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3D Printed Electrochemical Sensors

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Abstract

Three-dimensional (3D) printing has recently emerged as a novel approach in the development of electrochemical sensors. This approach to fabrication has provided a tremendous opportunity to make complex geometries of electrodes at high precision. The most widely used approach for fabrication is fused deposition modeling; however, other approaches facilitate making smaller geometries or expanding the range of materials that can be printed. The generation of complete analytical devices, such as electrochemical flow cells, provides an example of the array of analytical tools that can be developed. This review highlights the fabrication, design, preparation, and applications of 3D printed electrochemical sensors. Such developments have begun to highlight the vast potential that 3D printed electrochemical sensors can have compared to other strategies in sensor development.

INTRODUCTION

Three-dimensional (3D) printed electrochemical sensors have become highly popular in analytical chemistry since 2012 (1) owing to their advantages over conventional methods, such as inexpensive production as well as the ability to use a wide range of materials, fabricate a wide array of geometries, and interface with fluidic devices (2–4). The most widely used fabrication techniques include fused deposition modeling (FDM), stereolithography (SLA), and selective laser melting (SLM). The applications of 3D printed sensors have been widely presented in the field of biomedical science (5–8) and environmental monitoring, notably in heavy metal determination (9).

In this review, we discuss recent and impactful articles on 3D printed electrochemical sensors and their applications for bioanalytical and environmental monitoring. Readers are encouraged to consult several previous reviews focused on 3D printed sensors providing more thorough coverage of different aspects (3, 4, 6–8, 10–13). Here, we focus on key publications that have shaped the field and present future perspectives and current challenges.

STRATEGIES UTILIZED FOR FABRICATION OF 3D PRINTED ELECTRODES

3D printing (also known as additive manufacturing) is a process used to make 3D objects and is based on the digitally controlled deposition of successive layers of materials until a final structure is made.

To start the process of 3D printing an object, a virtual model first needs to be rendered. This is performed using what is known as computer-aided design (CAD) software. With this, a 3D model of the object is created and converted into a file format suitable for saving the information and that can be then used in any 3D printer, which in this case is known as an STL (STereoLithography) file format. This file format breaks down the model into small triangular sections, each with a set of coordinates. Once loaded alongside a specific printer, the file is then converted into another coded file, called a G-code, through a process of "slicing" or breaking this model from a 3D format into multiple 2D cross sections. These cross sections or layers are then generated by the 3D printer one above another to finally result in the chosen 3D object.

There are several 3D printing technologies available and widely used in a host of analytical chemistry applications (3, 14–16); however, only a few approaches have currently been adopted to fabricate electrochemical sensors. These printing approaches are described here in detail, giving insight into their strengths and limitations.

Fused Deposition Modeling

The process of FDM is the most utilized method of 3D printing owing mainly to its simplicity. Created first by Scott Crump in 1989, FDM uses thermoplastic materials that are heated and extruded from a nozzle to be deposited in layers (17). As they are heated to their semi-molten state, they are deposited as the material solidifies to form a hardened layer that then sits on top of the previously made layer (**Figure 1**).

With this printing approach, a layer thickness of 0.1 to 0.4 mm can be easily generated. Thermoplastic materials such as polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS) are most widely utilized. To generate conductive printable materials, PLA and ABS are commonly mixed with various carbon allotropes to generate carbon composite filaments (12, 18).

Today, this method represents the most common 3D printing technology in use owing to its simplicity and the availability of machines and materials at affordable prices. FDM is also able to print parts quickly and is thus useful for fast prototyping. This form of printing has reasonable resolution (~200 μm) but a poor surface finish (e.g., surface roughness and lack of precision in

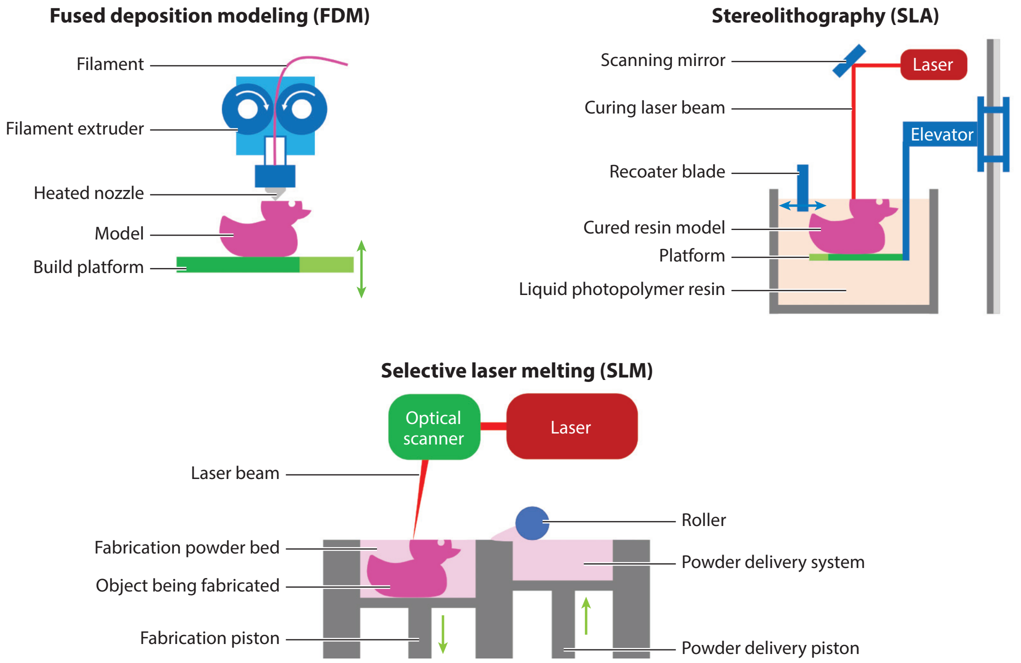

Figure 1

Different approaches to create 3D printed electrochemical sensors.

the thickness of the printed line). Additionally, postprinting the product is subjective to thermal shrinking, thus reducing the dimension of the final product. Lastly, depending on the geometry of the printed part, additional support material may be needed to benefit the structure.

