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Rough Gold Electrodes for Decreasing Impedance at the Electrolyte/Electrode Interface

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Abstract

Electrode polarization at the electrolyte/electrode interface is often undesirable for bio-sensing applications, where charge accumulated over an electrode at constant potential causes large potential drop at the interface and low measurement sensitivity. In this study, novel rough electrodes were developed for decreasing electrical impedance at the interface. The electrodes were fabricated using electrochemical deposition of gold and sintering of gold nanoparticles. The performances of the gold electrodes were compared with platinum black electrodes. A constant phase element model was used to describe the interfacial impedance. Hundred folds of decrease in interfacial impedance were observed for fractal gold electrodes and platinum black. Biotoxicity, contact angle, and surface morphology of the electrodes were investigated. Relatively low toxicity and hydrophilic nature of the fractal and granulated gold electrodes make them suitable for bioimpedance and cell electromanipulation studies compared to platinum black electrodes which are both hydrophobic and toxic.

Keywords

Electrode polarization; Dielectric Spectroscopy; Microfluidics; Bioimpedance; Fractal Electrodes

1. Introduction

Application of a constant electric potential to electrodes causes charge accumulation on electrodes that are in contact with an electrolyte solution. As a consequence of the accumulation, a large impedance forms at the electrode/electrolyte interface, and causes large electric potential to drop at the interface. This adverse effect is identified as electrode polarization (EP). Specifically, the sensitivity of dielectric spectroscopy (DS) is limited in sub-MHz range due to the EP effect. DS is a noninvasive and a label free technique to characterize biological samples. It was successfully used to investigate dielectric properties of cells, tissues and bacteria [1–7]. DS involves application of a small test voltage to the sample under study. Various types of approaches were used to reduce EP in DS. These

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include electrode separation distance variation technique [8], substitution technique [8], increasing effective surface area of electrodes [9–11] and four electrode techniques [12–16]. However, there is no single accepted method for the EP correction due to the complexity of the phenomenon. One of the most efficient techniques to minimize EP effect is to maximize the electrode/electrolyte interfacial area to produce rough, recessed and complex conductive structures on flat electrode surfaces. This technique is especially more suitable for microfluidic systems, where micro-electrodes are present, as portability and device size are important considerations.

Platinum black (PB) is widely used to create rough electrodes and to enable DS in sub-MHz range [17], and also in many distinct areas such as neural signal sensor [17–22], tissue sensor for orbicularis oculi muscle [23], gas sensor [24, 25], drug screening and thermal infrared detection [26] due to its unique contribution to reduction of EP. However, the fragility of electrodeposited PB under mechanical contact, poor reproducibility of deposition, its hydrophobic behavior and bio-toxicity [27] have limited its use in physiological measurements. Different electrode materials and fabrication techniques, including IrOX [9, 28, 29], TiN [9], Pedot-Nanotubes [22], conductive polymers [9, 22, 30–32] and activated carbon [33], were sought to reduce EP. However, the common disadvantage of these materials is their electrical stability under mechanical contact.

The gold based rough electrodes can reduce EP, since they can preserve its recessive structure under mechanical contact and its electrical behavior for long term electrical measurements. Plain gold electrodes have already been used extensively in impedance measurements owing to its biologically inert nature [34]. Recently, gold electrodes with fractal nanostructures were utilized in novel antennas [35], solar cells [36], and ultrasensitive biosensors [37–40]. Also the hydrophilic nature of the rough gold surface compared to PB surface offers a major advantage for microfluidic systems. Therefore, rough gold electrodes can be a favorable alternative to PB and other materials to reduce EP owing to its dendritic and recursively constructed shape to maximize effective surface area [41].

In this study, we developed two different kinds of gold nanostructured electrodes that have increased effective surface area compared to that of plain gold electrodes. While one of the techniques uses electrochemical deposition of gold to generate nanostructures, the other method depends on sintering of gold nanoparticles on plain gold electrodes. Electrochemical deposition requires expensive equipment, costly reagents, and can result in poor reproducibility, whereas nanoparticle sintering depends on a relatively simple procedure. In order to measure electrical impedance at the electrode/electrolyte interface a microfluidic channel was fabricated and filled with phosphate buffer saline (PBS), where the electrodes were at parallel plate configuration. Measuring PBS is relevant for physiological measurements, as PBS with physiological concentrations of ions cause strong EP. A constant phase element (CPE) model was used to model the impedance response at the interface. The parameters in the CPE model were used to characterize the electrodes. Surface topographies of each electrode were investigated with scanning electron microscopy (SEM) and a profilometer. Hydrophobicity of electrodes was measured using contact angles. Furthermore, the toxicity of the electrodes on a T-cell leukemia cell line was measured using Trypan Blue exclusion assay.

