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Brief Fine Polishing of Thin-film Gold Electrode Sensors Leads to Better Reproducibility than Electrochemical Pretreatment

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Surface roughness of the electrode and its activation is critical for improved reproducibility of biosensors. Electrochemical cycling has traditionally been the preferred approach for electrochemical activation with only a few reports of mechanical polishing as the surface activation technique. This study compares the efficiency of mechanical polishing and electrochemical activation of thin film gold electrode surfaces for electrochemical measurements. The effects of the approach on both the electrochemical activity and surface variations were studied. Our findings suggest that brief polishing with 50 nm alumina nanoparticles resulted in improved electrochemical activity and better electrochemical area and roughness control when compared to the electrochemically activated counterparts.

Keywords: Thin-film electrodes, bio-sensors, surface roughness, electrochemical sensors, gold electrodes

1. INTRODUCTION

Gold is the second most common electrode material used for sensor and transducer fabrication in biosensor studies [1]. Storage of electrodes prior to being used results in an insulating layer being formed on the sensor surface. This layer is formed by physisorption of molecules on the metal electrodes surface over time and is known to inhibit the electrochemical activity of the electrode surface. Hence the first step in designing electrochemical sensor platform is the pretreatment of the electrodes prior to any functionalization of the sensor surface [2-4].

There has been a continuing endeavor to establish a reliable and repeatable process for sensor surface activation. Numerous approaches including mechanical[5-6], thermal[7-8], chemical[9-10], electrochemical[5,7-9,11-14], UV ozone treatment[15] and O_2 plasma treatment[16] have been described for pretreatment of electrode surfaces.

Using a single technique or combination of more than one method is often resorted to ensure consistent electrochemical activation of the electrode surface. Combinations include mechanical and electrochemical methods[11,13,17-24], chemical, mechanical and electrochemical methods[21,25-29] in numerous sequences over the traditional approaches of mechanical only[30-32], electrochemical only[33-36], or chemical only[37-42] methods. Researchers occasionally have also used simple alcohol cleaning or acetone cleaning[43-44] in their sensor preparation, while some reported no pretreatment at all[45].

The vast range of approaches being tried showcases the fact that an optimal pretreatment strategy seems to be user and protocol specific. Pre-treatment of gold electrodes still continue to deliver inconsistent results when used by multiple groups. Studies have benchmarked different methods and reported on their effect on downstream procedures. Most commercial electrodes reccomend electrochemical pretreatment as the manufacturers' recommended protocol possibly due to the ease of implementation[47].

The electrochemical cleaning methods also have a wide variance - operating at different potential ranges, scan rates or different electrolyte media[11,18,25]. Likewise, a wide variety of mechanical polishing strategies have been used including manual[3-6,17,21-23] or machine polishing[13,25] with coarse (over 1 µm particle size)[6,17,21,23-24,26,28,30], medium-coarse (0.1 to 1 µm)[5,17-18,21-23,25,28,31-32] or fine (below 0.1 µm) grains of abrasives[6,16-17,22-23,28] in form of emery/sand-papers[6,17,30] or paste/slurries of alumina/diamond in water/oil[5,13,17,21-23,26,31-32]. While polishing with fine alumina, particles yield a better surface than coarse diamond particles or emery paper[5-6]. Many of the studies that describes mechanical polishing lacks the details either of application or polishing techniques, or of abrasive forms and grain sizes, making it difficult to compare the studies[4,17-18,21-25-28,30-32] or to group them under a single method, as "mechanical polishing".

