Diamond based relaxometry for biosensing

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Chapter 6

Summary

FNDs show spin-dependent fluorescence allowing quantitative detection of electron spins and in certain cases even nuclear spins at ambient conditions by optical readout. FNDs emit stable fluorescence without photobleaching or photo blinking, far-red emission, long lifetime, and high quantum efficiency. Recently nanodiamonds with NV^- centers (fluorescent nanodiamond particles, FNDs) opened a new window to detect free radical generation in intracellular environments and during chemical reactions at the nanoscale and at ambient conditions. This tool can be utilized to understand the role of free radicals in physiological and pathological conditions.

Chapter 1 focused on how free radicals cause pathological conditions and develop different diseases. We broadly discuss the role of free radicals in cardiovascular diseases, especially atherosclerosis. In the early stage of atherosclerosis development, cells produce more free radicals and gradually undergo cellular degradation. As a result, monocytes and low density lipoprotein (LDL) confine the cells and develop atherosclerosis. Consequently, it blocks blood circulation through the blood vessel. Additionally, free radicals are involved in liver damage. Free radicals may affect hepatocytes' energetic, respiratory, and regenerative pathways. These imbalances aggravate liver diseases. Free radicals influence the early stage of liver disease[1]. We also included a basic introduction of diamond magnetometry based on fluorescent nanodiamond particles and how NV centers convert magnetic signals to optical signals.

In chapter 2 we evaluated nanoparticle uptake in micro and macro environments. We used microfluidic channels to create a microenvironment and Petri dishes were used to create a macroenvironment. In the microenvironment, uptake was affected due to the production of CO_2 and pH in the culture medium. Cell morphology also influenced the nanoparticle uptake inside cells.

The culture medium of the microenvironment was three times lower than the macroenvironment. As a result, this low volume of medium quickly saturated with CO_2 and reduced pH. Morphology of cells also affects the nanoparticle

uptake inside the cells. For example, Hela cells and BHK21 cells spread on a surface while a macrophage cell's surface is circular.

Finally, modification of microfluidic channels with gelatin coating increased the cell's height. It showed an increased nanoparticle uptake compared to gelatin-coated Petri dishes, where cell's height and uptake remained the same as without coating Petri dishes.

In chapter 3 we estimated free radical generation in HUVECS cells under an arterial and venous range of shear stress with FNDs. FNDs were incubated in static conditions. Then cells were exposed short term 30 mins in venous and arterial range of shear stress 2, 10 and 20 dyne/cm2. Additionally, cells were exposed long term (4hrs) to gradually increased shear stress in the venus to the arterial range. Free radical generation was measured before, during and after flow conditions. When cells were exposed to a physiological range of shear stress, the low range of arterial shear stress or gradually increased from venous to arterial shear stress, the relaxation time (T1) decreased. This is due to an increased free radical generation than before the flow was applied. This shear stress helps maintain the physiological function of cells, such as alignment of fibers and nuclei with the flow direction and tight junction with other cells. However, exposure to shear stress beyond the physiological range of increased the T1 value due to reducing free radical generation. Under this shear stress, cells lost their tight junctions and failed to align with the flow direction.

In Chapter 4 we investigated free radical generation based on relaxation time in different organelles such as cytosol, mitochondria and the nucleus. We targeted different FNDs to the different organelles such as bare FNDs, Anti VDAC2 antibody coated FNDS (MIT-FNDs) and SV40 nuclear localization signal coated FNDs (NLS-FNDs) to the cytosol, mitochondria and the nucleus respectively. Higher concentrated Acetaminophen (APAP) was used to produce cellular toxicity. An overdose of APAP can lead to acute liver poisoning and death. Higher doses of APAP lead to critical depletion of glutathione, mitochondrial protein adduct formation and oxidative stress. This oxidative stress amplifies the formation of free radicals causing mitochondrial damage and cessation of adenosine triphosphate (ATP) synthesis. In addition, free radicals cause nuclear deoxyribonucleic acid (DNA) fragmentation. Together, the nuclear DNA damage and the extensive

mitochondrial dysfunction result in necrotic cell death. In this chapter, we found that cells produced huge amounts free radicals at the early stage before cell death. We noticed free radical formation depending on APAP concentration, treatment duration, and the specific organelles where the radical production was measured. In the cytosol, higher doses of 2 and 4mM of APAP decreased T1 due to an increase in the production of free radicals after a prolonged 18 hrs treatment. In the nucleus and mitochondria, T1 was significantly reduced after 3 hrs of APAP treatment due to increased free radical production compared to the control without treatment. An MTT assay showed that cells lost their metabolic activity after 18hrs of APAP treatment.

