Electroenhanced Antimicrobial Coating Based on Conjugated Polymers with Covalently Coupled Silver Nanoparticles Prevents Staphylococcus aureus Biofilm Formation

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The incidence of hospital-acquired infections is to a large extent due to device-associated infections. Bacterial attachment and biofilm formation on surfaces of medical devices often act as seeding points of infection. To prevent such infections, coatings based on silver nanoparticles (AgNPs) are often applied, however with varying clinical success. Here, the traditional AgNP-based antibacterial technology is reimagined, now forming the base for an electroenhanced antimicrobial coating. To integrate AgNPs in an electrically conducting polymer layer, a simple, yet effective chemical strategy based on poly(hydroxymethyl 3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT-MeOH:PSS) and (3-aminopropyl)triethoxysilane is designed. The resultant PEDOT-MeOH:PSS–AgNP composite presents a consistent coating of covalently linked AgNPs, as shown by scanning electron microscopy and surface plasmon resonance analysis. The efficacy of the coatings, with and without electrical addressing, is then tested against Staphylococcus aureus, a major colonizer of medical implants. Using custom-designed culturing devices, a nearly complete prevention of biofilm growth is obtained in AgNP composite devices addressed with a square wave voltage input. It is concluded that this electroenhancement of the bactericidal effect of the coupled AgNPs offers a novel, efficient solution against biofilm colonization of medical implants.

Indwelling devices have become key elements in the treatment of a wide variety of medical conditions with uses spanning from complementing or substituting compromised body functions to the targeted delivery of diagnostic and therapeutic agents.[1–3] These benefits are, however, compromised by the risk of device-associated infections. Bacteria may colonize the surfaces of indwelling devices in the form of biofilms. These resilient sessile communities anchor bacteria to the implant by embedding them in an extracellular polymeric matrix.[4] As the biofilm protects bacteria from the action of antibiotics, there is a prominent risk of biofilms to serve as persistent foci of infection.[5] Biofilm contamination of indwelling devices is often of nosocomial origin, with Staphylococcus aureus (S. aureus), ubiquitously present in the skin of patients and healthcare workers, listed as one main cause.[6] Infections associated to biofilm contamination of indwelling devices result in high morbidity, mortality, and economic losses.[7–9]

Intense research has been devoted to develop materials that minimize biofilm colonization.[10,11] Solutions are diverse, ranging from antifouling surfaces with specific physicochemical properties to bactericidal coatings with attached or releasable antimicrobial compounds.[10,11] One of few strategies that have been commercially translated is the use of silver nanoparticles (AgNPs), which upon oxidation release Ag⁺ ions. Based on the continuous release of Ag⁺ ions, catheters coated with AgNPs show improved protection against microbial colonization.[12–16] However, several studies have shown the potential toxicity of Ag⁺ ions, such as induction of DNA damage, denaturation of proteins, and stimulation of the production of reactive oxygen species.[16,17] These negative effects have, however, not been observed in studies of commercially available products.[5,18] One explanation may be that silver is released at very low levels, as shown by the accumulated release of 0.38–0.45 µg silver per cm length of subcutaneous catheters after 10 d in mice.[18] However, there is a strong consensus in the field that silver toxicity may be a concern, and that the antibacterial efficacy of AgNP-based coatings is far from optimal.[16]

Recently, the application of an electrical addressing has been shown to increase the bactericidal effect of certain compounds, particularly when targeting bacterial biofilms.[19,20] Electrical addressing is achieved by applying an electrical signal to conducting materials in contact with bacteria. Whereas the exact mechanism of action remains unknown, several hypotheses have been proposed to explain the joint effect of antimicrobials and an electrical addressing. These include an increase in bacterial membrane permeability by electroporation, increased delivery of the antimicrobial agent throughout the extracellular matrix via electrophoretic transport, electrochemically triggered changes of the components in the extracellular matrix, and enhanced antibiotic susceptibility due to the increase of bacterial metabolism by electrochemically generated oxygen, to
mention a few. Despite the great potential of this combined strategy, clinical translation of this technology is hindered by the current use of metal electrodes, which is incompatible with the requirement of mechanical flexibility of most indwelling devices. In addition, only antimicrobials applied directly in solution form have been tested so far, which is impractical due to the lack of sustained release. Novel, chemically versatile conducting materials are thus needed to enable clinical application of this combined technology.

Conducting polymers are electrically conductive and mechanically flexible materials with a rich surface chemistry. Of particular relevance is the poly(3,4-ethylenedioxythiophene) (PEDOT) family of conducting polymers. With high electrical conductivity, chemical stability, and a wide palette of functional variants, PEDOT has shown great potential for medical applications. Moreover, PEDOT is compatible with mass-production techniques such as dip-coating and roll-to-roll printing, rendering it suitable for commercial applications.

