3D printed electrochemical devices for sensing and biosensing of biomarkers

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Summary

Abstract

In this chapter, we explore the concepts of obtaining 3D devices, as well as the most relevant results regarding applications of these electrochemical devices for the determination of biomarkers. Some procedures and trends for obtaining 3D biosensors, new cell geometries, surface treatments, and applications in fully printed devices are described. Furthermore, the applications of the devices for biomarkers determination and their advantages will be discussed in detail, as well as the perspectives for the use of these devices.

Keywords: 3D-printing; electrochemistry; biomarkers; sensors; biosensors.

1. Introduction

As explored in the previous chapter, electrochemical sensors obtained by 2D printing techniques are widely used for the monitoring of biomarkers in general, and their surface can be modified with different types of biomolecules, or treatments depending on the final application purpose. However, some limitations on the sensor geometry, on the inks used in the process of obtaining the strips, in addition to limitations on the 2D-printing methodology, can restrict the scope of application of these sensors for biological applications. [1,2] Thus, as a way to get around and even improve performance, 3D-printing comes as a promising approach, allowing the devices obtained by this methodology to be more robust, stable, and enabling more stable measurements when in biological environments, such as fluids biomarkers in which the biomarkers are found [3].

3D-printing is one of the most innovative technologies available today, and it has established itself as a popular and powerful tool in many different fields. If we look at the last 30 years, it is possible to observe the growth of this technology in the most different niches of society [4,5]. The main motivation in using 3D printing is to obtain customized prototypes for different applications, quickly, with decentralized manufacturing and easy integration with the other parts of the system. This is possible because, in a very simple way, 3D printing is an additive manufacturing method that can build objects from a computational model, a model that is custom designed for the system in question.

In a very recent review, Su [6] makes a survey of 3D analytical devices with biological applications, in which the author concludes that the increasing use and replacement of conventional devices by 3D devices comes from a series of characteristics that can be changed when these devices are manufactured, which ultimately generate a result far superior to conventional devices, precisely because of the synergism that is generated when all the best features are placed in one device.

As a brief search in the literature, it is possible to note that a large portion of the application of this technology is for scientific research, and just as 3D printing offers a huge potential for devices from different areas, for systems and research in electroanalytical it would not be different. In the last 10 years, is possible to note an exponential growth in the publications numbers in the analytical field, especially for the electroanalytical devices (Fig. 1).

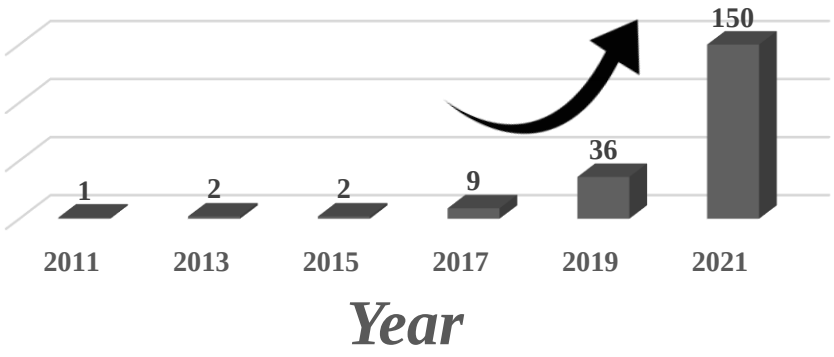

Figure 1. Publications from the last 10 years in electroanalytical field. Keywords: “3D-printed electrode”. Results from Science Direct database.

For most electroanalytical devices and applications the 3D devices were obtained using the extrusion of melting thermoplastics, as the fused deposition modeling (FDM) [1,2]. And although it seems that these devices obtained by FDM, have application limitations that are directly related to the most common thermoplastics, the acrylonitrile butadiene-styrene (ABS) and the polylactic acid (PLA), what can be observed, and will be discussed in detail throughout the chapter, is that different types of surface treatments, functionalizations and changes in the composition of thermoplastics can be carried out, thus improving the interface of these devices with biological environments, making the application range only increase.

As described by Abdalla and Patel [7], electrochemical devices obtained by 3D printing are a new horizon for measurements of biologically relevant molecules, and it is to demonstrate this new horizon that the following discussions were made.

2. 3D-printed electrochemical devices

The electrodes used at electrochemical sensors and biosensors are based on solid substrates manufactured by traditional processes. Carbon-based electrodes, especially glassy carbon and carbon paste electrodes; metallic electrodes such as platinum and gold, provide robust surfaces with good electronic conductivity and are versatile for the incorporation of modifiers. Despite the expressive success of the aforementioned working electrodes, currently, there is a new field of research in wide expansion, which aims to manufacture electrode materials and complete electrochemical devices for electroanalysis using a simple, low-cost, and reproducible methodology. Such research makes use of 3D-printing technology, developed at the end of the last century and with significant advances in the current 21st century.

3D-printing technology is based on the fabrication of solid objects from the sequential deposition of layers of a given material. In a typical procedure, the first step consists of drawing the desired object in a virtual environment using CAD software, when all dimensions, shapes, and geometries are defined [8]. The file is then converted into a format that is universally recognized and read by the 3D-printer software, the STL (StereoLithography) format [8]. In the printer's software, the 3D model is subjected to a slicing process, which consists of generating several layers of a 2D cross-section of the entire object. Finally, the printer starts to deposit the material after successive sequencing of these 2D layers, which are built on top of each other, until the desired 3D object is fully printed [8]. There are several 3D-printing technologies available, the most popular being that based on Fused Deposition Modeling (FDM). This technology makes use of an extrusion method, in which a thermoplastic filament is heated to its semi-molten state before extrusion by a movable heated nozzle, which deposits the polymer onto a substrate [1,9]. Some examples of thermoplastics for use in 3D-FDM printing are polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS). Once deposited, the material solidifies creating a layer that stacks on top of the previous layer. This step is repeated layer-by-layer until the entire object is printed [9].

