INTRODUCTION

Physiologically, $\text{H}_2\text{O}_2$ is the longest lifetime reactive oxygen species (ROS), a natural by-product of mitochondrial aerobic respiration and cellular metabolism (Aran et al., 2015; Chandan & Sashwati, 2008). $\text{H}_2\text{O}_2$ is vital in modulating redox homoeostatic processes, hormones production, cell signalling, phagocytosis, apoptosis, and immuno-response to pathogenic infection. $\text{H}_2\text{O}_2$ is also produced in compromised tissue oxygenation due to disrupted vasculature in wound repair process and is associated with meat discoloration (Chandan & Sashwati, 2008).

Due to stress activating factors such as UV light, chemical agents, or compromised enzyme antioxidants radical scavengers, ROS cellular buildup could result. The increase in cellular ROS induces carbonylation reactions on unsaturated fatty acids, proteins, and DNA (Aran et al., 2015; Barrera, 2012; Chandan & Sashwati, 2008). These reactions trigger irreversible damage to neurons, inactivation of enzymes, and DNA damage via breakage and crosslinking. Redox medicine an emerging innovative field suggests elevated cellular $\text{H}_2\text{O}_2$ is a biomarker of oxidative stress—culpable for pathologies such as Alzheimer's, Parkinson's, cardiovascular disease, ageing, obesity, chronic inflammation, cancers, and viral infections (Barrera,

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**Abstract**

We report a flexible redox responsive polymer-based sensor for detection of reactive oxygen species (ROS). The sensor comprises multilayers of silver nanoparticles (AgNPs), carbon nanotube/cellulose nanocrystal (CNT/CNC) and a redox responsive poly (glycidyl methacrylate-co-ethylene glycol dimethacrylate-co-vinyl ferrocene), herewith called poly (GMA-co-EGDMA-co-Fc) nanoferrogels. These sensor layers were printed on a micropillared polydimethylsiloxane (PDMS) substrate. The nanoferrogel sensor versatility has been demonstrated in its effective detection of ROS species, specifically $\text{H}_2\text{O}_2$, peroxylipids, and oxidative deriving species such as cholesterol and unsaturated triglycerides. The nanoferrogel ROS sensor rapidly (~1 min) responds to both $\text{H}_2\text{O}_2$ and peroxylipids with a limit of detection (LOD) of ~0.060 ± 0.001 µg/ml and 0.012 ± 0.001 µg/ml, respectively. The cholesterol oxidase and lipoxygenase-based nanoferrogel sensor were successfully evaluated for the detection of cholesterol (LOD of 0.12 ± 0.02 µg/ml) and glyceryltrilinoeate (LOD of 1.8 ± 0.2 ng/ml) standards, respectively. These enzyme-loaded ROS nanoferrogel sensors were also evaluated for quantification of cholesterol and glyceryltrilinoeate in bacon lard and olive oils. The fabricated flexible ROS sensors are versatile for quantitation of oxidative stress biomarkers, useful in myriad applications including clinical, environmental, food, and plant physiology.

**KEYWORDS**

cholesterol detection, $\text{H}_2\text{O}_2$ detection, peroxylipids detection, poly (GMA-co-EGDMA-co-Fc) nanoferrogels, redox responsive polymers, ROS flexible capacitive sensors
Conventional amperomeric devices for H$_2$O$_2$ detection require Horseradish peroxidase enzyme, thus the sensors are unstable with short shelf life (Amiri & Arshi, 2020; Hossain & Park, 2017; Nguyen & Kasi, 2015). Similar to peroxidases, Prussian Blue ROS scavenging ability is associated with hydroxyl radicals affinity (Zhang, Hu, et al., 2016). As such, non-enzymatic amperometric systems that use Prussian Blue have predominantly been demonstrated for H$_2$O$_2$, and not other ROS (Komkova et al., 2017). Spectrophotometric, spectroscopic, electrochemiluminescent methods are common, however they have limited dynamic range, sensitivity, and require extensive sample preparation and derivatisation steps (Gu et al., 2016; Mátaï & Hideg, 2017). There is need for inexpensive sensitive devices for real-time in-vitro, and in-vivo ROS detection. At the heart of such a device, would be a molecular recognition element that allows for selective response to the ROS.

