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Conductive gels based on modified agarose embedded with gold nanoparticles and their application as a conducting support for *Shewanella oneidensis* MR-1

Sindhu K. Suravaram,^[a] David K. Smith,^{*[a]} Alison Parkin^{*[a]} and Victor Chechik^{*[a]}

Dedicated to Professor R. M. Crooks on the occasion of his 65th birthday.

Abstract: *Shewanella oneidensis* is an electrogenic microbe which could be more widely applied in biosensing and fuel cell applications if better methods existed to promote electrode-biofilm formation. This paper reports a simple procedure that converts agarose, a cheap and readily available polymer, into a modified “MAGarose” material which will form biocompatible hydrogels that embed gold nanoparticles (AuNPs) along the fibers to yield a composite material with a conductivity ca. 80 times higher than an unmodified agarose-AuNP gel. Proof-of-concept bioelectrochemical experiments using *Shewanella oneidensis* show that when these MAGarose-AuNP gels are used to coat carbon veil there is a 10-fold increase in oxidative microbial current production when tested in a 3-electrode cell set-up. Microscopy results show that this can be attributed to the ability of the composite hydrogel to support MR-1 growth throughout the 3D matrix.

The first report of electricity generation from microbial activity was made in the early 20th century by Potter, who recorded the reducing capability of bacterial cultures of *Escherichia coli* and *Saccharomyces* with platinum electrodes.^[1] A number of electrogenic bacteria have since been reported,^[2] including *Clostridium beijerinckii*, *Geobacter sulfurreducens*, *Rhodospirillum rubrum*, *Shewanella putrefaciens*, *Streptococcus lactis* and the focus of this study, *Shewanella oneidensis*.^[3]

Shewanella oneidensis is a facultative aerobe, meaning it can use molecular oxygen as the terminal electron acceptor in its respiratory chain, but it will also grow in the absence of O₂.^[4] It is under such anaerobic (O₂-free) conditions that *Shewanella oneidensis* “wires” itself to solid support media and passes electrons out of the cell (electron transfer is facilitated by mediators such as flavins).^[5] This electrogenic ability is thought to have evolved to allow the microbe to reduce metals oxides within minerals, and therefore acquire essential trace elements such as Fe.^[6] In technological applications, the electrogenic properties of *Shewanella oneidensis* are often harnessed in microbial fuel cells. Such electricity generating devices consist of an electrogenic bacteria-containing anodic chamber where the oxidation of the

“fuel” substrates is microbially catalyzed and the resultant electrons are transferred to a supporting electrode which essentially acts as a current collector. MFCs have several applications including bio-sensing,^[7] bioremediation,^[8] and metal recovery.^[9] In related microbial electrolysis cells (MECs), the process is reversed, and an input of electricity enables fuel production in the form of H₂.^[10]

One of the difficulties in using *Shewanella oneidensis* in bio-electrochemical technological applications is that these bacteria are not good at forming biofilms on gold and planar carbon, and studies have focused on modifying the surfaces and/or the bacteria to increase binding.^[11] Several studies^[12] have reported the use of conducting hydrogels in microbial bio-electronic devices. We aimed to build on the well-known biocompatibility of agarose, a non-ionic biocompatible polysaccharide consisting of D-galactose and 3,6-dianhydro-l-galactopyranose. Herein, we describe a protocol that yields an agarose-derived hydrogel able to support a conductive network of gold nanoparticles (AuNPs) and an electron-generating colony of *Shewanella oneidensis*.

We hypothesized that agarose would be an ideal building block for improving the biotic-abiotic interface between *Shewanella oneidensis* and an electrode because in addition to supporting cell culturing, the thermo-responsive properties of this non-ionic polysaccharide allow its hydrogels to be molded and reshaped as required, and the exposed hydroxyl groups in the repeat unit allow for modification.^[13,14]

Initial experiments were carried out on AuNP-containing 2.88% (w/v) hydrogels made from commercially available agarose, referred to as Agarose-AuNP. In agreement with the findings of Faucher et al.,^[15] transmission electron microscopy (TEM) images showed that large uncontrolled aggregates of AuNPs were localized in the water pockets within the agarose gel (ESI, Fig. S1). Compared to an Au-free agarose control, the composite Agarose-AuNP gels were only 30-fold more conductive (Table 1 and ESI, Fig. S2). An agarose modification protocol was therefore designed with the aim of introducing Au-binding amine groups into the gel. Agarose was reacted with 2-chloroethylamine following a literature^[16] method, with the resulting product referred to as modified agarose, “MAGarose” (Scheme 1). TEM analysis of a composite MAGarose-AuNP gel indicated well-dispersed nanoparticles (Fig. 3 and ESI, Fig. S3-5). Uranyl acetate staining followed by TEM imaging (Fig. 3) and SEM analysis confirmed the clustering of the AuNP along the modified agarose fibers (ESI, Fig. S6). The conductivity of the top part of a composite MAGarose-AuNP gel was ca. 75 times greater than that of an

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Table 1. Conductivity values of 2.88% (w/v) hydrogels with and without AuNPs

