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News and Views

Joint International Conference on EPR Spectroscopy and Wound Healing

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ABSTRACT

The International Conference on Electron Paramagnetic Resonance Spectroscopy and Imaging of Biological Systems (EPR 2005) was held September 4 through 9, 2005, at Columbus, Ohio, U.S.A. Nearly 200 participants from 16 countries presented recent advances on the use of EPR technology to study biologic processes, with an emphasis on human health. For the first time, the EPR conference allied with the Wound Healing Conference, and the alliance opened an avenue for successful amalgamation of the basic biomedical and clinical aspects of wound healing with EPR technology and *vice versa.* This should lead to emerging applications of EPR technology in biomedical research and clinical practice. *Antioxid. Redox Signal.* 8, 1385–1387.

The International Conference on Electron Paramagnetic Resonance Spectroscopy and Imaging of Biological Systems (EPR 2005) was held on September 4 through 9, 2005, at the Easton Hilton Conference Center, Columbus, Ohio, U.S.A. This was a combined meeting of the "8th Spin Trapping EPR Spectroscopy" and "11th *In Vivo EPR Spectroscopy and Imaging"* conferences hosted by the Center for Biomedical EPR Spectroscopy and Imaging of The Ohio State University, Columbus, Ohio. This conference served as a venue for scientists from 16 countries, in which nearly 200 participants presented recent advances in the use of EPR technology to study the biologic processes related to human health. The conference supported 32 young investigators, enabling them to attend and participate in the meeting. The conference was organized by Dr. Periannan Kuppusamy and supported by the National Institutes of Health and the College of Medicine of The Ohio State University.

The EPR 2005 covered a wide spectrum of the recent advances in all areas of EPR as applied to biologic systems. This included the instrumentation, software, and spin probes for the detection of oxygen free radicals, nitric oxide, and oxygen; spin-labeling; and imaging. This maximized the cross-fertilization of ideas, allowing experts in different fields to learn from each other, spread information, and catalyze rapid advances. The organizers promoted this by integrating plenary talks from different areas and by designing symposia that addressed interfacial topics. The oral sessions were in the following areas: spin-trapping applications with respect to reactive oxygen radicals, protein radicals; spin labeling–protein structure/function; *in vivo* imaging applications; oximetry/dosimetry and clinical applications; hardware/software/methods; data acquisition, image reconstruction, and simulation; probe development; redox, oximetry, spin labels, and spin-traps; and emerging technologies. The highlight of the conference was the talk given by Prof. Albert W. Overhauser of the Physics Department of Purdue University, in which Prof. Overhauser gave a detailed account of how he discovered the phenomenon of dynamic nuclear polarization 52 years ago. This discovery has revolutionized MRI technology.

Three eminent EPR scientists were recognized at the conference. Prof. Lawrence J. Berliner, previously Professor of Chemistry at The Ohio State University and currently Professor and Chair of the Department of Chemistry and Biochemistry at the University of Denver, Denver, Colorado, was honored with a Lifetime Achievement Award for his 35 years of pioneering research in the field of biologic magnetic resonance spectroscopy. Prof. P. T. Manoharan, Professor Emeritus of Chemistry, Department of Chemistry–Sophisticated

Center for Biomedical EPR Spectroscopy and Imaging, and Comprehensive Wound Care Center, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio.

Analytical Instruments Facility, the Indian Institute of Technology, Chennai, India, was honored with a Lifetime Achievement Award for his outstanding service as a mentor, scientist, and promoter of EPR spectroscopy. Professor Harold M. Swartz of the Dartmouth Medical School, Hanover, New Hampshire, has been elected as a member of the International EPR Society (IES) and was awarded a Gold Medal for serving as the founding president of IES.

On September 8, 2005, the Inaugural Conference for the Comprehensive Wound Center (CWC), The Ohio State University, Columbus, Ohio, was held. Dr. Chandan Sen, Director of the CWC, delivered the welcoming address. The conference was inaugurated by Dr. Karen Holbrook, President, The Ohio State University, and Dr. Christopher Ellison, Chairman, Department of Surgery, OSU. Amalgamation of the wound-healing conference with the EPR conference fostered the interdisciplinary and translational aspects of both disciplines.

In the evenings, throughout the conference, after the lecture sessions, posters on various topics were displayed for viewing.