Other than H$_2$O$_2$, peroxylipids are ROS common intermediates in plant and animal physiological processes (Schoemaker et al., 1997). Peroxylipids are oxidation intermediates of unsaturated triglycerides. Peroxylipids can therefore be ROS indicator for quality of unsaturated oils (Aykas et al., 2020; Li & Wang, 2018). It is known the deficiency of essential fatty acids (EFAs) has been linked to cardiovascular and inflammatory disorders, and thus the need to quantify EFAs separate from total fats (James et al., 2000). Conventionally, lipids detection is carried by chromatographic and spectroscopic methods which are tedious (Lukic et al., 2020; Rejeb & Gargouri, 2011).

Responsive polymers are versatile materials gaining prominence as analyte specific molecular receptors for drug delivery, bioseparations, artificial actuators, tissue engineering, biochemical sensor devices, etc. (Culver et al., 2017; Zhang et al., 2015). Responsive polymers change their volume dimensions, morphology, shape, densities, or self-immolate in response to physical or (bio)chemical stimuli. Common stimuli include temperature, pH, light, redox potential, mechanical force, magnetic field, ionic strength, redox, enzymes or other small biomolecules. Of these polymer scaffolds, pH and temperature responsive polymers are the most studied (Culver et al., 2017; Zhang et al., 2015, 2016; Zhang, Hu, et al., 2016).

Compared to pH receptive, redox responsive polymers are more versatile and rapid to chemical and electrochemical reversible response, and thus ideal for ROS detection. In the literature, some redox responsive polymer scaffolds include supramolecular based (non-covalent), pendant located, and covalent crosslinked polymers. Common redox functional linkers integrated in responsive polymers include organochalcogen (Se or Te), arylboranes, tetrathiafulvalene, transition metal ions, sulphur-based moieties (e.g. thioethers, thiol, vinylthioether), and ferrocene scaffolds (Bas et al., 2014; Saleem et al., 2015; Senel et al., 2010; Wu et al., 2017). Of these, arylboranes and ferrocene linkers are the most promising due to their ease of integration as functional monomers in polymerisation, high responsivity to ROS, excellent electron transfer, commercial availability, relative environmental stability, low biotoxicity and cost of integration (Bas et al., 2014; Saleem et al., 2015; Senel et al., 2010; Wu et al., 2017). Improving the redox polymers for responsivity to ROS remains an area of interest. Redox polymers for ROS sensing should have the redox moiety within a polymer network that has polarity compatibility to analyte of interest. In this paper, Full Text investigated a redox responsive poly (glycidyl methacrylate-co-ethylene glycol dimethacrylate-co-vinyl ferrocene), herewith called poly (GMA-co-EGDMA-co Fc) nanoferrogels as a molecular receptor. The poly (GMA-co-EGDMA-co Fc) polymer embodies the appropriate hydrophobic character for lipophilic analytes compatibility. In addition, the pendant epoxy groups of the polymer play the role as immobilisation linkers to the amine groups of enzymes (Bas et al., 2014; Senel et al., 2010). Briefly, the ROS sensor comprises polydimethylsiloxane (PDMS) substrate coated with AgNPs, carbon nanotube/ cellulose nanocrystal (CNT/CNC) to create a conductive flexible substrate, which was then coated with the nanoferrogels. The nanoferrogels based flexible ROS sensor was evaluated for detection of H$_2$O$_2$, cholesterol, and peroxylipids.

## 2 | EXPERIMENTAL SECTION

### 2.1 | Materials and reagents

All aqueous solutions were prepared using >18 MΩ Milli-Q water (Millipore). Glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EDGMA), 4,4′-azobis(4-cyanopentanoic acid) (ACPA,) vinyl ferrocene (Fc), (3-glycidyloxypropyl trimethoxysilane) (GOPS) H$_2$O$_2$, cholesterol, 1-propanol, Tween 20, ammonium persulfate, carboxylic acid functionalised multiwalled carbon nanotubes (CNT) (diameter 9.5 nm × 1.5 µm), glycercyl trilinoleate, lipoxygenase, cholesterol, and peroxylipids.