In this study, we combine AgNPs and a PEDOT-based conducting polymer to generate surface coatings with electrophysical and antimicrobial activity. We based our approach on the combination of market-established products in order to produce a feasible solution for clinical applications. To construct the conducting polymer–AgNP composite, we employed the commercially available hydroxymethyl 3,4-ethylenedioxythiophene (EDOT-MeOH) monomer (Figure 1a). Whereas this compound is mainly used as precursor in the synthesis of more elaborate functional chemical constructs, we analyzed its use as a functional material on its own, utilizing the hydroxyl moiety for custom tailoring. First, we generated a semitransparent, robust, electrically conducting poly(3-hydroxymethyl 3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT-MeOH:PSS) coating by electropolymerizing a mix of EDOT-MeOH and the thoroughly tested counterion PSS (Figure 1b) on top of a PEDOT:PSS-coated polyethylene terephthalate (PET) (Orgacon) (Figure 1c, lower part). In the electropolymerization reaction, monomers are electrochemically oxidized to their radical cations, which leads to the formation of dimers and subsequently oligomers. This is followed by nucleation and growth of the oligomers in the working electrode, eventually forming an insoluble polymer layer.

Next, an amino-functionalized surface was obtained through a standard, aminosilane-based functionalization via the hydroxyl moieties of the PEDOT-MeOH:PSS coating. When this method is applied in glass and metal oxides, the hydroxyl groups must first be generated by oxygen plasma or chemical pretreatment. The intrinsic presence of hydroxyl moieties in PEDOT-MeOH eliminated the need of such pretreatment, thereby avoiding potential degradations in polymer conductivity. We were therefore able to couple a layer of (3-aminopropyl)trimethoxysilane (APTES) linker to the polymer surface using just the standard hydrolysis and heat-curing steps (Figure 1c, middle part). This resulted in an expected, marked increase in the hydrophobicity of the surfaces (data not shown). AgNPs were next attached to the APTES linker by immersing the amino-functionalized surfaces in a dispersion of citrate-coated silver nanospheres with a reported size distribution ranging between 40 and 70 nm and centered at 50 nm. To prevent agglomeration of the nanosphere dispersion during the chelation reaction, incubation was performed in the dark at 4 °C. By allowing the chelation reaction to proceed for 4 d, coordinate bonds between AgNPs and the amines in APTES were formed, thereby generating the AgNP-functionalized PEDOT-based conducting polymer PEDOT-MeOH:PSS–AgNPs (Figure 1c). This material is referred to as the “AgNP composite” throughout the text.

Next, we evaluated the surface morphology of the AgNP composite, starting by distinguishing the particular features of the AgNP composite in comparison to the control PEDOT-MeOH:PSS, which is referred to as “plain conjugated polymer” throughout the text. We also included a second control surface referred to as “pseudocomposite control” to evaluate the nature of the AgNP functionalization. The synthesis scheme of this material was exactly the same as for the AgNP composite except for the replacement of APTES with absolute ethanol. In the absence of the APTES linker, covalent coupling of AgNPs to the surface was eliminated, and AgNP functionalization relied only on physisorption and physical entrapment to remain in the surface. To assess the surface morphology, we first applied scanning electron microscopy (SEM). The AgNP composite showed exposed silver nanospheres mostly in the 100 nm range (Figure 1d). The size increase from ≈50 nm of AgNPs in dispersion to ≈100 nm when surface-bound produced a maximum reduction of the surface-to-volume ratio of ≈50%. Despite this decrease, nanoparticles of 100 nm are known to present very large surface-to-volume ratio, which is key in the distinct behavior of nanoparticles compared to bulk material. We also noted a homogeneous AgNP coverage, with no major uncoated gaps. When analyzing the pseudocomposite control surface with SEM, no silver nanospheres were observed (Figure 1e). Instead, the pseudocomposite control showed similar surface inhomogeneity as what is naturally present in the plain conjugated polymer (Figure 1f). Collectively, this shows that there is very little, if any, unspecific adsorption and physical entrapment of AgNPs in the conducting surfaces.

