Electrochemical Sensors for Oxidative Stress Monitoring

Aaditya S. Deshpande,[a] Wayne Muraoka [b] and Silvana Andreescu [a*]

[a] Department of Chemistry and Biomolecular Science, Clarkson University, Potsdam, NY 13699

[b] U.S. Army Institute of Surgical Research, Blood and Shock Resuscitation, Fort Sam Houston, TX 78234

Corresponding Author- eandrees@clarkson.edu

Abstract

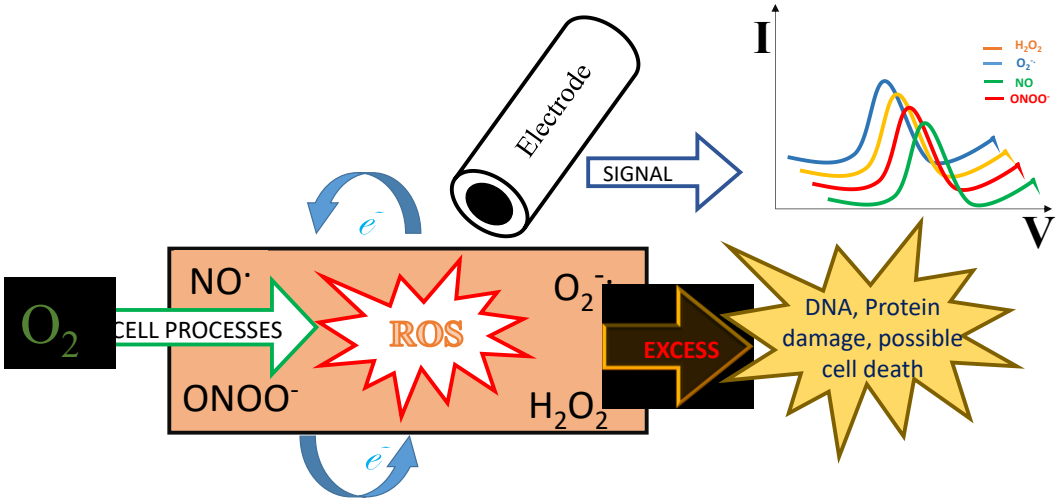
Electrochemical sensors are ideally suited for the detection of reactive oxygen and nitrogen species (ROS and RNS) generated during biological processes. This review discusses the latest work in the development of electrochemical microsensors for ROS/RNS and their possible applications for monitoring oxidative stress in biological systems. The performance of recent designs of microelectrodes and electrode materials are discussed along with their functionality in preclinical models of drug efficacy, mitochondrial distress, and endothelial dysfunction. Challenges and opportunities in translating this methodology to study the pathophysiology associated with various diseases are discussed.

Keywords: Oxidative stress, ROS/RNS, Nitric Oxide, Hydrogen Peroxide, Superoxide

Highlights

- Recent developments in electrochemical sensors for detection of ROS/RNS in biological systems
- Recent designs and performance of materials and electrodes for in *vivo/in vitro* monitoring of oxidative stress
- Discussion of the remaining challenges for measurements in biological systems
- Highlights of biological applications and translational aspects

Graphical Abstract



1. Introduction

Reactive oxygen and nitrogen species (ROS and RNS) are formed by redox reactions of molecules that contain oxygen or nitrogen. ROS include superoxide ($O_2^{\cdot-}$), hydroxyl ($HO^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), hydrogen peroxide (H_2O_2), hypochlorous acid/hypochlorite (HOCl/$^-$OCl) and singlet oxygen ($^1O_2$) [1,2]. RNS include nitric oxide ($^{\bullet}NO$), nitrogen dioxide ($^{\bullet}NO_2$), and peroxynitrite ($ONOO^-$). ROS/RNS are continuously produced during normal cell processes like oxidative phosphorylation, catabolism of fatty acids, phagocytosis, breakdown of macromolecular compounds, and protein folding. Classified as radical (e.g., $O_2^{\cdot-}$, $NO^{\bullet}$, and $OH^{\bullet}$) or non-radical (e.g., HOCl and H_2O_2), each type of ROS has a different reactivity rate, biological activity, and role within the cell [3,4]. Collectively, both radical and non-radical ROS contribute to the overall oxidative burden of the cell [2,4], and when in excess, the highly reactive nature of ROS/RNS can damage cell components, leading to oxidative stress [5]. While the significance of ROS is in general well recognized, some aspects of ROS in distinguishing the physiological and pathological processes are still debated.

Despite the importance of ROS/RNS, there is still a lack of suitable analytical tools to selectively monitor ROS/RNS evolution to address the cell's oxidative status in situ. Using custom designed microelectrodes, electrochemistry provides unique opportunities to detect reactive species in living tissues [6], thereby providing direct and real time evidence of ROS levels with high spatial resolution [7-9]. In this review, we discuss electrochemical detection methods and microsensors for ROS/RNS, with focus on H_2O_2, NO and $ONOO^-$ and $O_2^{\cdot}$. Finally, we conclude with a perspective on future advances of this technology for the development of electrochemical probes for real time monitoring of these species in biological systems as well as translational aspects for medical applications.

2. Cell oxidative stress: relevance and significance

ROS play an essential role in redox signalling, allowing the cell to rapidly adapt to environmental or nutritional perturbations [3,10,11]. Under homeostatic conditions, both enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (e.g., glutathione, thioredoxin, ascorbate) antioxidant mechanisms tightly regulate ROS levels and prevent excess accumulation [11,12]. Dysregulation of the balance between pro- and anti-oxidants is associated with physiological and developmental derangements. Insufficient H_2O_2 restricts neuron growth, induces stem cell quiescence and thwarts wound healing, while insufficient $O_2^{\bullet-}$ impairs immune cell clearance of pathogens [13-15]. However, excessive ROS damages DNA, proteins, and lipids, and can lead to cell death, tissue damage, and if not corrected, organ failure [16-18]. Indeed, oxidative damage to lipid (8-isoprostaglandin F2α and malondialdehyde), DNA (8-hydroxy-deoxyguanosine), and protein (3-nitrotyrosine) are often used as biomarkers of oxidative stress in clinical samples [19,20]. Oxidative stress is an imbalance of cellular redox where a pro-oxidant state is favoured and is implicated in a myriad of diseases [21,22]. Different types of ROS can impact cell physiology and oxidative stress in different ways, and it is important to measure individual species to understand the impact of a specific ROS within each (patho)physiologic setting [2]. Therefore, monitoring a specific ROS in different disease conditions and experimental treatments is necessary to investigate disease mechanisms and drug efficacy. However, ROS are highly reactive and extremely short lived in the body, making its direct measurement in live tissues and organisms difficult.

