Electrochemical lactate biosensor based upon chitosan/carbon nanotubes modified screen-printed graphite electrodes for the determination of lactate in embryonic cell cultures

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Abstract.

L-lactate is an essential metabolite present in embryonic cell culture. Changes of this important metabolite during the growth of human embryo reflect the quality and viability of the embryo. In this study, we report a sensitive, stable, and easily manufactured electrochemical biosensor for the detection of lactate within embryonic cell cultures media. Screen-printed disposable electrodes are used as electrochemical sensing platforms for the miniaturization of the lactate biosensor. Multi-walled carbon nanotubes/Chitosan composite have been employed for the enzymatic immobilization of the lactate oxidase enzyme.

This novel electrochemical lactate biosensors analytical efficacy is explored towards the sensing of lactate in model (buffer) solutions and is found to exhibit a linear response towards lactate over the concentration range of 30.4 and 243.9 μM in phosphate buffer solution, with a corresponding limit of detection (based on 3-sigma) of 22.6 μM and exhibits a sensitivity of 3417 ± 131 μA M^{-1} according to the reproducibility study. These novel electrochemical lactate biosensors exhibit a high reproducibility, with a relative standard deviation of less than 3.8 % and an enzymatic response over 82 % after 5 months stored at 4 ºC. Furthermore, high performance liquid chromatography technique has been utilized to independently validate the electrochemical lactate biosensor for the determination of lactate in a commercial embryonic cell culture medium providing excellent agreement between the two analytical protocols.

Keywords: Chitosan, MWCNT, screen-printed graphite electrode, lactate oxidase, lactate, embryo cell culture medium

1. Introduction

Metabolomics changes within cell culture media *in vitro* human reproduction after the retrieval of the human embryo or during its development, the uptake or formation of new metabolites may reflect the quality and thus the viability of the embryo to be transferred . L-lactate is an essential metabolite present in a wide number of cell culture medium used in the development and growth of human embryonic cells in vitro under advanced reproduction techniques. Moreover, lactate is vital during the first days of the embryo development therefore the tracking of its concentration can be utilized as a tremendous biomarker upon adequate cellular proliferation . Thus, the *in-situ* and real time monitoring of lactate allows the embryologist to have additional and complementary data for the selection assessment of the embryo.

High performance liquid chromatography (HPLC) , mass spectrometry (MS) or nuclear magnetic resonance (NMR) , are well established techniques employed for the detection and quantification of lactate present in human embryo culture cells and generally biological systems; however, such techniques are costly and possess the inability for real-time or *in-situ* measurements. To overcome these limitations, researchers continually strive to source analytical techniques that can be utilized within clinical applications that focus upon the human reproduction system. In this regard, (bio)sensors based upon screen-printed platforms are an extremely promising and alternative method that allow for real-time, *in/ex-situ* monitoring of the metabolites present within cell cultures. Additionally such platforms allow for the creation of a low cost, robust, highly reproducible and sensitive, that can be utilized as non-intrusive point of care sensors, due to the possibility of handling small sample volumes (between 25 and 50 microliters). Moreover, screen-printed

electrodes (SPEs) are appropriate platforms for the immobilization of biomolecules, *e.g.* nucleic acid , enzymes or antibodies onto the underlying working electrode surface in order to obtain a sensitive, selective, disposable electrochemical biosensor. SPE platforms are mostly based on carbon materials in which the nature, structural and physic-chemical properties of the carbonaceous materials have paid significant attention to the performance of the electrochemical (bio)sensors. In this regard, carbon materials are being utilized for biological electrochemical sensing, *e.g.* carbon nanotube , graphene and nanoporous carbons to just name a few examples since the increase in the electrode active area is reported to enhance the sensitivity, selectivity and improvement of biomolecules immobilization and electron transfer.