Surface roughness is described as a significant constraint in the reproducibility of biosensors[37,48-52] and the surface properties are shown to affect the thin-films to be coated on pretreated electrodes during functionalization procedures[15-16,53-57]. Evaluating the efficacy of the methodologies for improving the surface roughness continues to be a challenge. A traditional approach to measure the efficacy of surface treatment procedures is by measuring the electrode roughness factor (γ) as a function of charge transfer[46 58]. Imaging techniques such as AFM micrographs[3,27], STM[6,42,50] or SEM photographs[24,27,41,59] as representative of the whole electrode surface have also been attempted to visualize the surface roughness. Efforts have been made to compare the effects of fine and coarse polishing of glassy carbon electrodes however no significant effect of electrode surface roughness on cyclic voltammetry results were observed[60]. Carralero, et al. reported that a simple polishing of gold electrodes with a coarse abrasive paper could lead to the RSD values of 2.8-3.6%[30], while others reported no causality between the reproducibility of sensors and surface treatment methods. As summarized above, most of the available studies have

diverging results and when commercial electrodes are used, manufacturers rarely comment on the effect of recommended surface treatments on eventual reproducibility of the measurements. Thus this paper focuses on comparing the effect of manufacturer recommended procedures to a variety of mechanical polishing protocols with an emphasis on surface roughness as well as the reproducibility of the electrochemical response of gold electrodes.

2. EXPERIMENTAL

Commercial three-electrode electrochemical sensors with thin-film gold electrodes (ED-SE1-Au, Micrux, Asturias, Spain) were used for this study. All electrodes in the sensor were made of gold. The fabrication process results in the substrates coated with a 50 nm Ti adhesion layer topped with a 150 nm gold film as the electrode. The working electrode diameter is 1 mm and the electrochemical cell diameter is 2 mm[61]. A stylus profilometer (Veeco Dektak-8, NY, United States) was used for surface profiling of the thin-film electrodes with a tip diameter of 2 µm, The measurements were made under a constant force mode with a force of 3 mg (29.4 µN) contact force. The scan rate was 66 µm/sec. Due to semi-manual operation, moving the stylus through the exact same trajectory on all sensors was not possible so instead, the scanning trajectory for each sensor was noted.

Two different potentiostats were used for electrochemical measurements. Both the potentiostats were compact and portable versions (CHI1230B, CH Instruments, Texas, United States and GalvanoPlot-GX201, SolarBiotec, Turkey). For polishing, low viscosity solutions of polycrystalline wet-milled acidic aluminum slurry of 300nm and 50nm (Pace Technologies, AZ, United States) were used. Chemicals were procured from Sigma-Aldrich (Merck KGaA, Germany). 5 mM ferrocyanide/ferricyanide solution was used as redox probe for performing the cyclic voltammetry (CV) studies. CVs were performed between -0.6 V and +0.6 V at a step resolution of 1 mV and a scan rate of 50 mV/sec.

Sensors were classified into 5 groups for the 5 different studies performed. The first group (Group 0) served as the control group and was subjected to the manufacturer recommended activation protocol meanwhile the other groups underwent different mechanical polishing protocols. The recommended protocol given by the manufacturer was electrochemical cleaning using cyclic voltammetry in 0.05 M H_2SO_4 scanned between the voltage range -1.0 V and +1.3 V, at a sweep rate of 100 mV/sec for 12 complete cycles[47]. The next three groups of sensors were treated using mechanical polishing that was performed manually by scrubbing the electrode surface with a drop of aluminum slurry in a circular motion at a velocity of about 10 cm/sec. Mechanical scrubbing protocols were grouped on the basis of slurry size used, as coarse, heavy and fine polishing; (i) electrodes polished with 300 nm slurry were subjected to 30 cycles at 30 N average pressure (Group I), (ii) electrodes polished with 300 nm slurry followed by 50 nm slurry for 100 cycles each at 30N average pressure (Group II) and (iii) electrodes polished with only 50 nm slurry for 10 cycles at 5 N (Group III), respectively. The final group (Group IV) was subjected to cleaning by rubbing the electrode surface with absolute ethanol followed by a rub of absolute isopropanol. These electrodes were

evaluated for surface roughness profiling and electrochemical performance both prior to any treatment and after cleaning,

3. RESULTS

When subjected to a electrochemical studies; new and unused electrodes exhibited uneven levels of oxidization and loss of surface properties. For the unused and untreated sensors, cathodic peak currents (I_p) were observed to be below 5 nA at the peak potential of 40 mV (E_p). This value was about 2,000-fold lower than the expected value of the manufacturer's test results[61] (**Fig 1A**).

