Published in final edited form as:

ACS Sens. 2016 ; 1(2): 197–202. doi:10.1021/acssensors.5b00156.

Electrochemiluminescence at Bare and DNA-Coated Graphite Electrodes in 3D-Printed Fluidic Devices

Gregory W. Bishop^{†,‡}, Jennifer E. Satterwhite-Warden[†], Itti Bist[†], Eric Chen[†], and James F. Rusling^{†,‡,§,||,*}

[†]Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269-3060, United States

[‡]Institute of Materials Science, University of Connecticut, Storrs, Connecticut 06269-3136, United States

[§]Department of Surgery and Neag Cancer Center, University of Connecticut Health Center, Farmington, Connecticut 06030, United States

^{||}School of Chemistry, National University of Ireland at Galway, Galway, Ireland

Abstract

Clear plastic fluidic devices with ports for incorporating electrodes to enable electrochemiluminescence (ECL) measurements were prepared using a low-cost, desktop three-dimensional (3D) printer based on stereolithography. Electrodes consisted of 0.5 mm pencil graphite rods and 0.5 mm silver wires inserted into commercially available 1/4 in.-28 threaded fittings. A bioimaging system equipped with a CCD camera was used to measure ECL generated at electrodes and small arrays using 0.2 M phosphate buffer solutions containing tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate ($[\text{Ru}(\text{bpy})_3]^{2+}$) with 100 mM tri-*n*-propylamine (TPA) as the coreactant. ECL signals produced at pencil graphite working electrodes were linear with respect to $[\text{Ru}(\text{bpy})_3]^{2+}$ concentration for 9–900 μM $[\text{Ru}(\text{bpy})_3]^{2+}$. The detection limit was found to be 7 μM using the CCD camera with exposure time set at 10 s. Electrode-to-electrode ECL signals varied by $\pm 7.5\%$. Device performance was further evaluated using pencil graphite electrodes coated with multilayer poly(diallyldimethylammonium chloride) (PDDA)/DNA films. In these experiments, ECL resulted from the reaction of $[\text{Ru}(\text{bpy})_3]^{3+}$ with guanines of DNA. ECL produced at these thin-film electrodes was linear with respect to $[\text{Ru}(\text{bpy})_3]^{2+}$ concentration from 180 to 800 μM . These studies provide the first demonstration of ECL measurements obtained using a 3D-printed closed-channel fluidic device platform. The affordable, high-resolution 3D printer used in these studies enables easy, fast, and adaptable prototyping of fluidic devices capable of incorporating electrodes for measuring ECL.

Graphical Abstract

*Corresponding Author: Tel.: 860-486-4909. Fax: 860-486-2981. james.rusling@uconn.edu.

[‡]Present Address: G.W.P.: Department of Chemistry, East Tennessee State University, Johnson City, Tennessee 37614.

Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.5b00156.

Comparison of electrochemical responses of pencil graphite electrodes obtained inside and outside 3D-printed channels via cyclic voltammetry and cyclic voltammograms of bare and DNA-film-modified electrodes in the presence of $[\text{Ru}(\text{bpy})_3]^{2+}$ (PDF)

Keywords

3D-printed fluidics; electrochemiluminescence; stereolithography; DNA oxidation; biosensing

Three-dimensional (3D) printing provides a simple, fast route to prototyping and fabrication of objects directly from computer-aided design (CAD) files. Printer instructions are generated by processing the CAD file using a slicer program.¹ After uploading the instructions to the printer, the object is fabricated, typically in a layer-by-layer fashion involving the deposition of viscoplastics or heated thermoplastic filament, by sintering powdered materials, or by selective exposure of photocurable resins or inks to a light source. The simple work-flow of 3D printing enables iterations of object designs to be prepared relatively quickly without the need to produce masks or molds for each design as in traditional lithographic approaches.

