Diamond based relaxometry for biosensing

Sharmin, Rokshana

DOI:
10.33612/diss.229110585

Chapter 1

General Introduction

The role of free radical in Diseases:

In recent years there is an increase in curiosity for studying the role of free radicals in biology. They play a crucial role in various physiological conditions and are involved in a diverse range of diseases. Free radicals are reactive atoms or groups of atoms containing one or more unpaired electron(s). They are highly reactive and stabilize by obtaining electrons from outside in order to fill up empty orbitals. Some examples of free radicals are superoxide ($O_2^{\cdot-}$), oxygen radical ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), alkoxyradical ($RO^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), nitric oxide (nitrogen monoxide) ($NO^{\cdot}$) and nitrogen dioxide ($NO_2^{\cdot}$) [1]

The first time in 1900, Moses Gomberg, Professor of Chemistry at the University of Michigan, proved the existence of an organic free radical, triphenyl methyl radical ($Ph3C^{\bullet}$) in a living system [2]. The electron paramagnetic resonance (EPR) studies by Commoner et al. 1954 [3] confirmed the presence of free radicals in biological materials. The free radicals can be both beneficial and toxic compounds to the living system. At moderate or low levels free radicals are responsible to maintain various physiological functions such as in immune function (i.e. defense against pathogenic microorganisms), a number of cellular signaling pathways and mitogenic response and redox regulation [4,5]. But at higher concentration, free radicals generate oxidative stress causing damage to biomolecules such as nucleic acids, proteins and lipids. The oxidative stress is developed when there is an excess production of free radical due to pathological condition on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other side.

Due to very short lifetime and high reactivity free radicals attack other biological molecules and degrade biomolecules. This process plays an important role in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular diseases, liver and neurodegenerative diseases [6].

Atherosclerosis is a cardiovascular disease which develops in the arterial wall where a lipid plaque is formed and narrowing the arterial wall restricts the

blood flow. Atherosclerosis develops in area of acute curvatures, branch points and bifurcations that are associated with disturbed flow resulting in low and reversal vascular wall shear stress [7]. In the very early stage of Atherosclerosis development, vascular endothelial cells increase the production of free radicals due to low or disturbed shear stress. Subsequently endothelial cells lose their tight junction and increase the permeability for blood components including monocytes and molecules like low density lipoprotein (LDL) through the junctions. Under normal conditions, monocytes move through the blood and do not attach to the endothelial cells. However, when endothelial cells are damaged, they produce adhesion molecules that catch the monocytes causing morphological changes and allow monocytes to squeeze between the endothelial cells. Monocyte cells are capable to produce free radicals which oxidize the LDL. Immune cells engulf these oxidized LDL and form foam cells. Foam cells ultimately die and form a plaque. These plaques cover the endothelial cells, narrow the blood vessel and cause Atherosclerosis. From the
Atherosclerosis development pathway, it is clear that cells first increase the production of free radicals and subsequently undergo cellular damage and develop Atherosclerosis.

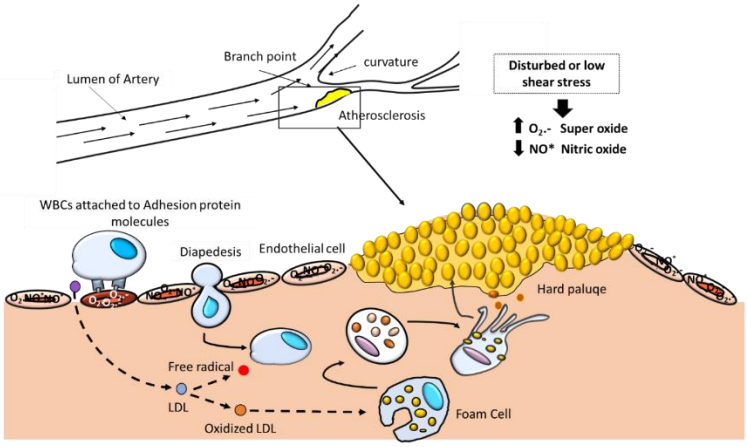

Figure 1: A schematic diagram showing the progression of Atherosclerosis when endothelial cells are exposed to a disturbed or lower shear stress. Lower or disturbed shear stress causes the endothelial cells to increase the production of superoxide (O2*-) and decrease nitric oxide (NO*) production. As a result, endothelial cells lose their tight junctions and start Atherosclerosis progression.

