



June 22, 2013 – June 28, 2013

The Founding of ISOTT: The Shamattawa (Confluence) of Engineering Science and Medical Science

Dr. Duane F. Bruley, Society Historian

Synthesizer, Inc.

Study Goal: To assemble the fundamental engineering and medical science elements that was bases in the founding of ISOTT.

Abstract: This paper expands upon past writings and reflects the truism stated by Confucius, "Study the past if you would define the future." The founding of ISOTT was based upon the blending of Medical and Engineering sciences. Beginning with Carl Scheele's discovery of oxygen, the medical sciences advanced the knowledge of its importance to physiological phenomena. Meanwhile, engineering science was evolving as a mathematical discipline used to define systems quantitatively from basic principles. In particular, Adolf Fick's employment of a gradient led to the formalization of transport phenomena. These two rivers of knowledge were blended to found ISOTT at Clemson/Charleston in 1973. The future will be determined.

Conclusion: The blending of the engineering and medical sciences has led to an inter- and cross disciplinary international society that promotes collaboration in the pursuit of knowledge regarding oxygen transport and utilization from source to single cells.

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Cognitive function in Gambian infants using optical topography

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Study Goal: To investigate the feasibility of using optical topography (OT) as a novel neuroimaging technique to study the effects of under-nutrition in cognitive development in infants in the rural Gambia.

Abstract: Dietary insufficiency in the first 1000 days of life can contribute to poor cognitive development. We used OT as a compact, non-invasive technique for measuring the changes in brain oxygenation associated with localized brain function. Data from a total of 42 Gambian infants, aged 4-8 months, were acquired. The study involved measuring the response of brain activity during visual and auditory stimulation by replicating the protocols followed previously in UK infants. Initial analysis reveals that the Gambian infants showed a preferential response to the human (rather than non-human) visual and auditory stimuli with activation clearly seen in the temporal cortex. These observations are in agreement with the studies in the UK infants.

Conclusion: OT is a feasible neuroimaging technology for this resource poor setting and may be a useful tool in assessing nutritional-specific interventions. We have set the ground for longitudinal brain development in a large cohort of infants and children.

Acknowledgments: This work is supported by the Bill and Melinda Gates Foundation



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Hypoxia and Brain Oxygen

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Abstract: The mammalian brain relies completely on oxygen to generate ATP from glucose for its function. Yet, oxygen stores are non-existent and brain tissue oxygen tension, on average, are low relative to venous levels. Thus, the brain depends on a close relationship between oxygen delivery through parenchymal capillaries to match demand from neuronal activity. This dynamic dependence on blood flow bringing oxygenated hemoglobin to activated brain tissue regions underlies the technique of functional magnetic resonance (fMRI).

Chronic changes in oxygen availability, such as with acclimatization to altitude, result in long term compensatory changes in capillary structure that restore brain tissue oxygen and maintain the dynamic relationship between flow and activity. Hypoxic environmental conditions result in angiogenesis and increased capillary density; hyperoxic conditions lead to capillary rarefaction.

The dynamic changes in capillary structure and function have led us to propose the concept of “angioplasticity”. This concept comprises the processes of angiogenesis and angiolytic which are the basic structural responses to changes in the energy demands and/or oxygen/substrate delivery at the level of the neurovascular unit, which comprises of neuron, astrocyte and endothelial cell. Prolonged mild hypoxia results in an increase in brain capillary density (angiogenesis) as an integral part of the acclimatization process. There are 2 major pathways responsible for brain angiogenesis: a hypoxia-inducible factor-1/2 (HIF-1/2 α) dependent up-regulation of vascular endothelial growth factor (VEGF), and a HIF-1/2 α independent up-regulation of cyclooxygenase-2 (COX-2) and angiopoietin-2 (Ang-2). In the CNS there appears to be a continuous balance between angiogenic and angiolytic signals, responding to the tissue metabolic energy demands and the availability of oxygen and substrate (glucose); and this balance is assessed at the local level of the neurovascular unit.

Conclusion: The mammalian is maintained in a low oxygen environment by regulated angioplasticity.

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The use of NIRS to assess the effect of training on peripheral muscle oxygenation changes in elite rugby players performing repeated supramaximal cycling tests

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Study Goal: Assess the usefulness of near-infrared spectroscopy (NIRS) in the identification of individual changes in peripheral muscle oxygenation in elite rugby players.

Abstract: Eight UK premier league academy level rugby players were assessed. During both cycle tests all subjects experienced a drop in muscle oxygen saturation (Pre Δ -12.39% (6.01), Post Δ -14.83% (3.88)). Post training, there was an increase in the extent of desaturation (drop in TSI %). Additionally seven out of eight players showed a significant increase in Δ HHb (Pre Δ +4.80 (3.87), Post Δ +7.58 (4.36)) ($p < 0.05$). Players who exercised at the highest power tended to decrease their muscle oxygenation to a greater extent. A non-significant increase in reoxy rate post exercise ($p > .05$) was noted, with some variation amongst individuals. There was also a tendency to an increase in the recovery half time.

Conclusion: In conclusion NIRS is able to measure positive training effects on muscle oxygen extraction. The similar responses seen by all players in this study, suggests that NIRS may indeed be a useful tool for optimising training in individual subjects.



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Group Analysis of hypoxia-ischaemia in piglets using a computational model

Tharindi Hapuarachchi

Study Goal: Neonatal cerebral hypoxia-ischaemia (HI) can result in severe disabilities. To investigate HI, we've developed a computational model of neonatal brain metabolism based on physiology, using data from piglet experiments to run and test the model.

Abstract: We previously reported case studies from HI experiments. We're now investigating group data from over 22 piglets, providing a more accurate overview of the metabolic and haemodynamic changes that occur during HI.

Our BRAINPiglet model is able to simulate near-infrared spectroscopy (NIRS) and magnetic resonance spectroscopy (MRS) measurements, in particular quantifying changes in concentrations of oxy- and deoxyhaemoglobin, pH and Cytochrome-C-Oxidase – responsible for oxygen consumption in mitochondria.

Composed of approx 25 variables and 100 parameters, the model also simulates indeterminable quantities such as the cerebral metabolic rate of oxygen. Parameter optimisation further offers an insight into the effect of HI on the brain.

Conclusion: We will present a group analysis of NIRS and MRS data from HI experiments in piglets and compare these with corresponding simulations of haemodynamics and metabolism from our model.



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A broader perspective on cerebral autoregulation

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Study Goal: Continuous monitoring of cerebral autoregulation (CA) might predict “optimal” arterial blood pressure (ABP) targets, to avoid cerebral hypoperfusion after brain injury. We examined pitfalls of this approach using model based analysis of clinical data.

Abstract: If cerebral blood flow (CBF) falls below the lower pressure limit of CA there is a risk of brain ischaemia. It is not easy to measure CBF, so indices of CA, derived from slow waves of neuromonitoring, have been used to determine the lower limit of CA, to guide “optimal” ABP management. Multiple modalities may form the basis of this analysis, including intracranial pressure, transcranial Doppler flow velocity, near infrared spectroscopy and brain tissue pO₂. However, these modalities are influenced by factors other than ABP, including CO₂, intracranial compliance and metabolism. Model based analysis of multimodal monitoring will be presented highlighting the effect on this method of determining optimal ABP by physiological confounders.

Conclusion: We show that CA indices have modality specific limitations, and that model informed multimodal monitoring of CA might overcome these constraints, delivering relevant clinical information at the bedside.

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Changes of Cerebral Tissue Oxygen Saturation at Specific Sleep Transitions in Adolescents

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Study Goal: In adults, cerebral oxy- and deoxyhaemoglobin concentrations showed characteristic changes at sleep transitions. The aim was to assess these changes in adolescents and additionally to measure tissue oxygenation by near infrared spectroscopy.

Abstract: It was reported that cerebral oxyhaemoglobin concentration ([O₂Hb]) increased and deoxyhaemoglobin concentration ([HHb]) decreased at the transition from non-rapid eye movement (NREM) sleep to REM sleep and wakefulness. Transitions to NREM sleep (from REM sleep, wakefulness) led to a decrease in [O₂Hb] and an increase in [HHb].

We measured [O₂Hb], [HHb] and tissue oxygenation (StO₂) of the left prefrontal cortex in 12 healthy adolescent male subjects (age 10-16 years) during sleep. The direction and magnitude of changes in [O₂Hb] and [HHb] were comparable to the ones observed in adults. StO₂ increased at the transitions from NREM to REM sleep and decreased from REM to NREM sleep and sleep onset (all $p < 0.01$, linear mixed effects model).

Conclusion: The changes in oxygen metabolism during sleep stage transitions in adolescents are similar to the ones observed in adults. In addition, we show for the first time changes of StO₂ at sleep transitions.



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Psychological mechanism of increases in deoxy-hemoglobin concentration during neuronal activation in patients with cerebral ischemia: a simulation study with the Balloon model

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Study Goal: To clarify the physiological mechanisms of the atypical evoked cerebral blood oxygenation response in cerebral ischemia or brain tumor (i.e., increases in deoxy-hemoglobin during activation), we performed a simulation study with the Balloon model.

Abstract: Patients with cerebral ischemia or brain tumor have been reported to exhibit an increase of deoxygenated hemoglobin (deoxy-Hb) with an increase of oxygenated hemoglobin (oxy-Hb). However, the physiological mechanisms underlying these hemodynamic response patterns are unclear. In this study, we performed a simulation with the Balloon model (Buxton et al., 1998). We hypothesized that the oxygen extraction rate during the rest period (E₀) in the patients is larger than normal, because the cerebral blood flow and the speed at which the blood passes through the brain tissues are lower in the patients. The simulation result showed an increase of deoxy-Hb as well as oxy-Hb, especially when E₀ is extremely high.

Conclusion: The results of our simulation suggest that the increase of deoxy-Hb during activation in patients with ischemia or brain tumor is caused by an increase in the oxygen extraction rate at rest, compared with that of healthy adults.

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The challenge of the influence of paCO₂ on brain hemodynamics and oxygenation

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Study Goal: We performed several functional brain studies investigating effects of speech related tasks using fNIRI. We will present the results and highlight the impact of paCO₂ as a confounding factor and the importance to monitor paCO₂ during such studies.

Abstract: In two initial fNIRI studies of our research group we found a decrease in cerebral oxygenation and hemodynamics during speech tasks. We hypothesized that changes in paCO₂ caused the signal changes which was proven to be correct by performing three subsequent studies. In these studies, we could show that indeed paCO₂ drops during different speech tasks (active speech, inner speech and - new - even heard speech), causing also a drop in the fNIRI signals. These findings highlight the importance of monitoring CO₂ changes when performing fNIRS/fNIRI studies that involve task that could alter respiration.

Conclusion: paCO₂ is an important confounder in fNIRI and probably generally in functional brain studies that may alter respiration. The implications of the conclusion, possible future research directions and approaches for solutions will be discussed.



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Usefulness of NIRS in prevention of stress-induced diseases

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Study Goal: The incidence of stress-induced psychological and somatic diseases has been increasing rapidly in advanced countries. In order to prevent stress-induced diseases, it is important to establish a non-invasive method for assessment of stress response.

Abstract: We have been using a two-channel near-infrared spectroscopy (NIRS) for assessment of mental stress and relaxation, and found that asymmetry of prefrontal cortex (PFC) activity measured by NIRS correlated with behavioral and somatic responses to mental stress. In addition, relaxation by fragrance altered the dominant side of the stress induced PFC activity from the right to the left side, and reduced the hyperactivity of the HPA system. Recently, we have developed a new analyzing method to evaluate the asymmetry of PFC activation at rest without any task. We found that asymmetry of spontaneous oscillation of hemodynamic changes measured by NIRS in the PFC correlated with STAI-1 (state anxiety) score but not STAI-2 (trait anxiety) score.

Conclusion: NIRS may be helpful in prevention of stress-induced diseases, since it allows us to assess conveniently and noninvasively the level of mental stress and the effectiveness of various relaxation methods.

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False Positives in fNIRS: Identifying and Quantifying Systemic Influences in fNIRS Data during Cognitive Tasks

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Study Goal: Investigate the relationship between task related brain fNIRS measurements and systemic changes during cognitive functional tasks and discuss methods to uncouple them.

Abstract: Brain functional studies of task-specific activation using functional neuroimaging rely on the existence of a close coupling between neuronal electrical excitation and regional changes in brain metabolism and regional cerebral blood flow, sometimes referred to as neurovascular coupling. Regional haemodynamic changes are used as a surrogate marker for changes in regional brain function that occur due to changes in metabolism during excitatory or inhibitory neurotransmission. Functional near-infrared spectroscopy (fNIRS) is used to non-invasively measure the changes in oxygenated and deoxygenated haemoglobin ([HbO₂], [HHb]) and hence investigate the brain haemodynamic changes, which occur in response to functional activation at specific regions of the cerebral cortex.

Neuronal excitation causes oxidative metabolism increases that lead to brain vascular responses; blood vessels dilate causing an increase in cerebral blood flow and cerebral blood volume. This oversupply of oxygenated blood causes the [HbO₂] to increase and the [HHb] to reduce. In order for this response to be monitored unambiguously it is important that the haemodynamic task related activity is occurring on top of an unchanged global systemic and brain resting state. However, changes in cerebrovascular dynamics due to task related systemic cardiovascular changes can lead to results that are unrelated to neuronal activity; in addition to producing extracranial vascular changes that can lead to fNIRS signal contamination.

For several years now we have investigated the relationship between task related brain fNIRS and systemic changes in a large group of young healthy adults during cognitive functional tasks that included anagram solving and video gaming. Here we will present fNIRS and systemic data from these studies and discuss novel methodological tools to investigate their relationship.

Conclusion: Task related changes seen in systemic variables such as blood pressure in some volunteers might contribute to the changes in the brain fNIRS signals leading to false positives.

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Influence of Subjective Happiness on the Prefrontal Brain Activity: An fNIRS Study

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Study Goal: By investigating the relationships between brain activities and levels of subjective happiness, we aim at prevention of mental disorders and physical illnesses.

Abstract: Focusing on the relationship between subjective happiness (SH) and emotional changes, we examined influences of SH on emotion-related prefrontal activities by using multichannel NIRS. International Affective Picture System (IAPS) was used to evoke emotional changes. Subjects were a total of 18 right-handed healthy undergraduate students. Frequency of picture-induced increases in oxygenated hemoglobin (oxy-Hb) was evaluated. Subjects with high SH-score were accompanied by higher frequency of increased oxy-Hb in the left prefrontal cortex (LPFC) while viewing pleasant pictures (PPs), whereas they showed lower frequency in the right PFC (RPFC) while viewing unpleasant pictures (UPPs).

Conclusion: It is well known that the LPFC and RPFC are differently engaged in the emotional processes. Although further investigations are required, the present results indicate that the SH level influences the right-left differences in emotion-related prefrontal cortex.



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Diffuse NIRS under dynamic pressure application

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Study Goal: Applied pressure to the skin surface can be used to empty and refill the capillary bed. Use of diffuse reflectance spectroscopy to observe the effect of this refilling on near infrared absorbance may provide useful information on capillary bed perfusion.

Abstract: Apparatus recently developed at the University of Nottingham allows near infra-red diffuse reflectance spectroscopy of the skin surface at large source-detector separation to be conducted during rapid transitions in surface applied pressure. A novel spectrometer design using a cluster of light emitting diodes at discrete wavelengths and a single photodiode will be described, along with a force feedback controlled linear actuator system. This allows applied pressure to be varied between arbitrary points in the 0 to 5psi range over a time-scale of around 50 milliseconds. Preliminary results from healthy volunteers will be presented and discussed in detail.

Conclusion: Preliminary results demonstrate the practicality of the hardware. Blood deoxygenation during passage through the capillary bed can be observed, and different vascular compartments can be separated using low applied pressures. Further modeling is required to fully understand the results.

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Bayesian Prediction of Anxiety Level in Aged People at Rest using 2-Channel NIRS Data from Prefrontal Cortex

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Study Goal: The aim of this study was to predict mental stress levels of aged people at rest from two-channel near-infrared spectroscopy (NIRS) in the prefrontal cortex (PFC). We used the State-Trait Anxiety Inventory (STAI) for the mental stress index.

Abstract: We previously constructed a machine learning algorithm to predict mental stress level using two-channel NIRS data from the PFC in 19 subjects aged 20–24 at rest [1]. The present study attempted the same prediction for older subjects aged 61–79 (women, 10; men, 7). The mental stress index was STAI. After subjects answered the STAI questionnaire, the NIRS device measured oxy- and deoxyhemoglobin concentration changes during a 3-minute resting state. The algorithm was formulated within a Bayesian machine learning framework and implemented by Markov Chain Monte Carlo. Leave-one-out prediction was performed.

[1] M. Sato, W. Ishikawa, T. Suzuki, T. Matsumoto, T. Tsujii and K. Sakatani, *Adv.Med.Biol.*, 765, 251-256, 2013

Conclusion: Average prediction error was 5.27. Prediction errors of 12 subjects were lower than 5.0. Since the STAI index ranged from 20 to 80, the algorithm appeared functional for aged subjects also.

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Development of a hybrid microwave-optical thermoregulation monitor for the muscle

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Study Goal: This paper presents the latest development of the hybrid microwave-optical thermoregulation monitor for the muscle. It is capable of warming the muscle and measuring the subsequent blood volume changes, using a novel microwave applicator and NIRS.

Abstract: Current thermoregulation studies in the muscle are performed by warming the site using microwave applicators, and measuring the blood flow change using invasive/radioactive methods. The aim here was to combine both the heat source and the blood volume monitor into a single thermoregulation monitor. It consists of a microwave applicator which raises the muscle temperature by 2-3 °C, causing vessels to dilate and blood flow to increase. This is monitored by an integrated NIRS optical probe and a laser Doppler probe to monitor the blood volume change in the deep tissue and the skin blood flow, respectively. The device can be used to monitor the condition of the vasculature with potential applications such as assessing the amputation level.

Conclusion: In vivo human results will be presented in terms of changes in oxy/deoxy-Hb concentrations and skin blood flow in response to local warming. They will be helpful to assess the nature and location of the thermal response, i.e, muscle and/or skin.

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Reduced Adenosine A2a Receptor-Mediated Efferent Arteriolar Vasodilation Causes Diabetes-Induced Glomerular Hyperfiltration

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Study Goal: To clarify the involvement of adenosine A2a receptor signaling in mediating the increased glomerular filtration rate (GFR) and filtration fraction (FF) in diabetes and thereby increased tubular Na⁺ transport in relation to renal blood flow (RBF).