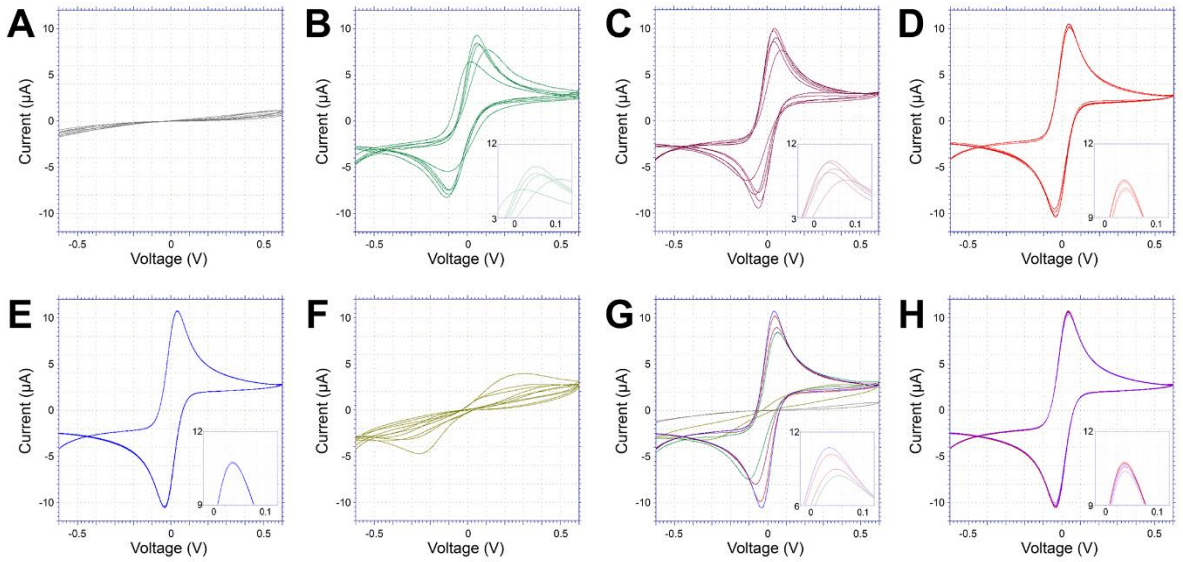

Figure 1. Electrochemical behavior of ferro-/ferri-cyanide on untreated gold electrodes and after various surface treatments, measured through cyclic voltammetry. A. Untreated unused; B. Electrochemically treated; C. Mechanically polished, coarse; D. Mechanically polished, heavy; E. Mechanically polished, fine; F. Alcohol rubbed; G. All treatments compared; H. Fine polished, After 1 week. Insets: Magnification of positive peaks in corresponding chart.

The manufacturer's recommended protocol led to a good recovery of the electrochemical behavior of the electrodes, with cathodic peak currents averaging 8.1 µA. However, the electrodes in this group showed a 14.9% relative standard error of reproducibility (RSD) (Fig 1B). Surface profiling results showed changes in the physical properties of the treated electrodes. Untreated electrodes had a surface roughness of Ra: 13-18 nm/ Rmax: 80 nm, which increased to Ra: 15-22 nm/ Rmax: 170 nm after electrochemical treatment (Fig 2A, 2B).