Another area where radicals are crucial, that is relevant for this thesis, is the role of free radicals in liver toxicity. Free radicals cause liver diseases by damaging hepatic tissue. Paracetamol (acetaminophen) is a safe and effective analgesic and antipyretic drug when used at therapeutic doses. However, an overdose causes severe liver injury both in experimental animals and in humans. Physiologically, APAP is metabolized in the liver, by cytochrome P450s [3,5] of the active metabolite N-acetyl-P-benzoquinone imine (NAPQI) which is efficiently detoxified by conjugation with glutathione [6,7]. Excess amounts of paracetamol cause the depletion of glutathione, and covalent binding of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) to cellular proteins. This pathway is the main cause of hepatic cell death. Cell death include mitochondrial dysfunction and, importantly, the formation of ROS and peroxynitrite. Oxidant stress of mitochondria triggers mitochondrial membrane permeability, loss of the membrane potential of the mitochondria, depletion of ATP, and release of intermembrane proteins that are responsible for the typical nuclear DNA fragmentation of APAP-induced cell death [8]

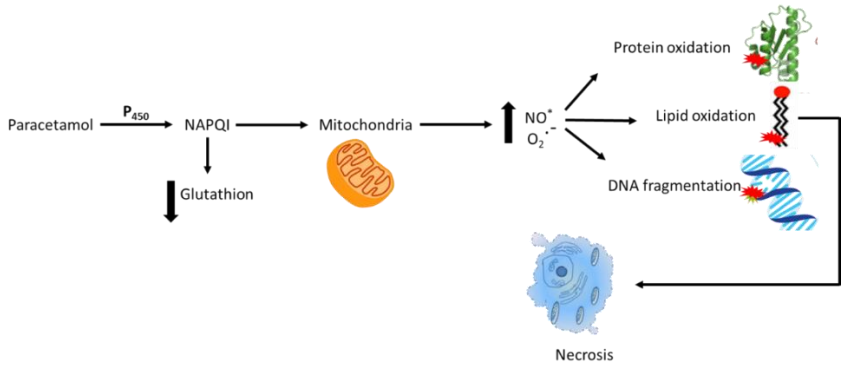

Figure 2: Schematic representation of overdose paracetamol(APAP) induced cell necrosis.

As a result, detection of excess amount of free radical generation in cells helps to understand pathological conditions.

Methods for detecting radicals

It is very difficult to determine the accurate location and quantify free radicals due to their instability, short lifetime and mutual interference with other molecules.

Many methods are available to detect free radicals such as spectrophotometry methods, fluorescent and chemiluminescence probes, and electron spin resonance (ESR/EPR). Furthermore, recently developed Diamond magnetometry, based on fluorescence nanodiamond particles, has been used for detecting intracellular free radicals.

Fluorescent probes are non-fluorescent before being oxidized by oxygen species. The most widely used members are dihydroethidium and dichlorodihydro fluorescein. Most of these probes are oxidized by a one-electron free radical mechanism, yielding a probe radical intermediate and resulting in fluorescent products. For example, 20 ,70 -dichlorodihydro fluorescein diacetate (DCFH-DA) is regularly used for the direct measurement of intracellular redox states. It is able to enter cells and is hydrolyzed by esterase to membrane impermeable DCFH, which diffuses inside the cell and is oxidized by nonspecific oxygen radicals to generate fluorescent DCF, through a nonfluorescent intermediate free radical DCFHc.

Early research studies have proved that the fluorescence intensity of DCF is correlated with the levels of oxidative stress in cells. However, there are limitations to the use of DCFDA. First of all, DCFDA cannot be attributed to any specific free radical and is also sensitive to non-radical reactive molecules. Secondly, the probe radical intermediate DCF easily reacts with oxygen to form additional superoxide which amplifies the actual ROS levels[9]. Thirdly factors, like cytochrome, hematin, peroxidases, ferrous ions and horseradish peroxide (HRP) promote the oxidation of DCFH even in the absence of reactive species [10,11].

