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NIST and Standards for Tissue Engineered Medical Products

Reference: Tesk, J.A., and Karam, L.R**., "NIST and Standards for Tissue Engineered Medical Products,"** *Tissue Engineered Medical Products (TEMPS), ASTM STP 1452,* E. Schutte , and G. L. Picciolo, and D. S. Kaplan, Eds., ASTM International, West Conshohocken, PA, 2003

Abstract: On June 13-14, 2001, the National Institute of Standards and Technology (NIST) convened a workshop of high level representatives from industry, federal agencies, and standards organizations to identify standards-related needs of the biomedical materials and devices industry. There were individual breakout sessions on standards for: Biomaterials, Therapeutic and Drug Delivery Devices, Auditory Diagnostic and Prosthetic Devices, Manufacturing of Prostheses, and Tissue Engineered Medical Products (TEMPs). Cross-cutting issues of Harmonization of Standards, Data, and Sterilization were also addressed. The session on Standards for TEMPs placed its most significant needs on the development of test methods and materials characterization. Action items for NIST to consider were separated into those relative to the Food and Drug Administration (FDA) approval process and those relative to industry needs not directly related to the FDA approval process. This paper summarizes the needs identified for TEMPs and the status of NIST-related activities.

Keywords: standards for tissue engineering, TEMPs, test methods for tissue engineering, measurements for tissue engineering, scaffolds, cell-material interactions, measurement technologies, characterization of surfaces, characterization of materials, characterization of cells, NIST, standard reference materials, SRM, DNA, bioinformatics.

Introduction

TEMPs are products that may use scaffolds, alone or in combination with biological components such as proteins or cells. From the enormous number of issues that had

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evolved from the ongoing ASTM effort on standards for TEMPs, it became apparent that there were many opportunities for NIST to make unique contributions to the standards processes. During the TEMPs breakout session of the 2001 NIST workshop, significant emphasis was placed on the need to develop new test methods and methods for characterizing biomaterials [1]. The group determined that NIST could play a meaningful strategic role and overwhelmingly urged NIST to take a leadership position, working with collaborators in industry, the National Institutes of Health (NIH), FDA, clinicians, and standards organizations. A list of action items was developed and the group suggested appropriate roles for NIST in carrying out those actions. It was, and is, NIST's intent to respond to these calls for action, consistent with its mission and resources. This paper presents current NIST activities that are relevant to the action items that were identified.

Actions Identified for Standards for Tissue Engineered Medical Devices

The following is a complete list of the actions that the medical-device-industry workshop participants identified as those most needed. Detailed elucidation of the roles suggested for NIST and others, the kinds of standards needed (consensus standard, standardized test method, standard practice), priorities, and underlying rationale, is given in the NIST Internal Technical Report, NISTIR 6791 [1].

High-Priority TEMP Action Items Addressing Industry Needs Relative to NIST and the FDA Approval Process

- 1.1 Develop rapid, highly-accurate, phenotypic determination methods (also see 2.1 & 2.2).
- 1.2 Develop methodology to characterize human-animal co-cultured TEMPs, (particularly with respect to determining the percentage (number-fraction) of each cell type).
- 1.3 Develop criteria for assessing the persistence of degradable material.
- 1.4 Develop non-invasive imaging methods.
- 1.5 Pool information useful for facilitating approvals of TEMPs by the FDA.

The High-Priority TEMP Action Items Addressing Industry Needs Relative to NIST

- 2.1 Develop test methods for phenotype analysis and characterization.
- 2.2 Develop stem-cell analysis/characterization methods. (This is a separate, special consideration of action item 2.1 .)
- 2.3 Develop consensus test-standards for temporary components of TEMPs; cells, bioactive agents and solid phase materials.

- 2.4 Identify or develop standards for preservation of cells.
- 2.5 Develop biomarkers for the identification of potentially infectious agents or xenogenic cells from co-cultures that are employed in the manufacture of TEMPs
- 2.6 Develop *in vitro* safety and efficacy assays for individual components of a final TEMP.
- 2.7 Develop standard, non cell-based safety and efficacy assays (e.g. *in vitro* calcification model) and reference materials for detecting residuals on TEMPS.
- 2.8 Develop genomic/proteomic assessments.

