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High sensitivity carbon nanotube based electrochemiluminescence sensor array

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Abstract

Ink jet printed carbon nanotube forest arrays capable of detecting picomolar concentrations of immunoglobulin G (IgG) using electrochemiluminescence (ECL) are described. Patterned arrays of vertically aligned single walled carbon nanotube (SWCNT) forests were printed on indium tin oxide (ITO) electrodes. Capture anti-IgG antibodies were then coupled through peptide bond formation to acidic functional groups on the vertical nanotubes. IgG immunoassays were performed using silica nano particles (Si NP) functionalized with the ECL luminophore [Ru(bpy)\textsubscript{2}PICH\textsubscript{2}]\textsuperscript{2+}, and IgG labelled G1.5 acid terminated PAMAM dendrimers. PAMAM is poly(amido amine), bpy is 2,2′-bipyridyl and PICH\textsubscript{2} is (2-(4-carboxyphenyl)imidazo[4,5-f][1,10]phenanthroline). The carboxyl terminal of [Ru(bpy)\textsubscript{2}PICH\textsubscript{2}]\textsuperscript{2+} (fluorescence lifetime \(\approx 682 \pm 5\) ns) dye was covalently coupled to amine groups on the 800 nm diameter silica spheres in order to produce significant ECL enhancement in the presence of sodium oxalate as co-reactant in PBS at pH 7.2). Significantly, this SWCNT-based sensor array shows a wide linear dynamic range for IgG coated spheres (\(10^{6}\) to \(10^{12}\) spheres) corresponding to IgG concentrations between 20 pM and 300 nM. A detection limit of 1.1 \pm 0.1 pM IgG is obtained under optimal conditions.

Keywords

Electrochemiluminescence (ECL); Ruthenium polypyridyl complex; Ink-jet printing; Single walled carbon nanotubes; IgG; Biosensor

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Supplementary data available

Detailed experimental methodology and six additional figures showing schematic diagram of the ECL apparatus used, CV of [Ru(bpy)\textsubscript{2}PICH\textsubscript{2}]\textsuperscript{2+} coupled printed vertically aligned SWCNT forests, fluorescence microscopic images of the ruthenium dye coated silica spheres, schematic of the coupling procedure, various labelling schemes and UV–Vis absorption spectra of the Cy-5 labelled IgG with and without ruthenium coated spheres have been provided in Appendix A. A tabular column with calibration data is also provided.
1. Introduction

Micro arrays of biological capture agents such as antibodies or nucleic acids are emerging as powerful diagnostic tools for genomics and proteomics since they allow screening of biologically important molecules in a parallel and high throughput manner (Schena et al., 1998a). A significant number of approaches for biomolecule immobilization have been explored including polymer films (Venkatanarayanan et al., 2008), spontaneously adsorbed monolayers (Forster et al., 2003; Gooding and Ciampi, 2011), dendrimers (Benters et al., 2002) and hydrogels (Rusmini et al., 2007). However, achieving a high packing density of active biomolecules continues to pose a significant challenge.

