Workshop Summary: "ASTM Scaffolds Workshop - Standards & Measurements for Tissue Engineering Scaffolds: What Do We Have & What Do We Need?", May 21, 2013, ASTM Standards Week, Indianapolis, IN

Date Workshop Summary Was Completed: August 15, 2013

Workshop Organizing Committee: Carl Simon (NIST), Michael Yaszemski (Mayo Clinic), Anthony Ratcliffe (Synthasome), Paul Tomlins (European Standards Consultant), Reto Luginbuehl (RMS), John Tesk (Consultant)

Sponsoring ASTM Committees: F04.04 (Tissue Engineered Medical Products) & F04.42 (Biomaterials & Biomolecules for TEMPs)

Link: http://www.astm.org/F04Wrshhp0513.htm

Summary: The objective of the workshop was to identify the highest priority items for future standards work for scaffolds for tissue engineered medical products (TEMPs). The workshop was attended by 79 participants and highlighted many potential areas for scaffolds standards activity within the membership of ASTM Committee F04. The key next step is to discuss and prioritize the standards needs at the sub-committee level so that task forces and working groups may be initiated to develop the documents. A key finding is that the TEMPs groups have many guide documents for educational and advisory purposes, but that very few standard tests or procedural methods have been written. Overwhelmingly, the most clearly identified need was standards for measuring scaffold structure. The second was standards for biological characterization including in vitro testing, animal models and cell-material interactions. Standards for scaffold mechanics, degradation, reference materials, composition, clinical outcomes, reporting, fixation and sterilization effects were also discussed. Below, the needs identified are listed in a bulleted fashion. The needs are roughly ordered in terms of which ones received the most discussion, where items receiving more discussion will come first. A number of discussion items that were not strictly standards needs are presented at the end of the report. Many scaffold standards needs have been identified and the real challenge of writing those standards is ready to begin.

1. Standards Needs

1.1. Scaffold Structure: The greatest need identified at the workshop was for standard *methods* for assessing scaffold structure. Many *guides* exist for solid scaffolds (polymers, ceramics, metals) that identify measurement methods that can be used for obtaining porosity, pore size, fraction of open cells, pore uniformity, pore size distribution, pore connectivity, etc. However, there is a strong need for consensus *methods* that instruct users on how to specifically perform these measurements on a specific type of scaffold.

- Standard methods for measuring porosity, pore size, fraction of open/closed pores, pore uniformity, pore connectivity, permeability, pore size distribution

- Uniformity of nanofiber scaffold fiber diameter, pore size and nanofiber alignment; nanofiber scaffolds have become very important in the past several years
- Standard methods for describing the shape and size of irregular/asymmetric pores, when do connections become large enough to be considered pores?
- Strong need for 3D methods, since scaffolds are 3D and 3D structure is important for providing the proper microenvironment for cells and for guiding regeneration
- Standards for assessing uniformity of printed scaffold structure, identifying structural defects
- Methods are needed for assessing 3D scaffold structure at 1 μm resolution since this size scale is the most relevant for guiding cell function
- Standards in addition to ASTM F2122-11 are required for collagen for assessing collagen microstructure, which is critical for directing cell function and tissue regeneration in matrix-guided therapies
- Need guides/methods for measuring structure for different types of scaffolds including solid polymer, metal or ceramic scaffolds, hydrogels, fiber-spun, self-assembled, etc.
- A task force is needed to develop a guide for defining measurement hierarchies; the number of measurements that should be made to uniquely characterize scaffolds and differences between scaffolds; too many measurements will raise the costs unnecessarily; what measurements are needed for archival publication versus regulatory purposes?
- Standards for BET (Brunauer–Emmett–Teller) gas adsorption methods for characterizing scaffold surface area are needed since this approach can be sensitive to small pores (< 1 μm) not detected by other approaches

1.1.2. Comments:

- Need methods that are simple and low cost
- μCT can be effective in some cases, but it is very expensive
- Histology is very slow and could not be used on a production line
- Confocal microscopy is effective but quantitative methods for 3D image analysis are currently difficult to implement
- Collinear optical coherence has potential but is difficult to implement and is expensive
- Time/cost improvements on measurement methods would be helpful: confocal, μCT, collinear optical CT

1.2. Scaffold Toxicity, Biocompatibility & Cell-Material Interactions: This was the second most discussed area for standards needs for scaffolds.

