Bio-inspired patterned networks (BIPS) for development of wearable/disposable biosensors

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Bio-Inspired Patterned networkS (BIPS) for the development of wearable/disposable biosensors

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ABSTRACT
Here we demonstrate a novel approach for fabricating point of care (POC) wearable electrochemical biosensors based on 3D patterning of bionanocomposite networks. To create Bio-Inspired Patterned network (BIPS) electrodes, we first generate fractal network in silico models that optimize transport of network fluxes according to an energy function. Network patterns are then inkjet printed onto flexible substrate using conductive graphene ink. We then deposit fractal nanometal structures onto the graphene to create a 3D nanocomposite network. Finally, we biofunctionalize the surface with biorecognition agents using covalent bonding. In this paper, BIPS are used to develop high efficiency, low cost biosensors for measuring glucose as a proof of concept. Our results on the fundamental performance of BIPS sensors show that the biomimetic nanostructures significantly enhance biosensor sensitivity, accuracy, response time, limit of detection, and hysteresis compared to conventional POC non fractal electrodes (serpentine, interdigitated, and screen printed electrodes). BIPS, in particular Apollonian patterned BIPS, represent a new generation of POC biosensors based on nanoscale and microscale fractal networks that significantly improve electrical connectivity, leading to enhanced sensor performance.

1. INTRODUCTION
There is a critical need for rapid, cost-effective, accurate and easy-to-use biosensors that can operate at the point-of-care (POC) and subsequently replace conventional laboratory-based detection methods (e.g., ELISA, PCR) that are tedious, costly, and time-consuming. Although recent developments in materials and nanofabrication techniques have transformed the development of nanobiosensors, a major technological hurdle that deters progress is the low reliability and poor performance of most nanobiosensors in field conditions or POC applications. Screen-printed electrodes, serpentine electrodes, and interdigitated electrode technologies are currently the standard platform for many electrochemical POC biosensors, and most state of the art electrodes are functionalized with conductive nanomaterials to enhance signal transduction. The development of nanomaterial-functionalized electrodes in the last decade has improved the performance compared to standard electrodes, and resulted in faster biosensors with detection limits superior to devices based on traditional (i.e., non-nanomaterial coated) electrodes. Many labs, including ours, have developed a variety of nanobiosensors based on this concept, and inclusion of graphene-nanometal hybrids has been shown to significantly enhance sensor performance [1]–[9]. However, problems with stability in POC or field applications are a persistent problem. Other researchers such as Soleymani [10] and co-workers have begun to piece together the relationship between biodetection sensitivity and controlled nanostructuring of electrochemically deposited metallic nanoparticles (e.g., nanostructured palladium microelectrodes) and nucleic acid detection/biosensing. Yet, the link between electrode nanostructuring and the transport of target molecules, transduction signals (e.g., electrons), and byproducts in hybrid bionanomaterials remains relatively unexplored in the literature. Filling this knowledge gap is particularly important for “real world” POC applications where accuracy and reliability are paramount. To further understand the fundamental aspects of hybrid bionanomaterials in field applications, we couple optimal scale free network patterns with state of the art nanomaterial-mediated biosensors.
Biomaterial patterning is one of the most promising techniques for improving biosensor stability. The first publications on microscale biomaterial patterning date back to the 1980's [11]–[13]. Recent work to pattern biomaterials for sensing applications has focused on use of DNA, quantum dots, or graphene as a molecular template, leading to development of nanoscale patterned biomaterials [14], [15]. Work in the characterization of biological transport networks and complex socio-technical systems [16]–[19], have realized a number of bio-inspired scale free network models that generate networks optimizing transport. The optimization of tree-like networks is obtained by models that minimize the energy required to transport fluxes globally in the network. Equivalently, for 3D packing structures the energy that is minimized is related to the packing energy of objects that are assembled together. We propose a new concept for patterning conductive networks of hybrid bio-nanomaterials based on these established in silico network models. The structure of these space filling networks is linked to universal scaling laws that regulate the size of parts to the function of the system resulting into scale-free patterns [16], [20], [21]. The existence of scaling laws proves the fractal character of these networks. Apollonian networks are special space-filling fractals in which components have meaningful dimensions in two or three coordinate directions. These networks can be constructed using models from Apollonian patterns that generate triples of circles, where each circle is tangent to the other two and all newly constructed circles are iterated to produce triples of tangent circles. Space-filling tree networks can be considered as a special case of Apollonian networks that maximize the coverage of space with links and nodes rather than with circles. Both space-filling networks and Apollonian patterns have been shown to maximize the coverage of space with highly inter-connected links and circles, respectively, across multiple physical scales, from microscale to macroscale networks. We use both Apollonian patterns and tree-like networks to create optimal biosensing patterns.