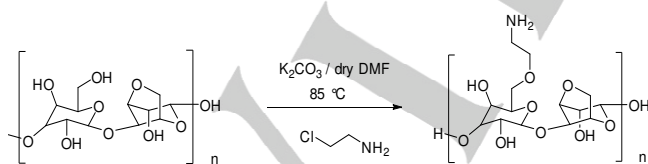
| Material | Conductivity / S m^{-1} ^a |
|---------------------------------|---|
| Agarose only | $(5.40 \pm 0.08) \times 10^{-7}$ |
| Agarose-AuNP (21% Au by mass) | $(1.68 \pm 0.02) \times 10^{-5}$ |
| MAGarose only | $(5.76 \pm 0.43) \times 10^{-7}$ |
| MAGarose-AuNP (5.8% Au by mass) | $(1.27 \pm 0.21) \times 10^{-3}$ |

^a Averaged values and their respective standard deviations. Individual measurement values are shown in ESI, Table S1.

Agarose-AuNP (Table 1) despite ICP-MS analysis showing a lower gold content (5.8% w/w vs 21%, respectively; Table 1 and Table S1). Together, this supports the conclusion that a more conductive composite hydrogel matrix has been produced by chemically modifying the agarose to support the formation of AuNP “wires” along the gel fibers, rather than clustered within the water pockets.

As in Scheme 1, the aim of the modification procedure was to convert secondary alcohol functionalities into amine groups. However, characterization of MAGarose by elemental analysis (ESI, Table S3), NMR (ESI, Fig. S7) and FTIR (ESI, Fig. S8) indicated a negligible nitrogen content, and fluorescence studies also quantified a low amino-group content of approximately 0.4% (ESI, Fig. S9-S10 and Table S4). The bulk material properties of the MAGarose material were therefore measured. Compared to hydrogels formed with agarose, those made using MAGarose had a decreased maximum gelation temperature (T_{gel} dropped from 98-100 °C to 80-83 °C); smaller fibre widths (ESI, Fig. S11); Newtonian rather than non-Newtonian viscosity and ca. 11 times lower viscosity-average molecular weight (ESI, Table S5-S7, Fig. S12-S13). These changes are consistent with MAGarose containing fewer polymer units than agarose.

Control experiments showed that hydrolysis of agarose in the presence of K_2CO_3 but absence of 2-chloroethylamine is not enough to generate composite gels of the same high conductivity as MAGarose-AuNP (ESI, Scheme S2-S3, Table S8). Therefore, although the specific chemical change cannot be determined, it is shown that all aspects of the described modification procedure play an important role in yielding a gelator which will form a highly conductive AuNP containing gel.



Scheme 1. Synthetic modification procedure^[16] and intended structure of MAGarose. Characterization of the product showed some hydrolysis and only a very limited amount of amine incorporation, indicating the real structure of MAGarose deviated from the intended outcome (see Discussion).

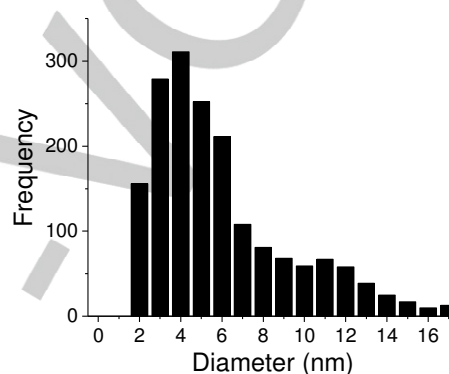
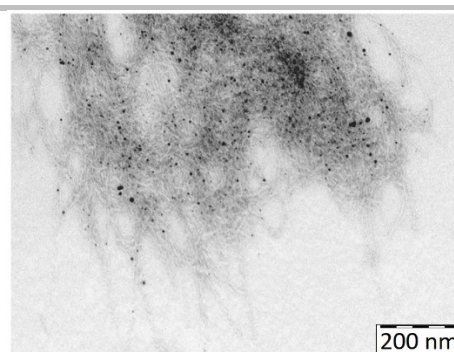


Figure 3. (Top) TEM image of the top part of the MAGarose-AuNP gel, that has been stained with uranyl acetate to show the gel fibers and (Bottom) histogram of AuNP size distribution obtained from analysis of a TEM image of the top part of the MAGarose-AuNP gel, see ESI, Fig. S4.

Bio-electrochemistry experiments (ESI, Fig. S14-S17) were conducted to show that the addition of MAGarose-AuNP gel to a carbon veil support results in a substantial increase in *Shewanella oneidensis* MR-1 oxidative current collection. This was quantified by the average oxidative current measured at the end of 24-hour chronoamperometry experiments conducted in triplicate (Fig. 4; current vs time plots are in the ESI, Fig. S18-S19). Qualitatively, cyclic voltammetry experiments (ESI, Fig. S20) also support a substantial increase in microbial oxidation current when a MAGarose-AuNP electrode is used instead of just carbon veil.