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### 2.2 | Fabrication of the nanoferrogel-based ROS sensor

The nanoferrogel-based sensor assembly is illustrated in Figure 1a. The multilayered capacitive flexible nanoferrogel sensor comprised of poly (GMA-co-EGDMA-co-Fc) nanoferrogels, carbon nanotube/ cellulose nanocrystal (CNT/CNC) and silver nanoparticles (AgNPs) casted on a micropilled PDMS patch.
The PDMS/Ecoflex substrate was prepared as follows: The Sylgard 184 PDMS was prepared by mixing the cross-linker/curing agent in a 1:10 (wt/wt) mix ratio. The Ecoflex™ 00-30 rubber was prepared by mixing Ecoflex elastomer and hardener in a 1:1 (wt/wt) ratio. The PDMS blend and Ecoflex mixture were mixed in a 1:1 (wt/wt) ratio. A PDMS/Ecoflex mixture (8 g) was casted on a Petri dish (9 mm diameter) with the base glued with 100 µm grain size sandpaper. On curing at 72°C for 4 h, a PDMS/Ecoflex substrate ~2 mm was formed.

A 200 µl aliquot of AgNPs/CNC suspension deposited on the micropillared PDMS patch (1.5 cm × 2.0 cm) and air dried at room temperature resulting in a conductive film of 0.5 cm radius. The AgNPs suspension was prepared as described elsewhere (Mugo & Alberkant, 2020). To stabilise the AgNPs layer on the PDMS, a 10 µl mixture of 20% GOPS dissolved in 10 M glacial acetic acid. To the AgNPs film, a 100 µL aliquot of 0.0167% CNT in 0.067% CNC aqueous suspension was deposited and air dried.

To prepare the nanoferrogel polymer film, a mixture of 130 µl GMA, 10 µl EDGMA, 10 mg ACPA initiator and 2.6 mg vinyl ferrocene, were dissolved in 1 ml cyclohexanol. A 50 µl of this prepolymer suspension was deposited onto the CNT/CNC@AgNPs@PDMS. Polymerisation ensued in an 80°C oven overnight, resulting in the nanoferrogel@CNT/CNC@AgNPs@PDMS sensor electrode.

To prepare sensors for cholesterol and essential fatty acids detection, the nanoferrogel-based sensors, were modified by addition of a 50 µl aliquot of 1 µg/ml cholesterol oxidase and lipoxygenase enzymes (in 0.1 M phosphate buffer, pH 7.4), respectively. Following the addition of the enzymes, the ROS sensors were dried under nitrogen flow and stored in the fridge until they were ready for testing.

### 2.3 Morphology characterisation of nanoferrogel-based ROS sensor

The multilayered sensor morphology and the synthesised nanoferrogels were characterised using Zeiss EVO scanning electron microscopy (SEM) with LaB6 electron source (resolution ~100 nm) and equipped with a Bruker energy dispersive X-ray spectroscopy (EDX). The nanoferrogel@CNT/CNC@AgNPs@PDMS sensor was connected to the alligator clips of the potentiostat as shown in set up depicted in Figure 1a. During the sensor fabrication the CNT/CNC@AgNPs@PDMS, nanoferrogel@CNT/CNC@AgNPs@PDMS, and enzyme-loaded nanoferrogel@CNT/CNC@AgNPs@PDMS sensor films, were evaluated by cyclic voltammetry (CV), to probe the surface area and electrochemical properties changes. All electrochemical measurements were acquired using Palmsens 4 potentiostat with

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**Figure 1** (a) Schematic of multilayered assembly of the nanoferrogel@CNT/CNC@AgNPs@PDMS ROS sensor; (b) Reaction schematic of the nanoferrogel polymer preparation.
PSTrace software (PalmSens BV), with Bluetooth integration and capability for use of smartphone as the interface for data acquisition.