To confirm the findings from the SEM analysis, we studied the surface plasmon resonance (SPR) response of the synthesized surfaces. When excited with an incident light of a particular wavelength, metal nanoparticles exhibit resonant oscillations of conduction electrons. As this generates a characteristic absorption, shown as a peak at the resonant wavelength, SPR measurements have become a standard method for identification of the size and morphology of nanoparticles both in the colloidal and surface-bound state. To characterize the absorption profiles of the produced surfaces, strips (3.2 cm × 1 cm) from the AgNP composite, the plain conjugated polymer, and the pseudocomposite control were placed individually in wells of a 6-well plate (Figure 1g). By covering the strips in a sodium citrate solution, the dispersion medium of the original nanoparticle colloid, the refractive index of the liquid phase interfacing surface-bound nanoparticles and nanoparticles in suspension was equalized. This allowed direct comparisons of the absorbance profiles to be made. After positioning the 6-well plate in a microplate reader, the single-point absorbance spectra were recorded. A distinct resonant peak at 426 nm was observed in all three replicates of the AgNP composite (Figure 1h). This peak closely matched the peak
of colloidal AgNPs in dispersion, which appeared at 418 nm in all replicates. The 8 nm difference between surface-bound and colloidal AgNPs is accounted for by the small variations in particle size and the presence of the APTES coating. The difference in absorbance between the resonant peak of the AgNP composite and the plain conjugated polymer at 426 nm was 0.396 \pm 0.029 arbitrary units (a.u.) (mean \pm standard error of the mean (SEM), n = 3), whereas the difference in absorbance of colloidal AgNPs and the sodium citrate solution at 418 nm was 0.776 \pm 0.001 a.u. (mean \pm SEM, n = 3). Importantly, the pseudocomposite control and the plain conjugated polymer control showed overlapping curves with no resonant peaks. This demonstrates that nanoparticles do not become physiosorbed or physically entrapped on the PEDOT surface. Taken together, our results confirm the utility of our synthesis scheme to generate a PEDOT-based surface functionalized with AgNPs through covalent coupling.

As our goal is to generate an antimicrobial coating that can be applied over large areas, the synthesis of AgNP-functionalized PEDOT-based conducting polymer must generate close to complete coverage. To assess the presence of AgNPs on the complete strip, we performed spatial absorbance scans at 426 nm. By showing the results as a topological heatmap, we were able to generate a macroscopic picture of AgNP coverage. A high predominance in red color (high absorbance at 426 nm) was observed along the surface of the AgNP composite, which contrasted with the dark blue (negligible absorbance) of the transparent bottom of the polystyrene well (Figure 1i, AgNP composite). This indicated the presence of resonance peaks across the entire AgNP composite strip, confirming an
extensive AgNP coverage. Conversely, a light blue/green predominance (low absorbance at 426 nm) was found across the pseudocomposite control (Figure 1i, Pseudocomposite) and the plain conjugated polymer (pCP) strips (Figure 1i, pCP). This result, as can also be observed in the single-point absorbance spectrum, corresponded to the intrinsic absorbance of PEDOT-MeOH:PSS, thus indicating a lack of AgNPs. Taken together, these data validate that our novel, simple, and inexpensive procedure generates conducting polymer surfaces with a consistent coating of covalently linked silver nanospheres across large areas.

Next, we characterized the electrochemical behavior of our synthesized surfaces under conditions relevant for experiments with bacterial cultures. A series of cyclic voltammetry analyses were performed, using the rich culturing medium tryptic soy broth (TSB) as supporting electrolyte and each of the three surfaces under study as working electrodes. We first analyzed the electrochemical behavior of the plain conjugated polymer. A stable, slow-varying response was obtained, showing a monotonically increasing current in the anodic direction that accounted for the progressive oxidation of the polymer chains (Figure 2a). The curve then shifted into a plateau, which signified the saturation of the oxidation process. Polymer oxidation was then reverted in the cathodic direction, with the monotonically decreasing current indicating the return of the polymer chains to their neutral state. At $-0.3\, \text{V}$, polymer reduction became noticeable as the current magnitude dropped due to the increased amount of poorly conductive, neutral polymer chains. No steep current peaks were observed in the voltammogram, indicating that no electrochemical reactions occurred in any redox couple within the supporting conducting polymer or in the TSB medium.

We then investigated whether coupled AgNPs affected the electrochemical behavior of the polymer using the AgNP composite as working electrode. A series of peaks were found in the anodic and cathodic directions (Figure 2b). To determine the origin of these peaks, we constructed a simpler system where a AgNP suspension was physically adsorbed by evaporation over an indium tin oxide (ITO) electrode, known to present little reactivity on the potential window under study. The AgNP–ITO construct presented a large irreversible anodic peak at 0.15 V followed by smaller cathodic and anodic peaks at $-0.16$ and 0.16 V, respectively (Figure 2c). This pattern closely matched the distinct features of the AgNP composite, suggesting oxidation of AgNPs into Ag$^+$ as the cause of the differences with respect to the plain conjugated polymer. We can thus interpret the AgNP composite response shown in Figure 2b as a Ag$^+$ ion delivery system with two different dynamics. The first is represented by the large, anodic irreversible peak at 0.23 V during the initial voltammetric cycle, which accounts for an intense, rapid oxidation of AgNPs into Ag$^+$ ions and their subsequent diffusion from the electrode. The second corresponds to a slower, steady release, as indicated by the subsequent series of smaller peaks at 0.17 V with amplitude decreasing on every cycle. Certain