Chemiluminescence-derived methods

Chemiluminescence is generally used for detection of superoxide. This probe reacts with superoxide to form a photon which is captured by a photometer or scintillation counter without the requirement of an excitation light source [12]. The main limitation of this probe is that not only superoxide but also other reducing molecules or nucleophiles like alkaline hydrogen peroxide induce luminescence [13].

Chromatography methods are used in the separation and identification of hydroxyl radicals and their reaction products. Chromatography methods are rapid, efficient and sensitive in the detection of hydroxyl radicals. Since the reaction process and products are complicated, and the treatment of pre-detection is complex, this technique is less widely used than other methods. Additionally, the technique is destructive and does not offer spatial resolution.

Electron spin resonance (ESR), also known as electron paramagnetic resonance (EPR), is used for direct identification of free radicals. The electron spin resonance method is widely applied in the identification of oxygen, nitrogen or organic free radicals generated from non-cellular and even non-biological systems. In this method specific spin traps are required to stabilize free radicals by incorporating radicals into their structures or by oxidizing them to a relatively stable radical. The role of the capturing agents in this process is critical.

There are many kinds of spin traps for the analysis of free radicals, among them the nitrone compound 5,5-dimethyl-1-pyrroline N-oxide (DMPO) is the most popular. Its adducts with superoxide and hydroxyl radicals form DMPO/$^{\cdot}$OOH and DMPO/$^{\cdot}$OH, respectively[12]. Both are captured using the ESR spectrometer to generate specific spectra. The DMPO-based method is used to analyze radicals produced by cellular or cell-free systems.

However, several factors influence the detection of spectra by ESR identification. For example,the pH value has an influence on radical formation and mutual transformation, and temperature affects the energy level of radicals, while the liquid environment of substrates is responsible for the resonant state of radicals [14]. Additionally, considering the high cost of the ESR assay, experiments must be performed rationally. Since the method is relatively insensitive, large amounts of radicals are needed to obtain a measurable signal.

Diamond magnetometry

From ancient time, diamond has been used by different cultures for healing processes. These applications were superstitious beliefs lacking any medicinal basis. They used diamonds as an antidote of poisoning, internal cleansing to ward off plagues, and even as antipsychotic drug.

Nowadays, it is proved that diamonds don't have any natural medicine properties themselves. However, diamonds are considered to be used in prosthetics, sensing and imaging devices, and drug delivery.

Recently Diamond magnetometry with nitrogen vacancy (NV^-) centers has been established which offers a new way of sensing radicals [15]. The NV^- centers are generated by replacing two carbon atoms by a nitrogen atom(N) and a vacancy (V) fig 3(a). The NV^- center has two electron and triplet spin state ms=0 and ms= ±1. Both ground state and excited state are spin triplets. These spin states can be readout optically, since their fluorescence depends on the position of spins and surrounding magnetic field.
There are two choices of electrons to come back ms=0 state. After excitation from the ms=0 state with a green laser, electrons directly come back from the excited to the ground state and the NV^- center emits roughly 90% of red photons with a lifetime around 15ns. This pathway is spin preserving and produces fluorescence. When an electron goes from the ms= ±1 to the excited state, not all electrons come back directly to the ground ms= 0 state. 20 to 40% of electrons return to the ms=0 state via a dark state. This pathway takes longer (approximately 300 ns).
This results in a decrease in fluorescence. In other words, when the spin is in the $m_s = \pm 1$, it emits less light and thus is darker. This difference in brightness allows us to readout the spin state of the NV^- center optically fig 2(b).

This way the NV^- centers act as sensitive sensors for monitoring magnetic changes in their environment. Via a quantum effect the magnetic signals of spins in their surrounding are converted into an optical signal. Since optical signals can be read out much more sensitively than magnetic signals the method is unprecedentedly sensitive. Even the small magnetic signal of single electrons or atomic nuclei can be detected [16-18]. Here we use a specific type of diamond magnetometry measurements called relaxometry or T_1 measurements [19].

