NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES (NIDDK)

NIDDK TECHNOLOGY IN UROLOGY AND NEPHROLOGY LOCAL WORKSHOP

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Summary Report

INTRODUCTORY REMARKS

Debuene Chang, M.D., NIDDK

Dr. Chang welcomed participants and said that this meeting is the first of a series of small regional meetings to gather ideas from those conducting research in urology and nephrology. A larger summit-type meeting will be held sometime next year depending on how people feel about this format and interaction. The small regional meetings are meant to be low-key and afford participants an opportunity to share ideas and think about how technologies can be used to address some of the research challenges everyone faces. The meeting includes clinicians, research scientists, physical scientists, and engineers to stimulate "out-of-the-box" thinking.

Dr. Chang described the Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs. The objective of SBIR/STTR is to encourage the development of technological advancements to diagnose and treat urologic and nephrologic disorders. Its mission is to support scientific excellence and technologic innovation through the investment of federal research funds in critical American priorities to build a strong national economy. SBIR is a set-aside program for small business concerns to engage in federal research and development with the potential for commercialization; STTR is a set-aside program to facilitate cooperative research and development between small business concerns and U.S. research institutions, with the potential for commercialization. Both programs have congressionally mandated percentages for federal grants from the Small Business Innovation Development Act of 1982. SBIR grants must represent 2.5 percent, and STTR grants must represent 0.3 percent, of all National Institutes of Health (NIH) grants.

Dr. Chang reviewed eligibility checkpoints for both SBIR and STTR. The 2001 reauthorization of STTR extended the program through fiscal year (FY) 2009 and set increases for set-aside percentages and award levels, and also required participating agency outreach efforts similar to those required for SBIR. Differences between SBIR and STTR include the relationship of the programs to institution partners and the percentage allowed for each type of partnership. In FY 2006, NIH funded $640 million for the SBIR/STTR mechanisms. More information about the programs may be found at http://grants.nih.gov/grants/funding/sbir.htm.

Discussion

A participant asked about the time periods for applying for these grants. Dr. Chang said that grant applications are accepted by December 5, April 5, and August 5. Response time for

awarding grants depends on the review groups. Dates and more information are found at the website listed previously.

A participant asked about the success rates for 2006. Dr. Chang responded that these rates are not known yet; however, competitive phase II grant awards have taken out some funds from 2006, mainly because of flat funding rates. In the past, SBIR was undersubscribed; this is no longer the case. It is hoped that the types of meetings being held, such as this, will stimulate new Requests for Applications (RFAs) or Program Announcements (PAs).

Dennis Matthews, Ph.D., Chair, University of California, Davis (UC Davis)

Dr. Matthews welcomed participants and asked that participants recognize the opportunity they have to brainstorm about new medical technologies and network with those in diverse fields of research. Objectives of the meeting include:

- Assemble experts in urology, nephrology, and bioengineering and physical scientists to discuss medical needs and state-of-the-art technologies and to develop new ideas for the medical application of these technologies.
- Stimulate a network of possible collaborators and develop means for them to share ideas and contacts.
- Stimulate concepts and interests for potential new RFAs and PAs from NIDDK.

Dr. Chang explained the difference between RFAs and PAs. PAs are meant to assess the interest level in particular types of projects. The RFA is issued when funds have been set aside for a specific project. New money for RFAs is going to be difficult to find in the current budgetary environment. Dr. Matthews added that this meeting should result in the creation of advocacy to NIDDK for funding new projects that are identified during the meeting. He asked that everyone consider how they can support this process.

Dr. Matthews reviewed the agenda for the day and suggested that follow-up of the meeting will be critical in developing a research agenda for the future in urology and nephrology. He reiterated the expected follow-up initiatives from the series of regional workshops, including the national summit at NIH after the workshops, and potential PAs and RFAs. One idea is to create a password-protected blog site to provide a forum for interested parties. A summary of principal findings from the workshops will be developed and distributed to presenters for their input.

UROLOGY INCLUDING: BPH, OAB, URGE INCONTINENCE, IC, MALE AND FEMALE SEXUAL DYSFUNCTION, STONES DISEASE, ETC.

Michael Chancellor, M.D., University of Pittsburgh

Dr. Chancellor provided background on societal issues related to urology, including the impact of adult incontinence. Important issues in urology include the following:

- Prostate health among men takes a substantial number of health care dollars, especially in an aging population. Benign prostatic hyperplasia (BPH) is significant for its impact on quality of life and physician attention.
- Bladder issues, such as overactive bladder, interstitial cystitis (IC), and urge incontinence, are significant medical issues in the population.
- The sphincter that controls urinary function is an important issue.
- Sexual dysfunction is a significant factor for both men and women.
- Urinary tract infections need research for diagnosis that is timelier for treatment.
- Kidney stone diseases are common: Up to 10 percent of Americans will have a kidney stone sometime in their lives. Improved diagnosis and treatment are areas of research that need attention.

Dr. Chancellor described each of the urologic issues just enumerated and current treatments and preventive strategies, although for most of these conditions, little is currently available for prevention or control. He described two NIH RO1s he administers for urologic disease. The first is in partnership with Cook MyoSite, Inc., on adult muscle stem cells for tissue augmentation in urinary tract and other indications, and the second is in partnership with Lipella Pharmaceuticals Inc. on development of intravesical liposomes, nanoparticles to coat the bladder and deliver drugs. He presented information on each of these projects.

