A contact active bactericidal stainless steel was synthesized in water utilizing phenol electrodeposition, followed by covalent attachment of quaternary ammonium salts. The approach minimizes the amount of the antimicrobial agent and avoids its release into the environment. Gram negative and Gram positive bacteria were inactivated upon contact with the modified surface.

Bacterial build up on steel surfaces is a serious problem in a number of industries, including food storage and processing devices, medical instruments, and ship hulls. Contact active antimicrobial materials provide a new, promising approach for reducing bacterial adhesion and proliferation. The approach involves a durable (usually covalent) linkage of an antimicrobial moiety to a material’s surface. Unlike superhydrophobic surfaces that do not allow for the attachment of bacteria and have proven to be an elegant solution in some recent reports, contact active materials can be used in applications where the physical elimination of pathogens is necessary. Being surface linked, the antimicrobial agent is not readily released into the bulk material that would be in contact with the coated surfaces, and is not released into the environment. The modified materials often retain their antimicrobial activity after multiple usages. In addition, the approach minimizes the amount of active material needed to achieve antimicrobial protection. Due to these environmental and operational advantages that satisfy an important green chemistry criterion, contact active antimicrobial materials are of high research and applicative interest for their possible use in a large number of innovative and non-toxic products. Quaternary ammonium salts (QAS) have long been recognized as a general solution for disrupting the membranes of a large range of bacteria. Their attachment to surfaces is a strategy for the synthesis of non-leaching antibacterial materials that minimize both the evolution of bacterial resistance and mammalian toxicity.

Recently, a number of techniques were developed for conferring antibacterial activity to stainless steel. However, usually such modifications require expensive substrates such as peptides or hyperbranched polymers, a good number of synthetic methods was also reported. For instance, an interesting report on creating QAS surfaces on steel with cold plasma led to excellent antimicrobial activity, however several modification steps were required. An additional interesting approach suggested a vapour polymerization of active materials.

In the present report, we aimed at conferring antibacterial activity to steel by surface modification with QAS, with as few synthetic steps as possible that utilized water as the solvent in order to address cost and environmental concerns, thus satisfying a green chemistry criterion in industrial applications. The QAS salt can be modified with a trialkoxysilyl moiety that can attach to a hydroxylated surface, in a stable polydentate fashion. Previous reports from our group have demonstrated the effectiveness of this substrate in antibacterial applications on plastic, glass, and naturally occurring polymer surfaces. In order to create a stainless steel metal surface with OH moieties, the steel would normally need to be activated by ozone or air plasma. In this work, we exploited electrodeposition to functionalize stainless steel and provide support for the subsequent covalent attachment of the active material.

Modification of stainless steel

In order to form an OH layer on the surface via a sustainable and easily accessible method, a layer of p-diazonium salt of...
phenol\textsuperscript{18} was electrodeposited on stainless steel via three CV scans to form an activated SS-phenol, \textsuperscript{1} (Fig. 1).

The reaction occurred in water in which the starting diazonium salt is soluble. As in previous reports on the electrografting of diazonium salts\textsuperscript{13,14,18} a small negative potential on the first scan (0.04 V vs. SCE) identifies the one electron reduction potential. As the surface of the steel becomes covered with phenol moieties, the current is further reduced (and a slightly greater reducing potential is required) on the second and third scans by half from 1.2 × 10^{-4} A to 6 × 10^{-5} A, which is typical of other reports where CV scans are provided for similar diazonium salt substrates that were deposited on various metal (chromium, gold, etc.) electrodes.\textsuperscript{17}

After cleaning via rinsing in deionized water and ultrasonication for 10 minutes, the surfaces were dipped for 24 h in a solution of silylated QAS\textsuperscript{2} to bind the substrate via a strong chelating effect to the phenol modified surface and thus form the functionalized SS-QAS, \textsuperscript{3} (Fig. 2).

The formation of a carbon–metal bond on surface utilizing electrodeposition offers an elegant and materially economical way to functionalize metal surfaces. The technique is very rapid and requires very little amount of the activating agent (diazonium salt in this case) and ultimately of the antimicrobial agent (QAS in this case). This approach results in the formation of a covalently grafted antimicrobial layer on the metal surface, invisible to the naked eye.

Characterization of the prepared materials

The prepared materials were characterized by X-ray photoelectron spectroscopy (Fig. 3). Fig. 3(left) shows the fitted high-resolution N 1s XP spectrum for the phenol activated stainless steel \textsuperscript{1}. The band at 397.9 eV refers to the substrate metal nitride Fe\textsubscript{3}N–Fe\textsubscript{2}N. The presence of nitrogen (metal nitride) in steel is a well-known phenomenon and this observation indicates that actually with XPS we are able to probe the whole film thickness. In addition, there is also some presence of the unreacted diazonium salt (N\textsubscript{2}\textsuperscript{+} BF\textsubscript{4}\textsuperscript{-}) used for the electrodeposition process (band at 399.6 eV). Fig. 3(right) shows the fitted high-resolution N 1s XP spectrum for the modified stainless steel-QAS \textsuperscript{3} material. The main component lies at 402.2 eV and is due to the quaternized nitrogen of the C\textsubscript{18}H\textsubscript{37}N\textsuperscript{+} group.\textsuperscript{19–22} The band at 398.2 eV is due to residues of metal nitride. The band at 399.5 eV is due to residues of the unreacted diazonium salt.