NIST Activities Relevant to Workshop-Identified Action Items

Research Addressing Action Items 1.1, 2.1 and 2.2

Measurements for Gene Responses to External Signals

Recently, specific genes have been shown to express different responses to the extracellular matrix. These genes produce proteins that guide cellular functions. Ongoing NIST research is directed toward coupling of fluorescent proteins to genes and utilizing fluorescence for specific indications of cell states and responses. These methods may be employed for tracking cellular activity and responses such as apoptosis, state of differentiation, proliferation, and adhesion to surfaces. This research has relevance to items 1.1, 2.1 and 2.2 (contact: Anne Plant, 301-975-3124, anne.plant@nist.gov). In a related activity, NIST is working on the development of measurement models and a concentration standard that consists of fluorescein immobilized on the surface of microspheres (contact Adolfas Gaigalas, 301-975-2873, adolfas.gaigalas@nist.gov).

Actions with Relevance to Action Item 1.5

Pooling of Data for Facilitating FDA Approvals

This action item called for the pooling of information useful for facilitating approvals of TEMPs by the FDA. Pooling of data presents an enormous challenge and involves not only data from biomaterials but also bioinformatics. Interoperability issues are involved that require consistent identifiers (labels or tags) of data along with software systems capable of accessing the data and extracting or exporting it for use in many other applications. Many activities at NIST support these kinds of efforts, but more extensive cooperation among all users in the health care community is needed. On August 1-2, 2002, NIST held a workshop, “Information Technologies for Healthcare: Barriers to Implementation”, as the first of a series of workshops aimed at medical informatics (contact Dr. Ram Sriram, 301-975-3507, ram.sriram@nist.gov, or Lisa Carnahan, 301-975- 3362, carnahan@nist.gov). NIST also co-sponsored the September 19-20, 2002, NIH-FDA workshop “Medical Implant Information, Performance, and Policies (NIST contact: John Tesk, 301-975-6799, john.tesk@nist.gov). These workshops are relevant to

item 1.5. They are among ongoing efforts across NIST aimed at bringing together stakeholders to address issues that will impact on the entire health care community, including tissue engineering. Details on other action items and issues may be found on pages 29-35 of NISTIR 6791; they are too numerous to be related here.

Research that Addresses Action Item 2.3

Measurements of Cell Responses to Material Surfaces

NIST is currently investigating the use of combinatorial methods for the development of test methods for the rapid assaying of cell responses to material surfaces. Gradients in material properties and features provide continuously varying physical, chemical, or biological characteristics, which induce varying degrees of cell responses. Both material characterization and cellular response are part of the program. Materials characterizations are carried out in the Polymers Division of NIST (contact Newell Washburn, 301-975-4348, newell.washburn@nist.gov). The cell responses will be assessed within NIST's Biotechnology Division. NIST and ASTM are planning an October 2003 workshop on "Metrology of Cell Signaling and its Impact on Tissue Engineering" (contact: Anne Plant, 301-975-3124, anne.plant@nist.gov). A poster on this effort (Washburn et al.) can be found elsewhere in this symposium.

Imaging of Cells

At the NIST Center for Neutron Research (NCNR) in Gaithersburg, MD, a collaboration of university and government scientists will use super-chilled neutrons to probe the structure and interactions of cell membranes and their components, gathering information that is key to improving disease diagnosis and treatment. Enhanced understanding may help guide the design of new tissue-engineered medical devices. "Neutron probes offer the only realistic hope for many challenges in medicine and biology," says Stephen White, the University of California Irvine (UCI) biophysicist who leads the Cold Neutrons for Biology and Technology (CNBT) partnership. White has organized the CNBT partnership, which includes researchers from UCI, NIST, the University of Pennsylvania, Rice University, Carnegie Mellon University, the Duke University Medical Center and the Los Alamos National Laboratory. The CNBT team is now building a unique instrument with dual capabilities: diffractometry and reflectometry. To be completed in 2003, this instrument will provide cell membrane scientists with access to powerful technologies well beyond those available from the resources of individual researchers. For more information on CNBT, go to www.nist.gov/public_affairs/releases/neutrons.htm. For technical matters contact Stephen White, UC-Irvine, 949-824-7122, SHWhite@uci.edu or Susan Krueger, NIST, 301-975-6734, susan.krueger@nist.gov.