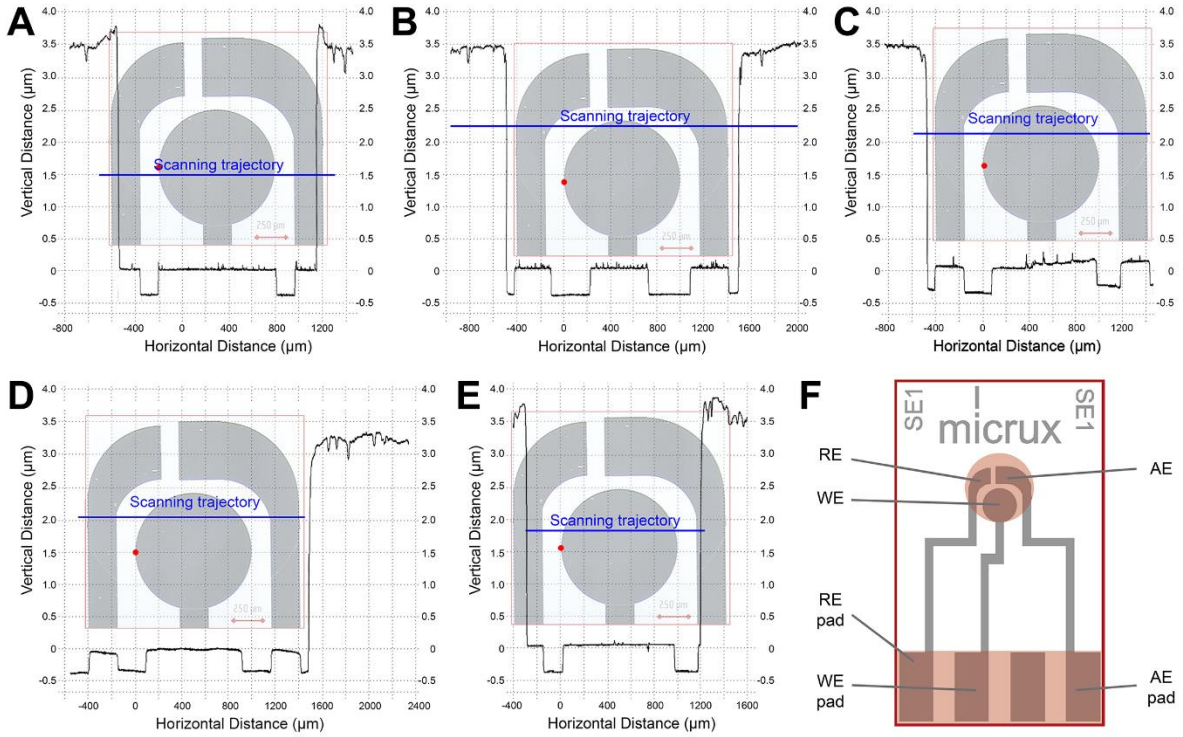

Figure 2. The surface roughness of untreated gold electrodes and after various surface treatments. A. Untreated unused; B. Electrochemically treated; C. Mechanically polished, coarse; D. Mechanically polished, heavy; E. Mechanically polished, fine; F. Electrode design

Table 1. Comparison of effects of various treatment procedures on electrode surface roughness, film thickness and maximum value and reproducibility of electrochemical response.

	Untreated	Electrochemical Procedure	Coarse Polishing	Hard Polishing	Fine Polishing
Max current with redox couple (I_p)	No peak, signal below <6 nA	8.10µA (6.41-9.72)	8.98µA (7.65-9.97)	10.36µA (10.11-10.54)	10.73µA (10.70-10.76)
Relative Standard Deviation (RSD)	N/A*	14.89%	10.46%	1.91%	0.24%
Surface Roughness, average (Ra)	13-18nm	15-22nm	8-16nm	<2nm	<0.5nm
Surface Roughness, max. (Rmax)	80nm	170nm	120nm	8nm	2nm
Film thickness	382-400nm	380-399nm	371-380nm	306-327nm	386-390nm

*: N/A: Not calculated

Mechanically polished electrode surfaces had a roughness of Ra: 8-16 nm/ Rmax: 120 nm, Ra <2 nm/Rmax: 8 nm and Ra<2um/Rmax:4nm for coarse, heavy and fine scrubbing, respectively (Fig 2C-2E). Coarse polished electrodes ended up having I_p values of almost 9.0 µA and a RSD of 10.5%, while the heavy polished electrodes had I_p values of 10.4 µA with 1.9% RSD. Fine polished electrodes showed the highest I_p values of 10.7 µA with the least variations (RSD: 0.24%) (Fig 1C-1E). Rubbing of the untreated surface with alcohol followed by acetone resulted in a very weak redox response with random peak currents, which are still below the one tenth of the target electrochemical character that is to be regained (Fig 1F).