Fluidic devices such as gradient² and microdroplet³ generators, flow-cells for analytical measurements,^{2,4-6} and components for modular microfluidics^{3,7} have recently been prepared using 3D printing methods. Channel structure, device durability, and utility are determined by the printing method and material used. Of the reported 3D-printed fluidic devices, most have been prepared using commercially available 3D printers based on fused deposition modeling (FDM), stereolithography, MultiJet, and PolyJet technologies. Commercially available 3D printers of these types can range in price from less than 1000 to hundreds of thousands of dollars, and materials compatible with these printers currently range from tens to hundreds of dollars per kilogram or liter.^{1,6}

Printers based on MultiJet and PolyJet technologies, which combine inkjet-printing of photocurable materials with instant curing using a light source, are typically among the most expensive (tens of thousands to hundreds of thousands of dollars).¹ These printers have been used to produce modular fluidic components,⁷ fluidic devices for various applications,^{5,8,9} as well as open channels (600 μm dia.) that can be filled with colloidal metal suspensions to produce microelectronic components for building circuits.¹⁰ Besides the relatively high cost of printers and materials, support material is required during the printing process to build layers on object; void spaces can also be difficult to remove from small channels prepared by these methods.

Most low-cost, consumer-grade 3D printers are currently based on fused deposition modeling (FDM), a principle that relies on the extrusion of thin threads of thermoplastic filament through a heated nozzle. The earliest examples of FDM-fabricated fluidic devices involved the production of reusable master molds for poly(dimethylsiloxane) (PDMS)-based microfluidics.¹¹ FDM can also be used to prepare sacrificial channel scaffolds from the

commonly used thermoplastic acrylonitrile butadiene styrene, which can be dissolved in acetone, for more complex PDMS-based microfluidics.¹² Direct printing of fluidic channels by FDM has also been reported.¹³ Both printer and materials costs are typically low for this method of printing. However, surface roughness of objects printed by FDM is often quite large (~8 μm), which limits utility and transparency of directly printed fluidic devices prepared using this technique.

There are a growing number of low-cost 3D printers based on stereolithography, which uses a light source (laser or projector) and photocurable resin to produce objects. Some printers based on this technology possess resolution to print objects with surface roughness and limiting dimensions that rival some of the more expensive professional-grade printers.¹⁴ Acrylate-based resins have been used to prepare fully 3D-printed fluidic devices² and fluidic devices that feature deformable membranes to integrate pneumatic valves.^{15,16} Similar to inkjet-based MultiJet and PolyJet technologies, preparing devices with channels < 250 μm can be challenging using stereolithography due to difficulty in removing uncured support material (resin) from the channels.² Also, devices prepared using stereolithography often require postprocessing to remove supports. These are usually necessary to ensure structural integrity of the object while it is built in the resin reservoir, to promote adhesion of the object to the build platform during printing, and allow removal after fabrication is complete.

Electrodes can be incorporated into 3D-printed fluidic devices for electrochemical sensing.⁴⁻⁶ This has been accomplished by affixing electrodes deposited on Si/SiO₂ substrates or embedded in epoxy to 3D-printed devices with three-sided channels.⁴ Alternatively, threaded ports or access holes can be included in the fluidic device design so that wire electrodes or threaded fittings equipped with wire electrodes can be positioned within the channels.^{5,6} The combination of low-cost, easy-to-prepare, easy-to-revise fluidic devices with electrodes that can be reversibly incorporated into channels results in inexpensive, adaptable platforms for electrochemical sensing.

In comparison to purely electrochemical sensing, strategies based on electrochemiluminescence (ECL) typically feature excellent sensitivity and specificity due to the nature of the electrogenerated optical signal, which eliminates background interference.¹⁷ We recently used FDM 3D-printing to develop a gravity-fed immunoarray device for three-protein using an open ECL detection channel,¹⁸ since the FDM polymers are opaque. Here, we show that electrodes incorporated into closed 3D-printed channels can also be used to measure analytical ECL signals using 3D-printed fluidic devices prepared from clear acrylate-based resin using stereolithography. Working electrodes were prepared from inexpensive pencil graphite rods incorporated into the printed channels via threaded ports. Transmittance of the cured acrylate resin was sufficient to observe ECL from solutions of tris(2,2'-bipyridyl)-dichlororuthenium(II) hexahydrate ($[\text{Ru}(\text{bpy})_3]^{2+}$) with tri-*n*-propylamine (TPA) coreactant. Experiments with multilayer poly(diallyldimethylammonium chloride) (PDDA)/DNA film-coated electrodes and $[\text{Ru}(\text{bpy})_3]^{2+}$ in solution suggest the utility of this strategy to detect intact or damaged DNA. The combination of transparent, 3D-printed flow-cells with reusable, removable electrodes opens the door to novel applications in sensing and diagnostics at very low cost.