Biomarkers have the limitation that their accumulation or removal will alter the quantity of the measured analyte but may not correspond to nascent ROS evolution. Further, biomarker measurements do not provide insight into the type of ROS that is dysregulated, and it is difficult to deconvolute the consequence from the cause of oxidative stress. Methods to measure specific types of ROS in tissues were described for electron paramagnetic resonance and ultra-weak photon emission spectroscopy [23,24] but their application for in vivo ROS monitoring are hampered by high cost and low temporal resolution. Therefore, there is a need to develop tools that have an extremely rapid response time, are sensitive and selective to individual species, and capable of real time detection. The accurate measurement of these species is still a bottleneck in understanding their physiological functions and a universal technique that can detect the wide variety of radicals is not available [25].

3. Electrochemical sensors and biosensors for ROS/RNS detection

Monitoring ROS/RNS using electrochemistry provides a valuable approach to quantifying oxidative stress generated by these species in situ and can help elucidate their biological roles. Although a variety of electrochemical sensors have been reported, relatively few studies demonstrate their use in cells or biological systems. The use of glassy carbon electrodes is common in literature; however, its bulky size restricts use to proof-of-concept work to develop new chemistries, and it is not suitable for live tissues. Smaller size electrodes that can measure ROS in the proximity of cells are most suited to explore biological mechanisms in situ. Therefore, this review focusses primarily on microelectrodes that have been used to measure reactive species at the cellular or tissue level. These include carbon fibre microelectrodes (CFME) with sizes from 5 to 10 μm and gold or platinum wire microelectrodes with a diameter of ~100 μm.

ROS/RNS can be measured using microelectrodes functionalized with chemical or biological coatings. Chemical sensors provide a direct measure of the reactive species at their characteristic potentials. Common examples are those measuring the oxidation of NO at ~ 0.8 V vs Ag/AgCl, or, the oxygen/superoxide redox couple at ~-0.33 V vs NHE [9]. Pioneering work done by the group of Christian Amatore and collaborators demonstrated the use of platinized carbon microelectrodes (~10 μm diameter) for monitoring ROS/RNS species produced by single cells [9,26-28] and their ability to measure reactive species inside single phagolysosomes of living macrophages using a four step chronoamperometric method [28]. Recent advances involve modification of microelectrodes with catalytic materials to enhance the detection sensitivity and tailor selectivity. In contrast, biological sensors are protein-functionalized electrodes that contain a redox protein immobilized at the electrode surface to selectively recognize the targeted species and convert the biorecognition into an electrochemical redox signal. A common example is the use of cytochrome c (Cyt C) as molecular recognition and electron transfer mediator for $O_2^{\bullet-}$ measurements [29]. In these sensors, the immobilized Cyt C reacts with $O_2^{\bullet-}$; the protein is then oxidized by direct electron transfer to/from the electrode, generating a biocatalytic current that is proportional to the $O_2^{\bullet-}$ concentration. Because biological sensors take advantage of the selectivity of biomolecules they tend to be more selective. However, they require immobilization of the biomolecule onto

the microelectrode surface and the long-term stability of these sensors might be an issue. **Table 1** provides an overview of microelectrode platforms for measuring superoxide $O_2^{\cdot-}$, H_2O_2. The following sections discuss the most recent representative examples of microelectrochemical sensors for measurements of ROS/RNS in biological systems.

Table 1. Details of some electrochemical sensors and biosensors and electrode modifications for detection of ROS/RNS released from cells and tissues.

#	ROS/ RNS	Electrochemical Technique	Electrode materials	Working electrode	LOD	Biological system	Ref
1	H_2O_2	Fast Scan Cyclic Voltammetry	1,3-phenylenediamine	CFME	20 µM*	Rat brain	[30]
2	H_2O_2	Chronoamperometry	Pt-Pd bimetallic nanocoral	CFME	0.42 µM	A549 living cells, milk	[31]
3	H_2O_2	Amperometry, CV	Hemoglobin, SWCNT	CFME	0.23 µM	HePG2 cancer cells	[32]
4	H_2O_2	Amperometry	Au-Pd alloy NPs, Graphene Quantum Dots	CFME	500 nM	Clinical breast cancer tissue	[33]
5	H_2O_2	Amperometry	Pt NPs, Nafion, PPD	CFME	0.53 µM	*In vitro*	[34]
6	H_2O_2	Amperometry	Platinized silica nanoporous membranes	CFME Or ITO	0.01mM	Rat brain	[35]
7	H_2O_2,	Chronoamperometry	Heat treatment to create nanopores to improve catalytic performance	Heat-treated CFME	1 µM	*In vitro*	[36]
8	H_2O_2	Chronoamperometry	Core-shell 2D VS_2,@VC@N-doped carbon sheets decorated by Pd NPs	CFME	50 nM	MCS-7 cancer cells, and breast cancer tissue	[37]
9	H_2O_2	Chronoamperometry	Pt–Pd NPs, graphene oxide	CFME	0.3 µM	Raw 264.7 cells secretion	[38]
10	H_2O_2	Chronoamperometry	Au-Ag bimetallic NPs / polydopamine	CFME	0.12 µM	HepG2 cells	[39]
11	HClO/ClO$^-$	DPV	Graphene Oxide, carbon nanotubes, MBS	CFME	0.5 µM	Body fluids	[40]
12	$O_2^{\cdot-}$	Chronoamperometry	MWCNTs,Ionic Liquid-Br, SOD, Prussian Blue NPs	CFME	0.42 µM	Alzheimer rat brains	[41]
13	$O_2^{\cdot-}$	DPV with ratiometric signal output	Diphenylphosphonate-2-naphthol ester, methylene blue SWCNTs	CFME	2 µM	Rat brain	[42]
14	NO	DPV	NiTSPc/nafion	CFME	0.34 µM	Zebrafish intestine	[8]

MWCNTs-Multi Walled Carbon Nanotubes, CV- Cyclic Voltammetry, BBY- Bismarck Brown Y, rGO- Reduced Graphene Oxide, FTO- Fluorine doped Tin Oxide, AgNPs- Silver Nanoparticles, CNT- Carbon Nano Tubes, DNA- Deoxyribose Nucleic Acid, APTES- (3-aminopropyl) triethoxysilane , Cyt C – Cytochrome C,Poly(5A1N)- Electropolymerized 5-amino-1-naphthol , XG- Xero Gel, PSS-Polystyrene Sulfonic Acid, BA- 5-(1,2-dithiolan-3-yl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pent-anamide, CFME- Carbon Fiber Micro Electrode, AuNP- Gold Nanoparticles, PDDA- Poly(Diallyl Dimethil Ammonium Chloride), D-cell - Plastic

disposable Carbon based Electrochemical Cell, a-NSGF- Taurine-functionalized Graphene foam, ITO-PET- Indium Tin Oxide supported on Poly-Ethylene-Terephthalate foil, CTS-Chitosan, MPNS-Microporous Polymeric Nanospheres, HTCMP-Hollow Tubular Conjugated Organic Microporous Polymer