![Figure 2](image-url). Electrochemical characterization of surfaces and devices. a–c) Cyclic voltammetry between $-0.5$ and 0.5 V in TSB medium of (a) PEDOT-MeOH:PSS plain conjugated polymer, (b) AgNP composite, and (c) AgNP–ITO–PET construct electrode using a scan rate 50 mV s$^{-1}$. d) Photograph of a connectorized culturing device fitted in a standard 90 cm Petri dish. e,f) Current response (red, left axis) upon the application of a 2 V step signal (black, right axis) in (e) a plain conjugated polymer device, and (f) a AgNP composite device. Insets illustrate the initial dynamics. g,h) Short-term and long-term current responses (left axis) of (g) a plain conjugated polymer device and (h) a AgNP composite device upon the application of a 5 Hz, 4 Vpp square signal between $-2$ and $+2$ V (black, right axis). The short-term time is depicted in the abscissa, while the long-term time is color coded from blue (0 h) to red (24 h).
degree of reversibility seems to be present in this second dynamic, as indicated by the series of small peaks at −0.18 V in the cathodic direction. In comparison, larger current responses were obtained in the AgNP composite than in the AgNP–ITO construct, as noted by the different axis scales. This indicated a higher concentration of exposed nanoparticles in the AgNP composite, which together with the higher chemical stability of covalently coupled AgNPs, evidenced the superiority of our chemical strategy compared to simple physical adsorption.

Next, we shifted our focus from single surfaces to complete electrochemical systems, as these would better mimic the events occurring on implemented clinical devices. To study whether AgNP electroenhancement can be applied to influence biofilm growth, we constructed a custom-designed culturing device in which *S. aureus* was statically cultured at 37 °C for 24 h under electrical addressing of the surfaces. S. aureus biofilms form biofilms at the bottom solid–liquid interface, which prompted us to place the material under study at the base of the culturing device (Figure 2d). Two parallel 1 cm × 3.2 cm strips of the test material were glued with 2 mm separation to a 5.5 cm × 5.5 cm glass square. A 30 cm inner diameter glass ring was glued on top to generate a culture well. Part of the strips remained unenclosed, enabling the external electrical addressing of the device. The culturing device was then fitted in a standard 90 cm Petri dish, connectorized, and thermally disinfected by overnight storage at 70 °C.

To design an addressing scheme suitable for our 24 h experiments, we initially considered a constant voltage input, since it provides the maximum energy and presents simple propagation dynamics. An amplitude of 2 V was used, similar to previous studies.\(^\text{[19,20]}\) This constant 2 V input was applied to plain conjugated polymer and AgNP composite devices containing TSB. Real-time measurements of the current revealed steep decreases occurring in the first few seconds of the experiment for both types of devices (Figure 2e,f). This was attributed to the rapid increase in resistance of the electrochemically reduced polymer acting as cathode.\(^\text{[35]}\) A large electrical resistance is known to severely reduce the energy delivered by an electrical input, which would compromise any potential antibacterial effect.\(^\text{[44]}\) This prompted us to discard the use of a constant input voltage signal and to search for alternative addressing schemes.

To prevent the current decay, we designed an addressing scheme with the amplitude periodically alternating between −2 and +2 V. We hypothesized that this 4 V\(_{pp}\) (peak-to-peak volts) square signal would constantly exchange anode and cathode before any electrochemical reduction would occur, preventing the increase in electrical resistance. A frequency of 5 Hz was used, aiming to both minimize polymer reduction and remain close to a constant voltage in terms of signal energy and propagation dynamics. The designed 5 Hz, 4 V\(_{pp}\) square wave voltage input was then tested in plain conjugated polymer and AgNP composite devices containing TSB using a Keithley source-meter controlled by a custom LabVIEW program. This program constantly applied a specified input voltage while recording the resultant currents and voltages with two combined procedures. To study possible current decays due to polymer reduction, a fast data acquisition procedure was designed. This performed brief on-demand recordings with a high sampling rate, capturing the short-term electrical response. The fast acquisition procedure was executed at least hourly along the 24 h experiment as dictated by a control procedure, generating a large collection of readings. Once compiled, these readings were used to identify the long-term response of the device in order to assess possible current decreases from polymer degradation during the experiment. The recorded electrical response was summarized as a four-parameter plot, with current and voltage in ordinate axes, the short-term time in the abscissa, and the long-term time in color-code varying from blue to red (Figure 2g,h). As observed in the short-term response, the 5 Hz, 4 V\(_{pp}\) square wave excitation minimized current decay from polymer reduction in both the plain conjugated polymer and AgNP composite devices. In addition, the current closely followed the transitions in polarity of the voltage input, indicating undistorted signal propagation in both devices. The long-term response in the plain conjugated polymer device revealed a 28 ± 4% (mean ± SEM, *n* = 3) current decrease after 24 h. Most of this decrease occurred during the first 16 h of the experiment, while the overlapping yellow and red curves revealed no substantial change in the last 8 h. This suggested an eventual stabilization of the current response. Conversely, the AgNP composite device showed a 14 ± 5% (mean ± SEM, *n* = 3) current increase. This occurred primarily during the first hour, reflecting the gain in conductivity as the system stabilized. This different long-term response could be explained by the smaller initial current with respect to the plain polymer device, possibly caused by the resistance of the silane layer as well as by a partial polymer reduction by amine groups\(^\text{[45]}\) in the APTES linker. In addition, the presence of AgNPs in the AgNP composite may provide an alternative electrical path that would alleviate the strain on the polymer backbone and minimize current decrease. Taken together, our data show that the 5 Hz, 4 V\(_{pp}\) square wave voltage input generated current responses stable for 24 h in both the plain conjugated polymer and the AgNP composite devices. This validated our addressing scheme, as it provided consistent experimental conditions.