2.4 | Electrochemical measurements for the ROS sensor

Cyclic voltammetry was used as the electrochemical technique of choice to evaluate ROS sensor performance. The nanoferrogel ROS sensor was used as the working electrode, platinum as auxiliary electrode and in-house fabricated Ag/AgCl reference electrode. The electrochemical sensing set up is shown in Figure 1a. The fabrication for the in-house Ag/AgCl has been described elsewhere (Mugo & Berg, 2019).

The CV method consisted of a start and end potential of −0.95 to 0.95 V and a scan rate set at 0.1 V/s. The CV data was used to calculate capacitance signal for the ROS sensor. At the 0–0.2 V range, the capacitance was calculated by taking the ratio of electrical current and the scan rate. The change in cathodic capacitance voltammogram was determined by the ratio of the capacitance of the sample less that of blank (0.1 M phosphate buffer (pH = 7), divided by capacitance of the blank. The nanoferrogel sensor was evaluated for response to 0–1.2 µg/ml H₂O₂ standards. To evaluate the nanoferrogel ROS sensor, a 50 µl aliquot of 0.1 M phosphate buffer (pH 7.0) was deposited on the sensor and the blank cyclic voltammogram acquired. To the 50 µl blank on the sensor, 10 µl aliquots of 10 µg/ml of H₂O₂ standard were successively spiked, with cyclic voltammograms acquired in triplicate after every addition. Before acquiring the cyclic voltammograms, a 1 min residence time was allowed for molecular interactions between the nanoferrogel polymer and the H₂O₂ to ensue. Similarly, the nanoferrogel sensor was evaluated for response to 0–0.04 µg/ml peroxylipids, another intermediate ROS of relevance to plant and animal physiology.

2.5 | Synthesis of peroxylipid standard

To test the nanoferrogel ROS sensor beyond H₂O₂, peroxylipids was synthesised. The oxidation reaction schematic is shown in Figure 2a. The enzymatic oxidation synthesis procedure was adapted from literature with modifications (Schoemaker et al., 1997). Briefly, standard peroxylipids were prepared by sonicating 100 mg of glyceryl trilinoleate with 5 ml of 1 mg/ml of soybean lipoygenase in 0.1 M phosphate buffer (pH 7.0), for 2 h at room temperature. The oxidation reaction was then stopped by addition of 150 µl of 1 M HCl for a final pH of 4. The peroxylipid fraction was extracted by liquid–liquid extraction (LLE) using 1 ml of chloroform, and the bottom lipid layer was extracted using by a Pasteur pipette. The LLE procedure was repeated four times. Subsequently, the water residue in the peroxylipid was removed by addition of anhydrous MgSO₄, followed by microfiltration to remove the salt. The chloroform in the peroxylipid was removed by evaporation using nitrogen gas. A working 1.0 µg/ml peroxylipid standard concentration in deionised water with 2% Tween was prepared.

2.6 | Testing the nanoferrogel ROS sensor for peroxylipid detection

To evaluate the nanoferrogel ROS sensor response to peroxylipid, a 50 µl of 0.1 M phosphate buffer (pH 7.0) was deposited on the sensor and the blank cyclic voltammogram acquired. To the 50 µl buffer on the sensor, 10 µl aliquots of 1.0 µg/ml of peroxylipid standard were successively spiked, with cyclic voltammograms acquired in triplicate after every addition. Before acquiring the cyclic voltammograms, a 1 min residence time was allowed for molecular interactions between the nanoferrogel polymer and the peroxylipid to ensue. The sensors were tested for detection of 0–0.04 µg/ml peroxylipids.
2.7 | Enzyme-loaded nanoferrogel sensor for detection of unsaturated oils and cholesterol

The cholesterol oxidase and lipoxygenase immobilised nanoferrogel ROS sensors were evaluated for their detection of cholesterol and unsaturated oils, respectively. As illustrated in Figure 2b, cholesterol oxidase oxidises cholesterol to H2O2, detectable by the nanoferrogel ROS sensor (Amiri & Arshi, 2020; Lee et al., 2018). The sensors were tested by first acquiring a blank cyclic voltammogram of a 50 µl of 0.1 M phosphate buffer (pH 7.0). This was followed by incremental spike of a 10 µl aliquots of 10 µg/ml cholesterol and 1.0 µg/ml peroxylipid standard to the corresponding enzyme-loaded ROS sensors, with cyclic voltammograms acquired in triplicate after every addition. For each standard, five incremental additions were made, and a standard addition calibration plot generated.