More specifically, multi-walled carbon nanotubes (MWCNTs) show a large number of advantages in miniaturized electrochemical platforms due to their unique properties such as high conductivity, large surface area, easy chemically modified surface by adding a wide number of functional groups, biocompatibility, fouling resistance and high electrocatalytic activity. Furthermore, the enhanced electrocatalytic electron transfer can be promoted by decorating the MWCNT with an ample variety of nano-particulate metals, leading either to a huge number of hybrid nanomaterials composites or hybrid nanomaterials with enzymes . Moreover, some authors combine nanomaterials with conducting polymers , where the hybrid material present special properties because of the synergic effect from the individual components. The polymer allows enzyme immobilization and connects the nanomaterial, whereas the nanomaterial interacts with the polymer film achieving aggregates, which are able to reduce ions interleaving distance, which improves charge transfer and increases the polymeric film conductivity. By following the above approaches, Pérez and Fábregas examined the combination of

MWCNTs and polysulfone polymer for the immobilization of the enzyme lactate oxidase (LOx) to produce a lactate electrochemical biosensor for the determination of lactate in wine and beer samples with a good sensitivity and concentration linear range . Nonetheless, there are some limitations relative to the biosensor stability due presumably to the deterioration of enzyme catalytic activity. Alternatively, the use of biopolymers for the immobilization of the LOx can be beneficial, thereby enhancing the enzymatic stability. In this regard, the natural polymer chitosan (CS) is a polysaccharide mainly obtained from the crustacean shells, with a low cost, eco-friendly and biocompatible polymer what make it a suitable and therefore an interesting material for a wide range of applications especially in biomedical, food, biotechnology and pharmaceutical fields. It has already demonstrated how the biocompatibility property of CS guarantees the improvement of the limit of detection and enzyme stability . Several authors mixed CS with MWCNTs to enhance electrochemical behavior of electrochemical biosensors regarding electrical conductivity and electrocatalytic activity properties

Encouraged by the improvement of the stability, reproducibility and repeatability of the lactate electrochemical biosensor, this study depicts the manufacture of a bio-enzymatic biosensor using both Horseradish Peroxidase (HRP) and the enzyme Lactate oxidase LOx together with a redox mediator (ferrocene methanol) for the determination of lactate, according to similar strategy described in the literature . Our bio-enzymatic system has been dropped cast onto a MWCNT modified screen-printed graphite electrode surface with the full optimization of the lactate electrochemical biosensor manufacture been undertaken and fully described. Moreover, in this work, the electrochemical lactate biosensor has been partially validated in terms of sensitivity, linear range of lactate concentration, limit of detection and quantification, repeatability, reproducibility, accuracy and the presence of

interferences in order to perform a simpler faster, and more manageable lactate biosensor for the determination of lactate in real time or in-vitro in complex embryonic cell culture media containing glucose, carbohydrates, organic acids such as pyruvic and lactic acid and the majority of amino acids, which have been independently validated by HPLC.

2. Material and methods

2.1. Reagents and chemicals

All solutions were prepared with double deionized water of resistivity not less than 18.2 MΩ cm. Lactate oxidase (LOx) was purchased from pediococcus sp (Sigma Aldrich, Spain, lyophilized powder, activity ≥ 20 units mg^{-1}, 100 units); ferrocene methanol FcMe from Sigma Aldrich ≥ 97 %, Spain; multi-walled carbon nanotubes (MWCNT) functionalized with carboxylic groups from DropSens, Spain; Bovine Serum Albumin, BSA (purity ≥ 98 %) from Sigma Aldrich, Spain; Horse Radish Peroxidase HRP (~150 units mg^{-1}) from Sigma Aldrich, Spain; Chitosan (CS) low molecular weight from Sigma Aldrich, Spain; Sodium L-lactate (≥ 99 % from Fluka, Germany; 4-aminoantipyrine (purity ≥ 99 %) from Sigma Aldrich, Spain, cell culture medium of human embryos from the pronucleate stage to day 2 and day 3 (G1), G1-plus, similar to G1 medium but with the presence of Human Serum Albumin (HSA) and a handling and manipulating embryo solution (G-MOPS) were obtained from Vitrolife. Unless otherwise stated, electrochemical experiments were performed in 0.1 M potassium phosphate buffer solution (PBS) at pH 7.4. All other chemicals were obtained from the highest analytical grade.

