June 22, 2013 – June 28, 2013

Differential Sensitivity of Near-Infrared Spectroscopy to Oxygenation Changes in Different Vascular Compartments.

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Study Goal: It is not yet clear whether NIRS is equally sensitive to oxygenation changes of hemoglobin in all of the vascular compartments .We determined cerebral blood oxygenation changes during cerebral angiography using NIRS.

Abstract: We investigated 18 patients undergoing cerebral angiography. The subjects included three patients with cerebral infarction, three with cerebral aneurysms, six with cerebral hemorrhage, and six with brain tumors. The NIRS optodes were placed over the frontal lobe on the side where the contrast medium (8 ml) for cerebral angiography was injected into the internal carotid artery (ICA). After injection of the contrast medium into the ICA, concentrations of Oxy-hemoglobin (Hb), Deoxy-Hb, and Total-Hb in the vascular compartments decreased in all cases due to washing Hb out of the cerebral vessels by the contrast medium. However, there were differences in the Hb concentration changes among the vascular components. In the arterial phase, Oxy-Hb started to decrease first, and then Deoxy-Hb started to decrease. The largest reduction of Hb concentration was observed during the capillary phase, and the second largest reduction was observed during the venous phase; the reduction during the arterial phase was considerably smaller, and this was followed by a recovery towards the control level. However, Oxy-Hb and Deoxy-Hb did not reach the control level during the venous phase.

Conclusion: Our results indicate that NIRS is sensitive to Hb oxygenation changes in all compartments within the illuminated area, but it is more sensitive to those in capillaries and veins than in arteries.

June 22, 2013 – June 28, 2013

Increased endothelin receptor A signaling causes intrarenal tissue hypoxia in diabetes

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Study Goal: Diabetic kidneys develop tissue hypoxia, which induces nephropathy. Endothelin ETA-receptors mediate vasoconstriction and oxidative stress. We therefore tested the role of ETA-receptors for the diabetes-induced intrarenal tissue hypoxia.

Abstract: Diabetes was induced in Sprague-Dawley rats two weeks before experiments and compared to corresponding controls. Kidney function and oxygen metabolism were measured during baseline and after infusion of the selective ETA-receptor antagonist BQ123 into the renal artery (final concentration 8 mmol/l). ETA-receptor blockade in diabetic rats resulted in increased renal blood flow (28.6±0.1%; $P<0.05$), increased cortical and medullary oxygen tensions (12.5±0.1% and 18.4±0.1%, respectively; both $P<0.05$) and reduced glomerular filtration rate (-62±1%; $P<0.05$) without affecting arterial pressure. In controls, BQ123 increased renal blood flow (29.4±0.1%; $P<0.05$), but did not alter any of the other investigated parameters.

Conclusion: In conclusion, increased ETA-receptor signaling causes hypoxia in the diabetic kidney, which may be a mechanistic explanation for the development of diabetic nephropathy. Thus, this may be a novel target to reduce the incidence of nephropathy.

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June 22, 2013 – June 28, 2013

Normobaric Oxygen Therapy for Ischemic Stroke Pre-conditioning: effect on infarct volume and MMP-9 levels

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Study Goal: We studied the effects of a convenient duration of normobaric oxygen therapy (NBO) pre-exposure on infarct volume and on the levels of matrix metalloproteinases, which have been implicated in exercise pre-conditioning.

Abstract: Background: Animal and human studies have shown that normobaric oxygen therapy (NBO) applied shortly after ischemic onset reduces stroke volumes and functional deficits. Recent animal studies suggest that NBO (4 h daily for 6 days prior to stroke) induces pre-conditioning by upregulating anti-oxidant enzymes. We studied the effects of a shorter, more convenient duration of NBO pre-exposure on infarct volume and on the levels of matrix metalloproteinases, which have been implicated in exercise pre-conditioning.
Methods: Adult male Sprague-Dawley rats (n=6) were pre-conditioned by exposure to 100% oxygen without anesthesia, for 2 h daily for 7 days. Control rats (n=7) were not pre-conditioned. On day 7, rats were subjected to 90-minute filament occlusion of the right middle cerebral artery under laser Doppler flowmetry. Infarct volumes (TTC, 24 h), and plasma MMP-9 levels (pre-MCAO, and 90-min and 24-h post-MCAO using Gelatin zymography) were assessed by blinded co-investigators.
Results: During surgery (MCAO), there was no significant difference in physiological parameters between Controls and NBO preconditioned rats. LDF monitoring showed successful ischemia-reperfusion in all rats. There was no significant difference in infarct volumes (Control, 248.7±57.3 mm3 versus NBO pre-conditioning, 217.8 ±68.9 mm3, p=0.39). Plasma MMP-9 level was comparable pre-MCAO, and showed up-regulation after MCAO in Control and NBO pre-conditioned rats. However there was no significant difference between groups (control vs NBO; pre-MCAO, 18.9±2.7 % vs 16.7±3.7 %, p=0.36; 90 min, 40.0±8.0 % vs 45.4±12.5 %, p=0.36; 24 h, 34.9±7.4 % vs 29.5±4.8 %, p=0.16).

Conclusion: In this study, NBO did not significantly affect stroke volume or MMP-9. Further studies are warranted to determine whether alterations in the ischemic model, or the duration or time frame of NBO pre-conditioning, prove more effective.

June 22, 2013 – June 28, 2013

Monitoring of filter patency during carotid artery stenting using near-infrared spectroscopy with high time-resolution

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Study Goal: We aimed to evaluate the usefulness of a newly developed, near-infrared spectroscopy (NIRS) device for monitoring hemodynamic changes during carotid artery stenting (CAS), as a means to detect filter obstruction due to distal embolism.

Abstract: We evaluated 16 patients with internal carotid artery (ICA) stenosis during the CAS procedure, using a NIRS system that can monitor not only changes in oxygenation of hemoglobin (Hb), but also the fluctuation of oxyhemoglobin (oxy-Hb) synchronized with heartbeat.
The NIRS system detected a marked decrease of oxy-Hb and an increase of deoxyhemoglobin (deoxy-Hb) during ICA occlusion in patients without anterior cross circulation (ACC). Patients with ACC showed much smaller changes. The analysis of oxy-Hb fluctuation made it possible to detect occurrence of no-flow in the absence of Hb concentration changes. The amplitude of oxy-Hb fluctuation in the no/slow-flow group was significantly smaller than that in the normal-flow group.

Conclusion: Our results indicate that the present high time-resolution NIRS device, which can measure oxy-Hb fluctuation, is superior to conventional NIRS for detecting filter obstruction.

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June 22, 2013 – June 28, 2013

Effects of Ginkgo biloba extract on cerebral blood oxygenation in the prefrontal cortex measured by near infrared spectroscopy

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Study Goal: In order to clarify the mechanism of Ginkgo biloba (EGb) on cognitive function, we examined the effect of EGb on cerebral blood oxygenation in the prefrontal cortex (PFC) and performance during working tasks using near-infrared spectrometry (NIRS).

Abstract: First, we evaluated differences in behavioral performance of Sternberg working memory test (ST) and activation patterns of the PFC during ST between 15 young and 19 middle-aged healthy women. Then, we examined the effect of EGb (120 mg/day for 6 weeks) on ST performance and PFC activation pattern in the middle-aged group. The middle-age group exhibited a longer reaction time (RT) in ST than the young group and showed a different PFC activation pattern during ST, i.e., the middle-aged group showed bilateral activation while the young group showed right-dominant activation. In the middle-aged group, administration of EGb for 6 weeks shortened the RT of ST and changed the PFC activation pattern to right-dominant, like that in the young group.

Conclusion: The PFC plays a role in the mechanism of the cognitive function-enhancing EGb. EGb might improve working memory function in middle-aged individuals by counteracting the occurrence of aging-related hemispheric asymmetry reduction.

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June 22, 2013 – June 28, 2013

Changes of cerebral blood oxygenation induced by active standing test in children with POTS and NMS

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Study Goal: To clarify the pathophysiology of POTS and NMS, we investigated changes in cerebral blood flow and oxygenation during active standing test.

Abstract: We studied 31 children (15 boys and 16 girls ; mean age, 14.0 ±1.7 years) who presented with OD at Department of Pediatrics and Child Health, Nihon University School of Medicine between 2009 and 2011. OD was diagnosed using the Japanese clinical guidelines for juvenile orthostatic dysregulation. After the resting period for 10 min in the supine position, patients were asked to stand up actively by themselves and keep upright for 10 min. 19 patients were divided into POTS, and 12 were NMS. Cerebral blood flow and cerebral oxygenation were each measured using Transcranial Doppler sonography (TCD)(COMPANION Ⅲ, RIKO TRADING CO., LTD.) and near-infrared spectroscopy (NIRS) (TRS20; time resolved spectroscopy, Hamamatsu Photonicus).

Conclusion: POTS showed significant decrease of oxy-Hb and resistance index(RI), suggesting transient ischemia but maintainable cerebral autoregulation. In NMS, decrease of oxy-Hb and increase of RI, suggesting ischemia and impairment of autoregulation.

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June 22, 2013 – June 28, 2013

Assessment of the Sensitivity of Cerebral Autoregulation Modeling to the Quality of Data Recordings

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Study Goal: Model based data interpretation can provide unique insights into cerebral wellbeing. To consider the sensitivity of models to their input data we investigate the use of different preprocessing techniques and assess their effects on model output.

Abstract: We have previously shown that it is possible to use a modeling based approach to predict cerebral autoregulation using near infrared spectroscopy measurements which can be acquired noninvasively and continuously at the bedside. However, if we are to use such an approach in a clinical setting we must ensure that the input data is of sufficient quality to provide a reliable model output. To assess the accuracy of model based predictions of cerebral autoregulation we propose the use of several preprocessing techniques, e.g. filtering, artifact rejection. By using a combination of techniques we aim to improve the quality of the input data and hence the viability of the model as a clinical data interpretation tool in real time at the bedside.

Conclusion: By comparing the output of our model with a variety of preprocessing of the input data, we can evaluate the sensitivity of the model to the data under consideration. This allows us to set requirements for the input data and ensure accurate modeling.

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June 22, 2013 – June 28, 2013

Slow wave analysis of cerebral haemodynamics and metabolism following subarachnoid haemorrhage

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Study Goal: Cerebral ischaemia is a major cause of poor outcome following subarachnoid haemorrhage (SAH). This study aims to investigate two key mechanisms: dysfunctional neurovascular coupling (NC) and pressure autoregulation (AR).

Abstract: Slow waves in near infrared spectroscopy (NIRS) derived oxyhaemoglobin, deoxyhaemoglobin and cytochrome c oxidase concentrations ([HbO2], [HHb] and [oxCCO], respectively) might describe NC and AR. 8 patients with poor grade SAH were investigated using broadband NIRS, brain tissue oxygen monitoring and transcranial Doppler ultrasonography. Wavelet analysis of coherence and phase difference revealed pressure-passive oscillations of NIRS signals and other neuromonitoring, highlighting impaired autoregulation. Distinct oscillations (0.1–0.03Hz) of NIRS demonstrated opposite oscillations of [HbO2] and [oxCCO] (-2.6rad) - consistent with ischaemia.

Conclusion: Impairment of NC and AR is measurable non-invasively through slow wave analysis of NIRS signals. [oxCCO] contributes crucial metabolic information and might be used clinically to identify the underlying cause of brain ischaemia.

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June 22, 2013 – June 28, 2013

Active standing test, autonomic function, near-infrared spectroscopy, orthostatic dysregulation, postural tachycardia syndrome

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Study Goal: To investigate variability autonomic function related with Oxy-Hb changes induced by active standing in children with postural tachycardia syndrome

Abstract: Near-infrared spectroscopy (Pocket NIRS DuoTM, Hamamatsu Photonics, Shizuoka, Japan) is enable to recognition brain condition based on the result of some factors such as oxygenated hemoglobin (Oxy-Hb). We have been trying to estimate status of the autonomic function in children with orthostatic dysregulation since July 2012 in Nihon University Itabashi hospital in Tokyo, Japan. We enroll consecutive 23 children diagnosed as postural tachycardia syndrome (POTS), which is subtype of orthostatic dysregulation with an age of 7 to 16 year-old. All of them are right hander. We can perform active standing test to diagnose POTS. POTS is defined as following in this study; 1. heart rate indicates more than 115 bpm during active standing after supine position, 2. elevation of heart rate results more than 21 bpm during active standing compared with that during supine position. We compared Oxy-Hb changes in the right and left frontal cortices during active standing. Oxy-Hb resulted in a downward response in the frontal cortices during active standing. The results of Oxy-Hb changes gave asymmetry with findings of significantly larger fall in the left frontal cortices rather than that in right.