3D Printing Pens

3D printing pens work in a similar fashion to FDM in that they allow for the extrusion of thermoplastic filaments from a heated nozzle onto a colder surface to create a 3D object. To date, these devices have mainly been used by hobbyists and artists to make 3D artwork and tools. The main difference between FDM and a 3D printing pen is that the precision in printing is governed by the operator of the 3D printing pen. Therefore, this approach is not widely suitable if fabrication of highly reproducible electrochemical sensors is needed.

Commercial 3D printing pens are widely available, portable, easy to use, and inexpensive, making this approach to sensor fabrication highly accessible. Most of the available devices provide a constant or limited range of printing speeds and nozzle temperatures (60–220°C), which narrow the variety of thermoplastics that can be utilized. However, 3D printing pens can be very useful when used to make electrochemical sensors through molding. 3D printing pens have only recently been utilized as a strategy for development of electrochemical sensors and, thus, the true potential of this approach is yet to be fully understood (19–21).

Selective Laser Melting

SLM is a form of powder-based 3D printing, in which solid materials are generated from metal material particulates (usually 50–100 μm in size). In this form of printing, a thin layer of metal powder is distributed onto the building stage by a roller. A laser beam is then directed onto the metal powder layer, welding the particles together according to the model design (**Figure 1**). Once a solid layer has been formed, the stage is lowered, allowing distribution of a second layer of metal powder on the top layer in preparation for the binding process to occur. Within SLM printing, the most used metal and alloy particles are steel, aluminum, nickel, titanium, and bronze or precious metal–based alloys.

One major advantage of this approach is that all of the nonbonded powder acts as a support material during the printing process so no materials are wasted. SLM provides printed parts with good resolution (~100 μm) and has an excellent surface finish. The printed electrodes using SLM have high durability. With this approach, a layer thickness of 0.02 to 0.10 mm is commonly employed. The high cost of the printer is one major limitation that makes this approach less accessible than FDM (22).

Stereolithography

SLA, or photopolymerization, was first created by Hideo Kodama in 1981 (23) who built a 3D printing technique around the use of photo-hardening polymers that are cured or hardened through employing ultraviolet (UV) light. As each layer is solidified, the platform on which the model is being built moves down to allow more of the immersed polymer resin to be hardened by the UV light, which is followed by utilizing a CAD program to make the final model. This controlled stacking of the layers as they are hardened allows successive layers to be made with good adhesion between the stacked layers. Different sources of UV light can be employed, including lasers or a digital mirror that acts as a digital light projection. The laser works by following the CAD design/instructions to move across the surface of the liquid polymer to harden the polymer according to the set design, which is called direct laser writing. The mirror works through projecting the UV light according to set design once for each of the layers to be cured (**Figure 1**). The platform where the design is built also has two different arrangements: In the bath configuration, the platform is at the liquid surface and moves downward after each layer is cured, whereas in the other arrangement, the platform is made to come in contact with the polymer solution. Through the use of an optically transparent bottom, the layers are hardened and the stage moves up after each layer is complete. This configuration is more advantageous when using limited quantities of resin because the required volume is smaller than that in the bath configuration.

SLA printing has excellent resolution (25–100 μm), with very accurate surface finishes of the printed product. With this printing approach, a layer thickness of 0.05 to 0.15 mm can be easily constructed. Isotropic materials are widely used, which means their properties are not dependent on their direction. There is, however, a restriction in the materials that can be used at present, as most liquid resins are not conductive, so complex postprocessing is required to create electrochemical sensors (24).

EXPANSION OF 3D PRINTABLE MATERIALS

The most widely used electrochemical sensors are based on solid conductive materials such as allotropes of carbon or noble metals. Therefore, initially, the use of metals as 3D printing materials was common in earlier studies of 3D printing sensors owing to their wide range of advantages (3, 25). These metals were printed mostly into helical sensors made from a base of stainless steel that was then electroplated with other metals, dependent on the analytical application, such as platinum, gold, iridium, or bismuth (25–30). Although very advantageous and promising, the use of metals was limited because of its high cost and the small range of materials that can be utilized for these applications. Hence, carbon-based electrodes garnered much attention and growth in 3D printing. To make conductive carbon filaments that can replace metal-based filaments, materials such as carbon black, graphene, and carbon nanotubes were mixed in to form a composite with the different thermoplastics utilized, namely PLA and ABS. These different filaments were used successfully in a range of studies, including those published in 3D printing using graphene/PLA sensors (31–33), ABS/carbon black filaments (34, 35), polypropylene/carbon black filaments (36), polybutylene terephthalate/carbon nanotube/graphene filaments (37), and nanofiber/graphite/polystyrene composite filaments (38, 39). With this success, more growth and innovation are being implemented toward the creation of more carbon-based conductive filaments using the different allotropes of carbon (12).