2.2. Activity assay of Lactate Oxidase enzyme

Enzyme activity of LOx decays quickly when removed from its natural matrix , therefore it is necessary to store it correctly, and for that reason periodical enzymatic activity measurements were carried out. 100 units (U) of LOx were dissolved in 0.1 M PBS and separated in 50 eppendorfs of 20 µL each (2 U) and stored at -20 ºC . This procedure was performed under aseptic conditions. The oxidation activity of LOx was determined periodically by a chromogenic assay . UV-Vis spectrophotometer (UV probe 2.21 Shimadzu) was employed for the determination of LOx activity assays.

2.3. Preparation of the electrochemical lactate biosensor MWCNTs/FcMe/CS/ HRP/BSA/LOx/SPBGE biosensor.

The basal-plane like screen-printed graphite electrodes (SPBGE) were fabricated at Manchester Metropolitan University utilizing appropriate stencil designs using a microDEK 1760RS screen-printing machine (DEK, Weymouth, UK). For each of the screen-printed sensors a carbon–graphite ink formulation (Product Code: ED5020, Electra Polymers Ltd, UK) was first screen-printed onto a polyester flexible film (Autostat, 250 µm thickness). [24,25]. This layer was cured in a fan oven at 60 degrees Celsius for 30 min. Next a silver/silver chloride (40:60) reference electrode was applied by screen-printing Ag/AgCl paste (Product Code: C2040308P2; Gwent Electronic Materials Ltd, UK) onto the plastic substrate. This layer was once more cured in a fan oven at 60 degrees Celsius for 30 min. Last a dielectric paste ink (Product Code: D2070423P5; Gwent Electronic Materials Ltd, UK) was printed to cover the connections and define the 3 mm diameter graphite working electrode. After curing at 60 degrees Celsius for 30 min the screen-printed electrode is ready to use. An edge-connector was used to ensure the reproducibility of the electrochemical connections throughout the studies .

For the preparation of the biosensor we have followed a approach where the enzyme immobilization technique consists in the phase-inversion . Phase-inversion method is a process where the liquid state polymer becomes solid state in a controlled manner. In our case the biopolymer chitosan is dissolved in an organic solution 1:1 ethanol / dimethylformamide and the aqueous solution displaces the organic solution, then the biopolymer precipitates and finally a porous membrane is formed which is appropriate for the immobilization of LOx enzyme. The manufacture for the preparation of the biosensor comprised the following steps: 2.5 mg MWCNTs, 12.5 mg FcMe and 5.25 mg of CS were mixed in a 500 μL Dimedthylformamide (DM)F/ethanol (EtOH) (1:3 v:v) organic solution and sonicated for 1 hour (as shown in the Electronics supporting information Figure ESI-1, step I). Then, 0.6 μL of the above mixture was dropped cast onto the graphitic working electrode from the SPBGE platform (as shown in Figure ESI-1 step III) and then 5 μL volume from an enzymatic solution made of 0.5 mg HRP and 0.5 mg BSA dissolved in a 20 μL aliquot enzymatic solution comprised LOx in 0.1 M PBS solution pH 7.4 (as shown in Figure ESI-1, step II). Then, 5 μL volume of the above enzymatic solution were immediately dropped cast upon the still wet MWCNTs/FcMe/CS/DMF-EtOH paste composite (as shown in Figure ESI-1, step IV). The above procedure was designed for the performance of four equivalent electrochemical lactate biosensors. Finally, electrochemical lactate biosensors were dried under ultra-high vacuum conditions for 15 minutes at ambient temperature and then stored at 2-4 ºC without the need of any specific protection. Prior to use, the biosensor is thoroughly washed immersing the electrochemical biosensor in 0.1 M PBS buffer solution pH 7.4 under stirring conditions for 5 minutes.

2.4. SEM, electrochemical and HPLC instrumentation.

Scanning electron microscope (SEM) from Hitachi S3000N with an X-ray detector Bruker XFlash 3001 for microanalysis (EDX) and mapping was used for the morphological characterization of the biosensor film.