Conclusion: Oxy-Hb changes almost show asymmetry which includes larger fall in the left frontal cortices compared with that in the right one during active standing in Children with impaired cerebral autoregulation diagnosed as POTS.

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June 22, 2013 – June 28, 2013

Effect of blood in the cerebrospinal fluid on the accuracy of cerebral oxygenation measured by near infrared spectroscopy

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Study Goal: In this work, we used computer simulations to examine the effect of haemoglobin in the cerebrospinal fluid following intracranial haemhorrage on the accuracy of cerebral oxygenation measured by near infrared spectroscopy.

Abstract: Near infrared Spectroscopy (NIRS) is a powerful optical technique used to obtain information about the cerebral oxygen saturation. Due to the strong scattering within tissue, it is necessary to accurately model the transport of light in order to quantify the depth sensitivity of NIRS measurements. The cerebrospinal fluid (CSF) is a clear layer of fluid that surrounds and supports the brain. It has previously been shown that accounting for the cerebrospinal fluid in the head models has a profound effect on the predicted propagation of NIR light and the sensitivity profile of NIRS measurements. Furthermore, it is known that CSF may become discolored by a number of contaminants in disease, including but not limited to haemoglobin, the principal NIR chromophore. A simulated MRI image was first segmented into a four-layer model of the head and a 3D mesh was then generated. Typical values for the optical properties of the head tissues were used in the simulations, which were based on the diffusion equation to model the light transport within the head and allowed generation of sensitivity profiles for any source/detector combination. The presence of haemoglobin in the CSF was then simulated by increasing the absorption coefficient of the CSF in line with haemoglobin concentrations found in lumbar puncture following intracranial haemhorrage. The sensitivity of NIRS to the brain tissue was quantified in terms of the partial pathlength of the NIR light within the brain layer as a proportion of the total mean pathlength. This was measured by changing the absorption coefficient of each layer of the model by a small amount, and recording the change in intensity predicted at the detector. This gives the partial pathlength of the light within the layer, and the sum of these values across all layers gives the total mean pathlength. This approach allows the sensitivity to each layer to be quantified.

Conclusion: Blood in the CSF led to a marked decrease in the sensitivity of NIRS to absorption changes in the brain tissue layer, and may have a significant effect on the validity of NIRS for measuring cerebral oxygenation in patients.

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Effects of enriched environment on hippocampal neuronal cell death and neurogenesis in rat global ischemia

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Study Goal: Enriched environments have been reported to show neuroprotective effects. Here, we evaluated the effect of an enriched environment prior to cerebral ischemia on neuronal cell death and neurogenesis in rats.

Abstract: Male SD rats were housed under standard conditions (SC) or in an enriched environment (EE), then subjected to global ischemia. The Y-maze test was used to evaluate behavior before and after ischemia. At 7 days after ischemia, we evaluated hippocampal neuronal cell death with Fluoro-Jade B staining and neurogenesis with BrdU staining. Phosphorylated cAMP response element-binding protein (phospho-CREB) was assessed with immunohistochemistry. The EE rats showed a significant decrease of cell death compared with the SC. There was no difference in neurogenesis between SC and EE. The EE rats showed a significant increase of performance compared with SC rats. Phospho-CREB-positive cells were significantly increased in EE compared with SC rats.

Conclusion: EE suppressed hippocampal cell death due to global ischemia, and decrease of cognitive function after ischemia was ameliorated in EE rats compared with SC rats. The CREB pathway may play an important role in protection of cognitive ability.

June 22, 2013 – June 28, 2013

R24 Regulates ROS Production and Angiogenesis

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Study Goal: To improve the activity of flavonoid, our team designed and synthesized R24. We evaluated the effect of R24 on cancer cell proliferation, reactive oxygen species (ROS) production, and angiogenesis.

Abstract: Flavonoid and its derivatives, common ingredients in the human diet, reportedly have anticancer effects and therapeutic benefits. Although many strategies have been developed to improve the activity of flavonoid, its bioavailability remains a challenge. Thus, we examined the effect of R24 [3, 6-bis (2-oxiranylmethoxy)-9H-xanthen-9-one], a flavonoid derivative synthesized by our team, on tumor cells. The antiproliferative effect of R24 was measured by MTT assay in several cancer cell lines, including A549 (lung cancer), AsPC-1 (pancreatic cancer), HCT-116 (colorectal cancer), and PC-3 (prostate cancer). R24 exhibited an antiproliferative effect with an IC50 of 3.44 μM, 3.59 μM, 1.22 μM, and 11.83 μM, respectively, each cell line. Cell cycle analysis and Annexin-V/propidium iodide (PI) staining showed that R24 induced cancer cell apoptosis.

ROS play an important role in a variety of biological functions and pathological processes. Studies show that excessive ROS accumulation can induce apoptosis. To explore this apoptotic mechanism, we also investigated the effect of R24 on ROS production. R24 regulated intracellular ROS production in a dose-dependent manner. CM-H2DCFDA staining indicated that intracellular ROS production increased as the R24 dose increased. Furthermore, a chicken embryo chorioallantoic membrane assay showed that R24 disrupted neovascular formation. Fewer dendrites were captured and overall dendritic length was shorter in the R24-treated chicken embryos, as compared to the controls.

Conclusion: We found that R24 exhibited a potent antiproliferative effect, induced apoptosis through ROS production, and disrupted vascular formation. Further studies are warranted to examine its therapeutic potential for malignancies.

June 22, 2013 – June 28, 2013

Skin temperature in lower hind limb subjected to distal vein arterialization in rats

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Abstract: Vascular surgery for distal vein arterialization, DVA, has been adopted clinically as a strategy for saving arteriosclerotic lower limbs from amputation. To gain more detailed information on DVA, the present study investigated the procedure in hind limbs of rats under isoflurane anesthesia.

Since successful DVA requires destruction of venous valves, a coronary angioplasty catheter guide wire was used to destroy valves either solely in the femoral vein or in both femoral and popliteal veins.

The femoral artery was then anastomosed to the femoral vein with sutures under binocular microsopic control. Changes in the distribution of skin blood flow in the hind limbs were studied with a thermal camera. Skin temperature increased in the thigh and knee after femoral venous valve destruction, but hyperthermia was observed in the distal leg and foot only when the valves in the popliteal vein were also disrupted. These results showed that increased arterial blood flow could be established by DVA surgery in both the proximal and distal regions of the hind limbs.

June 22, 2013 – June 28, 2013

Acousto-optic tissue phantom imaging

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Study Goal: An acousto-optic (AO) imaging system has been developed which exploits both diffuse light and focused ultrasound for tissue phantom imaging, resulting in a better spatial resolution than the diffuse optical imaging (DOI) system.

Abstract: DOI has been performed in the neonatal brain, adult cortex and breast to obtain tissue oxygenation maps. Most DOI systems are based on launching and counting photons (time resolved) at multiple locations surrounding the measurement site. An AO imaging system exploits the interaction between ultrasound (US) and light, and requires fewer light source-detector (SD) pairs. We have developed an AO imaging system consisting of a coherent laser, a single photon counter, an autocorrelator and a 1 MHz ultrasound transducer. It has been used to image a tissue phantom embedded with an absorber. The AO data have been processed by an image reconstruction algorithm based on the correlation diffusion equation. The reconstructed absorption map shows the location and shape of the embedded object.

Conclusion: The AO imaging system allows a better spatial resolution of the tissue oxygenation map. It has the potential to be developed into a tissue imaging system, e.g., for the breast, in the future.

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Cellular-scale imaging of glucose transfer in cerebral microvasculature with fluorescent glucose analogue in anesthetized mouse cortex

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Study Goal: Chronic hypoxia increases uptake of glucose in the brain, suggesting the hypoxia- enhanced glucose transfer for energy usage. The present study examined in vivo imaging of glucose transfer in the cellular scale of mouse brain microvasculature.

Abstract: Fluorescent glucose analogue (2-NBDG, 2 mM, 0.2 mL) was intravenously injected (0.04 mL/min) in the anesthetized Tie2-GFP mice in which vascular endothelium expressed fluorescent proteins. Time lapse imaging was conducted on the cortical parenchyma with two-photon microscopy. The time-intensity curve of 2-NBDG was analyzed for vasculature and tissue compartments. We observed that 2-NBDG signal increased monotonically in the vasculature for a period of the injection, and rapidly declined following its cessation. In tissue compartments, however, a signal intensity change was small and slow relative to the vasculature. Spatiotemporal analysis of 2-NBDG intensity over the cross-sections of the vessels further showed distinct change of the 2-NBDG intensity at the boundary of the vessel wall (endothelium), which indicates a regulation site of tissue glucose influx.

Conclusion: Intravenously-injected fluorescent glucose analogue was slowly transferred into parenchymal tissue under normoxia condition of anesthetized mouse cortex, which could be regulated at the endothelium cells irrespective of the vessel types.

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Effects of brain temperature, energy metabolism and vasospasm on post-ischemic reperfusion injury in gerbil brain

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Study Goal: Acute ischemia induces sever reduction of brain energy level and results in histological damage. In the present study we investigated effects of brain temperature, energy metabolism and vasospasm on post-ischemic reperfusion injury in gerbil brain.

Abstract: Two phases of vasoconstriction with the possibilities that the immediate vasoconstriction likely contributed to transient improvement of cortical oxygen/energy metabolism, and the second extensive vasoconstriction was an index of tissue energy failure and imminent neuronal damage. Mild ischemic stress can induce improvement in oxygen metabolism during subsequent ischemia, which might be causally related to the phenomenon known as "ischemic tolerance," in which a protective effect toward ischemic/postischemic injury is induced by earlier mild ischemic pretreatment.

Conclusion: Mismatch recovery of regional cerebral blood flow and brain temperature during reperfusion after prolonged brain ischemia in gerbils initiates metabolic derangement in brain tissue, leading to the electrochemical dysfunction and mortality.

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June 22, 2013 – June 28, 2013

Multiparametric monitoring of rat cortex based on NADH fluorescence in different causes of hypoxia

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Study Goal: Our study is to prove the combination of NADH fluorescence and cerebral blood flow is sufficient to distinguish the four types of hypoxia death models, and to support NADH fluorescence as one of the sensitive indices to clinic diagnosis of hypoxia.

Abstract: Hypoxia is a common pathological condition appears among diseases in clinic. Compared to other organs, brain is the most active one in metabolic processes, and thus needs the highest amount of oxygen. When hypoxia happens, body will protect vital organs such as brain and heart by transporting oxygen prior to minor organs. Once basic oxygen demand cannot be reached, hypoxia of vital organs might cause death. Therefore, monitoring of cerebral hypoxia is an important measure in intensive care. On the other hand, cerebral hypoxia might be caused by various factors. Acute cerebral hypoxia happened for 4-6 minutes might lead to irreversible damage in the brain tissue and even make the brain death. Prompt and correct diagnosis of the cerebral hypoxia causes can help on the selection of treatment protocols, and plays a key role in clinic. According to the different stages of oxygen transport in body, hypoxia can be classified by the cause of the reduced brain oxygen: hypoxic, hypaemic, circulatory, and histogenous hypoxia. Current clinical hypoxia measurements utilize blood oxygen indices targeted to the global blood oxygenation properties of the first three stages, but the regional histogenous hypoxia is hard to be recognized. Diagnosis of cerebral hypoxia also relies on the above systemic parameters, as well as Electroencephalography (EEG) and brain CT.
As Dr. Britton Chance concluded in 1973, NADH can be considered as an oxygen indicator in mitochondria and tissues. Several trials have been proceeded focused on the hypoxia models applied in rat cortex in vivo since 1962. We have tried to establish the four different pathological models caused from the four hypoxia types, monitored the change of NADH fluorescence, cerebral blood flow and other parameters during the course beginning from induced hypoxia to death, and summarized the multiparametric patterns for specific types of hypoxia.

Conclusion: Our study indicated the combination of NADH fluorescence and cerebral blood flow is sufficient to distinguish the four types of hypoxia death models. This method can be also applied in monitoring other organs.

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June 22, 2013 – June 28, 2013

Cortical depth-dependent morphology of cerebral microvasculature revealed with automated image analysis of two-photon microscopic data.

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Study Goal: Cerebral microvasculature consists of 3-dimensionally-organized networks which can be visualized with two-photon microscopy in vivo.This study was aimed to characterize 3D morphology of cerebral microvasculature captured with two-photon microscopy.

Abstract: 3D microvascular images were obtained with two-photon microscopy in living mouse cortex. The images were reconstructed off-line, and the vascular networks were manually separated into five vascular segments; surface artery and vein, penetrating artery and vein, and other parenchyma micro-vessels. Diameters and lengths were then measured in each vessel segment with custom-written software by applying either variable or constant intensity thresholds for defining the edge of the vessel lumen across the different cortical depths. We observed that both parenchyma artery and vein had variable cross-section diameters across cortical depths. An accuracy of the quantification was further evaluated with tissue-mimic gelatin phantom that contained fluorescent micro-beads whose size was pre-determined.