FABRICATING AND OPTIMIZING SENSING-BASED DEVICES

A key step in making effective 3D printed electrochemical sensors is to gain an understanding of the process of 3D printing and its capabilities. This is initially based on understanding how the printing process can influence the electrochemical properties of the fabricated electrode and creatively considering how 3D printing can make complete analytical devices encompassing 3D printed electrodes. There is also potential to optimize the printed electrode through postprinting optimization, where multiple strategies have been utilized to enhance the electrochemical properties of the electrode.

Single-Step Fabrication

One major advantage of making electrochemical sensors with 3D printing is the ability to go one step further and make complete devices. This process is known as single-step 3D printing, where devices are fabricated using a mixture of conductive and nonconductive thermoplastics. These can be achieved using two approaches. The first method uses start-stop printing, but this approach is restrictive and can only place electrodes within insulative thermoplastic within specific printing layers. The more widely used approach, which provides a more creative scope, is to utilize a printer that contains a dual extruder and thus is capable of minting multiple materials (13).

Initially, entire devices were made using 3D printing, which were printed individually but could be interfaced together. Richter et al. (40) generated a sensing platform using parts made from 3D printing, where electrodes were interfaced in a device to make an electrochemical cell (**Figure 2*a***). The simplest form of the single-step devices developed thus far is electrodes that are embedded in insulative casings. Rymansaib and colleagues (38, 39) generated a 3D printed working electrode by fabricating a carbon nanofiber-graphite-polystyrene composite electrode that was embedded in polystyrene (**Figure 2*b***). This approach has been further enhanced with the development of complete electrochemical cells (40–42), as shown in **Figure 2*c*,*d***. Lastly, studies have focused on the fabrication of electrochemical flow cells (43–45) (**Figure 2*e*,*f***). Li et al. (44) created a new fluidic device with a printer where four different materials were extruded to make the single device (**Figure 2*f***). These devices highlight that 3D printing can be used creatively to generate complete electrochemical devices.

Optimization of Printing Parameters

3D printing of an electrode is a complex process comprising multiple parameters that can influence the quality of the surface finish of the printed part. These parameters include the printing speed, printing orientation, print layer thickness, infill printing anisotropy, and infill printing pattern. Some studies have explored how optimization of such printing parameters can enhance the electrochemical properties of the fabricated electrode. Zhang et al. (34) explored how the anisotropy in between layers of a 3D printed ABS/carbon black composite structure would alter the resistance