3.1. Electrochemical sensors for H_2O_2 and superoxide radicals

The electroactive H_2O_2 can be detected electrochemically using a chemically modified electrode [6]. Xu and co-workers modified a CFME with Au nanocones and a synthetic molecular receptor having affinity towards H_2O_2. The small size of the CFME coupled with the selectivity of the synthetic receptor enabled measurements of H_2O_2 in a single drop of blood. Measurements were performed by differential pulse voltammetry (DPV) in the range -0.5 - 0.6 V *vs* Ag/AgCl electrode and the sensor was able to measure H_2O_2 in the 0.5 -400 μM range [43]. André Afonso and co-workers used a disposable plastic carbon-based electrochemical cell with a chemically modified electrode coated with Ag nanoparticles and δ-FeOOH. The reduction of H_2O_2 catalyzed by Ag nanoparticles (NPs) lead to increased sensitivity for H_2O_2 detection in fetal bovine serum [44]. Non-enzymatic detection of H_2O_2 is achieved with electrodes functionalized with chemical mediators such as Prussian blue (PB), used alone or in composite forms with Au nanoparticles or graphene oxide. A PB-AuNPs-graphene oxide deposited on a GCE enabled detection of H_2O_2 down to 1.3 μM [45]. Similar strategies can be used to increase selectivity of CFMEs (Table 1). Most sensors use Pt-based structures that take advantage of the catalytic activity of PtNPs for H_2O_2; these are often combined with Au, Ag, carbon nanotubes or graphene oxide for enhanced performance. In some cases, the growth and self-assembly of a multidimensional structure on the surface of CFMEs reduces the oxidation potential minimizing interferences. A CFME functionalized with VS_2,@VC@N-doped carbon sheets decorated by PdNPs enabled detection of H_2O_2 at -0.05 V with no interferences from dopamine, uric acid, ascorbic acid and nitrite [37].

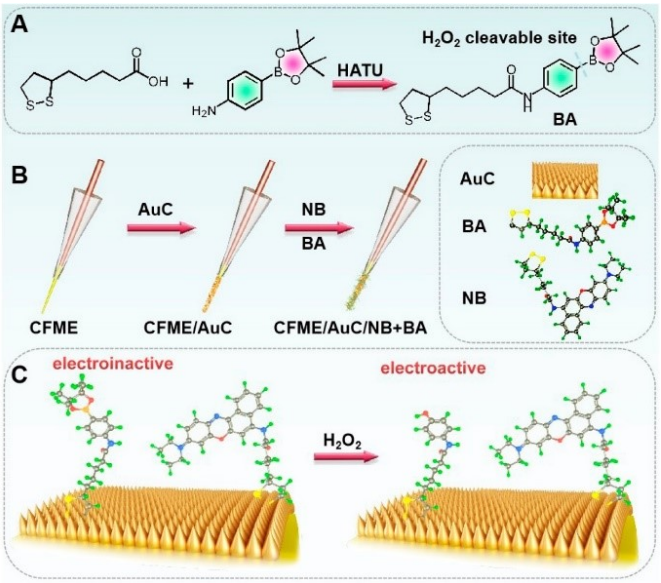


Figure 1 – Example of electrochemical H_2O_2 sensors used using a chemically modified CFME. Reproduced with permissions (ref [43] with permission).

The standard redox potential of the $O_2/O_2^{\bullet-}$ redox couple is between from 330 mV to 140 mV vs NHE and thus this can be determined by direct electrochemical oxidation using platinized microelectrodes, or electrodes modified with nitrogen-doped carbon AgNPs [46] or porous carbon networks [47]. To improve selectivity, a common approach is to immobilize Cyt C or superoxide dismutase onto AuNP-functionalized microelectrodes [6]. A recent trend is to use enzyme mimetic materials such as $MnTiO_3$ microdiscs, [48] manganese phosphate [49] or nanostructured Mxenes [50] and graphene/AgNP/CeO_2/TiO_2 [51] as alternative to natural enzymes. However, specificity of measurements is not always demonstrated, raising questions about the accuracy of such configurations. Thick-films Cyt c-based nanoporous gold electrodes with a detection limit of 1.9 nM and a sensitivity of 1.9 $nM.nM^{-1}cm^{-2}$ enabled the online detection of $O_2^{\bullet-}$ in skeletal muscle tissue [52].

3.2 Electrochemical sensors for nitric oxide and peroxynitrite

NO is a highly diffusible short-lived species, which can interact with $O_2^{\bullet-}$ to form peroxynitrite, a highly reactive and toxic species that can damage DNA, proteins and lipids. Because NO has reduced stability, NO sensors must have a short response time, be sensitive, and have a wide linearity range. The electrooxidation of NO takes place at a potential >0.8V vs Ag/AgCl that overlaps with the oxidation potential of other electroactive species. To prevent interferences,

CFMEs are commonly functionalized with blocking membranes such as Nafion [8], o-phenylene diamine (o-PD) and chitosan [8,53]. Electrochemical NO sensors have been reviewed by Brown and Schoenfisch [54].

Long term electrochemical measurement of NO released from cultured pro-inflammatory macrophages was demonstrated using an Pt disk electrode (6 mm diameter) modified with an electropolymerized 5-amino-1-naphtol (Poly(5A1N)) and fluorinated xerogel to prevent degradation (**Figure 2**) [55]. The xerogel provided permselective properties imparting selectivity and preventing biofouling. A detection limit of 1 nM and a dynamic range 0.01-10 µM was reported. Detection of NO in human serum (detection limit of 52 nM and linear range of 0.25-40 µM) was reported with an electrode coated with reduced graphene oxide and PtNPs [56]. A microsensor enabling detection of $NO^{\cdot}$ in the presence of H_2O_2 in static or flow conditions was achieved with a dual-electrode set up. Poly(eugenol) coating enhanced the selectivity of the Pt electrode and was superior to bare Pt and Pt-Pt black [57].

