Fluorescent nanodiamonds quantum sensing free radicals in bio-samples

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General Introduction

Chapter 1

The role of free radicals in the immune system

Free radicals are defined as atoms or molecules with an unpaired electron. Free radicals are produced during the natural metabolism. Most of them are unstable and readily react with all kinds of other molecules. Thus, they are damaging cellular proteins, DNA, lipids, and membranes. However, when free radicals are generated in large amounts, they cause oxidative stress. Oxidative stress plays a key role in a lot of human diseases, such as immune system dysfunctions, cardiovascular diseases, Alzheimer's disease (AD)[1]. Free radicals can also affect immune cells functioning. They are involved in maturation, activation and cytokine production. Radicals further contribute to the defense against pathogens. It is important to know what free radicals are generated under certain condition.

The immune system is the first line for infection defense. Immune cell metabolism is related with cell growth, differentiation, proliferation and function[2]. Immune cells recognize and engulf pathogens, this can trigger high production of reactive oxygen intermediates ("oxygen burst" or "respiratory burst"), such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\bullet$)[3]. These are used by the cell to kill pathogens. Mitochondria are always considered as cellar energy centers, but they are also involved in cell signaling[4], apoptosis, and immune function[5-7]. Mitochondria can regulate the innate and adaptive immunity[8,9]. Mitochondria are also considered as main source of free radicals. These radicals are created during electron transfer in the Citric acid cycle (TCA cycle). Thus they are a target organelle when investigating free radical generation.

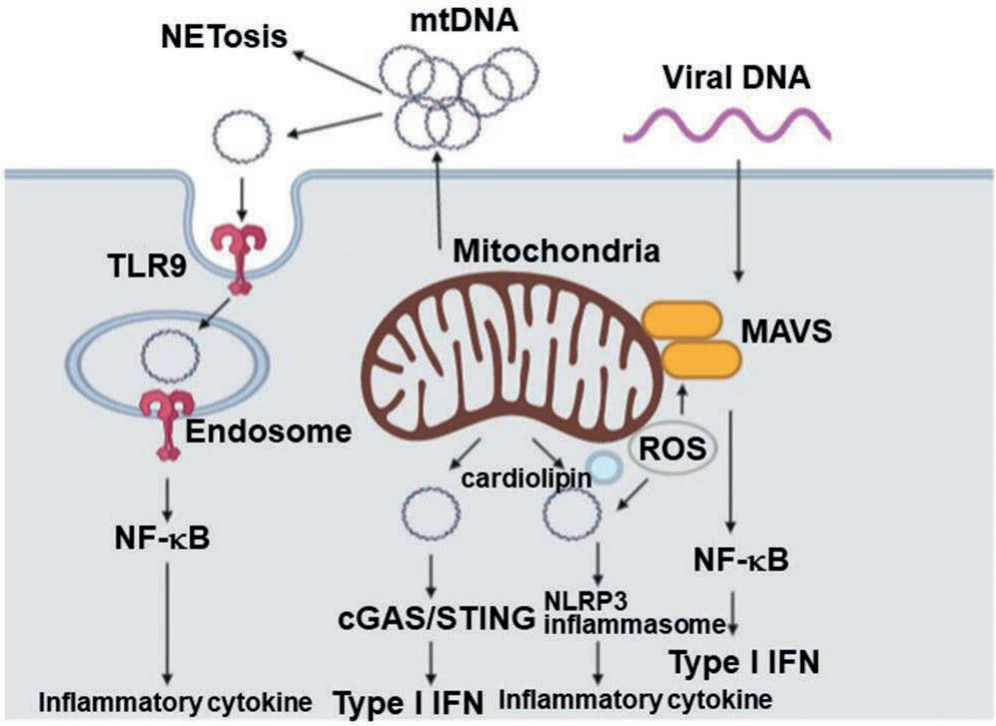

Figure 1. Mitochondrial regulation on immune system. (Reprint from Iwasaki Y et al, 2020)

However, free radicals have a short lifetime and are highly reactive and thus difficult to measure. Current measurements are mostly based on fluorescent probes. These usually measure the total amount of cellular reactive oxygen species (ROS) (including radicals and non-radicals) or specific species (as for instance H_2O_2). However, these assays do not allow real time measurements and usually require large ensembles of cells. Also since non-radical ROS are more abundant these usually dominate the signals that are obtained by these methods. Diamonds potentially offer a solution to this problem.

Diamond as a tool for mapping free radicals

When it comes to diamonds, the first impression that always comes to people's minds is their shining, pretty appearance. However, diamonds have many more beautiful qualities than their good looks. Nanodiamonds, a carbon-based material, now is widely used in biomedical fields, biology, and material science due to its outstanding features.

Nanodiamonds are commercially available, which makes them ready to use for researchers. Scientists' interest has been attracted by the fluorescent nanodiamonds (FNDs) over the years. FNDs contain specific defects called nitrogen-vacancy (NV-) centers, which cause them to fluoresce in the red spectral range. They are widely used for labelling[10], tracking and imaging due to their excellent optical properties. Unlike most conventional dyes, they are not bleaching over time. Several studies have reported that FNDs are excellent biocompatibility in several cell line, like macrophages[11], Hela cells[12], yeast[13,14] and HT29[15], and in in-vivo experiments.

The fluorescent nanodiamond (FNDs) based quantum sensing allows to measure nanoscale MRI. Since free radicals have an unpaired electron they produce a strong signal, which can be detected this way. The NV- centers inside diamonds, sense this magnetic noise and convert it into optical signals. Thus diamond magnetometry offers a high-resolution measurement for radicals with unprecedented sensitivity.

Aim of the thesis

This thesis aims to detect free radical generation in cells in specific organelles using a diamond magnetometry based quantum sensing technique. In order to achieve our goal, in **chapter 2**, we bring FNDs to mitochondria by using Anti-VDAC2 antibodies (target mitochondrial outer membrane) coating. With these mitochondria targeted FNDs, we demonstrate free radicals detection in a single J774A.1 macrophages. We further performed the first nanoscale MRI measurements of single mitochondria. In **chapter 3**, we investigate free radical production in human primary dendritic cell while NADPH (NOX2) activation at phagosome subunits. These are the first measurements of this kind in primary cells. **In chapter 4**, we investigated the FNDs temporal subcellular location inside Hela cells. In **chapter 5**, we studied FNDs conjugation with bacteria, we established a protocol as an initial step for detecting free radical generation while

immune cells (J774A.1 macrophage cells) combat the impact of a pathogen. Finally, in **chapter 6**, we discussed the importance of diamond magnetometry and potential use in the future.

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