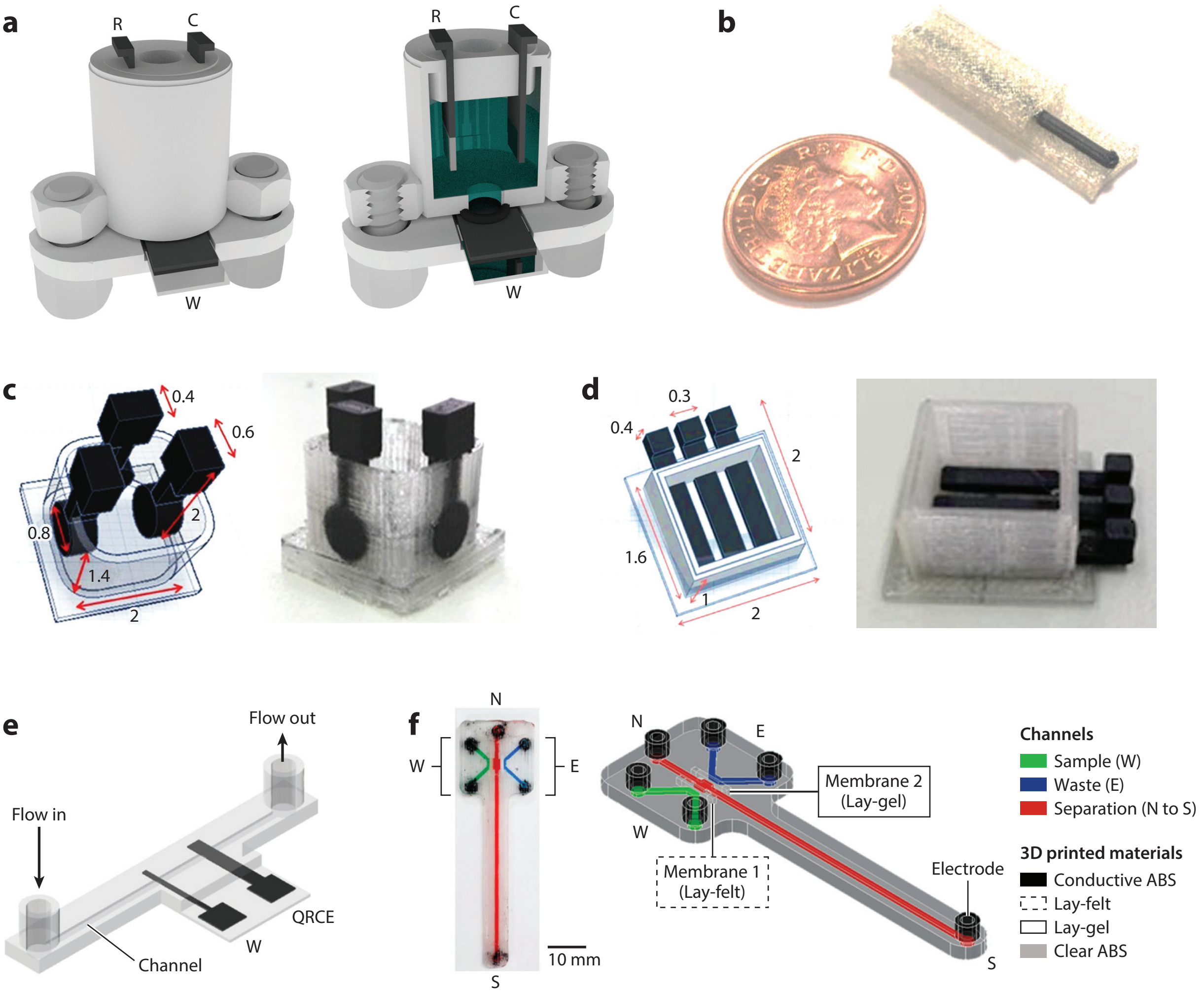

Figure 2

(*a*) 3D printed electrochemical cell that allowed linking 3D printed electrodes together in one device. Panel adapted with permission from Reference 40; copyright 2019 American Chemical Society. (*b*) Completely printed carbon nanofiber-graphite-polystyrene working electrode (W). Panel adapted with permission from Reference 38; copyright 2016 Wiley. (*c*) Single-step 3D printed electrochemical cell (measurements in centimeters). Panel adapted with permission from Reference 41; copyright 2020 Elsevier. (*d*) Single-step 3D printed electrochemical cell (measurements in centimeters). Panel adapted with permission from Reference 42; copyright 2020 Elsevier. (*e*) Single-step fabricated electrochemical flow cell. Panel adapted with permission from Reference 43; copyright 2019 Elsevier. (*f*) Single-step fluidic device made from four different materials. Panel adapted with permission from Reference 44; copyright 2019 American Chemical Society. Other abbreviations: ABS, acrylonitrile butadiene styrene; C, counter electrode; QRCE, quasi-reference counter electrode; R, reference electrode.

of the material. This study showed reduced resistivity in a vertically printed direction compared with the horizontal direction.

Hamzah et al. (35) showed that printing the electrode in a vertical orientation, rather than a horizontal one, can provide aligned conductive pathways of the print layers between the electric connection and solution interface, which enhances the number of conductive pathways and conductivity of the electrode (**Figure 3*a***). Abdalla et al. (46) also showed that the thickness of the

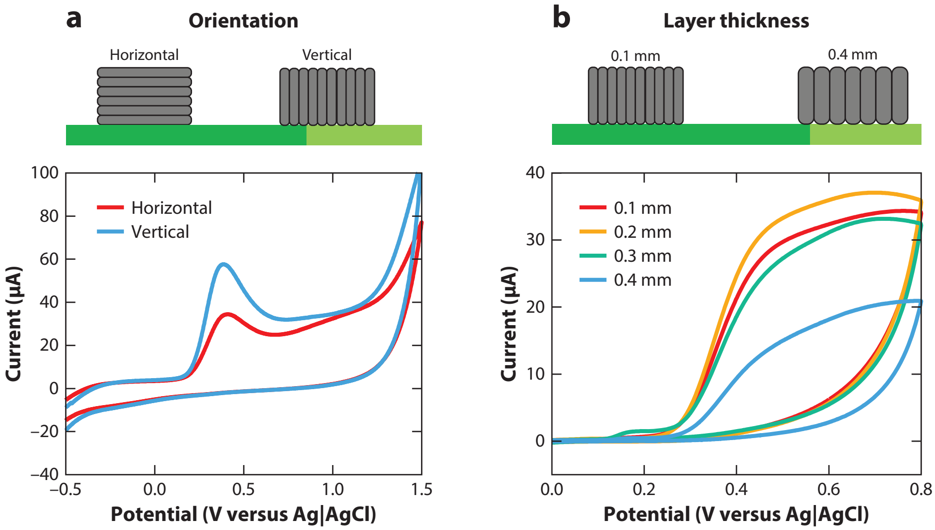

Figure 3

(*a*) Influence of printing orientation on voltammetric response of serotonin. Panel adapted with permission from Reference 35; copyright 2018 Springer Nature. (*b*) Effect of print layer thickness on the voltammetric response of serotonin. Panel adapted with permission from Reference 46; copyright 2020 Elsevier.

print layer plays a critical role in conductivity of the electrode. These findings showcased that 3D printing at a vertical orientation at lower print layer heights results in electrodes with the fastest electron transfer kinetics and lowest charge transfer resistance (**Figure 3*b***). This was due to smaller air voids between print layers and a more compact arrangement of the dense carbon black particles to the entire print layer in smaller print layers. These studies highlight the importance of defining and appropriately designing the manufacturing process of electrodes depending on their application when using 3D printing.

Electrochemical Pretreatment

The presence of insulating thermoplastics in large quantities in 3D printing filaments, along with the comparatively low conductive content (4), generate sensors printed from these filaments with sluggish kinetics and subpar performance when compared with other carbon-based electrodes (12, 40). Hence, different methods of postprinting pretreatment are utilized to enhance the electrochemical performance. One of these strategies is electrochemical pretreatment. This involves the application of a highly positive potential, called anodization, a highly negative potential, called cathodization, or cycling between the two potentials. The main aim of this pretreatment is to increase the presence of oxygen and amine functional groups on the surface, such as carboxylate, which in turn is well known to enhance the electrochemical performance of carbon sensors and increase the electron transfer kinetics (47–50). Based on this theory, many groups have attempted different variations of electrochemical pretreatment (21, 39, 46, 51–53) on 3D printed electrodes, ranging in time from 50 s (54) to 180 min (55) in many different electrolytes such as PBS (54), or NaOH (13) for different kinds of filaments (40, 56).

