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Bioengineering and Imaging Research Opportunities Workshop V: A Summary on Imaging and Characterizing Structure and Function in Native and Engineered Tissues

William R. Hendee,[1]* Kevin Cleary,[2] Richard L. Ehman,[3] Gary D. Fullerton,[4] Warren S. Grundfest,[5] John Haller,[6] Christine A. Kelley,[7] Anne E. Meyer,[8] Robert F. Murphy,[9] William Phillips,[10] Vladimir P. Torchilin[11]

[1]Department of Radiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

[2]Department of Radiology, Imaging Science and Information Systems Center, Georgetown University, Washington, DC

[3]Radiology Department, Mayo Clinic, Rochester, Minnesota

[4]Radiology Department, University of Texas Health Sciences Center San Antonio, San Antonio, Texas

[5]Bioengineering, Electrical Engineering and Surgery, University of California Los Angeles, Los Angeles, California

[6]National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, Maryland

[7]Division of Discovery Science and Technology, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, Maryland

[8]Biomaterials Graduate Program, University of Buffalo, Buffalo, New York

[9]Lane Center for Computational Biology, Carnegie Mellon University, Pittsburgh, Pennsylvania

[10]Radiology Department, University of Texas Medical Center at San Antonio, San Antonio, Texas

[11]Department of Pharmaceutical Sciences and Center for Pharmaceutical Biotechnology and Nanomedicine, Northeastern University, Boston, Masschusetts

• Abstract

The Fifth Bioengineering and Imaging Research Opportunities Workshop (BIROW V) was held on January 18–19, 2008. As with previous BIROW meetings, the purpose of BIROW V was to identify and characterize research and engineering opportunities in biomedical engineering and imaging. The topic of this BIROW meeting was Imaging and Characterizing Structure and Function in Native and Engineered Tissues. Under this topic, four areas were explored in depth: 1) Heterogeneous single-cell measurements and their integration into tissue and organism models; 2) Functional, molecular, and structural imaging of engineered tissue in vitro and in vivo; 3) New technologies for characterizing cells and tissues in situ; 4) Imaging for targeted cell, gene, and drug delivery.

• Key terms

tissue engineering, functional, molecular and structural imaging, imaging of engineered tissues, targeted cell, gene, and drug delivery, single-cell measurements, emerging imaging technologies

THE Fifth Bioengineering and Imaging Research Opportunities Workshop (BIROW V) was held on January 18–19 in North Bethesda, Maryland. The name of the workshop was changed slightly from preceding BIROW workshops (which were known as Biomedical Imaging Research Opportunities Workshops) to emphasize biomedical engineering as well as imaging. BIROW V was sponsored by the Academy of Radiology Research (ARR), American Association of Physicists in Medicine (AAPM), American Institute for Medical and Biological Engineering (AIMBE), International Society for Analytical Cytology (ISAC), and the Society for Imaging Informatics in Medicine (SIIM). Partial financial support for the meeting was furnished by the National Institute of Biomedical Imaging and Bioengineering. The purpose of BIROW V (as of BIROWs I–IV which were held in 2003, 2004, 2005, and 2006) (1–4) was to identify and characterize opportunities for scientific research and engineering development in biomedical engineering and imaging.

The topic of BIROW V was Imaging and Characterizing Structure and Function in Native and Engineered Tissues. The meeting focused on four areas of scientific research that offer opportunities for major developments in biomedical engineering and imaging. The four areas are as follows:

1. Heterogeneous single-cell measurements and their integration into tissue and organism models.
2. Functional, molecular, and structural imaging of engineered tissue in vitro and in vivo.

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*Correspondence to: William R. Hendee, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226

Email: whendee@mcw.edu

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3. New technologies for characterizing cells and tissues in situ.
4. Imaging for targeted cell, gene, and drug delivery.