The research conducted in this project with relaxometry based on fluorescence nanodiamond particles (FNDs) offers a powerful tool for detecting intracellular free radical generation under shear stress conditions and in the different organelles of a cell. Detection of free radical generation in different organelles and under different shear stress conditions may increase our understanding of the pathological conditions in the early stage of disease development.

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Samenvatting

Hoofdstuk 6

FND's tonen fotoluminescentie afhankelijk van spin die kwantitatieve detectie van elektronen en in bepaalde gevallen zelfs nucleaire spins in atmosferische condities mogelijk maakt met behulp van optische uitlezing. FND’s beschikken over een stabiele fluorescentie zonder fotobleken of knipperen, emissie in rood, lange levensduur en hoge kwantumefficiëntie. Recentelijk openen nanodiamanten met NV-centrum (fluorescent nanodiamond particles, FND's) een nieuw venster om de generatie van vrije radicalen op nanoschaal te detecteren in intracellulaire en chemische reacties en onder atmosferische condities. Met als gevolg dat het belangrijk is om de rol van vrije radicalen in fysiologische en pathologische omstandigheden te begrijpen.

Hoofdstuk 1 focuste op de rol van vrije radicalen bij het ontwikkelen van pathologische aandoeningen en de ontwikkeling van verschillende ziekten. In grote lijnen bespreken we de rol van vrije radicalen bij hart- en vaatziekten, met name atherosclerose. In het voor stadium van atherosclerose produceren cellen meer vrije radicalen waardoor ze geleidelijk afbreken. Als gevolg van diapedese door monocyten en low density lipoprotein (LDL) ontwikkelen de cellen atherosclerose. Gevolgd door het blokkeren van de bloedcirculatie door het bloedvat. Bovendien zijn vrije radicalen ook betrokken bij leverschade. Vrije radicalen beïnvloeden mogelijk de energetische, respiratoire en regeneratieve routes van hepatocyten. Deze onevenwichtigheden verergeren de leverziekte. Vrije radicalen beïnvloeden het vroege stadium van leverziekte om het te verergeren[1]. Ook werd hier een basisintroductie gegeven van diamantmagnetometrie welke gebaseerde is op de fluorescentie van nanodiamanten en hoe het NV center een magnetische signaal omzet in een optisch signaal.

In hoofdstuk 2 evalueerden we de opname van nanodeeltjes in micro- en macro-omgevingen. We gebruikten microfluïdische kanalen om een micro-omgeving te creëren en petridishes om een macro-omgeving te creëren. In de micro-omgeving werd opname beïnvloed door de productie van CO_2 en pH in het kweekmedium. Het volume van kweekmedium in de micro-omgeving

was drie maal kleiner dan dat van het macromilieu; met als gevolg dat dit medium met een klein volume snel verzadigt met CO_2 en een verlaagde pH. Ook de morfologie van cellen beïnvloedt ook de opname van nanodeeltjes. Bijvoorbeeld, Hela- en BHK21-cellen spreiden zich over een oppervlak waar de morfologie van de macrofaag cirkelvormig is.

Ten slotte beïnvloedde de modificatie van het microfluïdisch kanaal met een gelatinelaag de hoogte van de cel. Hier werden een hoger aantal nanodeeltjes opgenomen in vergelijking met een petridish met een gelijke gelatinelaag, waarbij de hoogte en opname gelijk bleven in vergelijking met petridishes zonder de gelatinelaag.

In hoofdstuk 3 schatten we de generatie van vrije radicalen in HUVECS-cellen onder arterieel en aderachtig bereik van schuifspanning met FND's. FND's werden geïncubeerd onder statische omstandigheden. Vervolgens werden cellen voor korte duur (30 min) blootgesteld aan schuifspanning in het bereik van aderachtig en/of arterieel van 2, 10 en 20 dyne / cm2. Daarnaast werden de cellen langdurig blootgesteld (4 uur) aan geleidelijk verhoogde schuifspanning van aderachtig tot arterieel. De generatie van vrije radicalen werd gemeten voor, tijdens en na de stroming. Wanneer cellen werden blootgesteld aan een fysiologisch bereik van schuifspanning of het beginbereik van arteriële schuifspanning of geleidelijk verhoogd van aderachtige naar arteriële schuifspanning werd een verminderde relaxation time (T1) als gevolg van verhoogde vrije radicalengeneratie NO* in vergelijking met voor- en nastroomomstandigheden waargenomen. De schuifspanning handhaaft de fysiologische functie van cellen, zoals uitlijning van vezels en celkern met de stroomrichting en nauwe pakking van deze cellen. Echter, blootstelling buiten het fysiologische bereik van schuifspanning verhoogde de T1-waarde door een vermindering van de generatie van vrije radicalen. Onder deze schuifspanning verloren cellen hun nauwe pakking en konden ze zich niet uitlijnen met stroomrichting.

