Biosensors and Bioelectronics 25 (2010) 1908–1913

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Single domain antibody templated nanoparticle resistors for sensing

Joseph M. Slocik[a], Sang Nyon Kim[a], Tyler Auvil[a], Ellen R. Goldman[b], Jinny Liu[b], Rajesh R. Naik[a,*]

[a] Nanostructured & Biological Materials Branch, Materials & Manufacturing Directorate, Air Force Research Lab, Wright-Patterson Air Force Base, OH 45433, United States
[b] Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington DC 20375, United States

ARTICLE INFO

Article history:
Received 13 October 2009
Received in revised form
31 December 2009
Accepted 6 January 2010
Available online 11 January 2010

Keywords:
Single domain antibodies
Gold naoparticles
Toxin
Sensing
Conductivity

ABSTRACT

The diversity of biomolecules enables materials to be synthesized at ambient conditions into complex shapes and structures while affording improved control over nanoparticle synthesis, structures, properties, and functionality. Also, biomolecules can impart recognition and sensing capabilities to metal nanoparticles. Here, llama single domain antibodies selected towards the β-subunit of cholera toxin were used to template the synthesis of gold nanoparticles and subsequently assembled onto electrodes via dielectrophoresis. These assemblies were then tested for the detection of cholera toxin via changes in the conductance of antibody coated gold nanoparticles.

Published by Elsevier B.V.

1. Introduction

The integration of biomacromolecules such as antibodies with nanoparticle based sensing platforms has led to significant advances in sensitivity, greatly increased specificity towards antigens/pathogens, and enhanced signal transduction via the nanoparticle reporter. For example, IgG antibodies have been widely used to functionalize various types of inorganic nanoparticles (Wang et al., 2002; Medintz et al., 2005; Koh et al., 2009; Perez et al., 2003), carbon nanotubes (Shao et al., 2008; Erlanger et al., 2001; Zhang et al., 2007), peptide nanotubes (Nuraje et al., 2004), and substrate surfaces for biosensing, labeling cells or viruses, nanoparticle assembly, and in diagnostic assays (Medintz et al., 2005; Bentzen et al., 2005). However, in each case, the large size of IgG antibodies limits the number that can be functionalized onto a nanoparticle or nanotube surface, and moreover the antibodies are easily denatured when subjected to high temperatures or adsorbed onto metallic surfaces.

Alternatively, with only ~120–150 amino acid residues, small-sized single domain antibodies (sdAb), derived from heavy chain antibodies found in sharks and llamas, offer numerous advantages over traditional IgG antibodies (Wesolowski et al., 2009). These recombinantly produced antibody fragments have been demonstrated to possess high affinity and to show excellent thermal stability at elevated temperatures (>90 °C).(Nutall et al., 2003; Lauwereys et al., 1998; Van der Linden et al., 1999; Dooley et al., 2003; Goldman et al., 2008; Goldman et al., 2006; Liu et al., 2007). Consequently, these qualities are appealing for the use as biosensing elements and have been recently exploited for imaging cancer cells by conjugating quantum dots to llama sdAbs (Zaman et al., 2009). For materials synthesis, sdAbs are attractive as scaffolds to direct the synthesis and functionalization of gold nanoparticles due to their potential to form finite particle domain, amino acid composition, and similarities (template sizes, metal binding groups) to other biomineralization templates (Slocik et al., 2009; Dickerson et al., 2008). As a result, the use of these antibodies affords the ability to synthesize and process inorganic materials at ambient conditions, introduce bio-functionalities, assemble materials into complex structures and shapes based on the architecture of the bioscaffold, to impart molecular recognition and specificity for sensing, and to achieve a pure homogeneous surface of antibodies on gold (vs. a heterogeneous mixture obtained via post-functionalization). Biomolecules can be immobilized onto nanoparticles, and the biomolecules-nanoparticle hybrid can be used in bioelectronic and sensing applications (Willner et al., 2007; Zhao et al., 2008). In this study, we demonstrate the first use of single domain antibodies derived from llamas for the synthesis of gold nanoparticles and the detection of β-subunit of cholera toxin using the antibody functionalized gold nanoparticles. Here, the sdAb templated gold nanoparticles were capable of detecting antigen binding (β-subunit) based on conductivity changes of gold-antibody assemblies, meanwhile their IgG-Au counterpart exhibited no sensitivity.

* Corresponding author.
E-mail address: rajesh.naik@wpafb.af.mil (R.R. Naik).

0956-5663/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.bios.2010.01.004

2. Materials and methods

2.1. Synthesis of gold nanoparticles using llama sdAb

10 μL of sdAb (4 μg/mL) or anti-cholera toxin β-subunit goat polyclonal antibody (Calbiochem) was added to 500 μL of 50 mM tetraborate buffer pH 8.0 (Amresco) along with 2.5 μL of 0.1 M $HAuCl_4$ in a 750 μL microfuge tube. After 15 min, the gold-sdAb solution was reduced by addition of 20 μL of 0.1 M $NaBH_4$ and left for 1 h. sdAb coated gold NPs were purified from excess sdAb by centrifuging particles at 14,000 rpm for 10 min. The supernatant was removed and the pellet redissolved in double deionized water and repeated two more times.