Conclusion: The methods allow for quantification of large volume data of microvascular images, which will contribute to further understanding the energy demand-supply balances between cortical tissue cells and vessels in the functioning brains.

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June 22, 2013 – June 28, 2013

Skeletal muscle deoxygenation responses during treadmill exercise in children

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Study Goal: The purpose of this study was to examine muscle deoxygenation responses during graded treadmill exercise in prepubertal boys and young men in order to characterize the circulatory response in activating muscle in children.

Abstract: Nine prepubertal boys (9 ± 1 years) and nine young men (23 ± 2 years) performed graded treadmill exercise until exhaustion. Muscle oxygen saturation (SmO2) and blood volume (BV) were continuously monitored during exercise at the belly of the gastrocnemius medialis muscle (GM) by near infrared spectroscopy. While BV was significantly increased in the men with increased exercise intensity ($p < 0.05$), no significant increase in BV was observed in the boys. Even though SmO2 was significantly decreased at 40~100% of peak VO2 from rest in the men ($p < 0.05$), significant deoxygenation response was observed only at peak exercise in the boys.

Conclusion: Both BV and deoxygenation responses in activating muscle were minor in prepubertal boys, compared to young men. The blunted deoxygenation response in prepubertal boys may be caused by unenhanced diffusive oxygen transport.

Acknowledgments: We thank Masayuki Konishi and Hiroki Tabata (Waseda University, Japan) for their helpful technical assistance.

June 22, 2013 – June 28, 2013

Short-term Hypoxic Preconditioning Improved Survival Following Cardiac Arrest and Resuscitation

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Study Goal: We investigated the effect of short-term (3 days) and long-term (21 days) hypoxic preconditioning on recovery following cardiac arrest and resuscitation.

Abstract: Male adult rats were exposed to hypobaric hypoxia (380mmHg, equivalent to 10% oxygen normobaric) for 3 or 21 days before undergoing 12-minute cardiac arrest. The control rats were exposed to normoxia before cardiac arrest. Overall survival rates and hippocampal neuronal counts were determined at 4 days following cardiac arrest and resuscitation. Our data showed that the overall survival rate in the short-term hypoxic preconditioning groups was significantly improved compared to the controls (86%, 6/7 vs. 55%, 11/20); no obvious protection was observed in hippocampal neuronal preservation 4 days after resuscitation. However, the survival rate in the long-term hypoxic preconditioning group was decreased compared to that of the controls.

Conclusion: Hypoxic preconditioning provides protection after cardiac arrest and resuscitation more likely through increased accumulation of HIF-1 alpha and its target genes rather than through successful vascular adaption by hypoxia-induced angiogenesis.

June 22, 2013 – June 28, 2013

Real-Time, In Vivo Determination of Dynamic Changes in Lung and Heart Tissue Oxygen Levels Using Electron Paramagnetic Resonance (EPR) Spectroscopy

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Study Goal: The goal of the study was to determine if implantable resonators could be used for temporal oximetry measurements in lung and heart tissues in a closed-chest model.

Abstract: Implantable resonators with LiNc-BuO crystals were surgically placed in the lung and heart tissues of Fisher F-344 rats for temporal oximetry measurements. For comparison, bare LiNc-BuO crystals were also implanted in a separate group. EPR oximetry was performed on the rats 3 - 4 days post-implant using a modular L-band EPR spectrometer, and repeated 4 days later. During EPR scanning, the inhaled oxygen level was varied from 10 - 100% mixed with isoflurane using a specially-designed chamber. Tissue pO2 was determined using a standard calibration curve and the OxyScope curve-fitting program. Retention of the implantable resonators within the heart tissue was confirmed on Day 10 post-implant by fluoroscopy.

Conclusion: We have demonstrated the feasibility of conducting EPR oximetry measurements in the hearts and lungs using implantable resonators under variable oxygen exposure conditions to examine the tissue response.

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

Application-specific evaluation of oxygen measurement accuracy of EPR probes

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Study Goal: The study goal is to develop an algorithmic tool to quantify application-specific pO2 measurement accuracy of EPR oximetry probes.

Abstract: Development of EPR oximetry probes has been a subject of several studies; yet, a methodology to evaluate the probes in terms of their application-specific oxygen measurement accuracy has been absent from the literature. For a given probe, the accuracy of oxygen measurement depends on the spin density, anoxic linewidth, and oxygen sensitivity of the probe (defined as change in the linewidth per unit change in pO2). Individually, high spin density, narrow linewidth, and high oxygen sensitivity improve pO2 measurement accuracy. However, the last two quantities are interconnected; an increase in linewidth due to oxygen-induced broadening lowers SNR. Therefore, an optimal selection of an oximetry probe heavily depends on the application. In this work, we adopt a principled approach for determining the relative performance of different probes for different oxygen ranges. First, we define each probe in terms of spin density, anoxic linewidth, and oxygen sensitivity. Second, a statistical sensitivity analysis based on Cramer Rao Bound (CRB) is employed to compute the pO2 estimation error across a predefined oxygen range, Omin to Omax. The probe with the lowest CRB bound averaged across Omin to Omax is declared a winner. Simulation studies were conducted to compare three commonly used oximetry probes: LiPc, LiNc, and LiNc-BuO for two different oxygen ranges.

Conclusion: The preliminary results indicate that the proposed algorithmic tool can be used to identify oximetry probes that are optimal for the given oxygen range, and this application-specific probe selection can significantly reduce the acquisition time.

June 22, 2013 – June 28, 2013

Modulation of hypoxia with magnetic nanoparticle hyperthermia to augment therapeutic index

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Study Goal: To investigate the effect of a hypoxic tumor microenvironment on nanoparticle (NP) uptake and efficacy of magnetic nanoparticle hyperthermia (mNPH)

Abstract: The hypoxic microenvironment in solid tumors is known to cause resistance to most standard therapies such as radiation and chemotherapy. In this study, the effect of a hypoxic tumor microenvironment on NP uptake and efficacy of mNPH is investigated in a human breast cancer orthotopic xenograft model. Electron paramagnetic resonance (EPR) oximetry was used to assess pO2 in tumors in vivo. Magnetic NPH was found to increase tumor pO2 from 3.5 to 68.8 mmHg for up to 10 days. Tumors treated with mNPH to CEM60 showed growth delay. On TEM, NPs were localized intracellularly in multiple vesicles in the cytoplasm of cells within the tumor. With IHC, NPs did not colocalize with hypoxic areas within the tumor.

Conclusion: A hypoxic microenvironment decreased intracellular uptake of NP in vitro. Magnetic NPH increased tumor oxygenation in vivo and resulted in decreased growth of hypoxic tumors. Future studies will establish tumor pO2-guided multimodal therapies.

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June 22, 2013 – June 28, 2013

EPR, MRI and bioluminescence imaging to track metastatic cell migration

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University of Louvain

Study Goal: The purpose was to use ex vivo EPR to accurately quantify iron oxide-labeled cancer cells that colonize distant organs and to correlate with MRI and bioluminescence imaging (BLI).

Abstract: Cancer cell tracking consists in labeling cells with iron oxides (SPIO) in order to visualize these cells using MRI. Metastasis detection is one of the main application of cancer cell tracking. Luciferase-expresing cancer cell lines with different metastatic patterns (homing in lungs, liver or brain) were labeled with SPIO. MRI and BLI were performed after injection and the iron oxide content in organs was assessed using X-band EPR. We found that MRI was sensitive to detect SPIO-labeled cells in the brain after injection whereas we could not detect SPIO-labeled cells in the liver. We observed a correlation between iron oxide quantification results using EPR and MR images. EPR was shown to detect tumor circulating cells labeled with SPIO entrapped in distant organs shortly after injection. Both MRI and BLI confirmed that macrometastases developed in iron oxide-positive organs.

Conclusion: The complementary role of EPR in MRI cell tracking studies was demonstrated. EPR assess quantitatively the distribution of SPIO-labeled circulating cancer cells after injection. BLI and MRI allow the assessment of subsequent macrometastases.

June 22, 2013 – June 28, 2013

Development and qualification of Phosphonated Triarylmethyl Radical as Prob to assess the extracellular pH in tumors.

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Study Goal: The aim of the study was to develop and qualify a new pH sensitive spin probe for the assessment of pHe in different tumor models characterized by different glycolytic phenotypes

Abstract: We synthesized a phosphonated tetrathiatriarylmethyl radicals (trityl) probe, which is a water soluble spin pH probe exhibiting a very narrow EPR linewidth therefore providing a high signal-to-noise ratio. The titration of this trityl revealed that our probe has two pKa at 1.3 and 7.1, this latter being particularly interesting to study the changes and acidification in physiological media. In addition, we measured the distribution of this probe in a sample of cells by adding a broadening agent (Gd-DTPA, 50 mM) which remains exclusively extracellular and so broadens the EPR signal in the extracellular compartment. The entire EPR signal was broadened so the probe is exclusively extracellular. We also used low frequency EPR to measure the pH in tumors (FSaII, TLT models) and in the muscle.

Conclusion: These characterizations demonstrate the validity of the phosphonated trityl probe for measuring extracellular pH in tumors.

June 22, 2013 – June 28, 2013

Mild hypothermia targets exacerbated metabolism of cancer cells and increases tumor oxygenation

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Study Goal: Most invasive cancers are constituted by highly proliferative cells displaying exacerbated metabolism. This work aims the effect of a hypometabolic state induced by mild hypothermia on the tumor oxygenation.

Abstract: Targeting cancer metabolism is increasingly regarded as a potent strategy in cancer therapies. Here, we studied the effect of a hypometabolism, induced by mild hypothermia (32°C). Oxygen consumption rates were measured in vitro by EPR using a neutral nitroxide as oxygen probe and glycolysis activity was assessed by enzymatic dosages. Tumor oxygenation was measured in vivo by low frequency EPR. We observed a major decrease in cellular respiration, reflecting the decrease in oxidative phosphorylation activity, in different cancer cell lines treated 1 hour at 32°C. In vivo, EPR oximetry showed a progressive improvement of intratumoral PO2 over time (two-fold increase after 2 hours) when mice were treated at 32°C.

Conclusion: Metabolic slowdown by the use of mild hypothermia leads to a reduction in tumor hypoxia. Future works will look to a possible potentiation of radiation therapy.

Acknowledgments: This work was supported by a grant from the FNRS-Télévie

June 22, 2013 – June 28, 2013

Boron tracedrug design for neutron dynamic therapeutics for Gc protein as a serum protein-quality-control treatment.

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Study Goal: We challenge to develop "beyond chemical" protein-quality-control therapeutics for a process termed proteotoxicity, such as Alzheimer's disease, and cardiac disease, based on neutron dynamic therapy against Gc protein using a boron tracedrug.

Abstract: We here describe our solution for removal of the serum albumin superfamily-member, Gc protein, vitamin D-binding protein, or actin-binding protein, which by neutron dynamic therapy using boron tracedrugs for NDT against BSA as a model protein. Thus we examined, among our developed boron tracedrugs, a boron-containing curcuminoid derivative UTX-51, to destroy a freshly isolated human Gc protein dynamically under irradiated thermal neutron (absorbed dose 2.0 Gy at the present of 100 nmol B-10 for 45 min) to obtain a decreased intensity of band of Gc protein treated with UTX-51 and thermal neutron irradiation in their SDS-PAGE and their electrophoresis analysis.

Conclusion: We present for the first time that the boron tracedrug UTX-51 causes the NDT-based destructive damage of serum Gc protein to be useful therapeutics for serum protein-quality-control in both treatment and prevention of pathological conditions.

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June 22, 2013 – June 28, 2013

Variation of RIF-1 tumor pO2 in response to hyperoxygenation challenges accessed by repeated in vivo EPR oximetry

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Study Goal: The effect of hyperoxygenation with normorbaric oxygen (100% O2-NBO) or normobaric carbogen (95% O2 + 5% CO2-NBC) multiple or single administered on RIF-1 tumor pO2 is reported.

Abstract: Method: The temporal changes in the subcutaneous RIF-1 tumor pO2 were assessed by in vivo Electron Paramagnetic Resonance (EPR) oximetry in mice breathing 30% O2 and then NBO or NBC under different days.

Results: The tumors were hypoxic with a tissue pO2 of 6.2-8.3 mm Hg. NBO and NBC breathing significantly increased tumor pO2 on day 1 to day 5, with a maximum increase at 37.8±5.4 min and 30.2±4.9 min on day 1; 36.3±6.1 min and 33.6±6.5 min on day 5, respectively. However, the magnitude of increase in pO2 and percentages of pO2 changes from baseline declined significantly on day 10. NBC given only once on day 1, day 3, day 5 or day 10, respectively, the patterns of pO2 changes remain same as above.