2. Materials and Methods

2.1. Electrode Fabrication

Glass slides were cut into 2.5 2.5 cm² pieces using diamond cutter. The glass pieces were cleaned in an ultrasonic bath (FB11201, Fisher Scientific) at 37 kHz-at 25 °C sequentially in DI (deionized) water, 1 M KOH, and acetone for 10 min, followed by a rinse with DI water. The substrates were dried with nitrogen gas following the cleaning steps. The glass slides were then put into 150 °C oven to fully evaporate water. A masking tape was used as a negative mask to generate electrode patterns. The tape was cut with a craft cutter (Silver Bullet) to obtain 4mm diameter circular shapes as negative masks. After the tape was stuck on the glass substrates, the slides were sequentially sputter-coated (EMS300TD, Emitech) with chromium (120mA-60s) and gold (120mA-150s) or platinum (120mA-150s). Chromium layer was used as a seeding layer to provide a fine adhesion between Au/Pt and glass. Sputtering generated thin layers of metals, where the thickness was on the order of nanometers. Following the sputtering process, the tape was removed and electrodes were rinsed using DI water. Copper tapes (3M) were used as terminal leads. The tapes were bond using silver conductive epoxy. Following this step, the electrodes were ready to be modified using electrochemical deposition.

2.2. Electrode Modification

A three-electrode potentiostat/galvanostat system was used for electrochemical deposition (EZstatPro, Nuvant). In the setup, electric current flows between a platinum counter electrode (MW-4130, BASI) and working electrodes (Au/Pt sputtered electrodes), where the potential at the working electrode is controlled with reference to a Ag/AgCl electrode (MF-2052, BASI). All solutions for electrochemical deposition were prepared in ultrapure DI water (EMD Millipore, electrical conductivity 5×10^{-6} S/m at 25 °C). Only circular part of electrodes was immersed in the solution in order to have a fixed area of deposition. The following modifications were made on electrodes:

2.2.1. Platinum Black Electrodes (PBEs)—The PB electroplating solution contains 1% Chloroplatinic acid (Sigma Aldrich) and 0.08% Lead Acetate (Sigma Aldrich). The electrodeposition setup was used in galvanostatic mode, where the electrodes were coated with PB at different current densities (mA/cm²) and varying time periods. PB is coated on Platinum electrodes.

2.2.2. Gold Nanostructured Electrodes (GNEs)—Gold electrodes were electrochemically coated in 1 mg/ml Sodium Tetrachloroaurate (III) (AuCl₄ Na 2H₂O) (Sigma Aldrich) solution using the deposition setup in the potentiostatic mode. Depositions were conducted at varying durations and electric potentials.

2.2.3. Gold Granulated Electrodes (GGEs)—Gold nanoparticles that are in 10nm diameter (nominal) and made using tannic acid and citrate were purchased from Sigma Aldrich. In order to have gold granulated electrodes, gold sputtered electrodes were placed on a hot plate (Isotherm, Fisher Scientific) that is kept at a constant temperature. Gold nanoparticle suspension droplets of ~80 µl, which was in 0.1 mM PBS solution at a

concentration of 6×10^{12} particles/ml, was slowly pipetted on the electrodes sequentially as water of a previous droplet evaporated at the surface.

Following all the deposition processes, modified electrodes were kept in an oven at 100 °C for 10 min for dehydration.

2.3. Device Assembly

Microfluidic channels were utilized to measure low amounts of liquids. The channels were fabricated using double sided tapes and glass substrates that bear electrodes (Figure 1). Inlets and outlets were perforated with a diamond drill bit on glass substrates. Double sided tape was cut with a craft cutter (Silver Bullet) into the shape of a microfluidic channel. Double sided tape was stuck on one substrate. A second substrate was aligned so that the two electrodes were aligned on top of each other in the middle region of the microfluidic channel. The thickness of the microfluidic channels was 500 μm .

2.4. Characterization Studies

A high precision impedance analyzer (4194A, Agilent) was used to measure the impedance of the microfluidic channel that was filled with different fluids in 1 kHz – 20 MHz range. The microfluidic device was interfaced to a printed circuit board (PCB), which was connected to the impedance analyzer via a BNC port. An adapter was used to interface impedance analyzer to the PCB. Open-short-load compensation was performed to eliminate residual effects of the adapter.

Equivalent circuit analysis was used to analyze electrode/electrolyte interfacial impedance. The equivalent circuit model is shown in Figure 2. Electrodes and electrolytes were modeled using serial combination of CPE Z_{CPE} to model EP effect and parallel combination of R_{sol} and C_{sol} to model electrolyte impedance. These are in series with resistance R_{lead} and inductance L_{lead} of the electrode leads. These elements are connected in parallel to a stray capacitance C_f . Faradaic effects were neglected considering the magnitude of the electric field, field frequency range and electrode materials. CPE model is given as $Z_{CPE} = \kappa^{-1} (i\omega)^{-\alpha}$, where κ and α are CPE coefficient and exponent, respectively, ω is angular frequency, and i is the imaginary unit. The parameter α changes from zero to one $0 \leq \alpha \leq 1$ corresponding to purely resistive ($\alpha = 0$) or purely capacitive $\alpha = 1$ electric double layer in the two limits.