Carbon nanotubes are attractive as nano scale building blocks for diagnostic devices because they possess a unique combination of excellent mechanical, electrical and electrochemical properties (Yu et al., 2006; O’Connor et al., 2004; Wang, 2005; Balasubramanian and Burghard, 2006). Also, they can be easily derivitized with different functional groups for the attachment of biomolecules and they have a very high surface area to weight ratio (~300 m² g⁻¹) (Wohlstedter et al., 2003). Significantly, vertically aligned carbon nanotube “forests” are useful for sensor application since the carboxylated edges of the nanotubes are exposed. This orientation of the nanotube has been shown to exhibit the highest electrocatalytic activity as well as large heterogeneous electron transfer rate constants (Yu et al., 2003, 2005, 2007a). Vertically aligned carbon nanotube forests are generally fabricated using in situ synthetic methods (Kim et al., 2007), electron beam lithography (Yu et al., 2007b), or chemical vapour deposition (Ghavanini et al., 2008), techniques which are tedious and/or technically demanding. A much simpler and alternative approach was demonstrated by Chattopadhyay et al. (2001) where SWCNT forests were self assembled on conducting substrates by selectively localising the carboxylate groups of the oxidatively shortened carbon nanotubes onto thin iron hydroxide decorated nafion films. Previous research (Yu et al., 2006; O’Connor et al., 2004) with these vertically aligned single walled carbon nanotube (SWCNT) forests demonstrated that electronic communication between glucose and peroxidase enzymes conjugated at tube ends is efficient. These platforms combined with multilabel strategies have enabled highly sensitive detection of cancer biomarkers in serum with a detection limit of 100 amol/mL (4 pg/mL) for prostate specific antigen (PSA) (Yu et al., 2006), and 25 fM (0.5 pg/mL) for interleukin-6 calf serum (Malhotra et al., 2010a). PSA was also measured in laser microdissected cancer cells from tissue samples with ±5% accuracy which outperformed the ELISA gold standard (Yu et al., 2006). When compared to commercially available PSA assays like ELISA, Elecsys, IMX, and Tandem-R, the SWCNT forest sensors required ten times less sample, gave 100 times better mass detection limit (Vessella et al., 1992; Woodruma et al., 1997; Ward. et al., 2001) and showed very low (≤3.3%) chip to chip variation (Chikkaveeraiah et al., 2011). SWCNT forests can accommodate up to 17-fold larger amounts of antibodies than smoother pyrolytic graphite electrodes (Malhotra et al., 2010b). Arrays featuring SWCNT forests prepared by manual self-assembly have been used for accurate measurement of up to four cancer biomarker proteins in patient serum with very good correlation with ELISA (slope of correlation plot close to 1) using amperometry (Chikkaveeraiah et al., 2009) or electrochemiluminescence (ECL) (Sardesai et al., 2011).

The SWCNT platforms have proved to provide very good sensitivity for serum biomarkers in clinically relevant range. They have been shown to accurately identify and detect single and multiple protein biomarkers in complex samples (Kim et al., 2007; Woodruma et al., 1997; Chikkaveeraiah et al., 2009). These studies have laid the groundwork for further development of these arrays. Different approaches to form these self assembled forests have been attempted such as using thiol surface functionalization on gold (Liu et al., 2000; Diao et al., 2002) or on nafion, SiOₓ/Si and graphite substrates by layer by layer assembly (Wei...
et al., 2006). However, these studies did not allow precise control over pattern of forest, led to low surface coverage and required long incubation times (i.e., several hours to form a good monolayer of thiol molecules on gold or 18 h to vacuum dry SWCNT forests on graphite) and hence there is a need to develop alternative, rapid and reproducible methods.

Ink-jet printing is a recently introduced non-contact deposition technique to form micro patterned surfaces which has been used to create biosensors (Setti et al., 2005), as well as DNA (Schena et al., 1998b) and protein arrays (Hart et al., 1996). Significantly, it allows very small volume droplets i.e., a few picoliters, to be deposited on a wide range of surfaces with high precision and reproducibility. An excellent resolution, of the order of 5000 drops per inch (dpi), with a mean dot diameter of about (15–40) μm can be achieved (Setti et al., 2005). Furthermore, inkjet printing eliminates time consuming adsorption and drying processes, results in precise, highly reproducible pattern and any desired pattern can be printed.

Electrochemiluminescence has received great attention in recent years because of its simplicity of operation, speed and wide linear dynamic range (Knight, 1999; Richter, 2004; Hu and Xu, 2010; Bertoncello and Forster, 2009; Venkatanarayanan et al., 2011). It is an electrode driven luminescence process where electrochemically generated intermediates undergo highly exergonic reactions to produce an electrically excited state that then emits light. While immunoassays play a central role in detecting low concentrations of analytes, achieving sufficient sensitivity, e.g., pM concentrations in nL volumes, can be a major challenge (Li et al., 2008; Rusling et al., 2010). Signal amplification strategies help to overcome these limitations and amplify the immunological interaction so as to result in an enhanced response. Particle based assays are important in high sensitivity electrochemical (Wang, 2007) and luminescence (Massay et al., 1995) based assays due to the large number of labels that can be attached (Rusling et al., 2010; Forster et al., 2009; Mani et al., 2009; Zhang and Dong, 2006). For example, Thomas et al. (2004) used inter-digitated array electrodes (IDA) and 2.8 μm diameter spheres to detect IgG whereas Rusling et al. used 1 μm diameter magnetic particles loaded with 200,000 enzyme labels to detect sub-pM levels of PSA and IL-6 in serum using a microfluidic system (Chikkaveeraiah et al., 2011).