To test whether our designed surfaces can influence biofilm growth, we prepared fresh cultures of *S. aureus* strain ATCC 25923 in TSB medium. This is a quality control strain used in antimicrobial susceptibility testing according to the CLSI guidelines.\(^\text{[46]}\) After inoculation, we placed the culturing devices without any electrical addressing inside a 37 °C incubator to promote bacterial growth. After 24 h, the culture was decanted, and the device gently washed in phosphate buffered saline (PBS). This was followed by crystal violet staining to allow for simple visual inspection of the biofilm macromorphology in the devices. To evaluate the biofilm-forming capacity of the strain, we began by studying biofilm growth on PET, the mechanical support of all of our conjugated polymer-based constructs. We observed a homogeneous, dark violet mat covering the practical thickness, as well as unstained areas indicating the absence of...
biofilm growth. These localized inhomogeneities suggested a contact-dependent antimicrobial effect from coupled AgNPs. However, decreased biofilm growth was also found on the glass bottom of the culturing device, which showed large unstained areas compared to the glass bottom in the PET and the plain conjugated polymer devices. This suggested a dual antimicrobial effect based on bacterial contact to surface-coupled AgNPs as well as an effect caused by released Ag⁺ ions.
Next, we analyzed whether the designed 5 Hz, 4 V_{pp} square wave voltage input could be used to electroenhance the observed antimicrobial effect. We first focused on the study of biofilm growth on the electrically addressed plain conjugated polymer device. We applied the voltage input right after inoculation and maintained it during the 24 h of the experiment. To ensure an appropriate addressing during the full length of the 24 h bacterial cultivation, we monitored the applied voltages and currents in real time (Figure 3b). In the presence of bacteria, the current closely followed the voltage input except for minor current decays, matching the short-term response in noninoculated TSB (Figure 2c). Marked differences were found, however, in the long-term response. A continuous, large current decrease was observed in inoculated polymer devices, which indicated a bacteria-triggered loss of conductivity. To investigate the cause of this result, the surfaces were visually inspected at the end of the experiment. A violet hue was observed in strips exposed to the bacterial culture (Figure 3c, E+pCP), which contrasted with the light blue color of surfaces from noninoculated TSB medium (Figure 3c, E+pCP, medium only). Subsequent quantification with a color intensity analysis revealed significant variations in the red and green color channels (Figure 3d). These changes, which are consistent with the known electrochromic response of PEDOT,[26] demonstrate the ability of bacterial cells to electrochemically reduce the PEDOT polymer due to S. aureus ability of bacterial cells to electrochemically reduce PEDOT,[26] which indicates the utility of the system as a real-time sensor of bacterial colonization.

Having analyzed the effects of bacterial growth on the polymer system, we next analyzed the influence of the voltage input on biofilm growth. Upon staining of the addressed plain conjugated polymer device with crystal violet (Figure 3e, E+pCP), we observed an extensive, thick biofilm coverage (+) in the nonaddressed case (Figure 3a, pCP). In the presence of bacteria, the current closely followed the voltage input except for minor current decays, matching the short-term response in noninoculated TSB (Figure 2c). Marked differences were found, however, in the long-term response. A continuous, large current decrease was observed in inoculated polymer devices, which indicated a bacteria-triggered loss of conductivity. To investigate the cause of this result, the surfaces were visually inspected at the end of the experiment. A violet hue was observed in strips exposed to the bacterial culture (Figure 3c, E+pCP), which contrasted with the light blue color of surfaces from noninoculated TSB medium (Figure 3c, E+pCP, medium only). Subsequent quantification with a color intensity analysis revealed significant variations in the red and green color channels (Figure 3d). These changes, which are consistent with the known electrochromic response of PEDOT,[26] demonstrate the ability of bacterial cells to electrochemically reduce the PEDOT polymer due to S. aureus ability of bacterial cells to electrochemically reduce PEDOT,[26] which indicates the utility of the system as a real-time sensor of bacterial colonization.

