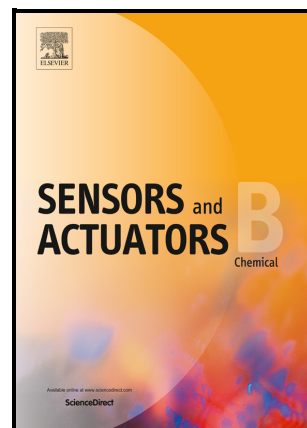


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3D printing of compact electrochemical cell for sequential analysis of steroid hormones

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Abstract

3D printing technology revolutionized the development of electrochemical sensors allowing the fabrication of custom devices that can be adapted to meet specific applications. This study describes the development of a miniaturized electrochemical cell containing integrated electrodes manufactured entirely by 3D printing for the sequential detection of steroid hormones. The electrochemical sensor was manufactured in a single step by combining two types of thermoplastic filaments. Polylactic acid-Carbon Black (PLA-CB) was used in the electrode region and acrylonitrile butadiene styrene (ABS) was used in the insulating region. Chemical/electrochemical treatments were used to remove the inert polymer matrix and expose the electroactive sites of the CB electrodes. These pre-treatment procedures significantly enhanced the electrochemical response of the printed sensor. The analytical performance of the activated 3D printed electrochemical cell was demonstrated in the sequential detection of estradiol (E2) and progesterone (P4) in artificial urine. The electrochemical cell offered good repeatability (RSD = 3.1%) and reproducibility (RSD = 10.7%) between analyses reaching detection limits of 0.11 and 17.8 $\mu\text{mol L}^{-1}$ for E2 and P4, respectively. In this sense, these results demonstrate that the use of 3D printing paves the way for the fast and economical fabrication of miniaturized electrochemical cells for hormone detection in biological fluids.

Keywords: Rapid prototyping; Multimaterial 3D printing; Fused deposition modeling; 3D printed electrodes; Estradiol; Progesterone.

1. Introduction

3D printing technology is a revolutionary tool gaining prominence for rapid prototyping of analytical devices containing complex structures. The growing popularity of 3D printing is due to its many advantages including the freedom of object design, the minimization of waste, and its capacity for fast and automated production [1–3]. Due to the high accessibility of 3D printers, and the great diversity of printable conductive materials, this technology has been adopted for the manufacture of electrodes included in customized batteries [4], microfluidic devices with integrated sensing electrodes for contactless conductivity detection [5,6] and printed electrochemical sensors [7–12].

The first studies that employed 3D printed electrodes were based on selective laser melting (SLM) printing technology [9]. Briefly, an incident laser melts a layer of metallic granules and promotes the union of particles creating a solid object. After the first layer is melted, a new layer of metallic material is deposited and the process is repeated, layer by layer until the piece is completed [1,13]. Typically, a stainless steel three-dimensional structure is first produced by SLM, and further galvanized with another metal such as gold, bismuth, nickel, platinum, or iridium oxide to suit different types of applications [9,14–18]. Meanwhile, this type of fabrication technology is fast and accurate, it employs expensive equipment and requires electroplating steps. On the other hand, fused deposition modeling (FDM) printing technology has stood out as an economical method with

great operational simplicity. This technology controls the deposition of semi-fused thermoplastic materials on a printing platform, building the object layer by layer [1]. In addition, FDM 3D printers with two or more extruders, also called multi-material 3D printers, allow the production of hybrid objects in a single step. This technology is becoming a trend in electrochemical sensor production because it enables the combination of a conductive and a non-conductive material in a single step, making the manufacturing process faster and less labor-intensive [12,19–27].

To produce electrodes through FDM, conductive filaments composed of carbon-based conductive particles and thermoplastic materials are used. Recently, several conductive polymeric filaments has been reported in literature such as polylactic acid (PLA)-graphene filament [11,28,29], PLA-carbon black (PLA-CB) [30–32], Acrylonitrile butadiene styrene (ABS)-CB [33], polypropylene-CB [34], polybutylene terephthalate-carbon nanotube-graphene [35] and carbon nanofiber-graphite-polystyrene [10,36]. Among all, PLA-CB filament has been increasingly explored to produce printable electrodes for electroanalytical applications. These printed electrodes have shown promising results in the detection of metals and biomolecules [30,37,38]. However, until now, 3D printed electrodes were not explored for hormones analysis.

Progesterone (P4) and 17 β -estradiol (E2) belong to the group of steroid hormones. These biomolecules are essential for the human body since they are involved in several metabolic pathways. E2 deficiency is related to heart disease and osteoporosis, in contrast, high amounts of E2 in the body can cause ovarian and breast cancers [39–41]. P4 is essential in maintaining the reproductive cycle of mammals by controlling the production of biologically active steroids [42]. Levels of P4 and E2 are important diagnostic indicators for possible disease states. Thus, new electroanalytical devices would provide important and useful diagnostic tools by monitoring steroid hormones such as E2 and P4 in the human body.