Impedance data was acquired through a GPIB (General Purpose Interface Bus) interface. The following steps were taken to calculate CPE parameters κ and α :

1. Admittance of the measured sample can be written in the following form for very low conductivity fluids, such as air and DI water: $Y = i\omega(\epsilon C_0 + C_f)$, neglecting the lead and the EP effects. The stray C_f and the unit capacitance C_0 of the measurement device were found using the measurements of air and DI water.
2. R_{lead} and L_{lead} of different electrodes were found using the PBS measurements obtained between 10–20 MHz. The impedance data was fitted to the equivalent circuit shown in Figure 2. However, the analysis

did not include the CPE element in this step, as in this frequency range EP effects are not present. The conductivity of PBS was measured using a conductivity meter (Con11, Oakton) before each experiment. The relative permittivity of PBS was taken as 78 in this frequency range. The optimum R_{lead} and L_{lead} were found using a nonlinear least squares fit to impedance data. Unit and stray capacitances of the measurement device, conductivity and permittivity of PBS were set as constants in the fitting routine.

3. In the last step, PBS measurements in 1 kHz – 20 MHz range were used to determine the interfacial impedance. The parameters characterizing the interfacial impedance are κ and α of the constant phase element model. The impedance data was fitted to all the elements of the equivalent circuit shown in Figure 2. In the fitting procedure, unit and stray capacitances of the measurement device; conductivity and permittivity of PBS; R_{lead} and L_{lead} were set as constants and κ and α were set to vary. Increase of κ decreases the interfacial impedance and decrease of α makes the interface less capacitive.

For all the fitting above the parameters that gave minimum difference between fitted and measured data (residual) were used. The fitting procedures were performed in MATLAB® (2013a, Mathworks) using the nested *lsqnonlin* function that utilizes an algorithm to minimize the sum of the squares of the residuals. The fitting procedure varied the values of the variables in the models until the difference between the model and the measurement was minimized. Impedance measurements were repeated at least six times.

SEM was used to observe structural details of the electrodes. PBE and GNE SEM images were taken with Leo-Zeiss 1450VPSE at 10,000× magnification and 30 kV resolution. GGE SEM images were captured with dual beam focus ion Beam/HRSEM (FEI Strata 400 Dual Beam FIB).

A device (Surface Analyst, SA 1001, Brighton Technologies) that uses 1.5 ml of purified water to determine surface contact angle was used to perform electrode contact angle measurements.

2.5.Toxicity

Toxic effects of PBEs, GNEs, GGEs, and gold electrodes were measured on T-cell leukemia Jurkat cell line (ATCC, Manassas, USA). Jurkat cells were grown in Roswell Park Memorial Institute Medium (RPMI; ATCC, USA). The growth medium was supplemented with glutamine, penicillin, streptomycin and 10% fetal bovine serum and cells were grown in a humidified atmosphere with 5% CO₂ at 37°C. Electrodes that are 8 mm in diameter were used in toxicity experiments. The electrodes were rinsed with 70% ethanol, deionized water, and air dried in a sterile hood. The electrodes were left under UV (ultraviolet) light overnight for sterilization. They were later placed in an 80 mm petri dish that also had sterile water in a separate small, open container (Fig. S4, supporting information). A droplet (~80 µl) of Jurkat cell suspension at a concentration of 4×10⁵ cell/ml was put on the electrodes. The petri dishes were placed inside a humidified incubator, and left for 24 hours. Cells suspension on the electrodes was transferred to centrifuge tubes after incubation for 24

hours. Toxicity is assessed by a Trypan Blue exclusion assay (Sigma-Aldrich, St. Louis, MO, USA).