During the last two decades, the role of free radicals has been well recognized in the pathogenesis of many diseases including cancer, atherosclerosis, ischemia–reperfusion injury, stroke, diabetic vascular disease, and in a variety of inflammatory diseases. The radicals, collectively termed "reactive oxygen/nitrogen species (ROS/RNS)," are generated in biologic milieus under stress and are propagated through a cascade of reactions leading to tissue damage. A great deal of progress has been made in the detection, characterization, and quantification of these radicals. EPR spectroscopy still remains the most sought-after technique that allows direct detection and quantification of these radicals *in vitro,* as well as *in vivo.* Obtaining spatially resolved information on the distribution of the radicals in tissue, when combined with the power of imaging, has become a unique feature of this technique. In spite of much potential and many advantages, the EPR technique is not yet completely recognized among investigators in the field of free radicals in biology and medicine. The main reason for this is a lack of awareness of the technique and its unique capabilities. The primary goal of this conference was to fill this void. The secondary goal of this meeting was to educate young investigators and non-EPR scientists about the potential applications of EPR technology. The symposia at this conference were designed to promote the fruitful cross-fertilization of ideas that has been the driving force behind the successful advances in this field.

ROS and RNS have been established to play a major role in neurodegenerative and cardiovascular diseases with the aid of many developments in the field of EPR spin-trapping. The generation of radicals in biologic macromolecules (DNA, proteins, and sugars) has been implicated in a number of pathologic conditions among humans. EPR spectroscopy, either directly, or in conjunction with spin-trapping, is the major method of analysis of such radicals. The formation and reactions of protein free radicals (PFRs) in biologic systems, which also play a critical role in several pathologic conditions, have been studied to a considerable extent. The detection of PFRs with the use of nitrone spin traps, in conjunction with an antibody that is sensitive and specific to the nitrone (*e.g.*, anti-DMPO), opens up the use of immunochemical assays as an alternate method to EPR spectroscopy. EPR spectroscopy has been a valuable tool in the field of enzymology for elucidating the mechanisms of superoxide generation catalyzed by endothelial nitric oxide synthase (eNOS) in real time and under turnover conditions. EPR and immuno–spin-trapping assays show that myeloperoxidase reacts with GSH, leading to the generation of superoxide radicals and formation of MPO protein radicals. By using EPR spectroscopy with a DEPMPO spin trap, the methylarginine enhancement of superoxide formation from NOS-1 has been established. With EPR spectroscopy, the regulation of superoxide generation catalyzed by eNOS through phosphorylation mechanisms has been elucidated. EPR spin-trapping has offered tremendous insights into the physical properties (adsorption) of surfactants at the gas–solution interface for better control of the effects of surfactants on chemical and biologic systems during exposure to ultrasound. Thus the application of the EPR spin-trapping method spans the detection and determination of radicals to studying of properties of materials and enzymology.

Biologic applications of functional EPR spectroscopy rely on functionally oriented probes (redox, pH, thiol, oxygen, microviscosity, and polarity). Linking of macromolecules with EPR probes can be an efficient tool for the studies of the structure/function of macromolecules, drug delivery, electron transfer, and biodegradation. The identification of ROS relies mostly on an EPR spin-trapping technique involving the use of nitrone spin traps. Continued spin-trap development uses the prediction of new probes based on computational chemistry, synthesis, and biologic applications. Dual fluorophore-nitroxide molecules that contain the fluorophore attached to the nitroxide possess all the properties of spin and fluorescent probes and serve as sensitive spin traps and redox probes. Cyclic hydroxylamines, which rapidly react with superoxide to form stable nitroxides with a much longer half-life and various charges, lipophilicity, and cell permeability, have been used to study intra- and extracellular superoxide. The nitroxides with pH-dependent EPR spectra have been successfully used as pH-sensitive spin probes for determination of local pH and for investigation of proton-related transport by using low-field EPR spectroscopy and site-directed labeling. Noncovalently linked nitroxides and spin labeling of polymer matrices and protein drugs are valuable tools in drug-delivery processes. The efficiency of the nitrone-cyclodextrins to trap superoxide and the capacity of the resulting nitroxide to withstand reduction offer possible biologic applications. The development of stable, selective, and long-lived spin probes is highly crucial for the application of EPR spectroscopy in biology and medicine.

Nitroxides have emerged as fascinating, novel drugs with exciting therapeutic and analytic applications. The use of nitroxide radioprotectors in preclinical studies supports their use in the selective radioprotection of normal tissue. Nitroxide-modified drugs and their amine precursors are extremely promising compounds in attenuating oxidative injury by scavenging ROS and RNS. The incorporation of paramagnetic amino acids into proteins opens new avenues in protein research. Paradoxically, nitroxides (antioxidants) like 2,2,6,6-tetramethylpiperidine-1-oxyl can act as the most potent prooxidants. The use of site-directed spin labeling of enzymes after interaction with substrates can reveal the conformational changes of the protein. The tertiary structure of the

proteins can be determined with the use of spin labels and EPR spectroscopy.