Conclusion: EPR oximetry could be used to repeatedly monitor tumor pO2 during hypoxia modifying interventions over course of 10 days and potentially enhance efficacy by scheduling hypofractionated radiations at times of increases in tumor pO2.

Acknowledgments: This work was used the facilities of the EPR Center for Viable Systems.

June 22, 2013 – June 28, 2013

Renal cortical and medullary in vivo oxygenation with L-band EPR

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Study Goal: No method today can repetitively monitor regional renal oxygenation using minimally invasive procedures. We therefore evaluated implantable lithium phthalocyanine (LiPc) probes, displaying a correlation between EPR linewidth and oxygen availability.

Abstract: LiPc probes implanted in kidney cortex and medulla and EPR spectra acquired using a L-band scanner during inhalation of air (21% oxygen) or a mixture of air and nitrogen (10% oxygen). In order to separate the signals from the two probes, a 1G/cm gradient was applied and the signaled averaged from 40 consecutive sweeps. Peak-to-peak comparison of the EPR line was used to convert the signal to an approximate oxygen tension in MATLAB. Over time, L-band EPR did not demonstrate a difference in cortical and medullary oxygenation (57±5 mmHg and 57±6 mmHg respectively), while in the hypoxic state both signals increased even more (35±2 mmHg $p<0.05$ and 37±6 mmHg $p<0.05$) compared to 21% oxygen. At anoxia, both signals increased (18 mmHg and 20 mmHg).

Conclusion: In conclusion, this method can be used to repetitively monitor renal oxygenation and is well suited for reduced intrarenal oxygenation since this increases the signal intensity which facilitates the quantification and conversion of the EPR signal.

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June 22, 2013 – June 28, 2013

Oxygen guided hypofractionated radiotherapy: potential for clinical translation using EPR oximetry

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Study Goal: To enhance treatment outcome of hypofractionated radiotherapy by irradiating glioma at times of optimal tumor oxygenation (pO2 > 15 - 20 mm Hg) observed during radiotherapy and/or hyperoxia with carbogen inhalation.

Abstract: Malignant gliomas are hypoxic (pO2 < 10 - 15 mm Hg) and aggressive brain tumors with rapid infiltrative growth. Despite multimodality approach of surgical resection followed by chemoradiation, the prognosis remains poor with a median survival of 14 - 18 months. New treatment strategies and optimization of the existing protocols are urgently needed to improve the outcome. Given the significance of hypoxia in resistance to chemoradiation, it is necessary to repeatedly measure glioma pO2 in order to test various hypoxia targeted strategies and optimize treatment protocols.

We have focused on EPR oximetry using particulate probes such as lithium phthalocyanine (LiPc) for pO2 measurement at depths of less than 10 mm, and implantable oxygen sensors (ImOs) for pO2 measurement at depths of up to 100 mm from the surface. We summarize the results of the repeated assessment of tissue pO2 in orthotopic experimental (9L, C6, F98, RG2) and human xenograft (U251) glioma established in syngeneic animal models.

The orthotopic C6, F98 and U251 gliomas were hypoxic (pO2 < 10 -15 mm Hg), whereas 9L and RG2 gliomas were well oxygenated (pO2 ~ 30 – 35 mm Hg). The contralateral brain pO2 of the animals bearing glioma were in the range of 40 - 45 mm Hg. Carbogen breathing led to a significant increase in the glioma and contralateral brain pO2; however, this effect declined during five days of repeated experiments. Irradiation of F98 glioma with 9.3 Gy x 4 fractions in rats breathing carbogen resulted in a significant decrease in growth assessed by MRI compared to controls. The baseline pO2 of the U251 glioma and contralateral brain measured using ImOs were 18 and 55 mm Hg respectively. The carbogen inhalation resulted in a significant increase in glioma and

contralateral brain pO2, however the magnitude of increase in pO2 declined in experiments repeated for five consecutive days.

Conclusion: The outcome of hypofractionated radiotherapy can be significantly enhanced if irradiation is scheduled when the glioma is oxygenated. EPR oximetry will be useful to test interventions being developed to modulate hypoxia for therapeutic benefit.

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June 22, 2013 – June 28, 2013

Checkpoint inhibition sensitizes cells to gemcitabine and inhibits glioma growth: an EPR, MRI and immunohistochemistry study

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Study Goal: To develop an effective treatment strategy by combining an antimetabolite with a checkpoint inhibitor to inhibit glioma growth and potentially increase pO2

Abstract: Experimental and clinical studies have demonstrated a significant role of hypoxia in resistance to chemoradiation. Additionally, cell cycle arrest following treatment further compromises therapy facilitating tumor recovery. Treatment with the antimetabolite gemcitabine depletes deoxynucleotides leading to arrest of tumor cells in S phase. The stalled replication forks are stabilized by Chk1, and therefore inhibition of Chk1 should lead to replication fork collapse and lethal DNA double strand breaks.
We have investigated the efficacy of gemcitabine with a Chk1 inhibitor (MK-8776) on the growth and pO2 of orthotopic U251 glioma xenograft. The orthotopic gliomas were established in immune suppressed mice and implantable oxygen sensors were used to repeatedly assess pO2 by EPR oximetry. The pO2 of the U251 glioma prior to any treatment was 13 - 19 mmHg, while the pO2 of the contralateral brain was 48 - 63 mmHg. No significant change in the glioma pO2 of the control or MK-8776 (20 mg/kg) group was observed. However, there was a significant increase in the glioma pO2 on day 4 following treatment with 150 mg/kg gemcitabine on day 1. A significant increase in pO2 was also observed on days 3 and 4 after treatment with gemcitabine + MK-8776. The pO2 of the contralateral brain did not change in either the control or treatment groups. A significant inhibition of glioma growth was observed in the gemcitabine + MK-8776 group compared to control. Ex vivo analysis of the glioma samples at 24 h after gemcitabine treatment showed that approximately 80% of the Ki67-positive tumor cells (i.e. proliferating cells) accumulated in S/G2 phase. The treatment with MK-8776 at 18 h post gemcitabine led to an increase in DNA double strand breaks (Y-H2AX). Arrest in S/G2 phase was also observed with a gemcitabine dose of 30 - 100 mg/kg indicating that lower doses can be used in combination with radiotherapy to minimize toxicity as observed in clinical trials.

Conclusion: A significant increase in U251 glioma pO2 and decrease in glioma growth was observed on treatment with gemcitabine + MK-8776. Ex vivo analysis confirms cell cycle arrest and DNA damage following treatment with gemcitabine + MK-8776.

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June 22, 2013 – June 28, 2013

Quantitative dose measurements for proton radiotherapy based on EPR/alanine dosimetry system

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Study Goal: The goal of the study was to develop alanine-based dosimetry system measuring and archiving the fraction and the total doses delivered to the tumour volume during proton and ion radiotherapy, as a routine dosimetry method for Quality Assurance (QA).

Abstract: The study has been divided in some sub-tasks.

The first step of the project was modification of Bruker ESP300 spectromer to perform quantitative dose measurements. It was equipped with a new cooling system which improved its performance and stability. A digital data acquisition module was also developed to facilitate quantitative analysis of the EPR spectra. Then some commercial available and some in-house manufactured alanine dosimeters were investigated in terms of their mechanical stability, radiosensitivity, linearity of dose response etc. in different radiation fields (proton beam, gamma radiation field) to choose the best one for the study purpose.

Finally the pellet-shaped detectors (4.8mm in diameter, 3.0mm height), consisting of 96% alanine and 4% of binder produced by Gamma Service have been chosen. These detectors were then tested in 60MeV proton beam at Institute of Nuclear Physic in Krakow (IFJ PAN) at the facility for eye treatment. It was found that they can be use for proton beam dosimetry as well as for patient dosimetry. In the first case they will be irradiated in the proton beam prior to treating the patient. For patient dosimetry they cannot be placed directly in the part of the beam which irradiates the target volum, as it would interfere with the planned dose distribution in the tumour volume, so it was decided to place them on the inner side of the individual patient collimator. In this location they register the entrance dose, which is correlated with the dose delivered to the isocenter of the facility, where the center of a tumor is placed. The correlation between the dose registered by alanine dosimeters and the dose delivered to the isocenter depends on the SOBP range and modulation and on the facility configuration.

The concentration of stable radicals generated in alanine by radiation, which is the linear function of absorbed dose, is then measured with EPR. The dose read-out do not “erase” the dosimetric signal from the detector.

Conclusion: Alanine pellet-shaped detector, placed on the reverse side of the individual patient collimator, can be successfully use as a form of in vivo dosimeter measuring fraction dose during proton eye radiotherapy in cumulative fashion.

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June 22, 2013 – June 28, 2013

Effects of rapamycin on energy metabolism, oxygenation, and angiogenesis in SCC tumor

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Study Goal: The purpose of this study is to elucidate effects of rapamycin on tumor energy metabolism and microenvironments (oxygenation and angiogenesis) non-invasively by using EPRI and MRI.

Abstract: The mammalian target of rapamycin (mTOR) is a protein kinase that is centrally involved in the control of cancer cell metabolism, growth, and proliferation, and therefore has become an attractive therapeutic target. Inhibition of mTOR by rapamycin causes diverse effects on tumor microenvironment, such as angiogenesis and oxygenation, as well as tumor energy metabolism. Such changes can serve as useful markers to assess tumor response to therapy. Recently, a novel methodology has been developed that can provide quantitative 3D maps of tissue pO2 and blood volume images in living animals by using electron paramagnetic resonance imaging (EPRI) and magnetic resonance imaging (MRI). Also recent development of 13C-MRI with hyperpolarized 13C-labeled compounds enabled us to monitor metabolic changes in tumors non-invasively. In this study, we investigated effects of rapamycin on tumor oxygenation, angiogenesis, and pyruvate metabolism of living mice by using EPRI and MRI. Squamous cell carcinoma (SCC) cells (5×105 cells) were implanted s.c. into a right hind leg of female C3H mice. Treatment with rapamycin and EPRI/MRI measurements were started after 8 days from implantation of the SCC tumor. EPRI measurements were done with a 300 MHz pulsed EPRI system, and MRI measurements were done with 4.7 T and 7 T scanners controlled with ParaVision 5.1 (Bruker Bio-Spin MRI GmbH). Tumor growth in rapamycin treated mice was delayed dependent on rapamycin dose. Blood volume in tumor region significantly decreased even after 2 days from beginning of the rapamycin treatment. Rapamycin treated mice showed higher tumor pO2 values after 2 and 4 days rapamycin treatment compared with non-treated mice. The median pO2 in the rapamycin treated group showed a small decrease after 6 days treatments, but it was at the same level as that before treatment. Hyperpolarized 13C-MRI revealed that pyruvate metabolism to lactate was significantly dropped by 2 days rapamycin treatments.

Conclusion: Hyperpolarized 13C-MRI can be used to monitor mTOR signaling inhibition by rapamycin, and EPRI/MRI co-imaging can provide non-invasive evidence of rapamycin-induced vascular renormalization and resultant transient increase in tumor oxygenation.

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June 22, 2013 – June 28, 2013

DNP Characterization of the Phosphonated Trityl Probe: Potential for *In Vivo* pH & pO_2 Mapping by PEDRI

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Study Goal: To evaluate applicability of the new dual function trityl probe for concurrent in vivo pH and oxygen tissue mapping using variable frequency proton-electron double-resonance imaging by characterization of its pH and oxygen DNP spectral response.

Abstract: New mono substituted trityl probe p_1TAM has 120 mG doublet hyperfine splitting in the DNP spectrum for its protonated and deprotonated states. At pH close to the pKa (6.95), the spectrum is represented by the superposition of the two variable intensity lines being a pH sensitive marker. Additionally, the linewidth of the probe is pO_2 sensitive giving the potential for concurrent pH & pO_2 mapping. The DNP characterization of the samples with variable pH and oxygen concentration values performed at different EPR irradiation powers. Optimum parameters for PEDRI were chosen. A new dual frequency resonator for the NMR/EPR was built. Less sample heating compared with the previous resonators observed.

Conclusion: The DNP probe characterization and in vitro PEDRI studies provide backgrounds for the *in vivo* application of the new dual function trityl probe. This will allow for concurrent pH & pO_2 mapping of living tissues independently of the probe distribution

Acknowledgments: This work is supported by NIH EB014542 grant.