MATERIALS AND METHODS

Materials

Clear resin was obtained from Formlabs (Somerville, MA). Pencil graphite (0.5 mm dia., Super Hi-Polymer HB) was supplied by Pentel (Torrance, CA). Au (0.1 mm and 0.5 mm dia.) and Ag (0.5 mm dia.) wires as well as potassium ferricyanide, tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate, tri-*n*-propylamine, salmon testes (ST) ds-DNA (2000 base pairs avg, 41.2% G/C), poly(diallyldimethylammonium chloride (PDDA), sodium phosphate dibasic, and sulfuric acid (98.0%) were obtained from Sigma-Aldrich (St. Louis, MO). NaCl and KCl, were purchased from J.T. Baker (Center Valley, PA). Potassium phosphate monobasic was obtained from Fisher Scientific (Pittsburgh, PA). All solutions were prepared using 18 M Ω -cm water purified by passing house-distilled water through a Hydro Service and Supplies purification system (Durham, NC).

3D-Printed Fluidic Device Preparation

Fluidic devices were designed using 123D Design software (Autodesk, San Rafael, CA). A 1/4 in.-28 threaded nut design file¹⁹ was modified and incorporated in the device models to define threaded ports (Scheme 1A). Fluidic device design files were converted into printer instructions using PreForm slicer software (Formlabs), and devices were fabricated using a Form1+ 3D printer (Formlabs) and clear methacrylate-based resin (Scheme 1B).

Uncured resin and device supports were removed in accordance with recommendations of the printer manufacturer. Briefly, isopropanol was forced through the device channel to remove any uncured resin. The fluidic device was then submerged in isopropanol for 10 min (Scheme 1C). Supports were snapped off of the base of the device, and the device was polished using P800 and P1500 abrasive papers (Scheme 1D). After rinsing with water and drying, devices were spray-coated with clear acrylic top coat (Krylon, Cleveland, OH). Commercially available 1/4 in.-28 threaded fittings were interfaced with polyetheretherketone (PEEK) tubing and ferrules (Upchurch Scientific, Oak Harbor, WA) and inserted into the 3D-printed threaded ports to provide access to the fluidic channels. Images of 3D-printed devices were obtained using a Firefly GT800 microscope (Belmont, MA). Transmission of 610 nm light through the bottom of the printed channel was measured using an Agilent Technologies Cary 60 UV-vis spectrophotometer (Santa Clara, CA).

Electrode Fabrication

Disk electrodes were fabricated from 5–10 mm long Ag (0.5 mm dia.) wires, and pencil graphite rods (0.5 mm dia.) as previously described.⁶ For multiworking electrode fittings, a ring-shaped (tubular) counter electrode was fashioned from a 16-gauge stainless steel needle. One end of each wire or graphite rod was connected to 24-gauge copper wire by soldering or through use of Ag/AgCl paste (Gwent, Pontypool, U.K.) to provide contacts for electrochemical measurements. The conductive wires and rods were covered with plastic wire insulator along their lengths to avoid short-circuiting and define disk-shaped electrodes at their ends.

Working, counter, and reference electrodes were grouped together and inserted into an opening of a threaded fitting. Gaps between fittings and electrodes were filled with epoxy. The resulting disk electrodes were polished using 600-grit abrasive paper (Buehler, Lake Bluff, IL). Ag electrodes were coated with AgCl using a 9 V battery and a 2.2 M Ω resistor to supply $\sim 4 \mu\text{A}$ to the Ag anode, which was immersed in a 3.5 M KCl solution along with an Ag/AgCl cathode. Prior to electrochemical and electrochemiluminescence measurements, fittings with bare graphite working electrodes were stored in ambient lab conditions.