Abstract: GFR was higher, but RBF was similar in diabetics compared to controls resulting in increased calculated FF. Infusion of the selective adenosine A2a-receptor agonist CGS21680 into the renal artery reduced GFR only in diabetics whereas renal blood flow was unaffected, resulting in a normalization of the FF. Additional groups were allocated to micropuncture studies where tubular free flow pressure (Pff) and stop flow pressure (Psf) were investigated. CGS21680 did not affect Pff in neither group, but decreased Psf in diabetics indicating reduced hydrostatic pressure in glomerular capillaries.

Conclusion: In conclusion, decreased adenosine A2a-receptor signaling in diabetes contributes to increased FF and GFR by increasing vascular resistance of the efferent arteriole.



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Pre-existing Hypoxia Sensitizes the Kidney to an Ischemia-Reperfusion Insult

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Study Goal: The aim of this project was to investigate if pre-existing tissue hypoxia sensitizes the kidney to an IR insult independently of hyperglycemia and oxidative stress.

Abstract: Hypoxia plays an important role in progression of kidney disease. Indeed, diabetic kidney develops pronounced hypoxia and is sensitive to an ischemia-reperfusion (IR) insult. However, diabetes is also characterized by hyperglycemia and oxidative stress. It is therefore important to delineate the role hypoxia per se. We therefore developed a protocol to induce intrarenal tissue hypoxia independently of the diabetic condition using the mitochondrial uncoupler dinitrophenol (DNP).

Rats with and without DNP-induced hypoxia were subjected to 40 min of kidney ischemia and function measured 4 weeks thereafter.

IR to DNP-treated rats resulted in 30% lower glomerular filtration rate, whereas it did not affect kidney function in untreated rats.

Conclusion: Pre-existing intrarenal hypoxia sensitizes the kidney to an IR insult. It may therefore be beneficial to target kidney hypoxia as a strategy to prevent or reduce the often detrimental effect of an IR insult to the diabetic kidney.



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Optical Imaging of the Tissue: Current State and Future of Instrumental and Methodological Approaches

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Study Goal: Optical methods are capable of imaging the oxygenation of tissue, a highly relevant diagnostic parameter. The aim is to present technical progress in near-infrared imaging (NIRI), future developments and compare NIRI to other methods.

Abstract: Near-infrared imaging (NIRI) currently achieves a spatial and temporal resolution of 10mm at 10min and is able to provide images of O₂ saturation of tissue. By employing novel single-photon avalanche diode (SPAD) arrays, the number of detectors is increased by several orders of magnitude. Time to digital converters determine the time of flight of each photon. In laboratory tests using phantoms, the greatly increased number of measurements enabled an unprecedented spatial resolution of 5mm. Simulations show that the potential resolution is in the order of millimeters. Further progress will be achieved by custom designed chips with properties optimized for NIRI. Advantages and limitations will be discussed and compared to other approaches.

Conclusion: The novel SPAD arrays have already shown to enhance NIRI and the potential is by far not exhausted. In the future NIRI will become a diagnostic tool for many clinical applications.

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A tale of two methods: the synergies of combining NIRS with MRI for studies of brain oxygenation and metabolic rate

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Study Goal: This paper discusses advantages of combining NIRS with MRI for studies of brain metabolism. We show how this multimodal method can be used for monitoring brain disorders with a hypoxic component and for measuring metabolic rate for oxygen.

Abstract: MRI has good spatial resolution and is sensitive to change in dHb content. Near-infrared spectroscopy has lower spatial resolution but has the advantage of being able to detect, and with specific technologies, to quantify oxyHb, tHb and dHb. By combining the two methods, excellent synergies result which can improve the interpretation of either method and can also provide additional functionality with respect to measuring brain oxygenation and metabolism. This paper discusses the application of the methods, both individually and combined into a multimodal technology, for assessing brain disorders that involve hypoxia. We also show data on how multimodal MRI and NIRS can be used to quantify metabolic rate for oxygen in the brain.

Conclusion: The combination of MRI and NIRS provides synergistic information. One significant advantage is that this multimodal approach can be used to measure metabolic rate

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Oxygen Diffusion: an enzyme controlled variable parameter

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Study Goal: Previous oxygen microelectrode studies have shown that the oxygen diffusion coefficient (DO_2) increases during extracellular PO_2 decreases, while intracellular PO_2 remained unchanged. The aim was to learn more about the DO_2 regulating mechanism.

Abstract: Oxygen dependency of complex multicellular organisms requires secure adequate oxygen supply to the cells while toxic concentrations have to be avoided. Oxygen brought to the tissue by convection diffuses through the intercellular and cell mucopolysaccharide membranes (diffusion barriers). PO_2 and DO_2 were measured by membrane-covered and by bare gold microelectrodes, as were also spike potentials, in gerbil brain cortex. Moderate respiratory hypoxia was followed by a primary sharp drop of tissue PO_2 that recovered to higher values concomitant with an increase of DO_2 . A drop of intracellular PO_2 recovered immediately. Studies on the abdominal ganglion of *aplysia californica* showed similar results.

Conclusion: Oxygen diffusion through membranes is variable securing an adequate extra-intracellular PO_2 . Cell-derived glucosaminooxidase seems to regulate the polymerisation-depolymerisation ratio of membrane mucopolysaccharides and thus diffusion.



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A Phase 1 trial of Sanguinate: An Oxygen transfer agent

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Prolong Pharmaceuticals

Study Goal: Sanguinate (PEGylated carboxyHb bovine) is an HBOC for the treatment of hypoxia. Its components inhibit vasoconstriction, decrease extravasation, prolong circulation and deliver oxygen. Phase I trials for safety and pharmacokinetics are completed.

Abstract: Toxicity studies found Sanguinate to be safe and well tolerated. No vaso-constrictive side effects were observed. The p50 of 12 falls between that of the RBC and the hypoxic tissue. Animal models of cerebral ischemia, peripheral ischemia, and myocardial ischemia demonstrated Sanguinate's efficacy in reducing myocardial infarct size, limiting brain death and neuronal damage from cerebral ischemia and promoting more rapid recovery from hind limb ischemia. In a Phase I trial, 3 cohorts of 8 patients received ascending doses of 80, 120 or 160 mg/kg Sanguinate as compared to a control saline group. There were no serious adverse events. Haptoglobin decreased with increasing dose. The T_{1/2} was dose dependent and ranged from 7.9 to 13.7 hours.

Conclusion: Phase I clinical results found Sanguinate safe and well tolerated. Pharmacokinetic results will be presented as well as findings from a compassionate use in a sickle cell patient. Considerations for Phase II trials will be discussed.

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cNO Nano-Therapeutic as a Universal Multifunctional Stroke Drug

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Study Goal: "Time is brain" is the mantra for treatment of all stroke patients. A drug safe for immediate and universal use in all stroke victims without the need for neuroimaging would be a major breakthrough and paradigm change in stroke therapy.

Abstract: The efficacy and safety requirements of an early treatment drug is that it can enhance or maintain cerebral blood flow for oxygen delivery to the ischemic region without reperfusion damage and inflammation injuries and can also reduce hemorrhagic transformation in ischemic stroke and the cerebral injuries from ongoing hemolysis in hemorrhagic stroke. SynZyme's cNO nano-therapeutic, polynitroxylated pegylated hemoglobin has been shown to be neuroprotective and to enhance blood flow in the ischemic penumbra while protecting the vasculature and reducing hemorrhagic transformation, which could expand the number of ischemic stroke patients treatable with lytic therapy beyond the current 2-3% after 16 years of tPA use per 2011 STAIR report.

Conclusion: SynZyme's cNO nano-therapeutic is a multifunctional vascular pegylated hemoglobin labeled with catalytic free-radical-based caged nitric oxide (cNO) to enhance its therapeutic index for the early treatment of both hemorrhagic and ischemic stroke.

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Haemodynamic and metabolic investigation of cerebral autoregulation in the newborn piglet

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Study Goal: To implement signal processing tools to assess cerebral autoregulation using NIRS with systemic data; and investigate this as a surrogate marker of histological outcome and protocol to assess the vulnerability of the neonatal brain to anesthesia.

Abstract: Newborn piglets were randomly assigned to receive either 6 hours of anaesthesia (isoflurane) or the same with an additional hour of minor surgery. The effect of spontaneous changes in mean arterial blood pressure (MABP) on cerebral blood flow was evaluated using NIRS signals. Transverse broadband NIRS measured the cerebral haemodynamic (oxy- and deoxyhaemoglobin, HbO₂ and Hb) and metabolic changes (cytochrome c oxidase, CCO). A marker for impaired cerebral autoregulation, concordance between MABP and intravascular oxygenation (HbD = HbO₂ – Hb) in the frequency domain, was assessed using cross-spectral analysis techniques (coherence and transfer-function gain). The relationship between MABP and CCO was also investigated.

Conclusion: Presence and severity of autoregulation impairment was compared with protocol (surgical exacerbation of impairment) and histological outcome (presence of cell death and microglial activation in the brain). Results will be presented in the meeting.

Acknowledgments: EPSPC, Wellcome Trust



June 22, 2013 – June 28, 2013

Observation of Cerebral Hemodynamic Changes and Metabolic Alteration in Severe Hemorrhagic Shock

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Study Goal: The biological mechanism of hemorrhagic shock is not totally clear. We aim to use optical imaging to study how the cerebral circulation and metabolism change during the long process of severe hemorrhagic shock, especially the decompensatory stage.

Abstract: We used a multi-parameter (blood pressure (BP), brain blood flow, functional vascular density, blood oxygenation and mitochondrial NADH signal) cerebral cortex optical imaging system for observation of cerebral hemodynamic change and metabolic alteration of rats in vivo for 4 hours. A severe hemorrhagic shock model was induced by rapid bleeding until BP down to 40 mmHg and maintained at that level for 2 hours, followed by intravenous fluid resuscitation. Cerebral circulation and mitochondrial metabolism can be well preserved in compensatory stage but impaired during decompensatory stage. Signature changes of brain hemodynamics and metabolism may provide sensitive markers for the transition from compensatory stage to decompensatory stage.

Conclusion: Our novel imaging observation of hemodynamic and metabolic signals in vivo indicates that rat brains under hemorrhagic shock suffer irreversible damage which can't be compensated by the autoregulation mechanism, probably due to injured mitochondria.



June 22, 2013 – June 28, 2013

An emerging paradigm in neuroscience: assessing inter-personal brain coupling using functional near-infrared imaging (fNIRI) hyperscanning

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Study Goal: The aim of the talk is to give a detailed overview about fNIRI studies performed so far using the hyperscanning paradigm.

Abstract: Since the first demonstration of how to simultaneously measure brain activity using functional magnetic resonance imaging (fMRI) on two subjects about 10 years ago, a new paradigm in neuroscience is emerging: the assessment of the inter-brain coupling between two or more subjects, termed “hyperscanning”. Especially the usage of near-infrared imaging (fNIRI) for hyperscanning is a promising new research methodology, opening a new field of neuroscience – “social neuroscience”.

First, a historical overview about the development of the hyperscanning methodology will be given. This includes a compressed explanation of fMRI and electroencephalography (EEG) studies using hyperscanning. After this overview, the talk will focus on studies using fNIRI for hyperscanning. Especially the own work to this topic will be presented. In a next block, methods for signal processing and data analysis (e.g. correlation, coherence, causality measured, phase coupling measures etc.) will be discussed that are necessary to analyze the signals when performing hyperscanning studies. In addition, we discuss how to extend the hyperscanning methodology to a multimodal hyperscanning (i.e. combining fNIRI with ECG, capnography and measurement of electrodermal activity). Finally, the challenges and potentials of fNIRI hyperscanning will be discussed.

Conclusion: The hyperscanning paradigm will change our view how the brain works and will reveal undiscovered brain functions that are at the moment hidden for neuroscience since the single-subject setting cannot capture them.



June 22, 2013 – June 28, 2013

Measurement of Cerebral Metabolism and Haemodynamics using Time Resolved Multi-Wavelength NIRS

Luke Dunne, Jem Hebden, Ilias Tachtsidis

University College London

Study Goal: To quantify metabolism and haemodynamics using a novel multi-wavelength time domain functional near infrared spectrometer (NIRS), measuring absorption and scattering of light independently.

Abstract: We have developed a new multi-wavelength time resolved optical spectrometer using a supercontinuum laser. The light is filtered into narrowband wavelengths and the time of flight of single photons is measured with photomultiplier tubes. The system is capable of independently measuring the absorption and scattering of light for 16 wavelengths between 650-890nm. This allows us to accurately measure the redox state of cytochrome-c-oxidase, the terminal electron acceptor of the mitochondrial respiratory chain. Therefore, in addition to measuring absolute concentrations of HHb and HbO₂ we are able to quantify mitochondrial metabolism and oxygen utilization in the brain.

Conclusion: Time resolved NIRS can provide a significant improvement over continuous wave measurements as can independently resolve scattering and absorption. We will present initial data of head baseline measurements from 10 adults.

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June 22, 2013 – June 28, 2013

A mouse brainwide metabolic imaging at cellular resolution using 3D optical cryo-imaging

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Study Goal: To provide the critical information for understanding the brain function and dysfunction at a single-neuron resolution, such as brainwide neuroanatomical architecture, blood vessels and metabolic distributions.

Abstract: Our group has reported a three-dimensional structural data set of a Golgi-stained whole mouse brain at the neurite level with a micro-optical sectioning tomography (MOST) system. We further obtained the a brainwide blood vessel data set of the Nissl staining mouse brain with MOST. Recently, we developed a fluorescence-MOST for obtaining the continuous, brainwide neuronal pathways in the fluorescent protein transgenic mice. To study the metabolic brainwide networks, with a homemade optical cryo-imaging system and measuring the optical redox ratio, which is the fluorescence intensity of FAD divided by the fluorescence intensity of NADH, we obtained a whole mouse brain metabolic atlas, all at one-micron voxel resolution.

Conclusion: The integral study of the brainwide morphology and spatial locations of neurons by MOST, long-distance neuronal pathways by fMOST, and metabolic networks by cryo-imaging, provides powerful tools for mapping the brain activity.

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June 22, 2013 – June 28, 2013

Angiotensin II reduces transport-dependent oxygen consumption, but increases transport-independent oxygen consumption in immortalized mouse proximal tubular cells

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Study Goal: To investigate the role of Ang II in regulating QO₂ in immortalized wildtype mouse proximal tubular cells in conditions with normal and increased levels of oxidative stress.

Abstract: Oxidative stress is closely associated with renal dysfunction following diabetes and hypertension. Angiotensin II (Ang-II) can activate the NADPH-oxidase, increasing oxidative stress that is thought to blunt proximal tubular electrolyte transport and thereby oxygen consumption (QO₂). We investigated the effect of Ang II on QO₂ in immortalized mouse proximal tubular cells over-expressing the NADPH-oxidase subunit p22phox; a model of increased oxidative stress. Cultured cells were exposed to Ang-II (10⁻⁷ mol/l, replaced every 12 hours) or H₂O₂ (2.5 10⁻⁵ mol/l, replaced every 12 hours) for 48 h. QO₂ was determined during baseline (113 mmol/l sodium; transport-dependent QO₂) and during sodium-free conditions (transport-independent QO₂). Ang-II reduced transport-dependent QO₂ in wildtypes, but not in p22phox which also displayed increased QO₂ at baseline. Transport-independent QO₂ was increased in p22phox and Ang-II had no additional effect, whereas it increased QO₂ in wildtype. Addition of H₂O₂ reduced transport-dependent QO₂ in wildtypes, but not in p22phox. Transport-independent QO₂ was unaffected by H₂O₂. The similar effects of Ang-II and H₂O₂ to reduce transport-dependent QO₂ suggest a direct regulatory role of oxidative stress. In accordance, the transport-dependent QO₂ was reduced in p22phox already during baseline. The effects of Ang-II on transport-independent QO₂ was not replicated by H₂O₂, indicating a direct regulation via Ang-II-receptors independently of oxidative stress. However, the Ang-II effect was absent in p22phox, suggesting that oxidative stress also modulates normal Ang-II signaling. I

Conclusion: Ang-II affects both transport-dependent and transport-independent QO₂ in proximal tubular cells and may be an important pathway modulating renal QO₂.



June 22, 2013 – June 28, 2013

Developing Novel Fluorescent Probe for Peroxynitrite: Implications for Understanding the Roles of Peroxynitrite and Drug Discovery in Cerebral Ischemia Reperfusion Injury

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Study Goal: Peroxynitrite (ONOO⁻) is a cytotoxic factor. As its short lifetime, ONOO⁻ is hard to be detected in biological systems. This study aims to develop novel probe for detecting ONOO⁻ and understand the roles of ONOO⁻ in ischemic brains and drug discovery

Abstract: MitoPN-1 was found to be a ONOO⁻ specific probe with no toxicity. With MitoPN-1, we studied the roles of ONOO⁻ in hypoxic neuronal cells in vitro and MCAO ischemia-reperfusion rat model in vivo. Low level of ONOO⁻ promoted neurogenesis whereas high level was cytotoxic. We also used the probe for drug discovery. Baicalin (BA) was found to be an effective ONOO⁻ scavenger. With mass spectrometer, we found that BA reacted with ONOO⁻ directly. BA inhibited extraneous ONOO⁻ induced cytotoxicity and hypoxia-induced cell death in vitro. BA inhibited nitration of tyrosine, reduced infarct size and attenuated apoptosis in MCAO rat brains in vivo, whose effects were similar to FeTMPyP, a peroxynitrite decomposition catalyst.

Conclusion: (1) MitoPN-1 is a valuable ONOO⁻ probe; (2) ONOO⁻ has dual roles in cerebral ischemia-reperfusion injury; (3) Baicalin is an effective ONOO⁻ scavenger.

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June 22, 2013 – June 28, 2013

Retrograde perfusion and venular valves

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Study Goal: Venular incompetence as the basis for the oxygen supply by the retrograde perfusion in AO patients

Abstract: The forced retrograde perfusion with the arterial blood (DVA surgery) provides oxygen to peripheral tissues through venous network at rest. Dissection of venous valves are essential at the first step for the surgery. Valves in the plantar vein can be dissected by the use of the coronary angioplastic catheter. However, valves in venules can not be dissected, since they are distributed deeply in tissues and too fine for the surgical procedure. Clinical observations suggested a quick recovery of the peripheral blood flow after the end of the surgery for the ischemic hind limb in patients suffering from AO. In the present study being based on Laplace's law we studied the mechanical characteristics of venular wall and valves.