An example is an experimental study conducted by dos Santos et al. (57), who investigated the effect of anodization and cathodization (in PBS) on the electrochemical performance of graphene/PLA sensors in dopamine. An anodic potential of +1.8 V was used followed by a

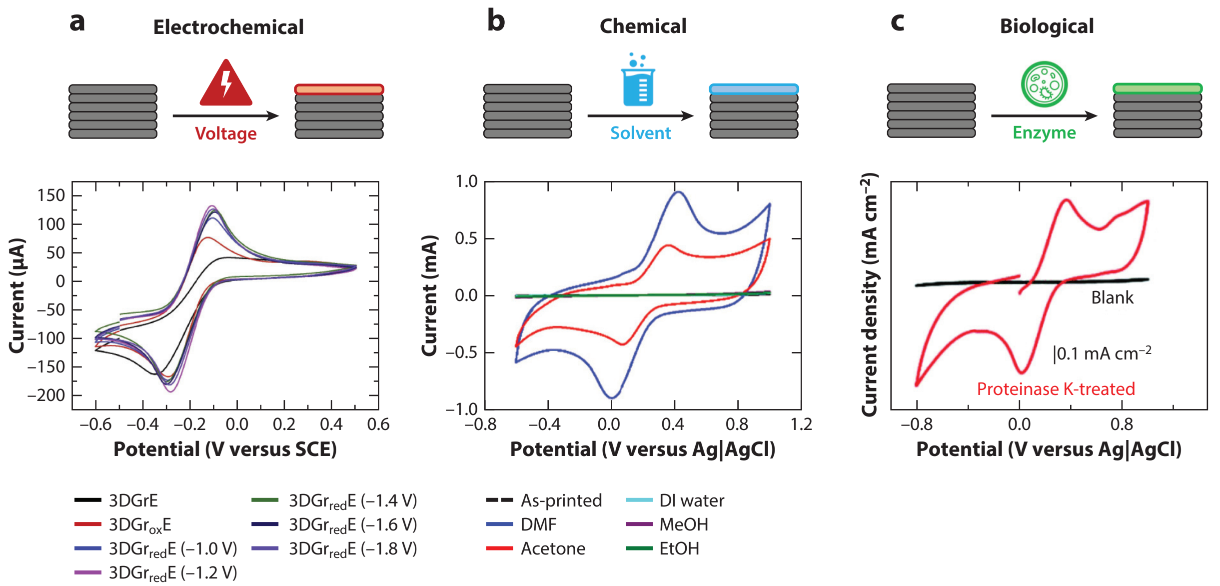

Figure 4

(*a*) Electrochemical pretreatment of graphene/PLA sensors, showing the effect of changing the cathodization potential on the ruthenium hexaamine response. Panel adapted with permission from Reference 57; copyright 2019 Elsevier. (*b*) Chemical pretreatment investigating the impact of varying solvents on electrochemical activity of ferri-/ferrocyanide on graphene/PLA sensors. Panel adapted with permission from Reference 62; copyright 2019 Elsevier. (*c*) Biological pretreatment, where proteinase K was used to digest the PLA to enhance electrochemical activity. Panel adapted with permission from Reference 64; copyright 2019 Royal Society of Chemistry. Abbreviations: DMF, dimethylformamide; EtOH, ethanol; MeOH, methanol; PLA, polylactic acid; SCE, saturated calomel electrode.

systematic change of the cathodic sweep between 0.0 V and −1.0 V to 0.0 V and −1.8 V. The electrochemical pretreatment was seen to remove some of the insulative PLA and increase the surface roughness, while the cathodization step in particular caused exposure of the edge planes of graphene sheets, thus further increasing the conductive area of the sensor. This resulted in an enhanced electrochemical response for all treatments when compared to untreated electrodes for the measurement of ruthenium hexaamine (**Figure 4*a***) and dopamine. The highest current density was seen for the −1.2 V pretreated sensor, whereas the smallest peak separation resulted from −1.8 V pretreatment (40). Electrochemical pretreatment is considered as the most straightforward and well-studied mode of pretreatment, making it feasible to manipulate the conditions (e.g., buffer, time, potential) depending on the material and molecule being measured.

Chemical Pretreatment

Similar to electrochemical pretreatment, chemical pretreatment is performed with the aim to enhance the electrochemical performance of the as-printed electrodes. Chemical pretreatment has been well known to enhance the electrochemical activity of carbon-based electrodes (58, 59). Solvents such as dimethylformamide (DMF), acetone, or NaOH are mainly used to dissolve the nonconductive binder, in most cases PLA, to better improve conductivity and hence improve electron transfer kinetics and electrochemical behavior (4, 20, 33, 51, 60, 61). In NaOH, the PLA undergoes saponification, and this increase in both electrode exposure and conductive sites results in performance enhancement (51). This is usually performed over a range of 1 min to 60 min using a range of solvents, either protic or aprotic, and either on its own or in tandem with electrochemical pretreatment. Gusmão et al. (62) approached this problem systematically by investigating the effects of graphene/PLA sensor immersion in three different protic solvents, namely water, ethanol, and methanol, as well as two aprotic solvents, namely DMF and acetone (**Figure 4*b***). Protic solvents showed minimal improvements in the sensors' response to ferri-/ferrocyanide redox couple, while immersion in the aprotic solvents resulted in the removal of PLA and enhancement of the electrochemical response. Comparison between the two aprotic solvents showed that acetone more greatly improved electron transfer kinetics, which was attributed to the rougher surface garnered by this treatment (62).

Although the use of chemical pretreatments is considered harsher because it tends to dissolve the insulative binder and makes drastic changes to the electrode geometry, it has shown great promise in enhancing the electrochemical performance of 3D printed sensors. This is especially true when used in combination with other pretreatment methods such as electrochemical or mechanical polishing.

