ACS APPLIED MATERIALS & INTERFACES

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Plasma-Polyplumbagin-Modified Microfiber Probes: A Functional Material Approach to Monitoring Vascular Access Line Contamination

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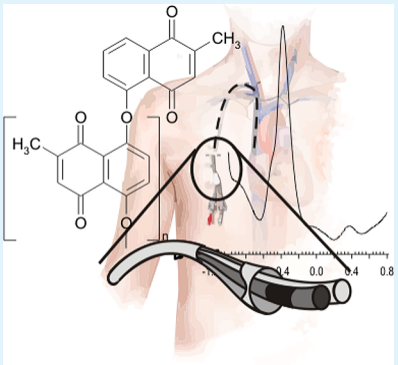
ABSTRACT: Atmospheric plasma treated carbon fiber filaments (10 micrometer) were used as the base substrate in the design of a probe intended for use within intravascular access devices. The microfiber probe was further functionalized with a polyplumbagin layer through which the line pH could be determined voltammetrically and therein provide the potential for obtaining diagnostic information relating to bacterial colonization of the line. The redox processes attributed to the immobilized polymer are characterized and a methodology developed to enable the acquisition of a redox signal that is selective and sensitive to pH. The applicability of the composite probe was demonstrated through examining the direct response in whole blood.



KEYWORDS: *carbon fiber, catheter, polyplumbagin, sensor biofilm, infection*

INTRODUCTION

Healthcare acquired infection (HAI) has been of such concern within the clinical community that most countries now have active surveillance programs to monitor the prevalence of the threat and the sources from which it can arise.[1,2] Intravascular catheter-related bloodstream infections (CRBSIs) are well-recognized as a particularly costly subset giving rise to major clinical complications and, according to recent figures from the US and European Centers for Disease Control, account for approximately 11% of all HAIs.[1,2] It has been estimated that some 300 000 cases occur in US hospitals each year.[3−5] The subsequent treatment regime is often complicated by the fact that bacteria associated with biofilm formation within central venous catheter (CVC) or peripherally inserted central catheter (PICC) surfaces benefit from an increased resistance to conventional antimicrobial treatments and leads to a prolonged hospital admission.[6,7] Healthcare costs attributed to the CRBSIs vary widely from one country to another and are often highly dependent on a large number of compounding factors but can range from $4000 to $56 000 per episode.[8−10] More importantly, the tenacity of the infection often presents life threatening complications[6−8] and gives rise to unacceptably high mortality rates that can reach 25%.[8] At present, infection is detected only once gross symptoms appear and has traditionally relied upon the expertise and vigilance of the patient to alert the clinical staff. This can be highly subjective and, as a consequence, presently gives rise to an unacceptably high mortality rate. There is a pressing need for a probe that could be integrated within a CVC or PICC line and which would periodically monitor the condition of the line and, where appropriate, alert the patient or the healthcare staff to the onset of biofilm formation.

It is known that the presence of a biofilm will induce changes in the local pH as a consequence of bacterial metabolism[11−14] and thus it could be anticipated that through monitoring such changes a warning system could be developed. The approach taken in the present communication therefore centered on the design of a probe capable of monitoring the line pH and which could be threaded within the internal lumen of the catheter (typically 0.6−0.8 mm internal diameter). A dual electrode system was adopted and consisted of a 10 micrometer diameter carbon fiber and 50 micrometer diameter chloridized silver wire as the sensing and reference-counter electrodes, respectively. A schematic of the probe design is shown in Figure 1.