Subtracting the average current after 24-hours of the carbon veil *Shewanella oneidensis* chronoamperometry experiment and the equivalent abiotic MAGarose-AuNP value away from the MAGarose-AuNP *Shewanella oneidensis* current suggests a value of 22.80 μA , i.e. 68%, can be attributed to the oxidative activity of bacteria encapsulated within the conducting gel matrix (Fig. 4). This suggests that the bacteria are colonizing the MAGarose hydrogel matrix. To visualize the dispersion of the *Shewanella oneidensis*, samples from MAGarose-AuNP bio-electrochemical experiments were analyzed using confocal microscopy (Fig. 5, ESI Fig. S21). This confirms that the porous, biocompatible structure enables the penetration and growth of bacteria throughout the electrode coating. The AuNP-free “MAGarose” structure would also be expected to support a large colony of microbes, but it is concluded

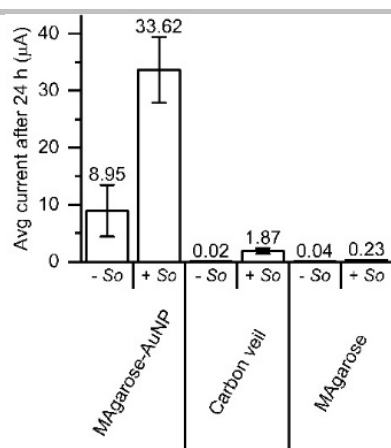


Figure 4. Average current obtained at the end of 24-hour, 30 °C, pH 7 electrochemical experiments using either MAgarose-AuNP on a carbon veil support, "MAgarose-AuNP"; carbon veil only; or MAgarose on carbon veil working electrodes in the presence (+ So) or absence (- So) of *Shewanella oneidensis* MR-1. A working electrode potential of 0.2 V vs. saturated Ag/AgCl reference electrode, equivalent to +0.388 V vs SHE was used in all experiments. All experiments were conducted in triplicate and the error bars indicate the standard error.

that the low electrical conductivity of this AuNP-free gel explains why it does not act as an effective current collector. Thus, the AuNP-component of the composite MAgarose-AuNP gel is necessary.

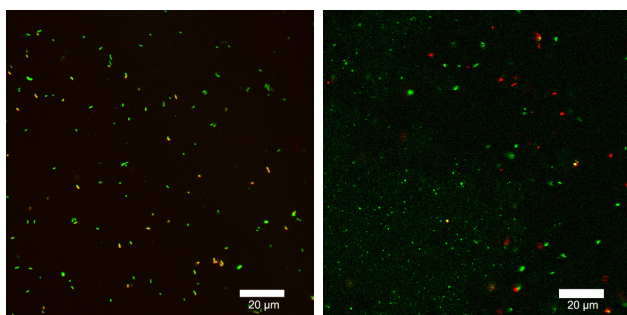


Figure 5. Confocal microscopy images of the (left) top and (right) carbon-veil touching bottom of an MAgarose-AuNP gel after a chronoamperometry bio-electrochemistry experiment. Green channel shows live *Shewanella oneidensis* stained by Styro 9 and red channel shows dead bacteria stained by propidium iodide. The scale bar is 20 µm.

This work establishes a method for synthesizing a conductive agarose-based hydrogel which can be used to improve the biotic-abiotic interface for *Shewanella oneidensis* electrochemical studies. It should be noted that the conditions of the bio-electrochemical experiments (zero electrochemical pre-conditioning, 24-hour experimental time, no stirring or media exchanges) have not been optimized for maximal *Shewanella oneidensis* current generation. Therefore, although the current densities from the *Shewanella oneidensis* carbon veil control experiments (approx. 3 µA cm⁻² at 0.2 V vs Ag/AgCl, ESI, Fig. S20) are in-line with a published^[17] biochemical study using a carbon rod electrode (8 µA cm⁻² at 0.2 V vs Ag/AgCl),

approximately 10-fold higher current-density values have been reported for a graphite foil electrode.^[18]

Significant improvements in current-production would therefore be sought in future experiments. Future work will also focus on optimizing the modification method and gold loading to improve the cost-effectiveness of this electrode-modification strategy.

4. Acknowledgements

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Keywords: microbial fuel cells • conductive gels • gold nanoparticles • agarose gels

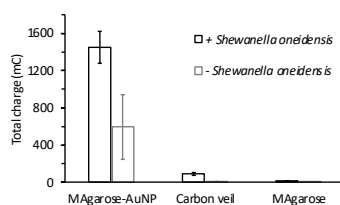
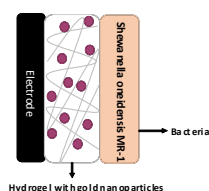
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ARTICLE

Entry for the Table of Contents (Please choose one layout)

ARTICLE



Sindhu K. Suravaram, David K. Smith,*
Alison Parkin,* Victor Chechik*

Page No. – Page No.

Conductive gels based on modified agarose embedded with gold nanoparticles and their application for bio-electrochemical cells using *Shewanella oneidensis* MR-1

A gel embedded with gold nanoparticles is used to modify a carbon veil electrode, leading to 10-fold enhancement of current generation in a simple bio-anode and providing long-term bio-stability and current output.