Diamond Magnetometry for localized free radical measurements in single sperm cell

C. Reyes-San-Martin[#1], T. Hamoh[#1], Y. Zhang[1], R. Li[1], A. Sigaeva[1], J. Kawalko[2], T. Vedelaar[1], A. Mzyk[1,3], R. Schirhagl[1]

[1] *Groningen University, University Medical Center Groningen, Antonius Deusinglaan 1, 9713 AW Groningen, The Netherlands.*
[2]*AGH University of Science and Technology, Academic Centre for Materials and Nanotechnology, Al. A. Mickiewicza 30, 30-059 Krakow, Poland*
[3]*Institute of Metallurgy and Materials Science, Polish Academy of Sciences, 25 Reymonta Street, 30-059 Krakow, Poland*

[#]These two authors contributed equally

thamirhamoh@gmail.com; claudiareyes13@gmail.com; romana.schirhagl@gmail.com

Introduction: The male factor in infertility has been correlated with an imbalance of free radicals that leads to damage in sperm cells. Free radicals (FR) are short-lived reactive chemical species with one or more unbound electron. Their imbalance has been linked with both physiological and pathological changes in sperm cells. Capacitation is one of the physiological processes in which free radicals generation has a significant impact on sperm cell behavior and fertilization capability. Several methods for free radical detection have been applied to sperm cells. Most of them are fluorescent probes that are consumed in interaction with FR. However, they usually bleach over time, and they are not sensitive enough for the small fluctuations in FR that trigger capacitation. Additionally, they are usually reacting to both radicals and other reactive species. Due to their higher abundance non-radicals dominate the signal in that case.
Diamond magnetometry potentially offers a complementary solution. This technique is based on defects in diamonds which change their optical properties based on their magnetic surrounding. Since optical signals can be read out more sensitively, this method offers nanoscale magnetic resonance signals with unprecedented sensitivity. Since free radicals have a free electron spin, they cause a spin noise which can be measured with this technique.
Aim: Here, we show for the first time that diamond magnetometry can be used to measure the free radical load in single sperm cells.
Materials and Methods: In this work, we use commercial 70nm oxygen terminated fluorescent nanodiamonds (FNDs, Adámas Nano, Raleigh, NC, USA). Boar sperm commercially available from *Varkens KI Nederland BV* was separated using Ficoll gradient, and selected motile sperm cells were plated on fibronectin coated dishes. Sperm cells were treated with FNDs suspension in Human Tubal Fluid (HTF) to induce the capacitated state and modified-HTF (HTF medium without bicarbonate, calcium salts and serum) for the uncapacitated state. Biocompatibility tests like MTT assay and DCFDA assay was performed. Confocal and scanning electron microscopy on fixed cells to were used to characterize the location of FNDs on sperm cells.
To evaluate the amount of free radicals in single sperm cells, T1 relaxometry a specific mode of diamond magnetometry- was performed in our home-built microscope. To this end we use a laser to pump the fluorescent defects in FNDs (so called NV centers) in the bright state and then probe after varying dark times. The presence of FR alters how fast the NV centres lose the bright state. This time to return to equilibrium, represented by the T1 value, is a measure for the concentration of radicals around the NV centres.
Results and discussion: To prove our concept, we first tested the biocompatibility of FNDs and the effect of them on sperm cells metabolic activity and reactive oxygen species formation. The results show no significant difference compared to the control group for FNDs at a concentration of 1 ug/ml in both capacitated and uncapacitated sperm cells. Confocal and scanning microscopy images show that FNDs located preferentially on the head of sperm cells and they attached to the extracellular side of the cell membrane. T1 relaxometry measurements comparing before and after capacitation in order to follow the FR production, show that T1 values dropped significantly over time, which indicates the increase in FR formation around the NV centers.
Conclusion: We have shown for the first time that fluorescent nanodiamonds can be used as innocuous selective labelling to perform localized free radical measurements in single sperm cells using diamond magnetometry.