In contrast to conventional potentiometric approaches, the detection methodology proposed was to exploit the pH dependent voltammetric response of a quinone moiety immobilized on the carbon surface. It has been previously demonstrated that the pretreatment of carbon fiber filaments with an atmospheric pressure plasma can enhance the electroanalytical performance of the underlying substrate[15] and therein the redox signature of the immobilized plumbagin layer. This results from the partial exfoliation of the fiber to create more edge plane sites and an enhanced population of

Received: July 15, 2013
Accepted: September 17, 2013
Published: September 17, 2013

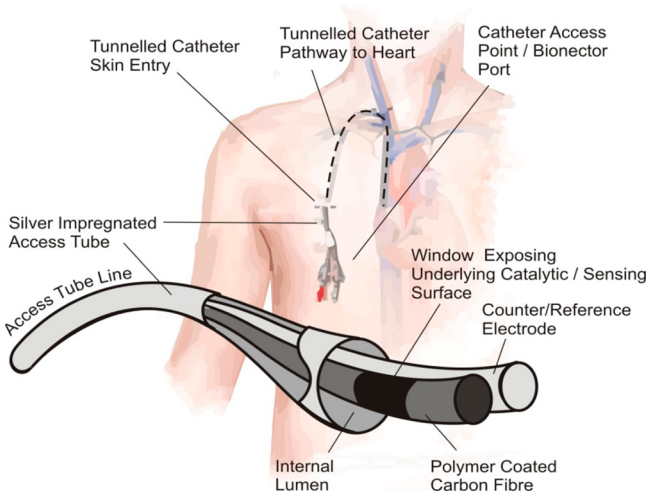


Figure 1. Schematic detailing the various components used in the construction of the catheter probe and the eventual application.

oxygen functional groups.[16,17] The electron transfer kinetics at edge plane sites is often assumed to increase electron transfer kinetics leading to a superior electroanalytical performance but there is some debate as to whether or not the oxygen functionalities that often accompany electrode modification is similarly beneficial.[18] There have been a number of studies that have shown, at least for some redox species (i.e., ferrocyanide), that the presence of such groups actually hinder electron transfer.[18] The authors have previously shown that the plasma treatment of carbon fiber leads to an overall enhancement[15] and thus the carbon filaments in this particular case were plasma pretreated under similar conditions. At present there are no technologies to enable in situ assessments of the line condition and therefore the component characterization presented herein is a prerequisite for laying the foundations of a probe that would address a pressing clinical need.

EXPERIMENTAL SECTION

All lab reagents were of the highest grade available and used without further purification. Carbon fiber thread were obtained from Goodfellow Research Materials and used as received. Unless stated otherwise, the electrochemical measurements were conducted at 22°C ± 2°C in Britton Robinson buffer (acetic, boric, and phosphoric acids, each at a concentration of 0.04 M and adjusted to the appropriate pH through the addition of sodium hydroxide). Electrochemical measurements were conducted using an Autolab PGStat computer controlled potentiostat (Eco-Chemie, Utrecht, The Netherlands). Electrochemical investigations used a two electrode configuration consisting of a carbon thread filament working electrode, a chloridized 50 μm diameter silver wire acting as a combined counter (Ag | AgCl) reference electrode. Atmospheric pressure plasmas were generated using a bespoke system (Lambert Equipment Engineering, Tadcaster, UK) designed for the roll-through processing of samples and were used to treat the fiber bulk in the first instance. Individual filaments were then separated and drawn through a solution of polycarbonate (Goodfellow) dissolved in dichloromethane. The filament was coated and leaving the drawn edge (4 mm) exposed to serve as the working/sensing probe. The probe was then adhered to the silver counter-reference. The polycarbonate film (80−100 micrometer) serves to isolate the two electrodes but also provides the single 10 micrometer carbon filament with a greater structural stability.

RESULTS AND DISCUSSION

Plumbagin, a natural product naphthoquinone derivative, was chosen as the quinone redox group which would provide the pH response. In addition to the core quinone redox functionality, Plumbagin has a 5-hydroxyl group that will, upon electrochemical oxidation, lead to the formation of a polymer film (through conventional head to tail coupling) at the electrode surface.[19] Cyclic voltammograms highlighting the response of the single carbon fiber probe in a solution containing plumbagin (0.2 mM, pH 7) are shown in Figure 2.