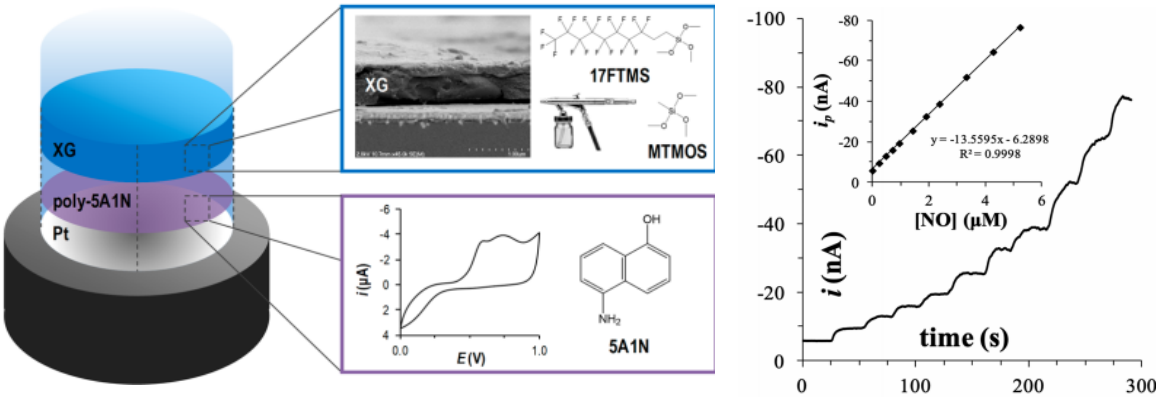


Figure 2 -NO sensor coated with gel to prevent degradation in biological medium (with permission from [55]).

Recent developments in microelectrode design integrate microelectrodes and wireless monitoring. Using a flexible transient electrode, real time monitoring of NO over 5 days was recorded in the hearth and joint cavity of rabbits [58]. The implantable sensor consisted of a biocompatible electrode constructed from polylactic acid and poly(trimethylene carbonate), an ultrathin Au membrane, and a poly(eugenol) film. This sensor had a detection limit of 0.97 nm and a 0.01-100 μM linear range.

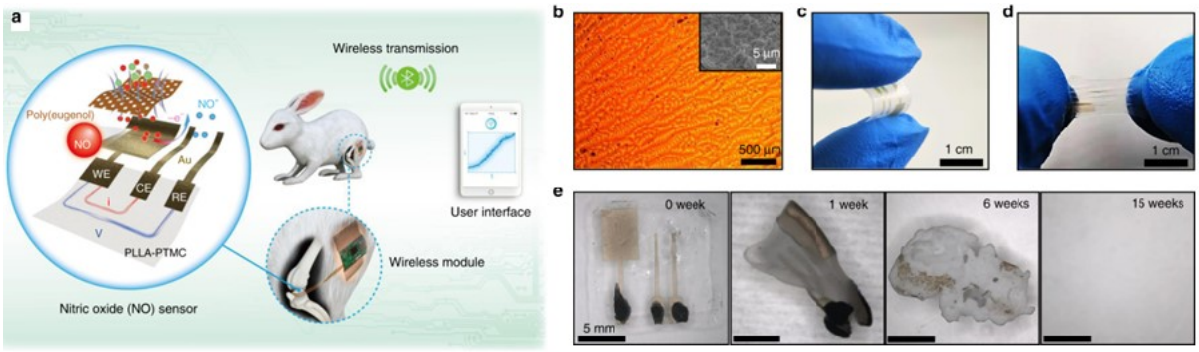


Figure 3 - **a** Transient NO sensor composed of a bioresorbable copolymer of poly(l-lactic acid) and poly(trimethylene carbonate (PLLA–PTMC) substrate, Au nanomembrane electrodes, and a poly(eugenol) thin film. NO concentration was measured through amperometry. The sensor can continuously detect NO concentrations in vivo and transmit the data to a user interface through a customized wireless module. **b** Optical image of the surface morphology of Au electrodes and SEM image of the surface morphology. **c** NO sensor under bending. **d** NO sensor in a stretched state. **e** Images at various stages (0, 1, 6, and 15 weeks) of accelerated degradation of a transient NO sensor in phosphate-buffered saline (with permission from refs [58]).

Peroxynitrite, the primary product formed in the fast reaction of superoxide radicals with NO, is an important but difficult to measure RNS [59]. The formal potential of $ONOO^-$ /$ONOO^{\cdot}$ is 0.27 V vs SSCE [60]. Electrochemical detection of transient concentrations of peroxynitrite was achieved with platinized, or nanostructured microelectrodes modified with conjugated Mn complexes (e.g., tetraaminophthalocyanine manganese (II)[61], MnO_2-Hemin [62] and PEDOT-Hemin [63] layers, and microporous polymeric nanospheres [64]) acting as electrocatalytic sites. Recent efforts are dedicated to simultaneous detection of multiple ROS/RNS released by cells by custom-designed microfluidic devices [65] and ratiometic measurements [66]. Such measurements can be effective at determining multiple ROS/RNS

species simultaneously and take into account issues of cross-reactivity. This approach is highly suited for the high throughput monitoring of cells.

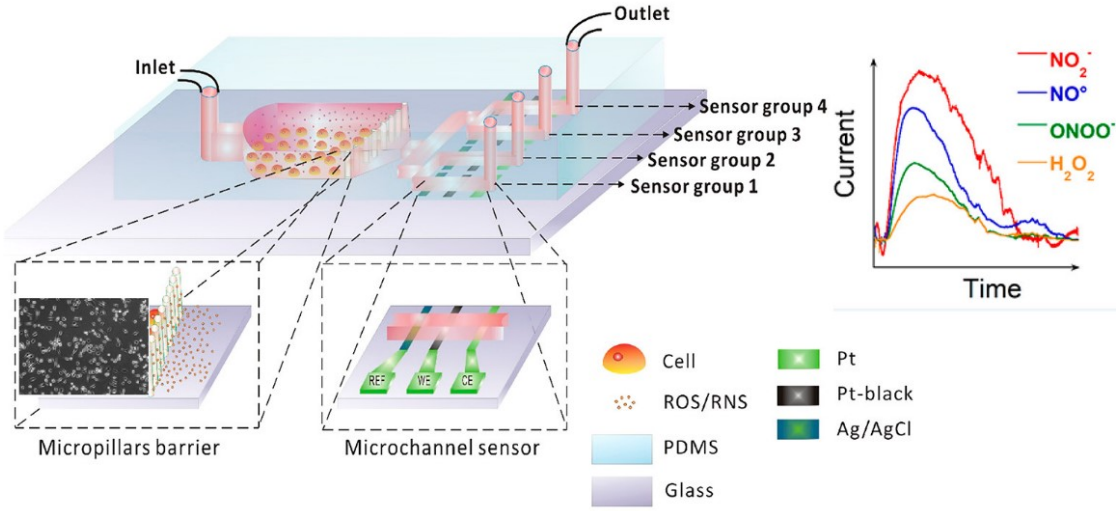


Figure 4 - Microfluidic platform integrating four parallel channels for H_2O_2, $ONOO^-$, $NO^{\cdot}$, and NO_2^- measurements (with permission from Ref [65]).

