

Intravital Multi-photon Microscopy to Visualize Renal Endothelial Cell Injury and Dysfunction

Bruce A. Molitoris

Division of Nephrology, Department of Medicine and the Indiana Center for Biological Microscopy, Indiana University School of Medicine^a, Indianapolis, Indiana, USA, and The Roudebush VA Medical Center^b, Indianapolis, Indiana, USA

Bruce A. Molitoris
Division of Nephrology/ Department of Medicine
R 2, Rm 202C
950 West Walnut St.
Indianapolis, IN 46202
Telephone: (317) 274-7453
Fax: (317) 274-8575
e-mail: bmolitor@iupui.edu

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ABSTRACT

New imaging technologies, such as multi-photon microscopy, have equipped researchers with extremely powerful tools to uniquely address biologically important questions intravitaly in whole organ studies with subcellular resolution. In parallel with this the development of fluorescent probes, the ability to fluorescently tag individual molecules, transgenic approaches and new delivery techniques have allowed the molecular engineering of unique intracellular approaches. Furthermore, the rapid development of computer sciences, and its application to imaging, has removed many of the obstacles previously limiting the ability to utilize microscopy to study and quantify dynamic cellular processes in three and four (time) dimensions. The quantification of physiological processes such as regional tissue perfusion and endothelial cell permeability are examples of the possibilities. Therefore, it is now possible to utilize these minimally invasive technologies within the functioning kidney to enhance our understanding of normal physiology and or disease processes. Researchers equipped with these unique, and ever improving, tools can utilize optical microscopy and digital image analysis to study events within cells, cell-cell interactions and integrative organ physiology, biochemistry, molecular and cellular biology.

Three major areas of development have now been combined to provide investigators with unique approaches to understanding biological processes in an organ specific fashion at cellular and subcellular levels. First, multi-photon microscopy offers the investigator a minimally invasive, high resolution, technique with increased depth of penetration and

markedly reduced phototoxicity for visualization of cell-cell and intracellular events intravitaly. The genesis of these advances was covered in the previous article and in our previous article. Second, improved high sensitivity detectors and cameras, enhanced and faster soft and hardware, and new computational algorithms for 3-D analysis and quantification have allowed for more rapid, sensitive and accurate data gathering. Finally, the revolution in fluorescent labels capable of reporting on a growing number of cellular processes has markedly improved the capabilities available to the investigator. Fluorescent labeling of proteins, either with genetic or chemical probe attachment, and the spectrum of colors now available allow for multi-colored imaging of multiple cellular processes at once

The pathophysiology of ischemic acute renal failure (ARF) involves a complex interplay between renal hemodynamics, tubular and endothelial cell injury, and inflammatory processes. A growing body of evidence supports the contribution of altered renal vascular function, especially at the microvascular level, in initiating and subsequently extending the initial tubular injury. The *Extension Phase* of ischemic ARF involves continued reduction in renal perfusion, ongoing hypoxia, and inflammatory processes that occur during reperfusion and contribute to continued tubular cell injury. Vascular endothelial cell injury and dysfunction play a vital part in this *Extension Phase*. With injury, the endothelial cell loses its ability to regulate vascular tone, perfusion, permeability and inflammation/adhesion. This loss of regulatory function has a detrimental impact upon renal function. Vascular congestion, edema formation, diminished blood flow, and infiltration of inflammatory cells have been documented in the corticomedullary junction

of the kidney. Specifically, early alterations in endothelial actin cytoskeleton result in cell swelling and are associated with increased permeability to macromolecules, enhanced WBC adhesion and vasoconstriction all resulting in reduced blood flow. These alterations are more pronounced in the cortico-medullary region and result in prolonged hypoxia and ischemia.

The approaches used to quantify these changes in vivo will allow for improved evaluations of preclinical therapies to understand these microvascular abnormalities. New diagnostic and therapeutic approaches to ischemic ARF must incorporate these findings to devise early recognition strategies and therapeutic approaches.

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