In this contribution, as illustrated in Fig. 1, we report a novel protein array featuring a patterned assembly of vertically aligned SWCNT forests formed using inkjet printing on transparent ITO substrates. ECL was generated with amine functionalized silica nano spheres and PAMAM dendrimers coated with [Ru(bpy)2PICH2]2+. PAMAM is poly(amido amine), bpy is 2,2′ bipyridine and PICH2 is (2-(4-carboxyphenyl)imidazo[4,5-f][1,10]phenanthroline). [Ru(bpy)2PICH2]2+ was chosen because its quantum yield (ϕ) is 0.067 which is 30% higher than ϕ for [Ru(bpy)3]2+ (Pellegrin et al., 2009; Pinter et al., 2007; Dennany et al., 2004; Wolf et al., 2004; Angenendt, 2005). Below, we report that this particle based electrochemiluminescence detection strategy allows IgG detection at picomolar concentrations.

### 2. Materials and methods

#### 2.1. Materials

[Ru(bpy)2(PICH)2]2+ was synthesized using a previously published procedure (Pellegrin et al., 2009). Single-walled carbon nanotubes (SWCNT-HiPco) were shortened and end-carboxylated as described elsewhere (Chikkaveeraiah et al., 2009). Silver-filled conductive epoxy and epoxy adhesive, Araldite Rapid, were from Radionics. 800 nm diameter NH2-functionalized silica microspheres were from Bangs Laboratories, Inc. Sodium oxalate (Sigma Aldrich) solutions were made in 0.01 M PBS buffer (pH 7.2) unless otherwise stated. G1.5 acid terminated PAMAM dendrimers, chemicals and solvents were from Sigma.
Aldrich and were of analytical grade. All solutions were made using purified water (18.5 MΩ cm⁻¹) and were deoxygenated for 20 min by purging with nitrogen before use.

### 2.2. Experimental methods

Electrochemical measurements were performed with a CHI660 electrochemical work station. Ink jet printing was done using a Dimatix DMP-2831 inkjet deposition system which has 16 nozzles with 254 µm nozzle spacing, single row and drop volume of 10 pL. Squares of 1 cm × 1 cm were printed with a printing resolution of 40 µm (cartridge angle 9.1°). Atomic force microscopy (AFM) was performed on Digital Instrument Bioscope II with a Nanoscope 7.30 controller operating in air, in tapping mode with a commercial non-conductive silicon nitride cantilever tip. ECL measurements were performed with Gene Gnome HR gel documentation system equipped with 16 bit CCD camera with a dynamic range of 4.8. Image analysis was done using Genesnap software with a high sensitivity acquisition setting and 40 s acquisition time. A schematic of the ECL setup is as shown in Appendix A, Supplementary data, Fig. S1. Electrochemical measurements were done with an aqueous Ag/AgCl/saturated KCl (3 M) electrode as reference electrode and a platinum mesh counter electrode. All potentials are quoted versus the Ag/AgCl reference electrode, and all measurements were made at ambient temperature (22 ± 2 °C).

### 2.3. Printing of SWCNT-ITO array electrode

Briefly, ITO slides were cleaned by first sonicating them in deionized water for 20 min, then in acetone for 20 min followed by 20 min sonication in chloroform. Electrical contact was made by fixing a copper wire to the ITO with silver-filled conductive epoxy. This was allowed to cure for 10 h. The contact was made waterproof by covering it with epoxy adhesive. Once cured and sealed, 1 cm × 1 cm squares of vertically aligned SWCNT forests were printed on the ITO slides using the ink-jet printer with a printing resolution of 40 µm (cartridge angle 9.1°). First, 5 ITO slides were placed on the graduated printer stage and 15 layers of nafion solution were printed to yield uniform films by optical microscopy. The exact location of the ITO slide was marked before removing them and washing them with deionized water. The ITO electrodes were placed back in the same position on the printer and 15 layers of FeCl₃ were then printed. Then, the slides were removed and washed with HCl and DMF. They were placed once again on the graduated stage and 8 layers of acid functionalized carbon nanotube dispersed in DMF (0.2 mg/mL) were printed. The ITO slides were finally removed and washed with methanol and air dried and carefully stored until further use. This resulted in the formation of uniform and precisely patterned assembly of vertically aligned SWCNT forests on ITO as shown in Fig. 2a.