Selected experimental treatments include Botox for BPH, neuromodulation and local therapy for bladder conditions, and stem cell research and tissue engineering for sphincter problems. As a practical matter, there are identified areas in urology that need attention to improve treatment, prevention, and diagnosis. For incontinence, better diapers with better absorption are needed. An experimental diaper using microchips to signal wetness is being developed. Comfort issues relating to catheter use, and reducing the rate of infections related to catheter use, are areas that need research attention. Help with sexual dysfunction and better treatments for kidney stone disease would have a positive impact on the population.

Discussion

A participant asked if there are biomarkers for risk for any of the urologic conditions. Dr. Chancellor indicated that there are some possible biomarkers, such as blood markers for possible kidney stone risk. For kidney stones, the issue is that there are few good treatments that are not painful or invasive. Dr. deVere White suggested that a big issue is having improved diagnosis but no better way to treat kidney stones. Dr. Chancellor said that the issues are complex and there really is no way to identify those individuals at risk. Although there are screening tests for hypercalciuria, it is impractical to recommend screening the entire population because of the costs associated with testing.

Dr. deVere White next suggested that it may make sense to screen those who have a history of kidney stones. Dr. Matthews cautioned that screening may cause undue alarm is some people. For example, the use of heart scan technology, which shows areas of calcification in arteries, causes patients to become overly stressed; although everyone has some arterial calcification, the presence of calcification does not necessarily indicate an increased risk of a heart attack or stroke.

OPPORTUNITIES FOR NEW TECHNOLOGY IN UROLOGY

Ralph deVere White, M.D., UC Davis Cancer Center

Dr. deVere White addressed opportunities for new technology in urology. New technologies are needed for the United States to maintain its pre-eminence in directing urologic cancer care. By 2012, spending on health care will account for 20 percent of the gross national product. The recent flat NIH and National Cancer Institute budgets will make development of new technologies challenging. The UC Davis Cancer Center partnership with Lawrence Livermore National Laboratory seeks to leverage existing resources to improve urologic cancer care. The Unique Biomedical Technology Program has as its goal promoting collaboration among physical scientists, engineers, life scientists, and clinicians to create advanced methods and instrumentation for cancer detection, diagnosis, therapy, and research.

Early detection of cancer will help improve survival; bladder cancer survival has, during the past 25 years, improved less than 1.25 percent. Molecular grading at the single-cell level using molecule-specific imaging analysis by time-of-flight secondary ion mass spectrometry (ToF-SIMS) permits analysis of images as specific peaks or average mass spectra of a defined area. By performing multivariate statistical analysis of spectra from cells, this technique could separate breast cancer cells from culture by type. Glycosylation, the most common post-translational modification, can be detected and used as a serum biomarker. For example, the prostate tumor cell lines LNCaP and PC3 produce glycan profiles that change when transfected with the p53 R273H mutant. These profiles can be analyzed using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) in the negative mode.

Compact proton accelerators can be used for diagnostics or for advanced radiotherapy. This technology is suitable for use in standard radiotherapy clinics. Proton therapy delivers protons directly to the patient in pulse format, which eliminates magnets or collimators, and this modality does not require substantial new shielding. The cost of pulse-by-pulse intensity-modulated proton therapy (IMPT) is between $10 million and $15 million.

Spectroscopic enhancement of cystoscopy can provide better imaging of cancer *in vivo* using near-infrared (NIR) optical spectroscopy. This technique can help identify whether lesions within the bladder are cancerous, determine stage, and also help determine if all cancer has been eliminated by resection. The system currently in development can simultaneously display conventional color images and cancer-enhancing NIR images and will be readily adaptable to any type of endoscope.

Invasive transitional cell carcinoma (TCC) accounts for 25 percent of all initial tumors of the bladder and has a poor prognosis (85 percent of TCC deaths). The UC Davis Intravesical UC Project uses NIR to improve visualization of lesions in the bladder (60-mm depth of visualization). Improvement in robotics also will help in treating this cancer. Currently, nearly 60 percent of the procedures involved in prostatectomy employ robotics. New technologies also are needed to improve care for conditions including urinary tract infections, urolithiasis, BPH, impotence, urinary incontinence, pelvic pain, and IC, which account for $11.5 billion in health care costs.

Discussion

Dr. Chang asked if it is possible to transfer technologies between cancer research and urologic research. Dr. deVere White indicated this should not be a problem. There are benefits to be found in many disease research areas.

A participant asked why bladder cancer has such a negative impact. Dr. deVere White responded that the bladder is muscle controlled, and failure in the muscle may lead to the use of urinary bags and catheters, which allow individuals to continue to live their lives but have many drawbacks.

Comments were made about biomarkers that may exist for urologic conditions. Biomarkers will only be useful if they change diagnosis and therapy.

BIODESIGN: FROM THOUGHT TO CLINICAL APPLICATIONS

Todd J. Brinton, M.D., Stanford University

Biodesign, from thought to clinical applications, was the focus of the presentation by Dr. Brinton. Development of leaders in biomedical technology innovation requires education and collaboration as well as innovation. In a real-world setting, innovation is taught using a needs-based approach, which first identifies the problem. Teams made up of experts in different disciplines address the problem to develop a solution. Needs are defined as problems identified through observations, and identification of needs, or "needs finding," begins with observation (generally for 2 months). Effective needs finding will involve examining the need from different perspectives (e.g., patient care and management perspectives). Two important principles of needs finding are to: (1) fully understand the need and specifications prior to embarking on the process of finding a solution and (2) differentiate needs from solutions; solutions are not discussed for 5 1/2 months to avoid overlap with needs finding.

