Detecting Disease Biomarkers Using Nanocavities and Nanoparticle Composites

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Abstract. The convergence of electrochemistry, materials, photonics and biomedical science at the nanoscale opens up significant opportunities for developing advanced sensors. In this contribution, we present examples of our use of nanometer dimensioned electrodes, nanocavities and nanoparticle-metallopolymer composites to create high sensitivity detection platforms and materials for detecting proteins and nucleic acids. The application of these approaches in the diagnosis and prognosis of cancers such as neuroblastoma, as well as point-of-care detection of infectious disease, will be discussed.

1. Introduction

The detection of disease biomarkers well in advance of clinical symptoms offers the possibility of improved patient outcomes and lower overall healthcare costs. However, the concentrations of these protein or nucleic acid biomarkers is typically very low, nanomolar to picomolar and for point-of-care applications using sample volumes of the order of a microlitre is desirable. Thus, the analytical challenge is often to detect a few thousand biomolecules in a complex sample. Success demands highly sensitive detection strategies and sensors that can address small sample volumes. Reducing the critical dimension of electrodes to the micron and smaller length scale offers unique analytical advantages in terms of the sample volume required, the speed of analysis and their high current density which can dramatically enhance the detection sensitivity.[1] Recessed micro-electrodes, offer further advantages and have the potential for single molecule detection limits. Moreover, the cavity can be functionalized, e.g., packed with an electrochemiluminescent nanocomposite,[2-4] to create sensitive sensors that are mechanically stable under real world conditions. However, typical recessed electrodes either have a rather simple geometry, e.g., a recessed planar disk, or the geometry is ill-defined, e.g., when microdisk electrodes are etched with acid, the resulting electrode shape depends strongly on the etch rate and irregular structures may be formed. Therefore, there is a significant need for new approaches that yield reproducible recessed ultramicroelectrodes of well defined geometry without using costly micro-fabrication techniques.

This contribution describes the use of nano-sphere lithography,[5] to create single conducting nanocavities, or arrays of cavities, and significantly shows how their shape can be controlled from spheres to cuboids. Moreover, a strategy to selectively modify their top and interior surfaces using self-assembled monolayers is described. Finally, the direct, electrochemiluminescent detection of biomolecules using a [Ru(bpy)$_2$PVP$_{10}$](ClO$_4$)$_2$ metallopolymer: gold nanoparticle nanocomposite is
demonstrated; bpy is 2,2’-bipyridyl and PVP is poly(4-vinyl pyridine). The limit of detection achieved using this combination of micro- or nano-electrodes and nanocomposites is several orders of magnitude lower than conventional systems.

2. Results and Discussion

2.1 Single and Arrays of Nanocavity Electrodes.

Figure 1 illustrates the approach used to construct single nanocavity electrodes.

![Diagram of nanocavity electrode construction](image)

**Figure 1.** Scheme showing the formation of a nano-cavity electrode and SEM images of (a) a single polystyrene sphere on a 10 µm gold electrode (b) gold which has been electrodeposited around the sphere to a thickness of 600 nm (c) electrode surface after sphere removal, showing a circular pore where the sphere was present and (d) higher magnification image of the pore.

First, a single polystyrene sphere, or an array of spheres, is deposited on an electrode and gold is electrodeposited around the sphere. A blocking layer of 1-hexadecanethiol is then self-assembled at the upper planar gold surface before sphere removal. This blocking layer confines the electrochemical reactions to the inside of the nanocavity. After sphere removal, a truncated spherical cavity is obtained, the dimensions of which is controlled by the templating sphere size and the thickness of electrodeposited gold. The choice of templating sphere limits the cavity size, while the thickness of gold deposited around the sphere controls the actual depth and pore opening produced. Also, by using an intermediate step in which a polydimethylsiloxane, PDMS, mould of the array is cast, the shape of the metal nanocavities can be altered by mechanical stretching. Figure 1 shows an example of a single sphere (r=410 nm) deposited on a 5 µm radius gold microdisk. Figure 1b shows the formation of the nanocavity following gold deposition to a depth of 600 nm. SEM reveals that the cavity opening is approximately 750 nm wide, which is consistent with the charge passed and a gold deposit of approximately 600 nm thickness.