In order to determine the total number of active acid sites on SWCNT array, the array was labelled with redox active [Ru(bpy)₂PICNH₂]²⁺ dye. The [Ru(bpy)₂PICNH₂]²⁺ dye was covalently coupled to the nanotube forests by first activating the carboxylated end groups of the vertically aligned SWCNT forest with N-hydroxysuccinimide-N-(3-dimethylaminopropyl)-N’-ethylecarbodiimide hydrochloride (NHS-EDC) (Bangs laboratories Inc., 2008a), followed by placing a 30 µL drop of 1 mM aqueous solution of [Ru(bpy)₂PICNH₂]²⁺. The electrode was incubated for 3 hrs at 37 °C to allow peptide bond formation to occur between the forest and the redox dye. The modified electrode was washed thoroughly with 0.01 M PBS buffer containing 0.05% Tween-20 to remove any physisorbed ruthenium pic complex. Cyclic voltammetry was then performed on this ITO sensor in 0.01 M PBS (containing no metal complex) and is shown in Appendix A, Supplementary data, Fig. S2. The surface coverage of the ruthenium complex, which in turn gave the total number of active acid sites, was determined by integrating the total amount of charge passed at 1.3 V under slow sweep rate (ν<10 mV s⁻¹).
2.4. Anti-IgG coupled SWCNT forest-ITO electrode

The carboxylated end groups of the printed vertically aligned SWCNT-ITO forests were activated by placing 30 μL drop of (NHS-EDC) (Bangs laboratories Inc., 2008a) (1:10 ratio in 1 mL PBS), for 10 min. The surface was then washed with deionized water and a 20 μL drop of 5 mg/mL anti-IgG in PBS was placed on the carbon nanotubes immediately. The electrode was then allowed to incubate for 3 h at 37 °C to allow peptide bond formation to occur between the carboxylated end groups of the vertically aligned SWCNT forests and the amine functionalities of the anti-IgG. The modified electrodes were then washed thoroughly with 0.01 M PBS containing 0.05% Tween-20. In order to prevent non-specific binding (NSB), these electrodes were further treated with 20 μL aqueous solution of 5% casein followed by thorough washing with 0.01 M PBS buffer containing 0.05% Tween-20. The atomic force micrograph showing the Anti-IgG modified SWCNT-ITO ECL sensor is as shown in Fig. 2c.

2.5. Preparation of IgG-G1.5 PAMAM-[Ru(bpy)$_2$(PICH)$_2$]$^{2+}$–Si NP bio-conjugates

In order to achieve high ECL signals, [Ru(bpy)$_2$(PICH)$_2$]$^{2+}$ was covalently linked to commercially available 800 nm diameter amine functionalized silica nano spheres using NHS-EDC coupling (Bangs laboratories Inc., 2008a) (Appendix A, Supplementary data). Fig. S3 in Appendix A. The Supplementary data shows confocal fluorescence images of ruthenium coated spheres. Care was taken not to saturate the surface of the silica particles with the ruthenium dye and surface coverage of the dye on the particle was limited to 80–90% by controlling the dye concentration or the conjugation time. The coverage was verified using UV–Vis absorption spectrum. As the silica particles and IgG antibody both contain amine groups, coupling between the sphere and IgG antibody was achieved using acid terminated PAMAM dendrimers. In order to couple IgG to the ruthenium functionalized spheres, a 20% (v/v) solution of G1.5 acid terminated PAMAM dendrimers was activated along with 400 μL of 25 mg/mL ruthenium coated spheres using NHS-EDC (Bangs laboratories Inc., 2008a). To this, 200 μL of 10 mg/mL IgG was added and stirred for 4 h, at room temperature. The G1.5 dendrimer has a total of 16 coupling sites (Kitchens et al., 2005) and the objective is to couple some of these sites to IgG, whereas others will couple to the remaining amine sites on the silica spheres. The functionalized particles containing 80% [Ru(bpy)$_2$(PICH)$_2$]$^{2+}$ dye and 20% IgG antibody attached through the dendrimer, were then washed, suspended in 1 mL PBS (pH 7.2), and used immediately for assay shown in Fig. 1. A schematic of the bio-conjugate preparation procedure is shown in Appendix A, Supplementary data, Fig. S4.

