Modification of a graphite electrode surface with nanomaterial for use in microbial fuel cells

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Abstract

As society grows, there is an increase in power demand, as well as in wastewater production. Wastewater is also a possible source of renewable energy, in that the waste products within the wastewater can be used as substrate for many strains of microbes. In the field of renewable energy, microbial fuel cells (MFCs) have a great potential in the treatment of wastewater. The ability of MFCs to be able to utilise many types of substrates, such as industrial and domestic wastewater, in order to generate power is of great interest. The bottlenecks in the up-scaling of MFCs to large-scale application in wastewater treatment has been the low power output. This can be dramatically increased when the material of the electrodes are adjusted and modified. New materials structured at the nanoscale has been shown to greatly enhance the electron collection of the MFC. The use of nanowires has been of great interest in modification of surfaces for purposes such as this. In our research, we investigate is the use of gold nanowires on the surface of graphite electrodes to improve the electrical conductivity and drastically increase the surface area of the anode. The gold nanowire modified surface drastically increases the surface area of the anode with uniform coverage; however the surface is not especially conducive to biofilm formation of the electrogen, *Shewanella oneidensis MR*-1, and thus would not translate into the performance of the microbial fuel cell. Further optimisation of the modified anodes is required for this application in order to further improve the power output of microbial fuel cells using this method.

Introduction

As society grows, there is an increase in power demand, as well as in wastewater production. High volumes of wastewater production in modern society today has many problems, chief of which the treatment of such high volume is of a priority, before wastewater can be discharged back into the environment. Further, wastewater is also a possible source of renewable energy, in that the waste products within the wastewater can be used as substrate for many strains of microbes[1–4]. In the field of renewable energy, microbial fuel cells (MFCs) have a great potential in the treatment of wastewater[1]. The ability of MFCs to be able to utilise many types of substrates, such as industrial and domestic wastewater, in order to generate power is of great interest. The bottlenecks in the up-scaling of MFCs to large-scale application in wastewater treatment has been the low power output[5]. This can be dramatically increased when the material of the electrodes are adjusted and modified. New materials structured at the nanoscale has been shown to greatly enhance the electron collection of the MFC[6,7]. Hence, we are exploring the possibility of using modifying the surface of graphite electrode material with gold nanowires to increase the electrical properties of the electrode.

Electrical and magnetic properties of noble metal wires with small diameter (<100 nm), also known as nanowires, have a variety of properties that are incredibly useful. According to classical boundary scattering, gold nanowires are less conductive when they have mean diameters smaller than 50 nm[8]. Radial diffusion dominates which results in rapid mass transport, and subsequently produce dramatic improvements in electrochemical performance compared to larger, macro-sized electrodes[9,10]. These improvements include higher current densities, steady-state behaviour, which has led to increased quality of signal measurements. Hence, modifying the surface of graphite with nanowires results in not only increasing the surface area, but effectively also generates a high density of nano-electrodes on the surface of the graphite.

As overall structure and placement of the nanowire network influences cell-engineering extensively, which is critical in applications such as microbial fuel cells and biomedical electrodes as the 3D structure is vital in cell growth and structure[11], the method used to form the nanowire array would be important as well. The nanowires can be tuned in terms of density and diameter which would in turn affect the properties of the nanowires as electrodes, and the system as a whole. Nanowires can be packed to a high density based on the orientation of the wires, and vertically orientated conductive nanowires are a good option. Vertically orientated conductive nanowires has been a goal for many applications especially in the use of 3D electronics, and miniaturised electrodes, as the world trends towards smaller, faster, more powerful personal electronics, energy conversion[12], in biofuel cells[12] as well as in biosensors[13,14]. Controlled diameter and length nanowires are of special interest as vertically orientated nanowires essentially create a high number of nanoelectrodes in a small footprint. When considered as nano-electrodes, conductive nanowires display very interesting electrochemical properties such as higher current densities, steady-state behavior which lead to improvements in the quality of the measureable signal [15], a lot of which can be attributed to the fact that their nano-sized diameters result in dominant radial diffusion and thus rapid mass transport of electrons [8–10] .