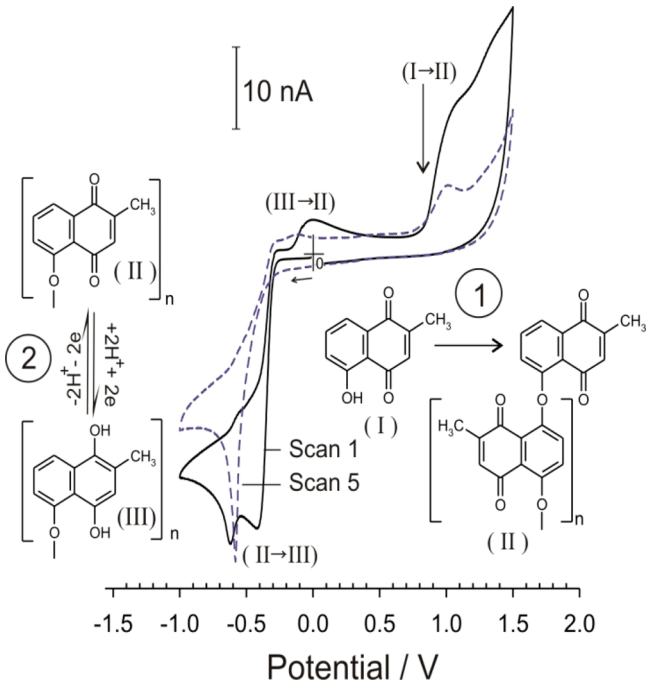


Figure 2. First and fifth cyclic voltammogram recorded at a carbon fiber filament immersed in a Plumbagin solution (0.2 mM, pH 7).

On the first scan the redox processes associated with the quinone redox functionality (-0.4 V) display a marked electrochemical irreversibility and can be attributed to the slow electron transfer at the fiber. Two reduction peaks are observed, the primary 2-electron reduction of the solution based quinone to the corresponding hydroquinone (−0.45 V) and a second, similar 2 electron reduction process, but attributed to adsorbed/surface bound quinone (−0.55 V).

The oxidation of the Plumbagin (Figure 2, I→II) results in an irreversible electrode process at +1.0 V that decreases in magnitude upon successive scans. The latter is attributed to the formation of a thin polymer on the electrode surface (Figure 2, II). The effect of the polymerization on the quinone redox profile is also marked with the reduction (Figure 2, II→III), displaying a single sharp process (−0.55 V) that is akin to the adsorbed species observed on the first scan.

Confirmation that a polymer was formed on the electrode was obtained by removing the probe, rinsing and placing in fresh buffer without the Plumbagin monomer. Initial investigations using cyclic voltammetry (not shown) found that the redox peaks associated with the quinone were retained and, in marked contrast to Plumbagin solvent cast onto the electrode surface,[20] the peaks were stable upon repetitive cycles.To obtain a more quantitative response, we adopted square wave voltammetry as the core detection methodology.

