PP I_C40

HETEROCELLULAR INTERACTIONS IN METABOLIC RESPONSES TO AIR POLLUTION FINE PARTICULATE MATTER ($PM_{2.5}$)

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Air pollution fine particulate matter ($PM_{2.5}$) uptake by alveolar macrophages impairs local redox metabolism and inflammatory pathways, which have been associated with cardiovascular risk factor development in humans. We and others have previously shown that $PM_{2.5}$ exposure promotes obesity by impaired thermogenesis in mice. However, transmission mechanism is unclear. Here, we aimed to study the heterocellular crosstalk between $PM_{2.5}$-exposed macrophages and pure primary adipocytes using co-culture systems. The Stromal Vascular Fraction (SVF) was isolated from the epididymal Visceral Adipose Tissue (epiVAT) of 6-week-old male C57BL/6 mice, and cultured in the lower compartment of 6.5 mm transwell plates (Corning). After 3 days in induction medium, remaining cells were differentiated to either white or brown adipocytes for 9 additional days. Appropriate adipocyte differentiation was confirmed by Oil Red O (ORO) staining or UCP-1 immunohistochemistry, respectively. In parallel, bone marrow derived macrophages (BMDMs) were isolated and cultured in RPMI supplemented with 100 ng/mL M-CSF. After 9 days, BMDMs were transferred to 0.4 μm pore polycarbonate membrane inserts, incubated with RPMI (control) or a transition-metal rich $PM_{2.5}$ surrogate (Residual Oil Fly Ash, ROFA) at 100 μg/mL, and co-cultured with differentiated white or brown adipocytes for 24 h. In BMDMs, $PM_{2.5}$ uptake was confirmed by bright field and transmission electron microscopy, and increased pro-inflammatory IL-6 and MCP-1 levels were detected in cell culture supernatants when compared to the control. In white adipocytes co-cultured with $PM_{2.5}$-exposed BMDMs, metabolic gene expression by qPCR showed a significant upregulation of *Cd36*, *Lipe*, and *Adrb3* mRNA levels. In brown adipocytes, thermogenic *Ucp1, Lipe*, and *Adrb3* mRNA levels were significantly decreased by 85%, 44%, and 58%, respectively ($p < 0.01$). Our findings provide a novel insight into the role of innate immune responses triggered by $PM_{2.5}$ exposure that are associated with metabolic derangements and the development of cardiovascular risk factors.

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PP I_D41

REDISULPHID: A STRUCTURAL BIOINFORMATIC SCREEN FOR IDENTIFYING REDOX-REGULATED DISULPHIDES

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Redox thiol modifications are key regulators of both pathological and physiological protein function. They also provide key targets for therapeutic intervention, as they can react with small molecule electrophiles. A prominent redox modification is disulphide bonds, which can have a potent impact on protein function by introducing structural constraints, thus representing important targets for therapy[1, 2, 3]. Despite their importance, there has been no broad identification of reversible redox-regulated disulphides due to limitations in their detection in complex biological samples. To address this, we have developed ReDisulphID, a bioinformatic tool designed to screen and identify novel reversible redox-regulated disulphides across the entire mammalian RCSB Protein Data Bank (RCSB PDB). ReDisulphID uses parameters derived from known redox-regulated disulphides to assign a redox-activity score to proximal cysteine pairs found in any mammalian structure. This method has scored over 50,000 potential redox-regulated disulphides, highlighting those likely to undergo this modification. Following its development, this approach was used in the discovery of a novel redox sensor in PEPD, which plays a key role in regulating p53 activation. These findings highlight the utility of ReDisulphID as a new platform for the discovery of novel sites for direct therapy, by providing a means to pinpoint redox-active disulphides.