Early steps to innovation begin with defining a clinical need and understanding disease state fundamentals. This process takes into account disease state fundamentals, existing treatment options, market evaluation, and stakeholder evaluation. Disease state fundamentals encompass physiology, pathophysiology, clinical presentation and outcomes, epidemiology, and economic cost. Consideration of treatment options must take into account existing options and clinical, economic, and utilization profiles to define the treatment landscape.

Because large numbers of needs often are identified, the crucial question is not "can we build it?" but rather "should we build it?" An addressable need must have an objectively measurable change in outcome. Part of the process of identifying needs that should be addressed includes development of a need statement. This statement isolates the single need that has the best chance of addressing the problem, driving a desired outcome, and supporting a reasonable market opportunity; it should be possible to capture this need in a one-sentence statement. The statement should focus on the goal or endpoint rather than the problem and should not reference current solutions. Need specification involves explaining the problem, describing the market, and identifying customer criteria. A list of specific criteria critical for a need could include cost,

market, features, intellectual property issues, and reimbursement. Screening of needs should consider the desired outcome, need category, market, clinical impact, technical and clinical feasibility, resources, and competition.

As an example, the following innovation process was applied to find an improved method for treating atrial fibrillation. Early research and development involved brainstorming focused on generating a large quantity of ideas. To understand the concept of a specific idea, the fundamental hypothesis (in this case, mechanical force, electrical current, or pressure) was considered. Tests to reduce uncertainty and risk were identified, including development of prototypes to test the fundamental hypothesis, that is, whether the necessary energy, force, or pressure can be achieved. Early prototypes tested the feasibility of the general concept, in this case addressing mechanical, electrical, biologic, or chemical hypotheses. Models used in testing the prototypes include bench (building anatomic or physiologic models), *ex vivo* (allows for near-real anatomy and physiology testing and minimizes assumptions), and *in vivo* (employs real anatomy or physiology but may assume no disease is present). Using these prototypes to answer fundamental questions will assist in validating the concept and provide the opportunity to develop further prototypes with more complete versions of the concept.

Discussion

A participant asked what percentage of projects was successful. Dr. Brinton explained that very few proposed projects are successful. This process eliminates ideas that are sound but not practical. Patent issues also must be considered because it can be difficult to obtain a patent on an idea.

Dr. Brinton was asked to define intellectual property (IP) and premarket approval (PMA). IP is patent protection, and once a person has research or methods designated as IP, that individual is able to work in that area exclusively for 20 years. After 20 years, there is an obligation to let everyone know how to use the idea for their research. Freedom-to-operate (FTO) means that you have the freedom to operate in that space because the idea may be more than 20 years old, but you can use it in any way you choose. New patents can emerge from FTO research if different from the PMA and the process for regulatory approval. A PMA requires that you prove that your process is safe and that there is nothing like it already approved.

CLINICAL NEEDS IN UROLOGY: IMPROVING OUTCOMES

Ken Peters, M.D., Beaumont Hospital, Royal Oak

Dr. Peters discussed clinical needs in urology, focusing on areas where outcomes could be improved. For example, vulvodynia occurs frequently in women, but there is no objective, reliable method for monitoring outcomes. An attempt was made to develop an Algesiometer, but this was unreliable. Dr. Peters stated developing a tool that would transmit the pressure applied when a cotton swab is attached and pressed on the vulva would be of benefit to objectively determine the pressure needed to elicit pain. Pelvic floor dysfunction also is common in patients with chronic pain, and this condition also lacks a reliable method for objectively measuring the pressure needed to cause the pain and assess outcome. Development of a device to measure the

pressure needed to create pain on the levator muscle complex is needed. The Palpometer is a similar device used for measuring pain associated with fibromyalgia and tracking response to treatment. It provides the examiner with objective information on how firmly or softly to press and thus standardizes the pressure stimulus of conventional manual palpation when eliciting tenderness responses. The Palpometer is small and cordless and fits conveniently on the examiner's finger. It employs a sensor that measures changes in electrical resistance caused by varying the pressure of the examining finger. The Palpometer beeps when a predetermined pressure level is reached. Dr. Peters felt that a similar device would be of benefit for the assessment of pelvic floor dysfunction, but that would give an exact value in pounds/square inch.

Pudendal nerve stimulation may be useful for treatment of pelvic pain. Neuromodulation involves placement of an implant such as the InterStim device at the nerve. Several studies have shown that a majority of patients receiving the implants experience decreases in symptoms, including pain. Currently, technology to guide insertion of the electrode through the ischiorectal fossa to the pudendal nerve is needed. Such an implant tool would be a single-insertion device that incorporates the tined quadripolar lead and allows stimulation of each electrode with a single pass.

Tibial nerve stimulation may be useful for treating overactive bladder or incontinence. Currently, a 30-minute treatment is performed weekly, but this is not practical for long-term use. An implantable device that subjects could stimulate themselves would be useful. A device similar to the BION device (see next section), currently used for pudendal nerve stimulation, could be considered for tibial nerve stimulation. Similar self-controlled implantable devices that stimulate the cavernous nerve could be useful in the treatment of erectile dysfunction.

Periurethral injection of collagen or Coaptite® injectable implant has been used as a treatment for incontinence, and adult stem cell injection also has potential as a therapy. Precise injection with intraurethral ultrasound and predetermined volumes is needed.

A skin–central nervous system–bladder reflex arc involving lumbar-to-sacral nerve rerouting has been developed to restore voiding in spina bifida and spinal cord injury patients. The procedure allows patients to control urination by scratching a leg. The surgery involves microanastomosis of L5 to S2 using traditional end-to-end anastomosis with 10-0 sutures. A nerve "glue" that allows nerve growth or nerve growth factors to speed axon growth would improve the success of this procedure. Also needed is a method to limit the effects on the lower extremities caused by sacrificing L5 in children with spina bifida.