3. Results and Discussion

3.1. Impedance Measurements

Microfluidic devices were filled with PBS and impedance measurements were performed between 1 kHz and 20 MHz for plain gold and platinum, platinum black, gold nanostructured, and gold granulated electrodes. Impedance data obtained for each electrode type were fitted to the equivalent circuit model shown in Figure 2. The complex dielectric spectrum of the solution and CPE model parameters were extracted. In Figure 3 measured and simulated spectra of relative permittivity and conductivity are shown for the electrodes. Comparison of simulated (lines) and measured (markers) values in Figure 3 indicates good fit between the measurement and the simulation. The relative impedance spectra in all cases start at values around 10^6 range in low frequency and end at the relative permittivity of water (~ 80) in the high frequency. Similarly, conductivity spectra start at values less than 0.1 S/m for plain electrodes at 1 kHz and end at conductivity of PBS (~ 1.6 S/m) at 20 MHz. The large differences in relative permittivity and conductivity spectra are due to large impedance of the electrodes/electrolyte interface. Modification of electrodes reduced the difference between low and high frequency permittivity and conductivity values as indicated by the comparison of dashed and solid lines in Figure 3. Reductions at the conductivity spectra with the introduction of the modified electrodes are much larger than the reductions in the relative permittivity spectra. CPE parameters κ and α were calculated for each electrode following the fitting procedure given above. The κ , α values, and electrode modification parameters are tabulated in Table 1. The mean and standard deviation on κ and α were calculated using consecutive measurements. The significant digits in Table 1 were given following an uncertainty analysis (Table S1 in the supporting information). In Table 1 the κ and α of modified electrodes were normalized using the κ and α of unmodified electrodes. The results in Table 1 indicate reduction in α and a major increase in κ . The increase in κ is largest for PBEs, followed by GNEs, and the least increase is in GGEs. According to Table 1, while for plain platinum and gold electrodes α is around 0.8 for both, modification of electrodes shifts α closer to zero. PB electrodes induced the largest reduction in α , followed by gold nanostructured electrodes, and the least reduction is in gold granulated electrodes. The slight variations indicated by the standard deviations could be due to minute differences in the wetted electrode surface area. The durability and stability of GNEs and PBEs were also tested by measuring κ and α every day for a time period of 15 days. In this time period the electrodes were stored in DI water. All measurements were taken using PBS. The variations in κ and α are depicted in Figure 4. The most drastic change in the CPE parameters occurred within the first day of measurements. Following this change, the CPE parameters converged to relatively stable values after 10 days. The most possible reason behind the sudden change in CPE parameters between first and second days could be peeling of weakly bonded grains on the electrode surfaces during introduction of PBS. Ultrasonic agitation [42], heat treatment [11] and polydopamine chemical reagent [43] were used in order to increase adhesion of coated materials on substrate. A baking step at constant temperature, which was also performed in this study, following electrochemical deposition is

a well-known technique to increase the adhesion strength [11]. While the baking step after electrode modification in this study could have helped for the stability of electrodes; the baking temperature was not increased beyond a point to prevent formation of larger grains, and hence, to prevent reduction of surface area [11].

3.2. Dielectric Spectroscopy Sensitivity Increase

Cell suspension capacitance is not constant with changing frequency but exhibit a dielectric dispersion at sub-MHz frequencies. This dispersion is known as the interfacial (β) dispersion, which occurs due to the mismatch between electrical properties of the cell membrane and the extracellular medium. In general, the dielectric dispersion of dilute suspension of cells and tissues can be described by the empirical Cole-Cole model [44]. A typical dielectric spectra of a cell (a T cell leukemia - Jurkat) suspension measured using PBEs are given in the supporting information (Figure S1). In dielectric spectroscopy, the measured dielectric spectrum is modeled using the serial combination of Cole-Cole and CPE models. In the modeling, the Cole-Cole parameters are extracted and related to cell dielectric properties. However, in sub-MHz frequencies the impedance due to EP is hundred folds higher than the impedance of cell suspensions; therefore, dielectric modeling of cells becomes inaccurate and impossible at some cases. The difference in the low frequency range is also evident at the spectra shown in Figure S1. Parametric modeling were undertaken in this study to evaluate if the developed electrodes are effective at reducing the interfacial impedance and alleviating cell suspension modeling. In our approach, first, total impedance spectrum was calculated using the serial combination of the Cole-Cole model and CPE impedance for each electrode type between 1 kHz and 40 MHz (forward model). The Cole-

Cole dielectric dispersion model is $\varepsilon_{\infty} + \frac{\varepsilon_s - \varepsilon_{\infty}}{1 + (j\omega\tau)^a} - \frac{\sigma_s}{\omega\varepsilon_0}$, where ε_s , ε_{∞} , σ_s , τ , ε_0 , a are low frequency permittivity, limiting high frequency permittivity, DC (direct current) conductivity, interfacial dispersion time constant, permittivity of vacuum, and a parameter that accounts for frequency broadening of the dispersion, respectively [44]. In constructing the total impedance spectrum, the Cole-Cole model impedance was constructed using the following parameters 800, 80, 1.3 S/m, 1.5×10^{-7} s, 0.87 for ε_s , ε_{∞} , σ_s , τ , ε_0 , a , respectively. These parameters are similar to what was obtained experimentally for Jurkat cell suspension (Figure S1). The quantities in Table 1 were used to construct the EP impedance for each electrode type. Second, using an optimization routine the input parameters were estimated (Inverse Model). Normalized error in the estimated Cole-Cole parameters using the inverse model for each electrode type are given in Table 2. According to the table, input parameters to the Cole-Cole model cannot be accurately predicted for plain gold and platinum electrodes due to the excessive EP effects, whereas, all of the modified electrodes yield very low error in the estimation of original Cole-Cole parameters. Therefore, all the electrodes developed in this study are effective performing dielectric spectroscopy for the given cell type. This condition is due to the fact that modified electrodes have less interfacial impedance than those of plain electrodes at low frequency. We also tested the DS sensitivity increase experimentally. We used gold, GG, and GN electrodes to measure *Saccharomyces Cerevisiae* (baker's yeast) cells' dielectric spectrum at 12% volume fraction in PBS (1.5 S/m conductivity). We used an inverse modeling approach to determine cell suspension dielectric properties. The inverse model that consists of serial