Each area was addressed in a plenary session in which several speakers presented their analysis of the subject and the research opportunities and challenges it presents, followed by audience discussion. Then, each area was the focus of discussion at one of four simultaneous breakout sessions. Each breakout session provided a forum for discussion of research opportunities from the perspective of the objectives of the Roadmap Program of the National Institutes of Health (5). These objectives are as follows:

1. Does it deepen understanding of fundamental biology?
2. Does it promote collaboration of multidisciplinary teams?
3. Does it reshape clinical research and promote discovery?
4. Does it improve people's health?

Breakout participants were also asked to identify challenges to the realization of the research opportunities. The four questions for this section of the breakout sessions are as follows:

1. What are the scientific challenges?
2. What are the primary obstacles to development?
3. What are the critical technologies that are lacking?
4. What are the impediments to translating the opportunities to improved health?

The plenary and breakout sessions yielded a wealth of information that has been synthesized and edited into the findings and recommendations presented in this article.

Session I: Single-Cell Measurements and their Integration into Tissue and Organism Models

A number of conventional technologies, including imaging methods, are available for assessing the structure and function of cells and organisms in vivo. These methods yield information averaged over a volume of tissue in which the characteristics of individual cells and small groupings of cells (cell subpopulations) are not revealed. New technologies are emerging, which provide an unprecedented ability to target and analyze the functions of individual cells both in vitro and in vivo. This capability presents a different challenge—how can information collected at the single-cell and cell subpopulation levels be interpreted in terms of the structural and functional integrity of the organism as a whole. This question must be addressed if the benefits of tissue engineering and regeneration are to be realized. A particular need and opportunity exists for development of methods to automatically integrate information from single-cell measurements into multiscale predictive modes.

Single-Cell Measurements: Opportunities

To understand the dynamics of a population of cells, the characteristics of individual cells and their interactions with other cells in the population must be known. This necessity demands that measurements and images must be obtained at the single-cell level, but with sufficient throughput to adequately sample large numbers of cells. Ongoing advances allow both flow cytometry and fluorescence microscopy to meet this need, and recent work illustrates how data on subcellular and cellular events from these technologies can be combined with machine learning methods to automatically build models (6–8). A major opportunity exists for building active learning systems that can collect a set of biological data, build a predictive model (or improve an existing model), determine what new data would be needed to test the predictions of that model, and repeat the cycle of collection and model improvement. Such systems are expected to enable both a greater understanding of fundamental cell biology and how tissue-level behaviors emerge.

The evolution of single-cell measurements into tissue and organism models is interdisciplinary by its very nature, and requires expertise from fields as diverse as physics, biology, chemistry, physics, optics, electrical and biomedical engineering, imaging and computer science, statistics, and mathematics. As an indication of the interdisciplinary nature of the enterprise, researchers from several different fields self-elected to participate in the breakout session concerned with single-cell measurements. These participants expressed the need for interdisciplinary training of persons interested in working on single-cell measurements and their integration into systems models.

Single-cell subpopulation measurements have the potential to greatly impact human health. Measurements in the lymphoid subpopulations of cells are critical to improved understanding of immune-mediated diseases, and have already revolutionized the treatment of acquired immune-deficiency syndrome. Simpler, less-invasive single-cell tests have the potential to improve patient compliance with screening tests, and may lead to changes in clinical practice through earlier and improved intervention in disease and disability. Automated creation of models from single-cell measurements may be a key to individualized medicine by enabling personalized diagnosis and monitoring of response to specific treatment regimens.

Single-Cell Measurements: Challenges

A major challenge to single-cell measurements is the need for improved methods for single-cell segmentation and validation, and for automated pattern characterization across cells and cell-types. Also needed are improved methods for imaging live cells at high resolution (especially in vivo), and compiling atlases of protein and RNA localization across tissues and disease states. A number of limitations were noted by breakout participants that are impeding the development of single-cell and cell subpopulation measurement techniques and their integration into clinical medicine. Among these limitations are 1) insufficient resources and mechanisms for sharing and annotating images and for compiling them into image collections for the purpose of training systems for machine learning; 2) omission of image analysis details in scientific publications so that reproduction of results is often difficult and sometimes impossible; 3) limited availability of state-of-the-art instrumentation, computational power, and interdisciplinary scientists needed to bridge the knowledge gap between single-cell measurements, their extrapolation to higher-order scales, and their integration into tissue and organism models. Also needed are improved labeling and sampling methods, better label-free imaging methods and in vivo sensors, more exacting standards for single-cell measurement technologies, enhanced techniques for tracking cells in the in vivo environment, and development of methods for active learning in hierarchical systems.