2.6. Immunoassay

The immunoassay shown in Fig. 1 was performed using a previously reported procedure (see Appendix A, Supplementary data 5 for detailed procedure) (Yu et al., 2003, 2005, 2006). A constant potential of +1.6 V was applied over the entire 40 s accumulation time and the ECL intensity recorded. Control experiments using SWCNT forests sensor without anti-IgG and ruthenium coated spheres without IgG were also performed.

3. Results and discussions

3.1. Nanotube array

In order to estimate the number of acidic binding sites that could bind anti-IgG, the SWCNT-ITO arrays were labelled with [Ru(bpy)$_2$(PICH)$_2$]$^{2+}$. The quantity of complex immobilized was determined using cyclic voltammetry at slow sweep rate (ν< 10 mV s$^{-1}$) in 0.01 M PBS buffer (pH 7.2) by measuring the charge passed under the Ru$^{2+/3+}$ couple after correcting for double layer charging. Assuming that for high concentrations in solution and long reaction times, all acidic sites are labelled, the surface coverage of active acid sites
was found to be $1.82 \pm 0.03 \, \mu \text{mol cm}^{-2}$. This result indicates that there is a high density of active binding sites for anti-IgG compared to the value obtained for a flat surface of same geometric area. Fig. 2b and c shows AFM images of printed vertically aligned SWCNT array before and after anti-IgG functionalization and show that following antibody deposition, a globular protein coating is observed and the apparent height increases approximately four fold. These images are consistent with successful immobilization of anti-IgG at a high surface coverage.

3.2. Si NP labelling for ECL enhancement

UV–Vis absorption spectroscopy was used to characterize the [Ru(bpy)$_2$(PICH)$_2$]$^{2+}$ functionalized 800 nm silica particles. Surface confinement of the label on the silica spheres does not significantly alter the spectrum. Assuming that the extinction coefficient for the MLCT transition at 450 nm is unaffected by immobilization, the concentration of ruthenium present on the surface of an 18 nM solution of the functionalized spheres was $10.6 \pm 0.2 \, \mu \text{mol}$. This result corresponds to approximately 600 metal complexes per particle. The surface coverage of amines on the particles surface is approximately $645.9 \times 10^3$ charge groups/nanosphere (Bangs laboratories Inc., 2008b).

In the case of the IgG-G1.5-[Ru(bpy)$_2$(PICH)$_2$]$^{2+}$–Si NP bio-conjugate, the number of IgGs attached to each [Ru(bpy)$_2$(PICH)$_2$]$^{2+}$ coated sphere through the dendrimer was also determined using UV–Vis absorption spectroscopy and a commercially available Cy-5 labelled IgG (dye to protein ratio of 3). See Appendix A, Supplementary data for schematic diagram of various labels and bio-conjugates synthesized, Fig. S5. Cy-5 labelled IgG was conjugated to the dendrimer first followed by coupling to the ruthenium coated spheres and washed thoroughly. Care was taken to minimize settling of the spheres by agitating the solution prior to recording the spectra. While the UV–Vis spectrum is complicated by scattering, the Cy-5 peak observed at 650 nm in solution shifted by only 5 nm after surface confinement indicating minimal changes in the local microenvironment (Appendix A, Supplementary data Fig. S6) (Hong et al., 1997; Clontech, 2008). The apparent extinction coefficient, $e$, for the Cy-5 dye Zhang and Dong, 2006; is approximately $2.5 \times 10^5 \, \text{M}^{-1} \text{cm}^{-1}$ and the absorbance at $\lambda_{\text{max}}$ indicates that the quantity of Cy-5 on the dendrimer coupled ruthenium coated silica spheres was 1.3 $\mu$mol. This result suggests that approximately 70 IgG molecules are coupled to the surface of one nanosphere through the G1.5 acid terminated PAMAM dendrimer.

