Designation: F3510 – 21

Standard Guide for Characterizing Fiber-Based Constructs for Tissue-Engineered Medical Products[1]

This standard is issued under the fixed designation F3510; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide is a resource for the characterization of fiber-based constructs intended for use in a tissue-engineered medical product (TEMP). There are existing standards that broadly cover scaffolds in a more generalized fashion (Guides F2150, F2450, F2900, F2902, ISO 21560). This guide focuses specifically on fiber-based constructs.

1.2 Fiber-based constructs may be fabricated by many different methods including, but not limited to the following: electrospinning, forcespinning, meltspinning, pneumatospinning, blowspinning, melt-electrowriting, melt extrusion, wet extrusion, fused deposition, liquid crystal deposition, electrochemical alignment, drawing, spinning, knitting, weaving, braiding, powder bed fusion (laser sintering), vat photopolymerization (stereolithography), binder jetting, directed energy deposition, self-assembly (for example, fibrillogenesis), and hybrid approaches. This document is intended to address fibers made by any of these methods, although electrospun fibers are addressed in greater detail in some sections.

1.3 This guide will focus on constructs made of fibers wherein the average fiber diameter is within the range of approximately 100 nm to 100 µm.

1.4 For the purposes of this standard, a "fiber-based construct" is defined as a construct composed of slender, elongated filaments.

1.5 *Units*—The values stated in SI units are to be regarded as the standard. No other units of measurement are included in this standard.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

C1559 Test Method for Determining Wicking of Fibrous Glass Blanket Insulation (Aircraft Type)

D257 Test Methods for DC Resistance or Conductance of Insulating Materials

D412 Test Methods for Vulcanized Rubber and Thermoplastic Elastomers—Tension

D638 Test Method for Tensile Properties of Plastics

D648 Test Method for Deflection Temperature of Plastics Under Flexural Load in the Edgewise Position

D695 Test Method for Compressive Properties of Rigid Plastics

D790 Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials

D792 Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement

D854 Test Methods for Specific Gravity of Soil Solids by Water Pycnometer

D882 Test Method for Tensile Properties of Thin Plastic Sheeting

D1388 Test Method for Stiffness of Fabrics

D1621 Test Method for Compressive Properties of Rigid Cellular Plastics

D1623 Test Method for Tensile and Tensile Adhesion Properties of Rigid Cellular Plastics

D1708 Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens

D1777 Test Method for Thickness of Textile Materials

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.42 on Biomaterials and Biomolecules for TEMPs.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

D1876 Test Method for Peel Resistance of Adhesives (T-Peel Test)
D1894 Test Method for Static and Kinetic Coefficients of Friction of Plastic Film and Sheeting
D2256/D2256M Test Method for Tensile Properties of Yarns by the Single-Strand Method
D2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics
D3039/D3039M Test Method for Tensile Properties of Polymer Matrix Composite Materials
D3418 Test Method for Transition Temperatures and Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry
D3786/D3786M Test Method for Bursting Strength of Textile Fabrics—Diaphragm Bursting Strength Tester Method
D3787 Test Method for Bursting Strength of Textiles—Constant-Rate-of-Traverse (CRT) Ball Burst Test
D4404 Test Method for Determination of Pore Volume and Pore Volume Distribution of Soil and Rock by Mercury Intrusion Porosimetry
D4496 Test Method for D-C Resistance or Conductance of Moderately Conductive Materials
D4833/D4833M Test Method for Index Puncture Resistance of Geomembranes and Related Products
D6420 Test Method for Determination of Gaseous Organic Compounds by Direct Interface Gas Chromatography-Mass Spectrometry
D6539 Test Method for Measurement of the Permeability of Unsaturated Porous Materials by Flowing Air
D6701 Test Method for Determining Water Vapor Transmission Rates Through Nonwoven and Plastic Barriers
D6797 Test Method for Bursting Strength of Fabrics Constant-Rate-of-Extension (CRE) Ball Burst Test
D7264 Test Method for Flexural Properties of Polymer Matrix Composite Materials
E96/E96M Test Methods for Water Vapor Transmission of Materials
E128 Test Method for Maximum Pore Diameter and Permeability of Rigid Porous Filters for Laboratory Use
E793 Test Method for Enthalpies of Fusion and Crystallization by Differential Scanning Calorimetry
E1868 Test Methods for Loss-On-Drying by Thermogravimetry
F316 Test Methods for Pore Size Characteristics of Membrane Filters by Bubble Point and Mean Flow Pore Test
F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices
F1249 Test Method for Water Vapor Transmission Rate Through Plastic Film and Sheeting Using a Modulated Infrared Sensor
F1306 Test Method for Slow Rate Penetration Resistance of Flexible Barrier Films and Laminates
F1635 Test Method for ***in vitro*** Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants
F1983 Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications
F2027 Guide for Characterization and Testing of Raw or Starting Materials for Tissue-Engineered Medical Products
F2150 Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products
F2212 Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)
F2256 Test Method for Strength Properties of Tissue Adhesives in T-Peel by Tension Loading
F2450 Guide for Assessing Microstructure of Polymeric Scaffolds for Use in Tissue-Engineered Medical Products
F2475 Guide for Biocompatibility Evaluation of Medical Device Packaging Materials
F2477 Test Methods for ***in vitro*** Pulsatile Durability Testing of Vascular Stents
F2529 Guide for *in vivo* Evaluation of Osteoinductive Potential for Materials Containing Demineralized Bone (DBM)
F2603 Guide for Interpreting Images of Polymeric Tissue Scaffolds
F2606 Guide for Three-Point Bending of Balloon Expandable Vascular Stents and Stent Systems
F2664 Guide for Assessing the Attachment of Cells to Biomaterial Surfaces by Physical Methods
F2739 Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds
F2791 Guide for Assessment of Surface Texture of Non-Porous Biomaterials in Two Dimensions
F2900 Guide for Characterization of Hydrogels used in Regenerative Medicine (Withdrawn 2020)[3]
F2902 Guide for Assessment of Absorbable Polymeric Implants
F2952 Guide for Determining the Mean Darcy Permeability Coefficient for a Porous Tissue Scaffold
F2997 Practice for Quantification of Calcium Deposits in Osteogenic Culture of Progenitor Cells Using Fluorescent Image Analysis
F3036 Guide for Testing Absorbable Stents
F3089 Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions
F3106 Guide for ***in vitro*** Osteoblast Differentiation Assays
F3142 Guide for Evaluation of ***in vitro*** Release of Biomolecules from Biomaterials Scaffolds for TEMPs
F3224 Test Method for Evaluating Growth of Engineered Cartilage Tissue using Magnetic Resonance Imaging
F3259 Guide for Micro-computed Tomography of Tissue Engineered Scaffolds
F3294 Guide for Performing Quantitative Fluorescence Intensity Measurements in Cell-based Assays with Widefield Epifluorescence Microscopy
F3369 Guide for Assessing the Skeletal Myoblast Phenotype