Biological Pretreatment

One unique approach toward pretreatment of the electrode has been to use biological entities such as enzymes to digest the insulative thermoplastic. PLA fiber can be biodegraded by four different enzymes: lipase, esterase, protease, and Alcalase (63). Manzanares-Palenzuela et al. (64) explored the potential of proteinase K as a pretreatment step for graphene/PLA electrodes. Their study found that proteinase K digests PLA in a controllable fashion, exposing electroactive graphene sheets embedded within the 3D printed electrodes and thus providing improved electrochemical performance (**Figure 4*c***). This approach is not only a natural controlled way of exposing the conductive material within the 3D printed electrode but is environmentally friendly, as it mimics the natural breakdown process of the PLA thermoplastic.

APPLICATIONS OF 3D PRINTED ELECTROCHEMICAL SENSORS

Owing to the ease of fabrication of electrodes with high flexibility in different geometries that can be performed with high precision and low manufacturing costs, as well as the ability to make complete electrochemical sensing devices, 3D printed electrochemical sensors are useful in many applications. Much of the early work in this field has been focused on environmental monitoring and determination of biomolecules; however, new research areas such as forensics (20, 30, 65) are emerging.

Environmental Applications

Environmental pollution is a rising global concern due to the impact pollutants can have on all types of life-forms. Chemical pollutants are often transported in water, soil, and air. Upon human exposure, chemical pollutants can cause health effects that include, but are not limited to, cancer, respiratory diseases, kidney disease, and damage to the nervous and skeletal systems (66). Among environmental contaminants, those of interest include pesticides, heavy metals, aqueous-based compounds such as perchlorate, various phenolic compounds, volatile organic compounds, and fluorinated compounds (67, 68). Because of the abundant environmental chemical contaminants in food, water, air, and soil, it is critical to have sensing platforms that are also reliable and reproducible. Herein, methods for detecting environmental contaminants with 3D printed electrochemical sensors are presented.

One of the earliest applications of 3D printed electrochemical sensors was in the monitoring of trace heavy metals for potential environmental monitoring. Many studies were performed using