June 22, 2013 – June 28, 2013

Methamphetamine(METH)- induced attenuation of brain tissue oxygen as measured by EPR oximetry

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Study Goal: This study investigates localized striatal tissue pO2 changes following administration of METH in a mouse model. While METH neurotoxicity has been studied for several decades, there are no in vivo studies on METH-induced changes in brain pO2 levels.

Abstract: EPR oximetry is a useful method for non-invasive, sensitive and repetitive measurements of pO2 in the brain in vivo. Experiments were devoted to establishing whether EPR oximetry is capable of measuring changes in brain tissue pO2 were they to occur after mice were administered METH. We demonstrate that after a single injection of METH, a significant decrease in striatal pO2 was observed and continued exposure further attenuates striatal pO2. More importantly, pO2 does not recover fully to control levels even 24 hrs after exposure to a single dose of METH and continual exposure exacerbates the condition. We also show a reduction in CBF suggesting that decreased brain pO2 may be associated with decreased CBF indicating an ischemic condition.

Conclusion: This study demonstrates that exposure to METH may lead to hypoxic insult, which could promote ROS formation and oxidative stress, thus playing a significant role in the mechanism of METH-induced brain injury.

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June 22, 2013 – June 28, 2013

Comparative EPR spin-trapping study with new cyclic nitrone spin traps on RAW 264.7 macrophages

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Study Goal: We compared the superoxide detection abilities of 6 spin traps of the cyclic nitrone series in the presence of PMA-stimulated RAW macrophages as a model. We analyzed the results based on previous in vitro studies of adducts' metabolic stability.

Abstract: Reactive oxygen species (ROS) including superoxide anion and hydrogen peroxide (H2O2) have a diverse array of physiological and pathological effects within living cells depending on the extent, timing, and location of their production. For measurement of ROS production in cells, methods based on dihydro compounds such as 2',7'-dichlorodihydrofluorescein that fluoresce on oxidation are used widely because of their sensitivity and simplicity. However, such probes react with a variety of cellular oxidants including nitric oxide, peroxynitrite, and hypochloride in addition to H2O2. The EPR spin trap technique, on the contrary, is specific to ROS radicals (such as superoxide).
In this study, a comparison of the spin trapping efficiency of six different new cyclic nitrone spin traps, i.e. DMPO, BMPO, DEPMPO, DIPPMPO, Mito-DIPPMPO, and CD-DIPPMPO, was performed on RAW 264.7 macrophages stimulated with PMA.
The results are discussed taking into accounts the results on metabolic stability of the adducts obtained in a previous in vitro study with subcellular fractions.

Conclusion: From these results, it was concluded that CD-DIPPMPO is superior to all other tested spin traps for detection of superoxide in living RAW 264.7 macrophages.

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

Development of four-channel surface coil array for 300 MHz pulsed EPR imaging

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National Cancer Institute, National Institutes of Health

Study Goal: The goal of this study is to demonstrate the feasibility of the surface coil array for 300 MH to extend the region of visualization.

Abstract: Surface coil resonator is limited in its ability to visualize a broader region of the sample. To make the region of visualization with the surface coil broader, we developed a surface coil array for 300 MHz pulsed EPR imaging. When multiple coils are placed in close proximity, mutual coupling of the coils causes a shift in the resonance frequency. To suppress the influence of mutual coupling, we shifted the resonance frequencies of individual resonators by shorting a part of resonator circuit, except for one excited resonator. The EPR image data acquisition is sequentially performed by each coil. The final EPR image is obtained by combining those images. We will report the results of EPR imaging of phantoms and a tumor implanted into mouse.

Conclusion: The surface coil array was found to be successful in extending the region of visualization in 300 MHz pulsed EPR imaging. We were able to suppress the influence of mutual coupling between the next coils, on the resonance frequencies.

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June 22, 2013 – June 28, 2013

Microacidity and Release Studies on Thermally-Induced Chitosan-Based Gelling Systems by Electron Paramagnetic Resonance Spectroscopy

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Study Goal: The aim of the present study was to gain a deeper insight into thermogelling chitosan/ β-Glycerol phosphate systems. The internal pH value as well as the release from these systems were supposed to be investigated with the help of EPR spectroscopy.

Abstract: Although *in situ* gelling Chitosan/ β-Glycerol phosphate formulations have been characterized quite well, the knowledge about their microstructure is still limited. We conducted EPR experiments in order to monitor the pH value inside these systems during sol-gel transition. For different compositions pH values in the physiologically tolerated range of 6.62 to 6.78 were found, which were not affected by the gelation process. Moreover, a model protein, Insulin, was incorporated into the gels to examine the release profile. While *in vitro* there was a controlled release for two weeks, which was influenced by the composition, *in vivo* studies showed an initial burst followed by a sustained release after completion of the gelation process.

Conclusion: By the use of EPR, microacidity was measured. It was independent from the Chitosan/ β-Glycerol phosphate ratio and remained unchanged during the gelation process. Furthermore, a controlled release was found, that is affected by the gel's composition.

June 22, 2013 – June 28, 2013

Synthesis of RGD and pRGD-Conjugated Triarylmethyl Radicals as Spin Label For EPRI

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Study Goal: The goal of this work is to synthesize a triarylmethyl radical coupled to a carrier moiety in order to confer a tissue selectivity to the spin label.

Abstract: Triarylmethyl (TAM) radicals of type para-carboxyltetrathiatriarylmethyl are favourite spin probes used in EPRI. In this work, we describe a straightforward synthesis of two TAM radicals covalently bond to a small peptide containing the RGD sequence and a peptidomimetic (pRGD) of this sequence. Since the RGD sequence is recognized by some integrin receptors, our TAM radicals should exhibit a selectivity to tissues where integrins are expressed.

Conclusion: A straightforward synthesis of RGD and pRGD-conjugated TAM radicals is described. These two new spin probes are stable and should possess a selectivity to tissue expressing integrin receptors.

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June 22, 2013 – June 28, 2013

Simultaneous imaging of an enantiomer pair by EPR using isotopic nitrogen labeling

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Study Goal: The goal of this study is to demonstrate CW-EPR imaging of an enantiomer pair using isotopic nitrogen labeling.

Abstract: This presentation reports the simultaneous imaging of chiral nitroxyl radicals using EPR. Chiral nitroxyl radicals could be simultaneously visualized with the labeling of isotopic nitrogen. Chiral nitroxyl radicals, hydroxylmethyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl, were visualized using the method of simultaneous EPR imaging, which refers to the visualization of two kinds of molecules with unpaired electrons in a single image scan. EPR spectra of a racemic mixture of chiral nitroxyl radicals and those of the respective R and S configurations confirmed labeling by isotopic nitrogen. Simultaneous imaging of solutions of chiral nitroxyl radicals was performed in three-dimensional EPR image acquisition manner.

Conclusion: The experimental results suggest that 14N- and 15N-labeled radicals can be used as labels for chiral molecules. If biologically important enantiomers could be visualized, this would represent a new approach to molecular imaging in biological studies.

Acknowledgements: This work was supported by a grant from the Japan Society for the Promotion of Science (NEXT Program Grant LR002 to H.H.).

June 22, 2013 – June 28, 2013

Skeletal muscular tissue and glioma tumor oxygenation following carbogen inhalation in rats by EPR Oximetry using single probe implantable oxygen sensors

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Study Goal: The feasibility of pO2 measurements by EPR oximetry using a single probe implantable oxygen sensor (ImOS) was tested in skeletal muscle tissue and ectopic 9L tumors in rats

Abstract: Single-probe ImOSs (50 mm length) were fabricated using nickel based alloy wire with lithium phthalocyanine (LiPc) loaded in the sensor loop and were coated with AF 2400 Teflon solution. These ImOSs then were implanted into skeletal muscle in the thigh and subcutaneous 9L gliomas. The dynamic changes in the tissue and tumor pO2 were assessed using EPR oximetry at baseline, during growth, and during repeated hyperoxygenation with normobaric carbogen (NBC) breathing.

The mean skeletal muscle pO2 of normal rats was stable (51.5±5.1 mmHg on day 7, N=8) and increased significantly during NBC inhalation (131.2±19.9 and 93.3±23.8 mmHg on day 14 and day 84, respectively) in experiments repeated every 1 or 4 weeks for 12 weeks. The pO2 of 9L glioma at 300 mm3 was hypoxic (15.0±7.4 on day 1, N=6). A significant increase in the glioma pO2 (36.8.6±28.8 mmHg, 49.0±29.7 mmHg, 54.6.6±29.5 mmHg, 43.2±25.4 mmHg and 36.0±13.9 mmHg on day 3, day 5, day 7, day 9 and day 14, respectively) was observed during NBC inhalation in experiments repeated for 14 days.

Conclusion: The ability to repeatedly assess skeletal muscle and tumor pO2 is likely to play a vital role in understanding the dynamics of tissue pO2 during NBC inhalation and other therapies designed to modulate tumor hypoxia.

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

June 22, 2013 – June 28, 2013

Uniform Spinning Sampling Gradient EPR Imaging

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Study Goal: The goal of this study was to improve the quality and speed of electron paramagnetic resonance imaging (EPRI) acquisition by combining a uniform sampling distribution with the spinning gradient (SG) acquisition.

Abstract: The historical Equilinear Spinning Sampling (ESS, Eq 1) distribution has a well-known bias of acquiring too many projections near one axis, which wastes approximately 30% of the acquisition time. We propose a new method, called Uniform Spinning Sampling (USS, Eq 2), which provides nearly uniform distribution of projections in a SG acquisition via an arcsin transformation of the elevation angle. A resolution assessment phantom was constructed from three 100 µl capillary tubes each containing 5 mg lithium phthalocyanine (LiPc). USS images (Fig 1d-f) had fewer radial streaking artifacts and lower mean-squared error (MSE) than ESS images (a-c) when compared to a high SNR fast-scan reference images (g-i).

Conclusion: The SG acquisition has the potential to produce a nearly unlimited number of projections very quickly, and the USG distribution makes each projection contribute equally to the image reconstruction.

June 22, 2013 – June 28, 2013

Magnetic Field Effect in the Reaction of Recombination of Nitric Oxide and Superoxide Radicals

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Study Goal: Main goal of the study is to investigate the influence of magnetic field on reaction of recombination of NO and O2.-, and describe observed magnetic field effect in terms of of the magnetic-field-enhanced recombination rate constant of NO and O2.-.

Abstract: As a source of the radical pair, we used SIN-1 that in air saturated buffer solution decomposes to form NO and O2.-. The radicals recombine with diffusion-controlled rate to produce a diamagnetic product, peroxynitrite ONOO-. The yield of peroxinitrite is quantified by measuring optical absorption of rhodamine (RH) formed in the reaction between ONOO- and dihydrorhodamine-123. The effect of magnetic field was monitored by comparing the efficiency of peroxynitrite production in exposed and identical control samples. Magnetic fields in the range of 4.5-18 T were used. Simulation of obtained results was based on the theoretical formalism of Gorelik at al. Chem. Phys. 2000.

Conclusion: In a magnetic field, the yield of RH is shown to increase linearly, the MFE reaching a value of 5.5% at 18 T. Magnetic properties of the radicals have been estimated and a theoretical simulation based on the g-mechanism has been proposed.

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June 22, 2013 – June 28, 2013

Structure and reactivity of nAChR ligand enantiomers labeled by 14N- or 15N-nitroxyl radicals for simultaneous EPR imaging

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Study Goal: The goal of study is to specify structure and reactivity of newly synthesized chemicals, which are (R)- or (S)-nAChR ligands labeled by 14N or 15N nitroxyl radicals and used as spin probes for simultaneous EPR imaging.

Abstract: (R)- or (S)-nAChR ligands, which indicate different binding constant with a receptor in vivo, were labeled by 14N- or 15N-nitroxyl radicals, respectively. To confirm structure of the new samples, FT-IR spectra of 14N-labeled (R)-nAChR ligand and 15N-labled (S)-nAChR ligand were measured and assigned by comparison between the IR spectra and results of DFT calculation. To investigate reactivity of the 14N-labeled (R)-nAChR ligand or 15N-labled (S)-nAChR ligand with ascorbic acid, which exist in vivo and quench radicals, changing of EPR spectra was observed after ascorbic acid was added to 14N-labeled (R)-nAChR ligand or 15N-labled (S)-nAChR ligand. By analyzing time variation of the EPR spectra, rate constants of the reaction were determined.

Conclusion: Vibrational structure of newly synthesized chiral nAChR ligands was confirmed. Some peaks derived from vibration related with isotpically-labeled nitrogen were shifted slightly. Reactivity of the radicals with ascorbic acid was same.

Acknowledgments: The authors are grateful to Prof. Dr. Hirotada Fujii and Dr. Miho Emoto (Sapporo Medical University) for helpful discussion. This work was supported by the NEXT Program (LR002 to H.H.) form JSPS.