For the first time, bio-inspired patterns were developed with space filling, scale free network models and the patterns were printed with graphene ink on disposable substrates (Fig 1). The long term goal of this project is to create affordable, accurate POC wearable biosensors that can be used for monitoring compounds in field or bedside applications using wearable/disposable substrates (e.g., Kapton®, nanocellulose paper). Here, we show proof of concept for the development of BIPS electrodes, and validation for electrochemical detection of glucose.

![Figure 1. a) Bio-inspired patterned sensor (BIPS) network electrodes are 2D printed using graphene ink. Biomimetic patterns are generated via an optimal network model, and printed using graphene ink. b) Fractal platinum nanocauliflower structures are formed on the graphene ink by pulsed sonoelectrodeposition. The metal surface is biofunctionalized and performance is compared to commercial electrodes (also coated with graphene-nanoplatinum).](image-url)
2. METHODOLOGY

In silico design of patterns

Four different bio-inspired patterns were tested, representing tree and gasket scale free structures (Fig 2). Two major types of structural network models were used: (i) Apollonian patterns (known as gaskets) (Fig 2 a-b), and (ii) networks with different topologies (Fig 2 c-d). The networks are generated by computer simulations that replicate the Apollonian packing and the energy minimization criteria of tree-like networks. To generate the networks, the energy landscape (total electrode surface) is divided into 1nm basins, and the network model guides the fill-packing of basins with sensing hypersphere aggregates. The basins of attraction of the energy landscape provide a fractal-like network solution, yet producing scale-free patterns over space. Apollonian gaskets optimize the positions of circles in the 2D domain from randomly placed circles, while minimizing distance between circles. The gasket in Fig 2a represents an even distribution of 2D circles, where the pattern in Fig 2b has relatively high anisotropy. The tree structure in Fig 2c represents a random network that can be assumed as the initial condition for optimal networks. Fig 2d represents an optimal tree network.

![Figure 2](image)

Figure 2. Nanohybrid electrodes are patterned using space filling models, and graphene-platinum ink is inkjet printed. The images represent network coverage of 80% relative to a flat disc. a-b) Two Apollonian gaskets, c-d) Two space filling networks (random and scale-free, respectively).

After printing the graphene ink, fractal nanoplatinum was deposited using pulsed sonoelectrodeposition ([6], [22] to create a transducer layer as the working electrode and electrodes were biofunctionalized for measuring glucose (described below).

Patterned graphene networks

Inks were printed using an Optomec aerosol jet 200 printer with a standard atomizer and 100mm s-1 print speed. Graphene-platinum inks was prepared based on a recipe developed by Pham et al [23]. Briefly, the ink was prepared by mixing thermally exfoliate graphite oxide, ethylene glycol and citric acid in a 1:2:8:30 molar ratio, and then reduced with ascorbic acid (2mg/mL) for two hours at 30°C. Prior to printing, the solution was filtered through a 0.45 μm syringe filter. Nanocellulose paper was prepared based on [24]. A 100 μm thick layer of cellulose nanocrystals (CNC) was formed by vacuum filtration of a 1:4 (CNC slurry: DI water) mixture through cellulose acetate filter paper. The filter with CNC retentate was removed and pressed between borosilicate glass slides, and then dried at 75°C for one hour. For printing all patterns in shown Fig 2, the “landscape size” (i.e., physical diameter of the working electrode) was 10 mm. A reference and counter electrode were printed using a silver ink, and a Ag/AgCl reference electrode will be formed following McLamore et al [25].

Nanometal deposition and biofunctionalization

Patterned graphene ink electrodes were decorated with nanoplatinum based on [6], [22]. Nanoplatinum was deposited for 60 seconds in a solution of 0.72% chloroplatinic acid and 0.001% lead acetate at 10V in DI water at 20°C at a pulsed frequency of 900 mHz. Prior to biofunctionalization, a 20 nm thick layer of Nafion will be coated on paper and flexible BIPS electrodes using the procedures in Vanegas et al [5]. BIPS electrodes were
biofunctionalized with glucose oxidase (GOx) by metal-his bonding. First, 2mg of his-tagged GOx (lyophilized powder, Asperillus niger) was mixed with 50 µL of HEPES buffer at 20°C. The solution was drop cast on electrodes, and allowed to bond for 5 minutes at room temperature. Next, the electrode was rinsed three times with HEPES buffer and allowed to dry at room temperature for an additional 5 min.

Material analysis, imaging and analytical chemistry
Morphological characterization was conducted using scanning electron microscopy (SEM) and ellipsometry. Each pattern was tested for fundamental electron transport and mass transport using cyclic voltammetry (CV), DC potential amperometry (D CPA), and electrochemical impedance spectroscopy (EIS). The electroactive surface area of each electrode was determined by the Randles-Sevcik theorem. To further evaluate electron transport and mass transport, EIS was conducted using an eDAQ analyzer (ERZ100). Nyquist and Bode plots were prepared to determine electrolyte resistance, charge transfer resistance, Warburg impedance, and double layer capacitance according to our recent work [22].