In hoofdstuk 4 onderzochten we de productie van vrije radicalen op basis van ontspanningstijd in verschillende organellen zoals cytosol, mitochondriën en celkern. We richtten verschillende FND's op de verschillende organellen met behulp van kale FND's, Anti VDAC2 antilichaam gecoate FNDS (MIT-FNDs) en SV40 nucleaire lokalisatiesignaal (NLS-FND's) naar respectievelijk het cytosol, mitochondriën en de celkern. Een hoge

concentratie van Acetaminophen (APAP) werd gebruikt om cellulaire toxiciteit te induceren. Overdosering van APAP kan leiden tot acute leververgiftiging met overleiden als gevolg. Een hogere dosering met APAP leidt tot kritische uitputting van glutathion, mitochondriale eiwit adduct vorming en oxidatieve stress. Deze oxidatieve stress versterkt de vorming van vrije radicalen die mitochondriale schade veroorzaken en stopt de synthese van adenosinetrifosfaat (ATP). Bovendien veroorzaken vrije radicalen fragmentatie van nucleaire desoxyribonucleïnezuur (DNA). Uiteindelijk resulteert de nucleaire DNA-schade en uitgebreide mitochondriale malfunctie in necrotische celdood. In dit hoofdstuk ontdekten we dat cellen in een zeer vroeg stadium van celdood enorme hoeveelheden vrije radicalen produceerden. We detecteerden de vorming van vrije radicalen op basis van APAP-concentratie, behandelingsduur en specifieke organellen. In het cytosol hij hogere doses van 2 en 4mM daalde de T1-waarde als gevolg van toenemende productie van vrije radicalen na een langdurige behandeling van 18 uur. In de celkern en mitochondriën was er een significante daling van de T1-waarde na een 3 uur durende behandeling met APAP als gevolg van een verhoogde vrije radicalen productie in vergelijking met de groep zonder behandeling. Een MTT-test toonde aan dat cellen hun metabolische activiteit verloren na 18 uur durende APAP-behandeling.

Het onderzoek in dit project met relaxometrie op basis van fluorescerende nanodiamanten (FND's) wordt gebruikt als een krachtig hulpmiddel voor het detecteren van intracellulaire vrije radicalengeneratie onder verschillende schuifspanningen en in de verschillende organellen van een cel. Detectie van vrije radicalengeneratie in verschillende organellen en verschillende onder verschillende schuifspanningen kan meer inzicht geven in het vroege stadium van de ontwikkeling van de pathologische aandoeningen.

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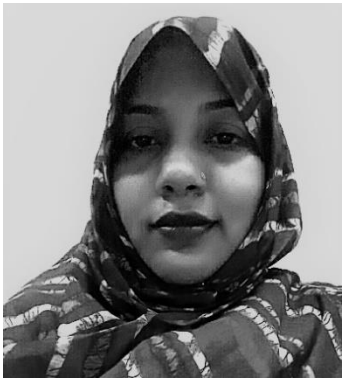
About the Author



Rokshana Sharmin completed a B. Pharm and M. Pharm from the University of Rajshahi, Bangladesh (2007). She started her career as a lecturer in the pharmacy department at the university of Gono, Bangladesh. In 2012, she joined the Jashore University of Science and Technology, Bangladesh in the same department. There she received an NSICT scholarship from the Ministry of Science and Technology, Bangladesh to conduct her M.Pharm thesis. In 2017, she secured the Bangabandhu Fellowship from the ministry of science and technology, Bangladesh to pursue her education (PhD) in the Netherlands. She started her PhD program in the Bioanalysis and Imaging group of Romana Schirhagl at the Department Biomedical Engineering, University Medical Center Groningen, Netherlands. Her research was focused on Diamond based relaxometry for detecting intracellular free radical generation under shear stress conditions and in specific organelles. She published several papers in different fields.

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