Cyclic voltammetry (CV) and chronoamperometry (CA) experiments were carried out using an Autolab PGSTAT X (Eco Chemie, the Netherlands) potenciostat/galvanostat and controlled by Autolab GPES software version 4.9 for windows XP. CV and CA experiments were carried out immersing the biosensor in a cell containing 5 mL of 0.1 M PBS buffer solution at pH 7.4 under continuous gentle stirring. Prior to CA measurements, the electrochemical lactate biosensor was subjected to -0.2 V vs the pseudo reference electrode for 120 s in 0.1 M PBS solution pH 7.4 and then consecutive aliquots of 10 mM L-lactate solution were performed in order to obtain certain lactate concentration in solution. CV experiments were carried out by cycling the working electrode between -0.1 to -0.4 V at a scan rate of 10 mV s^{-1}. Under gentle stirring conditions aliquots from 10 mM L-lactate solution were also added consecutively into the cell containing 0.1 M PBS pH 7.4. All electrochemical experiments were carried out at 22 ± 2 ºC under aerated conditions.

Lactate concentration present in a G1, G1 Plus and G-MOPS cell culture media were determined by HPLC (Agilent 1100 series, Santa Clara, USA) coupled with an UV-Vis detector. The mobile phase consisted of 20 mmol·L^{-1} NaH_2PO_4 aqueous solution adjusted to pH 2.5 with H_3PO_4. The column was a C18 Hypersil octadecylsilane(ODS). 4.0 internal diameter x 250 mm length, 5 μm particle diameter. The flow rate was 0.5 mL min^{-1} with a wavelength of 210 nm . Appropriate dilutions of the different cell culture media in 0.1 M PBS pH 7.4 were performed for the quantification of lactate by either the use of the electrochemical lactate biosensor or by the liquid chromatography technique. Alternatively, a certain aliquot of thedifferent cell culture medium was added into a 5.0 mL 0.1 M PBS

pH 7.4, and then an electrochemical lactate biosensor is immersed into the solution under a gentle stirring. Chronoamperommetric experiments were carried out by monitoring the current intensity versus time keeping the working electrode at a potential of -0.2 V versus the pseudo reference electrode of the SPE platform.

3. Results and discussion

3.1 SEM characterization and electrochemical response of the lactate biosensor.

depicts the SEM images of the basal plane like SPBGE surface unmodified, prior to the drop cast of the MWCNTs/FcMe/CS/ HRP/BSA/LOx composite (Figure 1a) and the morphology regarding the MWCNT/FcMe/CS/HRP/BSA/LOx/SPBGE biosensor (Figure 1b). The SEM image of the SPBGE reveals a homogeneous, smooth surface adequate to carry out an uniform film of the MWCNTs/FcMe/CS/HRP/BSA/LOx composite. The surface of the SPBGE platform provides sufficient chemical stability in contact with MWCNT composite mixed within a DMF/EtOH solution.

Figure 1b shows a MWCNT network immobilized upon the working graphitic surface of the SPBGE platform. MWCNTs seem to be well dispersed upon the underlying surface of the SPBGE platform, which is completely covered by the film formed by MWCNTFcMe/CS/HRP/BSA/LOx composite. The CS biopolymer acts as a binder to fix the MWCNTs and enzyme onto the basal like surface of the SPBGE platform. The films comprising MWCNTFcMe/CS/HRP/BSA/LOx composite do not appear to exhibit any cracks or fractures and are quite homogeneous suggesting a good mechanical stability and robustness upon manipulation.

The electrochemical lactate biosensor performed in this study works in accordance to the following reactions 1-4 displayed below, according to the literature :

$$L - lactate + O_2 \xrightarrow{LOx} pyruvate + H_2O_2 \quad (0)$$

$$H_2O_2 + (HRP)_{red} \rightarrow H_2O + (HRP)_{ox} \quad (0)$$

$$(HRP)_{ox} + (FcMe)_{red} \rightarrow (HRP)_{red} + (FcMe)_{ox} \quad (0)$$

$$(FcMe)_{ox} + e^- \rightarrow (FcMe)_{red} \quad (0)$$

Briefly, the enzyme lactate oxidase (LOx) reacts with the target analyte, lactate, in the presence of oxygen leading to pyruvate and H_2O_2 (reaction 1). Then the enzyme HRP (in its reduced state) reduces H_2O_2 to H_2O (as depicted in reaction 2), and then the enzyme HRP in its oxidized form oxidizes the redox mediator FcMe to the ferrocinium complex, according to reaction 3. Finally, the FcMe complex in its oxidized state is electrochemically reduced upon the electrode surface in accordance to reaction 4.