Many materials have been used in manufacturing nanowires, such as germanium[16], zinc oxides[17,18], and gold[9]. Gold is of particular interest because of the wide range of applications it can be used for, especially in biomedical and biofuel applications, since it is biocompatible. The methods of generating nanowires of any material have involved etching and lithography[19,20], the use of templates[18] and growth from seeds[21]. The first method, while very precise[19] , results in low density growth of nanowires, and is subject to equipment limitations. This method is a poor fit when increased electrical conductivity as high nanowire density is a requirement. Etching too, has its own limitation in terms of maximum density, since etching using electrical contact, for example, the wires cannot be allowed to touch[17] - this results in far less dense nanowires fabrication than desired.

The third method, having the nanowires grow from seeds fixed to the surface, is probably the best method for dense nanowire formation . Gold nanowire growth can be conducted at room

temperature as well. This, then influences the choice of substrate to grow the wires from.

For application in microbial fuel cell electrodes, a common substrate is graphite. Graphite is conductive, plentiful, relatively cheap, and particularly suitable for this application, especially considering its biocompatibility[22], and would not form oxides that might hamper attachment of gold seeds to the surface.

At the moment, current attempts to fabricate AuNW on substrates strongly rely on the use of templates. In applications that require high densities of nanowires, however, the density of nanowire formation is severely limited by templates - further, the fabrication of the template itself is an additional complication in the final production of AuNW. With all of the advantages of graphite, there is also a notable deficiency in the methods used to fabricate vertical AuNW growth on graphite substrates.

Methods

Graphite sheets were obtained from Graphitestore.com, made from exfoliated graphite flake and has no binders or resins, with density of 1.1gcm^{-3}.

Gold nanowires were grown on the graphite surface using a method that will be elaborated on in a future publication, using 18 nm gold seeds were synthsized using the protocol laid out by Huang & Kim (2011)[23]. In brief, 100 mL of 2.5 x 10^{-4} M $HAuCl_4$ solution was heated to 120 °C in an oil bath while vigorously stirred for 30 min. 10 mL of 1% sodium citrate solution was then added into the above solution while continuously boiled. After 20 min, the color of the boiled solution changed to ruby red, indicating the formation of 18n AuNPs in the solution. The solution was allowed to cool to room temperature and then used without further modification.

Graphite was immersed in growth solution for gold nanowire for 10, 15, 30 and 60 minutes before being incubated with *Shewanella oneidensis MR*-1 for 24 hours and assayed with a static biofilm assay as laid out by Merritt et al, 2005[24]. Samples with 60 minutes of AuNW growth solution were also stained with DAPI and examined using fluorescence microscopy to observe the biofilm formation on the surface. A static biofilm assay was also used to compare the mass of biofilm formed.

Results

Bare graphite substrate showed flakes or sheets of graphite under SEM. Under high magnification the surface is uneven and fairly rough, though each sheet is individually smooth (Figure 1).

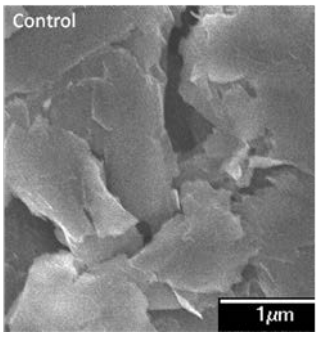

Figure 1. SEM image of bare graphite.

Compared to bare graphite (Figure 1), the surface area of the substrate is increased dramatically when modified with gold nanowire (Figure 2A-D). However, there is no visible difference in gold nanowire density or wire length between different growth times. The gold nanowires form a large textured three-dimensional surface on the graphite sheet. The gold nanowires grow only on the top-most graphite sheets of the substrate as seen in figure 1A, the grey bare graphite sheets which are lower than the other surfaces.