Conclusion: The vascular wall of small veins shows large distensibility. The retrogradely applied arterial blood pressure will distend venules and deform valvular leaflets. The arterial blood flows through the venular network of the peripheral tissues.



June 22, 2013 – June 28, 2013

Evaluation of a Textile Near Infrared Spectroscopy System in Calf Muscle Oxygenation Measurements

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Study Goal: Comfort and continuous measurement of oxygenation have always been aims of near infrared spectroscopy (NIRS). The goal was to test a novel textile NIRS system during venous occlusions on the gastrocnemius muscle.

Abstract: We recently introduced a novel textile integrated NIRS system (Tex NIRS). Here we tested the functionality of Tex NIRS on 8 subjects (12 legs, age: 28.5 ± 2.54 years, adipose tissue thickness (ATT): 4.14 ± 1.46 mm) and compared it to an established NIRS device in our lab (OxyPrem). Three venous occlusions at 50 mmHg were performed. Oxy/deoxy hemoglobin concentration changes were 3.47 ± 1.78 / 1.95 ± 1.13 μM , venous oxygen saturation was $74\% \pm 9\%$, and oxygen consumption was 0.90 ± 0.63 $\mu\text{mol}/100\text{mL}/\text{min}$. In subjects with more than 3 μM of change in total hemoglobin (tHb) after 3 minutes of occlusion, a good correlation of 94% between ATT and change of tHb was observed. The two instruments gave comparable results.

Conclusion: Tex NIRS showed a good mechanical stability and a high level comfort during the measurements. Oxygenation parameters were in agreement with the literature and OxyPrem.



June 22, 2013 – June 28, 2013

Hypoxia Stimulates Angiogenesis in Atherosclerosis: Is it Fact or Fiction?

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Abstract: The adventitial vasa vasorum become angiogenic during the atherosclerotic disease process. Media-intimal thickening was considered to be the driving force for neovascularization of the vasa vasorum. The rationale was that increase in the intima thickness (>0.5 mm) limits the nourishing effects of the luminal blood and that creates a hypoxic environment in the media. More recent studies in pigs show that the vasa vasorum become angiogenic prior to vessel wall thickening and detection of plaque development. Mouse studies show that hypoxia is predominantly determined by inflammatory cell content and not vessel wall thickness. Inflamed tissue is chronically hypoxic due to high metabolic needs, therefore the high metabolic demand of inflammatory cells in the vessel wall during atherosclerosis may stimulate hypoxia. Do we have the tools to rigorously investigate hypoxia in mouse and pig models of atherosclerosis?



June 22, 2013 – June 28, 2013

Influence of the Maternal use of Labetalol on the Neurogenic Mechanism for Cerebral Autoregulation Assessed by Means of NIRS

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Study Goal: The goal of this study is to investigate the influence of the maternal use of labetalol on the neurogenic mechanism (NM) involved in cerebral autoregulation. We hypothesized that the NM is impaired due to the labetalol effect on the α_1/β receptors.

Abstract: Labetalol is a drug that is normally used in the treatment of hypertensive disorders of pregnancy (HDP). In a previous study we investigated the effect of the maternal use of labetalol on the cerebral autoregulation (CA) mechanism of the neonates during the first three days of life. In that study, CA was assessed by means of transfer function between measurements of mean arterial blood pressure (MABP) and regional cerebral oxygen saturation (rScO₂). We found that during the first day of life, the gain values were higher in the neonates from the group treated with labetalol when compared with controls, indicating impaired CA. We hypothesized that this was due to a vasodilatory effect of the accumulated labetalol in the neonate. However, not strong evidence of this effect was found.

Conclusion: Impaired neurogenic mechanism was found in the labetalol group. In addition, this group had lower SA values during the first day, reaching control values by the third day. These indicate α_1/β receptor blockage due to the maternal use of labetalol.

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June 22, 2013 – June 28, 2013

Clinical EPR: Challenges and Progress

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Study Goal: The development and use of *in vivo* techniques for experimental applications of EPR in animals has been very successful and now has led to attractive clinical applications. This presentation provides an overview of the challenges, opportunities, and results as *in vivo* EPR is extended into use in human subjects.

Abstract The most widespread clinical (Medical) use is oximetry, where EPR can make repeated and accurate measurements of the actual pO_2 in tissues, which provides clinicians with information that bears directly on diagnosis and therapy, especially for oncology, peripheral vascular disease, and wound healing. The other area of importance in human subjects is the ability of *in vivo* EPR to measure clinically significant exposures to ionizing radiation 'after-the-fact', due to accidents, terrorism, or nuclear war. The unique capabilities of *in vivo* EPR to detect and characterize free radicals also could be applied to measure free radical intermediates from drugs and oxidative processes, including measurements of nitric oxide. These unique capabilities, combined with the sensitivity of EPR spectra to the immediate environment (e.g. pH, molecular motion, charge), have resulted in productive applications in animals that may be adapted for use in humans. The challenges for achieving full implementation in clinical research and practice include adapting the spectrometer for safe and comfortable measurements in human subjects, achieving sufficient sensitivity for measurements at the sites of the pathophysiological processes, and establishing a consensus on the clinical value of the measurements, e.g., for establishing the effectiveness of therapies involving oxidative processes or monitoring patients' progress or responsiveness to such treatments. Our experience with clinical EPR now extends to hundreds of measurements in human subjects, including a large number in patients.

Conclusion: As a result of the initial positive results, a number of other sites are now in the process of establishing clinical EPR instrumentation and carrying our collaborative investigations with the EPR Center at Dartmouth.

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June 22, 2013 – June 28, 2013

What we learn from oxygen images

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Study Goal: pO₂ distributions are critical determinants of normal tissue health and tumor aggressiveness and response to therapy. EPR oxygen images provide whole organ or tumor maps that give oxygen statistics and guide localized intervention.

Abstract: A number of studies show the value of normal tissue oxygenation images and tumor oxygenation. Oxygen images of the torso of wild type mice show initial reduction of lung, liver, visceral and muscle pO₂ with cyclic halving of FiO₂, but variation is blunted over an hour. pO₂ images of MCF7 tumors in nude mice with the pro-invasion gene BACH1 knocked down will be compared with those of WT tumors. Spontaneous breast cancers in MMTV-PyMT mice with BNIP3 KO, a major factor in promotion of mitochondrial autophagy will be compared with wild type. Preliminary studies in both show pO₂ differences. A strong correlation a single TCD50 radiation dose in 2 cancer types and hypoxic fraction with large pO₂ gradients is seen.

Conclusion: pO₂ images provide an important tool in understanding the relationship between microenvironment O₂ and a wide variety of crucial physiologic functions.

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June 22, 2013 – June 28, 2013

How EPR measures and images oxygen

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Study Goal: Tumor oxygen levels are major determinants of the response to cancer therapy. Developing accurate oxygen images assumes enormous biological, and medical importance. The success of imaging rests on instrumentation and acquisition methodology.

Abstract: Center for EPR Imaging In Vivo Physiology develops all aspects of technology relevant to Electron Paramagnetic Resonance oximetry. We designed bimodal resonators for full body mouse imaging and for high resolution imaging of superficial tumors. Resonators demonstrated robustness, high isolation and efficiency.

Most common EPR oxygen images are based on phase relaxation change due to spin exchange between spin probe and oxygen. We found those affected by the concentration-dependent self-relaxation. Spin-lattice relaxation imaging using inversion recovery sequence was found to have superior image accuracy.

High precision imaging was obtained by 4-fold increase of projection number and application of maximally spaced projection algorithm.

Conclusion: The improved precision and accuracy of the oxygen images due to technologies presented here, should further enhance animal studies of tissue and tumor oxygenation on animal models.

Acknowledgments: This work is supported by NIH, grants number P41 EB002034 and R01 CA98575.



June 22, 2013 – June 28, 2013

Overhauser Effect-based Functional Imaging

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Study Goal: To develop a proton-electron double-resonance imaging (PEDRI) for in vivo mapping of chemical environment (pH, O₂, redox status, etc.).

Abstract: PEDRI is based on the proton MRI acquired upon EPR irradiation of paramagnetic probes, the latter resulted in an enhancement of the NMR signal via transfer of polarization from electrons to protons by Overhauser effect. A conventional PEDRI does not acquire EPR spectral information and, consequently, lacks corresponding functional information. We proposed functional mapping with PEDRI using the probes with functionally-dependent EPR parameters. Spectral information at each pixel can be extracted from PEDRI acquisitions acquired at pre-selected EPR excitation fields [1] or frequencies [2]. In this work we performed PEDRI extracellular tissue pH (pHe) mapping in tumor-bearing mice using nitroxide pH probe with improved stability and range of pH sensitivity. The obtained data support both severe acidosis and high heterogeneity of tumor pHe. PEDRI with trityl probes has been used for oxygen mapping. An improved approach in which reference sample with known concentrations of a probe and oxygen are placed together with the measured sample was developed [3]. Two PEDRI acquisitions with different EPR irradiation powers are required to determine probe and oxygen distribution. An EPR-off image, which has low S/N is not required for the calculations, significantly reducing the total acquisition time while allowing for accurate pO₂ measurements in the range 0 - 240 μ M. Newly developed phosphonated trityl probes [4] for concurrent measurements of pH and O₂ were characterized for PEDRI applications. Studies performed at 200 G field (EPR \sim 560 MHz, MRI - 784.9 kHz) using home-made imager/spectrometer based on iron core Resonex 5000 resistive magnet. Specially design loop-gap double frequency resonator with coaxial B1 fields was developed for PEDRI and DNP experiments.

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Conclusion: PEDRI in combination with novel probes has potential as a useful tool for spatially-resolved in vivo noninvasive monitoring functional parameters like pH and oxygen.

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June 22, 2013 – June 28, 2013

EPR Image Based Oxygen Movies for Transient Hypoxia

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Study Goal: Dynamic EPR imaging studies of fluctuating pO₂ in vivo will provide oxygen movies necessary to help disentangle the relationship between chronic and transient hypoxia, to better understand their roles in therapeutic optimization and outcome.

Abstract: Chronic hypoxia strongly affects the malignant state and resistance to therapy for tumors. Transient hypoxia has been hypothesized, but not proven to be more deleterious. EPR imaging (EPRI) provides non-invasive, quantitative imaging of static pO₂ in vivo. Dynamic EPRI produces pO₂ movies, enabling noninvasive assessment of transient hypoxia.

Developments enabling Dynamic EPRI have been made: Hybrid T1/T2 imaging improves accuracy; maximally spaced projection sequencing gives more accurate and versatile EPRI acquisition when studying dynamic systems; principal component analysis filtering enhances SNR.

Conclusion: Temporally resolved pO₂ movies provide in vivo studies of physiologically relevant pO₂ changes in mouse tumors. These pO₂ movies will allow for localization/quantification of transient hypoxia and the eventual determination of its clinical relevance.

Acknowledgments: This work is supported by NIH, grants number P41 EB002034 and R01 CA98575 (Chicago).



June 22, 2013 – June 28, 2013

Multimodal imaging approach to detect the transient vascular normalization window following anti-angiogenic therapy in solid tumor

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Study Goal: We investigated if pulsed EPR oxygen imaging, redox sensitive MRI using a nitroxide probe, and hyperpolarized ¹³C MRI of pyruvate metabolism can be early surrogate markers for the transient vascular normalization following anti-angiogenic treatment.

Abstract: Anti-angiogenic therapies of tumor frequently proceed in two steps; transient normalization of the aberrant blood vessels with increased perfusion, followed by pruning the tumor blood vessels and resultant shutdown of blood supply. Daily sunitinib treatment of SCCVII tumor resulted in growth delay. EPR oxygen imaging showed transient increase in tumor oxygenation 2-4 days following treatment, while 45% decrease in microvessel density. Dynamic oxygen imaging showed the suppression of temporal fluctuations in tumor oxygenation. During this vascular normalization window, MRI of the redox state using a nitroxide and hyperpolarized ¹³C MRI of energy metabolic flux of pyruvate/lactate couple revealed the oxidative shift in tumor redox state.

Conclusion: Non-invasive imaging of transient shift in tumor oxygenation, redox state, and energy metabolism can be used for evaluating early tumor micro-environmental changes in response to anti-angiogenic therapy.

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June 22, 2013 – June 28, 2013

EPR oximetry as a tool to qualify or disqualify other oxygenation imaging methods

Bernard Gallez

University of Louvain - Louvain Drug Research Institute - Biomedical Magnetic Resonance Research Group

Study Goal: To apply EPR oximetry as a way to qualify or disqualify potential oxygenation imaging methods based on endogenous contrast in MRI or radiolabelled nitroimidazoles in PET

Abstract: EPR oximetry provides noninvasive measurements of the real pO₂ values in tissues. Despite the potential for using EPR oximetry in the clinic, its immediate application over a large scale is unlikely. To assess the oxygenation status of tissues, the medical community is increasingly using some "surrogate" markers of tissue oxygenation. In this context, EPR oximetry is an invaluable tool to qualify (or disqualify) other oxygenation imaging modalities. In this context, we applied hypoxic and hyperoxic challenges in order to modulate the tumor pO₂ values over a large range. We compared the pO₂ values obtained by EPR oximetry to several MRI parameters (T₂^{*}, T₁, Lipid T₁) and to the accumulation of several radiolabelled nitroimidazoles.

Conclusion: While the T₂^{*} (MRI) is non-predictive of the evolution of the pO₂, others parameters like Lipid T₁ seems promising. The range of pO₂ linked to the tissue accumulation of some nitroimidazole tracers was also established (i.e. for 18F-FAZA, 18F-EF5).



June 22, 2013 – June 28, 2013

Strong hypoxia correlates with lasting tumor inhibition after F2BMet-photodynamic therapy

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Study Goal: PDT effectiveness strongly depends on oxygen, vasculature, photosensitizer and a light dose. Tumor oxygenation and vascular effects after PDT with highly efficient bacteriochlorin, F2BMet were characterized.

Abstract: Lewis Lung Carcinoma tumors were grown in C57bl/J6 mice. 2 or 4 mg/kg BW of F2BMet solution was administrated i.v.; 15 min or 72 h later tumor illuminations were performed (86 J/cm², 750 nm). Vasculature was estimated by ultrasonography with Doppler and PW mode (VEVO 2100), Laser Doppler Perfusion Imaging, EPR oxymetry using LiPC, and immunohistochemistry.

PDT caused a decrease in total vessel area, vessel density and size of vessels, consistent with destruction of vasculature structure. Fluctuation in pO₂, blood flow and blood flow velocity reflected a loss of vessel function in tumors and surrounding tissues after PDT.

Conclusion: Lasting inhibition of tumor growth was achieved only when PDT caused very strong hypoxia, whereas partial dysfunction of blood flow did not guarantee a durable therapy effect and might even have a stimulatory effect on tumor growth.

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June 22, 2013 – June 28, 2013

Recurrent low dose chemotherapy to oxygenate and inhibit tumor growth

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Study Goal: Strategies that can reduce hypoxia will sensitize tumors to chemoradiation. The goal is to establish a recurrent low dose gemcitabine protocol to inhibit tumor growth and potentially enhance tumor pO₂ for radiosensitization.

Abstract: A lack of strategies to counteract hypoxia (pO₂ < 10 - 15 mm Hg) and techniques to repeatedly measure tumor pO₂ has restricted therapeutic optimization. We have investigated the potential application of a routinely used drug, gemcitabine, with a new approach of recurrent low dose (metronomic) chemotherapy to modulate hypoxia and tumor growth. The metronomic schedule is expected to prune the immature neovasculature and then remodel the remaining vasculature, leading to normalization of otherwise abnormal tumor vasculature. This should enhance drug delivery and oxygen into the tumors, thereby providing a therapeutic window which, if exploited, can improve treatment outcome. However, the efficacy of this approach will depend on the dose and schedule of the therapeutic agent.

The ectopic U87 (brain), AsPc-1 (pancreas) and FaDu (head and neck) tumor xenografts were established in immune deficient mice. EPR oximetry with a lithium phthalocyanine oximetry probe was used to follow temporal changes in tumor pO₂ during treatment. The solid tumors were hypoxic with a tissue pO₂ of less than 10 - 15 mm Hg prior to any treatment. A significant increase in the pO₂ of U87 tumors for 3 - 7 days post treatment with 150 mg/kg gemcitabine was observed. However, the treatment with metronomic schedule (25 mg/kg and 50 mg/kg x 3 times/week) led to a significant increase in pO₂ for up to 10 days. The growth of U87 tumors treated with 150 mg/kg gemcitabine was similar to that of control. On the other hand, tumor growth was significantly inhibited by metronomic gemcitabine treatment. A significant decrease in the growth of the AsPc-1 tumor was evident with metronomic schedule; however no change in pO₂ was observed. A significant increase in pO₂ of FaDu tumors and decrease in tumor volume was observed on treatment with metronomic gemcitabine. mCT and IHC results support vascular normalization and decrease in HIF-1-alpha on treatment with metronomic gemcitabine.

Conclusion: A consistent inhibition of tumor growth on treatment with metronomic gemcitabine was observed, however the increase in pO₂ was tumor specific. EPR oximetry can be used to follow the temporal changes in tumor pO₂ during metronomic treatment.

Acknowledgments: EPR Center, Geisel School of Medicine, Hanover, NH



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Life with less oxygen: how cancer cell mitochondria sustain membrane potential in hypoxic microenvironment

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Study Goal: The goal of this study is to provide evidence indicating that mitochondrial complex II may play an important role in sustaining mitochondrial membrane potential ($\Delta\Phi_m$) in deep hypoxia.

Abstract: In 2D tissue model of hypoxic microenvironment, $\Delta\Phi_m$ was abolished in cells locating $>500\ \mu\text{m}$ from the O₂ source (anoxic front, AF). This was consistent with the O₂ distribution determined by the redshift of GFP. The AF was extended to 1500~2000 μm when HIF-1 α is induced by a prolylhydroxylase inhibitor DMOG. In these cells, tissue O₂ gradients were substantially reduced indicating that HIF-1 α suppresses mitochondrial respiration. Then, a question arises how mitochondria sustain $\Delta\Phi_m$ with significantly reduced electron flux in the respiratory chain (reflected by reduced O₂ consumption). We demonstrated that inhibition of mitochondrial complex II abolished the effect of DMOG in extending the AF whereas the tissue O₂ gradients remained shallow.