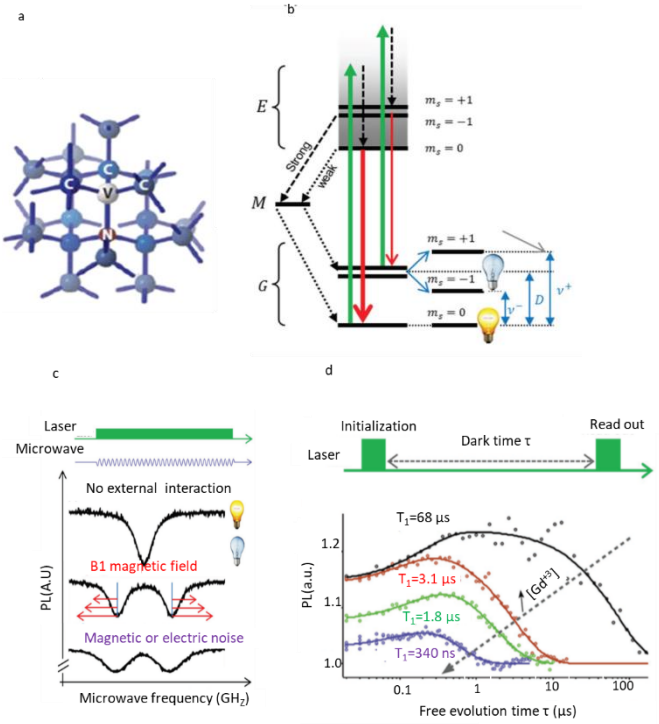

Figure 3: (a) structure of the NV-center [20] (b) energy diagram of the NV- center. If the NV- center is excited with a green laser it goes to the excited state. From there it loses some energy and decays to the ms=0 state of the ground state. If the NV^- center has been in the ms=+1 or -1 state it can also return to the ground state via a long-lived dark state. As a result, the NV^- center is brighter if it is in the ms=0 state than if it is in the ms=+1 or -1 states [21].
(c) if the NV- center receives energy in the microwave range, it can be manipulated to go to the +1 or -1 state. This results in a drop in fluorescence. The spectrum is influenced by external properties: (1) shows the spectrum of the NV^- center without external magnetic field. (2) In presence of an external magnetic field the +1 and the −1 state move away from each other. The larger the magnetic field, the further the lines separate (= Zeeman splitting). (3) Electric or magnetic fluctuation (or noise) also induces an uncertainty on the spin state energy and so a broadening of the resonance line. However, since this effect is quite small and the lines are also broadened by continuous optical and microwave radiations, measuring this noise requires relaxometry pulse sequences.
(d) shows the result of T1 (Relaxation time) measurements. Here, the NV^- center is brought to the ground state (= initialized) with a laser and then left to interact with the surrounding for a given dark time τ. During that time the NV^- center will go back to the initial state, the equilibrium of the states in ms = 0 and ms = ±1 is restored.

The more noise (in this example from Gd3+ ions) is present, the faster this process will occur. Varying the time τ before reading out the spin states results in the curves shown. If the NV^- center is still unperturbed in ms = 0, it will give a higher photoluminescence then when it has already interacted with the surrounding. As a result, a decrease in Gd3+ (or other substances with a nonzero spin) shifts the curve to the left. The decrease can be described as an exponential function with a characteristic time constant T1 [22].

For the measurement of surrounding magnetic field or intracellular free radical concentration by NV^- center based diamond particle (FNDs), it is important to internalise FNDs successfully inside the cell. Our group successfully internalised FNDs in living cells [23-29] evaluated their effect on metabolic activity [30], measured the intracellular free radical generation [31-34] and chemical reactions induced by biological environment [35,36].
In this thesis, A home-built diamond magnetometry setup [37] was used where a magnetic signal is converted to optical signal. This optical signal was measured as T1 relaxation time by applying a laser pulse to polarize the NV^- center and subsequently measuring the required time until relaxation.

Aim of this Thesis

The aim of this thesis is to detect intracellular free radical generation inside the cells and understand cell damage developed during pathological conditions. For this purpose, we used novel fluorescence nanodiamond particles (FNDs) which contain NV^- centers. FNDs were targeted to the cytosol, mitochondria and the nucleus where we can detect organelles specific free radical generation under normal and drug induced oxidative stress conditions. We also used FNDs for detecting free radical generation in HUVECS under shear stress.

In Chapter 2, we lay the foundation for intracellular quantum sensing by studying how FNDs can be ingested by cells. More specifically, we studied the impact of the microenvironment on nanoparticle uptake compared to the macroenvironment. FND uptake in the cells cultured in Petri dishes is significantly higher than the uptake in a microfluidic chip where the alteration in CO_2 environment, the cell culture medium pH, and the surface area to volume ratio seem to be the underlying causes leading to this observed difference.