We then investigated whether the voltage input could influence the antimicrobial properties of coupled AgNPs. Inoculated AgNP composite devices were electrically addressed for 24 h while recording the electrical response. Upon completion, crystal violet staining was applied. Only residual stained patches were found on the AgNP composite (Figure 3e, E+AgNP coroutine), indicating the nearly complete prevention of bacterial colonization. Large reductions in biofilm were also found on the glass bottom of the addressed AgNP composite culturing device compared to the nonaddressed case. This, together with the results of the electrochemical characterization (Figure 2a–c), points to the release of Ag ions as an important factor in the reduction of biofilm formation. As expected, no decrease in the long-term current, and accordingly no electron transfer, occurred (Figure 3f). This further confirms the use of the electrical recordings for real-time bacterial sensing.

To further analyze the results from the visual observations in Figure 3a,e, the biofilm mass was quantified. We cut a 1 cm × 1 cm square from each strip at a fixed position, extracted the crystal violet dye with acetic acid, and measured the resultant absorbance. Similar experiments performed in parallel using noninoculated TSB were used as blanks for the absorption readings of biofilm samples to allow for comparisons between the different types of surfaces. Quantification of the biofilm mass confirmed the results of the visual observations. Figure 3g shows that the plain conjugated polymer layer did not result in any appreciable differences with respect to the supporting PET substrate. Both promoted biofilm growth to a similarly high degree. However, a reduction in biofilm growth of ≈ 50% was achieved on the AgNP composite, clearly demonstrating the antibacterial effect of the coupled AgNPs. When analyzing the effect of electrical addressing, no reduction of biofilm growth was obtained in the addressed plain conjugated polymer. This confirmed that the designed 5 Hz, 4 V_{pp} square wave voltage did not induce any antimicrobial effect on its own. In contrast, an extremely low biofilm mass was found in the addressed AgNP composite. A reduction close to 90% was found with respect to the addressed plain conjugated polymer, and close to 80% with respect to the nonaddressed AgNP composite. These results demonstrate a synergistic effect of the coupled AgNPs and the applied electrical signal, as a result of the electroenhancement of the antibacterial effect of the coupled AgNPs.

Ag ions released from the surface into the medium may also exert antibacterial activity. To analyze a potential role for released Ag ions, we measured the concentration of Ag ions in the electrolyte after 24 h electrical addressing of the AgNP composite using a 5 Hz, 4 V_{pp} square wave. In this experiment, sodium nitrate was used as electrolyte to avoid formation of water insoluble silver salts occurring in complex TSB medium. Using square wave anodic stripping voltammetry (SWASV), the measured concentration of Ag ions in the electrolyte was 12.8 × 10^{-9} ± 6.3 × 10^{-9} M (mean ± SEM, n = 3). Interestingly, this concentration is well below the minimum inhibitory concentration (MIC, 100 × 10^{-9} M) and the minimum bactericidal concentration (250 × 10^{-9} M) reported for the studied strain.[48] Given that this number reflects the maximum Ag ion concentration appearing in the TSB medium of electrically addressed AgNP composites, one possible explanation of our data is that the antibacterial effect of Ag ions increases in the presence of an electrical current. Whether this electroenhanced antibacterial activity can be used to maximize the antibacterial effectiveness while minimizing silver-induced damage to mammalian cells remains to be analyzed.

In summary, we have developed an electrically conducting antibacterial coating where AgNPs were covalently bound to PEDOT-MeOH:PSS by an aminosilane linker. This constitutes a novel, simple method for conjugated polymer covalent functionalization where all the necessary reagents are commercially available. Major reductions in biofilm growth were obtained in the electrically addressed AgNP composite, surpassing the individual effects of electricity and coupled AgNPs. The cause seems to be largely based on the on-demand release of Ag ions, although other effects, like an electrically triggered increase in bacterial susceptibility toward silver,[19,20] cannot be discarded. Taken together, our results show the potential of electrical signals to improve current commercial AgNP-based antibacterial solutions, and offer novel, efficient technologies against biofilm colonization of implants.
Experimental Section

Materials: EDOT-MeOH, PSS, acetone, acetic acid, APTES, hydrochloric acid, sodium citrate, ITO-coated PET, TSB, crystal violet, sodium nitrate, and silver nitrate were purchased from Sigma-Aldrich (Stockholm, Sweden). Milli-Q water was purchased from Merck (Solna, Sweden). Orgacon, industrially produced PEDOT:PSS-coated PET was purchased from AGFA-Gaertner (Mortsel, Belgium). Absolute ethanol was purchased from Kemetyl AB (Jordbro, Sweden). PBS was purchased from Medicago AB (Uppsala, Sweden). Polydimethylsiloxane (PDMS) was prepared from Sylgard 184 Silicone Elastomer kit (base and curing agent), purchased from Dow Corning (Midland, Michigan, USA). AgNPs were purchased (NanoXact 50 nm, 0.02 mg mL−1 in 2 × 10−3 M sodium citrate solution) from nanoComposix (San Diego, California, USA). The 6-well plates (COSTAR 3516) were purchased from Corning Incorporated (Corning, New York, USA). The 96-well plates (83.9242) were purchased from Sarstedt. Glass squares and rings were purchased from Scientific Lab Class i Lund AB (Löberöd, Sweden).