3D-FDM printers have allowed the dissemination of 3D printing technology to various branches, due to their lower cost, relatively easy operation, versatility in the design of the most varied and complex objects, and rapid prototyping [10]. Currently, in the context of electrochemistry, 3D printing technology has already been explored in areas such as the development of Li-ion batteries, capacitors, electrocatalysis, and electroanalysis [8,11,12]. In the latter case, 3D printing has been applied in the

manufacture of electrodes for three-electrode systems, [13]. For this, the conductivity of the used material must be high for electrochemical/electroanalytical applications and, therefore, filaments based on polymers doped with carbonaceous conductive materials (graphene, graphite, and carbon black) are already commercially available, which enabled the manufacture of the electrodes.

In addition to the preparation of the electrodes themselves, an important advance reported is the use of 3D printing for the fabrication of the entire electrochemical device, that is, electrodes and electrochemical cells, the so-called all-in-one 3D-printed electrochemical devices [1]. In the case of the electrochemical cell, to avoid interference with the electrochemical transduction of the three-electrode system, naturally, a non-conductive filament is chosen to manufacture the electrochemical cells. The electrochemical cell and electrodes 3D printed by Richter et al. [14] are displayed in Fig. 2. The components were fabricated by FDM 3D-printing using a carbon black/PLA filament for the electrodes and insulant PLA filament for the other electrochemical cell parts.

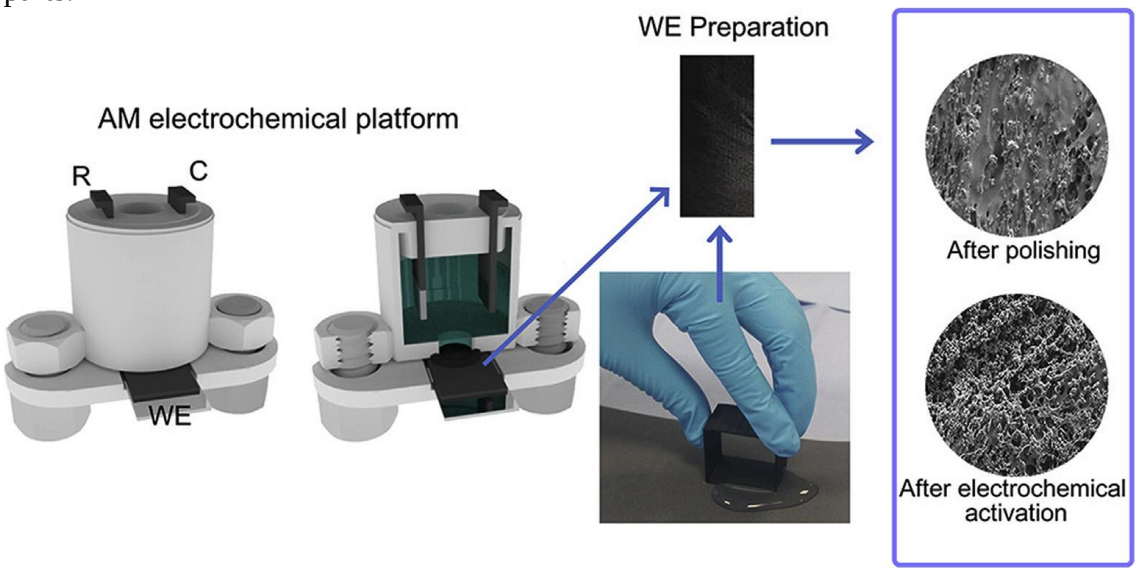

Figure 2. Schematic 3D-printed (AM) electrochemical cell and the 3D-printing working electrode preparation by polishing. On right, SEM images of the 3D-printed surface after polishing and after electrochemical activation. (Reprinted with permission from [14], Copyright (2019), American Chemical Society).

Although a conductive filament is used, the surface that will remain exposed to the electrolytic solution does not yet have fully adequate charge transfer characteristics. This is because conductive particles (such as graphene sheets or carbon black nanoparticles) are still occluded in the insulating polymer matrix. Thus, chemical, electrochemical, and/or mechanical treatment procedures are essential to active the electrodic surface [1]. A systematic study was recently reported by Kalinke et al. [15], in which the use of mechanical polishing, chemical, and electrochemical treatments, individually or in combination, of electrodes printed with PLA/graphene (PLA-G) was explored. The best electrochemical performance was achieved by applying the combined chemical and electrochemical activation steps: (1) chemical treatment by immersion in 1.0 mol L^{-1} NaOH solution during 30 min and (2) electrochemical treatment carried out by applying +1.8 V during 900 s followed by cyclic voltammetry (potential range of 0.0 to −1.8 V and scan rate of 50 mV s^{-1}), both in 0.10 mol L^{-1} phosphate buffer solution (pH 7.4). Fig. 3 compares the morphology of as-printed electrodes and those obtained from different treatments. Indeed, the SEM images suggest the presence of multilayer graphene

nanoribbons, free of the dense PLA layer in the case of the NaOH and EC treated electrodes, which led to a great increase in surface area, defects, electron transfer rate, and amount of edge site.