combination of CPE element and Cole-Cole models was used in tandem with an optimization routine to extract Cole-Cole model parameters of yeast cell suspension. The experimental data and simulated spectra that was produced using the inverse model are shown in Figure 5. The fit parameters obtained using the inverse model are also tabulated in Table S2 of the supporting information. According to the results in the figure, the interfacial (β) dispersion is not evident at the spectra for the gold electrodes due to higher levels of interfacial impedance, whereas the dispersion is visible around 2 MHz for GNEs and GGEs. While Cole-Cole parameters obtained using GNEs and GGEs are consistent among each other according to the Table S2, the parameters τ and ϵ_s obtained using gold electrodes are different than the parameters obtained using GNEs and GGEs by magnitudes of order. These support usage of GGEs and GNEs for increasing sensitivity of DS measurements.

3.3.Optimizing the Electrode Modification Processes

In the literature, various current densities were recommended for fabrication of PBEs using 1% chloroplatinic acid and 0.08% lead acetate [21, 45]. While, low current density was suggested to fabricate PBEs for relatively large electrode size [8], it was shown that larger platinizing current densities increased effective surface area for small electrodes [12]. In this study, we tested PBEs that were electrochemically deposited at different durations (60, 140, 300, 600, and 900 seconds) and at varying current densities (−30, −100, −170, and −250 mA/cm²).

Impedance measurements indicated achieving highest κ and the lowest α values at the highest current density and for the largest deposition duration. At the same current densities, the impedance measurements indicated that the roughness of the PBE surface increased with increasing time. However, the coating grew not only along the perpendicular direction to the electrode surface but also radially beyond the electrode perimeter for deposition durations larger than 600s (See supporting information, Figure S2). At deposition current densities higher than 170mA/cm² peeling of seed Pt electrodes was observed during PB coating. Therefore, deposition current density and duration of 170mA/cm² and 600s, respectively, for PB electrode modification were deemed to be the optimum parameters for the given conditions here. Effects of different electric potentials and deposition durations were tested on the performance of gold nanostructured electrodes to decrease EP effect. The electrodeposition potential has a great influence on the formation of fractal nanostructures since it is a major parameter in the kinetics of the deposition process [46, 47]. Increasing the electric potential accelerates the kinetics of electrodeposition, which promotes irregular growth of nanostructures. While relatively smooth structures are generated at low deposition potentials, fractal Au nanostructures can be obtained at higher deposition potentials [41]. In this study, varying deposition time (600 s, 900 s, 1200 s, 1800 s, 2100 s, 2700 s and 3600 s) and voltages (−0.3 V, −0.5 V and −0.7 V) were tested for generating gold nanostructured electrodes. The seed gold layers were peeled off when the deposition potential was increased over 0.7 V. We observed a monotonic decrease in the electrode/electrolyte interfacial impedance with increasing the deposition time. However, due to practical reasons the deposition periods over an hour was not feasible. Therefore, the constant potential of −0.7 V and deposition time of 3600 s were deemed to be the optimum parameters for decreasing the EP effect for the given conditions.

An activation energy barrier needs to be overcome to sinter gold nanoparticles. This barrier is dominantly dependent on the stabilizing ligand of gold nanoparticles. Gold nanoparticles in close proximity were shown to sinter via neck-like structures [48]. While gold nanoparticle sintering was observed even at 140 °C [49], higher temperatures accelerate sublimation of stabilizing agents. In this study, gold nanoparticles were sintered on a seeding gold layer to have gold granulated electrodes. Glass substrates were kept at constant temperatures on a hot plate while adding droplets of gold nanoparticle suspension on a plain gold layer. Effects of three parameters, namely, temperature, baking time, and total volume of dispensed gold nanoparticle suspension on interfacial impedance were investigated. Impedance measurements were conducted to determine κ and α of gold granulated electrodes that were prepared at varying conditions. Decrease of α and increase of κ was observed with systematically increasing the process temperature. Gold nanoparticle suspension immediately evaporated at high temperatures yielding rougher surfaces, whereas relatively smooth surfaces were obtained at relatively low temperatures. However, at hot plate temperatures exceeding 200 °C, evaporation induced cracks at the electrode regions, where the suspension was dispensed. The total volume of dispensed gold nanoparticle suspension was varied between 180 and 500 μ l. Systematic change of dispensed suspension volume indicated an optimum volume of 400 μ l for decrease of interfacial impedance. A similar optimum was found for baking time by systematically varying the time between 30 and 60 minutes. The optimum conditions (highest κ and lowest α) were deemed to be 200 °C, 400 μ l, and 1800s analyzing the impedance measurements.

