Electrochimica Acta 140 (2014) 42–48

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Electrochemical Devices for Monitoring Biomarkers in Embryo Development

Maria Gómez-Mingot[a,*,1], Sophie Griveau[b,c,d,e], Fethi Bedioui[b,c,d,e,2], Craig E. Banks[f], Vicente Montiel[a], Jesús Iniesta[a]

[a] Physical Chemistry Department and Institute of Electrochemistry, University of Alicante, 03690, San Vicente del Raspeig, Alicante, Spain
[b] Chimie ParisTech, Ecole Nationale Supérieure De Chimie De Paris, Unité de Technologies Chimiques et Biologiques pour la Santé, Paris, France
[c] CNRS, Unité de Technologies Chimiques et Biologiques pour la Santé, UMR 8258, Paris, France
[d] Université Paris Descartes, Sorbonne Paris Cité, Unité de Technologies Chimiques et Biologiques pour la Santé, Paris, France
[e] INSERM, Unité de Technologies Chimiques et Biologiques pour la Santé (N ◦ 1022), Paris, France
[f] Faculty of Science and Engineering, School of Science and the Environment, Division of Chemistry and Environmental Science, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, Lancs, United Kingdom

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form 26 February 2014
Accepted 7 March 2014
Available online 15 March 2014

Keywords:
Oxidative Stress
Human Embryo Development
Embryo Culture Medium
Radical Species
Electrochemical (Bio)sensors

ABSTRACT

Oxidative stress has recently became one of the most important and negative factors that affect favourable human reproduction and therefore plays a crucial role in the successful outcome rates on artificial reproduction techniques (ARTs). The effects of oxidative stress are strengthened in an ART setting since there are more sources of reactive oxygen and nitrogen species (ROS/RNS) generation and a more vulnerable physiological defence mechanism. Culture media, for this reason, have improved in their composition and are becoming more complex, above everything, in their antioxidant power to face ROS/RNS generation.

In this work, we suggest the use of electrochemical (bio)sensors to evaluate the presence and evolution of nitric oxide (NO) and superoxide anion (O_2^-), in a commercial culture medium compared to conventional phosphate buffer solution. The electrochemical monitoring of NO was followed by the real-time NO-release from a commercially available NO donor (diazeniumdiolate) under aerobic conditions in a surface-modified screen-printed linear platinum array electrode. Concurrently, superoxide anion detection was evaluated at a surface-modified screen-printed linear gold electrode by the real-time and *in situ* generation of O_2^-. The performances of both arrayed sensors were compared to those obtained at single modified ultramicroelectrodes. We deepen the study of the intrinsic and external interferents for the application of these electrochemical (bio)sensors in order to provide an oxidative stress index during embryo development.

1. Introduction

In vitro fertilization (IVF) techniques are recently booming as a result of the increasing problems of infertility in human reproduction. In the utilization of those techniques, the controversy is served as the blastocysts, which will become embryos, are cultured *in vitro* for a few days. Many efforts have been made to improve embryo culture media and to look for a correlation between the consumption of nutrients and the capacity of implantation of the embryo [1–3]. *In vitro*, culture media simulate the nutritional need of the embryo in the early stages of development. But, unluckily, Assisted Reproduction Techniques (ARTs) still have low success outcomes. The investigation of the negative causes that reflect the ARTs outcome is therefore gaining a lot of importance on this field. The knowledge of the factors that affect the low outcome might help to improve ARTs success rates. Oxidative stress has recently become one of the most important and detrimental factors that affect the successful human reproduction [4] and a proof of that is the incorporation of numerous antioxidants and radicals scavengers to the culture media [5,6].

Oxidative stress caused by the imbalance of the amount of reactive oxygen and nitrogen species (ROS and RNS, respectively) and the antioxidants that block them, is implicated in many of the

* Corresponding author. Nano-Electrochemistry Group, Université Paris Diderot, Sorbonne ParisCité, ITODYS, UMR 7086 CNRS, 15 rue Jean-Antoine de Baïf, 75205 Paris Cedex 13, France.
E-mail addresses: maria.gomez@ua.es (M. Gómez-Mingot), fethi.bedioui@chimie-paristech.fr (F. Bedioui).
[1] Tel.: +33 157 27 68 68.
[2] Tel.: +33 144 27 64 89.

http://dx.doi.org/10.1016/j.electacta.2014.03.012
0013-4686/

processes involved in human reproduction, such as oocyte maturation, folliculogenesis, sperm DNA damage, sperm quality and most importantly, in the post-transfer-pre-implantation step where the drop containing the embryo faces the endometrial cavity [7,8]. There exist two main sources of ROS and RNS generation: intracellular propagation, produced by gametes themselves during the selection and preparation of the samples with a less antioxidant-rich plasma or follicular fluid; or extracellular production, from the environmental factors since embryo cells are cultured *in vitro* and exposed to a relative hyperoxic conditions compared to the *in vivo* surroundings. Although it is important to note that similar to other living cells, oocyte and embryo cells use oxygen to produce energy through different pathways, and therefore they produce ROS/RNS, it has been found that the production of ROS/RNS of embryos cultured *in vitro* is higher than those cultured *in vivo* [9,10]. Elevated ROS/RNS production in culture media was associated with low blastocysts rate, low fertilization rate, low cleavage rate and high cell fragmentation [11]. Another important external factor is visible light which can induce photodynamic stress leading to oxidative damage of unsaturated lipids and sterols in cell membranes [12–14]. Further studies are required to implement a wider concept on the factors that provoke a higher overall oxidative stress balance, and consequently, the more feasible hypothesis is related to the combination of intrinsic metabolic changes of the embryo cells, higher pO_2 values and environmental factors due to micromanipulation.