2.8 | Enzyme-loaded ROS nanoferrogel sensor testing on real samples and interfering species.

ROS sensors immobilised with cholesterol oxidase and lipoxygenase were used to detect cholesterol ion in bacon lard and unsaturated triglycerides in olive oils, respectively. The bacon lard was obtained from bacon strips purchased from a local grocery store. The bacon strips were baked on a cooking pan and 0.5 mg of grease collected. The fat was dissolved in 7 ml dichloromethane, filtered and the solvent evaporated. A 10 µg/ml of bacon fat dissolved in 0.1% tween in 0.1 M phosphate buffer was prepared for analysis. A cholesterol oxidase immobilised ROS sensor was used to analyse the cholesterol in the bacon fat. First, a blank cyclic voltammogram of a 50 µl aliquot of 0.1 M phosphate buffer (pH 7.0). This was followed by spiking a 10 µl aliquot of 10 µg/ml of bacon fat and cyclic voltammograms acquired. Incremental spiking of 10 µl additions of 10 µg/ml of cholesterol standard were carried out and analysed by the sensor after each addition. A standard addition calibration plot generated.

The cholesterol oxidase immobilised ROS sensor was also evaluated for its specificity to cholesterol by evaluating its relative response to interfering species including 1.0 µg/ml each for estrone and estriol standards dissolved in deionised water with 0.1% tween.

The lipoxygenase immobilised ROS sensor was tested for detection of unsaturated triglycerides in olive oil. A 10 µg/ml of olive oil in 0.1% tween mixed in 0.1 M phosphate buffer was prepared. A blank cyclic voltammogram of a 50 µl of 0.1 M phosphate buffer (pH 7.0), followed by spiking a 10 µl aliquots of 10 µg/ml of olive oil sample and cyclic voltammograms acquired. Incremental spiking of 10 µl additions of 1.0 µg/ml of glyceryl trillinoleate standard were carried out and analysed by the sensor after each addition. A standard addition calibration plot generated.

3 | RESULTS AND DISCUSSION

3.1 | Surface morphology and electrochemical characterisation of nanoferrogel ROS sensor

The SEM was used to characterise the morphology of the multilayered ROS sensor, comprising of the PDMS micropillar substrate, AgNPs, CNC/CNT and the poly (GMA-co-EGDMA-co-Fc) nanoferrogels films. The PDMS/Ecoflex sensor substrate is a flexible and stretchable material with an excellent wearability for uneven surfaces (Mugo & Alberkant, 2020). The SEM in Figure 3a, show the high surface area nanoporous fibrillar network of the CNC/CNT conductive layer. The nanoferrogels polymer provided the dual function of responding to ROS, and as enzyme anchoring platform for cholesterol oxidase or lipoxygenase via the epoxide linkage (Figure 1a). The fabricated nanoferrogel sensor film was tested for structural stability even in presence of water, and was found to be stable, affording multiple use without peeling off. The stability could be attributed to hydrogen bonding effects of the CNC/CNT interpenetrating networked film to the nanoferrogel layer (Mugo & Alberkant, 2020). The sensor surface area is further increased due to the micropillared structured architecture of the PDMS substrate, evident in Figure 3b. The inset in Figure 3b, show the EDX spectra with a micrograph, which evidence of the ferrocene redox moiety distribution in the nanoferrogel polymer film.