4. Biological applications and translational aspects

Accurate ROS measurements are important to understand the relationship between oxidative stress and disease. Oxidative stress underlies cardiovascular diseases by impairing endothelial cell function, thereby influencing vascular tone and inflammation [67] [48]. How the mechanical forces from blood flow and smooth muscle contraction alter ROS production was addressed using a flexible electrochemical sensor [68]. This sensor allowed for the attachment of cells onto a compliant surface. By simulating in vivo conditions of mechanical stress, it was shown that circumferential stretch at normotensive strain induces $NO^{\bullet}$ production, whereas hypertensive strain promotes H_2O_2 production, possibly through NADPH oxidase. This sensor revealed new insight to the redox response of endothelial cells under different mechanical stressors.

Because oxidative stress is implicated in the progression of many pathological conditions, considerable effort has been made to affect ROS levels under various disease settings for therapeutic benefit. Insight into the actions of established and novel therapeutics

were recently addressed by electrochemical methods. Jiang et al. created nanowire electrodes capable of quantifying, at the subcellular level, ROS production in fibroblast and cancer cell lines [67]. This electrode identified the mitochondria, specifically complex IV, as the principal site of ROS production in response to chemotherapeutic. Higher levels of paclitaxel-induced ROS were detected in cancer cells compared with normal cells, suggestive of a selective cytopathic mechanism. Vaneev et al. used platinized nanoelectrodes to demonstrate rapid H_2O_2 evolution in single cells after treatment with chemotherapeutics [69]. The translational utility of this sensor was demonstrated in tumour-bearing mice treated with doxorubicin. In this application, ROS levels increased with increasing tumour depth, highlighting possible spatial heterogeneity within the tumour. Lastly, Gubernatorova et.al. evaluated the in vivo ROS scavenging ability of Europium-doped ceria NPs using a Cyt C-based electrochemical biosensor [70] [52]. This study linked $O_2^{\bullet-}$ formation with the induction of inflammatory cytokines during intestinal ischemia-reperfusion injury. Ultimately, a greater understanding of the mechanism by which (chemo)therapeutics exert their effects may facilitate the screening of new drugs that are based on redox dependence, while avoiding interference of redox signalling in normal cells.

Despite the desirable characteristics of electrochemical sensors for in vivo ROS monitoring, several technical challenges remain before these sensors realize clinical utility. Biofouling, or adsorption of biomolecules onto the probe, can reduce the sensor's detection capability. This was observed with a carbon nanofiber sensor that initially showed sensitive detection of $O_2^{\bullet-}$ in the rat brain, but sensitivity was reduced by ~60% after implantation [71]. Antifouling strategies that mitigate signal reduction are necessary. Interference by electroactive compounds poses another challenge for in vivo use of electrochemical sensors. This was recently addressed by electrodeposition of 1,3-phenylenediamine onto an electrode surface to create a perm-selective barrier. This modification allowed for specific measurement of H_2O_2 flux in the brain [72]. Notwithstanding these challenges, there is an increasing need to accurately measure oxidative status in the clinical setting. The recent COVID-19 pandemic demonstrated the need for platforms that have rapid response time to test clinical specimens. An electrochemical sensor that detects H_2O_2 was developed to screen human sputum for lung inflammation [73]. ROS measurements showed good agreement with computed tomography

scan of lungs, and infection status could be inferred from the applied potential sweep data. These recent studies highlight electrochemical detection of ROS as a powerful tool for mechanistic and translational studies, but also revealed challenges that are currently being addressed.

5. Future challenges and trends

Although electrochemical sensors for ROS/RNS monitoring are well-established, most reported work measures concentrations of reactive species in standard solutions or synthetically generated radicals with few reports of implementation in live tissues. Advances in electrode design, featuring increased sensitivity and real time capabilities, provide a solid foundation for future implementation in biological systems. Since ROS/RNS is fundamental to many processes and diseases, electrochemical sensors have great potential to facilitate an understanding of their production and removal in cells and tissues, establish the relation between free radical production and disease progression, and evaluate oxidative stress mechanisms. The challenge is to design robust probes and surface modifications that can maintain performance in complex biological environments without passivation or biofouling. While electrochemical methods for ROS/RNS detection have improved in recent years, their implementation requires further refinement to address issues such as robustness, selectivity toward specific ROS/RNS, and cross-reactivity. Improving the selectivity toward individual radicals, or developing ratiometric or multi-array sensors for simultaneous quantification of a broader range of radicals through parallel measurements is of particular interest for future research. Manufacturing of more robust and stable microelectrodes and biosensors using methods that enable large-scale production is also needed.

Most measurements have been done to study released kinetics in isolated cultures or cells, with few examples of implementation in tissues and organs. Adoption of electrochemical probes to address relevant pathological events relies on interdisciplinary research and close collaboration between electrochemists, biologists, immunologists and medical doctors. Given the maturity of these probes, future research is expected to explore the use of this technology in relevant cellular and animal models through implantation. An immediate use of implantable microelectrodes is for monitoring ROS/RNS species in real time to better understand their

interplay in the biological environment. Innovations in electrode design to increase biocompatibility is also expected. To improve the capabilities of electrochemical measurements, the following potential directions for future research are expected: 1) increasing the sensitivity through improving the electrochemical interface and immobilization strategy by using 2D and 3D nanostructures materials like MXenes, metal organic frameworks, perovskites, or multi-layered polymer layers, 2) scalable manufacturing of microelectrodes to enable large scale adoption and improve reproducibility through the use of additive manufacturing techniques such as printing, 3) multiplexed detection of different ROS/RNS species simultaneously placed along with sentinel or self-reference electrodes to improve accuracy of measurements and minimize interfering effects from coexisting spices, 4) electrode coatings to minimize the non-specific interaction and biofouling effects in biological environments, 5) integration of electrochemical measurements with chemometrics analysis, machine learning and artificial intelligence, as well as wireless connectivity to improve data processing and remote monitoring capabilities of electrochemical measurements. Finally, 6) *in vivo* studies with implanted microelectrodes should be validated with suitable biological manipulations to demonstrate usefulness and accuracy of measurements. Monitoring physiological and pathological events such as cancer, ischemia/reperfusion, traumatic brain injury, trauma, and hypovolemic shock, are all relevant models for future applications.