Cyclic voltammetry measurements were next performed in order to study the optimal working potential for the electrochemical reduction of the FcMe complex in its oxidized state. In this regard, Figure 2 depicts the voltammetric profiles of lactate biosensor in the presence and absence of CS biopolymer inside the enzymatic composite matrix. The corresponding voltammetric peaks of the oxidation and reduction of FcMe are readily observed when the biosensor is manufactured in the absence of CS giving a peak potential separation of 140 mV at a scan rate of 10 mV s^{-1}. However, upon the introduction of the natural biopolymer CS, the cyclic voltammetric measurements give rise to an undefined oxidation peak, though the reduction wave is well established at a peak potential of *ca*. -150 mV versus the *pseudo*-reference electrode. This is explained as the incorporation of CS biopolymer into the enzymatic composite matrix leads to a more resistive film, due to the low ionic conductivity of the CS biopolymer . Strikingly, the presence of CS leads to a higher current intensity or charge passed within the reduction peak of FcMe compared to

the electrochemical biosensor performed without the presence of CS, which clearly indicates that the addition of chitosan improves the adsorption of the FcMe mediator, upon the electrode carbonaceous surface or the entrapment into the biopolymer / carbon nanotubes matrix. Moreover, the presence of CS exposes edge plane sites of the MWCNTs, thereby resulting in an enhancement of electron transfer and thus in electrochemical activity.

The working potential of the lactate biosensor was set at ca. -0.2 V based on the cyclic voltammetric results presented in Figure 2 with the electrochemical response of the lactate biosensor explored in the presence of L-lactate through the use of hydrodynamic cyclic voltammetry and chronoamperometry techniques. Figure 3 shows the cyclic voltammetry response for the electrochemical lactate biosensor and the corresponding calibration plot of current intensity versus lactate concentration over a concentration range of 99-476 μM (see inset in Figure 3. The voltammetry reveals an increment of current intensity with lactate concentration. The calibration plot was obtained measuring the current intensity at -0.2 V. In this regard, the biosensor presents a linear slope with a correlation coefficient of 0.99 and a sensitivity of -3503 ± 243 μA mM^{-1}.

Chronoamperometric response of the electrochemical lactate biosensor was next studied at -0.2 V, as shown in Figure 4. It can be observed that as the addition of 25 μL of 10 mM lactate within 0.1 M PBS pH 7.4 provides an increase within the current intensity and well-shaped amperometric current steps are readily visible after each addition. Inset of Figure 4 shows the calibration plot regarding the amperometric response of the electrochemical lactate biosensor with a concentration range of 50-250 μM of lactate in which a linear slope is obtained with a correlation coefficient of 0.99 and a sensitivity of

-3201 ± 179 μA mM^{-1}. Our results demonstrated that there are no significant differences between both electrochemical techniques employed.

3.2. Reproducibility, repeatability and long-term stability of the electrochemical lactate biosensor.

The reproducibility of the electrochemical lactate biosensor is examined using eight biosensors. Figure 5 shows a linear calibration curve of the average current intensity versus the lactate concentration over the range of 30.4 and 243.9 μM. Such response exhibited a sensitivity of 3417 ± 131 μA M^{-1} (n=8)–a similar value to that obtained from figure 3- with a Relative Standard Deviation RSD of 3.8 % and a Limit of Detection (LOD; 3-sigma) of 22.6 μM. It is worth noting that from our reproducibility assessment of the electrochemical lactate biosensor, we find that the preparation method of our electrochemical biosensor is reproducible regarding MWCNTs drop casting and LOx enzyme immobilization onto the graphitic surface of the SPBGE. Hence the electron transfer and lactate biosensor activity behave very similar, leading to a good performance in terms of accuracy and precision of the electrochemical device. Thus, the repeatability of the electrochemical lactate biosensor is also studied by means of a consecutive test of the biosensor for a known lactate concentration solution in pH 7.4 0.1 M PBS to examine reutilization of the electrochemical biosensor. After seven consecutive measurements, the biosensor can determine lactate in solution with a RSD of less than 5 %.