Some of the essentials requirements for biologic (animal) applications of EPR are resonator design, automatic frequency control, automatic coupling control, and rapid-scan capability. More specialized systems are CW imaging and Overhauser-effect imaging in the VHF frequency range. Loop-gap resonators (LGRs) have been introduced to EPR from a standpoint of lumped circuits, which can be used for *in vivo* imaging. Commercial EPR imaging systems have been developed (L- and X-band) for heart imaging in small animals. EPR microscopy is an emerging technology that offers high-resolution ESR microscopic imaging of materials and biologic samples. Magnetic resonance force microscopy (MRFM), a novel technique, is a combination of the 3-D imaging capabilities of MRI with the high sensitivity and resolution of atomic force microscopy. The difficulties in obtaining good-quality images of live animals because of distortions caused by respiratory and cardiac movements can be overcome by applying the feedback-control methods. EPR imaging also has a fundamental limitation, the prolonged data-acquisition time, which can be corrected by accelerated EPR imaging. 4-D EPR spectral–spatial imaging offers a multistage reconstruction. Spin-echo EPR spectroscopic imaging is advantageous for imaging of oxygen. Taken together, the instrumentation technology of EPR spectroscopy is rapidly evolving for biomedical applications.

Paramagnetic agents (MRI image contrast agents and intensity enhancers) have been used to enhance proton-based image intensity significantly by using the dynamic nuclear polarization (DNP) first described by Overhauser (Polarization of nuclei in metals. *Phys Rev* 92: 411–412, 1953). This technique has been adapted to NMR as the nuclear Overhauser effect (NOE). DNP, in conjunction with trityl radicals, has been applied to map tissue oxygen quantitatively. The Overhauser effect has been used for the detection and imaging of free radicals, which is known as either "Overhauser imaging" or "proton-electron double-resonance imaging" (PEDRI). This distinctively offers the spatial resolution of the image independent of the line width of the free radical, as opposed to direct EPR imaging. The sensitivity of NMR is still limited by low thermal polarization, which can be improved by NMR and MRI agents prepolarized by the DNR-NMR method and can be applied for *in vitro* and *in vivo* studies. The Overhauser MRI (OMRI) technique greatly enhances the application of proton MRI on human subjects at very low field strength, as the existing magnetization is enhanced by the Overhauser effect. Thus these advances have offered significant enhancements in the resolution of EPR imaging and MRI.

EPR spectroscopy and imaging is an important tool for noninvasive *ex vivo* and *in vivo* measurement and spatial imaging of free radicals, redox state, oxygenation, and nitric oxide in cardiovascular physiology and disease. With *in vivo* spin-trapping and spin–probe techniques, free radical generation induced by diethylnitrosamine in the liver has been detected by EPR spectroscopy. The role of pulmonary-formed ROS on left ventricular dysfunction as a result of normothermic regional lung ischemia–reperfusion injury has been determined by EPR spin-trapping technique. EPR imaging along with MRI can also be applied to image registration for multimodality image-guided radiation-induced gene therapy. Thus the EPR spectroscopy and EPR imaging techniques are used in the studies of pathophysiology, disease, toxicology, and therapy.

Oxygen is essential for the homeostasis of energy metabolism in aerobic organisms. Imbalance in tissue oxygenation leads to hypoxic or hyperoxic states, as observed in several pathologic conditions. Oxygen is a critical determinant in the prediction of the treatment outcome of several diseases and therapies. Hence the clinical significance of tissue oximetry is immense. Although several methods are being used to measure oxygen concentrations, a suitable technique for noninvasive and repeated measurements of oxygen in the same tissue or cells on a temporal scale is warranted. EPR oximetry has advantages over the existing methods of oxygen determination and enables reliable and accurate measurements of the concentration of molecular oxygen in tissues. Recent novel developments in EPR oximetry (oxygen-sensing probes, instrumentation, and technology) have opened innovative avenues to perform noninvasive tissue oximetry in a variety of important applications, including myocardial ischemia/reperfusion injury, congestive heart failure, cellular cardiomyoplasty, organ transplantation, angiogenesis, cancer therapy, diabetes, and wound healing. Although the current measurements are limited to animal models, potential opportunities are emerging for the use of *in vivo* EPR oximetry in peripheral tissues (topical wounds, diabetic foot ulcers, peripheral vascular diseases) and extremities in humans in clinical settings. These remarkable advances in EPR oximetry have created an alliance between basic scientists and clinicians for a successful translational application of biomedical EPR research.

For the first time, the EPR conference created an opportunity for the basic scientists and clinicians actively investigating various aspects of wound repair/healing to interact with EPR experts for a successful interchange of ideas to develop clinical applications of EPR. Experts gave lectures on such diverse topics as the role of nerves in wound repair, significance of dead-cell clearance in wound healing, molecular basis of endothelial cell adhesion, angiogenesis from hemangioendothelioma, role of nitric oxide in wound healing, mechanisms of stress-induced impairment of wound healing, mechanical signals in healing, inflammation and wound healing, and transcriptional regulation of tissue repair. The wound-healing conference lectures opened new avenues for the EPR experts and wound-healing experts to encourage them to use emerging EPR technologies in the basic, as well as, clinical aspects of wound healing.

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