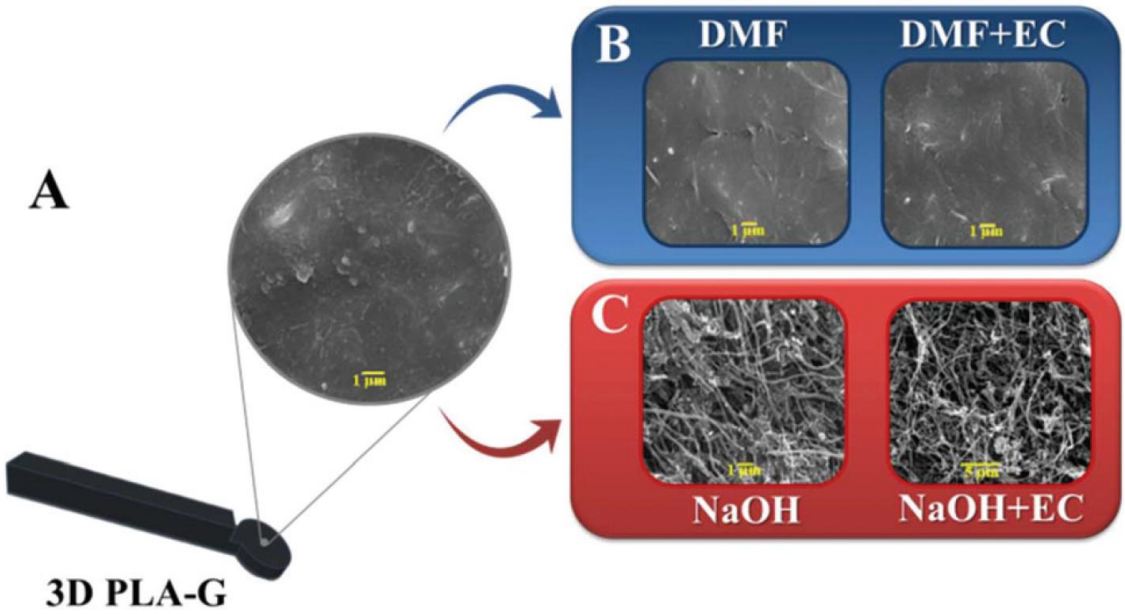

Figure 3. 3D PLA-G electrode design and SEM images with 10 000× magnification of the electrodes: **(a)** PLA-G, **(b)** PLA-G in DMF for 10 min and DMF followed by electrochemical treatment (EC), **(c)** PLA-G in 1.0 mol L^{-1} NaOH for 30 min and NaOH followed by electrochemical treatment (EC). (Reproduced from [15] with permission from the Royal Society of Chemistry).

The electrodes printed from the conductive filaments have been applied so far for the determination of analytes of pharmaceutical, environmental, and biological interest. In some cases, analytes with well-explored electrochemical behavior were determined, such as dopamine [10], uric acid [16], nitrite ion [16], heavy metals [17], among others. This is necessary to enable the comparison of data with those provided by electrodes conventionally used in electroanalysis. However, it opens up a great deal of space for the use of this new 3D (bio)sensors for the electrochemical study and quantification of more specific organic and inorganic analytes. 3D printed-electrodes can be used as a conventional non-modified electrode or as a platform for the preparation of electrochemical sensors or biosensors in electroanalysis. In addition to direct use, some recent works propose the modification of electrodes to improve electrochemical properties, such as electrochemically active area and charge transfer kinetics, or biological agents for the proposition of electrochemical biosensors. This modification can be performed by incorporating modifiers in the composition of the filament [18] or by modifying the electrode surface [19,20]. More examples of application specifically in the detection of biomarkers are presented in the following section.

3. Electrochemical determination of biomarkers using 3D printed devices

The use of 3D printing in electroanalysis is relatively new. Despite the use of this technology for decades, one of the first applications of 3D-printing for electrochemistry was demonstrated in 2010 [8], the first biomarker was detected only in 2018 using 3D printed sensors. Since then, to our knowledge, only 13 works have been reported for the detection of biomarkers. In this aspect, 3D printing for the analysis of biomarkers is a

growing field, and a lot has to be explored, improving the diagnostics of different kinds of diseases. Table 1 summarizing the above-discussed works is presented below, containing the possible biomarker studied, the material used for the fabrication of the sensors, the 3D printing method employed, as well as the analytical characteristics obtained in each work.

The precursor work employing 3D printed technology for the detection of a biomarker was reported in 2018 [21]. In this work, a 3D printed stainless steel electrode in helical shape was constructed for the detection of paracetamol and dopamine (DA). The 3D-printed stainless-steel electrode was surface-modified with a thin gold layer by electro-plating by applying a constant current (−20 mA for 90 minutes) to improve the electrochemical performance. The electrodes were obtained using selective laser melting SLM, based on the application of a focused laser beam of high energy which binds metallic particles deposited in a powder form in a printing stage, forming a previously established design, layer by layer. Fig. 4 presents an illustration of the design and 3D printed electrodes, including the Au-modified stainless-steel electrode. The simultaneous detection of the analytes was successfully performed, demonstrating that the 3D printed sensor is capable of detecting both without mutual interference. Following the same approach, Ho et al. used the Au-modified 3D-printed stainless-steel electrode for the detection of the biomarkers ascorbic acid (AA) and uric acid (UA) [22].