BIONIC TECHNOLOGY FOR NEURAL CONTROL OF MICTURITION

Gerald Loeb, M.D., NSF Engineering Research Center for Biomimetic Microelectronic Systems and Alfred Mann Institute for Biomedical Engineering, University of Southern California

Dr. Loeb described the BION device that is used for neural control in micturition. All neural actions in the body use the same basic digital signaling system, which involves action potentials in the form of ions and water; and modern digital electronics uses similar digital impulses, which are the flow of electrons in metals and semiconductors. For years it has been known how to

make devices for implantation into the body that can transfer information across the different forms of electricity without causing damage to tissue. The goal now is to determine which nerve impulses are missing (in a disease or physical condition) and replace those impulses with technologic solutions.

The concept of the BION device is neurostimulation of muscles and other tissue; the usual method is by transcutaneous or surgically implanted nerve stimulation of the motor neurons. The problem in all commonly used neurostimulating devices, such as the pacemaker, is that the electrical leads have to be attached to or routed within the body. Dr. Loeb described devices he is developing that provide leadless injectable microstimulation encapsulated in glass or other materials. These devices are similar to the microchips implanted in animals for identification purposes. He provided samples of the microcapsules for participants to view and handle. The capsules are implanted and activated by an externally applied radio-frequency magnetic field. It is like having a full-function laboratory stimulator in a grain of rice. The device has been tested in clinical trials and has been used in stroke and osteoarthritis patients. Commercialization of the devices should occur in the next few years.

For use in urology, neurostimulators may be most practical for micturition. Remediation of symptoms of various forms of incontinence is possible with implantable neurotransmitters. Dr. Loeb provided examples of the use of neurostimulators for strengthening the bladder sphincter for stress incontinence. In urge incontinence (i.e., spastic bladder), neurostimulators are being used to inhibit the excessive bladder activity that results from this condition. Stimulating the correct afferent pathways for bladder control has been the challenge in use of this technology.

For overflow incontinence, which occurs in patients with spinal cord injuries, a set of reflexes that are geared to emptying the bladder completely can be controlled by an implanted electronic stimulator. Dr. Loeb explained the method for controlling nerves that control this process. One challenge is to control bladder function without negatively influencing sexual function, as well as reducing the invasiveness of the surgery. For overflow incontinence, some of the same challenges are present, although the electrical pathways are somewhat different from those in urge incontinence. Progress is being made, but the limiting factor is finding urologists who want to work with electrophysiologists.

Discussion

A participant asked about the European devices mentioned in an earlier presentation. Dr. Loeb responded that these devices are still expensive and not readily covered by insurance. The devices can be used for each of the types of incontinence discussed. The technology is ready for use in clinical trials in this country, as well as for commercialization.

CONVENIENT OPTICAL METHOD FOR THE REAL-TIME MEASUREMENT OF RENAL FUNCTION

Carlos Rabito, M.D., Ph.D., Harvard Medical School/Massachusetts General Hospital

Dr. Rabito addressed technologies for real-time measurements of renal function. The high mortality rate (greater than 50 percent) of acute renal failure (ARF) has not changed in 50 years, largely because of delay in diagnosis; diagnosis of ARF commonly occurs hours or days after the initial organ insult. This occurs because ARF is initially asymptomatic and because current diagnostic techniques do not permit early diagnosis of organ dysfunction. Between 10 and 20 million people in the United States have chronic kidney disease (CKD), and diagnosis of this condition also is usually delayed; most CKD patients do not receive assistance until 70 percent of kidney function is lost. CKD also is initially asymptomatic, and current diagnostic technologies do not have the convenience and accuracy needed for detection of organ dysfunction. Methods to measure renal function (glomerular filtration rate [GFR]) in patients at risk for developing either ARF or CKD are needed.

Most methods of renal function assessment are inaccurate and have poor resolution time. Renal scintigraphy is accurate, but uses radioactive isotopes to show perfusion of the kidney. The nuclear renal monitor showed promise, but use of radioactive tracers was problematic, particularly in terms of acquiring venture capital to fund its development. This concern led to use of lanthanide chelates as probes for time-resolved fluorometric assays.

An optical method for real-time measurement of GFR has the accuracy of clearance techniques and the convenience of measuring plasma creatinine. This technique was used to measure renal function in rats as the rate of clearance determined from time-resolved transcutaneous fluorescence measurements of a new fluorescent glomerular filtration agent, the europium chelate of carbostyril-diethylene triamine penta-acetic acid (carbostyril-DTPA-Eu). This fluorescent glomerular filtration agent was calibrated against changes in plasma and urine concentration. This method provides a convenient and accurate means for performing real-time measurements of GFR and will permit early detection of kidney disease before renal function becomes irreversibly compromised.

Discussion

Dr. Matthews asked how a person would present with ARF in a clinical setting. Dr. Rabito explained that in about 85 percent of cases, ARF develops following an ischemic insult to the kidneys as a complication of one of many medical or surgical conditions. A participant asked how many patients a year present at his hospital with ARF. Dr. Rabito said that ARF patients represent approximately 5 percent of patients seen at his and other hospitals. The incidence of ARF in Critical Care Units could be even higher—around 20 percent or more.

Dr. Chuang asked if the slope described in one of his slides is proportional to GFR. Dr. Rabito said that GFR measurement in a patient using clearance techniques is usually corrected by the patient's body surface area to adjust for the variability in space distribution (patients with different body sizes). After correction, GFR and the slope of the natural logarithm of counts

versus time shown in the slide represent the same concept; this is the relative efficiency of the kidneys to clear the GFR agent from a standard-size body compartment.