Electropolymerized PEDOT-MeOH Surfaces: A mixture of EDOT-MeOH (0.02 m) and PSS (5 mg mL−1) in Milli-Q water was used as polymerization solution. Pieces of Orgacon were cut (4.5 cm × 3.5 cm, long × wide, with an immersed area of 12.6 cm2) and employed as working electrodes for the AgNP dispersion, and the sodium citrate solution was calculated at 418 nm. The resulting PEDOT-PSS polymer device is referred to as “plain conjugated polymer.”

Coupling of AgNPs: APTES was added (5%) to the 95% solution of absolute ethanol and Milli-Q water. The pH of the Milli-Q water was previously adjusted to 5 with acetic acid. The solution was added on top of strips (3.2 cm × 1 cm) cut from the plain conjugated polymer and left to incubate overnight in wells of a 6-well plate. Then, strips were washed in absolute ethanol and incubated at 70 °C for 24 h, followed by Milli-Q water rinsing and subsequent overnight incubation in Milli-Q water. The strips were then dried at 70 °C and covered with the suspension of AgNPs (4 mL of AgNP suspension per surface strip). The pH of the AgNP suspension in each strip was adjusted to ~4.5 with a 1:100 dilution of hydrochloric acid in Milli-Q water. The strips were left to incubate for at least 72 h at 4 °C, then thoroughly rinsed with Milli-Q water. The resulting PEDOT-MeOH:PSS–AgNP composite is referred to as “AgNP composite.” In addition, a similar procedure was performed where APTES was substituted with absolute ethanol. This material is referred to as “pseudocomposite control.”

Scanning Electron Microscopy: Pieces of AgNP composites, plain conjugated polymers, and pseudocomposite controls were glued to plastic supports using silver paint. A thin layer of gold was then deposited with an Agar sputter coater (Agar Scientific, Stansted, UK). The images were acquired with a Philips SEM 515 microscope (Philips, Amsterdam, Netherlands).

SPP Absorption Profile: The surface to be analyzed was placed into a well of a 6-well plate and covered with 5 mL of 2 × 10−3 M sodium citrate in Milli-Q water. The AgNP dispersion, and the sodium citrate solution were analyzed in a 96-well plate using 125 µL per sampled well. To determine the SPP absorption profiles, wavelength scans of the absorbance were performed using a Tecan Infinite M1000 PRO microplate reader (Tecan, Männedorf, Switzerland). Three replicates were analyzed for each experimental setup. The resonant wavelength of the AgNP composite (426 nm) and colloidal AgNPs in dispersion (418 nm) were found to be the same for all three replicates of respective sample. The difference between the resonant peak magnitude of the AgNP composite and the plain conducting polymer was analyzed at 426 nm, whereas the difference between the resonant peak of the AgNP dispersion and the sodium citrate solution was calculated at 418 nm.

Results are presented as mean ± SEM. A spatial absorbance analysis of the well was performed at 426 nm (the resonant wavelength of the AgNP composite) to determine the macroscopic AgNP coverage of the surfaces. To optimize the resolution of the generated heatmap, all values above 0.9 a.u. and below 0 a.u. (due to noise in the measurement) were cut off to 0.9 a.u. and 0 a.u., respectively. The same surfaces used for the absorption spectrum were employed for the spatial absorbance analysis. Three replicates were analyzed for each experimental setup, of which one representative recording is shown in Figure 1i.

AgNP–ITO Construct: Strips (3.2 cm × 1 cm) of ITO-coated PET were covered in the AgNP suspension and stored at 70 °C until the suspension evaporated.

Cyclic Voltammetry: The surface to analyze was used as working electrode (approximate immersed area of 0.44 cm2) together with a Ag/AgCl reference electrode and a platinum wire counter electrode. TSB medium (1 mL) was used as supporting electrolyte. The voltammograms were obtained from −0.5 to +0.5 V with a scan rate of 50 mV s−1 using a Gamry Reference 600 potentiostat.