Gan et al. reported the use of boron-doped diamond electrodes for the detection of a great number of estrogens such as E2, nonylphenol, bisphenol A, ethinyl estradiol, estrone, and estriol

[43]. The authors successfully demonstrated that the modification of diamond electrode with CB made it possible to reduce the LOD from $\mu\text{mol L}^{-1}$ to nmol L^{-1} range for all the estrogenic compounds tested. Smajdor et al. used glassy carbon electrodes modified with CB for the detection of ethinyl estradiol employing differential pulse voltammetry [44]. The analytical system offered high reproducibility (relative standard deviation (RSD) equal to 2.5%) and it reached a LOD of 130 nmol L^{-1} . Later, in another study, Smajdor et al. applied the modified electrode for the detection of E2 reaching a LOD of 92 nmol L^{-1} with RSD of 1.3% [45].

To the best of our knowledge, this paper describes for the first time the development of a fully 3D printed electrochemical cell designed with a novel and reduced architecture for sequential analysis of steroid hormones without the need for modification of the electrode surface to incorporate other materials before use. The analytical device contains integrated electrodes manufactured entirely with a 3D printer employing multi-material FDM technologies. The electrochemical cell was manufactured in a single step by combining two thermoplastic filaments (ABS and PLA-CB). After the fabrication procedure, the electrodes were treated to expose the CB particles and improve the performance of the electrochemical cell. The integrated electrodes were characterized by employing morphological, electrochemical, and structural analysis. The results revealed that the use of DMF followed by saponification-based reactions successfully activates the surface of the 3D printed PLA-CB devices. The sequential analysis of E2 and P4 was performed in artificial urine through differential pulsed voltammetry showing the potential application of the 3D printed electrochemical cell for hormones detection.

2. Experimental section

2.1. Chemicals and materials

Sodium hydroxide (NaOH), potassium chloride (KCl), potassium ferrocyanide ($\text{K}_4[\text{Fe}(\text{CN})_6]$), dimethylformamide (DMF), ethanol, as well as the 17β -estradiol (E2) and progesterone (P4) hormones were purchased from Sigma Aldrich Co. (Saint Louis, MO, USA). Glacial acetic acid was purchased from Vetec Química Fina LTDA (Rio de Janeiro, Brazil). Boric

acid and phosphoric acid were obtained from Merck. All chemical reagents were of analytical grade and they were used without further purification. The solutions were prepared using ultrapure water with resistivity $\geq 18 \text{ M}\Omega \text{ cm}$ obtained through a water purification system (Direct-Q® 3, Millipore, Darmstadt, Germany).

2.2. 3D printing of electrochemical cell

The electrochemical cell containing working electrode ($\phi = 3 \text{ mm}$), auxiliary electrode, and pseudo-reference electrode was designed in computer-aided design (CAD) software (Fusion 360, Autodesk, San Rafael, CA, USA), as shown in Figure 1. The multi-material 3D printer Original Prusa i3 mk2/S (Prusa Research sro, Prague, Czech Republic) was used to manufacture the electrochemical cell with the embedded electrodes. The electrode region was printed using a conductive PLA-CB filament, commercially named as Proto-Pasta, acquired from ProtoPlant (Washington, USA), while the insulating region was manufactured using ABS filament provided by 3D Fila (Belo Horizonte, MG, Brazil).

<Insert Figure 1>

2.3. Electrochemical/chemical pre-treatment

The electrochemical cell was exposed to DMF to attack the electrode surface [46]. The working electrode was electrochemically activated with NaOH applying anodic and cathodic potentials to drive the saponification of PLA to lactic acid [47]. First, 100 μL of DMF solution was placed on the printed cell for 170 s, and immediately cleaned three times with 96% ethanol. Once dried, the device was immersed in ultrapure water inside a becker and stirred with an ultrasound bath for 30 min. The electrochemical cell was dried for 30 min at 60 °C. Next, the working electrode was treated with 0.5 mol L⁻¹ NaOH applying 1.4 V for 200 s and -1.0 V for other 200 s. For simplicity, this procedure will be named of NaOH electrochemical treatment.

2.4. Electrochemical analysis

All the electrochemical analyzes were performed with a μ Stat 400 portable bipotentiostat/galvanostat from DropSens S.L (Oviedo, Spain). E2 and P4 solutions were prepared in Britton-Robinson buffer (0.1 mol L^{-1}) at pH ranges of 2-12, and 4-12, respectively. A volume of $100 \text{ }\mu\text{L}$ of hormone solutions (E2 and/or P4) was pipetted to the electrochemical cell and differential pulse voltammetry (DPV) was applied to analyze the samples through individual and sequential measurements. E2 was analyzed employing step potentials of 2 mV , pulse potentials of 8 mV , pulse times of 100 ms , and scan rates of 5 mV s^{-1} in a potential window from 0.1 to 0.85 V vs PLA-CB. P4 was analyzed employing step potentials of 2 mV , pulse potentials of 50 mV , pulse times of 100 ms , and scan rates of 5 mV s^{-1} in a potential window from -1.0 to -1.95 V vs PLA-CB. The above-mentioned DPV conditions were employed for the sequential oxidation of E2 and reduction of P4 in a single electrochemical experiment. All measurements were conducted at room temperature ($25 \pm 1 \text{ }^\circ\text{C}$).

2.5. Urine samples

Artificial urine samples were prepared in Britton-Robinson buffer (0.1 mol L^{-1} , pH 10) with or without E2 and P4, following a procedure described by Brooks and Keevil [48]. Recovery experiments were performed with urine standard solutions spiked with E2 and P4 to a final concentration of 25 and $100 \text{ }\mu\text{mol L}^{-1}$.