DOD disclaimer: The views expressed in this article are those of the author(s) and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DoD, or the U.S. Government. We also acknowledge funding from US -National Science Foundation grant (NSF 20425544) and Congressionally Directed Medical Research Programs (W81XWH2020054). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the US Army.

References

1. Chen XQ, Wang F, Hyun JY, Wei TW, Qiang J, Ren XT, Shin I, Yoon J: **Recent progress in the development of fluorescent, luminescent and colorimetric probes for detection of reactive oxygen and nitrogen species.** *Chem Soc Rev* (2016) **45**(10):2976-3016.

2. Murphy MP, Holmgren A, Larsson NG, Halliwell B, Chang CJ, Kalyanaraman B, Rhee SG, Thornalley PJ, Partridge L, Gems D, Nystrom T *et al*: **Unraveling the biological roles of reactive oxygen species.** *Cell Metab* (2011) **13**(4):361-366.

3. Sies H, Jones DP: **Reactive oxygen species (ros) as pleiotropic physiological signalling agents.** *Nat Rev Mol Cell Bio* (2020) **21**(7):363-383.

4. Schrader M, Fahimi HD: **Peroxisomes and oxidative stress.** *Bba-Mol Cell Res* (2006) **1763**(12):1755-1766.

5. Nash KM, Rockenbauer A, Villamena FA: **Reactive nitrogen species reactivities with nitrones: Theoretical and experimental studies.** *Chem Res Toxicol* (2012) **25**(8):1581-1597.

6. Calas-Blanchard C, Catanante G, Noguer T: **Electrochemical sensor and biosensor strategies for ros/rns detection in biological systems.** *Electroanal* (2014) **26**(6):1277-1286.

7. Malferrari M, Becconi M, Rapino S: **Electrochemical monitoring of reactive oxygen/nitrogen species and redox balance in living cells.** *Anal Bioanal Chem* (2019) **411**(19):4365-4374.

8. Dumitrescu E, Wallace KN, Andreescu S: **Real time electrochemical investigation of the release, distribution and modulation of nitric oxide in the intestine of individual zebrafish embryos.** *Nitric oxide : biology and chemistry* (2018) **74**: 32-38.

9. Amatore C, Arbault S: **Oxidative stress at the single cell level.** *Front Neuroeng* (2007) **1**: 261-283.

10. Chen Y, Azad MB, Gibson SB: **Superoxide is the major reactive oxygen species regulating autophagy.** *Cell Death Differ* (2009) **16**(7):1040-1052.

11. Checa J, Aran JM: **Reactive oxygen species: Drivers of physiological and pathological processes.** *J Inflamm Res* (2020) **13:** 1057-1073.

12. Gorlach A, Dimova EY, Petry A, Martinez-Ruiz A, Hernansanz-Agustin P, Rolo AP, Palmeira CM, Kietzmann T: **Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved?** *Redox Biol* (2015) **6**: 372-385.

13. Sies H: **Oxidative stress: A concept in redox biology and medicine.** *Redox Biol* (2015) **4**: 180-183.

14. Wilson C, Munoz-Palma E, Gonzalez-Billault C: **From birth to death: A role for reactive oxygen species in neuronal development.** *Semin Cell Dev Biol* (2018) **80:** 43-49.

15. Rada B, Leto TL: **Oxidative innate immune defenses by nox/duox family nadph oxidases.** *Contributions to microbiology* (2008) **15**: 164-187.

16. Burmeister DM, Gomez BI, Dubick MA: **Molecular mechanisms of trauma-induced acute kidney injury: Inflammatory and metabolic insights from animal models.** *Biochimica et biophysica acta Molecular basis of disease* (2017) **1863**(10 Pt B):2661-2671.

17. Collard CD, Gelman S: **Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury.** *Anesthesiology* (2001) **94**(6):1133-1138.

18. Eltzschig HK, Eckle T: **Ischemia and reperfusion-from mechanism to translation.** *Nat Med* (2011) **17**(11):1391-1401.

19. Andries A, Rozenski J, Vermeersch P, Mekahli D, Van Schepdael A: **Recent progress in the lc-ms/ms analysis of oxidative stress biomarkers.** *Electrophoresis* (2021) **42**(4):402-428.

20. Griendling KK, Touyz RM, Zweier JL, Dikalov S, Chilian W, Chen YR, Harrison DG, Bhatnagar A, Basic AHAC: **Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: A scientific statement from the american heart association.** *Circ Res* (2016) **119**(5):E39-E75.

21. Elbatreek MH, Pachado MP, Cuadrado A, Jandeleit-Dahm K, Schmidt H: **Reactive oxygen comes of age: Mechanism-based therapy of diabetic end-organ damage.** *Trends Endocrinol Metab* (2019) **30**(5):312-327.

22. Senoner T, Dichtl W: **Oxidative stress in cardiovascular diseases: Still a therapeutic target?** *Nutrients* (2019) **11**(9): 2090.

23. Prasad A, Balukova A, Pospisil P: **Triplet excited carbonyls and singlet oxygen formation during oxidative radical reaction in skin.** *Front Physiol* (2018) **9**: 1109.

24. Togashi H, Aoyama M, Oikawa K: **Imaging of reactive oxygen species generated in vivo.** *Magn Reson Med* (2016) **75**(3):1375-1379.

25. Wang HS: **Development of fluorescent and luminescent probes for reactive oxygen species.** *Trac-Trend Anal Chem* (2016) **85**: 181-202.

26. Amatore C, Arbault S, Guille M, Lemaitre F: **Electrochemical monitoring of single cell secretion: Vesicular exocytosis and oxidative stress.** *Chem Rev* (2008) **108**(7):2585-2621.

27. Hu KK, Liu YL, Oleinick A, Mirkin MV, Huang WH, Amatore C: **Nanoelectrodes for intracellular measurements of reactive oxygen and nitrogen species in single living cells.** *Curr Opin Electroche* (2020) **22:** 44-50.

28. Hu KK, Li Y, Rotenberg SA, Amatore C, Mirkin MV: **Electrochemical measurements of reactive oxygen and nitrogen species inside single phagolysosomes of living macrophages.** *J Am Chem Soc* (2019) **141**(11):4564-4568.

29. Beissenhirtz MK, Scheller FW, Lisdat F: **A superoxide sensor based on a multilayer cytochrome c electrode.** *Anal Chem* (2004) **76**(16):4665-4671.