dx.doi.org/10.1021/am402821c | ACS Appl. Mater. Interfaces 2013, 5, 9367−9371

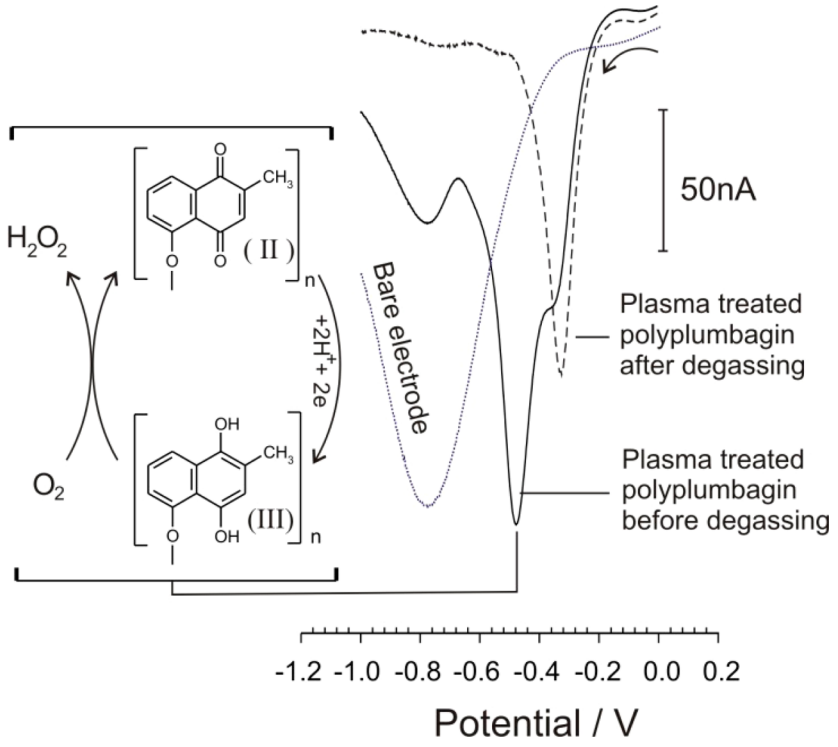


Figure 3. Square wave voltammograms detailing the response of a Plumbagin modified probe in the pH 7 buffer before and after degassing.

Square wave voltammograms detailing the reduction of the Plumbagin polymer in pH 7 buffer are shown in Figure 3.

Multiple peaks were observed and relate largely to the reduction of the quinone groups within the polymer (II→III) observed at −0.34 V and their subsequent electrocatalytic reduction of oxygen (−0.48 V). The latter was confirmed through degassing the solution with nitrogen to reveal a single reduction process (dashed line). In the presence of oxygen direct reduction occurs at −0.75 V. It is not, however, feasible to degass the line in situ and hence, from Figure 3, it can be seen that the initial reduction of the quinone, which could have been used to assess the pH, is obscured through the catalytic reduction process and hence induce an irrevocable error in any measurement process.

The general strategy, however, is still viable and can be rescued through simply switching the sweep direction from examining the reduction of the quinone to a system that involves the measuring the peak position of the quinone oxidation. In this case a reductive potential (−0.8 V) is applied in order to reduce the quinone (II→III) and the square wave voltammograms directed towards more positive potential whereupon the oxidation of the quinone occurs (III→II). Square wave voltammograms detailing the response observed at the modified Plumbagin fiber under various pH regimes are shown in Figure 4. It can be seen from Figure 4 that well-defined profiles are obtained over a wide range and, importantly, in the presence of oxygen.

The relationship between the oxidation potential and pH was found to be sub Nernstian with a 51 mV shift per decade pH (Ep_a/V = −0.051 (pH) + 0.005; N = 6; R^2 = 0.995). The analytical viability of the system was briefly assessed through examining the probe response in freshly struck whole blood (obtained through capillary stick sampling immediately prior to taking the measurement). A square wave voltammogram detailing the response is shown in Figure 5. The oxidation