Multilayer DNA film-modified graphite electrodes were prepared through a layer-by-layer deposition technique²⁰ based on electrostatic interaction between polycation PDDA (2 mg mL⁻¹ in 0.05 M NaCl) and DNA (2 mg mL⁻¹ in 10 mM Tris buffer pH 7.4 with 0.5 M NaCl). Three successive (PDDA/DNA) bilayers, (PDDA/DNA)₃, were deposited by sequentially pipetting 0.5 μL of each component on the pencil graphite electrode. Adsorption of each of the PDDA and DNA layers proceeded for 20 and 30 min, respectively in a humidifying chamber at room temperature. Between successive layers, the electrodes were washed with water and dried with N₂. Once layer deposition was complete, these film-modified graphite electrodes were used immediately or after storage at 4 °C for 48 h.

Electrochemical and Electrochemiluminescence Measurements

Electrode fittings were incorporated into 3D-printed fluidic channels through a threaded port located in the center of the device in a manner analogous to that which was reported using PolyJet- and FDM-printed fluidic devices.^{5,6} Electrochemical measurements were performed using a CH Instruments 1232 bipotentiostat or CH Instruments 1030 multipotentiostat (Austin, TX, USA). Electrochemiluminescence (ECL) measurements were obtained using a G:Box bioimaging system and GeneSnap software (Syngene, Cambridge, U.K.).²¹ Fresh solution was delivered to the electrode surface for each new experiment using a 3 cc syringe connected to the fluidic device inlet using a luer (Upchurch Scientific) and tubing. Multiple, sequential ECL measurements were obtained with bare graphite working electrode fittings, and no degradation of signal was observed during sets of 30 experiments. Electrode fittings were polished and processed as described above prior to each set of ECL experiments. ECL intensities for the images captured by the CCD camera were determined by integrating ECL signal over the area of the electrode using GeneTools software (Syngene). For illustrative purposes, ECL images obtained using the CCD camera were recolored using ImageJ software²² through application of the built-in Fire lookup table.

RESULTS

Incorporation of Electrodes in 3D-Printed Channels

3D-printed devices were designed to have 800 μm diameter channels that feature an oval opening with a 5.6 mm major axis and 2 mm minor axis to accommodate electrodes (Figure 1). At 730 (± 58) μm , the printed channel diameters were slightly smaller than the design size based on photographic measurements⁶ of channels filled with methylene blue ($n = 6$). The size of the oval opening in the center of the printed channel was true to the design and contains a volume of 7.9 μL . The electrode fittings incorporated into the channel contained 0.5 mm diameter pencil graphite working and counter electrodes and a 0.5 mm diameter Ag/

AgCl reference. The pencil graphite working electrodes exhibited good electrochemical response as evidenced by cyclic voltammetric (CV) experiments (Figure S1, Supporting Information).

Partially printed devices ($n = 3$) consisting of the bottom of the electrode chamber (oval opening) to the bottom of the device were prepared to mimic the window through which electrodes are viewed. Transmission of 610 nm light through these devices was 90.2 (± 0.10) %, indicating good transparency of the cured resin.

ECL in 3D-Printed Channels

ECL experiments were completed by introducing solutions containing 100 mM tri-*n*-propylamine (TPA) and various concentrations of $[\text{Ru}(\text{bpy})_3]^{2+}$ in 0.2 M phosphate buffer (pH 7.5) into the transparent 3D-printed fluidic device with integrated electrodes. CVs of 100 mM TPA gave the typical broad, chemically irreversible oxidation wave with a peak at +0.95 V vs Ag/AgCl (Figure S2, Supporting Information). Oxidation of TPA leads to the formation of cation radicals ($\text{TPA}^{\bullet+}$) and free radicals ($\text{TPA}^\bullet$) that proceed to react with $[\text{Ru}(\text{bpy})_3]^{2+}$ to generate excited state $[\text{Ru}(\text{bpy})_3]^{2+*}$ and ultimately result in light emission ($\lambda_{\text{max}} = 610 \text{ nm}$).²³ The 3D-printed channel with integrated electrodes was placed in a bioimaging system, which enabled measurement of ECL light emitted by $[\text{Ru}(\text{bpy})_3]^{2+*}$ (Figure 2). A potential of +0.95 V vs Ag/AgCl was applied to the working electrode, and images were taken with exposure time set to 10 s. Increasing concentration of $[\text{Ru}(\text{bpy})_3]^{2+}$ in the reaction mixture led to an increase in observed ECL signal.