The composition of commercial embryo culture media is devoted to reflect the nutritional need of the embryo in early stages of development, and sometimes a consecution of more complex media is recommended. The main components are necessary elements for the oocyte or embryo growth such as pyruvate, lactate and nonessential amino acids [15–17]. After the third day of cell culture the embryo consumes glucose [18] in an oxidative way or by aerobic glycolysis. It also needs in a huge extent nonessential and essential amino acids [19] for the cellular proliferation and differentiation since it has to produce high levels of proteins such as cyclins that are associated to cell cycle progression [20]. Some of the additives are thought to contribute to the generation of ROS/RNS throughout Fenton and Haber-Weiss reactions [21] such as metallic ions, while other additives form an elaborate defence system towards the most typical ROS/RNS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO) and hydroxyl radical ($^*OH$). This powerful defence system contains enzymes such as catalase, superoxide dismutase and glutathione peroxidase or reductase, and numerous nonenzymatic antioxidants among others such as vitamin C, vitamin A and vitamin E, pyruvate, taurine, glutathione. Another protein that requires special attention is human serum albumin (HSA) that is added to the culture media because of its powerful antioxidant properties while it is important to note that this enzyme preparation also commonly contains high levels of amine oxidase, which leads to a higher production of the amount of H_2O_2 [22].

Systematic monitoring of the complex and interrelated events in the microenvironment of *in vivo* biological cells represents a non-trivial challenge [23,24]. The detection of these species is often difficult due to the numerous cellular mechanism and pathways where they are involved in and their complicated nature and short lifetimes. Compared to other conventional techniques used for the direct or indirect detection of ROS/RNS such as immunochemistry, colorimetry, fluorimetry, chemiluminescence, mass spectroscopy or Electron Paramagnetic Resonance [25–28], electrochemical techniques are a logical choice for tackling the analysis of cellular redox communication since they offer non-destructive, low detection limits, label-free, selective and specific real-time analysis with spatial resolution [29–31]. Responses to signaling molecules are dependent on the identity, location, and state of the responding cells, and on the nature, concentration, as well as the spatial and temporal distribution of the soluble chemical signals [32,33]. Consequently, there are numerous electrochemical (bio)sensors designs for the detection of radical species in biological systems reported in literature [34,35] differing on the different architectures employed for the quantification of ROS/RNS.

In this work, we report for the first time on the design, fabrication and use of either gold or platinum disposable linear array screen-printed electrodes (LA-SPE) based on our experience in modified ultramicroelectrodes (UMEs) for the detection under *in vitro* oxidative stress conditions of nitric oxide (NO) and superoxide (O_2^-) [36,37]. Screen-printed electrodes indeed offer plenty of advantages compared to other electrodes since they are sensitive, easy to fabricate and use, rapid, cheap and portable. There are many applications where these platforms have potentiality in the field of sensing and monitoring since the increase in the number of electrodes on the platform induce an amplification of the signal and therefore higher responses can be obtained for the same concentration of analytes [38–41]. These characteristics confer to these platforms a very high potentiality to be used in a mass produced manner also in hospitals and IVF clinics in a simple way.

The electrochemical monitoring of NO and O_2^- was performed in phosphate buffer solutions (PBS) examining the interferences of a number of metabolites which are present in real culture medium. Finally, a commercial embryo culture medium was tested for the potential application of the ROS/RNS electrochemical (bio)sensors in real samples in order to evaluate the antioxidant power of the medium. The final aim of this work is to provide a simple electroanalytical tool for measuring the oxidative stress index during the embryo development that might be related to the metabolic rate and the viability of the embryo development.

2. Materials and methods

2.1. Reagents and chemicals

Cytochrome *c* (*cyt c*,horse heart), xanthine, xanthine oxidase (bovine milk, XOD), bovine serum albumin (BSA), superoxide dismutase (SOD), hyaluronic acid potassium salt (human umbilical cord, *HA*), eugenol, phenol, 3,3′-dithiobis(succinimidylpropionate) (DTSP), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$) and hydrogen peroxide (H_2O_2) were purchased to Sigma (Poole, UK). The DEA-NONOate (diethylammonium (Z)-1-(N,N-diethylamino)diazen-1-ium-1,2-diolate) was supplied by Cayman Chemicals (Michigan, USA). Stock solutions of 10 mM DEA-NONOate were prepared in 0.01 M NaOH and were stored at - 20 ^{0}C in the dark for two months maximum, and thawed to room temperature immediately prior to use and kept in an ice container during experiments.

Glucose, citric acid, pyruvic acid, lactic acid, alanine (Ala), alanylglutamine (Ala-Gln), arginine (Arg), asparagine (Asn), aspartate (Asp), cystine (Cys), glutamine (Gln), glutamate (Glu), glycine (Gly), histidine (His), isoleucine (Iso), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), taurine (Tau), threonine (Treo), tryptophan (Trp), tyrosine (Tyr) and valine (Val) were also obtained from Sigma (Poole, UK) and stock solution of these interferents were prepared freshly in PBS. All chemicals were reagent grade and were used without further purification. All aqueous solutions were made using ultrapure water with a resistivity of 18.2 MΩ cm. All experiments were achieved at room temperature ($25 \pm 3\,^\circ$C) in pH 7 PBS 0.1 mol L^{-1}. Embryo culture medium (G-1^{TM} v5 PLUS) was purchased from Vitrolife (Göteborg, Sweden), and was stored at–20 °C. Electrochemical experiments using the culture medium were performed by diluting 1:10 the medium in PBS in order to avoid viscosity effects.