The Chapter- 3 determined free radical generation in HUVECS under different shear stress conditions in real time. We investigated radical formation in HUVECS under different flow conditions ranging from those typically found in veins to those in arteries. Free radical formation was compared before, during and after flow conditions.

Chapter- 4 investigated drug induced cellular toxicity by targeting FNDs to different cellular organelles such as mitochondria and the nucleus. FNDs detected cellular toxicity in the early stage before cell death. For nucleus targeting we used SV40 NLS (nuclear localization signal) conjugated NLS-FNDs. For targeting mitochondria FNDs were coated with physically adsorbed anti-VDAC2 antibodies (MIT-FNDs).

In chapter- 5, we discussed the important findings of this thesis and future perspectives of relaxometry for detecting free radicals .

References:

1.Halliwell B. Free Radicals and other reactive species in disease. Nature Encyclopedia of life sciences. 2001. DOI: 10.1038/npg.els.0002269

2. Gomberg M. An Incidence of Trivalent Carbon Trimethylphenyl. *J Am Chem Soc*. 1900;22:757–771. [Google Scholar] https://doi.org/10.1021/ja02049a006

3.CommonerB, TownsendJ, Pake GE. Free radicals in biological materials. Nature. 1954;174(4432):689–691. [PubMed] [GoogleScholar]. https://doi.org/10.1038/174689a0

4. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84. doi: 10.1016/j.biocel.2006.07.001. Epub 2006 Aug 4. PMID: 16978905.

5. Nordberg J, Arnér ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med. 2001 Dec 1;31(11):1287-312. doi: 10.1016/s0891-5849(01)00724-9. PMID: 11728801.

6. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008 Jun;4(2):89-96. PMID: 23675073; PMCID: PMC3614697.

7. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. JAMA. 1999 Dec 1;282(21):2035-42. doi: 10.1001/jama.282.21.2035. PMID: 10591386.

8. Muriel P. Role of free radicals in liver diseases. Hepatol Int. 2009 Dec;3(4):526-36. doi: 10.1007/s12072-009-9158-6. Epub 2009 Nov 26. PMID: 19941170; PMCID: PMC2790593

9. Folkes LK, Patel KB, Wardman P, Wrona M. Kinetics of reaction of nitrogen dioxide with dihydrorhodamine and the reaction of the dihydrorhodamine radical with oxygen: implications for quantifying peroxynitrite formation in cells. Arch Biochem Biophys. 2009 Apr 15;484(2):122-6. doi: 10.1016/j.abb.2008.10.014. Epub 2008 Oct 19. PMID: 18976629.

10. Qian SY, Buettner GR. Iron and dioxygen chemistry is an important route to initiation of biological free radical oxidations: an electron paramagnetic resonance spin trapping study. Free Radic Biol Med. 1999 Jun;26(11-12):1447-56. doi: 10.1016/s0891-5849(99)00002-7. PMID: 10401608.

11. Tarpey MM, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol Regul Integr Comp Physiol. 2004 Mar;286(3):R431-44. doi: 10.1152/ajpregu.00361.2003. PMID: 14761864.

12. Münzel T, Afanas'ev IB, Kleschyov AL, Harrison DG. Detection of superoxide in vascular tissue. Arterioscler Thromb Vasc Biol. 2002 Nov 1;22(11):1761-8. doi: 10.1161/01.atv.0000034022.11764.ec. PMID: 12426202.

13. Maskiewicz R, Sogah DY, Bruice TC. Chemiliminescent reactions of lucigen.2. reaction of lucigenin with hydroxide ion and other nucleophiles J. Am. Chem.
Soc 1979; 101: 5347–5354. https://doi.org/10.1002/chin.197951139

14. Berliner LJ. Biomed. Spectrosc. Imaging, 2016, 5, 5–26.

15. Kalyanaraman B, Perez-Reyes E, Mason RP. Spin-trapping and direct electron spin resonance investigations of the redox metabolism of quinone anticancer drugs. Biochim Biophys Acta. 1980 Jun 5;630(1):119-30. doi: 10.1016/0304-4165(80)90142-7. PMID: 6248123.