2.6. Characterization

3D printed external and internal structures were characterized by scanning electron microscopy (SEM), Raman spectroscopy, and electrochemical measurements. SEM analysis was done with a microscope model JSM-6610 from JEOL (Akishima, Tokyo, Japan) using secondary

electron imaging mode and acceleration voltage of 5 kV. Raman spectroscopy was carried out in a confocal Raman spectrometer model LabRAM HR Evolution from HORIBA (Kyoto, Japan) using the laser with a wavelength of 532 nm and spot size of 2.6 μm . Electrochemical impedance spectroscopy (EIS) measurements were performed in a PGSTAT-100N potentiostat/galvanostat via a sinusoidal signal at frequencies ranging from 10^5 to 10^{-1} Hz, an amplitude potential of 20 mV and applied potential of 0.20 V (open-circuit potential) considering a redox probe 1.0 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{4-/3-}$ prepared in 0.1 mol L^{-1} KCl. Electrochemical analyzes involving cyclic voltammetry (CV) experiments were carried out using a mentioned above redox probe ($[\text{Fe}(\text{CN})_6]^{4-/3-}$) at scanning rates ranging from 10 to 200 mV s^{-1} .

3. Results and discussion

3.1. Fabrication of the electrochemical cell

The electrochemical cell was manufactured with a 3D printer, combining two different types of materials (ABS and PLA-CB) with multi-material FDM technology. The FDM printing method allows machines that contain one or more extruders to manufacture objects with different colors or materials [12]. To obtain multicolored/multi-material parts with a single extruder, it is necessary to interrupt the printing process to change the filament. However, this process can be laborious, and includes more steps during the printing process leading to increased manufacturing time. 3D printers with multi-material FDM technology contain two or more extruders and allow the construction of objects with different materials in an automated way in a single step. In this study, a multi-material 3D printer was used that contains four extruders capable of building objects with up to four different materials. The fusion and deposition process were previously configured with the aid of the computer-aided manufacturing (CAM) software. Thus the 3D printer recognizes the type and the exact amount of material needed for a specific region of the modeled object. Also, with the multi-material 3D printer, it is possible to print tens of electrochemical cells simultaneously. In this context, each device was built in about 12 minutes at a manufacturing cost of approximately \$ 0.08. The printed electrochemical cell can be seen in Figure 2A.

3.2. Morphological, structural and electrochemical characterization

The characterization of the electrochemical cell was performed through SEM microscopy and electrochemical approaches. These experiments were carried out upon the non-treated and treated electrodes surfaces.

As it can be seen in Figure 2, SEM analysis for non-treated WE surface revealed the absence of protrusion meanwhile the electrochemical results suggest large peak-to-peak separation (ΔE_p) values close to 1400 mV. This behavior is probably associated to the low presence of CB particles on the printed surfaces occasioning the mentioned above poor electron transfer. SEM images of DMF treated electrodes exhibited morphological alterations and the presence of plastic particulate clusters with diameters ranging from 1 to 3 μm . These voluminous particles formed on the surface can be associated to partial solubilization of the thermoplastic structures. The CVs responses revealed that after DMF treatment, the ΔE_p decreased upon values around 214 mV. On the other hand, the electrochemical treatment with NaOH produces a flat surface with superficial pores but without plastic spheres. The small ΔE_p value of 618 mV obtained shows that the NaOH produces the saponification of PLA promoting the exposure of the CB particles.

CVs experiments were also performed in the presence of a sensitive inner-sphere redox mediator $[\text{Fe}(\text{CN})_6]^{4-/3-}$ (see in Figure S1, available in the electronic supplementary material, ESM). As previously mentioned, pre-treatment methods involving NaOH and DMF reagents are interesting options to expose CB particles incorporated on printed electrodes [46,47]. In this way, the current study combines sequentially chemical and electrochemical treatments planned to degrade the superficial polymeric residues, plastic clusters, and internal PLA structures. The CVs experiments revealed that after the combination of treatments, a noticeable improvement in the reversibility process ($\Delta E_p = 180$ mV) was obtained when compared to non-treated electrodes ($\Delta E_p = 1400$ mV), DMF treated electrodes ($\Delta E_p = 214$ mV), or NaOH treated electrodes ($\Delta E_p = 618$

mV). The improvement of electrochemical behavior can be associated to the large presence of CB sites on the electrodes treated with DMF and NaOH, in agreement with recent studies [34,35,37,38].

<Insert Figure 2>

Raman spectra provide external surface information from carbon microstructures sp^2 and sp^3 [51]. For this reason, the proposed printed surface incorporated with CB structures was exposed to Raman mapping ranged from 1000 to 2000 cm^{-1} . The study was performed considering samples non-treated and treated via electrochemical/chemical methods, and the denoted results are summarized in Figure 3A. The spectra for both samples revealed domains at 1354 and 1589 cm^{-1} attributed to D and G bands, respectively. Based on the target peak intensity from obtained bands was estimated the structural defects (ratio I_D/I_G) of 0.75 and 0.81 were for non-treated and treated surfaces, respectively. These archived results suggest that the proposed surface exhibits high structural defects as expected to CB nanoparticles [39–41]. As it can be denoted in Figure 3A, the responses involving Raman analysis show that the activation treatment did not statistically compromise the microstructures hybridization from CB materials (t-test; $p = 0.05$).