Characterization of Scaffolds

Another research project involves measurement technology for the characterization of porous scaffolds and the development of reference tissue scaffolds. Well-characterized scaffolds (materials composition, structure, surfaces, porosity, and mechanical properties) and the measurement technologies by which they are characterized were requested also by Picciolo et al. in the July 2000 NIST workshop, "Reference Data for the Properties of

Biomaterials" [2]. Reference scaffolds can be employed for validating procedures and for providing materials for specific, known cellular responses against which new materials may be checked in the course of development of new TEMPs. For more on this topic refer to a paper earlier in this session from NIST (Washburn et al.) and one from the National Physical Laboratory and University of Brighton, Middlesex, United Kingdom (Mikhalovsky et al.). There is also a relevant poster at this symposium from NIST by Cicerone et al. on functional imaging of cell-material interactions.

Standards and Measurements for Mitochondrial Proteomics

NIST recently (September 17-18, 2002) held a workshop, "Systems Biology Approaches to Health Care: Mitochondrial Proteomics," that addressed underlying proteomic measurement technologies for detection and diagnosis of disease. Because the human mitochondrion is central to basic life functions for the generation of cellular energy, and as such is the site of key components of the biosynthetic pathways, as well as the cellular decision points leading to apoptosis (i.e., programmed cell death), the underlying measurement technologies have particular relevance to TEMPs. Since the mitochondrion is a discrete subcellular organelle with a non-nuclear genome that is comprised of about 1000 or more different protein species with tissue-specific features, it can be a tangible model system for the development of clinical standards with direct health care implications. The focus of this workshop was to provide: (1) Assessment of the mitochondrion as an integrated model for systems biology studies, (2) Assessment of emerging proteomics technologies, (3) Identification of the standards needs for proteomic applications in the clinical diagnostics industry and, (4) Guidance for determination of appropriate data elements (common data elements, or CDEs) for health care proteomics (contact Peter Barker, 301-975-5402, peter.barker@nist.gov or Gregory Vasquez, 301-975-4195, gregory.vasquez@nist.gov).
The web site is: http://www.cstl.nist.gov/biotech/mito/mitoproteomics.html

Test Methods and Standard Reference Materials for DNA Sequencing

Human mitochondrial DNA (mtDNA) has become an important tool in forensic and medical studies as well as anthropological and evolutionary research. NIST has a long history of working with the medical communities and helping in the development of test methods and Standard Reference Materials (SRMs) to provide quality control and assurance that scientific and testing results are accurate [3]. NIST has available a Standard Reference Material (SRM 2392) to provide quality control for the amplification and sequencing of human mitochondrial DNA (mtDNA) or any DNA [4]. One of the reasons that SRM 2392 was developed is to provide assurance that the diagnoses of mtDNA diseases being conducted by the medical community were correct. The reference material also is useful for assessing the quality of cells and tissues that may be used for TEMPs. NIST also has developed a simple methodology to detect low levels (number fractions of 0.1 % to 20 %) of the single base pair heteroplasmic mutation MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) (A3243G) in total DNA extracted from blood [5]. This methodology could be useful in the detection of other low frequency hetroplasmic disease mutations. Barbara C. Levin was an organizer and speaker at the Mitochondrial Standards Workshop held in Dallas Texas in June of 2002 (contact Barbara Levin, 301-975-6682, barbara.levin@nist.gov).