The inset of Figure 2 shows the voltammetric response for a 5 µm radius microelectrode in contact with a 2 mM [Fe(CN)₆]³⁻ dissolved in aqueous 0.2 M Na₂SO₄ as the supporting electrolyte. The electrode corresponds to that shown in Figure 1(b) whereby gold has been electrodeposited around a single templating sphere but no blocking of the surface with a monolayer has taken place and the sphere remains in place. Consistent with a response under radial diffusion control, a well defined steady state current, iersist, is observed.[6]
Figure 2. CVs obtained at the nano-cavity electrode in 2 mM [Fe(CN)₆]⁴⁻ with 0.2 M Na₂SO₄ as the supporting electrolyte. Curves A and B were obtained before and after sphere removal. The exposed gold surface was blocked with a C₁₆SH layer before sphere removal and the inset shows the voltammetric response of the unblocked electrode.

Figure 2 (curve A) shows the response of this electrode after deposition of the C₁₆SH self assembled monolayer, but before polystyrene sphere removal, corresponding to the electrode shown in Figure 1(c). In sharp contrast to the response illustrated in the inset of Figure 2, no well defined steady state current is observed and the current is approximately 350 times lower than that observed before monolayer formation. The blocking response seen here is typical behavior for an alkanethiol layer and a hydrophilic probe such as [Fe(CN)₆]⁴⁻. When the sphere is removed, but the alkanethiol remains assembled on the upper gold surface, corresponding to Figure 1(d), and the solution phase probe can access the cavity. Figure 2 (curve B) shows the voltammetric response obtained in 2 mM [Fe(CN)₆]⁴⁻ with 0.2 M Na₂SO₄ as the supporting electrolyte after the sphere has been removed by sonication in THF for 30 minutes. Here, steady state behavior, reminiscent of that seen at the unmodified microelectrode (inset), is clearly observed. However, the current magnitude is significantly smaller than observed at unblocked surface, which reflects the small electrochemically active area of the nanocavity electrode.

2.2 Nanocomposites for High Sensitivity Electrochemiluminescence Detection of Biomolecules.

As well as developing physically small sensor platforms capable of addressing small sample volumes, highly sensitive detection of biomolecules also requires the development of advanced sensor materials. Electrochemiluminescence (ECL), an electrochemically-induced process of generating light at the electrode surface and produces a high signal to noise ratio because an excitation source is not required. Specifically, metallopolymers of the form [M(bpy)₂PVP₁₀]²⁺(ClO₄)², where M may be Ru or Os, bpy is 2,2ʹ-bipyridyl, PVP is poly (4-vinylpyridine); are attractive due to their relatively long emission wavelength and stability in both oxidized and reduced forms.[7] Polymers in which the metal complex is coordinated are attractive because they eliminate the need for the luminophore to diffuse to the electrode surface and thin films can be easily formed. Enhancing the electrochemiluminescence intensity generated by these metallopolymer films relative to the background is a significant approach to improving the limits of detection. We recently demonstrated that incorporating 4-(N, Nʹ-dimethylamino) pyridine protected gold nanoparticles (DMAP-AuNPs) enhances the ECL emission intensity of the [Ru(bpy)₂PVP₁₀]²⁺ metallopolymer by up to 7 fold depending on the nanoparticle diameter where the co-reactant is oxalate.[8] This enhancement arises predominantly from a greater
film conductivity rather than plasmonic effects. However, the ability of nanocomposites to improve the signal-to-noise ratio and provide lower limits of detection remains under explored.

Figure 3 shows the ECL response for electrodes modified with the nanocomposites at different concentration of β-NADH. It is observed that the ECL emission intensity increases with an increasing β-NADH concentration. The nanocomposite of the metallopolymer and 12.5 nm DMAP protected gold nanoparticles dramatically lowers the detection limit of β-NADH due to the enhanced ECL emission intensity and fM concentrations can be detected.

![Graph showing ECL intensity versus concentration of β-NADH](image)

**Figure 3.** Dependence of ECL emission intensity of ruthenium metallopolymer-gold nanoparticle composite with various concentrations of β-NADH ranging from (top to bottom) 1 mM, 750 µM, 500 µM, 1 µM, 1 nM, 1 pM, 100 fM and 10 fM, respectively.

3. Conclusions

Nanocavity electrodes are attractive for biosensing including electrochemical detection of molecules in flow systems and offer greater sensitivity through metal enhanced emission. The small volume of the cavities (less than 300 picolitres) is also useful for self-metering of sample volumes. The ability to fill the cavity with fluid or to selectively functionalize its interior with reagent whose release can be electrochemically triggered, may open up new applications in sensing and localized drug release. Combinations of these novel platforms with metallopolymer nanocomposites ought to enable the detection of ultralow concentrations (femto molar) of biomolecules.

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