ECL light was integrated over the area of the working electrode to convert image data into numerical values proportional to signal intensity. The ECL signal obtained using the pencil graphite electrodes incorporated in the 3D-printed fluidic device exhibited good linearity from 9 to 900 μM (Figure 3). ECL intensity varied by <4% for replicate experiments ($n = 4$) of each $[\text{Ru}(\text{bpy})_3]^{2+}$ concentration carried out using a single electrode. Signals obtained using different similarly prepared graphite working electrodes ($n = 3$) under the same conditions differed by $\pm 7.5\%$. The detection limit for $[\text{Ru}(\text{bpy})_3]^{2+}$ using these electrodes and an exposure time of 10 s was 7 μM ($S/N = 3$).

ECL Emission from $[\text{Ru}(\text{bpy})_3]^{2+}$ with Oligonucleotides

Guanine bases within DNA strands can act as coreactants for ECL emission by $[\text{Ru}(\text{bpy})_3]^{2+}$.²⁴ Single pencil graphite electrodes with multilayer films, $(\text{PDDA}/\text{DNA})_3$, exhibited ECL emission due to oxidation of $[\text{Ru}(\text{bpy})_3]^{2+}$ that leads to catalytic oxidation of guanines in the DNA at +0.95 V vs Ag/AgCl²⁴ (Figure 4). ECL was not observed when $(\text{PDDA}/\text{DNA})_3$ films were exposed to buffer alone.

For the $(\text{PDDA}/\text{DNA})_3$ -coated graphite electrodes in 3D-printed fluidic devices, intensity of ECL emission from $[\text{Ru}(\text{bpy})_3]^{2+}$ increased linearly with increasing $[\text{Ru}(\text{bpy})_3]^{2+}$ concentration from 180 to 800 μM (Figure 4D). ECL intensity varied by <5.6% for replicate measurements using the same $[\text{Ru}(\text{bpy})_3]^{2+}$ concentration ($n = 3$). DNA-film-modified electrodes exhibited good stability when stored at 4 °C for 48 h with ECL signal intensities for stored electrodes and freshly prepared electrodes differing by only 2% (Figure S3).

Electrode Arrays Integrated into 3D-Printed Channels for ECL Measurements

In addition to fittings equipped with single working electrodes, fittings with three working electrodes were also prepared and integrated into 3D-printed fluidic devices (Figure 5). In order to ensure that all electrodes would contact the solution in the channel, another fluidic device with an oval opening in the center of the channel of 12 mm major axis and 4.6 mm minor axis, corresponding to volume of 39 μL , was designed and prepared. ECL was generated at all three working electrodes simultaneously and differed by 1.3 to 6.2% from electrode-to-electrode in different three working electrode arrays ($n = 3$). The average ECL response differed by $\pm 4.5\%$ among different electrode fittings ($n = 3$).

DISCUSSION

Fast design-to-object production times offered by 3D printing have enabled rapid prototyping of fluidic devices suitable for low-volume production²⁵ as well as customizable components for specific applications.^{3,7} Electrodes have been incorporated into 3D-printed fluidic devices prepared by PolyJet technology, fused deposition modeling, and stereolithography for electrochemical sensing.⁴⁻⁶ The devices described here for ECL measurements can similarly be used for biosensing, flow injection analysis, and other purposes that require relatively low sample volumes.

The fluidic devices prepared here each required $\sim \$2.50$ in resin and took ~ 3.5 h to print at a layer resolution of 50 μm . The transparent fluidic devices (90% transmission at 610 nm) enabled measurement of ECL from solutions containing $[\text{Ru}(\text{bpy})_3]^{2+}$ in 0.2 M phosphate buffer with 100 mM TPA via integrated pencil graphite working electrodes with Ag/AgCl reference electrodes. Single electrodes displayed good electrochemical response (Figure S1). ECL output was linear for 9–900 μM $[\text{Ru}(\text{bpy})_3]^{2+}$. The detection limit for $[\text{Ru}(\text{bpy})_3]^{2+}$ in buffer was 7 μM for an exposure time of 10 s. ECL from $[\text{Ru}(\text{bpy})_3]^{2+}$ was also generated at arrays of three working pencil graphite electrodes. The array design in these studies featured three 0.5 mm pencil graphite working electrodes positioned in a semicircular fashion around a central ring-shaped stainless steel counter electrode encircling a Ag/AgCl reference electrode. Electrode-to-electrode ECL generation differed by $< 7\%$.