Session II: Functional Molecular and Structural Imaging of Engineered Tissues In Vitro and In Vivo

Imaging methods have the potential to offer fast, noninvasive, and accurate assessments of cell growth, cell differentiation, and tissue development, including matrix development, in native and engineered tissues. Talks in this session covered two major topics: 1) molecular imaging in vivo, including non-invasive tracking and evaluation of implanted cells and the fate of three-dimensional (3D) engineered constructs; and 2) structural and functional imaging of 3D engineered tissue constructs in vitro. These topics encompassed a plethora of imaging techniques including magnetic resonance imaging, micro-positron emission tomography (PET), optical coherence tomography, multiphoton microscopy, and several multimodality imaging methods. The talks and discussion emphasized the importance of communication and interaction between tissue-engineering researchers and biomedical imagers in order to realize the potential benefits of imaging in tissue engineering and regenerative medicine.

Imaging of Engineered Tissues: Opportunities

Imaging is a critical element of tissue engineering and regenerative medicine. It has the potential to monitor tissue function and host response in vivo and to detect implant failure early enough to permit corrective action. Further, imaging could make the processes of tissue replacement and regeneration more effective and less invasive compared with conventional implants. Increased imaging characterization of both natural and engineered tissues would lead to improved design of tissue engineering therapies (9).

It is widely recognized that two-dimensional cell-culture systems are artificial and that cells raised in these systems are phenotypically different from those grown in a three-dimensional environment. The latter cells offer a rich resource for studying cell function and cell–cell, cell–matrix, and cell–medium interactions (10). Three-dimensional engineered tissue systems open new possibilities for studying complex physiological and pathophysiological processes in a controlled environment, including cell and tissue growth patterns and the reasons for success and failure of engineered tissues implanted in the body (11).

Tissue engineering is distinctly an interdisciplinary research effort that requires biological, engineering, and medical knowledge tempered with input from experts in bioinformatics, computational biology, embryology, and sensor technologies. Also needed are individuals who are highly knowledgeable about the technology-transfer process, translational research, and the protection and commercialization of intellectual property. Shortfalls were acknowledged in the ability of technology-transfer offices of many academic institutions to capitalize on promising new technologies, in part because these offices are often under considerable pressure to realize short-term profits by early sell-off of promising new technologies at prices well below their ultimate market value. Also recognized was the need for closer involvement of clinicians with biomedical engineers and others engaged in tissue engineering.

Engineered tissues hold great promise to supplement and even replace donor tissues and biological fluids that are perpetually in short supply (12). Three-dimensional engineered tissues may become highly useful tools for development of drugs and major incentives for better imaging methods, especially when compared with animal models currently used for evaluation of drugs and imaging techniques.

Imaging of Engineered Tissues: Challenges

Imaging of engineered tissues reveals many challenges, several of which are due to incomplete knowledge of cell physiology and dynamics, especially with regard to the integration of engineered and host tissues. Progress in tissue engineering requires a number of technological innovations, including: 1) noninvasive, real-time imaging methods to continuously monitor cell differentiation (e.g. molecular imaging of gene expression); 2) techniques for label-free imaging that are as sensitive as imaging using labels at molecular and cellular levels; 3) ways to identify and track individual cells and cell subpopulations in vivo; 4) procedures to enhance the likelihood that stem cells will seek the "proper" location when they are injected in vivo (13); and 5) processes to image cells at deeper levels within tissues and organs (14).