1.2.1. In vitro

- Guides and methods are needed for measuring cell seeding, morphology (in 3D and at 1 μm resolution), viability, adhesion, proliferation, migration, differentiation and cell distribution in 3D scaffolds
- Guides and methods for measuring cell-scaffold interactions are required, such as cell-collagen spatiotemporal interactions which are critical for matrix guided therapies

- A standard is needed for measuring the toxicity of catheters to cells which is an issue when using a catheter to deliver cells to a patient during a cell therapy; cells pumped into catheter at low shear, let them reside in catheter for 5 to 30 min, then elute and do tests such as cell adhesion, morphology, cell volume, proliferation
- Guides/methods needed for measuring ECM development in scaffolds, such as MRI for the amount of proteoglycans and collagen, or to quantify tissue functionality via molecular motion and tissue anisotropy
- Standard test for monomer toxicity
- Standard test for toxicity stemming from higher localized drug concentrations released from drug-loaded scaffolds
- Guides/methods/models for in vitro assessment of tissue regeneration that can be used for testing cell therapies and scaffolds
- Methods for measuring transmission of mechanical signals to cells
- Standards for collinear optical coherence and confocal fluorescence microscopy for 3D characterization of cell state in hydrogels
- Standard method for cell-adhesion assay using centrifuge (for stem cell isolation)

1.2.2. Animal

- Methods are needed to non-invasively map tissue growth and functionality
- Standards are needed for MRI methods to separate scaffolds from cells & ECM (relaxation, diffusion)
- Animal model standards are needed for *specific* clinical applications, such as the goat model under development for cartilage repair and articular cartilage fixation

1.2.3. Comments

- Standards activities identified must be carried out in the appropriate international committees so that there will not be a duplication of effort; must prevent overlap with the ISO 10993 series
- All of the above are required for different types of scaffolds including solid scaffolds, hydrogels and natural material scaffolds

1.3. Scaffold Mechanics: The following are needed for all types of scaffolds, especially hydrogels.

- Standard methods are needed for measuring mechanics of all types of scaffolds, including hydrogels (storage modulus), solid scaffolds, self-assembled scaffolds and natural material scaffolds
- Methods for measuring physical and chemical responses of hydrogels, polymer gels, physical gels, etc., to environmental stimuli (for example, changes in temperature or external stresses)
- Methods for assessing hydrogel polymerization kinetics, swelling, uniformity and residual stresses on global and localized levels
- Methods for measuring articular cartilage mechanics (compressive, tensile)

- Methods for assessing collagen mechanics and polymerization kinetics; what do these mean at microscales relevant to cells, especially for a material that is inhomogeneous and mostly water; what are the most appropriate mechanical models for either global or local stresses in response to externally applied loads
- For natural material matrices, including de-cellularized extracellular matrices and alginate gels, need methods for assessing batch-to-batch variability and differences in species and tissue source; consistent collagen properties are a challenge since commercial collagens are not designed for strength

1.4. Scaffold Degradation

- Standard guides and methods are needed for measuring 1) polymer degradation and 2) polymer degradation under dynamic loading and physiological conditions (temperature, aqueous, salt, pH, enzyme-mediated)
- Methods are needed for hydrogel degradation, measuring hydrogel leachables and degradation products
- Methods are needed for measuring cartilage degradation