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PP I_D42

QUANTUM SENSING OF FREE RADICALS IN SEMEN: INVESTIGATING THE ROLE OF OXIDATIVE STRESS IN IDIOPATHIC MALE INFERTILITY

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Male factor infertility contributes to 40-50% of fertility issues in couples, with approximately 40% lacking identifiable etiological factors. Oxidative stress (OS) is hypothesised to be a key element in the pathophysiology of idiopathic male infertility. Despite the crucial role of free radicals (FR) in sperm maturation and fertilisation, excessive levels can lead to OS-induced cell damage. Since FR are short-lived and reactive, current techniques lack the ability to measure real-time concentrations and pinpoint their origin. This study introduces T1 relaxometry, a quantum sensing technique using fluorescent nanodiamonds (FNDs) for nanoscale FR detection for the first time in living sperm cells and seminal fluids. FNDs exhibit unique properties, altering their optical properties in response to their magnetic environment without photobleaching, enabling long-term and real-time measurements of FR. Pre-clinical studies in boar spermatozoa assessed FND biocompatibility through viability assays. Furthermore, T1 measurements, utilising a custom magnetometry setup, were conducted to study real-time free radical dynamics before and during capacitation. Clinical studies were conducted on residual samples obtained following semen analysis from males seeking fertility treatment at the Centre of Reproductive Medicine (CRM) of the University Medical Centre Groningen (UMCG). Using T1 relaxometry, FR concentrations are measured in human seminal plasma and at spermatozoa to identify specific sources of FR production. Results demonstrate that we are able to detect FR generation with nanoscale resolution using T1 relaxometry in living sperm cells. Localised T1 measurements identified NOX5 as a primary FR source during capacitation in boar spermatozoa. Furthermore, our results show pioneering steps of T1 relaxometry measuring FR production in human spermatozoa and seminal plasma. In conclusion, this study marks pioneering progress in using T1 relaxometry to visualise free radical generation in boar and human semen, offering new prospects for clinical applications as a diagnostic tool for assessing OS in male infertility.

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PP I_D43

HYPER7 BIOSENSOR ASSOCIATE WITH PROTEOMIC SHIFTS RELATED TO OXIDATIVE STRESS IN ENDOTHELIAL CELLS

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Fluorescent proteins, such as GFP, undergo a pivotal maturation process that is associated with the generation of hydrogen peroxide (H_2O_2) at levels that were generally accepted as negligible. The genetically encoded GFP-based HyPer7 biosensor is widely utilized for intracellular H_2O_2 quantification. Here we investigate the potential implications of HyPer7 expression on proteomic profiles and overall cellular homeostasis, and explore its correlation with oxidative stress responses. Untargeted proteomic analysis of HyPer7 expressing EA.hy926

endothelial cells reveals substantial changes in their proteomics profiles. The significantly differentiated proteins were associated with cellular responses to stress and antioxidative related transcription factors. Assessment of Energy metabolism showed decline of oxidative respiration associated with disrupted mitochondria membrane potential. This data suggests that HyPer7 expression challenges cellular homeostasis. Hence, it is essential to exercise caution when using such tools, and to validate outcomes with complementary techniques to ensure the reliability of the obtained results.

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PP I_D44

ADVANCING SUSTAINABLE BIOFUNCTIONAL TEXTILES: EXPLORING THE ANTI-INFLAMMATORY, ANTIOXIDANT, AND UV-PROTECTION PROPERTIES IN ONION (ALLIUM CEPA L.) SKIN EXTRACT

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Skin acts as the body's primary defense against external aggressions. Hence, this study investigates the eco-sustainable production of biofunctional textiles using five cellulosic fabrics: cotton, linen, bamboo, hemp, and nettle. These textiles are produced from aqueous extracts derived from the outer skin of the Dorata di Parma onion variety (*Allium cepa L.*). As concerning dyeing experiments, pre-treatment processes were conducted both with and without the use of alum, tin chloride, or tannic acid. The results of UV-protection factor (UPF) measurements showed a significant enhancement in UPF values for all textiles when treated with tannic acid. Indeed, the values ranged from two times higher (in hemp) to six times higher (in bamboo) compared to the corresponding scoured fabrics. Furthermore, when immersing textiles in artificial sweat to simulate skin contact, cotton, hemp, and nettle showed a higher and uniformly distributed release of phenols, indicating greater biofunctional activity, in comparison to linen and bamboo. It was observed that cell viability of primary human dermal fibroblasts (HDF) and skin keratinocytes (NCTC2455) was not affected from the 'bio-functional sweat'. Phenolic components released by biofunctional textiles in artificial sweat were found to provide significant protection against heat shock in HDF cells.Moreover, in both HDF and NCTC2455 cells, the phenolic components from the textiles reduced ROS levels induced by H_2O_2 stress or LPS-induced inflammation. Cotton and hemp textiles, pre mordanted with tin chloride and tannic acid, exhibited higher effectiveness to reduce oxidative stress in vitro. In conclusion, this study highlights the potential of eco-friendly, coloured cellulosic fabrics containing phenol-rich onion peel as a natural shield against UV radiation. Additionally, these fabrics release antioxidants and anti-inflammatory agents onto the skin through sweat, showcasing their multifaceted benefits.