INVITED/CONTRIBUTED TALKS FROM PHYSICAL SCIENTISTS AND ENGINEERS

Use of Mesoparticles for Targeted Optical Imaging and Therapy

Bahman Anvari, Ph.D., University of California, Riverside

Dr. Anvari discussed mesoparticles for targeted optical imaging and therapy. He focused on the use of exogenous chromophores, which are introduced into the body. One of the most popular exogenous chromophores is indocyanine green (ICG), which has many clinical uses, such as in fluorescein angiography in eye tests or in cardiovascular testing for blood flow.

Encapsulation of ICG into mesoparticles has benefits that include the ability to place other compounds such as antibodies on the capsules. Coatings on the mesoparticles can potentially be designed to target specific organs or tissues. Examples of coating materials are Dextrin PEG–ferrous oxide constructs. Mesoparticles also have very rapid clearance from blood plasma, with the liver as the primary site of accumulation.

Ex vivo investigations of microcapsules with ICG indicate that they also can be used for photothermal therapy of tumors. These microcapsules enhance heat generation and have the potential for destruction of undesirable tissue, such as cancers or benign structures. Capsules can be coated with specific molecules to target specific organs, either for imaging or for therapeutic applications.

Upcoming studies using encapsulated chromophores include continued investigation of coating with different antibodies; assessment of microcapsule binding to tumor cells (*in vitro* studies); and optical imaging and destruction of tumors in animal models.

Discussion

Dr. Matthews asked if there have been studies on perfusion in *in vivo* systems. Dr. Anvari responded that these types of studies have been conducted with other types of chromophores, and perfusion becomes important in heat removal during relatively long times (on the order of minutes). Another participant asked if there is leakage from the capsules. Dr. Anvari said that very little leakage occurs up to 48 hours. These data come from distribution studies.

Dr. deVere White asked if the microcapsules have better delivery than other monoclonal antibody systems. Dr. Anvari related that delivery is about the same. He did not want to describe this system (i.e., microcapsules) as nanotechnology because the size of the molecules dictates that they are too big for this designation, although some of the methods used come from nanotechnology. Dr. deVere White followed up with a comment that he would like to know if it is possible to get enough of the microcapsules bound to a tumor to allow enough heat generation to destroy it. Dr. Anvari responded that it is unclear if this is possible at this stage because the

first step is to make sure that specific binding between antibody-coated mesocapsules and tumors occurs.

Dr. deVere White commented that the cell surface, as used in the system described by Dr. Anvari, may not be useful enough for urologic or nephrologic purposes because what is needed is entry into the cell. He suggested that the microcapsules be made smaller so they can enter the cell. Dr. Carlos Rabito added that he liked the microcapsule targeting concept.

Dr. Matthews summarized that it may be possible to conduct investigations on targeted tissue denaturation for nephrology.

Microendoscopic Methods for Visualizing Hydrodynamic Flow and Functional Histology
Frank Y.S. Chuang, M.D., Ph.D., National Science Foundation, Center for Biophotonics

Dr. Chuang provided a definition of biophotonics science—the study of the fundamental interactions of light and matter. It also is a term for the technology that utilizes light to visualize or manipulate biological systems. The National Science Foundation (NSF) Center for Biophotonics Science and Technology (CBST), based at the UC Davis, is investigating and developing new photonic tools and techniques for a wide variety of medical applications including cancer detection, drug discovery, disease diagnosis, and light-based sensors, assays, and medical devices. The Center has more than 30 projects in its research portfolio of collaborations between physical scientists and medical researchers and clinicians.

The role for biophotonics in medical diagnosis includes understanding the fundamental mechanisms of disease and identifying optical signatures or markers of disease. There are tools for each task. Dr. Chuang described a project using two-photon fluorescence microendoscopy for imaging cells in deep tissue. He described micro-optical probes that can image a few microns (e.g., in the brain). Two forms of microendoscopy are used: epi-fluorescence, which uses laser scanning at a level of two photons for greater depth of optical scanning, and a tool known as gradient refractive index (GRIN) fiber optic lenses, which is like a periscope and provides a window into tissue.

Dr. Chuang described other photonic tools currently in use or in development, including small neuron imagers that can be implanted into mice to measure optical nerve activity while the mice run through a maze. This technology could be used for any number of tissues and disease conditions. Another technology being developed will allow the visualization of neural stem cell migration during development.

Discussion

Dr. Matthews asked if this technology could be used for pain nerves or other tissue, such as those involved in IC. Dr. deVere White commented that if this technology could be used for real-time readouts during tests on nerves, it would be especially valuable. Dr. Chuang said there also may be applications at renal centers to investigate renal nerve actions. Other participants saw the possibility that this technology might have utility for nerve injuries involved in incontinence, nerve demyelination in multiple sclerosis, and spinal cord injuries.

Dr. Chancellor wondered if it is possible to differentiate between sensory and motor nerves. Dr. Matthews considered use of the technology to determine if sphincter malfunction (for incontinence or sexual dysfunction) was associated with conduction or physical perturbations.