To probe the surface area and electrochemical properties changes, CNT/CNC@AgNPs@PDMS, nanoferrogel@CNT/CNC@AgNPs@PDMS, and lipoxygenases-loaded nanoferrogel@CNT/CNC@AgNPs@PDMS films, were evaluated by CV using .1 M phosphate buffer (pH 7.0) as electrolyte. As shown in the overlapped CV data in Figure 3c, the addition of nanoferrogel and the lipoxygenase layers consistently decreased the electron conductivity of the conductive PDMS layer, attributed to increased electron transfer resistance. The electron conductivities between cholesterol oxidase ad lipoxygende enzymes were not different, and as such only lipoxygenase-based sensor is shown here.

3.2 | Nanoferrogel sensor electrochemical testing for H2O2

Using CV, the fabricated ROS nanoferrogel sensor film was tested for its responsivity to H2O2 standards. Following exposure to H2O2 or other redox species to the sensor, the nanoferrogel polymer entrained in the CNC/CNT layer is oxidised to a higher charge state with higher hydrophilicity, resulting in volume change and change in the ROS sensor film capacitance (Saleem et al., 2015; Zhang, Berg, et al., 2016; Zhang, Hu, et al., 2016). Figure 4a shows the
overlapped voltammograms of the nanoferrogel sensor exposure to a range, 0–1.1 µg/ml of H₂O₂. Figure 4b, show the corresponding linear calibration that resulted. Each H₂O₂ standard was analysed in triplicate. Determined as three times standard deviation of the blank divided by calibration sensitivity, the limit of detection (LOD) for H₂O₂ was determined to be 0.0600 ± 0.002 µg/ml, with a calibration sensitivity of 70.8 µF/µg ml⁻¹. The high responsivity of the nanoferrogel sensor indicates it can be used as a non-enzymatic platform for H₂O₂ sensing, a ROS metabolite of significant physiological relevance. The LOD of the nanoferrogel ROS sensor is in the µM range comparable to other fabricated devices reported in the literature (Peng et al., 2019; Shamkhalichenar & Choi, 2020). However, the nanoferrogel ROS sensor has the advantage of flexibility and reusability.
The nanoferrogel ROS sensor was evaluated for its reusability and stability with storage. A 0.1 M phosphate buffer blank and a 1.0 µg/ml H₂O₂ standard was analysed by the same sensor once every day (with each analysis done in triplicate) over 10 days. Four sensors were evaluated in this study. The sensors were stored in room temperature. Following each use, the sensors were electrochemically cleaned using phosphate buffer as described in the methods section. A representative capacitance data for the 1.0 µg/ml H₂O₂ standard and buffer measurement is shown in Figure 4c. Evidently, the capacitance barely changed over the 10 days of storage, usage, electrochemical cleaning cycling. Moreover, based on the data from the four different nanoferrogel ROS sensors, the inter-batch sensor reproducibility was 3.1% relative standard deviation (%RSD).

### 3.3 Nanoferrogel sensor electrochemical testing for cholesterol

The versatility of the nanoferrogel ROS sensor was evaluated by testing the response of cholesterol oxidase sensor to cholesterol detection. Cholesterol is a steroid ubiquitously present as a constituent in plasma membrane and acts as a precursor for bile acids, vitamin D and hormones etc. (Amiri & Arshi, 2020; Lee et al., 2018). As illustrated in Figure 2b cholesterol is oxidised to H₂O₂ by cholesterol oxidase. Figure 5a,b show the overlapped voltammograms resulting from the response of cholesterol oxidase-loaded and enzyme-free ROS sensor to cholesterol ranging 0–1.2 µg/ml, respectively. Figure 5c, show the correspondent linear calibrations from both ROS sensor response. The sensitivity of cholesterol oxidase base sensor was 53% times higher than the enzyme-free ROS sensor. The LOD for the cholesterol oxidase-based ROS sensor was determined to be 0.12 ± 0.02 µg/ml, well below the normal ≤2000 µg/ml blood cholesterol and ≤46 µg/ml in saliva (Amiri & Arshi, 2020; Lee et al., 2018). The cholesterol oxidase-loaded ROS sensor had a calibration sensitivity of 1.97 µF/µg ml⁻¹.