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PP I_D45

REVISITING THE ROLE OF H_2O_2 IN NRF2 ACTIVATION USING AN OXYGEN-INDEPENDENT BIOSENSOR

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NRF2 (Nuclear factor erythroid 2-related factor 2) is a crucial transcription factor that governs the expression of genes involved in oxidative stress response, cellular defense mechanisms, and energy metabolism. Impaired NRF2 function has been implicated in aging and various age-related diseases, including neurodegeneration. Despite its significance, the study of Nrf2 in living model systems has been hindered by the lack of suitable tools, leading to discrepancies in the literature. One unresolved question is whether and to what extent H_2O_2, a well-known reactive oxygen species (ROS), activates Nrf2. To address this, fluorescent protein (FP)-based biosensors are promising, yet the oxygen dependency of chromophore formation in prevalent FPs poses challenges under hypoxic conditions, potentially yielding inaccurate results. To overcome this limitation, we developed an oxygen-insensitive Nrf2 biosensor reporter based on miniGFP FP, which incorporates flavin to fluoresce, allowing us to monitor Nrf2 behavior regardless of pericellular oxygen levels. Our preliminary findings indicate that the administration of H_2O_2 whether exogenous (bolus; 1-300 uM, up to 24 hours) or endogenous (using mDAAO enzyme; 0.1-50 mM D-alanine, up to 24 hours) is insufficient to stimulate Nrf2 in human cerebrovascular endothelial cells (D3/hCMEC). More interestingly, treatment with Auranofin (0.1-1 uM, up to 24 hours), a TrxR inhibitor, led to significant increases in Nrf2 levels under normal cell culture oxygen conditions (18% O2). However, when replicating the experiment in cells adapted to normoxia (5% O2), no Nrf2 activation was observed. Collectively, our findings underscore two striking facts. Firstly, for H_2O_2 to induce Nrf2 activation, abating the antioxidant machinery is essential. Secondly, in comprehensively studying redox biology phenomena, accounting for oxygen level is indispensable; otherwise, the translational relevance of results to clinical settings may be compromised.

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PP I_D46

DEVELOPMENT OF A NON-ANIMAL TESTING METHOD USING GENETICALLY ENGINEERED BIOSENSORS FOR ASSESSING SKIN SENSITIZERS

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Skin sensitizers refer to chemical substances that trigger allergic reactions upon skin contact. The industrialization of these products has led to a significant increase in the prevalence of allergic diseases. These all products need to pass through the assessment process before marketing. Due to substantial limitations in the use of animals for risk assessment, considerable efforts have been dedicated to developing non-animal testing methods such as *in silico* or *in vitro*. Some of these tests, approved by the Organization for Economic Co-operation and Development, OECD, rely on luciferase activation linked to Nrf2 expression. Furthermore, genetically encoded biosensors enable real-time measurement of Nrf2 or even hydrogen peroxide (H_2O_2) levels in direct response to oxidative stress increase in a single cell. This advanced technology also allows for the precise targeting of these sensors to distinct subcellular locales, thereby allowing an understanding of localized cellular responses. Therefore, we aimed to develop a drug screening system utilizing keratinocyte HaCat cell line stably expressing the genetically engineered biosensors that detect the oxidative stress level induced by skin sensitizers using the HyPer7 sensor for H_2O_2 measurement and pTRAF sensor for Nrf2 activity assessment. Our data demonstrated that the expression level of either HyPer7 or pTRAF is significantly increased when we treated these stable cells with a high yet not-toxic dose of sensitizer. Moreover, we explored the impact of oxygen levels on the sensitivity of HaCat cells to skin sensitizers. Remarkably, the concentration lethal under hyperoxia conditions (18 kPa) did not induce cell death under normoxia conditions (5 kPa). These preliminary results demonstrate the potential of our approach as a valuable tool for non-animal testing methods, emphasizing its significance in advancing the field.

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PP I_D47

HUMAN 3D FULL SKIN MODELS COUPLED WITH ADVANCED BIO-MARKERS ASSESSMENT FOR THE EFFICACY EVALUATION OF TOPICAL REDOX MODULATORS

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