1.5. Reference Materials

- Reference scaffolds are needed for interlab comparison and tests on new materials should be calibrated against the reference material
- Reference scaffolds are needed for traceability of measurements, especially as the basis for making secondary references that may be used routinely for research and product quality assurance (translates to lower expense)
- It was noted that reference scaffolds from NIST are too expensive for routine use and a task force should explore how reference scaffolds can be produced at lower cost and more appropriately for industry needs
    - Could reduce use of NIST RM's by comparing on a batch basis, only use the NIST RM's to validate new batches
- A 2D cell culture reference material is also needed for routine use like tissue culture polystyrene
    - Would need 100000 units
    - Take samples off the line and characterize for surface roughness (atomic force microscopy) and composition (X-ray photoelectron microscopy)
    - Should be inexpensive, widely available
    - An alternative would be reference material that is used sparingly to validate batches, and not used as a consumable
- Reference scaffolds also needed for MRI to enable cartilage measurements in vivo

1.6. Clinical Outcomes

- Need standards for assessing clinical outcomes including patient-reported outcomes, clinically relevant biomechanical functions, clinical endpoints, imaging articular cartilage and measuring function

- Note that the ASTM Planning Committee has added a Symposium for 2016 to its tentative schedule for Surgical & Tissue Engineering Clinical Outcomes (Greg Brown and Carl Simon, co-chairs)

1.7. Scaffold Composition

- Need standards for measuring scaffold composition including articular cartilage matrix components, bioactive ligands in hydrogels and characterizing collagen composition (different preparations, tissues, species, oligomers)
- A task force is needed to identify whether standard guides or methods should be developed

1.8. Reporting

- Need standards for reporting of scaffolds-based studies including minimal data sets required for characterization
- Need teams of biologists and materials scientists to enable proper material and cell characterization
- Requires efforts to include editors/publishers/granting agencies who would be the enforcers

1.9. Effect of Sterilization: Need standards for assessing the effect of sterilization on scaffold properties (including hydrogels) as well as drug payloads

1.10. Drug release: Need standards for measuring drug release from drug-loaded scaffolds

2. Additional Discussion Items

2.1. Promoting Use of Standards

- The use of standards must be promoted at universities through classwork and instruction
- Must demonstrate value to academics and industry to promote use
- Must have joint symposia with other societies to raise awareness (SFB, TERMIS, AAOS); standards are for all R&D - not just for FDA/regulatory/industry

2.2. Assess Use and Need of Standards for TEMPs

- There is a need to assess the use of standards by the TEMPS community; could be done by database searching (pubmed, patents)
- Need study to assess TEMPS needs for standards - few standard test methods exist globally but many TEMPS are on the market; a cost-benefit analysis is required for ASTM standards through case studies; a pubmed search for “ASTM” and tissue engineering terms yields only 5 hits; majority of TEMPS standards are guides and are unlikely to be cited

2.3. Specific Application: Each scaffold has to be tailored for a specific unmet need; there can be no universal cell or universal scaffold; each device must be application specific

2.4. Must Measure Value Added: Goal should be to add value = QLAY/TCOC; QLAY = quality of life adjusted years; TCOC = total cost of care; you have to valuate to ensure that the treatment both helps the patient and is worth the cost in terms of money

2.5. CLINICAL Is Most Important: BIOmaterials and BioMATERIALs are both incorrect

2.6. Quality Function Deployment: There was a study where the rotator cuff repair tore loose when the patient moved after surgery, yet movement immediately after surgery is mandatory or the shoulder will seize, this was over-looked

- Solution: Should always involve a Quality Function Deployment team (QFD) consisting of surgeons, physical therapists, device manufacturer where "must haves", "should haves", "would like to haves" are identified; the "must have" list should have included that the cuff be sturdy enough for movement immediately after surgery

2.7. DNA Patents: DNA patents will be troublesome for combination constructs that have 2 biologics, will require 2 license agreements

2.8. Standards Can Measure State: How will standards be used for lot release criteria? QC can measure the state of something, does not have to predict efficacy, meeting a standard indicates that it is same as before

2.9. Length Scales: What are the important scaffold-based metrics at different length scales (nano/micro/macro) that influence clinical outcomes?