3.4. Surface Morphology of Modified Electrodes

SEM images of the PBEs, GGEs, and GNEs are shown in Figure 6. The high magnification SEM images were also captured for the each sample to acquire information on the grain size (Supporting information Figure S3). Images were analyzed using Image J [50] and sizes of ten grains were measured to yield an average value. In comparison with the platinum electrodes, surface morphology of the PBEs is enormously complex (Figure 6a). Images show 3-D assembly of rounded grains on a smooth platinum electrode to yield a porous, cauliflower like structures with a high effective surface area. The rounded grains are on average 200 nm in radius (Figure S3a). The SEM images of GNEs indicate a fern leaf type self-similarity. As opposed to larger grains in the 3-D structure of PBEs, GNEs are composed of smaller grains, on average at 40 nm level (Figure 6b and Figure S3b). GGE surface which consists of 15 nm diameter grains are shown in Figure 6c and Figure S3c).

Surface roughness of PB, GN, and GG electrodes was measured using a profilometer (PS50, Nanovea) to provide a quantitative measure of roughness. The profilometer used has 6 nm vertical resolution and 1 μ m lateral resolution. The surface roughness is expressed in terms of average surface roughness (R_a) that is defined as arithmetic mean of height deviations of valleys and peaks with reference to a mean line [51]. In Table 3, average surface roughness of different electrodes is tabulated. Results indicate the following order for average roughness PB>GN>GG electrodes, which is also in agreement with the order of CPE parameter α for these electrodes.

CPE is an empirical relationship describing the frequency dependent dispersion at the electrode electrolyte interface. Electrode surface topology has long been known to influence the CPE exponent α , and linear relationships between the CPE exponent and the fractal dimension of the electrode were found in various studies. Nyikos and Pajkossy derived a relationship between fractal dimension and CPE exponent for an ideally polarizable electrode (i.e. metal electrode in contact with an electrolyte) using a scaling approach in the absence of Faradaic reactions and specific adsorption [44]. Liu using a Cantor bar model for the electrode-electrolyte interface formulated the following relationship for the CPE exponent for a 3 dimensional measurement cell $\alpha = 3 - d$, where d is the fractal dimension of the electrode [52]. Fractal dimension of the gold nanostructured electrode can be estimated considering that gold electrodeposition is a diffusion limited aggregation process. 3 dimensional computer models of diffusion limited aggregates revealed the fractal dimension of 2.4 for such structures [53]. Using this dimension in the above formula for the CPE exponent, we obtained $\alpha = 0.6$, which is close to what was observed experimentally (0.55). Similarly, assuming a fractal dimension of the PB electrode the same as the fractal dimension of a cauliflower [54], we obtain $\alpha = 0.2$, which is close what was observed experimentally (0.25).

Hydrophobicity of electrode materials could be critical for microfluidic applications. Hydrophilic materials are usually desirable for microfluidic components considering practical aspects such as elimination of air bubbles. Contact angle of PB, GN, and GG electrodes was measured using an automated device that places a droplet of water on substrates. The contact angle measurement results are tabulated in Table 4. Results show high contact angle of PBEs (134°) indicating its hydrophobic behavior. According to the measurements, the most hydrophilic electrode was gold nanostructured electrodes with a contact angle of 54°. Hydrophilic gold nanostructured electrodes have a major advantage over hydrophobic PBEs for microfluidic applications. In order to visualize the surface behavior of the electrodes, pictures of DI water droplets on PB, GN, and GG electrodes were taken. These are shown in Figure 7. The relative hydrophobicity of PBEs can be linked to surface roughness; however, the relation between the wettability and surface roughness is not simple, and currently there is no single universal theory that can establish a solid link between these two properties. Classically, Wenzel and Cassie – Baxter theories explain wetting of rough interfaces. According to the Wenzel theory, a rough surface amplifies or diminishes specific energy of the liquid-solid interface proportional to a roughness factor that is the ratio of the actual surface to the geometric surface area [55]. Therefore, a rough hydrophobic surface is more hydrophobic relative to its planar form and a rough hydrophilic surface is more hydrophilic relative to its planar form according to the Wenzel theory. On the other hand, Cassie – Baxter theory considers gas bubbles that are trapped in between solid and liquid phases[56]. Therefore, the Cassie – Baxter theory involves a reduction in the solid-liquid interfacial area by a factor f . For a given rough surface, increase of f (decrease of solid/liquid interfacial area) increases hydrophobicity of the rough interface according to the Cassie – Baxter theory. Recently, other wetting regimes such as Lotus and rose petal effects, which correspond to different levels of surface roughness, were identified and reported [57]. Roughness at different levels (micrometer or nanometer) and chemical composition of the surface work in tandem to modify surface wettability behavior. Other studies investigated