Conclusion: We conclude that prolylhydroxylase inhibited (or HIF-1 α induced) cultured cells can sustain $\Delta\Phi_m$ in otherwise anoxic microenvironment by reducing the tissue O₂ gradients while activating O₂ independent electron flux at the same time.

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Redox imaging of the p53-dependent mitochondrial redox state in colon cancer *ex vivo*

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Study Goal: To investigate whether the redox state is associated with p53 status in colon cancer.

Abstract: Employing the Chance redox scanner we imaged the redox state of wild-type p53 and p53-null colon tumor mouse xenografts followed by H&E staining. Our results show: 1) both tumor lines have significant degree of intratumor heterogeneity of the redox state, typically exhibiting a distinct spatial core-rim pattern or the “hot/cold” oxidation-reduction patches; 2) the p53-null group is significantly more oxidized and more heterogeneous in the redox state; 3) the tumor size dependence of the redox indices is significant in the p53-null group with the larger ones being more oxidized and more heterogeneous; 4) the H&E staining images of tumor sections grossly correlate with the redox images.

Conclusion: The study revealed the intratumor heterogeneity of the mitochondrial redox state and its p53 dependence in colon tumors. The findings aid our understanding on colon cancer pathology and developing new imaging biomarkers for clinical applications.



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Assessing cerebral tissue oxygenation alteration in neurological disorders by EPR

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Study Goal: Oxygen is required to maintain neuronal metabolism and function in the brain. We have developed EPR techniques to assess cerebral tissue oxygenation (pO_2) and investigated the role of pO_2 alteration in the pathophysiology of neurological disorders.

Abstract: Cerebral ischemia causes heterogeneous changes in tissue oxygenation and cellular metabolism. Using EPR spectroscopy and imaging techniques, we measured both absolute values and temporal changes of localized interstitial pO_2 following ischemia and reperfusion. Our results showed that ischemic tissue pO_2 level could be modulated by changing the percentage of oxygen content in the breathing gas, and normobaric hyperoxia treatment not only increased tissue pO_2 , but also decreased free radical generation, contrary to common expectation. We also studied the alteration of cerebral tissue pO_2 in the white matter of a rat model of hypertensive vascular cognitive impairment, and methamphetamine-induced hypoxia in the brain.

Conclusion: These results demonstrate that alteration of tissue pO_2 , and the resulting oxidative stress, play important role in the mechanism of brain injury. EPR oximetry provides a unique tool to contribute to the better understanding of the complex processes.

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Boron nanoparticles for oxygen sensing using window chamber tumor models

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Study Goal: The goal of this study is to develop luminescent boron nanoparticles as oxygen sensors to characterize tumor hypoxia. We have developed imaging and quantification techniques and performed some initial toxicity and cell loading studies.

Abstract: Oxygen sensing nanoparticles composed of difluoroboron β -diketonate (BF₂bdk) dyes and biocompatible poly(lactic acid) (PLA) have been developed. These novel materials exhibit stable blue fluorescence and room temperature phosphorescence that is quenched by oxygen. This enables ratiometric oxygen sensing in which the same dye serves as both the reference and sensor. The nanoparticles are non-toxic over a usable concentration range as assessed by WST-1. The nanoparticles were also applied to the dorsal skin fold window chamber in which a tumor was implanted and visualized using wide-field and confocal microscopy. We have used these to assess tumor hypoxia in two settings: 1) fluctuating hypoxia in untreated tumors, and 2) tumors treated with hyperthermia. In the first case, we have successfully demonstrated the presence of fluctuating hypoxia at a microscopic scale, and in the second case, we have observed enhanced oxygenation following hyperthermia treatment in preliminary studies.

Conclusion: In conclusion, we have developed a quantitative means of assessing tumor oxygen tension at a microscopic scale using relatively simple ratiometric sensing techniques.



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Real-time monitoring of ischemic and tumor pO₂ during stroke and tumor growth by EPR oximetry using multiple probes implantable oxygen sensors

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Study Goal: Multi-site electron paramagnetic resonance (EPR) oximetry, using multi-probe implantable oxygen sensor (ImOS), was used to investigate the dynamic changes in the ischemic and tumor pO₂ during stroke and tumor growth in rodents.

Abstract: Methods: (1) ImOSs with 4 sensor loops were used to follow the temporal changes in cerebral pO₂ at two sites in each hemisphere during ischemia induced by left middle cerebral artery occlusion (MCAO) in rats breathing normobaric 30% O₂ or 100% O₂ (NBO). (2) ImOS with two sensor loops was tested in normal mice and mice bearing orthotopic human xenograft U251 glioma. The dynamic changes in the tissue pO₂ were assessed during repeated hyperoxia with normobaric carbogen (NBC) breathing.

Results: (1) A similar pO₂ at two sites in each hemisphere prior to the onset of ischemia was observed in rats breathing 30% O₂. However, a significant decline in the pO₂ of the left cortex and striatum occurred during ischemia but no change in the pO₂ of the contralateral brain was observed. A significant increase in the pO₂ of only the contralateral non-ischemic brain was observed in rats breathing NBO. (2) The brain pO₂ of normal mice was stable and increased significantly during NBC inhalation in experiments repeated for 2 months. The pO₂ of U251 glioma declined gradually, while the pO₂ of contralateral brain essentially remained the same. A significant increase in the glioma pO₂ was observed during NBC inhalation over days.

Conclusion: EPR oximetry with ImOS can repeatedly assess changes in the ischemic and tumor pO₂ at multiple sites during stroke and tumor growth, also can be used to test and develop interventions to rescue ischemic tissue and tumor tissue by modulating brain pO₂

Acknowledgments: This work was supported by Hitchcock Foundation Program Project Grant and the Prouty grants from the NCCC at Dartmouth-Hitchcock Medical Center



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Role of tissue oxygenation for onset and progression of kidney disease

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Study Goal: The role of altered renal oxygen metabolism resulting in hypoxia has emerged as a unifying pathway for several conditions associated with the development of kidney dysfunction, including diabetes, hypertension and ischemia-reperfusion injury.

Abstract: The kidney is unique in the sense that increasing oxygen delivery, i.e. blood flow, not necessarily will result in increased oxygenation since renal blood flow also influences the glomerular filtration rate, and thus also the energy demand for tubular sodium transport. This is in vast contrast to e.g. the brain where an increased oxygen demand rapidly is compensated by a similar increase in blood flow. We have just recently started to understand the intricate balance between oxygen utilization and oxygen supply in the kidney.

Conclusion: It is apparent that importance and the resources directed to understand of the role of defective oxygenation for development of kidney disease.

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Comparison of regional renal blood flow using non-invasive ASL measurements with Gadolinium in patients before renal nephrectomy

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Study Goal: The present study aimed to compare the non-invasive magnetic resonance (MR) perfusion measurement in the renal cortex, outer and inner medulla using arterial spin labeling (ASL) with gadolinium perfusion in patients investigated before renal surgery

Abstract: Methods and Materials: Eight patients with known renal carcinoma and planned for nephrectomy or resection of the tumor (3 female, 5 male) underwent examinations using a 3T scanner (Achieva, Philips; FOV = 267x260 mm, resolution 1.8x1.8x5 mm). From T1 and T2 anatomical images cortex, outer and inner medulla were identified and MR parameters were determined in these regions of the kidney.

Results: Total renal blood flow (RBF) (ml/min/kidney), as determined by phase-contrast, was 720±40. Regional renal blood flow (ml/min/100 g of tissue) determined by ASL was 236 ±27 in the cortex, 50±31 in the outer medulla, 21±24 in the inner medulla. Regional renal blood flow measured with gadolinium (evaluated by Nordic Ice software in ml/min/100 g of tissue) was 248 ±27 in the renal cortex, 138 ±42 in the outer medulla, 80±34 in the inner medulla. Regional tissue oxygenation as determined by T2* (ms) from BOLD contrast was 15.3±1.8, 20.9±1.8 and 22.8±1.7, respectively. Water diffusion measured as ADC (mm²/sec) was (2.2±0.2)x10⁻³, (2.1±0.1)x10⁻³ and (1.9±0.1)x10⁻³, respectively.

Conclusion: Non-invasive MR measurements using ASL is in accordance with Gadolinium measurements. ALS measurements can be used as a valuable tool to further our understanding of human renal physiology.



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Electron Paramagnetic Resonance (EPR) Oximetry to Monitor Regional Renal Oxygenation

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Study Goal: Intrarenal hypoxia has been linked to several conditions associated with developing kidney disease. Methods to monitor regional renal oxygenation in vivo are needed to further our understanding of how defective oxygenation affects kidney function.

Abstract: EPR oximetry using implantable lithium phthalocyanine (LiPc) probes has previously been used to monitor tumor oxygenation however, several issues need to be overcome and the technique thoroughly validated before applied in the kidney. We have recently started such validation project, we tested the technique in vivo in mice over a 45-day period during which we repetitively measured renal regional oxygenation during normal conditions and after acute interventions to manipulate oxygen availability by changing oxygen content in the inspired air. Results show that EPR oximetry yields similar absolute oxygen concentrations as the gold-standard invasive technique and that the technique is able to detect rapid changes in intrarenal oxygenation.

Conclusion: In conclusion, EPR oximetry using LiPc probes has the potential to fill the need for a minimally invasive technique to continuously and repetitively monitor regional renal oxygen availability in vivo.

Acknowledgments: We are deeply grateful to Dr. Harold Swartz and the EPR Center in Hanover, New Hampshire, USA for technical assistance.



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In Vivo EPR Oximetry using Isotopically-Labeled Nitroxides: Potential for Quantitative Measurement of Tissue Oxygen

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Study Goal: In light of the significance of O₂ in brain physiology, O₂ measurement in brain has remained problematic. This study investigates whether isotopically-substituted nitroxides are more sensitive in vivo than their nonisotopically-substituted analogs.

Abstract: Variations in brain O₂ concentrations can have profound effects on brain physiology. Thus, the ability to noninvasively quantitate local O₂ concentrations in vivo could significantly further our understanding of several brain pathologies. However, quantitative O₂ mapping in the brain has proven difficult. The electron paramagnetic resonance (EPR) spectra of nitroxides are sensitive to molecular O₂ and can be used to estimate O₂ concentration in aqueous solution. We recently synthesized labile-ester-containing nitroxides, which can accumulate in cerebral tissue after in situ hydrolysis, and thereby enable spatial mapping of local O₂ concentration in mouse brain by EPR imaging. In an effort to improve O₂ quantification, we synthesized ¹⁵N-perdeuterionitroxides, which proved to be more sensitive O₂ probes in vitro. EPR spectroscopic measurements demonstrate that these doubly isotopic-labeled nitroxide markedly improves signal-to-noise ratio (SNR) and thus, detection limits. Additionally, these nitroxides are more sensitive to changes in local O₂ concentration, which will enable more accurate O₂ measurement in tissues. We also demonstrate that these isotopically-substituted nitroxides are likewise more sensitive (~3 fold) in vivo than their nonisotopically-substituted analogs. Moreover, their spectral response to local O₂ concentrations in cerebral tissue is greater, making them excellent O₂ sensors for in vivo O₂ quantitation.

Conclusion: This study demonstrates that isotopically-substituted nitroxides are superior for O₂ quantitation in vivo and motivates their use for higher-resolution O₂ mapping in pathological states.

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Oxygen therapy for acute myocardial infarction: Take deep breathe & Relax

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Study Goal: The goal of the study was to determine the effect and mechanism of action of periodic administration of hyperoxygen (oxygen cycling; OxCy) on ischemia/reperfusion (IR)-induced myocardial infarction and dysfunction.

Abstract: Myocardial infarction (MI) is one of the most common causes of heart failure in adults. MI results in the irreversible and progressive loss of viable cardiomyocytes, leading to cardiac dysfunction and heart failure. Periodic administration of hyperoxygenation (OxCy; 100% oxygen; 2 ATA; 90 min/day) has been shown to inhibit MI damage and restore myocardial function in a rat model of acute ischemia/reperfusion (IR) injury. The goal of the present study was to determine the optimal conditions of OxCy (% oxygen; exposure time, etc) needed to reverse the myocardial damage after IR injury. MI was induced in Fischer-344 rats by ligating the left-anterior-descending (LAD) coronary artery for 60 min, followed by reperfusion by releasing the ligature. After 72 hours of post-surgical rest, the rats were subjected to OxCy treatment by placing them in a custom-built chamber for a defined duration of time per day for 5 days. The study was divided into three groups. The first group was subjected to 60 min of OxCy for 5 consecutive days with 100% O₂ at 1, 1.5 or 2 ATA pressure. The second group was subjected to 60 min of OxCy, for 5 days, using 40% O₂, 70% O₂ or carbogen (95% O₂+5% CO₂) at ambient pressure. The third group received carbogen breathing for 60, 30 or 15 min at ambient pressure. The analysis of myocardial O₂ and echocardiography showed that 5 days of OxCy using carbogen gas for 30 min at ambient pressure resulted in optimal recovery of cardiac function after IR injury. Similarly, histochemical and western-blot analysis showed a marked reduction in the myocardial infarct area and increased expression of pro-survival markers NOS3 and pAkt. While p53 was significantly upregulated in the OxCy hearts, the pro-apoptotic marker BAX was downregulated, suggesting a pro-survival role for p53 during OxCy-induced myocardial recovery. Our findings will be valuable in reevaluating and redesigning the existing oxygen therapy guidelines for patients with MI.

Conclusion: Periodic administration of hyperoxygen for brief periods of time showed significant reduction in the myocardial infarct size, and dysfunction in the MI heart. NOS3 and p53 have been identified to play a prosurvival role leading to cardioprotection.



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Chronic versus Acute Hypoxia in Tumors: Pathogenesis and Related Tumor Biologies

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Study Goal: Tumor hypoxia can lead to adaptive processes, development of aggressive phenotypes and treatment resistance. Based on underlying mechanisms and their duration, 2 main types of hypoxia have been identified coexisting with complex spatial heterogeneity.

Abstract: Chronic hypoxia is mainly caused by diffusion limitations due to enlarged diffusion distances and adverse diffusion geometries, and - to a lesser extent - by hypoxemia (e.g., anemic patients, in liver tumors supplied by portal vein), HbCO formation (smokers) and a compromised perfusion or flow stop due to sustained interstitial hypertension. Acute hypoxia mainly results from transient disruptions in perfusion (e.g., vascular occlusion by cell aggregates), fluctuating red blood cell fluxes and short-term contractions of the interstitial matrix. In each of these hypoxia subtypes oxygen supply is critically reduced, but perfusion-dependent nutrient supply, delivery of anticancer drugs, and repair competence can vary or may not be affected.

Conclusion: This detailed differentiation of tumor hypoxia may impact on our understanding of tumor biology and may aid in the development of novel treatment strategies, tumor detection by imaging and tumor targeting, and is thus of great clinical importance.



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Heterogeneity in Tissue Oxygenation: From Physiological Variability in Normal Tissues to Pathophysiological Chaos in Malignant Tumours

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Study Goal: In this presentation, the anatomical/functional basis for the physiological heterogeneity of blood flow, local and regional regulatory mechanisms in normal tissues is revisited, and the pathophysiology of the failure of regulation will be examined.

Abstract: The predictable, physiological heterogeneity of O₂ supply was a “hot topic” in ISOTT in the 1980s. Under physiological conditions, regulation of blood flow distribution at global, regional and micro-regional levels play coordinated roles in ensuring adequate O₂ supply to all tissue cells. How this is achieved may be organ/organ layer-specific, depending on its function and priorities to match local O₂ delivery to uptake. Examples where these regulatory mechanisms break down under conditions of ischaemia and shock will be given. In contrast, pathological heterogeneity in tissue oxygenation resulting from uncontrolled, chaotic growth as seen in malignant tumours represents a status that is not predictable, and will therefore be discussed.

Conclusion: Heterogeneity of blood flow at all levels, and local regulation of microcirculatory flow patterns are vital elements in the maintenance of adequate oxygen supply to tissues. Their disruption, such as in malignant tumours, can have fatal consequences.



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Cerenkov emission in radiation therapy to estimate skin dose and monitor tissue oxygenation

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Study Goal: The focus of this study has been to examine Cerenkov emission during radiation therapy, and exploit the potential to use this signal for sampling of radiation dose and tissue oxygenation in vivo. Basic Monte Carlo radiation transport studies were carried out, along with phantom validation studies. The first ever human measurements will be presented to assess their value in mapping skin dose.

Abstract: The signal is directly proportional to surface dose and images have been captured by gated ICCD imaging during Linear accelerator (LINAC) pulses incident on tissue. These images are linearly proportional to the deposited skin dose ($R^2=0.97$), and so they can be used to image the dose during each fraction of treatment. This has been validated by direct comparison with ionization chamber measurements and Monte Carlo modeling.

The ability to monitor tissue oxygenation with luminescent dye from the surface of tissue is shown, up to several centimeters into the tissue. A dendritic platinum-based phosphor (PtG4) has been used at micromolar concentrations to monitor Cerenkov-induced signals, and through gating the signal to monitor the lifetime, the partial pressure of oxygen can be estimated by the Stern-Volmer equation. Tomographic recovery of a region of low oxygenation was shown in a tissue phantom, with high accuracy in pO₂ estimation.

Conclusion: Taken together the surface dose and tissue oxygenation could be monitored for subjects undergoing standard fractionated radiation to track changes which occur during the month long treatment process. The monitoring of tissue oxygenation in animals appear feasible, and the extension to human use would require FDA approval for phosphor use. Imaging of skin dose in breast cancer patients is ongoing presently and the latest clinical data will be summarized as the time of the conference.

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Repeated clinical measurements of tumor oxygen to determine the therapeutic potential of hyperoxic therapy

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Study Goal: We have developed a method to provide direct repeatable measurements of tumor oxygen under clinically applicable conditions. Initial measurements characterize baseline pO₂, effects of hyperoxic interventions, and changes during courses of treatment.