Interventional Medical Device Applications of Shape Memory Polymers
Duncan Maitland, Ph.D., Lawrence Livermore National Laboratory

Shape memory polymers (SMPs) are a class of polymeric materials that can be formed into a specific primary shape, reformed into a stable secondary shape, and then controllably actuated to recover the primary shape. When the polymer is deployed *in vivo*, it can be manipulated through desired spaces, and when it is temporarily heated it will revert to its "memorized" shape. Dr. Maitland described the types of polymers that can be used as SMPs. Current technology allows SMPs to be heat- or electricity-sensitive, with many applications in medical science. In addition, photothermal (optical) actuation of SMPs is being designed using ICG, but platinum-based photothermal dyes are being developed. SMPs are biocompatible and are similar to stents.

Interventional applications have mostly been used for cardiovascular disease (CVD) but may be adapted to uses in urology or nephrology. For CVD, SMPs have been designed that can be used to pull blood clots out of arteries and veins. They can "grab" the clot as they are pulled out. One challenge is how to create a shape that does minimal damage to the vessel endothelium (as do catheters), but this challenge should be able to be met. Biodegradable SMPs are in development and could have important future applications in many fields.

SMPs are being investigated for many medical applications, including the lumen of the bile duct for treatment or removal of gallstones. Livermore National Laboratory is developing a laser-activated polymer that will take a memory shape. These polymers could be used in the bladder and kidney. One of the ongoing studies is to determine the damage done by thermal exposures at the levels used for heat- or laser-activated polymers.

Discussion

A discussion of SMPs for use in dialysis ensued. Anything that could reduce either the number of times per week a person needed to undergo dialysis (as many as six times for most patients), would be of great interest and benefit to the nephrology community. Dr. Chang said she would speak to the nephrology community about these techniques to get an idea of the interest. Dr. deVere White commented that if it would be possible to apply this technology to develop a portable dialysis machine it would be life-changing for many patients.

Parallel Image Acquisition for Spectroscopic Cancer Detection through Conventional Endoscopes
Stavros Demos, Ph.D., Lawrence Livermore National Laboratory

Dr. Demos presented material on endoscopy for cancer detection. Endoscopy has many uses, including helping guide surgeons during internal procedures, as well as allowing surgeons to tell whether all cancer has been removed from a site. New techniques and technologies can

revolutionize these procedures and make for more successful surgeries. The parallel image acquisition technology involving simultaneous display of a conventional color image with cancer-specific spectroscopic images is one such imaging device. Resolution is high and subsurface imaging can be added using one of the methods under development.

New optical techniques using conventional endoscopy offer an array of possibilities. The use of multimodal imaging techniques is being investigated. Dr. Demos described an investigation using conventional endoscopy devices (cystoscopes, laparoscopes, colonoscopes, etc.), but at the output, the image is relayed to an image processing unit via an image-preserving fiber. Using modified illumination including white light and laser sources, the different image components are separated in the image processing unit and simultaneously displayed on different monitors. One imaging method is based on native fluorescence of porphyrins, which are part of hemoglobin and are found in all cells. A second method is based on the spectral polarization difference imaging (SPDI) technique, which provides subsurface imaging. Dr. Demos showed images of bladder cancer with conventional endoscopy and the new imaging technique. He described optical systems that were tried with conventional endoscopy that were similar to the diagnostic autofluorescence endoscopy (DAFE) system and the Xillix MicroImager system and discussed the significant advantages offered by his new approach. Through use of different wavelengths of light, deeper subsurface imaging at higher resolution was achieved. Using these prototypes, Dr. Demos showed images from laparoscopic surgery and contrast agents in bile ducts and arteries.

Clinical issues include improvements in specificity for localized areas, which are encouraging from investigations using auto-fluorescence microscopy. Design features for improving imaging with micro-endoscopy include single-pulse laser, ultraviolet excitation, multi-image acquisition, and structured illumination. Each of these could be used in concert with conventional endoscopy. The goal will be to build technology that does everything at once in real time and noninvasively.

Discussion

Dr. Matthews asked if clinicians could use this method. Dr. deVere White said that these methods could be used with endoscopy, with the advantage of not having to use dyes or special equipment. They could be useful in many procedures, including resections. One participant said he has seen similar methodologies used in Europe, and the advantage is that the extent of the tumor or diseased tissue is shown. Dr. deVere White interjected that the European methods only show surface features but no depth. They also require the use of a contrast agent. Dr. Demos added that his methods are very utilitarian because they only require the use of a conventional endoscope. Additional comments regarding the usefulness of the process were made.

A participant asked if there is a disconnect between the contrast-agent supporters and this new approach to imaging, which does not use contrast agents. Dr. Demos responded that it is difficult to build these machines, and often the biology-oriented researchers have difficulty in becoming excited about the new technologies. The benefit is that instead of having to look for tumors, use of this technology allows the tumor to light up so it can be readily seen.

Dr. Matthews asked if there is a need in the urologic and nephrologic communities for *in vivo* pathology techniques. Dr. Chang added that she wanted to know if there is a role for this technology in IC. A participant said that one of the difficult things when training residents is teaching them to recognize subtle changes in structure or in the matrix that are characteristic of IC. Dr. Chancellor described IC as a disease of hypersensitivity in the bladder area or other areas near the bladder. In diagnosing IC, the bladder is drained; in most cases there are specific glomerularizations and hemorrhages present. IC appears to be a condition of hypersensitivity, although there is no clear pathology or diagnosis.

In Vitro **Multiplex Detection of Serum Protease Activity Using Bioluminescence**

Stephen Lane, Ph.D., UC Davis and Lawrence Livermore National Laboratory

Dr. Lane described a biophotonics project that may result in a more accurate measure of prostate-specific antigen (PSA). He described the multidisciplinary team that is working together on the project in a collaboration among Stanford University, Livermore National Laboratory, and UC Davis.