3. RESULTS AND DISCUSSION

Fig 3 shows confocal imaging and subsequent tracing analysis of protein networks formed on graphene-nanoplatinum modified electrodes (panel a), and an electrode printed using a scale free network model (panel b). Fluorescamine (FITC)-conjugated, his-tagged glucose oxidase was immobilized on a BIPS electrode as a proof of concept (scale bar denotes 5µm). The image on the right of each panel shows the result of a tracing model (Moore’s neighbor modified for 2D spheres). The tracing model was used to identify connectivity between aggregates (structures larger than 1µm) and nanostructures (smaller than 1µm). Connectivity (denoted as white circles) was defined as at least three neighboring structures in direct contact. Fig 3a shows high aggregation, and these aggregates did not destabilize after washing or light sonication. Poor network connectivity (denoted by gray circles) results in local diffusion limited transport and poor sensor performance. Fig 3b shows a bionanocomposite network 3D printed based on an “Eden” scale free network model. As shown in the tracing model, protein nanostructures are highly connected within the bio-inspired pattern, resulting in improved biosensor performance.

![Figure 3. Protein patterning at the network scale is vital to biosensor performance. Confocal microscopy and tracing analysis of glucose oxidase nanocomposites as a demonstration of the proposed principle. a) Protein network formed on a non-patterned electrode shows relatively poor connectivity b) Protein networks patterned using a scale free network model are highly connected. Scale bar represents 5µm.](image)

Fig 4 shows proof of concept data demonstrating the validity of the proposed hypothesis. For this demonstration, we printed all four networks shown in Fig 2 at a network coverage of 80%; his-tagged glucose oxidase was then functionalized onto the BIPS electrode. Fig 4 shows the electroactive surface area, sensitivity, and LOD for GOx biosensors prepared on different patterned electrodes (including a bare Pt/Ir electrode coated with rGO-nPt for comparison). In addition to these initial trials with glucose, biogenic amine BIPS sensors were developed and tested in buffer. Patterning of graphene hybrid nanomaterials using model-derived biomimetic networks significantly improve sensitivity (29%), selectivity (17%), and accuracy (31%). Based on ANOVA, the Appolonian gasket in Fig 2a had the highest sensitivity (p<0.001; α=0.05), followed by the gasket in Fig 2b and the optimal tree in Fig 2d. The lowest sensitivity was for the random network in Fig 2c, which was still at least 6% higher than a commercial screen printed electrode. Patterning of graphene hybrid nanomaterial reduces electroactive surface area by 7% when compared to a flat disc electrode, but uses 28% less ink; clearly indicating an increase in network transport efficiency.
Corson, Banavar et al., and other authors evidenced that tree-like structures are optimal transport network structures independently of any initial and boundary conditions, and intra-domain configuration of links filling the space. This comparison allowed us to tease out differences in transport for concentric loops versus connected branches among Apollonian patterns, trees, and gaskets. In this work, we show a clear trend, where the Appollonian gasket in Fig 2a is clearly more connected than the other networks, and the random network (albeit better than a screen printed electrode), had the poorest connectivity amongst the BIPS. Results prove that for biosensing purposes the Appollonian gasket (that is amenable to a looping scale free network) the sensitivity is the highest. This sensitivity is higher than loopless and directed scale free networks and it is also dependent on the spatial arrangement of spheres. We speculate that the directed scale free network does not capture the potential multiplicity of fluxes occurring in the sensed domain and impose an unnatural flow direction where fluxes accumulate toward the outlet. These results may be dependent on glucose; however, the increased sensitivity in biosensing for scale free networks versus non fractal networks seems a quite universal result independent of the sensed agent and biosensing potential.

Graphene is well suited as a transducer layer for biosensing because it is mechanically robust, extremely flexible, chemically stable and highly conductive. We are currently in the process of developing the ability to inkjet print graphene inks on Kapton tape to expand these results for including wearable biosensors. Our unpublished data (not shown here) indicate that we can print graphene ink/insulators to develop wearable biosensors based on the proof of concept data shown here.
4. CONCLUSIONS

In order to improve field/bedside applications of biosensors for detecting small molecules (or even whole cells/proteins), fabrication techniques are needed that are simple, inexpensive, durable, reproducible, and reliable. Development of flexible electrodes is a critical need for POC biosensors, since the flexible aspect permits development of technologies that can adhere to the contours of a biological surface (e.g., animals, plants). Furthermore, disposable POC biosensors are needed to facilitate rapid field diagnostics for one-time use applications. This approach is a way to directly engineer biomaterial-nanomaterial connectivity resembling optimal networks in nature, and experiments are designed to further improve understanding of nanoscale and microscale transport in biosensors. We tested the optimality of scale free patterns, in particular Apollonian gasket, for biosensing sensitivity in contrast to the patterns generated by random placement of sensing agents. Here, we demonstrate proof of concept on disposable (nanocellulose) substrates, and we are currently expanding this concept to include wearable biosensors.

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6. REFERENCES