Abstract: We have developed an approach, based on electron paramagnetic resonance (EPR) that now makes it feasible to provide direct repeatable measurements of tumor oxygen under conditions compatible with clinical routines. These in vivo measurements are made using low frequency (1.2 GHz) EPR spectroscopy and surface loop resonators. This technology enables measurements to be made in vivo at superficial sites, providing direct, non-invasive (after placing the ink in the tissues), repeatable measurements of tissue pO₂. In particular, we have assessed the effectiveness of a simple but potentially powerful and effective method to increase tumor oxygen levels: breathing enriched oxygen mixtures. We measured tumor pO₂ in patients before, during and after breathing enriched oxygen mixtures. The results from the initial set of patients indicate that tumors differ markedly in their response to such interventions. Tumor types include melanoma, basal cell, soft tissue sarcoma, and lymphoma, and measurement sites have ranged from the feet to the scalp. We found that the response to 100% O₂ was variable across subjects. In some patients increases in the fraction of inhaled O₂ led to observed increases in tumor pO₂ values. Other tumors had more modest increases in pO₂ over this duration, which may still be radiobiologically significant. Variation in tumor pO₂ was also observed during the course of radiation treatment, where changes due to vascular disruption and changes in O₂ consumption can result in radiobiologically significant effects. These preliminary data permit several significant tentative conclusions to be drawn: 1) it is feasible to make measurements of pO₂ in tumors in human subjects under conditions compatible with clinical practice; 2) dynamics of tumor pO₂ can be followed with EPR oximetry; 3) repeated measurements can be made over many days; 4) human tumors differ markedly in the extent to which they respond to changes in inhaled breathing gases.

Conclusion: These results indicate that it should be feasible to repeatedly and directly measure tumor pO₂, to more adequately determine the effectiveness of hyperoxic treatments, optimize such therapies, and provide more effective individualized care.

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The assessment of cancer treatment therapy in animal experiments via regrowth curve statistical analysis

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Study Goal: The goal of the study is to demonstrate how mathematical modeling of tumor response to treatment allows rigorous definition of the tumor treatment endpoint that substantially improves statistical significance and usually leads to smaller p-value.

Abstract: One of the aims of the EPR oxymetry is to correlate of tumor oxygenation with treatment outcome. Usually the study involves the comparison of tumor volume at several days after treatment in different animal groups using the t-test. There are two major problems with statistical approach: (1) typically the day-by-day comparison of tumor volume leads to an irregular pattern of the p-values, so on one day the tumor volume is different statistically significant but on another it is not; (2) the t-test implicitly assumes that the distribution of tumor volume observations is normal which contradicts the actual data leading to large p-values and lacking of statistically significant findings. We develop a mathematical model for tumor regrowth and propose several treatment endpoints including doubling time (DT), tumor growth delay (TGD), and surviving fraction (SF) to correlate with the tumor oxygenation and group comparison. We use a mixed model for statistical estimation of tumor regrowth curves that addresses usually considerable the animal-to-animal variation when it comes to measuring the response to treatment. This approach solves the two problems which faces the t-test and typically improves the results of statistical analysis. Our theory is illustrated with the photodynamic tumor treatment in several groups of mice. The R code to run the tumor regrowth curve estimation and compute the three endpoints with their standard errors is available online at the website www.dartmouth/~eugened.

Conclusion: Mathematical modeling of tumor regrowth curves as a response to cancer treatment, combined with the mixed modeling technique, allows rigorous definition of treatment outcomes such as DT, TGD, and SF, and typically reduces p-values.

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Applications of EPR-imaging and Dynamic Nuclear Polarization (DNP) Based Metabolic Imaging in Cancer. Pre-clinical and Clinical studies

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Abstract: Most imaging modalities provide volumetric and morphological information on tumors and tumor response to treatment. However these changes manifest several days-weeks to manifest. Molecular/physiological changes in tumors appear within a day or two after treatment initiation in responding tumors. Imaging techniques which can determine molecular physiological properties in in vivo in tumors will be useful in drug discovery research or potentially for human use. New techniques are being explored for the ability to provide biomarkers for diagnosis and treatment response. Among them, already PET (Positron Emission Tomography), MRI based techniques such as Dynamic Contrast enhanced MRI (DCE MRI), diffusion weighted MRI, and Magnetic Resonance Spectroscopic Imaging are being used clinically for diagnosis and treatment response.

EPR imaging is an emerging technique for in vivo applications. The availability of stable, non-toxic trityl radical probes such as Oxo 63 has made EPR imaging possible in live animals with potential for human applications¹. Electron Paramagnetic Resonance based imaging has been shown to provide three-dimensional maps of absolute pO₂ in small animals with useful spatial, spectral and temporal resolution and demonstrated to have the capability to study dynamics of tumor oxygen changes. It has also been shown to be useful in monitoring treatment response in experimental animals and identify vascular renormalization window in tumors treated with anti-angiogenic drugs.^{2,3} Similarly, the implementation of dynamic Nuclear Polarization methods to polarize ¹³C labeled tracers to greater than 10% polarization and injecting them in vivo has made metabolic imaging in vivo possible.⁴⁻⁶

Conclusion: In this presentation, the basics of these methodologies, practical implementation and applications in experimental animals and humans will be discussed.

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June 22, 2013 – June 28, 2013

Development of DNP-MRI for Free Radicals in Living Animal

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Abstract: Dynamic nuclear polarization (DNP)-MRI, also called proton electron double resonance imaging (PEDRI) or Overhauser MRI, is a new imaging method for observing free radical species. MRI image intensities from water proton can be enhanced up to 10^2 fold by irradiating at the EPR resonance frequency of the free radical prior to applying the MRI pulse sequence. The major advantage of DNP-MRI is that the spatial resolution of free radical spectra is similar to that in MRI. We succeeded in simultaneous dual images by using nitroxyl radicals labeled with ^{14}N and ^{15}N nuclei and changing the external magnetic field for EPR irradiation in DNP-MRI¹. Synthetic nitroxyl or trityl radicals have provided unique information regarding redox status², vascular permeability/oxygen concentration³ and whole body-pharmacokinetics⁴ in living animals. However, the development of DNP-MRI for human applications is still challenging. If endogenous free radical intermediates were used for imaging, DNP-MRI could become a promising technique to add metabolic/biochemical dimensions to anatomic images. Here, we demonstrate a novel redox molecular imaging (ReMI), which can simultaneously visualize various endogenous free radical intermediates derived from redox transformations in a single experiment. In the present study, two DNP-MRI scanners were also developed. One is a DNP-MRI scanner using a commercial EPR spectrometer. The system operates at 20 mT with corresponding frequencies of 850 kHz and 565 MHz for NMR and EPR modes, respectively, and free radical distributions were visualized *in vitro* and *in vivo*. The large difference of gyromagnetic ratio between electron and proton spins restricted EPR excitation and the proton detection fields. By transporting the sample between EPR (20 mT) and MR magnets at 1.5 T (or 0.4 T), we have developed a high sensitive DNP-MRI scanner, the spatial resolution of which was less than 0.2 mm.



June 22, 2013 – June 28, 2013

Multifunctional EPR-based applications *in vivo*

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Study Goal: To develop EPR-based spectroscopic and imaging approaches for *in vivo* assessment of functional parameters of tissue microenvironment.

Abstract: EPR-based techniques possess a unique functional sensitivity when applied in combination with specific paramagnetic probes. The recent progress in the development of advanced soluble paramagnetic probes of two major classes, nitroxides (1) and trityl radicals (2), makes possible non-invasive magnetic resonance monitoring of various physiologically relevant parameters, *in vivo*. The exemplified applications include concurrent monitoring of ischemia-induced myocardial oxygen depletion and acidosis in isolated rat hearts (3), and multifunctional (pH, redox, oxygen and glutathione content) monitoring of tumor tissue microenvironment (2,4), including pH mapping of living tissues using low-field EPR imaging and innovative proton-electron-double-resonance imaging (PEDRI). For two leading causes of mortality in the United States, cancer and ischemic heart disease, tissue hypoxia is well documented and is accompanied by changes in glycolysis resulting in tissue acidosis and tissue redox changes. In regard to tumor, we hypothesized that low oxygen, acidic extracellular pH and high reducing capacity of tumor microenvironment and high intracellular GSH of cancer cells, acting in an orchestrated way, favor cancer cell development while they are highly toxic and mutagenic for the normal cell types, in part resulting in changing the cell phenotype in favor of the malignant type. The discussed hypothesis is supported by preliminary data in the mouse tumor breast model using developed EPR-based multifunctional approaches.

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Conclusion: The developed multifunctional EPR approaches based on advanced paramagnetic probes provide an important tool for *in vivo* concurrent monitoring of tissue oxygenation, pH, redox and glutathione content in animal models of disease.

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June 22, 2013 – June 28, 2013

***In vivo* tumor extracellular pH monitoring using electron paramagnetic resonance**

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Study Goal: In this study, we assess the viability of CW-EPR spectroscopy with a pH sensitive nitroxide to measure extracellular tumor pH (pHe) in the mouse model.

Abstract: 750-MHz CW-EPR spectroscopy of C3H HeJ mice hind leg squamous cell tumor was performed after intra-venous tail vein injection of pH sensitive nitroxide (R-SG) during stages of normal tumor growth, and in response to a single 10 Gray (Gy) dose of X-ray irradiation. An inverse relationship was observed between tumor volume and pHe whereby during normal tumor growth a constant reduction in pHe was observed. This relationship was disrupted by X-ray irradiation, and from 2- 3 days post exposure, a transitory increase in pHe was observed.

Conclusion: In this study, we demonstrated the viability of CW-EPR spectroscopy using R-SG nitroxide to obtain high sensitivity pH measurements in mouse model tumour with an accuracy < 0.1 pH units.

Acknowledgments: This work was supported in part by the NEXT program (LR002) from JSPS.



June 22, 2013 – June 28, 2013

Impact of hypoxia related tumor acidosis on cytotoxicity of different chemotherapeutic drugs in vitro and in vivo

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Study Goal: Acidosis in tumors leads to an activation of the p-glycoprotein (Pgp) drug transporter. In the study the cytotoxicity of different chemotherapeutic drugs and its dependence on the Pgp activity during acidosis were analyzed in vitro and in vivo.

Abstract: Treating R3327-AT1, Pgp-positive tumor cells at pH 7.4 with daunorubicin, cisplatin or docetaxel led to marked apoptosis induction and cell death. Under acidic (pH 6.6) conditions cytotoxicity of daunorubicin or docetaxel was significantly reduced whereas cisplatin chemosensitivity was almost unaffected. Inhibiting Pgp with verapamil reversed the acidosis-induced chemoresistance against daunorubicin and docetaxel. The Pgp expression was unaffected by pH. In vivo the cytotoxicity of daunorubicin and docetaxel was also pH dependent. When acidifying the tumors by forcing glycolytic metabolism, apoptosis induction decreased significantly indicating a reduced chemosensitivity. The cytotoxic effect of cisplatin was unaffected by the tumor pH.

Conclusion: Since daunorubicin and docetaxel (but not cisplatin) are substrates of the Pgp, these results underline the influence of the tumor acidosis on the Pgp-mediated chemoresistance which can be counteracted by inhibition of the drug transporter.

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June 22, 2013 – June 28, 2013

Imaging redox state in cancer

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Study Goal: Our long term goal is to develop and utilize redox imaging techniques to probe the role of redox state in cancer transformation/progression, and identify redox imaging biomarkers for tumor diagnosis and prognosis. Here is a summary of progress.

Abstract: Employing the Chance redox scanner, i.e., cryogenic NADH/flavoprotein fluorescence imager, it was demonstrated for the first time that cancer transformation and progression to metastasis are correlated with a more oxidized and heterogeneous redox state in mitochondria in various xenografts and transgenic mouse models of cancer including human melanoma, breast, colon, and pancreatic cancers. More recently data were obtained from breast cancer patients indicating redox indices may provide diagnostic/prognostic biomarkers. To facilitate the clinical translation of redox imaging, we have also been developing non-invasive NMR methods to image redox state in vivo.

Conclusion: The study results indicate that redox state may play an important role in cancer transformation and progression. Redox imaging indices may have great significance for cancer management.

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June 22, 2013 – June 28, 2013

Quantitative hypoxia imaging for treatment planning of radiotherapy

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Study Goal: This study presents a clinical approach for including tumour hypoxia measurements into treatment planning for radiotherapy. It also explores theoretically the issue of quantitative measurements of hypoxia through combined PET and EPR measurements.

Abstract: Tumour oxygenation is an important determinant of radiotherapy outcome as it could modulate cellular radiation sensitivity. Advanced PET imaging able to characterise in vivo this microenvironmental aspect holds many promises for devising counteracting therapies as it could provide both the extent and the spatial distribution of the hypoxic regions. This study reviews the advantages and the limitations of PET for imaging and quantifying tumour hypoxia and proposes a novel approach to obtain absolute levels of hypoxia from PET images through the use of absolute EPR measurements. This offers a significant advantage over most other proposals that are unable to absolutely quantify the hypoxia levels from the relative intensities in PET images.

Conclusion: Tumour hypoxia must be taken into account at the stage of treatment planning for photons and particle therapy by accounting for its extent and severity through the use of PET imaging combined with absolute EPR measurements.



June 22, 2013 – June 28, 2013

Role of Microvascular Shunting in the Loss of CBF and ICP Autoregulation

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Study Goal: To describe the progressive increase in microvascular shunting and loss of CBF and ICP autoregulation in parallel with brain injury induced by a progressive increase in ICP. In brain injured patients, CBF and ICP autoregulation varies daily.

Abstract: Historically, CBF autoregulation and the critical CPP was identified by decreasing arterial pressure to lower CPP which produced a critical CPP of 60 mmHg. However, when CPP was decreased by increasing ICP the critical CPP was lowered to 30 mmHg. Using two-photon laser scanning microscopy, we showed that the decrease in CPP was due to microvascular shunting with high ICP resulting in a pathologically high CBF at a lower CPP. The development of microvascular shunting was associated with tissue hypoxia, brain edema and increased blood brain barrier permeability. The loss of CBF and ICP autoregulation due to microvascular shunting differs from that due to hyperemia as at high CO₂.

Conclusion: We have shown for the first time, the progression from normal capillary flow to microvascular and thoroughfare channel shunt flow in the injured brain where previously only two stable states of normal capillary flow and microvascular shunt flow.

Acknowledgments: The two-photon facility was supported by the UNM HSC COBRE program (P20 RR15636). This research was supported by NIH Grants NS061216 and NS051639



June 22, 2013 – June 28, 2013

Revising Fame for The Discovery Of Oxygen

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Michael Servetus, born in Spain in 1511, studied medicine and law, was a bio-scientist, poet, politician, cartographer, and biblical scholar who disputed the church doctrines of Trinity and infant baptism. He was called a heretic and lived in hiding with a change of name. In 1553 he published his anatomic and physiologic finding that blood flows through the lung, discharging waste and getting something from air that brightens its color. Unfortunately, this was hidden in a book titled “Christianismi Restitutio”. He boldly sent Calvin a copy. He visited Calvin’s church in Geneva incognito but was discovered, arrested and jailed, and after 2½ months, declared a heretic and was burned at the stake. Calvin and others burned all available copies of his book. Only 3 copies have survived. His discovery of pulmonary circulation and lung blood-gas exchange remained unknown to science historians.

In 1668, after working in Robert Boyle’s Oxford laboratory for several years, John Mayow published two books. He found that air had two components of which 1/5th was partly consumed during breathing, and in fire, and thus was used to provide both body heat and energy. He named it “spiritus igneo aereus”. He was ignored because at the same time the erroneous theory of fire and life, phlogiston became accepted by all scientists, not needing air. It was Science’s worst blunder.

In 1771, Carl Wilhelm Scheele, in Uppsala, by heating the calc (oxides) of manganese generated a new gas (O₂) that increased a candle flame. He called it “fire air” “Swedish llds Luft. He delayed publishing it since it didn’t fit the phlogiston idea. In 1775, he learned that Priestley had independently published the same method so he couldn’t claim priority. He published a book of his work in 1777 with a note that, on September 30, 1774, he wrote Lavoisier about fire air but Lavoisier had not answered.

Joseph Priestley, a Unitarian minister, fired by 3 churches as too liberal, became a self-trained scientist-teacher, wrote the best textbook about electricity, and discovered how to make soda water for which he was awarded the Copley medal. By 1772, he had discovered nitrous oxide and many other gases.

Priestley, again by heating calc of mercury, independently discovered O₂ on August 1, 1774 and named it “dephlogisticated air”. He found that mice could live in it and it caused a glowing splinter to burst into flames. He informed the Royal Society and showed the method to Lavoisier in October. Lavoisier never acknowledged Priestley’s help. Lavoisier, studying the new gas, in 1777 named it principe oxygen but couldn’t prove what it was.

In 1766, Henry Cavendish (1731-1810), by mixing iron filings with acid, made a gas he called flammable air (H₂) that, when burned, made pure water. No one believed him. On June 24, 1784, hearing that the Royal Society had invited Cavendish to review his strange report, Lavoisier with observers repeated Cavendish’s experiment. Water appeared. Lavoisier declared that flammable air and oxygen are elements. Water is a

compound made of them. He named flammable air hydrogen and then proved that the phlogiston theory was false.

Lavoisier, although a brilliant, meticulous scientist, tended to claim credit for work by others. He became very rich as a tax collector and gunpowder manufacturer. He was hated for building a wall around Paris to force incoming merchants to pay taxes. During the terror, the Revolutionary Tribunal tried, convicted and beheaded him, all on the same day, May 8, 1794.

In the 1780s, Priestley was repeatedly attacked by Church, Crown and press for supporting the French and American revolutions. On July 14, 1791 (Bastille day), drunken rioters destroyed his Birmingham home and laboratory, and many homes and churches of the Unitarian dissenters. Priestley and his family fled to Pennsylvania in 1793 and built a home and laboratory in Northumberland where he died in 1804.

In 1890, the French historian, Edouard Grimaux, claimed that he had been shown Scheele's letter to Lavoisier but was doubted because he was unable to show it to any historian. The Swedes erected a Scheele sculpture in Stockholm based on his claim.

In 1993, the French Academy of Science obtained Marie Anne Lavoisier's personal belongings containing the letter written by Scheele to Lavoisier, dated 30 Sept 1774, hidden for 219 years by family presumably because it confirmed plagiarism by Lavoisier.

In 1903, Auguste Dide, author of a book on heretics and revolutionaries, was elected a French Senator. He proposed to erect a monument in Geneva in honor of Servetus. Supporters commissioned Clothilde Roch to sculpt Servetus. The Geneva Calvinist town council refused but named an alley "rue Michel-Servet". Senator Dide then arranged for it to be mounted in France on the Annamasse town hall square 4 km east of Geneva. The French Vichy (Nazi) rulers melted it down during WW II. In 2011, Servetus' 500th birthday, a recast Roch sculpture was erected near the site of his execution beside Geneva University Hospital.

In 2009, a Greek cardiologist and 2 medical historians in Athens University translated and published in the Hellenic Journal of Cardiology "Michael Servetus and the discovery of pulmonary circulation".

