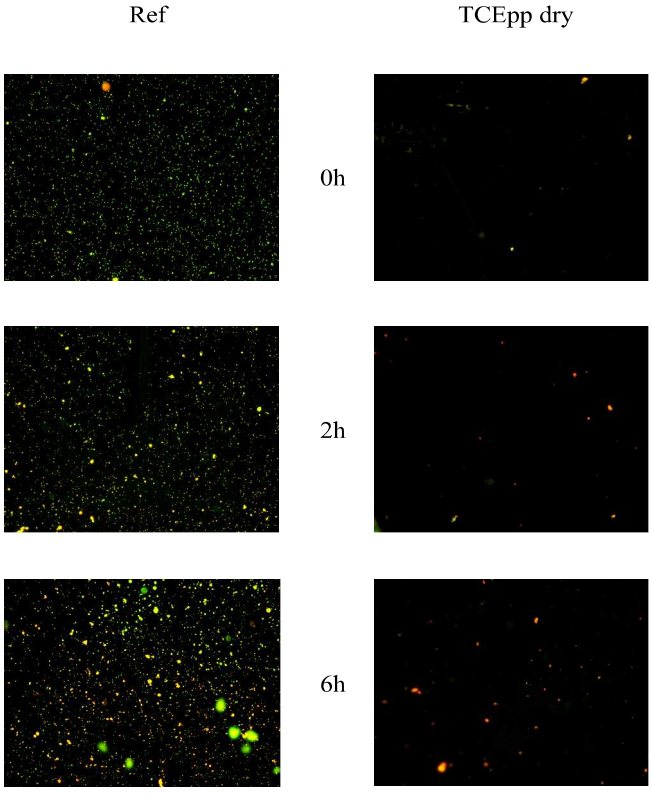

Figure 3: BacLight time lapse of Ref and TCEpp dry after 0, 2 and 6 h (10x magnification)

These coatings undergo chemical changes upon contact with water as could be seen from the XPS and Tof-Sims results; nevertheless, this did not affect their excellent short and long term antibacterial properties showing that TCEpp performs well even after extensive washing. Time lapse bacterial testing revealed the immediate reduction of bacteria, an effect that persisted even after 24 h. This most likely hints at a fast killing mechanism, dispatching the bacteria before any adhesion to the surface can take place. The beneficial qualities of plasma polymerization, combined with the affordability of the monomer and potential for scale-up make this method commercially attractive. Possible applications could be the coating of surgical gowns or air filters, to name a few.

In summary, this study showed that "one step" plasma polymerization of TCE leads to robust antibacterial surface coatings, which exhibit excellent, fast acting, antibacterial activity regardless of being washed with water or not. The mechanism of action, as well biocompatibility and range of other chlorinated monomers will be published in a follow up study.

Notes and references

[a] Ian Wark Research Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[b] Mawson Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[c] Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, QLD 4059, Australia

/
†
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

1. H. C. Flemming and G. Schaule, *WERKSTOFFE UND KORROSION-MATERIALS AND CORROSION*, 1994, **45**, 29-39.
2. I. Banerjee, R. C. Pangule and R. S. Kane, *Advanced Materials*, 2011, **23**, 690-718.
3. B. Q. Liao, D. M. Bagley, H. E. Kraemer, G. G. Leppard and S. N. Liss, *Water Environment Research*, 2004, **76**, 425-436.
4. T. Desmet, R. Morent, N. D. Geyter, C. Leys, E. Schacht and P. Dubruel, *Biomacromolecules*, 2009, **10**, 2351-2378.
5. S. N. Jampala, M. Sarmadi, E. B. Somers, A. C. L. Wong and F. S. Denes, *Langmuir*, 2008, **24**, 8583-8591.
6. S. Sarghini, S. Paulussen and H. Terryn, *Plasma Processes and Polymers*, 2011, **8**, 59-69.

7. C. R. Arciola, D. Campoccia, P. Speziale, L. Montanaro and J. W. Costerton, *Biomaterials*, 2012, **33**, 5967-5982.
8. K. Vasilev, S. S. Griesser and H. J. Griesser, *Plasma Processes and Polymers*, 2011, **8**, 1010-1023.
9. M. Rossberg, W. Lendle, G. Pfleiderer, A. Tögel, E.-L. Dreher, E. Langer, H. Rassaerts, P. Kleinschmidt, H. Strack, R. Cook, U. Beck, K.-A. Lipper, T. R. Torkelson, E. Löser, K. K. Beutel and T. Mann, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2000.
10. M. Vásquez, G. J. Cruz, M. G. Olayo, T. Timoshina, J. Morales and R. Olayo, *Polymer*, 2006, **47**, 7864-7870.
11. W. Lu, T. Cao, Q. Wang and Y. Cheng, *Plasma Processes and Polymers*, 2011, **8**, 94-99.
12. H. J. Griesser, *Vacuum*, 1989, **39**, 485-488.
13. T. M. Blättler, S. Pasche, M. Textor and H. J. Griesser, *Langmuir*, 2006, **22**, 5760-5769.
14. U. Oran, S. Swaraj, A. Lippitz and W. E. S. Unger, *Plasma Processes and Polymers*, 2006, **3**, 288-298.
15. M. Lejeune, F. Brétagnol, G. Ceccone, P. Colpo and F. Rossi, *Surface and Coatings Technology*, 2006, **200**, 5902-5907.
16. T. R. Gengenbach, R. C. Chatelier and H. J. Griesser, *Surface and Interface Analysis*, 1996, **24**, 271-281.
17. K. Vasilev, L. Britcher, A. Casanal and H. J. Griesser, *The Journal of Physical Chemistry B*, 2008, **112**, 10915-10921.
18. A. Barnett, B. Dave, G. A. Ksander and L. M. Vistnes, *Journal of Surgical Research*, 1986, **41**, 326-332.
19. J. Friedrich, *Plasma Processes and Polymers*, 2011, **8**, 783-802.
20. C. M. Chan, T. M. Ko and H. Hiraoka, *Surface Science Reports*, 1996, **24**, 1-54.