2.2. Characterization of sdAb coated gold nanoparticles

UV/vis spectroscopy was obtained on a Varian Cary 5000 UV–vis–NIR spectrophotometer. For each sample, 500 μL of solution was placed in a 750 μL quartz cuvette. HRTEM was performed on a Phillips CM200 transmission electron microscope with a field-emission gun operating at 200 kV by depositing 10 μL of antibody-gold NP sample onto a 3 mm diameter copper grid coated with carbon film (Ted Pella). After DEP collection of sdAb coated NPs across electrodes, NP networks were imaged on a Phillips XL series FEG eSEM operating in environmental mode at 10 kV and a working distance of 10 mm. Circular dichroism spectra was collected on a Jasco J-815 CD spectrometer; while FT-IR spectra was collected on a Perkin Elmer Auto image microscope system on a double sided polished silicon wafer. XPS was performed on a Surface Science M-Probe Surface spectrometer operating at 10 kV using charge neutralization.

2.3. Dot blot binding assay

Circular hydrophobic wells were painted on the surface of SuperAldehyde Substrates (Arrayit) using a 15% polystyrene solution in toluene. 20 μL of the β-subunit of cholera toxin (4 μg/mL) were spotted onto the substrates within the wells to promote covalent attachment to the slides. To remove unbound β-subunit, the substrates were rinsed in triplicate with double deionized water. Following immobilization, antibody coated gold particles were added to each respective well, incubated for 1.5 h, and washed with double deionized water. Images were taken with a Canon Powershot G2 digital camera.

To enhance the colloidal gold signal bound to the immobilized subunit, a "silver enhancing solution" was prepared by adding 400 μL of 1 M HEPES pH 6.8 to 1 mL of 50% Acacia (Aldrich) and agitating (Burry et al., 1992). 300 μL of 9.4 mM *n*-propyl gallate (NPG) (Aldrich) was then added, followed by agitation. Finally, 300 μL of 36.5 mM silver acetate (Strem) was added to the mixture, agitated, and incubated for 1 min. The entire surface of the substrate was then coated with the "silver enhancing solution" and allowed to incubate for 20 min. After the incubation period the substrates were washed in triplicate with double deionized water.

2.4. AC dielectrophoresis (DEP) of sdAb coated particles

2-wire gold electrodes with a 2 μm gap were photolithographically fabricated on a silicon wafer with 1 μm thermal oxide using typical "lift-off" techniques. Two-layer (Shipley's S1805 and PMGI) photoresists were spin-coated on the silicon wafer. Using Karl Suss MA6 mask aligner, mid-UV was exposed to the photoresist layer after contacting with photomask, and the top layer was developed while leaving patterns of S1805 on top. Patterns of PMGI covered with S1805 layer were acquired by subsequent deep-UV exposure and development. 15 nm titanium (adhesion layer) and 35 nm gold were thermally evaporated on the photoresist-patterned silicon wafer. Final electrodes were acquired by developing the PMGI layer and exposing the wafer to oxygen plasma for the removal of residual photoresists. For DEP, 5 μL of aqueous solutions of antibody coated gold nanoparticles were carefully dropped on the fabricated electrode in 100% humidity chamber to prevent water evaporation. An AC frequency of 100 kHz was applied at 6 V peak-to-peak amplitude for 5 min using an HP 31320A waveform function generator. The DEP samples were copiously washed with deionized water and isopropyl alcohol and dried in a vacuum oven at 30 °C for 30 min.

2.5. Electrical measurement of toxin binding using sdA-Au hybrids

The *I–V* curves and tunneling current of gold-antibody networks was measured on a Keithley semiconductor characterization

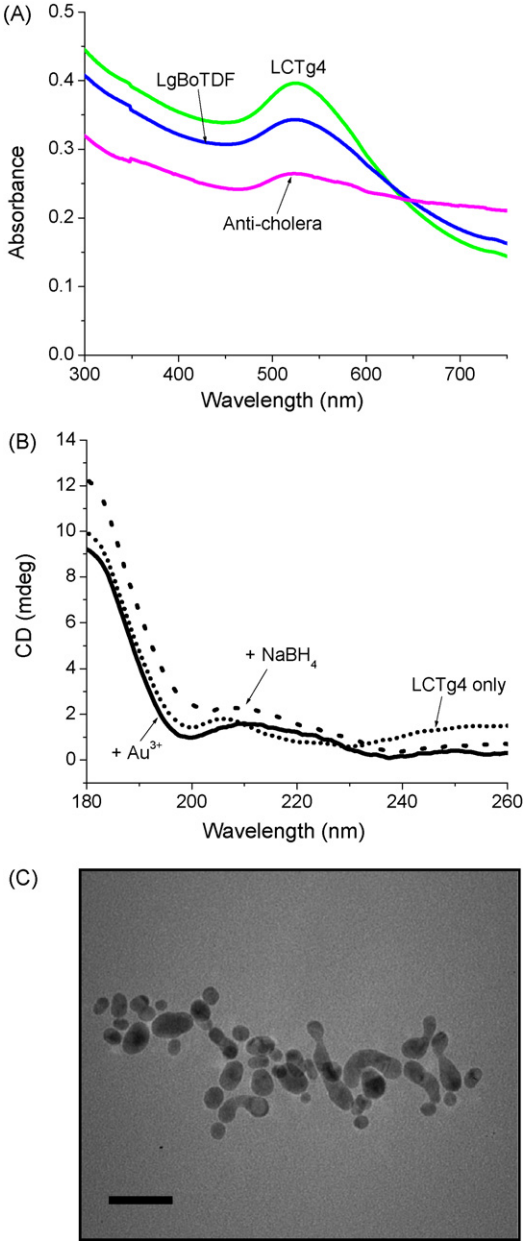

Fig. 1. (A) UV–vis spectra of antibody templated gold nanoparticles. (B) Circular dichroism spectra of sdAb-Au (LCTg4) hybrid particles before and after templating. (C) TEM micrograph of sdAb-Au (LCTg4) Scale bar equals 20 nm.