Other imaging advances that would accelerate the translation of tissue engineering from the laboratory to clinical use include: 1) better exogenous markers and identification of additional endogenous biomarkers; 2) improved three-dimensional image analysis and quantifica-

tion; 3) methods to evaluate the evolution of scaffold degradation and tissue replacement over time; 4) improved imaging procedures for automated edge detection, deeper penetration into tissues without excessive loss of spatial resolution, and use inside or outside a bioreactor; 5) multimodality imaging facilities and customized imaging methods, including imaging laboratories for larger animals; and 6) better ways to compile, store, and mine imaging data. Additional concerns include the cost and nonportability of many imaging methods, the regulatory environment that inhibits the translation of new technologies into the clinic, and the need for physician awareness and acceptance of the potential of engineered tissues to address a variety of human disorders.

Session III: New Technologies for Characterizing Cells and Tissues In Situ

Emerging technologies are offering new approaches to quantitative assessment of tissue properties that heretofore could not be measured in situ. Many of these technologies utilize imaging methods that exploit interactions of energy with tissues, and some employ the conversion of energy from one form to another (energy transduction). Examples include magnetic resonance elastography (15,16), photoacoustic tomography, (17) thermoacoustic tomography and ultrasound-modulated optical tomography (18), and ultrasonic elastographic (19) and acoustic radiation force based methods (20). Other approaches bring sensors and microscopic imaging techniques into contact with tissues of interest by minimally invasive, image-guided methods. Many of the emerging technologies are as adaptable to imaging tissue constructs as they are to imaging cells and cell subpopulations in vivo.

Characterizing Cells and Tissues: Opportunities

Emerging technologies improve biological understanding by offering new methods to characterize the properties of tissues at the multicell level. They also help reveal the interplay between a target tissue and its surroundings, leading to greater knowledge of differences between normal and diseased tissue. At the microscopic scale now achievable for tissue characterization, variations in normal tissues can be measured to yield a range of normal tissue characteristics rather than just an average. Participants in the breakout session emphasized that in situ characterization of tissues could radically improve the process of clinical trials of new therapies by early monitoring of changes at cellular and tissue levels as they occur. They also recognized that emerging imaging technologies could lead to new low-cost screening methods that would be accessible to all, including populations that are currently underserved or deprived of adequate health care. Examples of such technologies include elastography for liver fibrosis (16) and endomicroscopy for in vivo cancer diagnosis (21). Realization of the potential of emerging technologies requires a multidisciplinary effort that includes physicians from the clinical arena as well as scientists and engineers from the laboratory.

Characterizing Cells and Tissues: Challenges

Tissue characterization with emerging technologies encounters many challenges, including: 1) distinguishing transient from chronic phenomena; 2) applying the technologies across multiple scales, from cells to the whole organism; 3) characterizing the interactions between focal lesions and surrounding tissues; 4) recognizing precancerous states in cells and tissues, gene modulation by the cellular environment, and early stages of mental illness; and 5) determining the importance of cell and tissue variables that are currently inaccessible (e.g. hydrostatic pressure). In addressing these challenges, it is essential to put preconceptions aside and to think in novel ways to arrive at solutions.

Developing breakthrough technologies requires an investment of money and time beyond that awarded through the traditional funding mechanisms of federal agencies. This issue has been a perpetual problem in research funding that is slowly being addressed by federal agencies. Technological innovation is an extremely valuable characteristic in research that should be nurtured and supported by both funding agencies and academic institutions.

To realize the potential of new approaches used for characterizing cells and tissues, better tools are needed, such as physical and chemical sensors that offer higher sensitivity, improved spatial and temporal resolution, and greater penetration of tissues. Multimodality and multiparametric probes would be helpful in measuring complementary cell and tissue characteristics, many of which currently are immeasurable. Physicians should be brought into this effort, so that the potential of these approaches can be exploited in the clinic. Finally, the cost-effectiveness of early detection and treatment of disease through methods such as cell and tissue characterization by imaging technologies should be emphasized as an avenue to reduction of health care costs.