<Insert Figure 3>

Other electrochemical characterizations were also performed employing EIS and CV measurements to investigate the interfacial phenomenon upon electrodes sequentially treated with DMF and NaOH. These experiments were also carried out in the presence of $[Fe(CN)_6]^{4-/3-}$. The obtained EIS results were fitted based on the Randles equivalent circuit obtaining a solution resistance (R_s) of 1100 Ω and a charge transfer resistance (R_{ct}) of 18804 Ω (see Figure S2). These results upon internal electrical resistivity involving the activated electrode could be explained by the enhanced exposure of CB nanoparticles in the thermoplastic binder. Sensing materials with higher conductivity properties reduce the electrostatic repulsions with the redox species at the surface of the electrode, improving the charge transfer [42,43].

The CV experiments were performed at scan rates ranging from 10 to 200 mV s⁻¹. The obtained results demonstrated well-defined anodic (I_{pa}) and cathodic (I_{pc}) peak potential signals, as denoted in Figures 3B and 3C. Considering the CV analysis ($R^2 \geq 0.99$), it was possible indicating that the mass-transfer is diffusion-controlled, as expected for quasi-reversible systems [56]. In this way, the value of $k_s = 0.2 \times 10^{-3} \text{ cm s}^{-1}$ was estimated by the Nicholson method [57]. The estimated value of k_s is similar to electrodes based on the 3D printed cells and it suggests that the proposed integrated 3D printed sensor exhibits adequate electron transfer to perform relevant electrochemical applications as in the sensing field.

3.3 Analytical application

The activated 3D printed electrode was dedicated to perform individual and sequential analysis of molecules E2 and P4. In this way, the redox activities of target hormones were carefully investigated through CV experiments (Figure 3D). These electrochemical measurements were recorded in the presence and absence of 50 $\mu\text{mol L}^{-1}$ E2 and P4 at potentials ranging from -1.7 to 1.0 V and scan rates of 50 mV s⁻¹. The obtained screening indicated typical anodic peak potential at 0.58 V involving oxidation of biomolecule E2 and the cathodic peak potential at -1.55V associated to reduction of hormone P4. The oxidation peak of E2 is attributed to irreversible oxidation of the hydroxyl group present in the aromatic ring to give the corresponding ketone derivative [58], as shown in Fig. S3A. On the other hand, for the electrochemical mechanism of P4, the obtained result can be explained using the mechanism proposed by De Boer et al. [59] and also elucidated by Lima and Spinelli [60]. In an alkaline medium, occurs dimer formation, first of all, one electron reacted with the reduction of the C-3 keto group of P4 and formed an unprotonated radical. Afterwards, the radical undergoes protonation and reacts with another unprotonated radical. The electrochemical mechanism and dimer formation is shown in Figure S3B. Accordingly, E2 and P4 hormones exhibit distinct redox activities and can be detected sequentially. The electrochemical

reactions of E2 and P4 agree with the redox activities values of steroid hormones previously reported in the literature [49,50].

The effect of pH on the electrochemical behavior of both hormones was evaluated using activated 3D printed electrodes. DPV was employed in the presence of $50 \mu\text{mol L}^{-1}$ E2 and P4 prepared in Britton-Robinson buffer at different pH values ranging from 2.0 to 12. Figures S4A and S4B summarize the obtained results involving the oxidation of biomolecule E2, while Figures S4C and S4D exhibit the reduction of hormone P4. Considering the behavior of E2 hormone, the results indicated that the oxidation process exhibit higher positive potential as the pH decrease. The above-mentioned result could be attributed to the higher H_3O^+ concentration in the medium which limited the oxidation of the target molecule, as highlighted in studies reported in the literature [49]. On the other hand, the DPV results associated to P4 hormone indicated that the reduction process occurs at higher negative potentials and low pH values. Considering electrochemical results for both steroid hormones, the peak current (I_p) response and pH value has shown that the best chemical condition for hormones measurements was at pH 10 (Britton-Robinson buffer). Since display redox activities with well-defined peak potentials and pronounced improvement of faradaic performance. Then, the individual and sequential analysis of E2 and P4 hormones were established under ideal electrochemical and chemical conditions. The obtained electrochemical experiments and analytical curves involving the E2 and P4 hormones are summarized in Figure 4.