3.3. Analytical performance of immunoassay

Following incubation of the anti-IgG modified CNT array with the IgG functionalized Ru-spheres, the sensor was thoroughly washed with PBS to remove non-specifically bound spheres. By holding the electrode potential at +1.6 V, the Ru$^{2+}$ centres present on the spheres are oxidized to Ru$^{3+}$ which are then chemically reduced in the presence of the sodium oxalate co-reactant to produce the excited Ru$^{2+*}$ state. The Ru$^{2+*}$ then emits a photon and returns to the Ru$^{2+}$ ground state. Fig. 3 shows the CCD response obtained after the washing steps when the SWCNT-ITO sensor was incubated with different concentrations of IgG coated ruthenium functionalized spheres. The images were collected after 40 s accumulation time at a constant voltage of 1.6 V in 0.01 M PBS buffer (pH 7) in the presence of 50 mM sodium oxalate co-reactant. The concentration of IgG on an individual sphere was used to calculate the concentration of IgG for various sphere concentrations. This figure shows that the intensity of the ECL signal increases with increasing concentration of IgG. When the concentration of IgG is relatively high, i.e., above approximately 1.6 nM, the ECL intensity is not uniform across the surface and “hot-spots” of high intensity are observed due a high local concentration of functionalized spheres. However, as the concentration of IgG is gradually decreased, a more homogeneous
emission is observed. The uniformity of the response is not as good as that seen for commercially available fluorescent antibody arrays most likely due to non-uniform deposition of the anti-IgG on the SWCNT forest (Wolf et al., 2004; Zhou et al., 2010). However, the key advantage of ECL is that a light source is not required, auto-fluorescence and interference due to luminescent impurities are minimized and light scattering is negligible (Angenendt, 2005). Moreover, ECL assays can be more sensitive since they exhibit essentially zero background as the electrode potential replaces the source radiation to control the generation of excited states.

Fig. 4 shows a calibration graph of the number of IgG-Ru-spheres added vs. ECL intensity while the inset shows the dependence of the ECL intensity on IgG concentration. The SWCNT-ITO sensor shows a wide dynamic range and is able to detect IgG at concentrations from approximately 20 pM to at least 300 nM. However, the inter-array variability was approximately 10% which is higher than the ≤3% reported earlier for these arrays (Chikkaveeraiah et al., 2011). The origin of this inter-array variability is not fully understood but preliminary measurements suggest that briefly heat curing the nafion layer at 100 °C prior to deposition reduces the variability to approximately 2%. Under these conditions, a calibration plot for IgG is linear with an LOD of 1.1 pM IgG.

For the analysis of real world samples, the overall performance of the sensor will be influenced by non-specific adsorption. The non-specific adsorption of ruthenium coated spheres onto the anti-IgG functionalized CNT array surface was determined. The anti-IgG-SWCNT-ITO sensor surface was blocked with 2 mL of 2% casein +0.05% Tween-20 and the ECL intensity measured after incubating with 5.30 × 10^9 and 1.00 × 10^8 Ru-spheres separately followed by thorough washing with PBS buffer. As the ruthenium coated spheres did not contain any bound IgG, the ECL signal produced reflects the extent to which the spheres bind non-specifically to the surface of the SWCNT-anti-IgG-ITO sensor. The average ECL intensity was 1.3 ± 0.3 A.U, which is a factor of approximately 2.5 times lower than that observed for the addition of 30 pM IgG. Also, the ability of the IgG labelled ruthenium spheres to non-specifically bind to the SWCNT-ITO, electrode surface was assessed, i.e., where no anti-IgG is present. In this case, the SWCNT-ITO electrodes were blocked with 2 mL of 2% casein + 0.05% Tween-20 and incubated with 1.59 nM and 30 pM IgG separately for one hour at room temperature. The electrode surface was then washed thoroughly and the ECL response measured. Significantly, the ECL signal due to the non-specific adsorption of the IgG coupled spheres to the surface of SWCNT-ITO in the absence of capture antibody (anti-IgG) was found to be significantly lower, 0.5 ± 0.3 A.U, than that obtained for non-specific adsorption in the presence of capture antibody.