Session IV: Imaging for Targeted Cell, Gene, and Drug Delivery

A major challenge in the delivery of cells, genes, and drugs to tissues is the present uncertainty about where the substances localize within cells and tissues after administration. These substances may prove to be ineffective because they do not reach the intended target in adequate amounts, or because they are not retained in the target long enough to deliver the hoped-for impact. The development of new therapeutic approaches using cells, genes, and drugs, and the improvement of existing moieties, depends heavily on better methods to identify and track the migration, deposition, and elimination of these substances in cells and tissues in the body. New imaging technologies that offer these capabilities would be a major contribution to development and application of new diagnostic and therapeutic modalities.

Imaging for Target Delivery: Opportunities

Imaging methods to track the delivery of cells, genes, and drugs in situ may contribute to a heightened understanding of basic cellular processes such as transporter and receptor kinetics, cell-membrane structure, biochemical and signal

pathways, endocytosis, apoptosis, etc. These methods may also reveal new information about disease biology, specific biomarkers for disease processes, and the reasons why a treatment may succeed or fail based on the delivery of the therapeutic agent to the target. Imaging studies are important to the development of new therapeutic entities and have the potential to greatly reduce the time and cost of bringing new therapies to the market and to patients. Ultimately, imaging may help match the characteristics of individual patients with the properties of specific therapeutic regimens, leading to realization of the vision of "personalized medicine" through better patient characterization and improved therapeutic products (22). To realize this potential, a multidisciplinary effort is required that includes broadly educated physicians working with scientists and engineers in the research and development of new therapies, delivery systems for them, and methods to monitor the responses of patients after the therapies are administered.

Imaging for Targeted Delivery: Challenges

A major shortcoming of current therapeutic regimens involving drugs and other internally administered therapies is the lack of knowledge of exactly where the therapies localize after administration, how much of the therapies are concentrated and retained in the target tissues, the uniformity of distribution of the therapies within the targeted tissues at the local level (23), and what happens to the remainder of the product from the standpoint of toxicity in normal tissues. Inadequate delivery of a particular therapeutic agent to its targeted site may frequently be the cause of ineffective therapy. To solve this problem, new drug delivery systems are needed to improve uptake and distribution of a therapeutic agent within the targeted disease process (24). Imaging of the distribution of the drug can be a key tool for development of new drug delivery systems (25,26). A major challenge in the development of gene therapies is the need to track the presence, migration, and replication of viruses in the body. This challenge must be met if virus-mediated gene therapy is ever to succeed.

Several specific challenges impede the development of materials for targeted delivery in cells and tissues. These challenges include: 1) inadequate standards for image guidance of therapeutic intervention in animal studies, causing difficulties in replication of results and their ultimate application in the clinic; 2) absence of quantitative imaging methods for stem cell tracking, which are required for successful development of stem-cell therapies; 3) high cost of some commercially available imaging agents and other markers that are useful in animal and clinical studies; 4) regulatory hurdles in gaining approval for labeling a specific therapy to permit imaging during initial clinical evaluation.

There are technologies that are essential to the evolution of more effective drugs and other therapies. Among these technologies are: 1) combined imaging systems (e.g. PET/CT, SPECT/PET, PET/MRI, US/CT) for evaluation of drug delivery, localization, and monitoring and for image-guided therapy; 2) small and large animal imaging facilities that are accessible to researchers exploring new cell, gene, and drug therapies; 3) optical and other new imaging modalities that can be used to guide the delivery of new therapies in animals and that can be translated into imaging techniques for the clinic; and 4) automation technologies for quantitative labeling and for image analysis.

The development of new therapies delivered by cells, genes, and drugs is expensive, and additional research funding is needed to realize their potential to alleviate human disease and suffering. In addition to core imaging facilities for small and large animals, more funding is needed to support Phase 1 studies of new therapies, and for translational research, in general. Finally, a major effort should be directed to the interdisciplinary education and training of scientists and clinicians, so that they have the integrated knowledge necessary to work productively in this new arena of medicine.

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