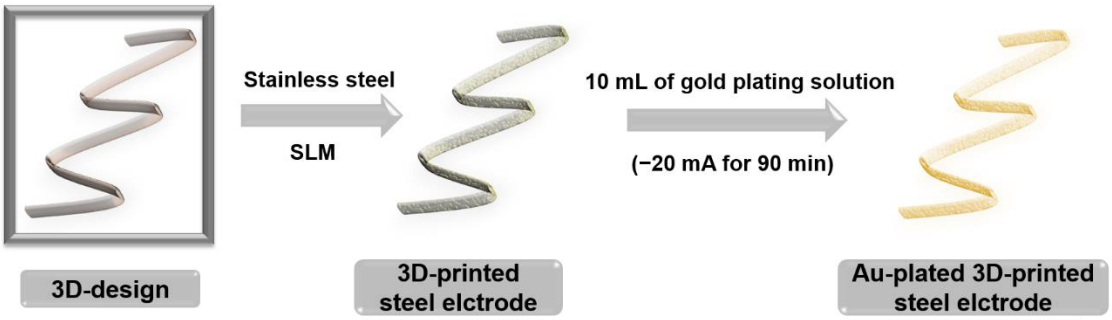

Figure 4. Schematic representation of the metal 3D-printed electrode fabrication and modification. (Adapted from [21]).

Regarding the use of commercial conductive filaments, Santos et al. [23] developed inexpensive and reproducible 3D-printed graphene electrodes employing the FDM printing technique with conductive polylactic acid/graphene (PLA/graphene) filaments for electrocatalytic detection of dopamine (DA). The electrodes were 3D printed using an extrusion temperature of 190 °C in the form of disks with diameters of 5 mm and thickness of 1 mm. A 2 mm thick strip was designed to allow the electrode to be connected to a connector. An illustration of the working electrode can be seen in Fig. 5.

Figure 5. (a) The design drawn in the Tinkercad platform and **(b)** the digital photo of the 3D graphene electrode. (Reprinted from [23] with permission of Elsevier).

Still employing the FDM technique, an interesting work that presents a way to manufacture the full electrochemical assembly (electrochemical cell and electrodes) in a single printing step was presented by Katseli et al. [24]. Its applicability was tested facing the determination of caffeine and glucose. The three conductive electrodes (working, counter, and pseudo-reference electrodes) were printed from a conductive PLA/carbon filament, and an electrode holder was printed from a non-conductive PLA filament. Fig. 6 presents an illustrated diagram of the printer used as well as the final printed electrodes.

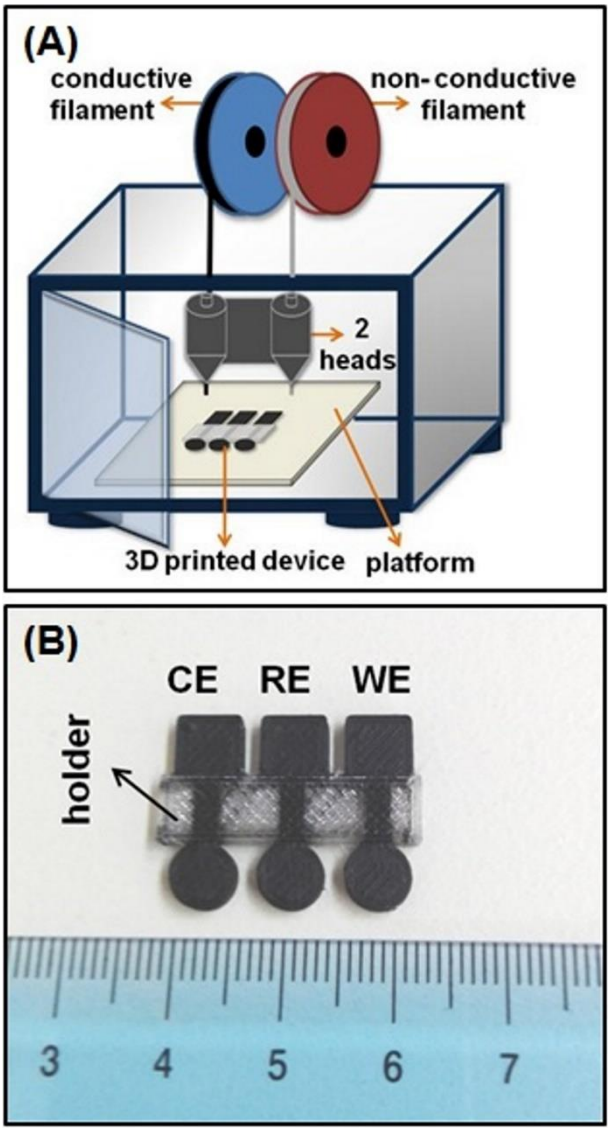

Figure 6. (a) Schematic illustration of the3D-printing processing of a 3D printer equipped with two heads. **(b)** Photograph of the 3D-printed integrated device. (Reprinted from [24] with permission of Elsevier).