<Insert Figure 4>

Similar electrochemical behaviors were presented by sequential and individual steroids detection. Oxidation peaks for E2 were observed during anodic potential scanning, at a potential of 0.32 V (Figure 4A) and 0.43 V (Figure 4C), which indicates that the oxidation process exhibits a lower positive potential when this hormone had been detected in the presence of P4. Reduction peaks for P4 (Figure 4 B and 4D) during cathodic potential scanning were close to -1.70 V, also a higher resolution of the P4 peak was evidenced when this hormone was detected without a presence

of E2. As it can be observed in Figures 4A and 4B, for sequential detection, the current values increased linearly when the concentration ranged from 2.5 to 50 $\mu\text{mol L}^{-1}$ ($I(\mu\text{A}) = -0.127 + 0.098786 [\text{E2}], R^2 = 0.979$) for E2, and 12.5 to 75.0 $\mu\text{mol L}^{-1}$ ($I(\mu\text{A}) = 0.107 - 0.039781 [\text{P4}], R^2 = 0.997$) for P4. The achieved sensitivity values for E2 and P4 based on the slopes of calibration curves were 0.099 $\mu\text{A}/[\mu\text{mol L}^{-1}]$ and 0.040 $\mu\text{A}/[\mu\text{mol L}^{-1}]$, respectively. When the detection of E2 and P4 was performed separately, the linear regressions are $I(\mu\text{A}) = -0.06455 + 0.051\mu\text{A} [\text{E2}]$ in the range from 2.5 to 75 $\mu\text{mol L}^{-1}$ and $I(\mu\text{A}) = -0.497 - 0.0339 \mu\text{A} [\text{P4}]$ in the range from 50.0 to 150.0 $\mu\text{mol L}^{-1}$, showing a higher range linear detection for both steroids. The limits of detection (LOD) were estimated using the ratio between three times the standard deviation for the blank signal and the analytical sensitivity (slope of analytical curve). The found values are listed in Table 1 and they are compared to other works reported in the literature for the same target analytes using electrochemical sensors.

<Insert Table 1>

As summarized in Table 1, it can be seen that the developed 3D-electrodes exhibited a simple method in comparison with other reported approaches. Simultaneous detection of E2 and P4 was reported by Arvand et al. with low LOD of 0.23 and 0.31 nmol L^{-1} [53]. In their work, both hormones were detected using a conventional electrochemical cell and the working electrode was modified with graphene quantum dots and poly(sulfosalicylic acid). The sensor proposed here is not able to detect concentrations of E2 and P4 as low as those reported by Arvand et al. However, the 3D printed electrochemical cell and the methodology proposed here offer important advantages over the current electrochemical sensors for hormones detection such as the possibility to perform a sequential determination of two hormones dispensing the use of biomolecules (antibodies, aptamers, enzymes) and the need for laborious steps like nanomaterials synthesis (graphene, nanotubes, gold nanoparticles, and others), combination of them or device surface modification for incorporation of these materials, fast and low-cost fabrication, portability and low consumption of reagents in the electrochemical measurement (100 μL). For this study, reproducibility and

repeatability of the electrochemical devices were evaluated using pulse differential with $20 \mu\text{mol L}^{-1}$ E2. The devices indicated good reproducibility and repeatability (Figure S5) which were expressed as RSD values for seven different fabricated electrodes (RSD = 10.7%, $n = 7$) and three measurements for the same electrode (RSD = 3.1%, $n = 3$), respectively.

The electrochemical devices were evaluated for application in synthetic urine. In this way, the sample was diluted to 1:1 (v/v) in BR buffer solution (pH = 10.0) before analysis. Addition and recovery tests (shown in Table 2 and Figure S6) were performed through external calibration of the sample spiked with 25 and $100 \mu\text{mol L}^{-1}$ progesterone with a recovery of 84 and 98%, respectively ($n = 3$ replicates). Also, the proposed electrode was applied for 5 and $25 \mu\text{mol L}^{-1}$ E2, which has shown recovery of 80 and 96%, respectively. The results demonstrated not only the accuracy of the proposed method but also the potential tool to detect both hormones in healthcare analysis.

<Insert Table 2>

4 Conclusions

The combination of conductive (PLA-CB) and non-conductive (ABS) materials associated with 3D printing technology has allowed the fabrication of a miniaturized electrochemical cell with a complex geometry that integrates the sample reservoir and the three electrodes. The electrochemical cell was manufactured in 12 min in a single step, fully automated, and at a low cost (US\$ 0.08 per device) which would not be possible without the aid of multi-material 3D printing. It is worth noting that due to the small size of the proposed electrochemical cell and the practicality of multi-material 3D printing technology it was possible to achieve a scalable manufacturing process allowing the production of dozens of devices simultaneously. A simple chemical and electrochemical process promoted the degradation of polymeric residues incorporated on the surface of the printed electrode, allowing a considerable increase in its electrochemical properties. The treated 3D printed sensor has been successfully employed for the sequential detection of E2 and P4 in artificial urine samples achieving a LOD of 0.11 and $17.8 \mu\text{mol L}^{-1}$ in a linear range of

2.5 to 50 $\mu\text{mol L}^{-1}$ and 12.5 to 75.0 $\mu\text{mol L}^{-1}$, respectively. Also, the 3D printed electrochemical cell showed good repeatability and reproducibility. Thus, based on the results demonstrated here, we believe that the proposed device has great potential to be used as an important new analytical tool in clinical health studies capable of aiding in diagnostics by monitoring steroid hormones, as well as other biomolecules, in biological fluids such as urine. In addition, future research directions should address the limitations of sensitivity in hormone determination through the synthesis of new printable materials or even through device surface modification suitable for the target analyte.

Author contributions

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript. The authors declare no competing financial interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

The following is Supplementary data to this article:

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Figure Captions

Figure 1. Projected design of the electrochemical cell containing working electrode (WE), auxiliary electrode (AE), and pseudo-reference electrode (RE). The electrodes were printed using the PLA-CB conductive filament and the insulating region was manufactured with ABS.