To evaluate the cholesterol oxidase-based ROS sensor for selectivity to cholesterol, the sensor was evaluated for its detection of estrone and estriol as interfering species. Both these hormones bear structural similarity to cholesterol. As shown in the overlapped voltammogram in Figure 5d, exposure of the cholesterol oxidase ROS sensor to 50 µl aliquot of 1 µg/ml estrone and estriol resulted in minimal detectable signal, compared to a similar concentration spike of cholesterol standard. This confirms the cholesterol oxidase nanoferrogel ROS sensor specificity and sensitivity to cholesterol detection.

### 3.4 Nanoferrogel-based sensor by electrochemical testing for peroxylipids

The nanoferrogel ROS sensor was tested for its response to other redox species beyond, H₂O₂. The enzyme-free and lipoxygenase-loaded nanoferrogel sensors were evaluated for their response to peroxylipids and their precursor glyceryl trilinoleate. These compounds were tested in the 0.00167–0.0367 µg/ml range, with each standard tested in triplicate. The lipoxygenase immobilised on the sensor results in in-situ oxidation of glyceryl trilinoleate to peroxylipids. Figure 6a,b show the overlapped voltammograms for enzyme-free and lipoxygenase-loaded nanoferrogel sensors for their response to peroxylipids and glyceryl trilinoleate standards, respectively. Figure 6c show the corresponding linear responses for both sensors. The nanoferrogel sensor response to peroxylipids, thus useful in proving ROS species beyond H₂O₂, of relevance in foods and medical diagnostics. While the response of the nanoferrogel ROS sensor to both H₂O₂ and peroxylipids could put into question the specificity of the sensor, in general these species would rarely be present simultaneously. In addition, in both food and medical
diagnostics, the focus is often less on speciation but in general the sample ROS intermediate bulk quantitation. Comparable to $H_2O_2$, the peroxylipids induces oxidation of nanoferrogel, resulting in a volume change of the nanoferrogel and a concomitant change in capacitance of the entire film. The nanoferrogel ROS sensor LOD for peroxylipids was determined to be $0.012 \pm 0.002 \mu g/ml$.

Notably, the nanoferrogel ROS sensor can be employed to quantify the quality of the unsaturated oils, of importance due to their utility as fatty acids (EFAs), for example, linoleic acid and \( \alpha \)-linolenic acid. Quantitation of EFAs are especially relevant as their deficiency has been linked to cardiovascular and inflammatory disorders (Schoemaker et al., 1997). Determining the EFAs is an important benchmark for quality of high value oils such as extra virgin oil. The quality of extra virgin oils deteriorates with storage due to oxidation of unsaturated oils to peroxylipids and their oxylipin degradation products, resulting in inferior quality and unpleasant taste. Oxylipins are known to be harmful to cells by inducing DNA damage (Aykas et al., 2020; Li & Wang, 2018; Schoemaker et al., 1997). Some unscrupulous merchants deodorise olive oils and mislabel as extra virgin oils. As such, rapid analytical methods to quantify polyunsaturated oil quality is essential.

As shown in Figure 6c, the lipoxygenase immobilised nanoferrogel sensor responds linearly to different concentrations of glyceryl trilinoleate standards. The lipoxygenase on the nanoferrogel oxidises the glyceryl trilinoleate to form peroxylipids, the redox species. The LOD of the lipoxygenase-based nanoferrogel sensor to glyceryl trilinoleate was $1.8 \pm 0.2 \mu g/ml$ and demonstrated good linearity in the tested concentration range. The sensor % relative standard deviation was below 3.2%, indicative of the sensor precision and reliability. As shown in Figure 7c, the lipoxygenase-based nanoferrogel sensor had a calibration sensitivity of $377 \mu F/\mu g ml^{-1}$.