In summary, 6 scientists deserve part of the credit for the most important discovery of science. The first 5 did crucial experiments while Lavoisier needed years to understand and proclaim it was an element. But his insight led to all modern chemistry.

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June 22, 2013 – June 28, 2013

Segmental Acquisition of Pure Absorption and Pure Dispersion EPR Spectra Using Time Averaging

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Abstract: An arbitrary waveform generator (AWG) allows one to sweep the resonance condition over a segment of an EPR spectrum at a uniform rate, to fly back to the origin of the sweep practically instantaneously, and to sweep again. If the signal is digitized during each sweep, and the digitized sweep signals are added, an EPR spectrum of the segment can be obtained with high signal to noise (SNR) ratio. This is called “time averaging.” Over a wide range of assumptions concerning noise, the SNR increases as $n^{1/2}$, where n is the number of averages. If the main magnetic field is shifted, a second segment can be acquired. Repeat of the process allows combining of spectral segments to produce to a complete spectrum that can be either the pure absorption or the pure dispersion, or both, depending on the microwave bridge set up.

In general, the information of interest is not uniform across the complete spectrum. It may be distributed across the spectrum in a complicated way in accordance with the spin Hamiltonian, or there may be a superhyperfine coupling present, or a dipolar coupling from the Pake doublet, or a linewidth associated with a Heisenberg exchange event – each of over-riding interest. In general, it is simplistic to speak of the SNR of the spectrum; it is uncertainty of the information of interest arising from the presence of noise that matters. In general, the information of interest is extracted from the complete spectrum by application of a filter that is based on *a priori* information.

Conventional CW EPR using sinusoidal magnetic field modulation effectively applies a filter to the spectrum and then adds noise. Field modulation is a filter that smears data within the width of the field modulation amplitude, which inherently decreases the detectability of information of interest.

We have written three papers within this framework: improved spin label sensitivity, which we call non-adiabatic rapid sweep (NARS) [1], measurement of the Pake doublet produced by two interacting spin labels [2], and study of superhyperfine coupling of nitrogen ligands in square-planar copper complexes [3]. These papers led to this abstract where the concepts are generalized. Extension to adiabatic magnetic field sweeps is foreseen.

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June 22, 2013 – June 28, 2013

Improving Sensitivity of Detection of Biologically-Generated Radicals by Rapid-Scan EPR

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Study Goal: We seek to determine the extent to which the sensitivity of EPR measurements of spin-trapped superoxide radicals can be improved by using rapid scan EPR instead of continuous wave (CW) EPR.

Abstract: The low flux and short lifetime of superoxide in vivo makes detection challenging. Spin trapping with BMPO to form the BMPO-OOH adduct converts the very short-lived superoxide radical into a more stable spin adduct. In rapid scan EPR the magnetic field is scanned through resonance in a time that is short relative to electron spin relaxation times, and data are processed to obtain the absorption spectrum. To validate the methodology superoxide was generated by the reaction of xanthine oxidase and hypoxanthine with flux rates of 0.1 to 6.0 micromolar/min and trapped with BMPO. The technique was used to detect superoxide produced by *Enterococcus Faecalis*.

Conclusion: The signal-to-noise obtained by rapid scan EPR is substantially higher than obtained by CW EPR for the same data acquisition time. The improvement in sensitivity permits detection of BMPO-OOH at concentrations that are otherwise undetectable.

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June 22, 2013 – June 28, 2013

T2 Estimation for Spin-Echo EPR

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Study Goal: The study goal is to improve the accuracy or acquisition time for spin-echo (SE)-based EPR oximetry by developing new data processing methods.

Abstract: In pulsed EPR oximetry, the SE approach allows direct measurement of intrinsic T2 relaxation time, which can be readily converted to the underlying oxygen concentration in the sample. For spectroscopy, traditional methods to estimate T2 include fitting an exponential to the peaks or the integrated areas of multiple noisy echoes measured at different time delays. These methods are suboptimal and result in lower estimation accuracy for a given acquisition time. Here, the maximum likelihood estimator (MLE) of T2 from SE-EPR data is presented; the technique allows for an arbitrary and unknown echo shape. The underlying rank-one structure of SE-EPR data allows estimation of T2 by a computationally inexpensive one-dimensional line search. Additionally, we consider the MLE of T2 for the case of an assumed parametric shape for the echo; this second estimator requires a non-linear least-squares fit to the data. Interestingly, the first method, despite being agnostic to the echo shape, provides practically the same estimation accuracy (for a wide range of parameters and noise powers) as the maximum likelihood estimator that is endowed with perfect prior knowledge of the echo shape. The methods are validated using statistical sensitivity analysis as well as both simulation and experimental data.

Conclusion: The proposed estimate of the T2 relaxation time provides significant reduction in acquisition time versus traditional methods. For the experimental parameters considered, a time savings of 3:1 were observed versus the echo integration approach.



June 22, 2013 – June 28, 2013

EPR Spinomics for Diagnostic and Mechanistic Studies of Mitochondria in Disease

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Study Goal: To develop EPR into a diagnostic and mechanistic tool for the study of the role of mitochondria in disease.

Abstract: Mitochondrial disease (MD) is a devastating and incurable disease. MD is heavily under-diagnosed and, where clinically present, the mechanism of 80 % of patients' disease is unknown. In addition, mitochondrial dysfunction is implicated in many other diseases, including neurological diseases. Mitochondrial insult, whether through primary MD, environmental causes, or as a secondary effect of another disease process, can cause disease pathology through either energy deficiency (depleted ATP synthesis) or oxidative stress (ROS, RNS). Western blots and electron transfer chain (ETC) assays of biopsy tissue can determine the amount and activity, respectively, of the mitochondrial respiratory chain components. Additional and complementary to this information, EPR can provide information on the individual redox centers in the ETC and also provide a "redox snapshot" of the state of the mitochondrion. However, there are significant challenges to overcome, and we describe our efforts to develop EPR into a robust diagnostic and mechanistic tool for mitochondria in disease.

Conclusion: We have developed sample collection, data collection, and data analysis methods for EPR spectroscopy of biopsy tissue that we will develop into a mechanistic tool for mitochondria in disease.



June 22, 2013 – June 28, 2013

Quantitative EPR

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Study Goal: What is the “best” method for determining the electron spin concentration and spectroscopic parameters for biomedical applications such as in vivo oximetry and dosimetry? How can one quantitatively estimate and calibrate spectrometer performance?

Abstract: Continuous wave (CW), rapid scan, and pulsed EPR each can be used to measure the number of spins in a sample of interest, the relaxation-time-determined line shape, and other parameters. Each method has different dependence on, and possibility to exploit, relaxation times, and each method has different background interferences. Short or long T1 and/or T2, broad or narrow distributions of resonances, large or small samples, etc., favor different methods. For example, since many radiation defect centers exhibit a distribution of relaxation times, there are important tradeoffs in signal-to-noise and quantitation of various subpopulations. As always, the key question is “what do you want to learn from the EPR of the sample?”

Conclusion: The measurement of electron spin concentration is inherently a quantitative physical method, but accurate and reproducible EPR measurements require attention to many aspects of the sample as well as spectrometer design and experimental methodology.

Acknowledgments: Discussions with Dr. Ralph Weber (Bruker) facilitated our studies. Partial support of this work by NIH P41 grant EB002034 to GRE (Howard Halpern, PI), grants EB002807 and EB000557 to GRE and SSE, and an NSF graduate Fellowship to DGM, are gratefully acknowledged.



June 22, 2013 – June 28, 2013

Routine Quantitative Spin Concentration Measurements

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Study Goal: How to routinely measure spin concentrations or number of spins without an external standard.

Abstract: CW EPR concentration measurements have historically been considered as not being particularly quantitative unless much effort was invested. There are many experimental details such as sample position and size, B1 (microwave magnetic field), instrument gains and losses, etc. to measure concentration without an external standard. With careful EPR spectrometer characterization and keeping track of all relevant parameters, EPR concentration and spin count measurements can be performed in a routine fashion.

Low SNR (Signal to Noise Ratio) may offer difficulties for quantitation. Simulation of the spectra greatly increases the reliability of such measurements. Multi-species simulations facilitates kinetic studies of reactions.

Conclusion: Spectrometer characterization and parameter tracking facilitate reliable and routine concentration measurements with minimal user input. Simulation greatly improves reliability of measurements and opens the door for multispecies kinetic studies



June 22, 2013 – June 28, 2013

Spatial 3D EPR Imaging with Compressed Sensing Reconstruction

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Study Goal: The goal of this study was to produce 3D EPR images with only a small number of projections using a compressed sensing (CS) reconstruction and to test the reconstruction on a typical EPR phantom.

Abstract: Continuous wave EPR spatial imaging is a form of tomography where a 3D object is recovered from projections blurred by a known point spread function (the zero gradient spectrum). CS has been shown to recover tomographic images from only a small number of the projections. A sparse basis for EPR images would overcome the historical difficulties due to a lack of projections and limited SNR. The traditional deconvolution and Filtered Back-Projection reconstruction (FBP) was compared to a CS reconstruction (Eq 1) of 19 bottles of 1 mM triarylmethyl radical (Fig 1a,b). When only 441 (25%) out of the full 1,764 projections were used (c,d), the FBP reconstruction degraded whereas the CS reconstruction was still visually acceptable.

Conclusion: Spatial 3D EPR images are compressible on both the image itself and also on the Total Variation (TV) of the image. The reduction in projections can be used to greatly accelerate the EPR acquisition.



June 22, 2013 – June 28, 2013

Formation of a New Paramagnetic Hybrid Intermediate in the Reaction of Nitrite with Deoxyhemoglobin

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Study Goal: Deoxyhemoglobin chains can reduce the nitrite ions. This has been implicated in red cell induced vasodilation. However, no convincing mechanism for this process has been brought out thus far. It is intended to establish a mechanism.

Abstract: Earlier works have shown that the nitrite reaction with deoxyhemoglobin involves a hybrid intermediate of the type 'Hb(II)NO⁺ and Hb(III)NO' leading to potential NO bioactivity. To explain the stability of the latter which prevents the release of NO we had suggested the transfer of an electron from beta-93 thiol to NO⁺ to produce an intermediate .SHb(II)NO. In order to identify the species we have investigated the changes in the EPR spectral characteristics of deoxyhemoglobin-nitrite reaction. Two procedures, namely attaching of NEM to block the thiol and use of CPA to stabilize the R-state of Hb, reveal the formation of .SHb(II)NO by the observation of the the EPR spectrum expected for the NO bound heme in the beta-chain plus that of a thiyl radical. This new species is in equilibrium with the earlier suggested hybrid intermediate, Hb(II)NO⁺ <--> Hb(III)NO. This will further inhibit the release of NO from the latter of the two. Furthermore the -20 C incubation studies reveal the formation of another NO related paramagnetic species other than the Hb(II)NO the EPR intensity of which decreases, shifting the equilibrium back to the above said hybrid intermediate. This new NO-hemoglobin species unrecognized earlier can not only explain the stability of the intermediate but also can explain the build-up of a pool of the potentially bioactive NO during the nitrite reaction. The build-up of new molecules and disappearance of others have been well characterized through incubation and subsequent EPR intensity studies at 8K. All the above can be explained by PES diagrams developed by other workers for these various molecules involved in these reactions.

Conclusion: The methodology for the formation of beta-93 thiol-nitrosylated hemoglobin suggests a pathway to induce vasodilation through a rapid radical-radical reaction of any free NO with the thiyl radical of this new paramagnetic intermediate.

Acknowledgments: We acknowledge the help from the NIH, the DST, Govt. of India and other workers.



June 22, 2013 – June 28, 2013

New functional Trityl Probes for Oxygen and pH Monitoring Using EPR-based Techniques

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The Ohio State University

Study Goal: To synthesize new trityl radical probes with EPR sensitivity towards oxygen and pH. To modify existing trityl probes to achieve optimal pharmacokinetic properties in vivo. To simplify the processes for the syntheses of trityl radicals.

Abstract: Trityl radicals are ideal probes for EPR imaging. Our work with trityl radicals is divided into three major components:

1. Synthesis in gram scale of Finland trityl-based radicals using processes that are reproduced with ease. 2. The synthesis of dual function pH and pO₂ trityl radical probes. The analysis of EPR spectra of the recently synthesized phosphonated trityl allows one to calculate both pH and pO₂, in vivo. Dual functionality is important to the study tumor microenvironment via EPR/PEDRI imaging, because it enables the monitoring of the interplay between acidosis and hypoxia, which are two important biomarkers of tumor progression. 3. The synthesis of trityl radicals with improved water solubility and stability in vivo.

Conclusion: We have achieved the synthesis of Finland trityl-based radicals in various modified forms in large scale. In recent EPR studies we demonstrated the capacity of our probes to measure oxygen concentrations and pH in vitro, and in vivo.

Acknowledgements: Funding: NIH, and Pulmonary Division of the Department of Internal Medicine, Ohio State University



June 22, 2013 – June 28, 2013

EPR investigations on drug delivery systems - from in situ forming implants to nanoscale systems

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Study Goal: The aim of the studies is to follow key processes of drug delivery by ESR in vitro and in vivo.

Abstract: Drug delivery systems (DDS) are an important tool to improve pharmacotherapy. They include a wide range of different sizes, materials and structures and cover local and systemic delivery. The performance of DDS is a result of complex interactions between the carrier matrix (e.g. polymer, lipid), the drug and the environment. As a result, quite frequently surprising and paradoxical release patterns are observed and poorly understood. EPR can be used to monitor key processes of drug delivery in vitro and in vivo. Application examples include preformed and in situ forming implants, but also nanoparticles, nanocapsules and self-emulsifying systems. Important information on microviscosity, micropolarity, micro pH and the heterogeneity of the DDS can be obtained. This information is of key importance to understand and to optimize DDS.

In situ forming implants can be made from polymeric solutions in polar biocompatible solvents. In the body, the diffusion of water into the solution leads to a solvent exchange and the polymer precipitation. EPR is capable to quantify the implant formation of in situ forming implants with respect to the kinetics of the solvent exchange and the polymer precipitation. Clinically used biodegradable might accumulate acidic monomeric degradation products and as a result, the pH might drop locally to very low values.

Other examples include the monitoring of digestion induced (pancreatin) degradation of lipid DDS which results in a translocation of lipophilic nitroxides into a more polar environment.

Conclusion: EPR spectroscopy and EPR Imaging are important tools to understand and to optimize both nanoscopic and macroscopic drug delivery systems.

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June 22, 2013 – June 28, 2013

EPR Spin trapping detection of lung lipid radicals in an animal model of acute pneumonia

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Study Goal: To demonstrate in vivo free radical production in the lungs of mice with *P. Aeruginosa* induced bacterial interstitial pneumonia. To determine the mechanisms involved in free radical generation by using EPR and in vivo spin-trapping technique.

Abstract: In the *P. aeruginosa* induced rodent bacterial pneumonia model, it is thought that free radicals are significantly associated with the disease pathogenesis. There has been no direct evidence of free radical generation in vivo. We used EPR and in vivo spin-trapping with a nitron compound to investigate free radical production in mice treated with *P.aeruginosa*. We detected and identified generation of lipid-derived free radicals in the lungs. To further investigate the mechanism of lipid radical production, we used modulating agents and knockout mice. We found that with GdCl₃ (phagocytic toxicant), NADPH oxidase knockout mice, allopurinol (xanthine oxidase inhibitor), and Desferal, generation of lipid radicals was decreased significantly.

Conclusion: Lipid-derived free radical formation is mediated by NADPH oxidase and xanthine oxidase activation, and metal-catalyzed hydroxyl radical-like species play role in lung injury caused by bacterial infection with *P. aeruginosa*.

Acknowledgments: Keizo Sato, JinJie Jiang, Ronald Mason.



June 22, 2013 – June 28, 2013

Developing nitroxides with long intracellular half-life

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Study Goal: The goal of the research is to design and synthesize nitroxides that, when loaded into living cells, will be resistant to active extrusion and thus be well retained intracellularly.

Abstract: To enable tumor visualization by electron paramagnetic resonance imaging (EPRI), we have previously targeted delivery of ionic nitroxides to tumors *in vivo*. Once loaded into tumor cells, the ionic nitroxides are cleared from the cells by active extrusion mechanisms. Extrusion limits the intracellular half-life of the loaded nitroxide and thus narrows the temporal window for EPRI. Preliminary studies suggest that resistance to active extrusion depends strongly on the total number of ionic functional groups on the nitroxide. We have therefore designed and synthesized a new nitroxide bearing a total of six ionic groups. The loading of this nitroxide into cells, as well as its intracellular retention, have been tested in a lymphocyte system.

Conclusion: Increasing the total number of charged functional groups on a spin probe enhances intracellular retention. This principle is expected to be generally applicable to molecular probes intended for intracellular applications.

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June 22, 2013 – June 28, 2013

pH-, redox- and GSH-sensitive nitroxide probes

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The Ohio State University

Study Goal: To develop EPR-based approaches for in vivo real-time assessment of tumor tissue extracellular pH (pHe), redox and intracellular glutathione (GSH) based on the application of specially designed nitroxide probes.

Abstract: Tissue pH, redox status and GSH content are among the most crucial parameters related to metabolism and physiology in tumors. Dual function pH and redox nitroxide probes which possess (i) pH-sensitive spectral properties in the pH range from about 6.5 to 7.5, and (ii) enhanced stability to survive a reducing tumor microenvironment were designed and characterized by EPR-based techniques. To ensure their targeting to extracellular space the probes were bound to membrane impermeable fragment. The use of GSH-sensitive paramagnetic analog of Ellman's reagent, namely nitroxide disulfide biradical, for in vivo intracellular GSH detection by L-band EPR spectroscopy has been optimized.

Conclusion: The functional sensitivity of the developed nitroxide probes to the physiologically relevant parameters of tumor microenvironment, namely pHe, redox and GSH, has been demonstrated in mouse model of breast cancer.

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June 22, 2013 – June 28, 2013

Ascertaining Spin-trapping Artifacts

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Study Goal: To study the role of the Forrester-Hepburn mechanism as artifact source in ESR spin trapping by dimethyl-1-pyrroline N-oxide (DMPO) and nucleophilic substrates under conditions close to biological samples.

Abstract: Free radical detection with ESR spin trapping relies on the specific addition of the radical to nitrene/nitroso compounds. It has been proposed that spin traps can react in biological systems with nucleophiles to give false-positive results. For nitrene spin traps, this reaction first described by Forrester and Hepburn has been discussed as the most critical source of artifacts. With the ESR preincubation method which uses isotopically marked spin traps to identify the artifact, we found that Forrester-Hepburn mechanism did occur in spin trapping experiments of cyanide oxidation and sulfite oxidation with ferricyanide. No evidence was found for the sulfite / horseradish peroxidase / hydrogen peroxide system or for thiol oxidation.