30. Wilson LR, Panda S, Schmidt AC, Sombers LA: **Selective and mechanically robust sensors for electrochemical measurements of real-time hydrogen peroxide dynamics in vivo.** *Anal Chem* (2018) **90**(1):888-895.

31. Li H, Zhao H, He H, Shi L, Cai X, Lan M: **Pt-pd bimetallic nanocoral modified carbon fiber microelectrode as a sensitive hydrogen peroxide sensor for cellular detection.** *Sensors and Actuators B: Chemical* (2018) **260**: 174-182.

32. Zhang H, Ruan J, Liu W, Jiang X, Du T, Jiang H, Alberto P, Gottschalk K-E, Wang X: **Monitoring dynamic release of intracellular hydrogen peroxide through a microelectrode based enzymatic biosensor.** *Anal Bioanal Chem* (2018) **410**(18):4509-4517.

33. Xu Q, Yuan H, Dong X, Zhang Y, Asif M, Dong Z, He W, Ren J, Sun Y, Xiao F: **Dual nanoenzyme modified microelectrode based on carbon fiber coated with aupd alloy nanoparticles decorated graphene quantum dots assembly for electrochemical detection in clinic cancer samples.** *Biosensors and Bioelectronics* (2018) **107**: 153-162.

34. Wang B, Wen X, Chiou P-Y, Maidment NT: **Pt nanoparticle-modified carbon fiber microelectrode for selective electrochemical sensing of hydrogen peroxide.** *Electroanal* (2019) **31**(9):1641-1645.

35. Li X, Zhou L, Ding J, Sun L, Su B: **Platinized silica nanoporous membrane electrodes for low-fouling hydrogen peroxide detection.** *ChemElectroChem* (2020) **7**(9):2081-2086.

36. Seven F, Gölcez T, ŞEn M: **Nanoporous carbon-fiber microelectrodes for sensitive detection of h2o2 and dopamine.** *Journal of Electroanalytical Chemistry* (2020) **864:** 114104.

37. Yuan H, Zhao J, Wang Q, Manoj D, Zhao A, Chi K, Ren J, He W, Zhang Y, Sun Y, Xiao F *et al*: **Hierarchical core–shell structure of 2d vs2@vc@n-doped carbon sheets decorated by ultrafine pd nanoparticles: Assembled in a 3d rosette-like array on carbon fiber microelectrode for electrochemical sensing.** *ACS Applied Materials & Interfaces* (2020) **12**(13):15507-15516.

38. Qi H, Song J, Fu Y, Wu X, Qi H: **Highly dispersive pt–pd nanoparticles on graphene oxide sheathed carbon fiber microelectrodes for electrochemical detection of h2o2 released from living cells.** *Nanotechnology* (2020) **31**(13):135503.

39. Sun W, Cai X, Wang Z, Zhao H, Lan M: **A novel modification method via in-situ reduction of auag bimetallic nanoparticles by polydopamine on carbon fiber microelectrode for h2o2 detection.** *Microchemical Journal* (2020) **154**(January):104595-104595.

40. Dong H, Zhou Y, Zhao L, Hao Y, Zhang Y, Ye B, Xu M: **Dual-response ratiometric electrochemical microsensor for effective simultaneous monitoring of hypochlorous acid and ascorbic acid in human body fluids.** *Anal Chem* (2020) **92**(22):15079-15086.

41. Peng Q, Yan X, Shi X, Ou S, Gu H, Yin X, Shi G, Yu Y: **In vivo monitoring of superoxide anion from alzheimer's rat brains with functionalized ionic liquid polymer decorated microsensor.** *Biosensors and Bioelectronics* (2019) **144**: 111665-111665.

42. Huang S, Zhang L, Dai L, Wang Y, Tian Y: **Nonenzymatic electrochemical sensor with ratiometric signal output for selective determination of superoxide anion in rat brain.** *Anal Chem* (2021) **93**(13):5570-5576.

*Recent report demonstarting ratiometric measurements for improving selectivity of superoxide measurements.

43. Dong H, Zhou Y, Hao Y, Zhao L, Sun S, Zhang Y, Ye B, Xu M: **"Turn-on" ratiometric electrochemical detection of h2o2 in one drop of whole blood sample via a novel microelectrode sensor.** *Biosensors and Bioelectronics* (2020) **165**: 112402-112402.

44. de Meira FHA, Resende SF, Monteiro DS, Pereira MC, Mattoso LHC, Faria RC, Afonso AS: **A non-enzymatic ag/delta-feooh sensor for hydrogen peroxide determination using disposable carbon-based electrochemical cells.** *Electroanal* (2020) **32**(10):2231-2236.

45. Liu X, Zhang X, Zheng J: **One-pot fabrication of aunps-prussian blue-graphene oxide hybrid nanomaterials for non-enzymatic hydrogen peroxide electrochemical detection.** *Microchemical Journal* (2021) **160**: 105595-105595.

46. Wu TD, Li L, Song GJ, Ran MM, Lu XQ, Liu XH: **An ultrasensitive electrochemical sensor based on cotton carbon fiber composites for the determination of superoxide anion release from cells.** *Microchim Acta* (2019) **186**(3): 198.

47. Gao QM, Zhao HL, Wang ZX, Cai X, Zhou LF, Lan MB: **Fabrication of hierarchically porous carbon networks for the electrochemical determination of superoxide anion released from living cells.** *Sensor Actuat B-Chem* (2021) **330**: 129309.

48. Zhao SF, Shi ZZ, Guo CX, Li CM: **A high-energy-state biomimetic enzyme of oxygen-deficient mntio3 nanodiscs for sensitive electrochemical sensing of the superoxide anion.** *Chem Commun* (2019) **55**(54):7836-7839.

49. Cai X, Shi LB, Sun WQ, Zhao HL, Li H, He HY, Lan MB: **A facile way to fabricate manganese phosphate self-assembled carbon networks as efficient electrochemical catalysts for real-time monitoring of superoxide anions released from hepg2 cells.** *Biosens Bioelectron* (2018) **102**:171-178.

50. Zheng JS, Wang B, Jin YZ, Weng B, Chen JC: **Nanostructured mxene-based biomimetic enzymes for amperometric detection of superoxide anions from hepg2 cells.** *Microchim Acta* (2019) **186**(2): 95.