Although the sensors were described to have a total film thickness of 200 nm, the film thickness of the untreated sensors was found to be between 382 and 400nm. The electrochemical treatment process (Group 0) and simple alcohol-acetone rubbing (Group IV) did not affect the film thickness significantly. However, it is observed that thin film thickness after polishing was decreased to 371-380 nm, 306-327 nm, and 386-390 nm, for Groups I through III sensors respectively (Fig 2C-2E). The effect of different types of pretreatment process on the electrochemical response of the thin-film sensors is visualized together in Fig 1G and study results are summarized in Table 1.

Sensors that revealed the preeminent surface and electrochemical properties were stored in individual air-tight containers and electrochemical characterization was repeated after 24 hours and 7 days. The measured currents were almost stable in a week, with less than 2% decrease in I_p values compared to the first experiment; while the variation slightly increased (RSD: 1.1% compared to 0.24%) (Fig 1H). Surface roughness was observed to be stable after 7 days, or after multiple cyclic voltammetry experiments performed in ferro/ferric-cyanide medium.

4. DISCUSSION

The electrochemical protocol is the best bet for many authors and is also recommended by the manufacturer as an effective protocol for recovering the expected electrochemical properties of the metal surface[9,33-36,47]; however, it yields a stunted reproducibility of about 15% RSD, which is lower than acceptable limit for sensor fabrication. Although the manufacturer suggested a RSD of 6% inter-electrode with electrochemical treatment[61], our measurments show that mechanical polishing yields much better electrochemical properties of the sensor, decreasing the RSD down to less than 0.25%. Another study had documented an insignificant effect of electrode surface roughness on cyclic voltammetry results[60] that was performed with glassy carbon electrodes with very high surface roughness compared to thin-film electrodes. Carralero, et al. reported a simple polishing of gold electrodes with a coarse abrasive paper yielded RSD values of 2.8-3.6%[30], which was coherent with our findings.

Polishing with fine alumina particles was shown to yield better surfaces than the coarse diamond particles or emery paper[5-6]. It is common practice to use a stepwise method of decreasing slurry sizes for polishing[17,19-23,25,28]. Although this improves the surface roughness, we have observed that a vigorous polishing may destroy the surface of the electrode by removing a deposited metal film thickness of about 90 nm. Some authors recommended electrochemical treatment over

mechanical polishing[3,18] for a better surface roughness, where they applied only a medium-coarse polishing with a 300-500 nm aluminum slurry. We have observed slightly better surface properties with coarse polishing over electrochemical treatmentbut none of the referenced studies investigated surface roughness by profilometer and the details for the extent of polishing can exactly be told. It is also possible that slight differences in electrochemical cleaning protocol might have had an effect on the results.

Moreover, Dutta et al. demonstrated that anodic potentials of the gold disk and plate electrodes that were polished coarsely decreased significantly even after one day of the treatment[3], whereas we observed only about a 2% decrease after seven days of the treatment with briefly fine polished electrodes. This work demonstrates the studies that employ various degrees of polishing with a variety of abrasives ranging from sand-papers to aluminum slurries by the manual or machine application will yield different results, and that they can not be simply compiled under the name of "mechanical polishing" as a single technique.

Surface roughness is suggested to be a significant parameter for biosensor behaviour[15-16,37,48-57] and is to be taken into consideration as a parameter for voltammetric response in the presence of a redox marker. The proposed technique is straightforward, manually operated, uses only one component, and specifically without using any tools. This precisely yields faster and better results than the manufacturer recommended procedure for first step surface preparation of downstream applications in biosensors.

5. CONCLUSION

Although the manufacturer suggests activating the surface of thin-film electrode sensors by various electrochemical protocols, it was observed that these protocols are actually incompetent to prepare the surface and may cause variations up to 15% among electrodes of the same batch. A brief manual polishing of thin-film gold electrode sensors with 50 nm aluminum slurry is effective to lessen the reproducibility error, as well as the surface roughness. This yields better results than the manufacturer recommended procedure when applicable. However, unwarranted more vigorous polishing may destroy the surface properties of thin film electrodes.

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