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Bactericidal contact active stainless steel via a sustainable process utilizing electrodeposition and covalent attachment in water

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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 allows minimizing 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 was 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 steps to synthesize and/or attach the active substrate, and the use of organic solvents. An activation of stainless steel by non-synthetic methods was also reported. For instance, an interesting work 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 this work we aimed to confer antibacterial activity to steel by surface modification with QAS with as few synthetic steps and utilizing water as solvent to benefit cost and environmental concerns, thus satisfying a green chemistry perennial concern in industrial applications. The QAS salt can be modified with a trialkoxysilyl moiety that can attach to a hydroxylated surface, in a stable tridentate 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 was electrodeposited on stainless steel via three CV scans to form an activated SS-phenol, 1 (Figure 1).
Figure 1. Electrodeposition of diazonium salt. Scan rate 0.25 mV/s.

The reaction occurred in water in which the starting diazonium salt is soluble. As in previous reports on the electrografting of diazonium salts, 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 1.2E-4 A to 6E-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. 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 2 to bind the substrate via a strong tridentate chelating effect to the phenol modified surface and to form the functionalized SS-QSA, 3 (Figure 2).

Figure 2. Grafting antibacterial QAS to a stainless steel surface.

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

Characterization of the prepared materials

The prepared materials were characterized by X-ray photoelectron spectroscopy (Figure 3). Figure 3a shows the fitted high-resolution N 1s XP spectrum for the phenol activated stainless steel (1). The band at 397.9 eV refers to the substrate metal nitride Fe\textsubscript{n}N-Fe\textsubscript{n}N. The presence of nitrogen (metal nitride) in steel is a well known phenomenon and this observation indicate that actually with XPS we are able to probe the whole film thickness. In addition, there is also some presence of not reacted diazonium salt (N\textsubscript{2} BF\textsubscript{4}) used for the electrodeposition process (band at 399.6 eV). Figure 3b shows the fitted high-resolution N 1s XP spectrum for the modified stainless steel-QAS (3) material. The main component lies at 402.2 eV and is due to the quaternized nitrogen of C\textsubscript{18}H\textsubscript{37}N\textsuperscript{+} group. The band at 398.2 eV is due to residues of metal nitride. The 399.5 eV is due to residues of non-reacted 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 emerges that the not reacted diazonium units are only 1/5 of the C\textsubscript{18}H\textsubscript{37}N(CH\textsubscript{3})\textsubscript{3} groups present on the substrate surface. This observation was substantiated by the XPS atomic concentration analysis of the Si peak was found to be identical to that of the quaternized nitrogen.

Figure 3. Al Kα excited XPS of left) SS-phenol, 1; right) SS-QSA, 3 in the N 1s energy region. The black dots represent the experimental profile. The red line superimposed to the experimental profile refers to the sum of the components. Left) The magenta and blue lines refer to the 397.9 and 399.6 eV components, respectively. Right) The magenta, blue and green lines refer to the 398.2, 399.5 and 402.2 eV components, respectively.

The contact angle of the modified QAS stainless steel 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 (Figure 4). The Rq of the non-modified surface was found to be 37.2 and the Rq of the modified surface 3 was 47.4.

Figure 4. Topographic imaging of the original stainless steel (left) and the modified SS-QAS, 3 (right) performed in the tapping mode with a scan rate of 1.0 Hz at fixed resolution (512 × 512 data points).

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 CFU was quantified and is presented as percentage of viable units with respect to the control sample in Figure 5.
Figure 5. Survival of E. coli ATCC 25922 after treatment with control and modified materials. The results represent the mean and standard errors (SD) of three independent biological experiments done in triplicates.

The antimicrobial effect of the modified SS-QAS, 3 surface was found to be dramatic for E. coli. The bactericidal count after contact with the SS-QAS, 3 surface was found to be 2.32 Log_{CFU/ml} and the bacterial count after contact with the untreated stainless steel was found to be 5.64 Log_{CFU/ml}, which point on 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_{CFU/ml} is 6.01).

Confocal microscopy studies were carried out on the unmodified stainless steel, phenol modified stainless steel 1 and the QSA-grafted stainless steel 3 (Figure 6). The QAS functionalized SS surfaces show a much drastic reduction of bacterial numbers that is consistent with the numerical data of Figure 5. Confocal microscopy studies were carried out on the B. subtilis cells (YC161 with P\text{~}_{spank}^{-}gf)\text{\textsuperscript{3}} strain that produces fluorescent GFP constitutively (Figure 6). The QAS functionalized SS, 3 shows drastic reduction of bacterial numbers that is consistent with the numerical data of Figure 5.

Figure 6. CSLM images of fluorescently tagged B. subtilis cells (YC161 with P\text{~}_{spank}^{-}gf)\text{\textsuperscript{3}} after 3 h contact with unmodified stainless steel (left) and SS-QSA, 3 (right) visualized under an Olympus IX81 confocal laser scanning microscope (CLSM, Japan).

The antimicrobial effect of the SS-QAS, 3 was examined on human pathogen B. cereus. The bacterial count after a contact with the modified surface was found to be 4.40 Log_{CFU/ml} and the bacterial count after contact with the untreated stainless steel was found to be 5.06 Log_{CFU/ml}, which point to the inactivation of 70% of bacteria. It was further shown that another pathogenic bacterium such as the Pseudomonas aeruginosa PA14\text{\textsuperscript{4,5}} which is considered as an extremely problematic drug resistant bacterium is affected upon contact with the modified stainless steel surface. Nearly the 90% of the bacteria was inactivated upon contact with SS-QAS, 3 surface, following the overnight incubation in LB medium, as compared with the control stainless steel sample. The bacterial count upon contact with the modified surface was found to be 6.79 Log_{CFU/ml} while the bacterial count after contact with the untreated stainless steel was 7.69 Log_{CFU/ml}.

A recent report has highlighted that resistance to QAS can be developed by some bacteria and fungi.\textsuperscript{26} 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 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 covalent attachment to functionalized surfaces.

Conclusions

To conclude, 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 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 a sustainable formation of antimicrobial contact active metal surfaces and could lead to further research and applications.

Notes and references

Acknowledgements

The research leading to these results has received funding from the ARO Director Strategic Program Grant, Fund No. 421-0281-14 and from The United States-Israel Agricultural Research and Development Fund, BARD Grant No. US-4680-13C. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, 690 /14. We would like 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.bacfoodnet.org/) and acknowledge this action for facilitating collaborative networking that assisted with this study.

Electronic Supplementary Information (ESI) available: [general procedures; methods and materials; description of experiments]. See DOI: 10.1039/c000000x/


A contact active bactericidal stainless steel synthesised in water utilizing phenol electrodeposition is effective against Gram negative and positive bacteria.