Conclusion: The Forrester-Hepburn mechanism can occur under mild conditions. Affected systems had significant hydroxylamine formation and thermodynamically unfavorable radical formation. Examples of misinterpretation in radical biochemistry are rare.



June 22, 2013 – June 28, 2013

Applications and Limitations Of TPP-Modified Nitrones in the Study of Mitochondrial Derived Free Radicals

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Study Goal: To test if TPP-modification of newly designed nitrone spin traps would facilitate superoxide radical anion ($O_2^{\bullet-}$) detection

Abstract: Two TPP-modified analogs of 5-(diisopropoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DIPPMPO) were tested, mito-DIPPMPO and mito-bis-DIPPMPO which contains two TPP groups. Superoxide-trapping efficiency of these analogs was comparable producing higher yields of spin-adducts than the analog 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO). Similarly to DEPMPO-OO(H) adducts, the mito-DIPPMPO and mito-bis-DIPPMPO adducts were reduced to the corresponding hydroxyl-adduct. The uptake of mito-DIPPMPO and mito-bis-DIPPMPO in incubations with energized mitochondria succinate or glutamate+malate as substrates, was shown to be 20 and 30% higher than non-TPP modified compound. Under these conditions, mito-bis-DIPPMPO did not generate radical signals. Complex III inhibition with antimycin A (AA) led to a detection of mito-bis-DIPPMPO-superoxide radical adduct. Mitochondrial incubations with DEPMPO lead to detection of a strong DEPMPO-hydroxyl radical adduct that was abolished by addition of cytochrome bc1 complex inhibitor myxothiazol. To control for nitrone effects on mitochondria respiration we used a XF-flux analyzer. Unlike DEPMPO, mito-bis-DIPPMPO readily inhibits mitochondrial ADP-stimulated respiration. Respiratory complex activity assay showed that mito-bis-DIPMPO inhibited complex IV>complex III>complex I.

Conclusion: We found that TPP-derived mito-bis-DIPPMPO has complex IV inhibitory activity, which may facilitate superoxide release from mitochondria. Potential applications in mitochondrial research will be discussed.

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June 22, 2013 – June 28, 2013

***In vivo, in situ* imaging of free radical adducts in disease models using immuno-spin trapping and molecular MRI**

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National University of San Luis, Argentina;
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Study Goal: To present a novel approach that allows in situ detection of nitrono-adducts in various oxidative-stress related diseases, such as diabetes, sepsis, ALS and gliomas, by incorporating a combination of molecular MRI and immunospin trapping.

Abstract: Combined immuno-spin-trapping (IST) and molecular magnetic resonance imaging (mMRI) was used to detect in situ levels of spin-trapped radicals in brain tissues from mice with gliomas, amyotrophic lateral sclerosis (ALS), or sepsis, as well as livers, lungs and kidneys of diabetic mice. The nitrono spin trap DMPO (5,5-dimethyl pyrroline N-oxide) was administered prior to injection of an anti-DMPO probe (anti-DMPO antibody covalently bound to an albumin-Gd (gadolinium)-DTPA (diethylene triamine penta acetic acid)-biotin MRI contrast agent) to trap disease-associated free radicals. mMRI detected the presence of anti-DMPO adducts by either a significant sustained increase in MR signal intensity or a significant decrease in T1 relaxation.

Conclusion: Using both mMRI and IST provides the advantage of in vivo image resolution and spatial differentiation of heterogeneous tissues or organs and the regional targeting of free radical mediated oxidation of cellular components in various disease models.

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June 22, 2013 – June 28, 2013

Revisiting spin trapping of superoxide radical in cells

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Study Goal: In the context of the implantation of EPR spectroscopy and imaging facilities dedicated to biomedical applications in Paris, we studied the cell metabolic pathways of superoxide adducts of new cyclic nitrones to guide future spin trap design.

Abstract: Reactive oxygen species (ROS) are by-products of aerobic metabolism and are involved in the onset and evolution of various pathological conditions including inflammation, carcinogenesis, and neurodegenerative diseases. Among them, superoxide radical is of special interest as the origin of several more damaging species such as H₂O₂, hydroxyl radical, or peroxynitrite (ONOO⁻).

Spin trapping coupled with EPR is a method of choice to characterise these species in chemical and biological systems. Since the 1970's, many efforts have been dedicated to improvement of the intrinsic stability of the spin adducts. New spin traps have been proposed such as cyclic nitrones substituted by a triphenylphosphonium or β -cyclodextrin moiety. However the historic spin traps, DMPO and DEPMPO, remain mostly used in the literature for spin trapping on cells, despite their drawbacks.

Here we will present a comparison of the metabolic stability of the superoxide adducts of various new cyclic nitron spin traps in the presence of subcellular fractions, purified enzymes, or biologically relevant reductants. This study was performed using an original set up combining a stopped-flow device and an EPR spectrometer. A simulation program was used to analyze the kinetics of the spin adduct decay.

We will show that the resistance of the adducts to some metabolic pathways depends on the structure of the spin trap.

Conclusion: These results should be helpful for the choice of the spin trap for cell studies and for the design of new spin traps that would form more metabolically stable spin adducts.

Acknowledgments: This work results from the collaboration with Olivier Ouari and coworkers in SREP team (Institut de Chimie Radicalaire, UMR CNRS 7273, Université Aix-Marseille) and was funded by Agence Nationale de la Recherche (ANR IRPE and SPINBIORAD)



June 22, 2013 – June 28, 2013

Translating from Bench to Disaster Sites: Special Considerations in Developing EPR for Use in Radiation Disasters

Ann Barry Flood, Benjamin B. Williams, Roberto J. Nicolalde, Holly Boyle, Paul Calderon and Harold M. Swartz

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Abstract: The life cycle from bench to clinical practice of usual medical devices is a complex but relatively straightforward process, which involves moving through the scientific stage of instrument development and feasibility analyses including evaluating its use in animal and clinical studies, often carried out in the context of patient care in academic medical center settings. This stage is followed by decisions to undergo the arduous regulatory processes to manufacture a prototype of the device to establish its safety and efficacy. Because this stage is particularly expensive and long and highly regimented, the developers also need to establish the business case to see if the business risks are outweighed by the potential for long-term success and profit.

In contrast, each stage in the life cycle of a medical device whose intended use is in a major radiation disaster, e.g., EPR for tooth dosimetry, requires a different set of assumptions about the needs for the instrument and its operators, the people who will be measured and the processes and circumstances for evaluating its safety and efficacy. There is no well-established regulatory process, no capacity to conduct usual clinical trials, and no usual 'business case'. The events are expected to be rare and the emergency setting is a public health disaster rather than a health care setting with experienced personnel, structured information flow and established reimbursement mechanisms. In short, the assumptions for developing, evaluating, testing and successfully manufacturing and using FDA-approved medical devices are often inappropriate or inadequate to apply to devices intended for use in a large-scale radiation disaster.

For EPR tooth dosimetry, a major challenge is to develop an instrument that can be deployed at a moment's notice and operated by untrained users on frightened strangers in a chaotic emergency field setting, while assuming the *unavailability* of usual sources of power, water, internet access, or medical or dental histories. This means that instruments need to operate successfully in extreme environmental conditions ranging from turbulent transportation to operating in a setting exposed to wind, rain, and vibrations from trucks and other types of instruments. The operators will be untrained and unfamiliar with the instrument or process, and so the instrument must be essentially 'idiot proof' with minimal demands on the operator and maximal safeguards for its accurate and reliable performance under continuous use with emergency power only. The 'patients' may be injured, nauseous, vomiting, untrusting and worried about an unknown process as well as contamination from previous patients (from germs or radiation). However, such environmental conditions are difficult to simulate in a lab or clinical study and require creative simulation models that can 'challenge' the instrument and process appropriately while still meeting the regulatory needs as well as the additional considerations for a public health uses, including with an accuracy dominated by minimizing false negatives instead of false positives, availability to 'all' people potentially exposed (e.g., mentally ill, children, aged, sick). Thus, the human factors development

is doubly important because it is not possible to assume that training and experience can overcome the complexity of the work, that the instrument can be protected from abuse or environmental extremes, or that usual supplies and expertise for maintenance or repair will be readily available within the time constraints of emergency use. Finally, the instrument needs to be used interdependently with other dosimetry methods and treatment triage plans. The capacity to relay the patient information and test results must not only be fast, accurate and well understood by decision-makers, it needs to be standardized and transmittable to a network of people and organizations—all daunting challenges requiring new ways to test and evaluate the instrument in addition to meeting the 'usual' standards for medical devices.

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June 22, 2013 – June 28, 2013

***Ex vivo* nail dosimetry for triage after a radiation event involving large populations**

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Abstract: *Ex vivo* nail dosimetry is being developed into a practical method for triage in large scale radiation exposure events. This is made possible due to the development of methods for removing the interfering mechanically-induced signal thus allow for the measurement of the radiation-induced signal (RIS) in nail clippings.

To achieve accurate and precise dose estimates based on the RIS in clipped nails, a number of approaches are being assessed to remove the interference of a mechanically-induced signal (MIS). A number of methods are being examined, which include spectral decomposition, chemical treatments of the nail clipping and the use of advanced and multi-frequency EPR techniques. In spectral decomposition methods, the variability of the individual spectral components in the nail sample preparation steps following clipping must be controlled by regulating the temperature, humidity, water content, and O₂ level. By utilizing proper modeling of the MIS, RIS and background spectral elements, regression-based fitting models are employed to calculate an estimate of the RIS in the experimental EPR spectra of the irradiated nail clipping. Initial validation of a multi-component EPR spectral fitting models have been conducted on irradiated (0-6 Gy) clipped nail spectra acquired from a large dataset (90 samples from 15 donors). The results of these studies showed a high degree of consistency between the pure RIS and the estimated RIS computed from spectral analysis. Chemical treatment of nails make use of the redox reagents in either aqueous or organic solutions to preferentially remove the surface distributed MIS while retaining the RIS, and is amenable for use in the field. In one approach a soaking of nail clippings in water (10 min) removes all the MIS and an unstable RIS signal component, retaining a smaller dose-dependent stable RIS signal. The measurement of this stable RIS signal in nail clippings has been shown to provide excellent dose estimates in individuals exposed to high IR doses (20+ Gy). Although not amenable at this time for field deployment, advanced EPR techniques, such as saturation recovery and multi-frequency analysis of the MIS and RIS components could be used to provide confirmatory dosimetry in off-site analysis facilities by offering alternative methods for extracting the RIS signal from the clipped nail spectrum. For example, small differences in the T1 relaxation times between the main MIS interfering (singlet) signal and the RIS as measured using saturation recovery techniques may offer an ability to differentiate these two signals in nail spectra. Multi-frequency EPR studies of the MIS singlet and RIS have shown slight differences in g-tensors of

the MIS singlet, RIS and background spectral components and are providing clues of the chemical nature of the radical centers underlying these signals

The use of spectral decomposition or chemical treatment techniques in ex vivo nail dosimetry to remove the interfering MIS signal are providing accurate estimates of the RIS in irradiated nail clippings that, with further validation, are expected to provide a reliable approach to estimating dose.

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June 22, 2013 – June 28, 2013

In vivo EPR tooth dosimetry for triage after a radiation event involving large populations

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Study Goal: In order to meet the potential need for large-scale retrospective radiation biodosimetry following an accident or attack, we have developed EPR instrumentation and methodology for the quantification of radiation-induced radicals within intact teeth.

Abstract: EPR tooth dosimetry has several very desirable characteristics for triage including independence from confounding biologic factors and concomitant injuries, a non-destructive and non-invasive measurement procedure, the capability to make measurements at any time after the event, and the ability to provide immediate estimates of individual doses. In an emergency setting, these measurements will provide an accurate determination as to whether the radiation dose is above or below the threshold for entering into the health care system because of a significant risk of acute radiation syndrome. The feasibility of the in vivo tooth dosimetry has been established and current efforts are focused on expanding the amount of data available to evaluate and optimize the technology, increasing the accuracy and precision of dose estimation via instrumental and analytic improvements, and refining instrumental features to facilitate use in the field by non-expert operators. Instrumental improvement efforts are focused on full automation of the spectrometer, optimization of the surface-loop resonator geometry, and systems for reliable resonator and subject positioning. Analytic developments efforts have focused on increasing the precision of radiation induced signal determination and considerations of the potential impacts of interpersonal variations so that concerns can be eliminated or appropriate correction factors can be applied. An assessment of the use of upper incisor measurements for dose estimation and screening has been performed with volunteer subjects who have not been exposed to significant levels of ionizing radiation and patients who have undergone total body irradiation as part of bone marrow transplant procedures. These measurements have also been performed under simulated field-conditions. Based on these measurements, with the current technology, the standard error of inverse predication is approximately 1 Gy.

Conclusion: Based on the features of EPR tooth dosimetry and considerations of the needs for triage after a large radiation incident, this technique will be an effective and valuable tool for screening for exposures associated with acute radiation syndrome.

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June 22, 2013 – June 28, 2013

Radiation Doses Received by Navy Personnel in Japan After Fukushima Accident. Applicability of EPR Dosimetry

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Naval Dosimetry Center

Study Goal: To evaluate applicability of EPR dosimetry to measure post-accident radiation doses for US Navy personnel in Japan

Abstract: After Fukushima nuclear accident there was immediate participation of US Navy personnel in humanitarian aid and disaster relief operations. All Navy personnel had dosimeters issued by the Naval Dosimetry Center. We analyzed received doses and compared them with historic data. Our results showed that the dose-contribution of the radiation and released radiological materials from the Fukushima nuclear accident to background radiation doses is less than $0.375 \mu\text{Sv}/\text{day}$ for shallow and deep photon exposure. The average dose measured for the Navy personnel was $57.4 \mu\text{Sv}$ and the highest measured dose was $510 \mu\text{Sv}$.

Conclusion: Current detection limits of EPR dosimetry are 100 mSv for in vitro and 1 Sv for in vivo techniques. The later makes difficult to expect broad use of EPR for dose measurements received by Japanese population as result of Fukushima accident.

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June 22, 2013 – June 28, 2013

Aperture resonators for *in vivo* EPR: modeling and experiment

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Abstract: The *in vivo* EPR nail dosimetry method relies on the measurement of a radiation-induced signal in fingernails and toenails to estimate exposure dose. To decrease the effect of lossy human tissue on EPR measurements *in vivo* usually require the use of lower EPR frequencies. Conversely, in order to detect the weak EPR signal produced at low doses (i.e. 2Gy) there is a need for a higher EPR frequency. These two contradictory requirements potentially could be satisfied by developing X-band EPR techniques that constrain the microwave to the nail and avoids the underlying lossy tissues. The goal of this study is to develop aperture resonators for X-band EPR Dosimeter that are capable of making measurements on human nails *in vivo* with sufficient sensitivity for retrospective radiation biodosimetry following a nuclear accident. Aperture resonators use a resonant EPR cavity that has a hole in a cavity wall to make available microwaves with a local distribution that minimizes energy deposition in the lossy tissue. The sample then is placed over an aperture or near a small transmitting antenna coupled to a cavity via an aperture in a cavity wall or positioned partially inside the cavity across an aperture. The results of HFSS simulations of critically coupled aperture resonator structures created based on different cavities and variety of apertures are presented. The interrelationships of aperture and iris sizes, Q-value, resonant frequency and effects of different inserts inside a cavity on signal amplitude are discussed. Results of bench tests of different fabricated aperture resonators and results of simulations are compared. Observed discrepancies between experiments and simulations do not allow direct use of simulation results to optimize aperture resonator. The discrepancies could be due to resonant features of the aperture that did not occur experimentally. Evaluation of achieved detection sensitivity using an *in vivo* fingernail model and the best but not fully optimized available aperture resonator shows that measurements made during 15-20 min acquisition intervals gave an EPR signal that is equivalent to at least 3Gy dose in nails.

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June 22, 2013 – June 28, 2013

Tooth dosimetry at S-band: design of the spectrometer and evaluation of performance in-vitro

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Study Goal: Design of S band EPR spectrometer and evaluation of feasibility for tooth dosimetry

Abstract: In-vivo tooth dosimetry is now being developed on L-band. This is mainly due to the availability of in-vivo magnets and due to the many years of experience collected during the development of in-vivo EPR. One of options for further development of the technique is to move to a higher frequency because higher frequencies are attractive at least due to such potential advantages as higher sensitivity. However at the moment we do not yet have an S-band magnet with a gap which is large enough to accommodate the human head. Design and implementation of such a magnet is a challenging and an expensive project. Therefore in-vitro performance at the S-band has to be estimated first. For such an evaluation we have designed and constructed an S-band spectrometer and carried out in-vitro measurements with an available electromagnet. Features of the design of the spectrometer will be presented and discussed. Results of careful, statistically proven comparison of performance of the spectrometers on S-band and L-band will be reported. Comparison of different types of magnets at same L-band frequency will be reported too since most L-band data has been acquired with permanent magnets while the S-band measurements were carried out with electro magnets.

Conclusion: The data justifies further developments towards an in-vivo measurements on S-band. In particular, the data suggests that design and implementation of an in-vivo magnet for S band is worthwhile.

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June 22, 2013 – June 28, 2013

A Clinical Analysis of Emesis as a Medical Screening Diagnostic For Radiation Exposure

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Study Goal: Current radiation disaster manuals list the time-to-emesis as a key indicator of radiation dose. These recommendations are based on variable condition radiation accident databases and, in the most widely used analysis, do not consider radiated subjects that did not vomit. Additionally, there has been no differentiation between high (initial detonation) and low (fallout) dose rate exposures. We are undertaking a systematic re-evaluation of the available data on time-to-emesis and adding in the experiences from therapeutic exposures. This initial study re-analyzes relatively recent, published clinical total body irradiation (TBI) data (Westbrook et al., Clinical Radiology, 1987) to evaluate emesis as a screening diagnostic for a low dose rate exposure.

Abstract: 305 patients were treated with TBI in a single fraction with total doses ranging from 9.5 to 11.5 Gy at dose rates ranging from 0.02 to 0.04 Gy/min. All patients fasted for 12 hours prior to TBI. 117 patients received anti-emetics. Chemotherapy (cyclophosphamide 1.8 G/m² or melphalan 110 mg/m²) was also administered prior to TBI.