In separate experiments, DNA immobilized on the surface of the graphite electrodes via layer-by-layer deposition with the polycation PDDA acted as the coreactant for the $[\text{Ru}(\text{bpy})_3]^{2+}$ ECL generation process. As expected from the relatively low concentrations of coreactant DNA on the surface, ECL signals were comparatively lower in relation to those obtained when 100 mM TPA was used as the coreactant. However, ECL was still easily detectable by the CCD camera using these DNA-modified electrodes. Thus, these clear plastic devices facilitate detection of ECL at relatively low levels and provide a path to develop microfluidic biosensors and arrays.

CONCLUSIONS

Improvements in the capabilities, affordability, and availability of 3D printing technologies have opened up a host of new solutions to problems in many fields. User-friendly, low-cost, commercially available 3D printers have increased the pace of innovation. Increasing

printing speed, without compromising printing accuracy, as recently demonstrated,²⁶ may well lead to 3D printing for mass production of bioanalytical devices.

Our results described above demonstrate successful fabrication of fluidic devices that enable incorporation of electrodes using a low-cost, stereolithographic 3D printer. The commercially available clear resin employed in the printing process yields transparent fluidic devices (90% transmission at 610 nm) that allow the visualization of electrodes within the oval-shaped channels of the 3D-printed flow-cell for ECL measurement. ECL generated by $[\text{Ru}(\text{bpy})_3]^{2+}$ in buffer with TPA in solution or for DNA on pencil graphite electrodes was readily observed using a CCD bioimaging camera. Our results suggest that this approach can serve as an excellent platform for developing more sophisticated low-cost, microfluidic ECL-based sensing strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported financially by Grant Nos. EB016707 and EB014586 from the National Institute of Biomedical Imaging and Bioengineering (NIBIB), NIH. E.C. acknowledges support from the University of Connecticut Department of Chemistry Summer Research Scholarship.

References

1. Gross BC, Erkal JL, Lockwood SY, Chen C, Spence DM. Evaluation of 3D Printing and Its Potential Impact on Biotechnology and the Chemical Sciences. *Anal Chem.* 2014; 86:3240–3253. [PubMed: 24432804]
2. Shallan AI, Smejkal P, Corban M, Guijt RM, Breadmore MC. Cost-Effective Three-Dimensional Printing of Visibly Transparent Microchips within Minutes. *Anal Chem.* 2014; 86:3124–3130. [PubMed: 24512498]
3. Bhargava KC, Thompson B, Malmstadt N. Discrete Elements for 3D Microfluidics. *Proc Natl Acad Sci U S A.* 2014; 111:15013–15018. [PubMed: 25246553]
4. Snowden ME, King PH, Covington JA, Macpherson JV, Unwin PR. Fabrication of Versatile Channel Flow Cells for Quantitative Electroanalysis Using Prototyping. *Anal Chem.* 2010; 82:3124–3131. [PubMed: 20329754]
5. Erkal JL, Selimovic A, Gross BC, Lockwood SY, Walton EL, McNamara S, Martin RS, Spence DM. 3D printed Microfluidic Devices with Integrated Versatile and Reusable Electrodes. *Lab Chip.* 2014; 14:2023–2032. [PubMed: 24763966]
6. Bishop GW, Satterwhite JE, Bhakta S, Kadimisetty K, Gillette KM, Chen E, Rusling JF. 3D-Printed Fluidic Devices for Nanoparticle Preparation and Flow-Injection Amperometry Using Integrated Prussian Blue Nanoparticle-Modified Electrodes. *Anal Chem.* 2015; 87:5437–5443. [PubMed: 25901660]
7. Lee KG, Park KJ, Seok S, Shin S, Kim DH, Park JY, Heo YS, Lee SJ, Lee TJ. 3D Printed Modules for Integrated Microfluidic Devices. *RSC Adv.* 2014; 4:32876–32880.
8. Anderson KB, Lockwood SY, Martin RS, Spence DM. A 3D Printed Fluidic Device that Enables Integrated Features. *Anal Chem.* 2013; 85:5622–5626. [PubMed: 23687961]
9. Chen C, Wang Y, Lockwood SY, Spence DM. 3D-printed Fluidic Devices Enable Quantitative Evaluation of Blood Components in Modified Storage Solutions for Use in Transfusion Medicine. *Analyst.* 2014; 139:3219–3226. [PubMed: 24660218]
10. Wu S-Y, Yang C, Hsu W, Lin L. 3D-Printed Microelectronics for Integrated Circuitry and Passive Wireless Sensors. *Microsys & Microeng.* 2015; 1:15013.