Dr. Lane discussed an example of bioluminescence based on the luciferin-luciferase enzymatic reaction that is responsible for the light produced by fireflies. Luciferase has been used to track specific cells for cancer, gene expression, and drug discovery studies in transgenic animals. As part of a project funded by the NSF Center for Biophotonics, researchers alter luciferin to extend the bioluminescent imaging times.

In the past few years, the focus of the project has been on producing an assay for protease enzymes by using luciferin with an attached peptide sequence specific for certain protease enzymes. Luciferin-peptide conjugates have been developed that are specific to the protease enzyme PSA, which has led to the development of a new protease assay for PSA. The amount of light produced has been correlated to specific PSA levels. Current work is aimed at making the test more specific and more sensitive. The hope is that if specificity for active protease can be improved, this method can be used to more accurately assess prostate cancer. (Current antibody-based assays respond to inactive, fragmented, and complexed PSA as well as the active form.) Preliminary tests have been encouraging.

Using the methods developed for the PSA test gives encouragement for future development of protease panels for use in many diseases and conditions that are characterized by known proteolytic enzymes.

Discussion

Dr. Matthews asked if there are urologic or kidney diseases that have proteolytic activity. Dr. Lane responded that it was likely and he would get the specifics.

Advances in Separation
David Clague, Ph.D., California Polytechnic State University

Dr. Clague described the biomedical engineering program at the University of California Polytechnic State University (Cal Poly), which has a strong tie to the biomedical industry through St. Jude Medical. His work with the Defense Advanced Research Projects Agency (DARPA), U.S. Department of Defense, focused on separation preparation. DARPA was interested in knowing what sample preparation requirements and research needs exist for clinical and forensic analysis.

Research at Cal Poly involves separation technologies related to renal function through development of a multifunctional dialysis membrane. Artificial membranes are not perfect but could add much to the science of kidney dialysis if they can be developed. Challenges include developing membranes that filter out specific molecules without having them contact the membrane; this is first a manufacturing problem, then an engineering problem. The current approach is to have fluid pass through a microfiltration system before encountering the dialysis membrane. Dr. Clague described some of the applications of microfluid and separation technologies that have defense and anti-bioterrorism applications.

At the Cal Poly microfluidics laboratory, Dr. Clague is working on a disease quantification system using single-cell analysis. Work in this area could be enhanced if his laboratory could develop single-cell impedance spectrometry to assess the degree of biologic exposure.

Discussion

A participant asked how easily molecules can be excluded in membranes. Dr. Clague explained that each species has its own signature based on polarizability. If the DEP spectra have been identified for a species, it is relatively easy to develop a screen for that species. This technique has been used at M.D. Anderson Cancer Center, Houston, TX, to identify breast cancer cells in samples. The spectra and impedance are very sensitive to the conditions.

Combined Time-Resolved Optical/Intravascular Ultrasound Methods for Intravascular Tissue Analysis
Laura Marcu, Ph.D., Biomedical Engineering, UC Davis

Dr. Marcu described her work with fluorescence lifetime spectroscopy and imaging techniques for diagnosis in tissues. Fluorescence can be either endogenous (structural proteins, enzyme cofactors, etc.) or exogenous (fluorescent molecular probes).

Fluorescence spectroscopy and imaging provide information about biochemical, functional, and structural changes of bio-molecular complexes in tissues that occur as a result of either pathological transformation or therapeutic intervention. As fluorescence-based devices allow light delivery and collection using fiber optic probes, they can facilitate non-invasive or minimally invasive investigations of tissues with catheters or endoscopic probes and enhance the diagnostic capability of traditional clinical devices. In principle, steady-state (spectrally resolved) and time-resolved (lifetime) fluorescence spectroscopy techniques can be employed for

retrieving quantitative and qualitative information with respect to the compositional makeup and pathophysiology of any biological system.

The use of time-resolved fluorescence for biological systems studies offers several distinct advantages:

(1) The measurements are sensitive to various parameters of the biological microenvironment (including pH, ion concentration and binding, enzymatic activity, and temperature), thus allowing these variables to be analyzed.
(2) Biomolecules with overlapping fluorescence emission spectra but with different fluorescence decay times can be discriminated.
(3) A complete fluorescence emission spectrum (steady-state) can be obtained by recording the time-resolved fluorescence emission at a number of wavelengths across the emission spectrum. Numerous studies have suggested that the use of fluorescence lifetime not only improves the specificity of fluorescence measurements but also has potential for a more robust evaluation of data collected in the clinical environment.

Despite these recognized inherent advantages, the potential value of fluorescence lifetime information has not been broadly implemented in clinical settings due to barriers including complexity of instrumentation, lengthy data acquisition and analysis, and high instrumentation costs.

Research in Dr. Marcu's lab addresses needs related to the translation of the fluorescence lifetime techniques into clinical settings including: (1) construction of two clinical-compatible systems based on time-resolved laser-induced fluorescence spectroscopy (TR-LIFS) and fluorescence lifetime imaging microscopy (FLIM) techniques, (2) data acquisition and analysis methods conducive to near–real time tissue diagnosis based on fluorescence data, and (3) development of multimodal diagnostic platforms including optical and ultrasonic detection.