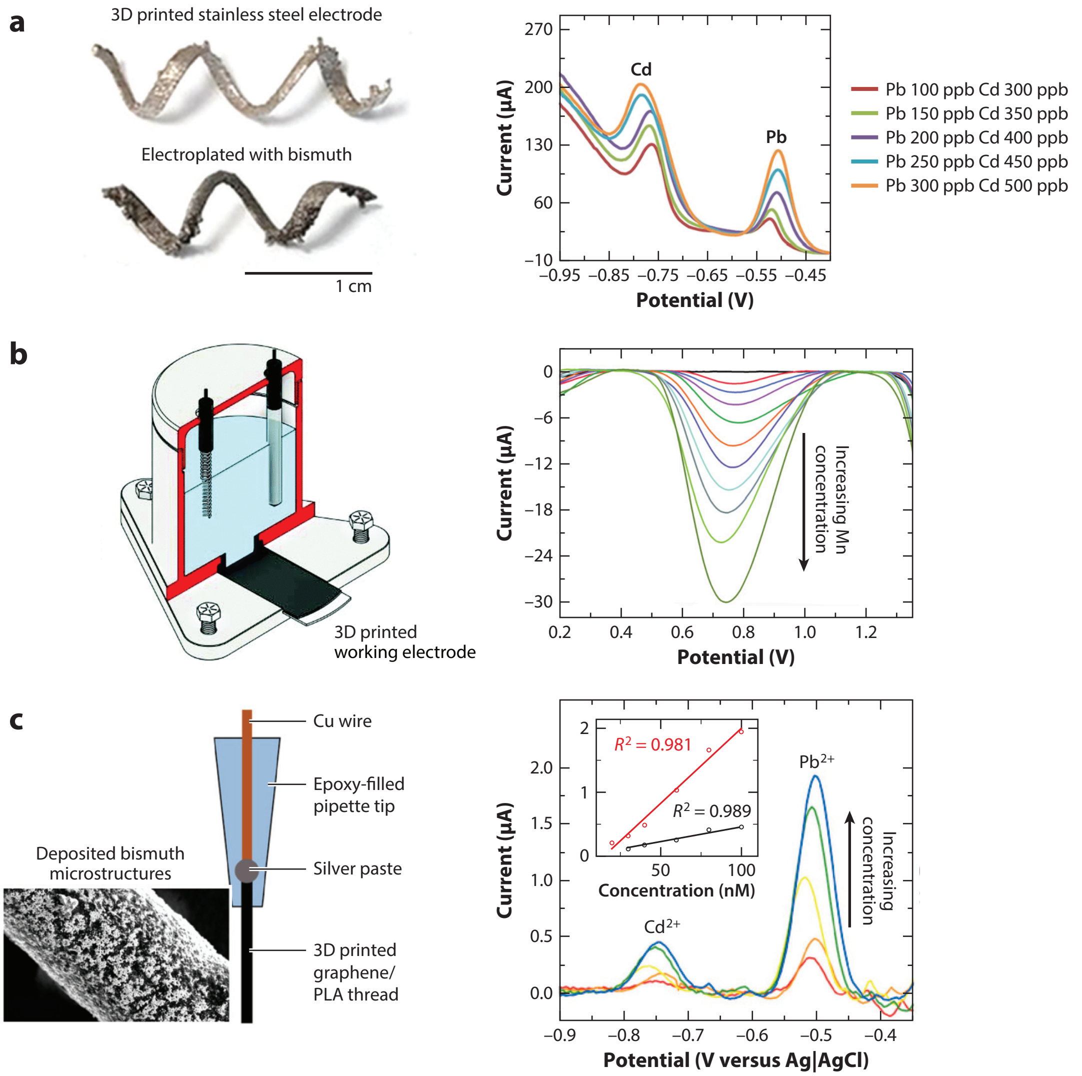

Figure 5

(*a*) 3D printed stainless steel electrodes electroplated with bismuth for determination of cadmium and lead. Panel adapted from Reference 28; copyright 2017 Wiley. (*b*) 3D printed electrochemical sensing platform for trace manganese detection. Panel adapted from Reference 71; copyright 2020 Royal Society of Chemistry. (*c*) Bismuth-modified graphene/PLA (polylactic acid) sensors for measurement of lead and cadmium from tap water. Panel adapted from Reference 69; copyright 2020 Wiley.

stripping voltammetry to showcase the ability of 3D printed electrodes to monitor a range of trace heavy metals such as lead, mercury, manganese, and cadmium (9, 28, 39, 51, 69–71).

Lee et al. (28) utilized 3D printed steel electrodes that were modified by electroplating with bismuth films to measure Pb and Cd in tap water (**Figure 5*a***). They found that the 3D printed electrodes showed enhanced performance when compared to a glassy carbon electrode. Rocha et al. (71) utilized a completely additively manufactured electrochemical well, introducing the ability to conduct portable measurements using their 3D printed sensing platform. Within this well, they utilized a nanographite/PLA electrode to monitor manganese levels in tap water samples and showed comparable responses to screen printed electrodes (**Figure 5*b***).

Walters et al. (69) also focused on the measurement of lead and cadmium using graphene/PLA cylindrical electrodes deposited with bismuth microstructures (**Figure 5*c***). These electrodes were able to monitor trace levels of mercury, lead, and cadmium in the parts per billion (ppb) range. This sensor was then able to detect these heavy metals at levels below those recommended by the US Environmental Protection Agency for drinking water.

Other studies have also explored the ability of 3D printed electrodes to monitor organic pollutants in water (27). Manzanares-Palenzuela et al. (64) developed a 3D printed electrochemical biosensor, where immobilization of the enzyme alkaline phosphatase was utilized for the electrochemical detection of 1-naphthol in aqueous media. These studies highlight the benefits of 3D printed sensors and their potential as a more economical, portable, and customizable alternative to measuring contaminants in environmental applications.

Biological Applications

Biomolecules play an essential role in human physiology, and an imbalance in the levels of these molecules can often lead to altered function, which can manifest as a disease. Determination of chemical biomolecules provides a profound understanding of how our bodies work and has served to contribute important insights into the diagnosis and treatment of diseases. Electrochemical detection has over many years been the most widely used approach owing to its ability to conduct spatial and temporal measurements in a host of biological environments (72). The mass production of electrodes at low cost using 3D printing represents a unique approach to making sensors for bioanalytical measurement.

Initial studies showcase the ability of 3D printed electrodes to monitor important biological molecules such as dopamine and serotonin. To expand the range of detectable analytes, electrochemical biosensors have been developed using 3D printed electrodes to monitor analytes such as glucose (73–75). López Marzo et al. (76) developed an enzymatic biosensor for the determination of hydrogen peroxide by immobilizing horseradish peroxidase onto the surface of graphene/PLA sensors coated with gold nanoparticles. This resulted in a selective sensor that successfully measured peroxide from human serum and that was also stable for 7 days (**Figure 6*a***). Another advancement in the use of 3D printing sensors for biomolecules is the fabrication of a DNA biosensor. A helical stainless steel electrode was first 3D printed, after which gold was electrodeposited on the surface. The thiol single-stranded DNA was subsequently attached to the gold surface of the sensor, which resulted in an excellent biosensor that was able to selectively identify complementary DNA when other noncomplementary DNA was present (29).

The emergence of 3D printed electrodes has provided the ability to create distinctly shaped electrodes that can interface with biological tissues (6). This aspect is clearly highlighted by Hamzah et al. (77), who developed a 3D printed electrochemical anorectum sensor. The shape of this sensor was unique, as it was fabricated to imitate the shape of a fecal pellet. This was an important detail because this shape causes a natural physiological stimulus, the result of which can then be measured. This stimulus caused an overflow of serotonin that was then measured simultaneously alongside the contractility of the circular muscle through insertion of the probe into the lumen of the anorectum (77) (**Figure 6*b***). This study thus showcases the advantage of 3D printing in creating electrodes with unique geometries that are best made to interact and interface with the biological system in which the measurement takes place.