Already in 2020, the number of works employing 3D printing for the detection of biomarkers increased. A good example is the work proposed by Kalinke et al. [15], which exploited different types of surface treatments on FDM 3D-printed graphene electrodes for the determination of DA in synthetic urine and serum. The electrodes used in this work also presented a disc shape with 5.0 mm in diameter and 1.0 mm in thickness, and with a connector of 20.0 mm in length and 2.0 mm in thickness. A series of surface treatments and its combination were exploited, including the electrochemical activation, mechanical treatment (polishing), and chemical treatments by direct immersion in different types of solvents (dimethylformamide - DMF, NaOH, HNO_3, and H_2SO_4). The optimized surface treatment was a combination of direct immersion in NaOH for 30 minutes with electrochemical treatment, that consist of applying a constant +1.8 V potential for 900 s in the presence of 0.1 mol L^{-1} PBS (pH = 4). The same strategy was used for the determination of L-methionine [25]. The developed sensor was tested in the analysis of biological samples (serum) enriched with L-methionine and presented adequate recovery values, indicating a great potential of the produced 3D printed sensors for the determination of this biomarker.

Rocha et al. have reported the modification of a commercial conductive filament (PLA/graphene) with nickel microparticles (NiG-PLA) [26]. for the non-enzymatic determination of glucose. For the production of NiG-PLA, a mixture of 30 g of the commercial conductive filament (cut into small pieces) was solubilized together with 3 g of $Ni(OH)_2$ in 250 mL of acetone and chloroform compound solution (3:1 v/v). The material obtained was dried at 100 ºC in an oven for 12 h and then cut into small pieces. Finally, the material was extruded at a temperature of 220 ºC and a speed of 30 rpm to form the desired filaments (Fig. 7 (a)). The new filament was used to print 3D hollow square boxes (4 cm × 4 cm × 2 cm) with a wall thickness of 0.72 mm in a vertical orientation (Fig. 7 (b)). Fig. 6C shows the coupling of the 3D printed electrodes to the bottom of the batch injection analysis cell (BIA). Thus, the method employed in conjunction with the PLA/graphene 3D printed electrode with nickel microparticles was a great alternative for the fabrication of sensors.

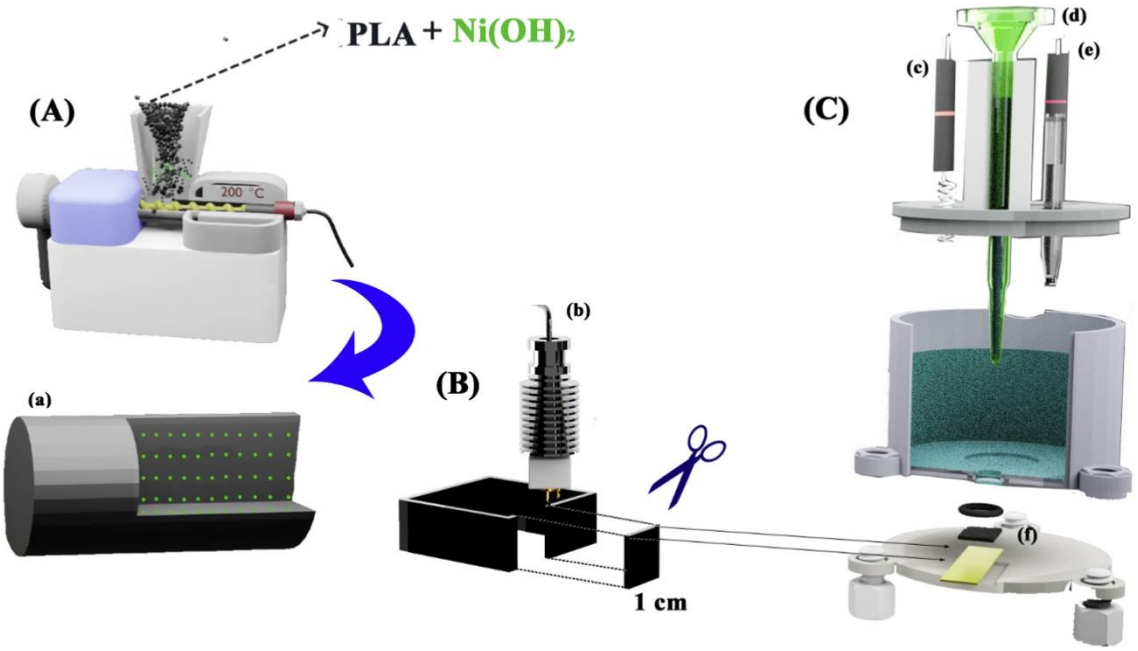

Figure 7. Schematic diagrams: **(A)** Production of the Ni-G-PLA filament using the 3D extruder; **(B)** 3D-printing of a hollow square box (4 cm × 4 cm × 2 cm) with the wall thickness of 0.72 mm; **(C)** The 3D-printing electrode (1 × 1 cm) is positioned at the bottom of the BIA cell on a metal plate (electrical contact); **(a)** illustration of Ni-G-PLA filament; **(b)** printer nozzle; **(c)** Pt counter electrode; **(d)** micropipette tip; **(e)** reference electrode (Ag/AgCl); **(f)** 3D printed Ni-G-PLA working electrode (1 × 1 cm). (Reprinted from [26] with permission of Elsevier).