11. McDonald JC, Chabinyc ML, Metallo S, Anderson JR, Stroock AD, Whitesides GM. Prototyping of Microfluidic Devices in Poly(dimethylsiloxane) Using Solid-Object Printing. *Anal Chem.* 2002; 74:1537–1545. [PubMed: 12033242]
12. Saggiomo V, Velders AH. Simple 3D Printed Scaffold-Removal Method for the Fabrication of Intricate Microfluidic Devices. *Adv Sci.* 2015; 2:1500125.
13. Kitson PJ, Rosnes MH, Sans V, Dragone V, Cronin L. Configurable 3D-Printed Millifluidic and Microfluidic ‘Lab on a Chip’ Reactionware Devices. *Lab Chip.* 2012; 12:3267–3271. [PubMed: 22875258]
14. Comina G, Suska A, Filippini D. Low Cost Lab-on-a-Chip Prototyping with a Consumer Grade 3D printer. *Lab Chip.* 2014; 14:2978–2982. [PubMed: 24931176]
15. Rogers CI, Qaderi K, Woolley AT, Nordin GP. 3D Printed Microfluidic Devices with Integrated Valves. *Biomicrofluidics.* 2015; 9:016501. [PubMed: 25610517]
16. Au AK, Bhattacharjee N, Horowitz LF, Chang TC, Folch A. *Lab Chip.* 2015; 15:1934–1941. [PubMed: 25738695]
17. Forster RJ, Bertonecello P, Keyes TE. Electrogenenerated Chemiluminescence. *Annu Rev Anal Chem.* 2009; 2:359–385.
18. Kadimisetty K, Mosa IM, Malla S, Satterwhite-Warden JE, Kuhns T, Faria RC, Lee NH, Rusling JF. 3D-printed supercapacitor-powered electrochemiluminescent protein immunoarray. *Biosens Bioelectron.* 2016; 77:188–193. [PubMed: 26406460]
19. [accessed March 17, 2015] Zinc-plated grade 5 Steel Hex Coupling Nut: Thread Size 1/4"-28. <http://www.mcmaster.com/#90977a140>
20. (a) Lvov YM, Lu Z, Schenkman JB, Zu X, Rusling JF. Direct Electrochemistry of Myoglobin and Cytochrome P450_{cam} in Alternate Layer-by-Layer Films with DNA and Other Polyions. *J Am Chem Soc.* 1998; 120:4073–4080. (b) Lvov, Y. Protein Architecture: Interfacing Molecular Assemblies and Immobilization Biotechnology. Lvov, Y.; Mohwald, H., editors. Marcel Dekker; New York: 2000. p. 125-167.
21. Sardesai N, Kadimisetty K, Faria R, Rusling JF. A Microfluidic Electrochemiluminescent Device for Detecting Cancer Biomarker Proteins. *Anal Bioanal Chem.* 2013; 405:3831–3838. [PubMed: 23307128]
22. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012; 9:671–675. [PubMed: 22930834]
23. Miao W, Choi JP, Bard AJ. Electrogenenerated Chemiluminescence 69: The Tris(2,2'-Bipyridine)Ruthenium(II), (Ru(bpy)₃²⁺)/Tri-*n*-propylamine (TPRA) System Revisited– A New Route Involving TPRA^{•+} Cation Radicals. *J Am Chem Soc.* 2002; 124:14478–14485. [PubMed: 12452725]
24. Dennany L, Forster RJ, Rusling JF. Simultaneous Direct Electrochemiluminescence and Catalytic Voltammetry Detection of DNA in Ultrathin Films. *J Am Chem Soc.* 2003; 125:5213–5218. [PubMed: 12708874]
25. Au AK, Lee W, Folch A. Mail-order microfluidics: evaluation of stereolithography for the production of microfluidic devices. *Lab Chip.* 2014; 14:1294–1301. [PubMed: 24510161]
26. (a) Tumbleston JR, Shirvanyants D, Ermoshkin N, Januszewicz R, Johnson AR, Kelly D, Chen K, Pinschmidt R, Rolland JP, Ermoshkin A, Samulski ET, DeSimone JM. Continuous liquid interface production of 3D objects. *Science.* 2015; 347:1349–1352. [PubMed: 25780246] (b) [accessed May, 14, 2015] <http://carbon3d.com/>