Figure 2. (A) 3D printed miniaturized electrochemical cell image. SEM images obtained with the proposed electrode surface before and after the activation treatment. (B) Non-activated surface, (C) activated surface with DMF, (D) activated surface with NaOH and (E) activated surface using the strategy DMF following by saponification method.

Figure 3. (A) Raman spectra associated to 3D printed surface before and after the combined activation treatment; (B) Cyclic voltammograms recorded in the presence of 1.0 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{4-/3-}$ prepared in 0.1 mol L^{-1} KCl with scanning rates ranging from 10 to 200 mV s^{-1} using the activated electrode (C) Plots peak current signals vs square root of scan rate considering electrochemical experiments using the activated electrode in the presence of 1.0 mmol L^{-1} $[\text{Fe}(\text{CN})_6]^{4-/3-}$ prepared in 0.1 mol L^{-1} KCl and (D) Cyclic voltammograms of 3D printed electrodes in 0.1 mol L^{-1} Britton-Robinson buffer solution (pH = 10.0) at 50 mV s^{-1} with potentials ranging from -1.7 to 1.0 V. Black and blue lines represent absence and presence of $50 \text{ }\mu\text{mol L}^{-1}$ E2 and P4 hormones, respectively.

Figure 4. DPV voltammograms for (A) E2 and (B) P4 sequential detection; (C) E2 and (D) P4 individual detection in different concentrations. Inset of analytical curves.

List of Tables

Table 1. Comparison among the proposed sensor and other electrochemical platforms dedicated to the detection of estradiol and progesterone.

| Hormone | Electrode material | Electrode modification | LOD $\mu\text{mol L}^{-1}$ | References |
|---------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------|----------------------------|------------|
| | Glassy carbon | Carbon black | 0.092 | [45] |
| | Boron-doped polycrystalline diamond | Carbon black nanoparticles | 0.0021 | [43] |
| | Laser-scribed electrodes | Electrochemical treatment with 1.0 mol L ⁻¹ KCl | 0.094 | [61] |
| β - Estradiol | 3D printed electrodes (individual detection) | Chemical treatment with DMF and Electrochemical treatment with 0.5 mol L ⁻¹ NaOH | 0.31 | This work |
| | 3D- printed electrodes (sequential detection) | Chemical treatment with DMF and Electrochemical treatment with 0.5 mol L ⁻¹ NaOH | 0.11 | This work |
| Progesterone | Glassy carbon | Sn nanorods. | 0.12 | [62] |

| | | | |
|-----------------------------------------------|---------------------------------------------------------------------------------------------|--------|-----------|
| Glassy carbon | Fe ₃ O ₄ @GQD(*)/f-MWCNTs* | 0.0022 | [64] |
| Glassy carbon | GO-IMZ | 0.068 | [65] |
| 3D- printed electrodes (individual detection) | Chemical treatment with DMF and Electrochemical treatment with 0.5 mol L ⁻¹ NaOH | 4.18 | This work |
| 3D- printed electrodes (sequential detection) | Chemical treatment with DMF and Electrochemical treatment with 0.5 mol L ⁻¹ NaOH | 17.8 | This work |

Table 2. Results of β -estradiol and progesterone determination in simulated urine samples by DPV measurements.

| [PRO] added/ $\mu\text{mol L}^{-1}$ | [PRO] found/ $\mu\text{mol L}^{-1}$ | % Recovery | [EST] added/ $\mu\text{mol L}^{-1}$ | [EST] found/ $\mu\text{mol L}^{-1}$ | % Recovery |
|----------------------------------------|----------------------------------------|---------------|----------------------------------------|----------------------------------------|---------------|
| 25 | 21 \pm 1 | 84 | 5 | 4 \pm 1 | 80 |
| 100 | 98 \pm 4 | 98 | 25 | 24 \pm 1 | 96 |

Biographies

Lucas C. Duarte received his graduated in Chemistry from the Federal University of Goiás (UFG), Goiânia, Brazil (2013) and M.Sc. (2016) in Chemistry at the same University. In 2016– 2020, he completed his Ph.D. at UFG. He is currently a postdoctoral at UFG. His project focuses on the development of microfluidic devices and electrochemical sensors employed 3D-printing method for bioanalytical and environmental applications. Thaisa Aparecida Baldo is graduated in Chemistry (2011) and received MSc (2014) from the São Paulo State University. She received her Ph.D. (2019) in Analytical Chemistry from the Federal University of São Carlos. Currently, she is a postdoc at the Federal University of Goiás under the supervision of Prof. Dr. Wendell Coltro. Her project focuses on the development of electrochemical and colorimetric devices using simple and affordable tools for disease diagnosis. Habdias A. Silva-Neto received his B.Sc. (2017) and M.Sc. (2020) in Chemistry at the Federal University of Goiás. Currently, he is a first-year Ph.D. student in Analytical Chemistry at the same University. His main research interests involve the development of electrochemical sensors, 3D printing electrodes, wearable sensors for bioanalytical, forensic and

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Lucas C. Duarte: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft.

Thaisa A. Baldo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft.

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Federico Figueredo: Conceptualization, Writing- Reviewing and Editing.

Bruno C. Janegitz: Conceptualization, Supervision, Writing- Reviewing and Editing.