Regarding the use of conductive filaments for the construction of biosensors, Marzo et al. [27] produced a 3D printed PLA/graphene electrode using the FDM technique. The obtained device was modified with horseradish peroxidase (HPR) to develop a biosensor capable of detecting hydrogen peroxide. Thus, initially, the complete activation of the surface (chemical and electrochemical - DMF-EC) of the 3D electrodes was performed, and later the sensors were modified with gold nanoparticles (AuNPs). An illustration of the whole process can be seen in Fig. 8. In the same way, Silva et al., 2020, presented an unprecedented procedure in which they have employed different chemical treatments to form reduced graphene oxide (rGO) in 3D printed electrodes made from conductive filaments of PLA/graphene printed in 3D by FDM for the determination of serotonin and catechol [28]. Tyrosinase enzyme was immobilized on the surface of the

working electrode to obtain a biosensor for catechol determination. Thus, to obtain the enzyme layer film, 1.0 mg of dihexadecyl phosphate (DHP) was dissolved in 1.0 mL of 0.2 mol L^{-1} phosphate buffer (pH 6.0), and 90 µL of this solution was mixed with the enzyme tyrosinase (10 µL/25 units) under constant stirring per 10 s. Next, a volume of 40 µL of this solution was placed, using a micropipette, upon the treated G-PLA surface, and the system was kept inside a refrigerator for 48 h for drying. The method employed for detection serotonin for catechol.

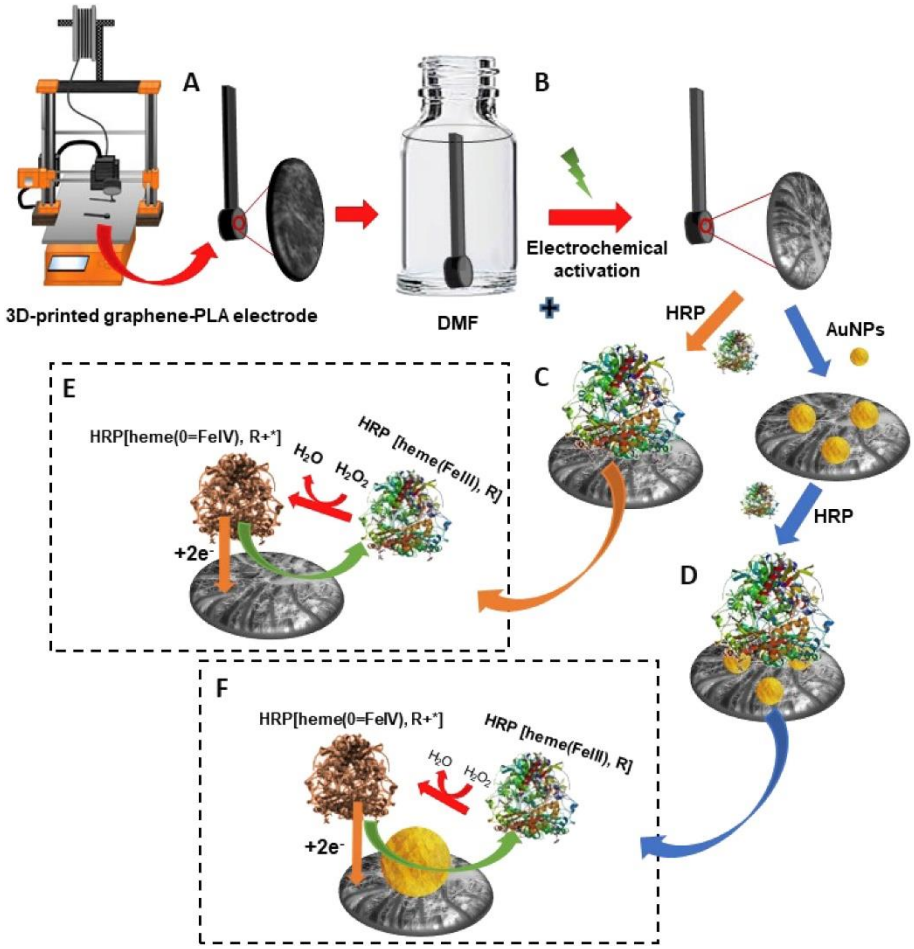

Figure 8. Representative scheme of 3D graphene-PLA biosensor fabrication: **(a)** 3D-printing of the electrode; **(b)** activation in DMF and by electrochemistry; **(c)** modification of 3D-printed electrode with HRP enzyme; and **(d)** modification of the 3D-printed electrode with gold NPs and, subsequently, with HRP enzyme. **(e)** and **(f)** are corresponding mechanisms of H_2O_2 detection. (Reprinted from [27] with permission of Elsevier).

Katseli et al. [29] have proposed an innovative conformation of 3D printed electrochemical microtitration wells (e-wells) based on direct quantum dots for enzymatic bioassays, employing a 3D printer equipped with a twin extruder fed with non-conductive (PLA) and conductive (PLA/CB) filaments. The sensors were printed at 60 ºC on the printing platform and 200 and 220ºC on the extruder nozzles for the PLA and PLA/CB filaments, respectively. Fig. 9 presents a summarized graphic illustration of the sensor production method and the final design. The bioanalytical applicability of the 3D e-wells was demonstrated by performing voltammetric bioassays in the detection of the C-reactive protein biomarker employing biotinylated reporter antibody and streptavidin-conjugated CdSe/ZnS QDs. In addition, due to the extension of its scope to the enzymatic

biosensing, the e-wells were applied for the determination of by-products of hydrogen peroxide, demonstrating universal applicability in electrochemical bioassays.