The long-term stability of the electrochemical lactate biosensors were addressed for five months by keeping the biosensors in the fridge at 4 ºC without any protection of the enzymatic composite film. Sixteen electrochemical lactate biosensors were fabricated and stored at 4 ºC and then lactate calibration plots (n=4) were recorded at days 2, 30, 62 and

150 after their fabrication. Our findings show that sensitivities obtained from the average calibration plots remains inside range of control limits (±3 x standard deviation from the slope value obtained on the first day) with an enzymatic response higher than 82 % after 150 days under our storage conditions.

Table 1 shows analytical data of different LOx biosensors based on screen-printed carbon electrodes found in the literature. It is worthwhile noting that our electrochemical lactate biosensor shows sensitivity higher than the majority of biosensors presented within Table 1 . Even though the LOD value of our electrochemical lactate biosensor is clearly of the order of some electrochemical biosensors based on the use of screen-printed electrodes, as shown in Table 1, LOD values are higher than compared to non-electrochemical approaches, see for example Minami et al who developed an organic field effect transistor. However, in their work, no real samples were explored limiting their work and sensor application. It is important to note that thelactate concentration within the culture media is among 10-15 mM, so for practical sensor applications, the requirement of a super low LOD value is not a limitation since our novel electrochemical lactate biosensor has the advantage of high stability, robustness and low cost effective production.

In the case of the long-term stability our electrochemical biosensor also offers excellent performances, better than others reported . Even when our electrochemical lactate biosensors are only stored at 4 ºC without any specific protection against *(i.e.* humidity or oxygen atmosphere) in contrast to other electrochemical lactate biosensors which needed the use of protected membrane films or preservation using a aqueous buffer solution. Finally, our reproducibility values of the electrochemical lactate biosensor are in the range reported for biosensors used for food and clinical applications.

3.3. Interference study.

An interferences study is carried out in order to ensure its applicability to real samples. Different substances present in embryonic cell culture such as glucose, pyruvate and Bovine Serum Albumin (BSA) are checked out by chronoamperometric measurements in a pH 7.4 0.1 M PBS buffer solution at a controlled potential of -0.2 V. Different amounts of the above substances are added successively, as shown in Figure ESI-2, and only the addition of lactate into the buffer solution leads to an increase in current intensity. Therefore, the presence of the different substances, even the presence of a large protein like BSA, does not modify the correct performance and selectivity of the electrochemical biosensor.

3.4. Determination of lactate in commercial embryonic cell culture.

The electrochemical lactate biosensor is employed to the determination of lactate present in an commercial embryonic culture medium from the pronucleate stage to day 2 day 3 (G1). Lactate quantification from the commercial sample is measured by chronoamperometric technique by diluting the G1 sample in a factor of 1:80 using a pH 7.4 0.1 M PBS. No matrix effects are observed and the value of the lactate concentration for the medium G1 is obtained by interpolation into calibration curve of the corresponding amperometric signals. Our results provide an average value of 11.8 ± 1.7 mM of lactate over four different electrochemical biosensors. In order to validate the new proposed methodology, the G1 medium was also analyzed by independent HPLC-UV according to the procedure mentioned in experimental section. Such liquid chromatographic methodology revealed a lactate concentration of 11.94 ± 0.10 mM for three repeats. To clearly demonstrate lactate analysis using our electrochemical lactate biosensor, lactate concentration in a different cell