June 22, 2013 – June 28, 2013

Optimization of Fitting Spectral Modeling Parameterization for Tooth Dosimetry

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Study Goal: The goal of this study was to improve the precision of the dose estimate output by the conventional linear least squares fitting routine by using multiple stages and model restriction.

Abstract: The impact of coherent noise and microphonic distortion on L-Band EPR spectra are major sources of error when using non-linear least square regression modeling. The size and shape of the radiation induced signal (RIS) are difficult to discern with these quasi-periodic anomalies which compromise the accuracy of parameter estimates. Strategic restriction of the spectral parameters can minimize the effect of these unwanted signals in a number of ways provided their values are known precisely. While the native signal (NAT) is defined and has been modeled, it does not provide greater accuracy with our current instrumentation's signal to noise ratio. In a general sense, using a two component model and varying restrictions on the parameters provides a means to examine the effect of each independently. Multiple combinations are explored for thoroughness with mixed results. Using a staged or weighted fitting approach by limiting the window and fitting a perdeuterated tempo (PDT) reference signal prior to the RIS, provides more accurate parameter estimates by serving as the first phase in a multi-stage fitting routine. This procedure selectively distinguishes between the expected signal and distortions by fitting components separately. These methods are founded in an effort to increase the precision of dose estimation for tooth dosimetry.

Conclusion: Multiple methods for optimizing fitting procedures were tested on various data sets. These routines were outlined and demonstrated mixed results with respect to overall dosimetric precision for tooth dosimetry.

Acknowledgments: EPR Center

June 22, 2013 – June 28, 2013

EPR Instrumentation and Resonator Advances for Aqueous Samples at W-band (94 GHz)

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Study Goal: This poster highlights a number of technical advances and enabling technology at W-band (94 GHz) for advanced quantitative EPR experiments using aqueous samples.

Abstract: First, an oversized WR-28 waveguide section with interfacing hyperbolic cosine tapers has been incorporated into the present W-band system [Mett et al., Rev Sci Instrum, Vol 82, 2011] replacing a 902 mm WR-10 waveguide probe connecting the bridge to the sample resonator. The WR-10 waveguide has a typical loss of 2.60 dB/m. In contrast, the oversized waveguide section exhibits a measured loss of 0.52 dB/m. By virtue of its lower power loss, the new section improves system Noise Figure by a measured 2.1 dB, and increases the available pump power by 2.1 dB.

Two resonators are featured: A new Seven-Loop–Six-Gap Loop Gap Resonator has been built and tested for improved quantitative EPR results. This LGR implements enhanced uniformity end sections [Mett et al., Appl Magn Reson Vol 31, 2007], long-slot iris coupling [Mett et al., Appl Magn Reson Vol. 35, 2009], and uniform 100 kHz field modulation slot optimization. With the end sections in place, the LGR rf field over the region of interest is calculated to be 67% uniform in H2, whereas the resonator without the end sections is 30% uniform. The rf magnetic field squared (H2) is directly proportional to EPR signal intensity. Additionally, for continuous-wave experiments, the 100 kHz applied field modulation is calculated to be 98% uniform over the region of interest. A TEU02 resonator has been designed and fabricated at W-band for the in situ sample irradiation by light. Using a uniform field resonator with a region-of-interest of 4 mm yields a factor of 2 over a traditional TE102 resonator. Additionally, using a uniform field (81% H2 uniformity) and a flat cell allows for very high (85%) sample irradiation and uniform excitation.

A fixed translational source at S-band (2 GHz) has been replaced with a Agilent N8241A Arbitrary Waveform Generators (AWG). They translate the rapid sweep waveform outputs nominally at 300 MHz or frequency modulation from the AWGs up to W-band frequencies.

Conclusion: Experimental data comparing conventional CW 100 kHz field modulation and CW Frequency Modulation is presented. Finally, frequency swept [Hyde et al., J Magn Reson Vol 205, 2010] power saturation experiments using uniform field resonators are shown.

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June 22, 2013 – June 28, 2013

Robust EPR-dosimetry standard - positioning of PDT, superabsorber polymers, importance of line width control

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Study Goal: Tooth dosimetry for the precise dose determination need a side reference, that is PDT solution in water. Due to good separation from tooth signal this PDT line gives good reference to field position of the tooth signal, thus allowing locked line shape fitting of the signal from irradiated tooth.

Abstract: Due to the design of the loop the PDT sample at present conditions is placed few millimeters away from the tooth. In this case the inherent small field inhomogeneity of the L-band magnet may lead to small shifts in the relative position of the lines of tooth signal and PDT signal thus spoiling the fit accuracy. In addition to this problem the possibility of use the PDT not just as field standard but also as an amplitude standard depends upon the position of the PDT standard with respect to the loop of the resonator: at upper positioning bending of the loop changes the signal from PDT from 1.52 V to 1.88 V (24%), while the same bending for positioning of the PDT sample under the wavequide holding the loop and capacitor lead to change of PDT signal from 1.73 to 1.81 V (5%).

One of the problems of the instability of the PDT amplitude standard due to the change of the position of possible trapped inside air bubble. In order to prevent such change of the position of the air bubble the idea of use of superabsorbing polymer was proposed. The attempts were made to put the water solution of PDT into the superabsorbing polymer, so the whole solution is hold within the same place. Despite the mobility of PDT in superabsorbing polymer is the same as for the water, the chemical stability of it turned out to be nonsatisfactory.

Conclusion: One more implication of the PDT use as an amplitude standard is connected with the necessity to control the line width of PDT line. Because of the computer fitting program random error in the PDT line width there are fluctuations of the tails of PDT line in the position of tooth, what in the case of too strong amplitude of PDT creates visible discrepancies in tooth signal amplitude (even in the absence of the signal from tooth the slope in the middle between two lines, being incorrectly fitted by the program due to bad choice of line width may lead to something that is signal like, thus increasing error).

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

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

Wei Tan

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June 22, 2013 – June 28, 2013

Comparison of dose-response of incisor teeth measured by EPR Dosimetry *in vitro* and *in vivo*

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Study Goal: To evaluate how anatomical or demographic variations impact *in vivo* tooth EPR measurements, studies conducted using extracted teeth and mouth models can systematically vary anatomical features and can provide results more readily and comprehensibly. However, it is important to first validate that spectra obtained *in vivo* and *in vitro* are comparable.

Abstract: We conducted two interrelated studies, using a volunteer who agreed to be measured prior to having an upper central incisor removed and again during the period of time before the gap would be permanently replaced with an implant. (1) Study 1 consisted of comparing several measurements on the same tooth made *in vivo* prior to extraction and *in vitro* afterwards. The *in vitro* measurements were made on the isolated tooth and the tooth embedded in a mouth model with lossy materials to simulate the anatomical features and lossy conditions of the mouth. Study 1 provides direct comparisons between the living tooth and extracted tooth--both when the extracted tooth is isolated and when it is embedded in a mouth model. Results (all at 0 gray [Gy]) were similar in all 3 comparisons. (2) In Study 2, the same volunteer agreed to be measured with several isolated teeth (including the volunteer's extracted tooth). Isolated teeth were measured *in vitro* and *in vivo,* i.e., placed in the gap of the volunteer's mouth via the use of a 'flipper' (a removable denture for a single tooth). This provided a direct comparison of the EPR spectra of an irradiated tooth measured *in vitro* and *in vivo*. The volunteer's tooth and the 5 additional teeth needed to fit into the dentition gap; roots were removed from all 6 teeth and each tooth was secured in a flipper, using the original flipper mold prepared by the volunteer's dentist. *In vivo* measurements were carried out on teeth irradiated *ex vivo* at doses of 0, 1, 2, 4, 6 or 10 Gy. To allow all in vivo measurements to occur on one day, 2 teeth (including the volunteer's original) were measured at 0 and again several hours later at 10 Gy; 4 were pre-irradiated to 1, 2, 4 and 6 and measured once. All teeth were also measured *in vitro* in isolated molds and in the mouth model at each dose. The time required for acquisition was about 5 minutes per tooth. Results show that dose-response curves obtained from *in vitro* mouth model are similar to that from *in vivo in situ* volunteer's mouth.

Conclusion: These data validate that measurements made *in vitro* of teeth in isolated molds and in mouth models can be used in clinical studies to investigate the impact of anatomical and demographic variations on *in vivo* EPR tooth dosimetry

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June 22, 2013 – June 28, 2013

MRI of tooth enamel

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Study Goal: To measure enamel thickness in irradiated teeth to determine whether it is a source of variation in EPR signal for tooth biodosimetry.

Abstract: *Introduction*
Conventional MRI sequences have an echo time (TE) that is too long to capture signal from regions of ultra-short T2 relaxation, such as teeth. Recently, zero echo time (ZTE) and ultrashort echo time (UTE-3D) sequences have been developed to rapidly capture these fast decaying signals to enable tooth and other musculoskeletal imaging. We image irradiated teeth and non-irradiated controls to measure enamel thickness and correlate it to EPR signal. The UTE-3D sequence has a non-selective RF excitation followed by image acquisition in the presence of imaging gradients. In the ZTE sequence, imaging gradients are switched on during the RF excitation and remain on for the acquisition period.

Material and Methods
Incisors were immersed in water and MRI performed. UTE-3D images were acquired with TR=8 ms, TE=20 us, one signal average and acquisition time of 18 min. ZTE images were acquired with TR=8 ms, TE=20 us, four signal averages and acquisition time of 7 min. 12 human teeth were scanned with both UTE-3D and ZTE. The images from both sequences show clear boundaries between the water and the enamel and between the dentin and the enamel. Therefore the volume of the enamel of the tooth can be accurately measured.

Conclusion: MRI resulted in clear delineation of tooth enamel, with much higher contrast-to-noise for ZTE images than with UTE images.

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June 22, 2013 – June 28, 2013

Radiation Biodosimetry Based on Amino Acid Decarboxylation

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Study Goal: We propose to develop a precise, rapid retrospective biodosimetric assay based upon the observation that ionizing radiation results in decarboxylation of glutamic and aspartic acid residues in a yield that is directly related to absorbed dose.

Abstract: Ionizing radiation (IR)-induced damage to amino acids (AA) with carboxylic acid side-chains, specifically glutamic acid (Glu) and aspartic acid (Asp), results in decarboxylation of the AA by elimination of CO2. We have recently demonstrated that the yield of the decarboxylated product (dAA) is linear over a broad range and is directly related to the absorbed dose. We are developing two retrospective biodosimetry assays based on quantification of dAA in in several easily obtainable blood proteins (e.g. human serum albumin, hemoglobin).

The assays, which will be developed in parallel, are (1) LC-MS/MS quantification of dAA in purified protein hydrolysates and proteolytic fragments and (2) Immunological detection of dAA in isolated proteins and proteolytic fragments. The primary focus will be evaluating the feasibility of each method for use as a reliable field assay for rapid triage and response in the event of mass radiation exposure.

The two proposed techniques are complementary. The LC-MS/MS quantification technique will provide precise measurements of product yield, and will allow us to analyze factors that affect the yield and recovery of dAA, such as biological environment and purification techniques. In contrast, detection of dAA by immunological methods will require the successful production of antiserum monospecific for peptides containing dAA residues. However, an immunological method could provide faster throughput, involve less sample preparation, and may ultimately be incorporated into an ELISA assay commonly used in diagnostic kits. These kits would not require sophisticated instrumentation and would be especially applicable to triage in the field. Both techniques benefit from the fact that the dAA analyte is produced immediately following irradiation and is stable. Generation of dAA is purely a physical process and does not rely on any biological response, eliminating the need for a waiting period before subjects can be reliably tested after exposure.

Conclusion: We determined the yield of dAA to be sufficient to detect it at clinically relevant IR doses, and have recently made advances in LCMS-MS sensitivity. We have begun analysis of protolytic digests of HSA to identify targets for immunological detection.

Acknowledgments: The authors would like to acknowledge funding from the National Institute of Allergy and Infectious Diseases (U19-AI091173).

June 22, 2013 – June 28, 2013

Investigation of EPR / CT image co-registration to extend the field of application of retrospective bone dosimetry

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Study Goal: EPR / CT coregistration imaging was used to reconstruct ex vivo the relative dose distribution in human bones taking into account the bone density

Abstract: Various tools are currently available for dose reconstruction in individuals after accidental exposure to ionizing radiation. EPR has proved its usefulness for retrospective dosimetry. Previous work in our lab (Med. Phys 2009, 36, 4223-4229) demonstrated the possibility to reconstruct the dose gradient in hydroxyapatite samples. When applying the method to bone samples, we found that the bone density distribution was a possible source of confound. In the present study, we co-register EPRI and CT in order to depict the dose more accurately, taking into account the influence of the bone density.