### 3.5 Analysis of bacon fat and olive oil by enzyme-loaded nanoferrogel ROS sensors

The enzyme-loaded nanoferrogel ROS sensors were evaluated for their detection of real samples. The cholesterol oxidase-loaded nanoferrogel sensor was used to detect cholesterol in bacon lard. A blank voltammogram of a 50 µl aliquot of 0.1 M phosphate buffer on the sensor was first acquired. A 10 µl aliquot of the 10 µg/ml bacon fat sample was then added to the ROS sensor and a cyclic voltammogram acquired. This was followed by five 10 µl incremental spike additions of the 10 µg/ml cholesterol standard. Cyclic voltammograms were acquired in triplicate after each addition. Figure 7a,b shows the overlapped voltammograms and the correspondent standard addition linear calibration graph. The concentration of cholesterol in the original bacon fat was calculated to be $0.050 \pm 0.03\%$ which is within the 1% accuracy to the nutritional label of the bacon tested.

The same procedure was used to evaluate the lipoxygenase immobilised nanoferrogel ROS sensor to quantify glyceryl trilinoleate in olive oil. A blank voltammogram of a 50 µl aliquot of 0.1 M phosphate buffer on the sensor was first acquired. A 10 µl aliquot of 10 µg/ml Petrelli Extra Virgin pure olive oil was then added to the sensor and a cyclic voltammogram acquired. This was followed by five 10 µl incremental spike additions of the 10 µg/ml glyceryl trilinoleate. Cyclic voltammograms were acquired in triplicate after each addition. Figure 7c,d show the overlapped voltammograms and

| FIGURE 6 | (a) Overlapped cyclic voltammogram responses for different concentration of peroxylipid on an enzyme-free nanoferrogel ROS sensor; (b) Overlapped cyclic voltammogram responses for different concentrations of glyceryl trilinoleate on a lipoxygenase immobilised nanoferrogel ROS sensor; (c) Corresponding capacitance (µF) linear calibrations for peroxylipid on an enzyme-free ROS sensor compared to that of glyceryl trilinoleate concentrations on a lipoxygenase immobilised nanoferrogel ROS sensor |
the correspondent standard addition linear calibration graph for triglycerides in olive oil. The concentration of unsaturated triglycerides in olive oil was calculated to be 68 ± 2% which is within the normal range of unsaturated triglycerides in olive oil (Lukic et al., 2020).

The ability of the nanoferrogel sensors to detect cholesterol and unsaturated triglycerides in both bacon and olive oil, respectively, validates the ROS sensor versatility for applications in food analysis.

4 | CONCLUSIONS

A capacitive sensor based on redox responsive poly(GMA-co-EGDMA-co-Fc) nanoferrogels has been demonstrated as effective in detection of redox species, specifically H₂O₂ and peroxylipids. The article demonstrates the nanoferrogel functional capacity as a scaffold to encapsulate enzymes, such as cholesterol oxidase and lipoxygenase, lending the versatility of the sensor in detection of cholesterol and glyceryl trilinoleate, which produce H₂O₂ and peroxylipids as oxidative products, respectively.

The ROS sensors were highly reproducible with an inter-batch precision of ≤3.2%RSD. The enzyme-based nanoferrogel ROS sensors were validated for detection of cholesterol and glyceryl trilinoleate in bacon fat and olive oil, respectively. With appropriate enzyme loading the versatile nanoferrogel ROS sensor could be expanded to other relevant biochemical such as biogenic amines, choline, lactate etc. In addition, the ROS nanoferrogel platform could be employed for drug encapsulation and controlled delivery systems, dynamic platforms of interest in theranostics.

ACKNOWLEDGMENTS

Mugo research group acknowledges funding from, the Natural Sciences and Engineering Research Council of Canada (NSERC), MacEwan University Research, and Alberta Innovates Summer Studentship Program. We also acknowledge the assistance of Lisa Mugo (St. Mary’s Elementary, Edmonton) in designing the graphical abstract.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Mugo SM, Lu W. A versatile reactive oxygen species-responsive gels sensor for analysis of metabolic species. Med Devices Sens. 2020;3:e10131. https://doi.org/10.1002/mds3.10131