culture medium, called G-MOPS, was performed. G-MOPS is designed to handling and manipulating of oocytes and embryos outside the incubator. G-MPS consists of amino acids, organic acids and antibiotics, according to the supplier. Lactate quantification within the sample G-MOPS was 10.34 ± 2.3 mM (n=3), whereas the liquid chromatography method revealed a lactate concentration of 9.82 ± 0.09 mM (n=3). "Figure ESI-3 depicts the calibration curve regarding current intensity versus lactate concentration present in sample G-MOPS after five successive additions (25 μL each). On the other hand, lactate concentration was also determined in the cell culture medium called G1-plus, a similar medium to G1 but with the presence of a estimated Human Serum Albumin (HSA) protein concentration of 5.0 mg mL^{-1}. In this case, lactate quantification within the sample G1-plus was 11.29 ± 1.3 mM (n=3), whereas the liquid chromatography method revealed a lactate concentration of 10.12 ± 0.10 mM (n=3). Hence, results clearly demonstrate the reliability of the lactate analysis in complex cell culture media using our electrochemical lactate biosensor. Hence the results obtained from both the electrochemical lactate biosensor and liquid chromatographic methodology shows no significant differences within the retrieval of lactate concentration, according to a t-test with a 95 % confidence level.

4. Conclusions.

This article reports the novel fabrication of an electrochemical lactate biosensor towards the determination of lactate within an embryonic cell culture. Such electrochemical biosensor provides a simple, fast and reproducible sensor (RSD of less than 3.8 %), which can be potentially utilized as a non-intrusive point of care sensor. The electrochemical lactate biosensor based upon a MWCNT and chitosan modified SPBGE provides a well-

defined bioelectrocatalytic response upon the presence of lactate. These electrochemical biosensors offer a linear range of 30.4 - 243.9 μM and a LOD of 22.6 μM. Moreover the hybrid composite biosensor presents an excellent sensitivity of -3417 ± 131 μA M^{-}μA M^{-1} due to the high electron transfer provided by the MWCNTs, in addition to the adequate immobilization of a LOx enzyme favored by the chitosan biopolymer. In terms of stability, the biocompatibility of the chitosan matrix with the LOx and the HRP enzymes makes the electrochemical lactate biosensor stable even after 5 months (with a retention of more than 82 % of the enzyme activity of the electrochemical biosensor) when stored at 4 ºC, which unlike current literature does not require any protection of the enzymatic composite.. In conclusion, this novel screen-printed electrochemical lactate biosensor is an ideal embryologist tool for determining lactate within the cell culture media of a human embryo during its cell development or after embryo retrieval. Such revelations have been validated by highly costly liquid chromatographic methods and possess no significant differences within the retrieval of lactate. Both the methodology of the fabrication of the electrochemical lactate biosensor and its applications are protected by the Spanish patent number P201431875.

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Figures and Table captions

Figure 1. (a) SEM image of the working electrode from a SPBGE platform; (b) SEM image of the film formed by MWCNTFcMe/CS/HRP/BSA/LOx composite film on the working electrode surface of the SPBGE electrochemical platform

Figure 2. Cyclic voltammograms of the electrochemical lactate biosensor in the absence of CS (solid line) and in the presence of CS (dashed line) in 0.1 M PBS pH 7.4 at 22 ºC. Scan rate 10 mV s-1. First scan recorded.

Figure 3. Linear sweep voltammetry response for the electrochemical lactate biosensor with a successive addition of 50 μL of a 10 mM lactate solution in 0.1 M PBS solution pH 7.4, under hydrodynamic conditions. Scan rate was 10 mV s^{-1}. Inset of figure: Calibration plot for the electrochemical lactate biosensor with lactate concentration in 0.1 M PBS pH 7.4 at a working potential of -0.2 V and 22 ºC.

Figure 4. Chronoamperometric response of the electrochemical lactate biosensor with a successive addition of 25 μL of a 10 mM lactate solution in 0.1 M PBS solution pH 7.4 at working potential of -0.2 V. Inset of figure: Calibration plot of the response electrochemical lactate biosensor with lactate concentration in 0.1 M PBS pH 7.4 at -0.2 V and 22 ºC.

Figure 5. Reproducibility study for the lactate calibration plot (R=0.99781, n=8 electrochemical lactate biosensors) in 0.1 M PBS pH 7.4 at 22 ºC and -0.2 V.

Table 1. Comparison of different electrochemical lactate biosensors reported in literature based on LOx immobilization on Screen-printed carbon electrodes SPCE

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