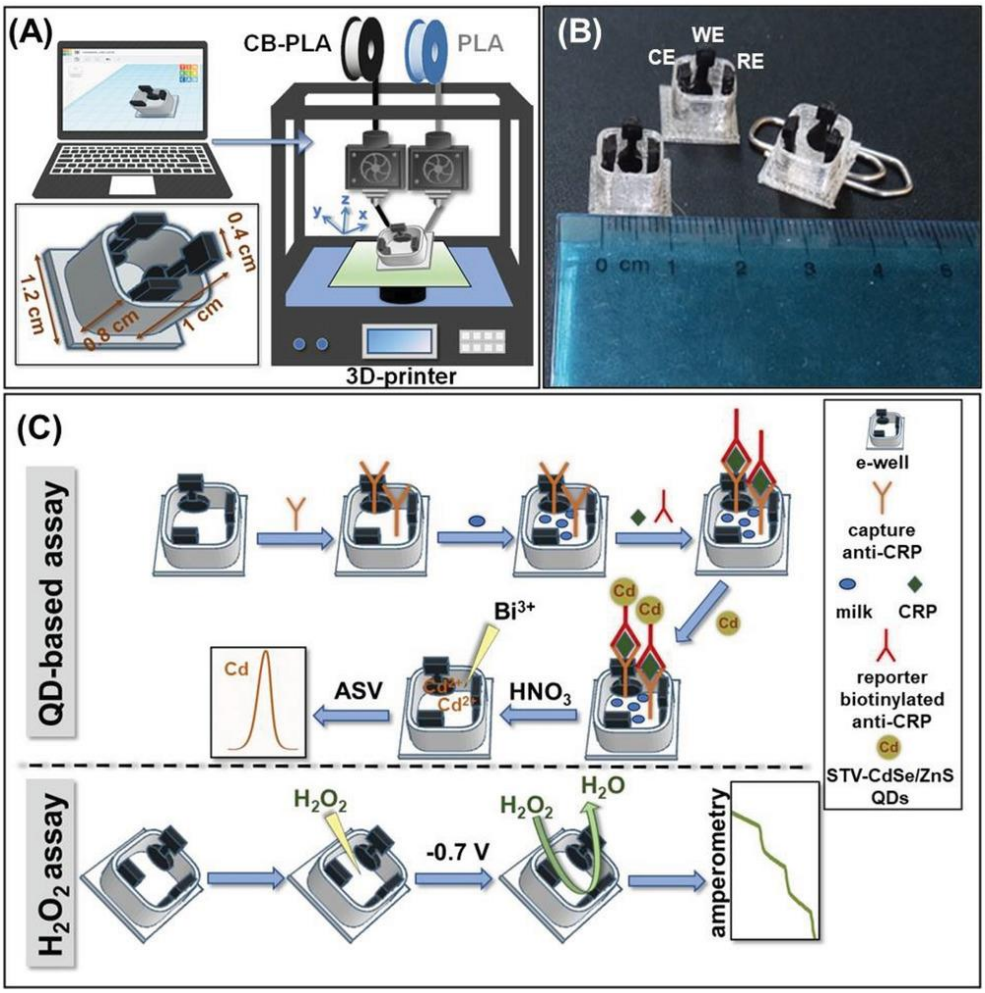

Figure 9. (a) 3D printing fabrication procedure of e-well and its dimension in cm. The conductive filament is PLA loaded with carbon black (PLA/CB). **(b)** Photograph of the 3D printed e-wells. **(c)** Schematic illustration of the immunoassay for the QD-based voltammetric determination of CRP and H_2O_2 amperometric assay in 3D e-wells. (Reprinted from [29] with permission of Wiley Analytical Science).

Finally, Martins et al. [30] have recently reported the first immunosensor constructed with the commercial 3D conductive filament of CB/(PLA) to detect Hantavirus Araucaria nucleoprotein (Np). In this work, the biorecognition element (antibody against Hantavirus) was directly immobilized on the 3D-printed electrodes using EDC/NHS chemistry (Please, see Fig. 10). To detect the hantavirus nucleoprotein (Np), the electrode's response towards the redox probe ($K_3[Fe(CN)_6]$) was compared in the absence and presence of Np. By applying this simple biosensing approach, it was possible to quantify the Hantavirus Araucaria, which was successfully applied in the analysis of diluted human serum samples.

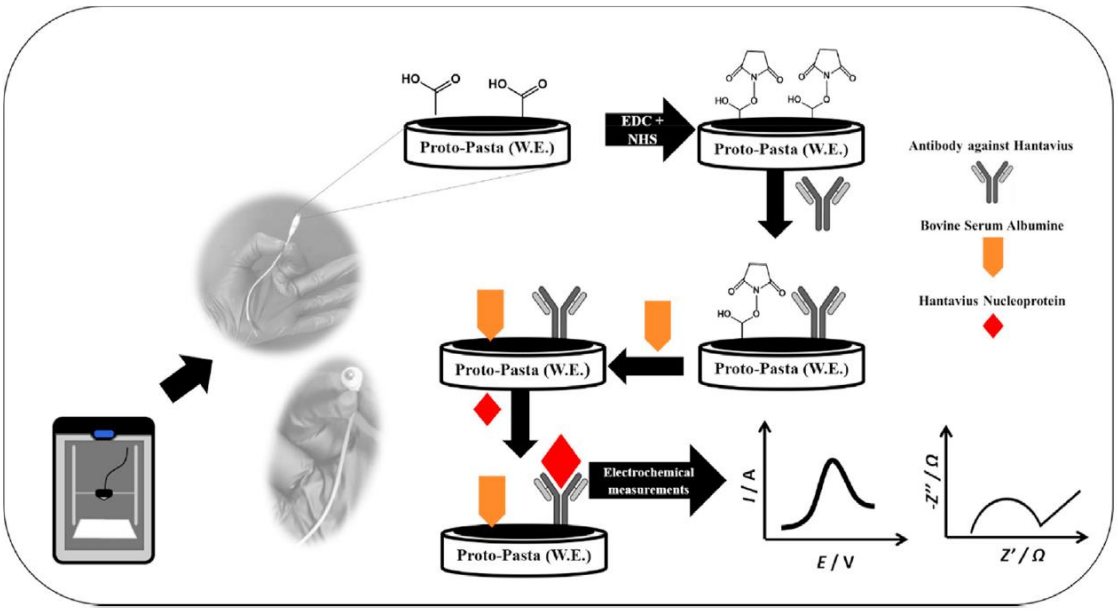

Figure 10. Immunosensor step by step buildup. (Reprinted from [30] with permission of Elsevier).