The radiation dose of greatest clinical interest is around 4 to 6 Gy as doses above this level would likely require medical intervention for survival. The sensitivity and specificity of emesis as an indicator of exposure above 4 Gy was 65.9% (range 50.5% to 74.8%) and 84.3% (range 59.8% to 99.1%), respectively and above 6 Gy was 71.3% (range 65.4% to 74.8%) and 71.2% (range 40.2% to 99.1%), respectively. Between 25% and 50% of patients did not vomit at any tested dose.

Conclusion: Diagnostic tests for large population screening must have high sensitivity to minimize the risk of false negatives and high specificity to minimize the risk of overwhelming the system. Based on the results of this work, it appears that emesis has inadequate sensitivity for mass population screening and does not provide the desired specificity for clinically relevant doses between 4-6 Gy. Efforts should focus on developing a more complete database consisting of data on emesis in subjects with well characterized radiation levels to the whole abdomen or whole body. The results could then be used with the development of new, high sensitivity screening tests for radiation exposure that are rapidly deployable, easy to administer and cost effective.



June 22, 2013 – June 28, 2013

Surface Resonator Array (SRA) and Bridge Development for *in vivo* Electron Paramagnetic Resonance Spectroscopy (EPR)

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Study Goal: In order to cope with a mass triage condition where large numbers of individuals may have been exposed to levels of nuclear radiation, it is essential to be able to measure ionizing dosages with the aid of one or more dosimetry devices that can be operated in the field with minimal training.

Abstract: Electron paramagnetic resonance (EPR) *in vivo* spectroscopy of human finger-nails at X-band (9.5 GHz) has shown promise in measuring the radiation induced by an individual mainly by using the stable free radicals formed in a potentially affected individual's tissues (teeth, fingernails and toenails, bone, and hair) to perform an EPR *in vivo* dosimetry measurement. In case of a triage, this method makes it feasible to determine immediately whether an individual has received at least 2 Gray since the EPR signal has been shown to be proportional to radiation dosage. This work will focus on obtaining signal from *in vivo* finger and toe nails.

The EPR system under development includes a microwave bridge being built in this laboratory, a Surface Resonator Array (SRA) structure, a small Varian electromagnet and a magnet power supply. The magnetic field will be controlled by a Bruker field controller, which will be interfaced with a computer.

This bridge has the capability to tune to the operating frequency of the resonator. It utilizes a tunable YIG (Yttrium Iron Garnet) oscillator (9.2 to 9.9 GHz) as the primary source. The bridge offers various AFC (automatic frequency control) and display options. The detected signal is then down-converted to L-band (800 MHz) at the receiver end. The receiver consists of two paths, an analog detection path and a digital detection path. The analog path uses an I/Q mixer and the digital path uses a Pentek (Model 71630) A/D module and DDC (Digital Downconverter). The digital detection path allows rapid data processing. This Pentek card is designed for digital detection in communications and radar systems, its features make it well suited for EPR signal acquisition and processing.

Conclusion: In order to reduce the losses associated with the tissue beneath the nail which yield no EPR signal, a novel Surface Resonator Array (SRA) structure consisting of an array of anti-parallel transmission lines has been designed to reduce the depth sensitivity. Modeling and design of the structure has been done using Wolfram Mathematica software and simulations performed using Ansoft High Frequency Structure Simulator (HFSS). A dose response curves using a finger equivalent model is obtained and results are presented.



June 22, 2013 – June 28, 2013

The development of the new design resonators for EPR spectroscopy

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Study Goal: Improving the sensitivity of the external surface coil resonator design for in-vivo L-band EPR tooth dosimetry and reducing base line distortion is described. The development of new surface coil resonator concepts are also described.

Abstract: Any metal surfaces of the resonator can be contaminated with material(s) that reduce the overall sensitivity of a given resonator by increasing baseline distortion (BLD). Many industrial methods for manufacturing the various components of the resonator have a high likelihood of being magnetic. Unfortunately, very pure metals (such as silver) are capable of absorbing other metals (process known as diffusion bonding) that could contaminate EPR spectra. The magnetic contaminations on the surface of the loop of the resonator causes local inhomogeneity of the magnetic field local to the sensing region of interest resulting in unwanted BLD of the resonator. Methods for constructing resonators utilizing non-magnetic components and processes, as well as cleaning of sensitive components will be discussed. Further improvements to previous resonator designs for in-vivo L-band EPR spectroscopy will also be discussed, which include increased relative sensitivity due to increased filling factor, and better coupling capability.

Improved resonator topologies and concepts utilizing modern manufacturing techniques, such as magnetron sputtering deposition, have shown increased rejection of low frequency (LF) modulating magnetic field from the collected EPR spectra, which can be explained by the skin effect. This results in a low impedance for the high-frequency (HF) EPR signal, and a high impedance for the LF modulation. Another advantage of this improved resonator topology and manufacturing method is that the materials used to make the resonator can be highly controlled, as a result of the magnetron sputtering deposition technique. This can result in a resonator with minimal contamination due to magnetic particles/materials. This deposition technique is also highly repeatable, and is capable of controlling thicknesses down to the sub-micron level.

Conclusion: New manufacturing methods for constructing EPR resonators gave significant improvements in the quality, and precision of EPR data for tooth dosimetry.

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Developing a mouth model as an alternative to human subjects for EPR tooth dosimetry

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Study Goal: To develop a mouth model which works as a vehicle for testing improvements in the EPR tooth dosimeter, and for testing the effects of variations in anatomy and conditions within the mouth such as the variation in the size of the tongue and lips.

Abstract: We have established the feasibility of collecting *in vivo* data using the EPR tooth dosimetry system and have developed prototypes capable of being deployed to nonclinical sites and operated by generator power. Nonetheless, to facilitate rapid development of instrumental and measurement techniques to improve dosimetric precision and to establish its intended use in clinical studies, more systematic and controllable studies need to be carried out. For this purpose we have designed and constructed mouth models that are stable over time and simulate the conditions of *in vivo* measurements, especially the presence of lossy materials and their anatomical variations in the human mouth.

The basic mouth model consists of human teeth placed in anatomically correct positions, held rigidly in place during measurements. All nonhuman materials used in the model are assessed to ensure that they have no EPR signal that could interfere with the radiation induced signal (RIS). Especially important for tooth dosimetry is the inclusion of lossy material, to represent the gums, tongue, cheeks and lips, because it needs to simulate the microwave properties of tissue in human mouths. Such lossy materials also need to be stable over time so that measurements can be carried out over the course of clinical and instrumental studies.

We first needed to establish the stability of our lossy material, made from gelatin and ethylene glycol, by evaluating the stability over time of its physical properties such as size and weight. The lossy material was then installed into a mouth model and its ability to simulate *in vivo* measurements was assessed by comparing the electrical properties, i.e., the quality factor (Q) and microwave frequency (f_{mw}), when a resonator is placed on the tooth surface in the mouth model and human subjects. Three other types of tests were performed: (1) using EPR to assess RIS in teeth in a mouth model following serial irradiation to 1, 2, 4, 6, and 10 gray with lossy material, (2) using EPR to assess the effect of lossy material on RIS using mouth model at 10 gray, and (3) using a network analyzer to assess the impact on Q when lip and tongue size is systematically varied.

We confirmed that, physical properties of the lossy material changed little (4-5 %) over 6 months, and the electrical properties did not change in this period. Measurements made in the mouth model following serial irradiation demonstrated that the expected linear relationship between doses and RIS was observed. In addition, the expected impact (a smaller signal amplitude) associated with lossy material was observed. Finally, we found that Q was changed by altering the size or amount of lossy material. These results confirm the usefulness of lossy material to simulate *in vivo* measurements.

Conclusion: We succeeded in making mouth models with lossy material that reflect the key microwave conditions *in vivo*. The stability of the Q and its variations are useful for both testing improvements in the instrument and simulating anatomy in human subjects.

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June 22, 2013 – June 28, 2013

Microstrip Resonator – Innovations and Refinements

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Study Goal: The goal of this project is to develop an in vivo resonator that when coupled with our existing RF bridges and spectrometer can detect radiation dose to within 0.5 Gy accuracy.

Abstract: The microstrip resonator was initially conceived as an electric field coupling based unit in contrast to the magnetic field coupling based conventional resonators. This effort has focused on developing a suitable device for the BARDA radiation dosimetry project at the EPR Center at Dartmouth. Recent innovations have allowed us to translate the technology from purely RF considerations to actual testing in the clinical spectrometer. We have thoroughly characterized a range of designs and developed robust strategies for quickly achieving good impedance match at the desired frequencies. The innovations include robust loop mounting strategies, and techniques for minimizing the RF baseline loss and enhancing Q – we now routinely achieve Q values over 300. The resonator operates best with the prototype vector compensation bridge, but new strategies are being explored to make it possible for testing on the standard bridge. While recent results indicate lower signal amplitudes than those observed for conventional designs, the white noise has typically been a fraction than that for the other and the signal standard deviation has been close to desired clinical goals.

Conclusion: Progress has been made with the microstrip resonator design and it is now approaching performance metrics competitive with more conventional designs. We will outline strategies for further improvements.

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Computer Aided Optimization and Evaluation of the EPR sensors

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Study Goal: Improving sensitivity of the L-band EPR external loop resonator without fabrication of the multiple prototypes. Estimation of the error related to positioning of the L-band resonator sensing loop on human incisor tooth sample.

Abstract: High Frequency Field Simulator - HFSS software is a primary tool used to simulate performance and electromagnetic field distribution of L-band EPR sensors used in EPR Tooth Dosimetry. We are able to visualize B1 field distribution, calculate relative EPR signal and scattering parameters for given sample and L-band resonator sensing loop.

As part of the design process we are utilizing HFSS to optimize geometry and test performance of our sensor design without physically making a prototype, here at Dartmouth we were able to test 250+ different designs loaded as parametric batch overnight, it was several times faster than the number that would have been possible with physical prototyping and testing.

Thanks to parametric optimization we are able to identify the effect of all design parameters and performance impact on L-band EPR sensor.

Introduced to the world in 1990 HFSS is now a mature software that allows engineers to understand the electromagnetic characteristics of the design, more over HFSS is a FCC approved Finite Element Method Analysis (FEM) in biomedical engineering and valid tool to simulate a medical device.

Conclusion: The conference poster will introduce in details to HFSS (High Frequency Field Simulator) computer simulation environment, process of computer design optimization, parametric model design and calculation related to EPR signal optimization.

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***In-vivo* radiation dosimetry using L band EPR - The measurement from volunteers in FUKUSHIMA Prefecture, Japan**

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Study Goal: Using in vivo EPR tooth dosimetry, to collect and analyze the data of EPR signals from volunteers who had potential exposures with radiation related to the nuclear power plant accident in FUKUSHIMA due to the Great East Japan Earthquake

Abstract: 35 volunteers were enrolled in this study. They were residents in Fukushima within 80km distance from the FUKUSHIMA Nuclear Power plant. These measurements were made using a clinical L-band EPR spectrometer which was originally developed in the EPR Center at Dartmouth. All measurements are performed using surface loop resonators that have been specifically designed for the upper incisor teeth. The EPR signals from the upper incisor of volunteers were detected. These signals include not only RIS but also background signals. The signals that were obtained were not significantly above those seen in volunteers who had not had radiation exposures. Therefore these results confirmed that the volunteers had not had a detectable radiation exposure.

Conclusion: We were able successfully to carry out, in vivo EPR measurements from human upper incisors in volunteers from Fukushima. There were no indications of radiation-induced signals above background.

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Calculation by the Monte-Carlo method of the conversion coefficients from dose absorbed in the fingernails to whole body dose at gamma irradiation

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Study Goal: Determine conversion coefficients from absorbed dose in nails to whole body dose in case of fallout radiation

Abstract: Absorbed doses to nails for standard homogeneous geometries of photon exposure in vacuum in the energy range of 0.05–3.5 MeV were obtained by Monte-Carlo calculations using MCNP4B code. The data was calculated for four mathematical models representing clenched and flat hand configurations in two detailization levels. The elemental composition and density of nails were determined using available data for composition of nail keratin.

The photon dose in fingernails dependences on the following factors were studied: geometry of irradiation; photon energy; finger position (from thumb to smallest finger); degree of mathematical model detailization; nail thickness; and hand configuration (flat and clenched).

Separate Monte-Carlo calculations were made to obtain the whole body doses for the same photon energy range and irradiation geometries using a standard mathematical (MIRD-type) computational model. Based on the calculated data, the conversion coefficients from dose in nails to whole body doses in case of radioactive fallout in different periods after nuclear explosion were assessed. Calculations with a slab phantom were made to find the optimal source parameters providing the conditions of electronic equilibrium in nails at irradiation in vacuum and in air.

Dose calculations in nails for the detailed hand models irradiated in air were made in the energy range of 0.05–3.5 MeV. The obtained data were used to assess the conversion coefficients from dose in nails to whole body doses for the energy spectra expected from fallout under conditions of electronic equilibrium in the .

Conclusion: The ratio of whole body dose to dose in nails at fallout radiation was calculated; it ranges from 1.0 to 1.4 depending on the configuration of irradiation.

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Three-dimensional thermal tomography as predictor for radiation-induced skin reactions

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Study Goal: To explore the potential use of thermal effusivity measured from 3D-thermal tomography for early detection of skin reactions in cancer patients undergoing radiation therapy and for population exposure monitoring following a radiation disaster.

Abstract: We have designed a three-dimensional thermal tomography imaging system (3DTT) for this study. The system consists of two high power (5000 W) photographic flash lamps with custom built infrared (IR) filters and a high performance IR camera (320 x 256 pixels resolution at 200 Hz frame rate). The system was calibrated with high emissivity materials and was optimized for our study. The 3DTT system produces three-dimensional effusivity images by 1) inducing a brief surface temperature change with flash lamps; 2) measuring the thermal response of the skin by taking a rapid set of images using the IR camera; and 3) calculating thermal effusivity-based cross-sectional images using a thermal diffusion model. Four to five week old female SKH-1 hairless mice were irradiated to different dose levels (20 Gy, 10 Gy, 5 Gy and 2 Gy) to the dorsal surface of the right hind thigh in a single fraction using a 1.0 cm Leipzig applicator with an Ir-192 radiation source. Images were obtained 30 minutes pre-irradiation and 0.5 hour, 1 hour, and daily post-irradiation. Circular regions of interest (ROI) with 1.0 cm diameter were drawn around the irradiated region on 100 slices of coronal cross-sectional images of approximately 20 μm thick. Mean and standard deviations of apparent effusivity in these ROIs were calculated for each time point. A baseline effusivity reading was also obtained from a plastic reference phantom placed next to the mice during every measurement. The relative effusivity was computed as ratios of effusivities from the mouse to that of the phantom. Each mouse's relative effusivity at different time points was normalized to that calculated from pre-irradiation images. The preliminary data showed 6-12% change in relative effusivity from 2-20 Gy radiation exposure as early as 30 minutes post-irradiation. Differences in effusivity were also observed several days post-irradiation.

Conclusion: Our preliminary data on skin effusivity changes following radiation exposure suggest application of 3DTT in early prediction of radiation exposure. More experiments are warranted to confirm these findings and to extend the study to human subjects.

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Application of Premature Chromosome Condensation (PCC) in mass casualty accidents: For rapid dose assessments following exposure to ionizing radiation

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Study Goal: The suitability of premature chromosome condensation assay using fusion method was examined for scenarios of mass casualties following exposure to X-rays at low and high dose levels immediately and at different post-exposure times

Abstract: In the case of mass casualties event it is essential that many individuals to be evaluated in a short period of time. Currently the gold standard technique is the dicentric assay. Due to the requirement for a culture time of at least 48 hours for stimulated lymphocytes, the assay takes at least 51 hours for sample preparation. The subsequent analysis effort for each case is 1-3 people per hour for the 50 cell triage mode for whole-body exposure. In case of high doses of exposure (>4 Gy), two important parameters of cell death and mitotic delay are playing an important role in reducing the capability of dicentric for a reliable dose estimation. We have standardized and validated the use of Premature Chromosome Condensation (PCC) technique for biological dosimetry in scenarios of mass casualties immediately and at different intervals after exposures to low and high doses of X-rays. A calibration curve is generated for doses of 1 up to 6 Gy and using peripheral blood lymphocytes from 10 donors. PCC was performed immediately (0 day) and after one and two days. The background frequency of PCC in different donors is found to be zero. The initial frequency (average) of induced PCC per cell per Gy of X-rays is estimated to be 4.5. This value is significantly higher (15-20 times) than those estimated for Dicentric in metaphases. Consequently, PCC assay is very valuable and suitable for a triage and analysis can be done just within three hours after receiving blood samples. In cases that PCC performed after one and two days, though the initial frequency of PCC is drastically (80%) reduced, but still a significant difference between exposed (to doses of 1, 4 and 6 Gy) and unexposed samples was evident. PCC applied to assess the effect of low doses (1-10 cGy). The shape of the dose response curve is found to be supra-linear, and linear at a dose range of 10 cGy to 6 Gy.

Conclusion: PCC assay has a great potential to be applied for scenarios of mass casualties to assess the effect of low and high doses of radiations of different qualities.

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A Self-hardening Carbonated Hydroxyapatite Cement as a Reference Material for EPR Biodosimetry Systems

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Study Goal: To develop a reference material for calibrating Electron Paramagnetic Resonance (EPR) retrospective biodosimetry systems. The material should have a uniform enamel-like composition and stable and reproducible EPR signal after gamma-ray irradiation.

Abstract: A self-hardening calcium phosphate cement was used to prepare carbonated hydroxyapatite (HAC) in the forms of cylinders and discs. The cement powder consisted of tetracalcium phosphate, dicalcium phosphate, and sodium bicarbonate to allow the HAC products to contain desired levels of carbonate. The cement liquid was 0.5 mol/L Na₂HPO₄ solution. Powder X-ray diffraction analysis showed that the hardened HAC specimens contained low crystalline HA. Fourier transfer infrared analysis revealed carbonate bands at 1413 cm⁻¹ and 1455 cm⁻¹ indicating that the HA contained type-b carbonate similar to the carbonate in apatitic biominerals. HAC samples with 3% carbonate produced a spectrally pure example of the enamel carbonate radical.

Conclusion: The results demonstrate the suitability of HAC cement materials for EPR biodosimetry. ADAF uniformly-manufactured, NIST dose-certified materials can be used for R&D, device manufacturing QC/QA, and on-site field calibrations at triage locations.

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