Commercial ECL sandwich assays available from Meso Scale Discovery (MSD) (MSD, 2010), make use of specially made microtiter plates containing carbon electrodes in each well. The sensitivity of the MSD has been found to be 100 fold better than the ELISA and 10 fold better than the magnetic bead based assays from BioVeris (Debad et al., 2004). The MSD assays report a linear dynamic range of 3–4 logs for IgG-anti-IgG coupling with 0.5–5 nM limits of detection (MSD, 2010). The assay reported by Thomas et al. (2004) detected 1000, 2.8 μm diameter spheres corresponding to 177 pM mouse IgG. The SWCNT-ITO arrays reported here not only allows much smaller beads to be detected but also exhibits increased sensitivity and two orders of magnitude lower detection limits when compared to the above mentioned commercially available assays.

4. Conclusions

A rapid, highly sensitive vertically aligned SWCNT forest-ITO ECL sensor array has been successfully developed. The present study describes a new approach to printed, nano-
structured antibody arrays using bead based electrochemiluminescence detection. This work is the first demonstration of using ink-jet printer to form vertically aligned SWCNT forest array platforms and is very promising for creating versatile ‘lab-on-a-chip’ devices as it is rapid, allows for automation and control over nanotube patterning. Similar to a previous manually fabricated ECL array (Sardesai et al., 2011), this array emits ECL upon application of suitable positive voltage and the CCD placed above the array captures the emitted light from the spot simultaneously. The printed SWCNT surface allows easy attachment of primary antibodies and increases the sensitivity of the sensor. The surfaces of the nanotubes were effectively blocked with 2% casein to result in very low nonspecific binding of IgG-ruthenium spheres. Precise control over the pattern of SWCNT forest was achieved by inkjet printing. G1.5 acid terminated PAMAM dendrimers were used as protein linkers and signal enhancement was achieved through [Ru(bpy)$_2$PICH$_2$]$_2^+$ coated silica nano spheres. Significantly, when compared to similar technologies, this transparent ITO sensor shows a wide linear dynamic range, a remarkably low detection limit of 1.1 pM for IgG and shows promise for future development of multiplexed assays.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.bios.2011.10.022.
Fig. 1.
Schematic diagram of the anti-IgG-IgG immuno assay.
Fig. 2. Digital image of inkjet printed ITO electrode with vertically aligned SWCNT forests (a). Sample is 1 cm × 1 cm, 40 μm resolution (cartridge angle 9.1°). The image on the right hand side shows zoomed in tapping mode atomic force micrographs of self assembled, vertically aligned SWCNT forests (b) and anti-IgG coupled self assembled, vertically aligned SWCNT forests (c) formed on ITO electrode by the inkjet printing method. A commercial non-conductive silicon nitride cantilever was used.
Fig. 3.
False colour CCD ECL response of 12 printed SWCNT/anti-IgG spots after incubation with concentrations of IgG coated ruthenium spheres. From 1 to 12, the concentrations of IgG are 318.0, 159.0, 79.5, 10.9, 6.4, 3.3, 1.6, 0.08, 0.01, 0.0015 and 0.0011 nM. The voltage was kept constant at +1.6 V, the accumulation time was 40 s and the co-reactant was 50 mM sodium oxalate in 0.01 M PBS buffer (pH 7.2).
Fig. 4.
Calibration plot of the integrated ECL intensity from Fig. 3 vs. the number of IgG-G1.5-
[Ru(bpy)$_2$ PICH$_2$]$_2^{2+}$ spheres. The voltage was kept constant at +1.6 V, the accumulation
time was 40 s and the co-reactant was 50 mM sodium oxalate in 0.01 M PBS buffer (pH
7.2). The inset shows the dependence of the ECL intensity on IgG concentration.