51. Wang ZX, Zhao HL, Gao QM, Chen KC, Lan MB: **Facile synthesis of ultrathin two-dimensional graphene-like ceo2 & ndash;tio2 & nbsp;mesoporous nanosheet loaded with ag nanoparticles for non-enzymatic electrochemical detection of superoxide anions in hepg2 cells.** *Biosens Bioelectron* (2021) **184**: 113236

52. Sadeghian RB, Ostrovidov S, Han JH, Salehi S, Bahraminejad B, Bae H, Chen MW, Khademhosseini A: **Online monitoring of superoxide anions released from skeletal muscle cells using an electrochemical biosensor based on thick-film nanoporous gold.** *Acs Sensors* (2016) **1**(7):921-928.

53. Ozel RE, Wallace KN, Andreescu S: **Chitosan coated carbon fiber microelectrode for selective in vivo detection of neurotransmitters in live zebrafish embryos.** *Anal Chim Acta* (2011) **695**(1-2):89-95.

54. Brown MD, Schoenfisch MH: **Electrochemical nitric oxide sensors: Principles of design and characterization.** *Chem Rev* (2019) **119**(22):11551-11575.

**A comprehensive review on electrochemical NO sensors

55. Brown MD, Schoenfisch MH: **Selective and sensocompatible electrochemical nitric oxide sensor with a bilaminar design.** *Acs Sensors* (2019) **4**(7):1766-1773.

56. Mathew G, Narayanan N, Abraham DA, De M, Neppolian B: **Facile green approach for developing electrochemically reduced graphene oxide-embedded platinum nanoparticles for ultrasensitive detection of nitric oxide.** *ACS omega* (2021) **6**(12):8068-8080.

57. Oliveira R, Sella C, Souprayen C, Ait-Yahiatene E, Slim C, Griveau S, Thouin L, Bedioui F: **Development of a flow microsensor for selective detection of nitric oxide in the presence of hydrogen peroxide.** *Electrochim Acta* (2018) **286:** 365-373.

58. Li RF, Qi H, Ma Y, Deng YP, Liu SN, Jie YS, Jing JZ, He JL, Zhang X, Wheatley L, Huang CX *et al*: **A flexible and physically transient electrochemical sensor for real-time wireless nitric oxide monitoring.** *Nat Commun* (2020) **11**(1): 3207.

* Illustration of a flexible transient electrode for wireless monitoring of NO in vivo.

59. Ferrer-Sueta G, Radi R: **Chemical biology of peroxynitrite: Kinetics, diffusion, and radicals.** *Acs Chem Biol* (2009) **4**(3):161-177.

60. Amatore C, Arbault S, Bruce D, de Oliveira P, Erard M, Vuillaume M: **Characterization of the electrochemical oxidation of peroxynitrite: Relevance to oxidative stress bursts measured at the single cell level.** *Chem-Eur J* (2001) **7**(19):4171-4179.

61. Xue J, Ying XY, Chen JS, Xian YH, Jin LT, Jin J: **Amperometric ultramicrosensors for peroxynitrite detection and its application toward single myocardial cells.** *Anal Chem* (2000) **72**(21):5313-5321.

62. Kalil H, Maher S, Bose T, Bayachou M: **Manganese oxide/hemin-functionalized graphene as a platform for peroxynitrite sensing.** *J Electrochem Soc* (2018) **165**(12):G3133-G3140.

63. Peteu SF, Whitman BW, Galligan JJ, Swain GM: **Electrochemical detection of peroxynitrite using hemin-pedot functionalized boron-doped diamond microelectrode.** *Analyst* (2016) **141**(5):1796-1806.

64. Liu FX, Li L, Zhang BY, Fan WZ, Zhang RJ, Liu GA, Liu XH: **A novel electrochemical sensor based on microporous polymeric nanospheres for measuring peroxynitrite anion released by living cells and studying the synergistic effect of antioxidants.** *Analyst* (2019) **144**(23):6905-6913.

65. Li Y, Sella C, Lemaitre F, Guille-Collignon M, Amatore C, Thouin L: **Downstream simultaneous electrochemical detection of primary reactive oxygen and nitrogen species released by cell populations in an integrated microfluidic device.** *Anal Chem* (2018) **90**(15):9386-9394.

* Recent report demonstrating microfluidic detection of multiple radicals sumultaneously.

66. Lin L, Qian L, Weizhou F, Lan D, Xue Z, Xiuhui L, Xiaoquan L: **A novel ratiometric electrochemical sensing strategy for monitoring of peroxynitrite anion released from high glucose-induced cells.** *Sensors and Actuators, B: Chemical* (2021) **328,** 129071.

67. Jiang H, Zhang XW, Liao QL, Wu WT, Liu YL, Huang WH: **Electrochemical monitoring of paclitaxel-induced ros release from mitochondria inside single cells.** *Small* (2019) **15**(48):1901787.

68. Jin ZH, Liu YL, Fan WT, Huang WH: **Integrating flexible electrochemical sensor into microfluidic chip for simulating and monitoring vascular mechanotransduction.** *Small* (2020) **16**(9):1903204.

69. Vaneev AN, Gorelkin PV, Garanina AS, Lopatukhina HV, Vodopyanov SS, Alova AV, Ryabaya OO, Akasov RA, Zhang Y, Novak P, Salikhov SV *et al*: **In vitro and in vivo**

electrochemical measurement of reactive oxygen species after treatment with anticancer drugs. *Anal Chem* (2020) **92**(12):8010-8014.

70. Gubernatorova EO, Liu X, Othman A, Muraoka WT, Koroleva EP, Andreescu S, Tumanov AV: **Europium-doped cerium oxide nanoparticles limit reactive oxygen species formation and ameliorate intestinal ischemia-reperfusion injury.** *Adv Healthc Mater* (2017) **6**(14): 1700176.

71. Peng Q, Yan X, Shi X, Ou S, Gu H, Yin X, Shi G, Yu Y: **In vivo monitoring of superoxide anion from alzheimer's rat brains with functionalized ionic liquid polymer decorated microsensor.** *Biosens Bioelectron* (2019) **144**: 111665.

72. Wilson LR, Panda S, Schmidt AC, Sombers LA: **Selective and mechanically robust sensors for electrochemical measurements of real-time hydrogen peroxide dynamics in vivo.** *Anal Chem* (2018) **90**(1):888-895.

73. Miripour ZS, Sarrami-Forooshani R, Sanati H, Makarem J, Taheri MS, Shojaeian F, Eskafi AH, Abbasvandi F, Namdar N, Ghafari H, Aghaee P *et al*: **Real-time diagnosis of reactive oxygen species (ros) in fresh sputum by electrochemical tracing; correlation between covid-19 and viral-induced ros in lung/respiratory epithelium during this pandemic.** *Biosens Bioelectron* (2020) **165:** 112435.