Human Serum Proteomics Standards and SRM 1951a

NIST is currently evaluating SRM 1951a with six outside labs as a prototype proteomics standard for human serum. This SRM is available as a lipid reference, but because the processing that was done for producing SRM 1951a was "minimal" as far as affecting other proteins, it is considered as having a protein composition that is very close to that found in human serum. Hence, it is considered as a viable candidate reference standard for other proteins. This is the first "proteomics standard" in actual field trials and will be discussed as a prototype proteomics standard at a meeting in Europe in November 2002. The lead NIST researcher, Peter Barker, chaired the Human Proteomics Standards session at the organizational meeting of the Human Proteome Organization, that was held in September 2002. (contact Peter Barker, 301-975-5402, peter.barker@nist.gov or Michaael Welch, 301-975-3100, michael.welch@nist.gov).

Research that Addresses Action Items 2.5, 2.6, and 2.8

Biomarkers for Inflammatory Responses to Polymer Substrates

NIST has research under way aimed at the development of measurement technologies that may be used to assess the cellular mechanism of how cells react to inflammatory stimuli from different polymer matrices. Rodriguez has noted that it is important to have biological safety check points in place during the manufacturing process of TEMPs [6]. NIST researchers are identifying biomarkers that have the ability to reveal oxidative damage to genomic DNA, mitochondrial damage, mutations, and chromosomal loss. Rodriguez has a poster at this symposium that describes NIST research comparing genomic and mitochondrial markers that can be used to detect cellular inflammation in tissue- engineered skin (contact Henry Rodriguez, 301-975-2578, henry.rodriguez@nist.gov).

Heightened Sensitivity to DNA Malignant Cells-NIST researchers Catherine O'Connell and Henry Rodriguez have been working on the detection of cellular mutations so as to identify malignant cells using recent technological advances in mutational scanning and chip technologies. These advances reduce the region of a gene that needs to be sequenced. This is a less time consuming and less costly process than the most commonly utilized method of DNA sequencing across the entire genome (the "gold standard"). At this symposium, a poster entitled "Molecular Biomarkers Used to Detect Cellular Genetic Damage in Tissue-Engineered Skin" describes this work in more detail. (contact Catherine O'Connell, 301-975-3123, catherine.oconnell@nist.gov).

Measurements for Assurance of Disease-Free Cells and Validation of Biomarker Assays

NIST has established an ongoing collaboration with the Cancer Biomarkers Group, Division of Cancer Prevention at the National Cancer Institute. One validation study involves quantification of chromosomal breaks within a specific chromosomal region. In normal peripheral lymphocytes from short-term cultures, induced breaks in this region have been shown to correlate with susceptibility to lung cancer. These validation and other technology evaluation activities support a large collaborative team of 35 discovery laboratories, eight clinical and epidemiological centers, and two other biomarker

validation laboratories, all focused on improving early detection of solid tumors in the American public [7]. This work, when extended to other cells and tissues, may find applications for helping to ensure the use of disease-free cells for TEMPs. (contact Peter Barker, 301-975-5402, peter.barker@nist.gov).

Research that Addresses Action Item 2.7

Tissue-engineered Biomaterial Surface Structure Characterizations

NIST has a project underway that utilizes nonlinear and linear optical methods for determining the properties of interfaces and of molecules found at the surfaces of materials. The materials include those that are important to tissue engineering. The measurement methods include vibrationally resonant sum frequency generation (SFG) spectroscopy. This method can provide surface specific measurements that exclude bulk effects and focus exclusively on the structure of molecules localized at the surface. Hence, this unique tool is a powerful addition to the armamentarium that is needed for making highly tailored surfaces for directing the responses of cells, localizing proteins, or attaching drugs in combinatorial medical devices. (contact Kimberly Briggman, 301-975-2358, kimberly.briggman@nist.gov, John Elliott, 301-975-8551, john.elliott@nist.gov, or John Stephenson, 301-975-2372, john.stephenson@nist.gov).

Summary

The activities described here are standards-related needs/recommendations made at NIST workshops. They represent a small, but significant, fraction of NIST health-care-related research. For more information, the reader is encouraged to visit the NIST Industrial Liaison Office web site: http://www-i.ilo.nist.gov/ and click on“Health Care”.

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