Conclusion: Co-registration of EPR and CT images is a key strategy to perform absolute dosimetry in irradiated heterogenous samples such as soft bone tissues

June 22, 2013 – June 28, 2013

Characterization of the EPR signal induced by radiations in dimethacrylate-based resins used for tooth restorations

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Study Goal: To characterise the EPR signal induced by radiations in dimethacrylate-based resins used for tooth restorations.

Abstract: We previously demonstrated that freshly polymerized resins used for tooth restorations gave a strong EPR signal, interfering with the RIS in enamel. This signal was negligible after a 6 months period.
We investigated here whether Ionising Radiations could also induce a signal in the resin itself. Old commercial resins and experimental compositions were irradiated at different doses. RI signal and decay kinetics were measured.

A RI signal was observed for high doses (100 Gy) in 14 out of 19 commercial resins tested. This signal was however almost undetectable only 1 week after irradiation. At low doses (≤10 Gy), there was no signal, except in 3 out of 19 resins.

In experimental resins, the signal was higher but decay kinetics were faster.

Conclusion: Below 10 Gy, the EPR signal induced by radiations in dimethacrylate-based resins was generally negligible, when detectable, and should not interfere by itself with the RIS arising from the enamel.

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June 22, 2013 – June 28, 2013

Influence of tooth restorations using commercial composites on the EPR dosimetric signal in tooth enamel

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Study Goal: To evaluate the influence of dental resins on the measurement of the dosimetric signal in teeth.

Abstract: Teeth frequently bear restoration made with resins, which can give a strong EPR signal, possibly causing interferences with the dosimetric signal from the enamel.
19 commercial resins were included in this study. Experimental compositions of monomers (Bis-GMA, TEGDMA, etc) were also evaluated. The occurrence, magnitude and decay kinetics of an EPR signal was investigated in each resin, and compared to a dosimetric signal in enamel (3Gy).
An EPR signal was observed in all samples tested. It ranged from 16 to 105 times the dosimetric signal. Decay kinetics generally followed a bi-exponential model. In most resins, the signal recorded six months after the polymerization was low enough not to interfere significantly with the dosimetric signal.

Conclusion: EPR signal arising from composites used for tooth restoration should not affect by itself the dosimetric signal, in most cases. Attention should be paid to recent restorations on teeth (less than 6 months), specially for doses lower than 3Gy.

June 22, 2013 – June 28, 2013

Improved *ex vivo* EPR nail biodosimetry: Removing the mechanically-induced signal from nail clippings through mercaptoethanol treatment

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Study Goal: We have found that mercaptoethanol (ME) treatment removes the interfering mechanically-induced signal (MIS) from nail clippings, leading to improved measurement of the radiation-induced signal (RIS).

Abstract: The ex vivo EPR biodosimetry method relies on the measurement of the RIS in nail clippings to estimate exposure dose. However, when the nail is harvested, an MIS that interferes with the direct measurement of the RIS is produced. In an attempt to remove the MIS from nail clippings, we explored the use of ME to preferentially eliminate the radicals underlying the MIS, which are on the surface of the cut edge, while preserving the more bulk-distributed radicals underlying the RIS. This treatment takes advantage of the thiol function on ME to react with and eliminate MIS radicals. By adjusting the water content of ME (0, 5, or 10%), we controlled the wetting properties of the ME-H2O mixture, thereby limiting the penetration of the mixture into the dense keratin fibers of the nail matrix and minimizing the reaction of ME with RIS radicals. The use of neat ME on nail clippings with soak times of 60 minutes reduced the MIS by approximately 60 - 70%, with smaller changes in the RIS intensity within this same timeframe. Adding water to the ME (5% or 10% v:v) reduced the MIS by 70 – 80% but required shorter soak times of 40 minutes. The presence of a background signal limited the extent of the reduction to 20% of the initial signal of the non-RIS in irradiated clipped nails. Although ME with 10% water reduced the RIS by 25% at 30-min soak times to 55% at 120-min soak times, there was sufficient RIS (75%) remaining after the 40-min exposure period that 90% ME treatment could be used to reduce the MIS while still retaining sufficient RIS for dose estimation.

Conclusion: The 90% ME treatment method not only is a viable approach for removing the MIS while retaining an acceptable level of RIS with minimal variability in the remaining signal but also can be easily integrated into ex vivo EPR nail biodosimetry methods.

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June 22, 2013 – June 28, 2013

Radiation induced signal dependence on measurement position and enamel thickness for human incisor teeth

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Study Goal: We assessed the effect of geometrical position of the surface loop on the anterior surface of the tooth and the dependence on enamel thickness to optimize measurements procedures, using L band EPR spectrometry with specifically designed surface loop (4mm in diameter) resonators and upper central incisor teeth.

Abstract: 10 intact human upper incisor teeth were used. The samples were irradiated with various doses (1, 5, 10, 20Gy) by X-rays. We made measurements with the surface loop placed at three positions over the tooth surface (upper part, middle part, lower part of the tooth crown). Following the EPR measurements, the enamel thickness of each tooth was measured by micro CT. The radiation induced signal was modeled as α×irradiation dose＋β, For the upper part ; α=0.063, β=0.211, the middle part ; α=0.056, β=0.235, the lower part ; α=0.049, β=0.0217. One-way analysis of variance showed that α was significantly dependent on measurement position (p=0.005). Multiple comparisons showed that there is a significantly difference between the upper part of the teeth and the lower part of the teeth (p=0.003).

Conclusion: Measurement position significantly affected the magnitude of the radiation-induced EPR signal. The upper and middle parts of the incisor are more optimal for the measurements using the surface type resonator.

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June 22, 2013 – June 28, 2013

Low-temperature longitudinally detected W-band ESR to study RIS in nail clippings

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Study Goal: The goal of the study is to measure and characterize the dose response of the residual RIS signal present following water treatment to remove the MIS signal, using low temperature longitudinally-detected W-band ESR.

Abstract: Electron spin resonance (or ESR) measurements of radiation-induced signals (RIS) in teeth and bone have been proposed as a viable physically-based dosimetric technique for retrospective measurements of radiation exposure in the event of accidental release of radiation or terrorist attack. Ex vivo ESR measurements of fingernail clippings for radiation dosimetry are complicated by the presence of so-called mechanically-induced signals (MIS) during clipping of the fingernails. While the MIS signal can be eliminated by washing the samples with distilled water, this procedure also reduces the RIS signal. However it has been observed that a fraction of the radiation induced signal persists after the water treatment, and remains stable even under further treatment with water.

Our goal is to measure and characterize the dose response of this residual ESR signal using longitudinally-detected CW and pulsed W-band ESR experiments. In this presentation we describe the design and setup of our home-built low-temperature W-band spectrometer, and outline our plans to characterize the changes in the RIS signal as a result of the water treatment used to remove the MIS signal.

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June 22, 2013 – June 28, 2013

The Impact of Demographic Variables on the RIS Dose Response of Ex Vivo Irradiated Nails

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Study Goal: To assess the potential influence of demographic variables on the radiation-induced signal (RIS) dose response of ex vivo irradiated nails, as measured through EPR biodosimetry.

Abstract: To achieve accurate and precise dose estimates based on the RIS in clipped nails, we have developed not only nail handling procedures to control the variability of the individual spectral components in the nail sample preparation steps following clipping but also spectral fitting models that use different combinations of the mechanically induced signal (MIS)broad, MISsinglet, MISdoublet, RIS, and background signals. We tested a large dataset with 90 samples from 15 donors to validate these sample handling and spectral analysis methods. A linear dose response in the RIS was obtained at doses of 0-6 Gy. Good agreement was found between the actual RIS and the estimated RIS computed from spectral analysis. However, receiver operating characteristic (ROC) analysis of the dose response data indicated a higher than desired false positive rate at the 2-Gy dose threshold point. This result was likely due to variations attributable to demographic variables, such as age, sex, or race. For example, well-known racial differences in the alpha and beta keratin ratios and sulfur content in nails may influence the stability of radical centers in the nail, thereby affecting the RIS dose response. Sex-related and age-related changes in the pliability of nails may also play a role in the RIS dose response. These and other demographic variables are currently being studied in the newest phase of our ex vivo nail dosimetry method and a summary of recent results will be presented.

Conclusion: Variances in the RIS dose-response that are due to demographic variables need to be identified and appropriate calibrations put in place to achieve accurate dose estimates based on the RIS.

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June 22, 2013 – June 28, 2013

New step in sample preparation procedure for ex-vivo EPR fingernail dosimetry

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Study Goal: The ultimate goal of this pilot project is to establish a procedure of sample preparation which will assure the dose measurements with accuracy and precision that are required for triage applications.

Abstract: It is well-known that radiation exposure induces radicals in finger- and toenails and that these radicals can be measured by electron paramagnetic resonance (EPR). The radiation-induced EPR signal persists for many hours and it is proportional to dose. If necessary, the signal can be preserved for months if not years by storage at low temperatures (on ice or in freezer). However, there is a known problem: cutting fingernails generates the formation of additional EPR signals, which makes it difficult to do dose measurements. A sample preparation procedure that allows the drastic reduction of the impact from mechanical stress in fingernails on dose measurement was developed. It consists of a brief (10-15 min) soaking of fingernails in water and following drying time.

Two sets of fingernails were collected from the same group of volunteers (10-15) with an approximately 2-3 week interval to allow fingernail re-growth. At the first collection volunteers soaked their hands in warm water (~40 ºC) for 10-15 min prior to fingernail cut to reduce stress at cutting (precutting soak). At the second collection they didn't have this procedure. Samples were weighed and measured with EPR. The fingernails were kept in a freezer between EPR measurements. The mechanically-induced EPR signals (MIS) were compared in samples with and without precutting soak.
At the next stages of the study, samples will be irradiated to 5 Gy, which will represent some accidental exposure. EPR spectra will be recorded and compared again; and then dose response curves will be obtained and used to reconstruct the 5 Gy "accidental" dose.

Conclusion: The soaking the fingernails before cutting was found to be useful means to reduce the intensity of MIS and improve accuracy of the dose reconstruction technique with fingernails.

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June 22, 2013 – June 28, 2013

Development and Optimization of L-Band Surface-Coil Resonator for EPR Tooth Dosimetry

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Study Goal: The goal of this work was to develop a surface-coil resonator for EPR-based tooth dosimetry, which has high sensitivity and stability. We optimized the sensitivity of the resonator using electromagnetic field simulator HFSS (ANSYS).

Abstract: We improved a previously reported surface-coil resonator for EPR tooth dosimetry to optimize coil size and parallel transmission line structure using HFSS. The surface coil was formed by 1.5 mm thick silver wire and 8.0 mm in outer diameter from the aspect of magnetic field distribution and mechanical robustness. The chip capacitor inserted between the surface coil and a quarter-wavelength parallel transmission line for impedance matching. The frequency of the resonator resonance was 1,151 MHz. The quality factor and the RF magnetic field generation efficiency were 366 and 384 μT/W^1/2, respectively. Measurements consisting of six sets of EPR spectra on a 30 Gy irradiated tooth using this resonator achieved a SEM of 0.44 Gy.

Conclusion: We optimized the resonator by using its numerical model and achieved high sensitivity and stability. This combination of modeling and then careful construction facilitates the development of improved resonators.

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June 22, 2013 – June 28, 2013

Spectral Deconvolution Methods for Separating the Radiation-Induced Signal from an Interfering Signal in the Ex Vivo EPR Fingernail Biodosimetry Method

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Study Goal: This abstract describes the key factors required for the successful spectral modeling and estimation of the radiation-induced signal (RIS) in nail clippings in the ex vivo EPR biodosimetry method and summarizes our validation studies.

Abstract: To achieve accurate and precise dose estimates based on the RIS in clipped nails, variability of the individual spectral components in the nail sample preparation steps following clipping must be controlled. We found that temperature, humidity, water content, and O2 level are the 4 key factors that should be regulated in order to stabilize the RIS and the mechanically-induced signal (MIS). By placing the nail samples into mylar bags containing both desiccants and oxygen scavengers immediately after clipping, we achieved sufficient control of the RIS and MIS to use regression-based spectral fitting models to estimate the RIS. In addition, we assessed various 2-component and 3-component spectral fitting models that made use of different combinations of the MISbroad, MISsinglet, MISdoublet, RIS, and background signals to determine which model provided the most reliable estimates of the RIS. A large dataset with 90 samples from 15 donors was tested to validate our sample handling and spectral analysis methods. Although we obtained a linear dose response in the RIS at doses of 0-6 Gy for all spectral models, the differences between the actual RIS and the estimated RIS computed from spectral analyses differed between the models. These differences were due to (1) remaining instabilities of the MISdoublet despite the special sample handling method, and (2) variability in the background signals of nail samples. Spectral models that allowed for individual fitting of the MISdoublet improved the precision of RIS estimates. The remaining background signal-induced variations in the RIS estimates can be addressed by understanding the demographic differences in nail samples.

