Free radical detection in living cells with relaxometry

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DOI:
10.33612/diss.180852826

Chapter

1. General Introduction

1.1 Free radicals in biological systems

Free radicals are chemically active molecules with one or more free unpaired electrons. This free electron causes free radicals to be reactive. They play many beneficial roles in biological systems. Some physiological processes such as cell signaling, homeostasis, cell death, immune responses, and induction of mitogenic response are dependent on proper free radical production and presence inside the biological system at low level [1,2]. However, when there is an accumulation of free radicals inside cells during metabolism or inadequate clearance, they start to cause damage to important cellular structures such as DNA, lipids, and proteins [3].

1.2 Free radical detection

The study of free radicals is crucial as it can be linked to a lot of diseases including cardiovascular diseases, cancer as well as viral or bacterial infections. However, detecting free radicals is very challenging due to the fact that they are produced in very small concentrations and have a short life span. Therefore, measuring radical concentrations is challenging for the state of the art. Free radicals can be evaluated using different methodologies based on the quantification of radicals directly or indirectly by measuring the response of a cell or damage caused by radicals [4].

For instance, the use of fluorescent dyes such as H_2DCFDA to measure radicals directly is a relatively easy and sensitive method. This dye fluoresces after reacting with free radicals. However, this technique is not specific and is sensitive to many types of reactive molecules and the dye is bleaching, which limits long-term use. Additionally, this method reveals the history of free radical generation in a sample, rather than the current status [5].

As free radicals have free unpaired electrons they can be detected by electron spin resonance (ESR) spectroscopy. However, this technique has limitations when it comes to high spatial resolution measurements. This is especially problematic when only a small amount of sample is available. Most radicals are also not stable long enough to be detected and thus it is often required to use spin labels [6]. These spin labels react with the radicals and form a relatively stable radical which can then be detected.

One of the gold standard techniques is magnetic resonance imaging (MRI). However, as in ESR the sensitivity of an MRI is limited to large spins ensembles, which limit the spatial resolution to millimeter to micrometer scales [7].

Since free radicals are short lived and reactive it is usually hard to detect them directly. Thus, measuring the damage they cause to (DNA, lipids, and proteins) or the response of the cells to their presence instead are practical alternatives. The methods that are currently used to assess radical and/or ROS production in clinical samples are summarized in Table1.

Technique	Method	Advantages	Disadvantages
2,4 dinitropheenylhydrazine (DNPH) [4]	Detection of Protein carbonyl	Accessible and inexpensive	Laborious and time-consuming because it requires protein precipitation
High-performance liquid chromatography (HPLC) [8]	Detection of lipids, separate, identify, and quantify each component in a mixture	Speed, high sensitivity and specify	Cost, and complexity
Gas chromatography-mass spectrometry (GC-MS) [9]	Separate chemical mixtures and identifies the components at a molecular level	High specificity, selectivity, sensitivity, identify a wide range of DNA base products	Limited to thermally stable and volatile compounds
Enzyme-linked immunosorbent assay (ELISA) [10,11]	DNA damage marker, based on antigen–antibody reaction	Easy, high specificity and sensitivity	Price, inaccuracy, and insufficient blocking of immobilized antigen
Capillary zone electrophoresis (CZE) [12]	Separation of charged molecules (DNA, RNA, proteins) and transport them by an electrical field	High separation efficiency, speed, low sample consumption, low waste, ease	Relatively poor accuracy
Quantitative polymerase chain reaction [13]	Evaluating the relative gene expression of oxidative stress genes	Sensitivity, ability to compare damage to nuclear (nDNA) and to mitochondrial (mtDNA) from the same sample	Susceptible to inhibitors present in some biological samples
RNA sequencing [14,15]	Quantify and sequence of RNA in a sample, analyse genes expression	High dynamic range, not reliant on previous sequence information, high accuracy	Requires high power computing facilities, cost, analysis can be complex.

Table 1 A summary of indirect methods for free radical detection. The table summarizes how different assay work, either by detecting the damage in a cell to DNA, lipids, or proteins or by measuring the response from a cell.

1.3 Diamond magnetometry for free radical detection

Diamond magnetometry uses nanodiamonds or bulk crystals containing defects called nitrogen-vacancy (NV) centres. These NV centers can convert magnetic noise into optical signals. The basic principle is shown in figure 1 [16]. The presence of this defect gives nanodiamonds unique optical properties. First, they are fluorescent and emit a stable red signal once excited by a green laser. This signal also changes depending on its magnetic surrounding. As a result, they can sense very small quantities of magnetic noise as optical signals are easier to measure than magnetic signals.

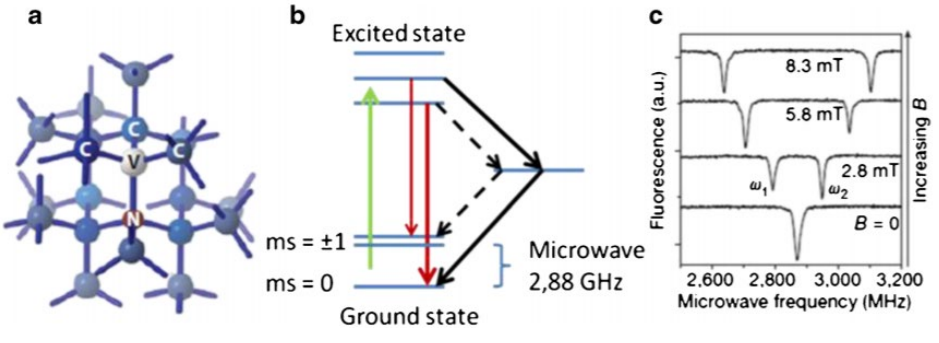

Figure 1 (a) Lattice structure of the NV centre in a diamond crystal. (b) The characteristics of the NV^- centre energy level diagram. Once an NV^- is excited by a green laser it emits red photons. When the electron was in the $m_s = \pm 1$ state there is a greater probability that the electron goes to the ground state via a dark state. As a consequence, fewer red photons are emitted and we will observe a decay of red-light intensity. (c) Electron paramagnetic resonance (EPR) spectrum of an NV centre with and without magnetic field. This phenomenon can be observed by applying a microwave frequency equal to the difference between the energies of the two states. This equals to 2.88 GHz at zero field (bottom curve in (c)). Once an external magnetic field is applied the energy between the two states is no longer equal. Therefore, there will be a split as shown in the other three curves in (c). This difference is related to the field (Zeeman splitting) and the magnetic field can be calculated from there.

1.4 Thesis objective and outline

The focus in this thesis is to use diamond magnetometry as a tool to measure free radicals in living cells and important and necessary milestones needed to reach this goal. The first step was to study the fate of nanodiamonds in cells. In order to get more meaningful information about radical formation intracellular targeting nanodiamonds to specific organelle is required. Since it is unknown what happens to nanodiamonds over time, in **chapter 2** we studied the fate of nanodiamonds in yeast cells. Then, we wanted to have better control of the location of our nanodiamonds. To this end, we modified our nano particles with specific antibodies to target them to cell nuclei described in **chapter 3**. In **chapter 4**, we performed diamond magnetometry measurements in living cells under different shear stress conditions. Low and high shear stress (in regimes relevant for venes and arteries) were applied to observe how shear stress affects the production of free radicals. In **chapter 5** we measured free radical generation using diamond magnetometry in boar sperm cells to study their capacitation process. In **chapter 6** we discuss the possibilities to valorise this technology, and its potential markets. Finally, in **chapter 7** we discuss the importance of the data in this thesis, and future perspectives of this research in different fields.

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