Free radical detection in living cells with relaxometry

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SUMMARY

Several diseases are correlated with an over production of free radicals in cells. These include infertility, cancer, cardiovascular diseases, Alzheimer's and many more. In addition to that, there is the free radical theory of aging which states that aging is caused by accumulation of damage inflicted by free radicals [1]. This topic has gained vast reputation lately, as an evidence the discussions about antioxidants available in food or supplement to scavenge free radicals. As free radicals are involved in nearly every disease it is valid to have optimized tools to assess the level of free radicals in biological environments.

In this research, we proposed the use of fluorescent nanodiamonds (FNDs) as biosensors to measure the level of free radicals. FNDs containing defects called NV centers are a promising tool for free radical detection due to their ability to convert magnetic noise to optical signals. Diamond magnetometry combines the advantage of fluorescent dyes and magnetic resonance methods. In this research, we use a specific type of magnetometry measurements called T_1 measurements. These are specific for spin noise and thus perfectly suited for free radical detection.

In **Chapter 1** a general introduction to the field and the topics discussed in this thesis is given. First, I introduced free radical generation, the role they play and damage caused in case of overproduction. Then, I briefly introduce different tools to detect free radicals directly and indirectly, their advantages and limitations. After that, an introduction to diamond magnetometry and fluorescence nanodiamonds as novel technique to detect free radicals in biological environment follows. Finally, the thesis objective and the topic are discussed.

Chapter 2 discusses the fate of nanodiamonds once they are introduced in cells. We chose yeast cells as a model to investigate the fate of FNDs during cell division. Since yeast cells can only undergo a limited number of divisions, this is one way to achieve aging. To study these processes with diamond magnetometry, it is important to know how FNDs behave during cell division. There are four possibilities of where FNDs end up after cell division. They can remain within the mother cell, move to the daughter, be excreted, or found in both mother and daughter cells. We observed that in most cases particles are found either in daughter cells or excreted. It is worth mentioning that the initial position of the particle has an influence on where the particle will end up. For example when a particle is found near the nucleus is most likely to remain with the mother cell, and in case of a particle is found close to the membrane it is likely to move to the daughter or to be excreted.

In **Chapter 3** we wanted to achieve targeting, as it is important to get accurate information about radical formation at specific locations. We modified the surface of FNDs with nuclear pore complex (NCP) antibodies specific for yeast cells. We compared bare-FNDs with FND-Antibodies. We found that with FND-Antibodies we achieved higher targeting. In this group 70% of particles were located at the nucleus after 24 hours. It is also worth mentioning that the longer the incubation time the higher the targeting rate.

An implementation of diamond magnetometry was described in **Chapter 4**. We investigated the effect of shear stress on free radical production in human umbilical vein endothelial cells (HUVECs). We exposed the cells to different shear stress and found that when cells are exposed to 2 dyne/cm^2 NO^* production is increased and that can be shown by recording changes in the T_1 values. At 20 dyne/cm^2 the NO^* level is lower while at 10 dyne/cm^2 the NO^* level remains the same. We also investigated increasing the flow rate gradually every 30 minutes for 4 hours. Using T_1 measurements, we found that free radical production increases over time.

In **Chapter 5** we investigated the capacitation process in boar sperms. We used diamond magnetometry to measure free radical production during the process. We measured the concentration of free radicals present on the acrosome part before capacitation. Then we stimulated the cells to capacitate using capacitating medium. First, we used nitrogen terminated FNDs to evaluate if they are more sensitive to free radicals. We found that bare-FNDs are more sensitive than NH_2-FNDs. Second, we did T_1 measurements in sequence to evaluate the level of free radical produced over time. We found that the T_1 values have dropped significantly over time which means more free radicals have been produced after capacitation.

In addition, we added different reagents to block or induce free radical production from the acrosome part or from mitochondria. We found that when we blocked free radical production from the acrosome part (NOX5), T_1 values remained unchanged. Moreover, once we blocked mitochondrial radical production T_1 values has dropped significantly. As a result, we confirmed our hypothesis that we are able to measure free radical production from localized organelles, and that NOX5 plays the major role in capacitation. Lastly, we induced the production of free radicals and we noticed a decrease in T_1 values which was expected.

In **Chapter 6** we talked about how to valorise this technique commercially and we discussed the advantages and disadvantages of the technique. Moreover, we exploit the possible markets that we can approach.

Finally, in **Chapter 7** we discuss the possible future applications and directions for diamond magnetometry briefly. We also suggest some development that could be implemented to improve the system and have higher sensitivity and accurate results.