effects of three dimensional surface topological parameters on wetting characteristics [55, 58]. In these studies, functional relations between standardized roughness parameters, such as developed area ratio and relative material ratio of roughness, were developed. Several experimental evidence were given in these studies that relate pitch and height of the surface structures to water contact angle. In the light of these studies, it is possible that water cannot wet PBE surface completely and gas pockets are present in relatively deeper parts of the rough PB surface. Several other studies also reported relatively hydrophobic behavior of PBEs [9, 59–61]. If this condition is true, however, presence of gas pockets on PB electrode surface did not cause a significant change in impedance characteristics of metal/liquid interface as inferred from the conductivity results in Figure 3. Replacing liquid by air on electrodes will decrease conductivity proportional to f . This suggests that air pockets cover relatively low amount of PB electrode surfaces. Therefore, in the light of this discussion, changes in electrodes' electrical characteristics should reflect the changes in the roughness not the air phase.

GNEs prepared in other studies had both hydrophobic and hydrophilic properties with water contact angles ranging from 10° to 176° . In general, contact angles below 90° were considered hydrophilic, and above hydrophobic. Also, super-hydrophobic and super-hydrophilic surfaces were prepared that have contact angles higher than 150° and less than 30° , respectively. The surface wettability of GNEs depends strongly on the chemical species used in chemical deposition, such as n-dodecanethiol to obtain hydrophobic surfaces[62]. Water contact angles for several different gold nanostructured electrodes are tabulated in the supporting information Table S3.

3.5. Bio-Toxicity of Electrodes

Toxic effects of electrodes should be minimal for bio-sensing applications. Toxicity of PB, GN, GG, and gold electrodes on a T-cell leukemia cell line was measured using Trypan Blue exclusion technique. Typan Blue is indicative of membrane poration and is generally used as a marker for dead cells. Cells were counted after 24 hours of incubation with electrodes. The dead over live cell ratio for the electrodes is shown in Figure 8. On Jurkat cells, PBEs yielded the highest mortality rate at 32%, followed by granulated electrodes at 19%, nanostructured electrodes at 10%, and plain gold electrodes at 8%. While the toxicity of PBEs could be due to the presence of low quantities of lead (0.08% lead acetate) involved in preparation of the electrodes, toxicity of GGEs could be related to potential toxicity of gold nanoparticles. Toxicity of 10 nm gold nanoparticles was previously tested on Jurkat cells over a time period of 24 hours [63]. The preparation of GNEs on the other hand involved 1 mM AuNaCl₄, which involves no known toxic chemicals. Relatively high toxicity of plain gold electrodes could be due to evaporation induced osmolar changes in the cell suspension droplet, which could have brought an offset to all toxicity measurements.

Toxicity of PBEs was previously reported. Platinum black coated electrode was used as a neural sensor and tested on mouse fibroblast cells [27]. The platinum black in the presence of lead showed a toxic effect on cells, and hence, it was not recommended for any biomedical application of chronic human implants. On the other hand, several studies utilized gold nanostructured electrodes for biomedical applications, and no toxic effects were reported for

gold nanostructured electrodes [40, 64, 65]. For instance, Zhang *et al.* fabricated gold nanostructures in order to enhance capturing efficiency of rare cells based on the similarity between cells and fractal electrodes' surface topographic features. Rare cells were trapped from whole blood, and consequently cells were released from electrode surface. High viability ratio was reported even after cells were released [66].

4. Conclusions

Fractal gold nanostructured and granulated gold electrodes were developed to decrease the interfacial impedance at electrode/electrolyte interface. The exponent (κ) and coefficient (α) of the constant phase element model for modified gold electrodes were measured and verified by surface morphology measurements. Modified gold electrodes were shown to increase the low frequency sensitivity of dielectric spectroscopy measurements. Contact angle measurements showed relative hydrophilic behavior of GNEs and GGEs. Toxicity measurements on Jurkat cells indicated relatively higher biocompatibility of nanostructured and granulated gold electrodes compared to PBEs. Relative biocompatible and hydrophilic nature of modified gold electrodes could be utilized to increase sensitivity of various biosensors that operates with an electrolyte solution and involve electric fields at low frequency to test biological samples. Relatively simple preparation technique of gold granulated electrodes compared to costly electro-deposition techniques could be favorable for preparing low-cost disposable biosensors. Furthermore, EP of microelectrodes that are used in single cell electro-manipulation tools, such as dielectrophoresis (DEP) and electro-rotation, is also a practical problem [67]. For instance, in DEP the low frequency ($f < 1$ kHz) positive DEP force is affected by EP, and at higher sub-MHz frequencies electric energy for particle manipulation is reduced by EP effect [68]. Therefore, electrodes developed here can also be used in electro-manipulation studies of cells. A future study will examine electric double layer on modified gold electrodes from the perspective of Nernst-Planck Equations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A schematic of the microfluidic channel. Two electrodes (4mm diameter) were aligned on top of each other. A double sided tape formed the microfluidic channel. The solutions were fed using a pipette through the inlet. Inset figure shows a glass substrate with an electrode.