16. Szychowski KA, Rybczyńska-Tkaczyk K, Leja ML, Wójtowicz AK, Gmiński J. Tetrabromobisphenol A (TBBPA)-stimulated reactive oxygen species (ROS) production in cell-free model using the 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) assay-limitations of method. Environ Sci Pollut Res Int. 2016 Jun;23(12):12246-52. doi: 10.1007/s11356-016-6450-6. Epub 2016 Mar 15. PMID: 26976009; PMCID: PMC4893053.

17. Gomes A, Fernandes E, Lima J. L. Fluorescence probes used for detection of reactive oxygen species. J Biochem Biophys Methods. 2005 Dec 31;65(2-3):45-80. doi: 10.1016/j.jbbm.2005.10.003. Epub 2005 Nov 4. PMID: 16297980.

18. Georgiou CD, Papapostolou I, Grintzalis K. Superoxide radical detection in cells, tissues, organisms (animals, plants, insects, microorganisms) and soils. Nat Protoc. 2008 Oct 9;3(11):1679-92. doi: 10.1038/nprot.2008.155. PMID: 18846095.

19. Juraschek DM, Meier QN, Trassin M, Trolier-McKinstry SE, Degen CL, Spaldin NA. Dynamical Magnetic Field Accompanying the Motion of Ferroelectric Domain Walls. Phys Rev Lett. 2019 Sep 20;123(12):127601. doi: 10.1103/PhysRevLett.123.127601. PMID: 31633948.

20 . Schirhagl R, Chang K, Loretz M, Degen CL. Nitrogen-vacancy centers in diamond: nanoscale sensors for physics and biology. Annu Rev Phys Chem. 2014;65:83-105. doi: 10.1146/annurev-physchem-040513-103659. Epub 2013 Nov 21. PMID: 24274702

21. Chipaux, M., Tallaire, A., Achard, J. *et al.* Magnetic imaging with an ensemble of nitrogen-vacancy centers in diamond. *Eur. Phys. J. D* **69,** 166 (2015). https://doi.org/10.1140/epjd/e2015-60080-1

22. Tetienne JP , Hingant T , Rondin L , Cavaill`es A , Mayer L , Dantelle G , Gacoin T , Wrachtrup J , J.-F. Roch JF, Jacques V. Spin relaxometry of single nitrogen-vacancy defects in diamond nanocrystals for magnetic noise sensing. Physical Review B 2013;arXiv:1304.1197v1.

23. Hemelaar SR, Nagl A, Bigot F, Rodríguez-García MM, de Vries MP, Chipaux M, Schirhagl R. The interaction of fluorescent nanodiamond probes with cellular media. Mikrochim Acta. 2017;184(4):1001-1009. doi: 10.1007/s00604-017-2086-6. Epub 2017 Jan 27. PMID: 28344361; PMCID: PMC5346409.

24. Morita A , Martinez F PP, Chipaux M, Jamot N, Hemelaar SR, van der Laan KJ, Schirhagl R. Cell uptake of lipid-coated diamond,Particle and Particle Systems Characterization 2019; 36: 1900116. doi.org/10.1002/ppsc.201900116

25. Hemelaar SR, de Boer P, Chipaux M, Zuidema W, Hamoh T, Martinez FP, Nagl A, Hoogenboom JP, Giepmans BNG, Schirhagl R. Nanodiamonds as multi-purpose labels for microscopy. Sci Rep. 2017 Apr 7;7(1):720. doi: 10.1038/s41598-017-00797-2. PMID: 28389652; PMCID: PMC5429637.

26. Hemelaar SR, Saspaanithy B, L'Hommelet SRM, Perona Martinez FP, van der Laan KJ, Schirhagl R. The Response of HeLa Cells to Fluorescent NanoDiamond Uptake. Sensors (Basel). 2018 Jan 26;18(2):355. doi: 10.3390/s18020355. PMID: 29373504; PMCID: PMC5855215.

27. Sigaeva A, Morita A, Hemelaar SR, Schirhagl R. Nanodiamond uptake in colon cancer cells: the influence of direction and trypsin-EDTA treatment. Nanoscale. 2019 Oct 7;11(37):17357-17367. doi: 10.1039/c9nr04228h. Epub 2019 Sep 13. PMID: 31517372.