Figure 1.

3D-printed fluidic device with incorporated electrodes. (A) Side view of device equipped with 1/4 in.-28 threaded nuts and tubing for inlet/outlet access to the fluidic channel and a threaded nut in the center through which Ag/AgCl reference and graphite working and counter electrodes are integrated into the channel. (B) Bottom view of device. (C) Close-up bottom view of channel depicting (from left to right) 0.5 mm diameter graphite working, Ag/AgCl reference, and graphite counter disk electrodes surrounded by white plastic insulator. Flow of solution is from left to right in C. Channels are filled with 0.1 mM methylene blue in 0.1 mM KCl for visualization.

Figure 2.

Photographs of electrodes integrated in a 3D-printed channel for ECL measurements. (A) Image of 0.5 mm working and counter pencil graphite electrodes and 0.5 mm Ag/AgCl reference electrode positioned in channel taken with bioimaging system CCD camera. ECL emitted by 18 (B), 45 (C), 90 (D), 180 (E), and 450 (F) μM $[\text{Ru}(\text{bpy})_3]^{2+}$ in 0.2 M phosphate buffer (pH 7.5) and 100 mM TPA. A potential of +0.95 V vs Ag/AgCl was applied to the working electrode, and the exposure time for ECL images was 10 s. Scale bar represents 3 mm.

Figure 3.

Relationship between average ECL response and concentration of $[\text{Ru}(\text{bpy})_3]^{2+}$ for a single graphite working electrode in a 3D-printed fluidic device filled with $[\text{Ru}(\text{bpy})_3]^{2+}$ in 0.2 M phosphate buffer (pH 7.5) and 100 mM TPA. Error bars correspond to one standard deviation ($n = 4$). Standard deviation is $<4\%$, making error bars smaller than data points at low concentrations of $[\text{Ru}(\text{bpy})_3]^{2+}$.

Figure 4.

ECL response from $[\text{Ru}(\text{bpy})_3]^{2+}$ with (PDDA/DNA)₃-coated pencil graphite electrodes in a 3D-printed fluidic channel. ECL emitted from oxidized guanine bases within the (PDDA/DNA)₃-modified electrode upon exposure to 180 (A), 450 (B), and 800 (C) μM $[\text{Ru}(\text{bpy})_3]^{2+}$. (D) Linear relationship between DNA and ECL response. Error bars correspond to one standard deviation ($n = 3$). Error bars are smaller than data points at low concentrations of $[\text{Ru}(\text{bpy})_3]^{2+}$.

Figure 5. Photographs of electrode arrays incorporated into 3D-printed channel. (A) Bottom view of 0.5 mm Ag/AgCl reference, stainless steel ring counter, and three 0.5 mm pencil graphite working electrodes. (B) ECL response from electrode array in 180 μM $[\text{Ru}(\text{bpy})_3]^{2+}$ in 0.2 M phosphate buffer with 100 mM TPA. Scale bars represent 3 mm.

Scheme 1.

Basic Steps Used to Prepare of 3D-Printed Fluidic Devices