Conclusion: By controlling the nail sample environment following clipping and using well-defined spectral components in a regression-based spectral fitting model, we achieved good estimates of the RIS in irradiated nail clippings.

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June 22, 2013 – June 28, 2013

The Use of Multifrequency EPR in the Analysis of MIS and RIS Spectral Components

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Study Goal: To examine multifrequency EPR methods used to characterize the spectral components in the radiation-induced, mechanically-induced, and background signals in clipped nail samples.

Abstract: The radiation-induced signal (RIS) in clipped nails, which is the basis of ex vivo EPR nail biodosimetry, cannot be directly measured due to the presence of an overlapping mechanically-induced signal (MIS). To help differentiate between the MIS, RIS, and background signals, we conducted multifrequency EPR analysis of the spectral components in nonirradiated and irradiated nails. Two of the MIS spectral components, the MISbroad and MISdoublet, were easily observed at X-band (9.5 GHz) due to their distinct spectral features outside of the g = 2.002-2.004 region. However, the MISsinglet, RIS, and background signals were indistinguishable at X-band within this region. Analysis of the MISsinglet and RIS at 4 other frequencies (1.8, 3.2, 34, and 95 GHz) showed that these 2 spectral components were again indistinguishable. Spectral simulations of the MISsinglet and RIS and possible radical assignments will be presented. A second RIS (deep trap) is currently being analyzed at 34 and 95 GHz to verify the findings of Romanyukha et al. (Radiat Meas 2011;46:888), which show a shift to a lower g-value for the weaker RIS at 34 GHz from the larger unstable RIS. Analysis of the background signal at 34 and 95 GHz showed that it could not be distinguished from the MISsinglet or RIS.

Saturation recovery studies of the MISsinglet and RIS at X-band found a 25% shorter T1 for the MISsinglet than for the RIS. This is likely due to the high concentration of spin centers underlying the MISsinglet at the cut edge, thereby resulting in some spin-spin interaction of the MIS radicals and the shorter T1. However, we found that the differential in the T1 was not sufficiently large to differentiate the MISsinglet and RIS using the saturation recovery method.

Conclusion: Multifrequency analysis of the MIS and RIS provided additional insights into the chemical nature of the underlying radical species and an additional RIS spectral component.

Acknowledgments: The work reported here is supported by a grant from NIH/NIAID (U19AI091173: Dartmouth Physically-Based Biodosimetry Center for Medical Countermeasures Against Radiation).

June 22, 2013 – June 28, 2013

Analysis of EPR line shape for the case of strong field inhomogeneities: implications for tooth dosimetry

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Study Goal: The resonator for L-band measurements has dimensions, comparable to the outer diameter of the magnet. Thus, the strong field inhogeneities are expected in the position of the resonator. Despite all the efforts are done to concentrate the RF field near the loop (which is much smaller compare to the size of the field homogeneity region in the center of the magnet) some power is still present on the outer surface of the resonator and waveguide. This microwave power combined with the presence of some unwanted EPR signals (copper oxide, for example), potentially may lead to the presence of unwanted EPR signal in the spectrum recorded.

Abstract: The problem is more complex due to the spreading of the possible contamination along the field inhomogeneity region. The theoretical calculations of line-shape of the resultant signal is provided. The analysis of possible contamination sources is outlined: how strong should be the signal from contamination in order to interfere perceptibly with the tooth signal.

RF analysis of resonator side bands allowed to understand the origin of the microwave power on the outer surface of the resonator box and waveguide, leading to the loop and capacitor. Simple experiments are described, which demonstrated how this problem may be solved.

Conclusion: Other sources of field inhomogeneities are also investigated: inhomogeneity of the modulation magnetic field and how it may lead to the distortion of the line shape of the tooth signal and temporal inhomogeneity of the sweep field – the excitations that may potentially lead to strong interference with tooth signal are demonstrated, discussed and eliminated.

June 22, 2013 – June 28, 2013

Double integration procedure combined with fitting for tooth dosimetry at L-band

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Study Goal: The presently used fitting procedure was found to work well for strong signals for tooth signal, but for the lower doses the presence of unwanted broad signal may potentially have an impact onto the accuracy of dose estimate. Instead of attempt to improve the fitting procedure by enhancing the regions of the most interest during calculations of the fit spectrum, the double integration procedure (which is usually considered as more "classic" way of calculation of the number of spins in the sample) was proposed with some special modifications.

Abstract: At first, the signal of PDT is used as a strong field reference and the integration interval is carefully chosen using the position in field of the PDT second line. At second, instead of the full integration of the spectrum the integration is processed in the narrow region near the tooth signal position. Since the tooth signal line shape is well known, the integral obtained is directly proportional to the full integral (the tooth line shape does not depend upon the dose). At the same time the integration in the narrow region successfully discriminates the tooth signal from broad underlying signal (possible origin is still under investigation), this broad signal is creating strong deviations of the amplitude for the case of fitting.

Conclusion: Several possible implications of the double integration procedure is considered: use of the Q values of the resonator as an amplitude reference (normalizing the tooth signal for different lossy materials present), frequency shift of the resonator due to the difference dielectric properties of different teeth and how it is reflected in the final amplitude of the tooth signal.

June 22, 2013 – June 28, 2013

Preliminary trial of neuron dosimetry of extracted tooth using L band EPR

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Study Goal: The purpose of this study is to make a research design for L band EPR neutron tooth dosimetry using the neutron field at neutron exposure accelerator system for biological effect

Abstract: In case of a nuclear accident such as atomic bomb attack by terrorists, a critical accident at a nuclear fuel plant and nuclear power plant, workers and first responder will be exposed by neutron. At that accident, victims will be exposed by neutron without certain notice. EPR dosimetry for neutron irradiation has disadvantage since the main composition of a tooth is hydroxyapatite. However neutron interaction in the surrounding soft tissue might result in appreciable radiation dose. To make a research plant to evaluate the responses for neutron irradiation, we are conditioning a neutron filed at NASBEE in NIRS. In this presentation, the preliminary results of neuron dosimetry of extracted tooth using L band EPR will be presented.

Conclusion: It was confirmed that EPR signals were observable for neutron-irradiated tooth using L band EPR. A vertical distribution of thermal neutron and photon dose in a flat plate was evaluated for assuming a dose distribution inside a human head.

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June 22, 2013 – June 28, 2013

Design of a Meanderline Surface Coil for tooth MRI

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Study Goal: To design and fabricate a meanderline radiofrequency coil comprising a serpentine array of parallel conductors for proton magnetic resonance imaging of tooth enamel.

Abstract: *Introduction*
RF surface coils with confined fields can increase detection sensitivity of samples that have essentially planar geometry. Meanderline coils are a serpentine array of parallel conductors. The B1 magnetic field from meanderline coils is confined to a region adjacent to the surface of the coil. Therefore, meanderline coils are more suitable for probing proximal surfaces with enhanced sensitivity as compared to conventional volume coils.

Methods
The meanderline coil functions as an RLC circuit; a meanderline was fabricated using photolithography on a 1/32 inch copper board that was presensitized with a positive photoresist. A mask was used to transfer the meanderline pattern to the board that was exposed to UV radiation using a Karl Suss MJB3 UV400 mask aligner. UV-exposed boards were developed and then etched using a copper etchant. We mounted capacitors in parallel and in series to resonate these meanderlines at 400 MHz for MRI and to impedance-match them to 50 ohms. Reflectance profiles were obtained using an Agilent Technologies E5071C network analyzer.

Conclusion: The Q value was 36.54 for the coil resonating at 401 MHz. MRI spin-echo images of an argarose phantom showed SNR profiles consistent with theoretical prediction.

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June 22, 2013 – June 28, 2013

Applying Constructs in EPR Instrumentation: Software Architecture

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Study Goal: The computational power of today's processing technology is staggering and its application can be a daunting task. We will discuss the use of software architecture as a means for making the most of our software application opportunities.

Abstract: The transistor has evolved in size from 3,000nm (Intel 8088 processor) in 1979 down to 32nm (Intel Core i7-975) in 2009 bringing with it vast new opportunities for computing. The 8088 was capable of executing several million instructions per second (3-5 MIPS) while the i7-975 has been benchmarked at 102270 MIPS. Clearly, the jump is tremendous, but the question that now begs answer; what does this mean to us as application developers and how do we go about making use of those 120270 million instruction executions per second? The short answer is that we use an interpreted language to capture our abstract thoughts and detailed specifications and then translate them to machine-level code for use. There exist many different types of interpreted languages (C, C++, BASIC, Java, Python, etc.) each comprised of a unique lexical command set and special rules for governing program flow during execution. With all of these different languages and the capability for processing hundreds of millions of instructions per second, one may conclude that our computing prayers have been answered. There is, however, a gap to fill between the user that wants the computer to do something computational and the ability for the computer to understand the user. This gap is much larger than the translation of high-level interpreted code into machine-language; it encompasses everything from the client application statement of need to the precise scheduling of time-sensitive deterministic sub-routines. In addition to minding the gap, there exist a range of practical business concerns; cost of maintenance, upgradeability, reliability, platform dependence, etc. The scope of this presentation will be limited to the application of software architecture, specifically the anatomy of architectural constructs formed to instantiate a greater overarching organizational precept for software development efforts.

Conclusion: The massive computational power of today's microprocessor technology can enable the seizing of great opportunities. Software architecture and its constructs can be used to help close the gap between client need and functional software product.

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EPR tooth dosimetry with single use, flexible resonant loops

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Study Goal: Evaluate the feasibility of using flexible, single use, resonant sensors for tooth EPR dosimetry. The potential benefits of such a sensor are better fit to variable tooth geometry, and improved workflow resulting from single use operation.

Abstract: The single use resonant loop was designed as a 10mm diameter loop with an integrated parallel plate capacitor for resonance at 1.15GHz (L-band). The design was modeled in HFSS and the size of the parallel plates was adjusted to achieve resonance. The dielectric material between the parallel plates was modeled as 89um thick layer of Polytetrafluoroethylene (PTFE, dielectric constant 2.1).
A flexible copper clad substrate was used in the manufacturing. Four frames of flexible loops, with each frame containing 36 parts, were manufactured.
For proof of concept, the resonant loop was soldered to half wavelength coaxial cable and connected to the microwave bridge of dosimeter under development at Dartmouth College EPR center. The resonant loop was placed on irradiated teeth samples (ex-vitro) and the radiation induced signal was measured. Each tooth was measured six times and representative peak-to-peak EPR signals were estimated. The results were plotted as a function of the administered (known) dose of radiation. The measurements were carried out for teeth with dose levels of 0Gy (un-irradiated), 10Gy and 30Gy.

Conclusion: The results showed a linear increase in signal amplitude as a function of the radiation dose, demonstrating the feasibility of using signal amplitude acquired from flexible resonator to predict radiation dose.

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Signal in the CW EPR Spectrometers with Reflection Resonators

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Study Goal: Signal in the CW EPR spectrometers with reflection resonators has been theoretical analyzed accurately, acceptable for any EPR frequency band

Abstract: Signal in the CW EPR spectrometers with reflection resonators has been theoretical analyzed accurately, acceptable for any EPR frequency band.

Long ago there is a huge number of publications analyzing basic equations of the detection of the EPR signal in the CW EPR spectrometer with reflection resonators. When studying dependence of the EPR signal from various factors, always the main attention were given to magnitude of the EPR signal and always it was at exact tuning the resonator to frequency of microwave source but it does not give the complete vector-picture of change of magnitude and phase of the EPR signal that is necessary for the proper understanding the EPR signal parameters. Moreover the resonator is never tuned to exact frequency of the microwave source because of leaking the circulator by means of which the resonator is connected to the microwave bridge. Always offset is 5 - 10% of half-width of the resonator dip and even more.

Presented general vector equations describe EPR signal phase and amplitude and make clear theirs dependence on coupling and tuning the frequency of the EPR sample resonator. From these equations, a graphical description of the resonator reflection coefficient is presented in order to describe qualitatively the EPR signal dependence on the matching and frequency tuning. Basing on the phase diagrams, the grids of the constant offset frequency, the constant coupling parameter and the constant magnitude of the EPR signal represented for use with Smith Charts on network analyzers that gives a direct view of theirs values during tuning the EPR resonators and EPR bridges.

Utilized approximations: 1) Variation of the microwave frequency are much less than value of the microwave frequency. 2) EPR signals are so weak that EPR variations of resonant the frequency and unloaded Q-factor are much less of their values.

Conclusion: Clear vector view of the CW EPR signal is represented

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