28. Damle VG, Sharmin R, Morita A, Nie L, Schirhagl R. Micro Versus Macro - The Effect of Environmental Confinement on Cellular Nanoparticle Uptake. Front Bioeng Biotechnol. 2020 Jul 24;8:869. doi: 10.3389/fbioe.2020.00869. PMID: 32793585; PMCID: PMC7393206.
29. Zheng T, Perona Martínez F, Storm IM, Rombouts W, Sprakel J, Schirhagl R, de Vries R. Recombinant Protein Polymers for Colloidal Stabilization and Improvement of Cellular Uptake of Diamond Nanosensors. Anal Chem. 2017 Dec 5;89(23):12812-12820. doi: 10.1021/acs.analchem.7b03236. Epub 2017 Nov 17. PMID: 29111679.

30. van der Laan KJ, Morita A, Perona-Martinez FP, Schirhagl R. Evaluation of the Oxidative Stress Response of Aging Yeast Cells in Response to Internalization of Fluorescent Nanodiamond Biosensors. Nanomaterials (Basel). 2020 Feb 20;10(2):372. doi: 10.3390/nano10020372. PMID: 32093318; PMCID: PMC7075316.

31. Nie L, Nusantara AC, Damle VG, Baranov MV, Chipaux M, Reyes-San-Martin C, Hamoh T, Epperla CP, Guricova M, Cigler P, van den Bogaart G, Schirhagl R. Quantum Sensing of Free Radicals in Primary Human Dendritic Cells. Nano Lett. 2022 Feb 23;22(4):1818-1825. doi: 10.1021/acs.nanolett.1c03021. Epub 2021 Dec 20. PMID: 34929080; PMCID: PMC8880378.

32. Sharmin R, Hamoh T, Sigaeva A, Mzyk A, Damle VG, Morita A, Vedelaar T, Schirhagl R. Fluorescent Nanodiamonds for Detecting Free-Radical Generation in Real Time during Shear Stress in Human Umbilical Vein Endothelial Cells. *ACS Sensors. 2021*; *6*(12): 4349-4359. https://doi.org/10.1021/acssensors.1c01582

33. Nie L, Nusantara AC, Damle VG, Sharmin R, Evans EPP, Hemelaar SR, van der Laan KJ, Li R, Perona Martinez FP, Vedelaar T, Chipaux M, Schirhagl R. Quantum monitoring of cellular metabolic activities in single mitochondria. Sci Adv. 2021 May 19;7(21):eabf0573. doi: 10.1126/sciadv.abf0573. PMID: 34138746; PMCID: PMC8133708.

34. Wu K, Vedelaar TA, Damle VG, Morita A, Mougnaud J, Reyes San Martin C, Zhang Y, van der Pol DPI, Ende-Metselaar H, Rodenhuis-Zybert I, Schirhagl R. Applying NV center-based quantum sensing to study intracellular free radical response upon viral infections. Redox Biol. 2022 Mar 18;52:102279. doi: 10.1016/j.redox.2022.102279. Epub ahead of print. PMID: 35349928; PMCID: PMC8965164.

35.Perona Martínez F, Nusantara AC, Chipaux M, Padamati SK, Schirhagl R. Nanodiamond Relaxometry-Based Detection of Free-Radical Species When Produced in Chemical Reactions in Biologically Relevant Conditions. ACS Sens. 2020 Dec 24;5(12):3862-3869. doi: 10.1021/acssensors.0c01037. Epub 2020 Dec 3. PMID: 33269596; PMCID: PMC8651177.

36.Li R, Vedelaar T, Mzyk A, Morita A, Padamati SK, Schirhagl R. Following Polymer Degradation with Nanodiamond Magnetometry. ACS Sens. 2022 Jan 28;7(1):123-130. doi: 10.1021/acssensors.1c01782. Epub 2022 Jan 4. PMID: 34982542; PMCID: PMC8809337.

37. Morita A, Hamoh T, Martinez FPP, Chipaux M, Sigaeva A, Mignon C, Laan KJV, Hochstetter A, Schirhagl R. The Fate of Lipid-Coated and Uncoated Fluorescent Nanodiamonds during Cell Division in Yeast. Nanomaterials (Basel). 2020 Mar 12;10(3):516. doi: 10.3390/nano10030516. PMID: 32178407; PMCID: PMC7153471