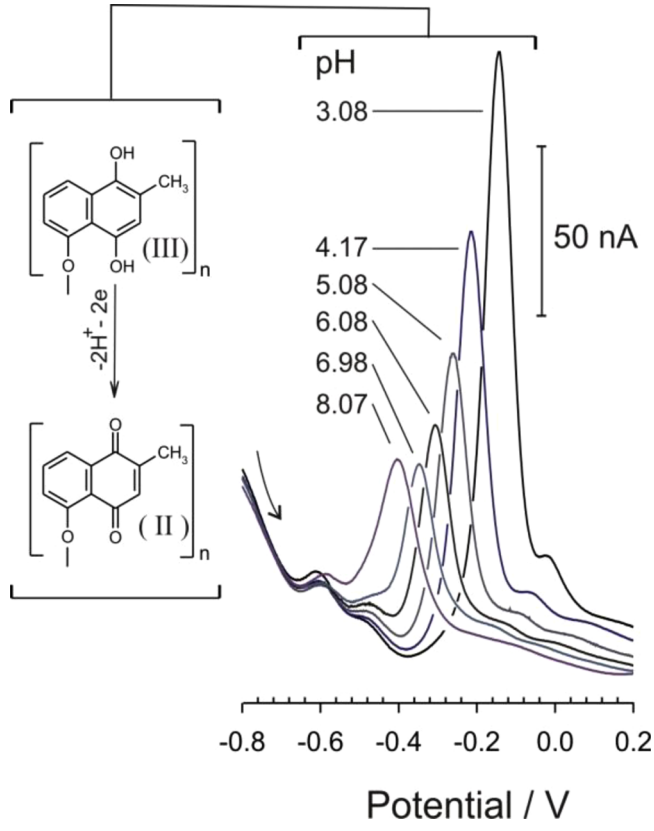


Figure 4. Square wave voltammograms detailing the influence of pH on the anodic scan record at the modified probe in the presence of oxygen.

peak for the Plumbagin film (-0.375 V) dominates the profile providing an unambiguous peak from which to determine the pH (calculated pH 7.46). Replicate scanning yields a ±3 mV

dx.doi.org/10.1021/am402821c | ACS Appl. Mater. Interfaces 2013, 5, 9367−9371

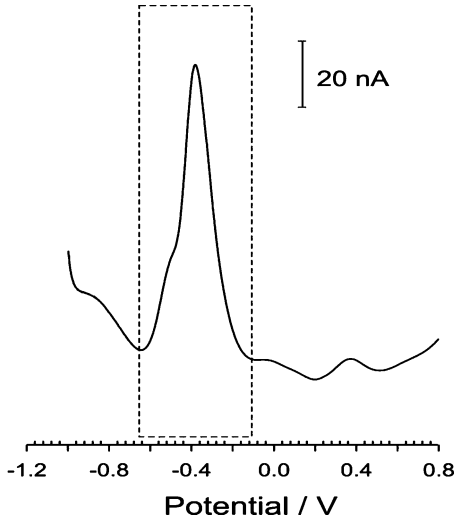


Figure 5. Square wave voltammogram detailing the response of the Plumbagin modified probe in whole blood.

range variability ($N = 5$) in the peak position assignment which would induce an error of ±0.06 pH. A second peak can be seen at +0.4 V and can be attributed to urate. The latter has been suggested as a possible endogenous biomarker through which to assess pH 7,[21,22] but it can be seen from Figure 5 that the Plumbagin signal is far superior in terms of resolution and magnitude.

The probe, in its present design, is reliant upon a stable chloride concentration. A number of previous studies examining voltammetric pH detection have employed a dual redox probe system in which one has a redox component (typically a ferrocene derivative) whose potential is unaffected by the prevailing pH that acts as an internal reference.[23−25] In this particular case, the biological system tightly regulates the concentration of chloride and the typical reference range is 95−105 mM. Moreover, the use of ferrocene as an internal standard can be complicated in biological systems as the oxidation peak can be obscured by the oxidation of other matrix components. This issue is highlighted in Figure 5, where the presence of urate within the blood results in an oxidative peak at +0.4 V. The fact that the Plumbagin polymer can be oxidised within the cathodic window deftly avoids many of the interferences common to biological systems and greatly simplifies the construction process.

CONCLUSIONS

The electropolymerisation of plumbagin onto the carbon fiber probe has been shown to provide an extremely versatile option when considering the development of an in situ sensor for use in the small confines of a CVC or PICC line. In selecting the oxidation process, then it is possible to obtain an unambiguous signal through which to evaluate the pH of the line and therein, assess the possibility of bacterial contamination. The low component count and design simplicity is particularly amenable to conventional ethylene oxide sterilization processes presently used for the CVC lines and, along with the small dimensions, provides the probe with a significant advantage over competing pH measurement systems. It is envisaged that the final device would require periodic measurements to assess the condition of the line. Thus the Plumbagin polymer has been shown to be a versatile functional material with considerable opportunities for the development of further theranostic applications.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thanks the Royal Society (London, JP100125)), Ministry of Science and Innovation (SAF-2009-10399), and the Engineering and Physical Sciences Research Council (EPSRC, U.K.) for supporting this work

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