XPS atomic concentration analysis reveals that the 402.2/399.5 eV band intensity ratio is 2.6:1. Since the band at 399.5 eV refers to the diazonium salt that has two nitrogen atoms per formula unit, it reveals that the unreacted diazonium units are only 1/5 of the C\textsubscript{18}H\textsubscript{37}N(CH\textsubscript{3})\textsuperscript{2}+ groups present on the substrate surface. This observation was substantiated by the XPS atomic concentration analysis of the Si peak which was found to be identical to that of the quaternized nitrogen.

The contact angle of the modified QAS stainless steel \textsuperscript{3} was measured (65.2° ± 1.61) and found to be similar to that of the original stainless steel (65.13 ± 1.06).

The stainless steel AFM morphology shows a typical turned metal surface. Therefore the evident surface pattern is just due to the starting surface material. AFM studies do not reveal drastic alternations on the surface morphology upon addition of the antimicrobial layer (Fig. 4). The R\textsubscript{q} of the non-modified surface was found to be 37.2 and the R\textsubscript{q} of the modified surface \textsuperscript{3} was 47.4.
Antimicrobial activity

The effect of the modified SS-QAS 3 on the survival of Gram negative E. coli bacteria was examined using a method that was specifically developed by us. A bacterial suspension was contacted with a material surface and samples were plated after 30 min. The number of CFUs was quantified and is presented as a percentage of viable units with respect to the control sample in Fig. 5.

The antimicrobial effect of the modified SS-QAS 3 surface was found to be dramatic for E. coli. The bacterial count after contact with the SS-QAS 3 surface was found to be 2.32log_{10}CFU ml⁻¹ and the bacterial count after contact with the untreated stainless steel was found to be 5.64log_{10}CFU ml⁻¹, which point to the inactivation of 99.9% of bacteria. The phenol modified steel 1 was not found to be active in diminishing the viability of bacteria, and in fact may have promoted bacterial growth (log_{10}CFU ml⁻¹ is 6.01).

Confocal microscopy studies were carried out on the unmodified stainless steel, phenol modified stainless steel 1 and the QAS-grafted stainless steel 3 (Fig. 6). The QAS functionalized SS surfaces show a drastic reduction of visible bacteria that is consistent with numerical data of Fig. 5. The studies were carried out on the B. subtilis cells (YC161 with P_{spank}gfp) strain that produces fluorescent GFP constitutively (Fig. 6).

The antimicrobial effect of the SS-QAS 3 on human pathogen B. cereus was examined. The bacterial count after contact with the modified surface was found to be 4.40 log_{10}CFU ml⁻¹ and the bacterial count after contact with the untreated stainless steel was found to be 5.06log_{10}CFU ml⁻¹, which point to the inactivation of 70% of bacteria. It was further shown that another pathogenic bacterium, Pseudomonas aeruginosa PA14, which is considered as an extremely problematic drug resistant bacterium, is affected upon contact with the modified stainless steel surface. Nearly 90% of bacteria was inactivated upon contact with the SS-QAS 3 surface, following overnight incubation in an LB medium, as compared with the control stainless steel sample. The bacterial count upon contact with the modified surface was found to be 6.79log_{10}CFU ml⁻¹, while the bacterial count after contact with the untreated stainless steel was 7.69log_{10}CFU ml⁻¹.

A recent report has highlighted that resistance to QAS can be developed by some bacteria and fungi. We believe that our approach can be tailored to overcome the resistance problem. Firstly, a number of different agents can be attached to a modified steel–OH surface at the same time. These agents may include modified antibiotic or other antimicrobial compounds that would work in concert with the QAS. Secondly, agents that involve toxicity and/or environmental concerns can be used since, in the case of covalently bonded surface units, leaching is minimized. In contrast, their use would be problematic if they were sprayed on surfaces. Thus we are confident that in future work, the toolbox of other acceptable antimicrobial and anti-fungi agents can be expanded via the covalent attachment to functionalized surfaces.

Conclusions

To conclude, a contact active antimicrobial stainless steel was prepared via a green and sustainable process. The synthesis was carried out by electrodeposition in water followed by covalent attachment of antimicrobial QAS in a neat solution of the QAS precursor material without the need for any solvent. The resulting modified surface thus obtained is active against a representative Gram negative and Gram positive bacteria species.

The covalently attached active layer has the advantages of minimizing release to the environment, and requiring little material to synthesize. We hope that the approach presented...
here may serve as a platform for the sustainable formation of antimicrobial contact active metal surfaces and lead to further research and applications.

Acknowledgements

The research leading to these results has received funding from the ARO Director Strategic Program Fund, grant no. 421-0281-14 and from The United States–Israel Agricultural Research and Development Fund, BARD grant no. US-4680-13C and contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, 690/14. We would like to thank Prof. Rony Wallach for help in contact angle measurements. E. Poverenov and M. Shemesh are members of the EU COST Action FA1202: a European Network for Mitigating Bacterial Colonisation and Persistence on Foods and Food Processing Environments (http://www.bactfoodnet.org/) and acknowledge this action for facilitating collaborative networking that assisted in this study.

Notes and references