Construction of a Custom-Designed Culturing Device: Two strips (3.2 cm × 1 cm) of either the AgNP composite, the plain conjugated polymer, or the PET back-side of Orgacon (referred to as “PET”) were glued with PDMS to a 5.5 cm × 5.5 cm glass square with a separation of 2 mm. A glass ring of 30 mm internal diameter and 1 cm height was glued with PDMS on top of the construct partially enclosing the strips. The device was enclosed into standard Petri dishes for easy, aseptic handling. When necessary, thin cables were connected to the nonenclosed sections of the surfaces using small flat clamps and attached to the exterior of the Petri dish for easy electrical addressing. The device enclosed in the Petri dish was thermally disinfected by overnight incubation at 70 °C.

Electrical Response to a Voltage Step Excitation: Both the plain conjugated polymer device and the AgNP composite device were characterized with a 2 V amplitude step voltage from 0 to 2 V. The experiments were performed at 37 °C. TSB medium was employed as supporting electrolyte. A Keithley 2600A source-meter (Tektronix, Beaverton, Oregon, USA) governed with a custom-made LabVIEW (National Instruments, Austin, Texas, USA) program was employed for both voltage excitation and signal recording.

Electrical Response to a Square Wave Excitation: Both the plain conjugated polymer device and the AgNP composite device were characterized with a 5 Hz, 4 Vpp from −2 to +2 V. For experiments with medium only, the characterization was performed at 37 °C employing TSB medium as supporting electrolyte. For experiments with bacterial cultures, the characterization was performed as indicated in the biofilm growth and analysis section. A Keithley 2600A source-meter governed with a custom-made LabVIEW program was employed for both voltage excitation and signal recording. For clarity in the figures, only the first 0.8 s of each measurement was shown.

Biofilm Growth and Analysis: S. aureus strain ATCC 25923 was employed in the experiment. A 1:100 dilution in TSB of an overnight culture was used to inoculate each culturing device (4 mL per device). The device was incubated at 37 °C for 24 h in static conditions for bacterial growth and biofilm formation. When required, a 5 Hz, 4 Vpp square wave voltage varying from −2 to 2 V was applied right after inoculation and maintained for the duration of the experiment. A second device was employed simultaneously as negative control, incubated with just TSB medium (4 mL), and treated similarly to the biofilm-incubating device. After incubation, the culture medium was decanted and the device rinsed in PBS, incubated in crystal violet (0.1% (w/v)) for 10 min, rinsed again in PBS, and left to dry. The device was then photographed and a 1 cm × 1 cm square (corresponding to >50% of the area exposed to the bacterial culture) was cut from each strip at a fixed position. Each square was immersed in acetic acid (1.5 mL) for crystal violet extraction. Biofilm growth was quantified by measuring the absorbance of extracted crystal violet at 595 nm using a Tecan Infinite M1000 PRO microplate reader. The background absorbance of the similarly processed negative
controls was measured and employed as blank to compare between the different materials analyzed.

**Electrochromism Analysis:** Plain conjugated polymer devices electrically addressed with a 5 Hz, 4 Vpp square wave voltage varying from −2 to 2 V were employed. After the electrical characterization, performed either with medium only or with a bacterial culture as indicated in the electrical response to a square wave excitation section, the devices were carefully rinsed with PBS and photographed using a backlight. Then, a region of interest was defined within each strip and the average intensities of the red, green, and blue channels were obtained.

**Characterization of Released Ag⁺ Ions:** To analyze the concentration of Ag⁺ ions released from the AgNP composite, SWASV was employed using sodium nitrate (1% w/v) as electrolyte. This electrolyte provided an adequate electrical conductivity of the system while preventing the released Ag⁺ ions from forming water insoluble silver salts. To prepare for sample collection, 4 mL of the sodium nitrate solution was added to the AgNP composite device, which was left at 37 °C for 30 min to electrochemically stabilize the system. The solution, which was retrieved and stored for later analysis, was then replaced by 4 mL fresh sodium nitrate solution, and the AgNP composite device was electrically addressed with a 5 Hz, 4 Vpp square wave for 24 h while maintained at 37 °C. At the end of experiments, which were performed in triplicates, solutions were analyzed for the concentration of Ag⁺ ions using SWASV. The SWASV setup employed an ITO working electrode, a platinum auxiliary electrode, and a Ag/AgCl reference electrode. An accumulation step was performed at −0.4 V for 10 min, followed by a square wave voltammetry analysis with 25 mV amplitude, 25 Hz frequency, and 2 mV pulse step between −0.4 and 0.8 V. Results from these recordings were compared to a standard curve, which was obtained using defined concentrations of silver nitrate added to a sodium nitrate solution (1% w/v). The concentration of released Ag⁺ ions (mean ± SEM) after 24 h incubation is reported in the main text. No detectable amount of Ag⁺ ions was found in the 30 min preincubation samples.

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**Conflict of Interest**

The authors declare no conflict of interest.

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