Given that FDM is the most commonly used 3D printing technique, most studies have used it for their different environmental and biological applications. However, FDM is not always most

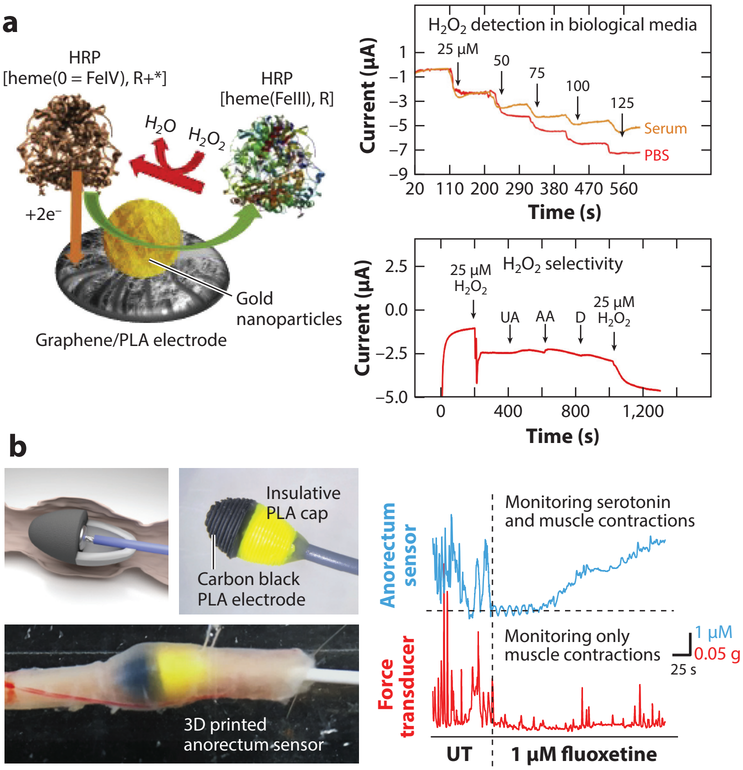

Figure 6

(*a*) 3D printed graphene electrode modified with gold nanoparticles for determination of H_2O_2. Panel adapted from Reference 76; copyright 2020 Elsevier. (*b*) 3D printed carbon black/PLA probe for the simultaneous monitoring of serotonin release and muscle contraction from ex vivo anorectum. Panel adapted from Reference 77; copyright 2019 American Chemical Society. (*c*) 3D printed nanostructures fabricated with two-photon lithography, which were pyrolyzed to make nanoelectrodes. Nanoelectrodes were utilized for measurement of dopamine from the adult fruit fly brain following stimulation using acetylcholine. Panel adapted from Reference 82; copyright 2020 American Chemical Society. Abbreviations: AA, ascorbic acid; D, dopamine; H_2O_2, hydrogen peroxide; HRP, horseradish peroxidase; PLA, polylactic acid; UA, uric acid; UT, untreated.

suited for applications that require sensors in the submicron dimensions. Thus, many studies have turned to two-photon lithography as an alternative method for developing sensors in those dimensions (microelectrodes) (78, 79).

This approach has also been utilized to make 3D printed microelectrodes capable of conducting in vivo measurements. In one study, two-photon lithography was utilized to fabricate 3D printed photoresist on the surface of metal electrodes. These were then subsequently carbonized to result in sphere microelectrodes (80) that were used to detect dopamine in vivo, specifically in the caudate region, in concentrations as low as 92 nM (81). To go one step further, Cao et al. (82) utilized this same approach to develop 3D printed nanoelectrodes (**Figure 6*c***). The nanoelectrodes were insulated with atomic layer deposition of Al_2O_3, and the nanotips were polished by a focused ion beam to form 600-nm disks. Using fast-scan cyclic voltammetry, the electrode was able to successfully detect the release of dopamine from the brain of an adult fruit fly. This approach provides the ability to revolutionize electrode fabrication by creating electrodes with geometries suitable to serve as implantable sensors.

The diverse range of studies discussed here shows that the ability to create electrodes of various shapes and sizes allows researchers to conduct bioanalytical measurements in a host of different biological environments. This varied electrode geometry has, however, not hindered the analytical performance of the sensors, but in most cases has been shown to be comparable to or better than widely used electrodes, such as screen printed carbon electrodes.

CONCLUSIONS AND FUTURE OUTLOOK

In this review, we described the most widely used methods to date for the fabrication of 3D printed electrochemical sensors and their current applications in environmental and biological monitoring. The strategies currently utilized to enhance the electrochemical activity of the printed electrodes were presented in detail. The findings presented here clearly highlight that 3D printing provides the ability to easily manufacture any geometry of electrode and convert this into complete analytical devices, which is not achievable by current approaches of electrode fabrication. Despite the advances made by using 3D printed electrochemical sensors, several challenges remain. The printed electrodes tend to have poor electrochemical activity and thus require extensive pretreatment strategies, which have been the focus of research within the field. The developed 3D printed electrodes have not been widely used in real samples and, thus, how such electrodes will cope in complex harsh environments and perform robust measurements is not yet fully understood. However, the significant efforts to develop new 3D printed electrodes and explore their potential in new areas will help showcase the benefits of these electrodes and expand their usage to make them a widely used type within all forms of electroanalytical measurement.

Although there is tremendous growth in the development of 3D printed electrochemical sensors, there remain many challenges and opportunities ahead. The range of materials that can be utilized to make 3D printed electrodes is limited, with mostly all studies to date having used commercially developed, conductive printable materials. Given that any conductive 3D printing filaments consist of a thermoplastic and conductive powder, expanding the range of conductive powders used will, in turn, broaden the range of electrodes developed and their applications. There is still much to learn about the fundamental behavior of printed electrodes to understand the best strategies for printing and pretreatment to provide enhanced electrochemical activity. Additionally, the true potential of 3D printed electrodes is not yet fully understood compared to other widely used electrodes, such as screen printed electrodes or carbon-based electrode materials. Robust studies comparing different electrode types will provide a better understanding of where 3D printed electrodes offer significant benefits compared to other electrode materials. Lastly, we have only begun to utilize 3D printed electrodes in biological and environmental measurements, an area where significantly more scope exists to showcase their unique features. Additionally, more emerging applications of 3D printed electrochemical sensors will help to illustrate their potential as the best future approach for mainstream electrode development.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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