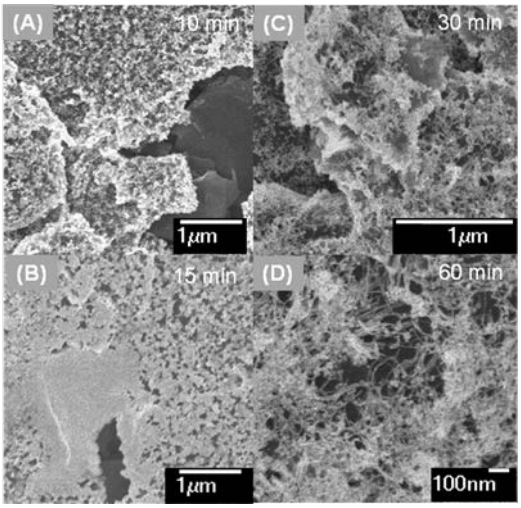

Figure 2. SEM images of gold nanowire on graphite surface with increased surface area, after being immersed in growth solution of (A)10, (B) 15, (C) 30 and (D) 60 minutes.

When incubated with *MR*-1, the biofilm formed (Figure 3B) did not appear to be thicker or better than compared to the control (Figure 3A). Bacteria however was alive and moving (due to the bright blue stain) when observed, which means that at least the modified graphite substrate was not toxic to the bacteria.

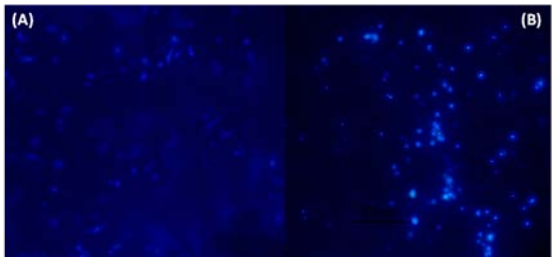

Figure 3. DAPI stains of *MR*-1 biofilm growth on bare graphite (A) and gold nanowire modified graphite surface (B).

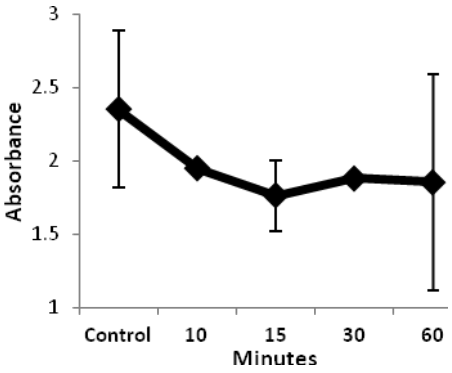

Figure 4. Absorbance spectra of static bioassay of *MR-1* biofilm mass on bare graphite (control) versus graphite substrate modified with gold nanowires grown for 10, 15, 30 and 60 minutes.

Absorbance spectra of the static bioassay showed that the *MR*-1 did form biofilms of comparative mass to that of bare graphite control. However the difference in mass of biofilm was not significant – and instead had a decreasing trend (Figure 4).

Discussion

Modifying the graphite substrate with gold nanowires resulted in a visibly obvious increase in surface area (Figures 1 and 2). This had achieved the goal we had set in increasing the surface area of a potential electrode substrate.

However, the surface of graphite is quite soft, and under the SEM it can be seen to be very uneven (Figure 1), hence the growth of gold nanowires is not as dense as it might have been since not all of the surface is exposed and can grow the gold nanowire (see for example, figure 2A). The gold nanowire does not increase in density or change in morphology despite different wire growth times, so it is unsurprising that the biofilm formation is not affected based on the nanowire growth times.

However, the surface of the modified graphite is both three-dimensional and has a forest-like structure at the nanoscale, and biocompatibility is not an issue with the biofilm formation. The morphology of the substrate surface being especially critical when trying to increase biofilm formation [25,26], this is still a promising material for development of better electrode materials in the use of microbial fuel cells.

While the biofilm formation on the modified graphite surfaces was not improved significantly compared to bare graphite, this could be due to the nanostructures forming pockets of unavailable space or air such that the bacteria was unable to utilise the increased surface area. There are still possible ways to improve biofilm formation – such as pre-soaking the material in growth medium

Conclusion

Modified graphite is still a promising electrode surface for the use in microbial fuel cells – using gold nanostructures on the graphite surface does not compromise on biocompatibility of the bacteria, compared to bare graphite. Further improvements can be made to increase biofilm formation and thereby increasing the power output of microbial fuel cells.

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