2.10. Scaffolds Standards Task Force: Need a task force to assemble scaffolds standards resources

2.11. Bedside to Bench and Back: We may need effective mechanisms to get devices to the market quicker, then do longer follow ups, since big issues tend to come up in the long term (issues with metal on metal hips took > 5 years to arise)

2.12. In Vitro vs. In Vivo: Can't do just in vitro studies, must also do animal studies to validate in vitro results

2.13. Interface Fixation: Interface fixation is key to successful implant therapies

3. Future

- This workshop report will be used as an outline for a manuscript that will be published in the archival literature in a relevant journal such as *Biomaterials* or *Journal of Biomedical Materials Research B: Applied Biomaterials*.

- This report has been generated to serve as a resource for advancing future F04 TEMPs standards activities starting immediately and with the November 2013 F04 TEMPS meetings in Jacksonville, FL.

ASTM Scaffolds Workshop
Tuesday, May 21, 2013, 9:00 am to 5:30 pm
Indianapolis, IN (JW Marriott Indianapolis)
Committees F04.04 & F04.42

Title: Standards and Measurements for Tissue Engineering Scaffolds: What Do We Have & What Do We Need?

Organizing Committee: Carl Simon (NIST), Michael Yaszemski (Mayo Clinic), Anthony Ratcliffe (Synthasome), Paul Tomlins (European Standards Consultant), Reto Luginbuehl (RMS), John Tesk (Consultant)

Link: http://www.astm.org/F04Wrshhp0513.htm

Part 1: Introduction (Carl Simon, NIST)
9:00 Jack Parr (Chairman ASTM Committee F04): Welcome & opening comments
9:05 Carl Simon (NIST): Goals of the workshop
9:10 Byron Hayes (Gore): Intro to ASTM documents (Classifications, Specifications, Test Methods, Practices, Guides, Terminology) and existing documents for scaffolds
9:25 Reto Luginbuehl (Robert Mathys Foundation): Guides to characterization methods for scaffolds
9:40 Questions

9:45 Break

Part 2: Needs Revealed by Clinical Research Experience (Ryan Roeder, Notre Dame)
10:15 Michael Yaszemski (Mayo Clinic)
10:30 Tony Ratcliff (Synthasome)
10:45 David Kaplan (FDA)
11:00 Discussion: Gregory Brown (Park Nicollet Health Services)

Part 3: Hydrogels (Paul Tomlins, Consultant)
11:15 Michael Dornish (FMC BioPolymer)
11:30 Kurt Kasper (Rice Univ.)
11:45 Discussion: Michael Dornish (FMC Biopolymer)

12:00 Lunch

Part 4: Solid Scaffolds (Stephanie Norris, Atex Technologies)
1:00 Barbara Boyan (VCU)
1:15 Jed Johnson (Nanofiber Solutions)
1:30 Wing Lau (3D-Biotek)
1:45 Kurt Sly (Exactech)
2:00 Discussion Leader: Barbara Boyan (Ga Tech)

2:15 Posters-on-Laptop Session (this is an opportunity for non-speakers to present abstracts on their personal laptops to small groups of attendees at tables)
- #1 - Sherry L. Voytik-Harbin (Purdue)
- #2 - Mrignayani Kotecha (U Illinois at Chicago)
- #3 - Sanjukta Guha Thakurta (U Nebraska-Lincoln)
- #4 - David Hoelzle (Notre Dame)
- #5 - Masood Machingal (Vanderbilt)
- #6 - Stefani Biechler (Bose)

2:45 Break

Part 5: Natural Scaffolds & Characterization (Eugene Smit, Stellenbosch Nanofiber Company)
3:15 Michael Hiles (Cook Biotech)
3:30 Ted Wakatsuki (InVivoSciences)
4:00 Paul Tomlins (Consultant)
4:15 Joy Dunkers (NIST)
4:30 Carl Simon (NIST)
4:45 Discussion Leader: Jayesh Doshi (Espin Technologies)

Part 6: Conclusion
5:00 Carl Simon: Final discussion
5:15 Warren Haggard (F04.04 Chair): Closing remarks