Examples of applications of these techniques were presented. They include the following:

1. A human study of carotid plaques in 65 patients that indicates that TR-LIFS may be useful for lesion classification and detection of features involved in plaque vulnerability (rupture).
2. A human study in 17 patients that demonstrates that TR-LIFS has the potential to evolve into a spectroscopic/image guided surgery technique for intraoperative detection of brain margins or infiltrations.
3. A more recent study to validate the applicability of FLIM for delineation of oral carcinoma. A study in hamster oral carcinoma *in vivo* was presented. Studies indicate that FLIM can distinguish between normal tissue and diseased tissue. Discrimination has been high in these studies.
4. The use of a hybrid system including TR-LIFS and ultrasound backscatter microscopy (UBM) also is being developed, and currently the hybrid system is validated on *ex vivo* human atherosclerotic plaque and in the hamster/oral carcinoma model. Current results show the importance of developing synergy among complementary technologies to provide an intersection between structural/anatomic and biochemical/functional factors.

Discussion

A participant asked for a summary of how these technologies have been useful. Dr. Marcu said that the images can show different structures, for example to identify basement membrane and other important structures. These technologies, however, must be used carefully.

PANEL DISCUSSION—PANELISTS INCLUDE SPEAKERS AND INVITED PARTICIPANTS

David Clague, Ph.D., California Polytechnic State University; Don Cohen, M.S., Vandolay, Inc.; Luiz Da Silva, Ph.D., Consultant; Dipankar Ganguly, M.S., BioTelligent; Steve George, M.D., Ph.D., University of California, Irvine; and Jim Trebes, Ph.D., Lawrence Livermore National Laboratory

Dr. Matthews introduced the panel session and said that this will be the most important part of the meeting—summarizing what has been learned today and putting technologies together with medical needs. The format of the discussion will be open but directed toward specific areas that relate to the goals of the meeting. The following is the resulting summary, provided in outline form by area of medical need.

BPH/Enlarged Prostate

- Diagnostic tests to rule out cancer with better, less invasive imaging for diagnosis and staging, such as development of the Smart Needle (BioTelligent).
- New, improved therapy, such as a biocompatible, comfortable stent; current technology produces pain and stone formation. Of critical importance is development of treatments that do not lead to sexual dysfunction.

Overactive Bladder

- Development of a biomarker for overactive bladder. Potential biomarkers include nerve growth factor (NGF), which is a candidate for overactive bladder and IC; the cytokine INF for cancer and overactive bladder; and interleukin-6 and antiproliferative factor for IC.
- Treatment of IC. Issues include diagnosis by cystoscopy and development of a slow-release therapy.
- Point-of-care test for INF. Issues include determining if overactive bladder is caused by a pathogen, what antibiotic to use, and drug resistance.
- Noncancer assessment of bladder structure and function, including biomarkers for assessment.
- Urge/frequency incontinence research for neurostimulation. Guidance using non–x-ray technology to address questions such as whether we can help the probe/neurostimulator find the foramen (e.g., optical coherence tomography [OCT] or ultrasound).
- Research is absent on issues of mixed incontinence (i.e., stress + urge). What new ideas for topics can we develop?
- Development of a wearable tibial nerve stimulator using a sensory glove or other technology.

- Research on neurogenic disorders (e.g., spina bifida), with possible understanding of the use of glue in nerve anastomosis, and a way to determine motor versus sensory nerves (i.e., an imaging system for nerves, this could possibly be done using Raman spectroscopy).

Urinary Tract Infection

- Need a point-of-care device for immediate diagnosis of urinary tract infections. Suggested ideas include a monitor system for detecting bacteria in catheters, as well as for drug delivery in catheters for compliance with prescribed dosages of medications.

Incontinence

- Development of stent technology (e.g., SMPs).
- Sensor-activated sphincter functionality.
- Better means to treat sphincter strength or reinforce with collagen or other artificial tissue or stem cells.
- Better catheters.
- Evaluation using indwelling urodynamic measurement.

Neuromodulation to stimulate voiding

- Research on function and control via the pudendal and sacral nerves.

Sexual dysfunction

- Research on vulvodynia, both sexual- and IC-based. Suggestions were for a quantitative, glove-based palpometer, with an easy readout.
- Pelvic floor pain (sexual- and IC-based and prostatitis)
- For male sexual dysfunction, research is needed for guidance of surgery to avoid nerves and for neuromodulation to create erection. Unanswered questions include: (1) How much impotence is vascular versus nerve damage? (2) Can we distinguish between motor versus sensory nerves? and (3) What are the issues regarding local vasodilators and local neuromodulators?
- For female sexual dysfunction, research issues need to concentrate on which nerves to focus on for stimulation—pudendal or other nerves—and which contribute to the dysfunction.

Stones and more stones

- A presymptomatic detection/diagnosis technique is needed to determine if there are biomarkers or microcrystals present in urine samples.
- Better methods to remove stones and prevent re-formation are needed.
- Catheter technology is needed to reroute within the ureter temporarily, yet it should be biodegradable or signal that it's time for it to be removed.
- Is it possible to change where calcium is going (e.g., to the gut instead of the ureter)?
- Is it possible to screen for hypercalciuria and/or oxalate?

SBIR funding

- How can we fund SBIR projects in the areas we've identified, especially because funding at NIH is primarily incremental?
- There is a need to develop science and technology that will grab the attention of policymakers and funders.

SUMMARY AND CLOSING REMARKS

Dennis Matthews, Ph.D., UC Davis; Debuene Chang, M.D., NIDDK

Dr. Matthews thanked everyone for attending and participating in lively discussions of the issues. He informed the group that the summary report will be developed, and each speaker will have an opportunity to review his/her section before it is submitted to NIDDK. This meeting, as one of a series of regional meetings, will add to the recommendations for future research or for potential RFAs and PAs issued by NIDDK.

Dr. Matthews asked speakers to submit copies of their presentation slides so they can be placed on the NIDDK website. With that, he closed the meeting and bid everyone a safe trip home.