Figure 2. Equivalent circuit model of the microfluidic channel. Impedance of various fluids was measured using the microfluidic channel. Electrode/electrolyte interfacial impedance is modeled using a constant phase element.

Figure 3.

Conductivity and permittivity spectra of PBS measured using modified and plain electrodes. The difference between the limiting low and high frequency values are due to electrode/electrolyte impedance. Figures (a) and (b) are the conductivity and permittivity spectra of plain gold, gold nanostructured, gold granulated electrodes, respectively. In (a) and (b), squares, triangles, and circles denote experimental data of gold nanostructured electrodes, gold granulated electrodes, and plain electrodes, respectively. Figures (c) and (d) are conductivity and permittivity spectra of plain platinum and PB electrodes, respectively. In (c) and (d), squares and circles denote experimental data of platinum black electrodes and plain platinum electrodes, respectively. In all figures lines are the best fits to the experimental data.

Figure 4.

Time lapse impedance measurements were performed with platinum black and gold nanostructured electrodes. The changes in κ versus time are shown in (a) and (c) for gold nanostructured (●) and platinum black (▲) electrodes, respectively. The changes in α versus time are shown in (b) and (d) for gold nanostructured electrodes (●) and platinum black (▲), respectively.

Figure 5.

Permittivity and conductivity spectra of *Saccharomyces Cerevisiae* (baker's yeast) cells' in PBS (1.5 S/m conductivity) at 12 % volume fraction. The spectra were measured using GE ((a) and (b)), GGE ((c) and (d)), and GNE ((e) and (f)) electrodes (circles). Using an inverse model Cole-Cole parameters of yeast cell suspension were extracted. The continuous lines show the total spectra of cell suspension and interfacial impedances calculated using the inverse model and the dashed lines show cell suspension dielectric spectra.

Figure 6. SEM images of platinum black (a), gold nanostructured (b), and gold granulated electrodes (c). Fig. 5a and 5b were taken at 30kV and 10000 magnification factor. The scale bar indicates 5 μm. The imaging conditions for (c) is 500 kV and 120000 magnification

Figure 7.

Pictures of a deionized water droplet on PB (a), GN (b) and GG (c) electrodes. Schematic of water droplet showing the contact angle (d).

Figure 8. Dead over live ratio of Jurkat cells following incubation with Platinum Black (PB), Gold Nanostructured (GN), Gold Granulated (GG), Plain Gold (PG) electrodes. Error bars show standard deviation over 3 repetitions.

Table 1

CPE parameters (κ and α) of electrodes. Measurements were taken in chamber where channel height is 500 μ m and electrode radius is 2 mm. Chamber was filled with PBS (1.62 S/m), and measurements are taken at 1 kHz –20 MHz.

Electrode	Method	Time[s]	$\kappa / \kappa_{\text{control}}$	$\alpha / \alpha_{\text{control}}$
Gold Nanostructured	Potentiostatic (–0.7V)	3600	389 $\pm$ 25	0.6693 $\pm$ 0.0115
Platinum-Black	Galvanostatic (–170mA/cm ²)	600	32857 $\pm$ 2470	0.3171 $\pm$ 0.0129
Gold Granulated	Sinter (200 °C, 400 μ l)	1800	48 $\pm$ 3	0.7626 $\pm$ 0.0131
Platinum: $\kappa_{\text{control}} = 1.75 \times 10^{-6} \pm 0.055 \times 10^{-6}$ $\alpha_{\text{control}} = 0.8012 \pm 0.006$				
Gold: $\kappa_{\text{control}} = 4.62 \times 10^{-6} \pm 0.1 \times 10^{-6}$ $\alpha_{\text{control}} = 0.8243 \pm 0.006$				

Table 2

The normalized error in the estimated Cole-Cole parameters (ε_s , ε_∞ , σ_s , τ , a) using the inverse model. Parameters with superscript shows values predicted by inverse modeling.

Electrode	$\frac{\varepsilon_s - \varepsilon_s}{\varepsilon_s}$	$\frac{\varepsilon_\infty - \varepsilon_\infty}{\varepsilon_\infty}$	$\frac{\sigma_s - \sigma_s}{\sigma_s}$	$\frac{\tau - \tau}{\tau}$	$\frac{a - a}{a}$
Gold	0.99	0.85	0.20	0.73	0.99
GN	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
GG	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Platinum	0.99	0.87	0.19	0.57	0.02
PB	$>10^{-6}$	$<10^{-6}$	$>10^{-6}$	$>10^{-6}$	$>10^{-6}$

Table 3

Surface roughness is given in terms of Ra (average surface roughness).

Material	R _a (μm)
GNE	1.05
GGE	0.42
PBE	3.2

Table 4

Contact angles are given for different materials.

Material	Contact Angle (θ°)
PBE	134°
GNE	54°
GGE	77°