Wendell K. T. Coltro: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing- Reviewing and Editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights •

- A miniaturized electrochemical cell was entirely manufactured by 3D printing.
 - Automated combination between ABS and PLA - Carbon Black allowed the fabrication of a multi-material electrochemical cell.
 - Chemical and electrochemical treatments were carried out to improve the analytical performance of the printed sensor
- • Pulse voltammetry was used for detection of steroid hormones.
 - Individual and sequential measurements of estradiol and progesterone in artificial urine has been reported with success

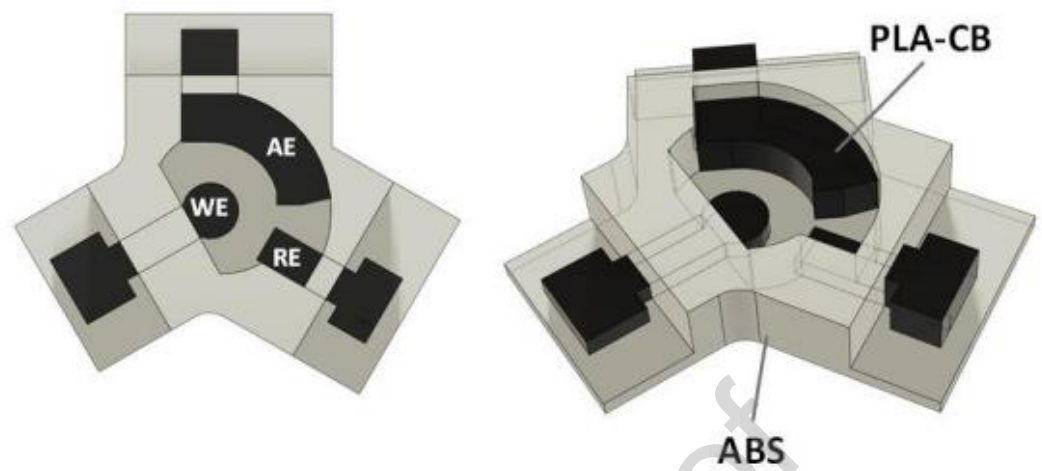


Fig 1

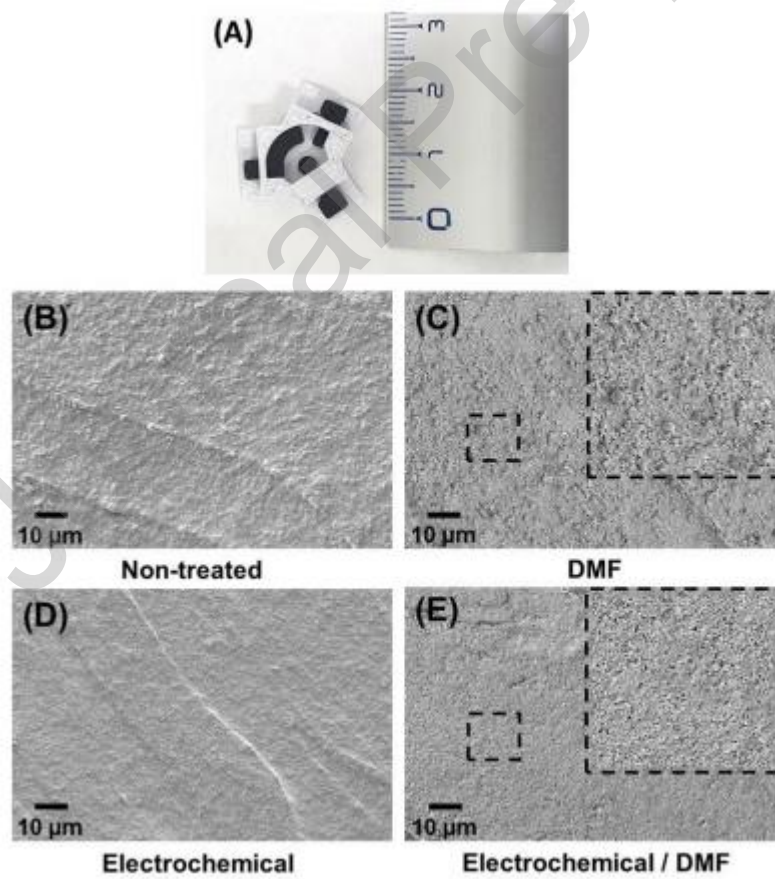


Fig 2

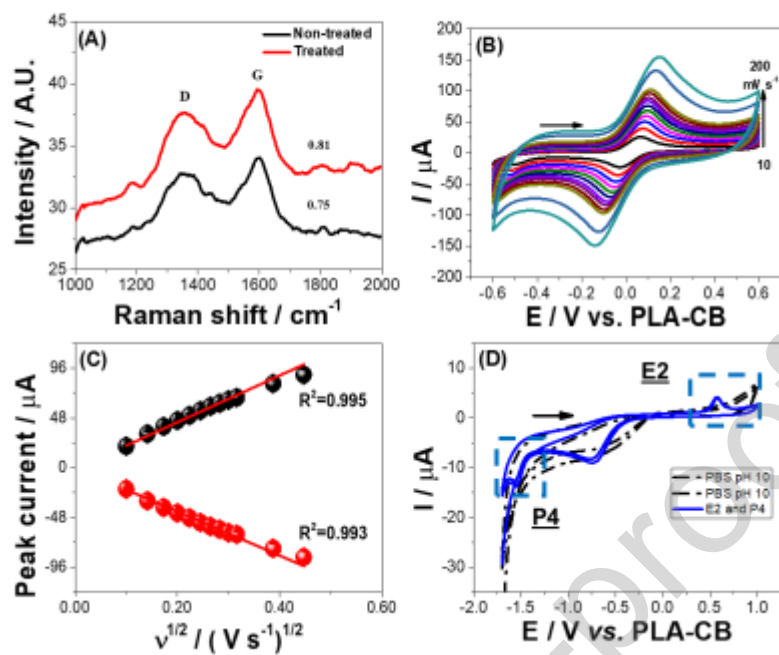


Fig 3

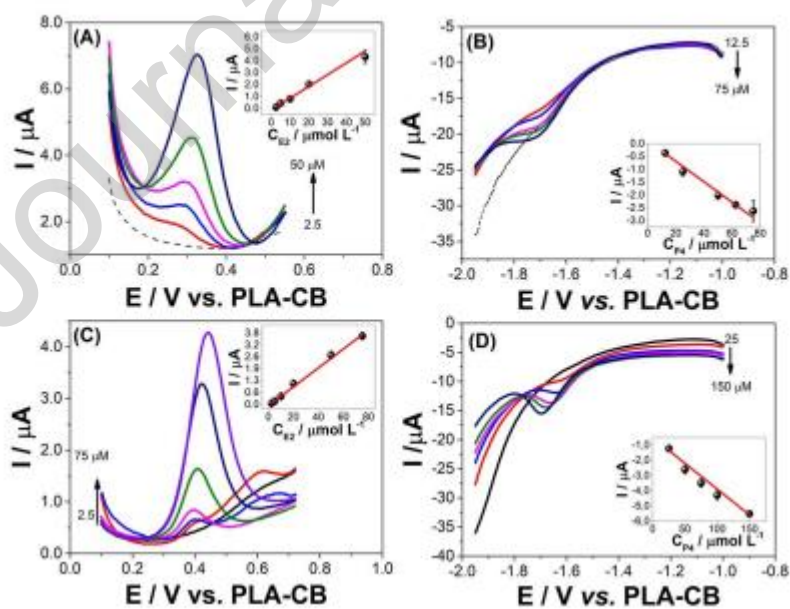
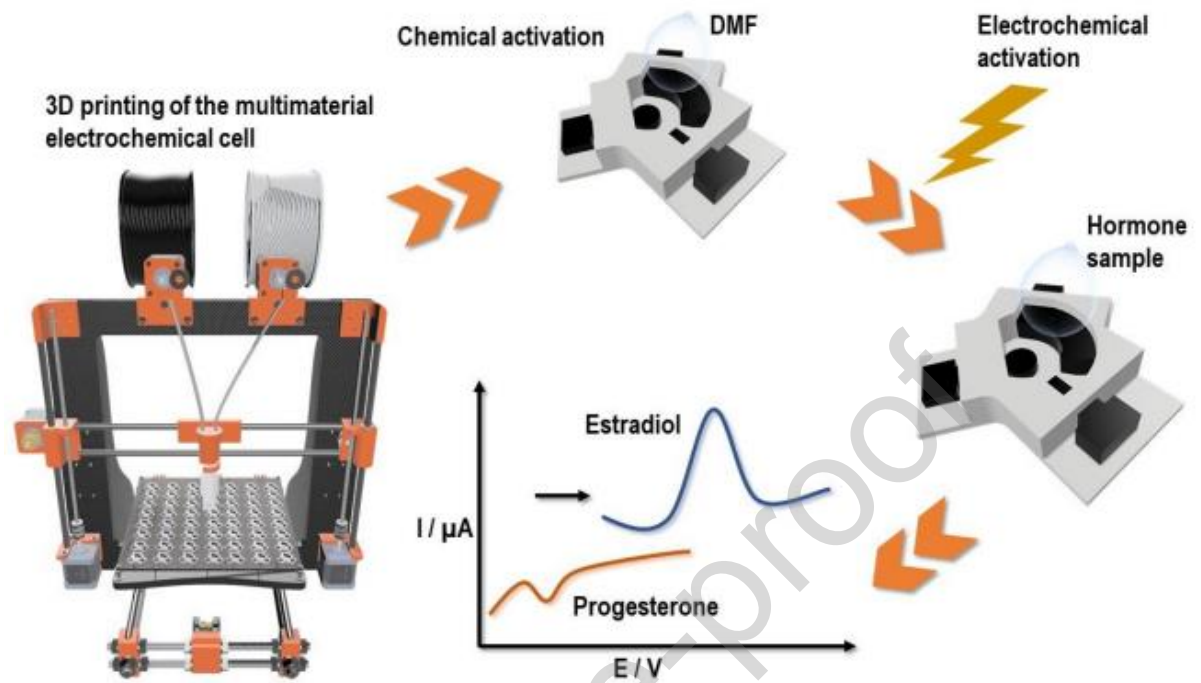


Fig 4



Graphical abstract