Table 1. Analytical features of 3D electrochemical (bio)sensors towards biomarkers determination

Biomarker	Material	Method	Technique	Concentration range	LOD	Sample	Ref.
Dopamine	PLA/graphene	FDM	SWV	5.0 to 1000 µmol L^{-1}	1.67 µmol L^{-1}	Synthetic human urine and serum	[15]
	PLA/CB	FDM	SWV	1 to 250 µmol L^{-1}	0.1 µmol L^{-1}	PBS (pH 7.2)	[14]
	PLA/graphene	FDM	DPV	2.0 to 93.8 µmol L^{-1}	0.24 µmol L^{-1}	PBS (pH 7.4)	[23]
	Stainless steel	SLM	DPV	50 to 250 µmol L^{-1}	–	PBS (pH 7.4)	[21]
Ascorbic acid	Stainless steel	SLM	DPV	0.1 to 1.0 mmol L^{-1}	2.1 µmol L^{-1}	Vitamin Cf	[22]
Uric acid	Stainless steel	SLM	DPV	0.1 to 1.0 mmol L^{-1}	84.0 µmol L^{-}	PBS (pH 7.1) and	[22]
	PLA/graphene	FDM	BIA-MPA	0.5 to 250 µmol L^{-1}	0.02 µmol L^{-1}	Urine and saliva	[16]
L-methionine	PLA/graphene	FDM	SWV	5.0 to 3000 µmol L^{-1}	1.39 µmol L^{-1}	Serum	[25]
Hydrogen peroxide	PLA/graphene	FDM	Amperometric	25 to 100 µmol L^{-1}	9.1	Human serum	[27]
	PLA/CB	FDM	Amperometric	1.5 to 13.5 mmol L^{-1}	–		[29]
C-reactive protein	PLA/CB	FDM	SWV	0 to 50 ng mL^{-1}	0.06 ng mL^{-1}	Artificial blood	[29]
Glucose	PLA/carbon	FDM	Amperometric	2 to 28 mmol L^{-1}	–	ABS (pH 4.5)	[24]
	PLA/graphene + $Ni(OH)_2$	FDM	Amperometric	75 to 1000 µmol L^{-1}	2.4 µmol L^{-1}	NaOH	[26]
	PLA/graphene	FDM	Amperometric	0.5 to 6.3 mmol L^{-1}	0.015 mmol L^{-1}	Bood plasma	[16]
Caffein	PLA/carbon	FDM	DPV	0 to 90 mg L^{-1}	1.8 mg L^{-1}	ABS (pH 4.5)	[24]

Nitrite	PLA/graphene	FDM	BIA-MPA	0.5 to 250 μmol L^{-1}	0.03 μmol L^{-1}	Urine and saliva	[16]
Serotonin	PLA/graphene	FDM	DPV	0.30 to 10.0 μmol L^{-1}	0.032 μmol L^{-1}	Synthetic urine	[28]
Catechol	PLA/graphene	FDM	SWV	30 to 700 μmol L^{-1}	0.26 μmol L^{-1}	Natural water	[28]
	PLA/graphene	FDM	CV	0.2 to 5.0 mmol L^{-1}	7.7 μmol L^{-1}	$HClO_4$	[31]

4. Conclusions and Perspectives

As presented in the previous chapter, the development of new types of devices for the sensing of biomarkers is of paramount importance, especially devices that aim to overcome the current problems of conventional methods of analysis. In this way, 3D printing technology is a fundamental tool to support the production of a new generation of electrochemical devices. This technology, coupled with the development of new electrochemical sensors, brings several advantages, such as automatization and large-scale production, development of complete and miniaturized systems (cell and electrodes) with a wide variety of designs, cost reduction, and application of new materials.

Given the aspects discussed in this book chapter, 3D printed electrochemical devices are increasingly being used in the monitoring of biomarkers, as the evolution of the produced sensors is constant, with new designs, fabrication methods, and surface treatments, facilitating the handling and employability of the sensors. In addition, with the advancement of 3D technology, whether employing simpler or more sophisticated printers, an advance in the production of increasingly robust electrochemical sensors is observed, whether in miniaturization, with greater specificity, sensitivity, applicability, or production with new materials.

Finally, the use of 3D printing strategies applied to the development of miniaturized electrochemical systems is a technology that fully adequates to the precepts of analytical chemistry. The possibility of integration between automated production with the freedom to model new designs and complete electrochemical systems is one of the most promising trends in current electroanalytic, considering the exponential growth of publications. Thus, this manufacturing strategy will become increasingly popular and with wide growth, aiming the production of new types of materials, new methods of surface treatment, or new architectures that brings beneficial advances both for the academic environment and society.

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