[3] The last approved version of this historical standard is referenced on www.astm.org.

2.2 *ISO Standards:*[4]

ISO 2758 Paper—Determination of bursting strength

ISO 2759 Board—Determination of bursting strength

ISO 7198 Cardiovascular implants and extracorporeal systems—Vascular prostheses—Tubular vascular grafts and vascular patches

ISO 9000 Quality management systems—Fundamentals and vocabulary

ISO 9001 Quality management systems—Requirements

ISO 9073-6 Textiles—Test methods for nonwovens Part 6: Absorption

ISO 9277 Determination of the specific surface area of solids by gas adsorption—BET method

ISO 10993-1 Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process

ISO 10993-2 Biological evaluation of medical devices—Part 2: Animal welfare requirements

ISO 10993-3 Biological evaluation of medical devices—Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity

ISO 10993-4 Biological evaluation of medical devices—Part 4: Selection of tests for interactions with blood

ISO 10993-5 Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity

ISO 10993-6 Biological evaluation of medical devices—Part 6: Tests for local effects after implantation

ISO 10993-7 Biological evaluation of medical devices—Part 7: Ethylene oxide sterilization residuals

ISO 10993-9 Biological evaluation of medical devices—Part 9: Framework for identification and quantification of potential degradation products

ISO 10993-10 Biological evaluation of medical devices—Part 10: Tests for irritation and skin sensitization

ISO 10993-11 Biological evaluation of medical devices—Part 11: Tests for systemic toxicity

ISO 10993-12 Biological evaluation of medical devices—Part 12: Sample preparation and reference materials

ISO 10993-13 Biological evaluation of medical devices—Part 13: Identification and quantification of degradation products from polymeric medical devices

ISO 10993-14 Biological evaluation of medical devices—Part 14: Identification and quantification of degradation products from ceramics

ISO 10993-15 Biological evaluation of medical devices—Part 15: Identification and quantification of degradation products from metals and alloys

ISO 10993-17 Biological evaluation of medical devices—Part 17: Establishment of allowable limits for leachable substances

ISO 10993-18 Biological evaluation of medical devices—Part 18: Chemical characterization of materials

ISO 10993-19 Biological evaluation of medical devices—Part 19: Physico-chemical, morphological and topographical characterization of materials

ISO 10993-20 Biological evaluation of medical devices—Part 20: Principles and methods for immunotoxicology testing of medical devices

ISO 10993-22 Biological evaluation of medical devices—Part 22: Guidance on nanomaterials

ISO 11137-1 Sterilization of health care products—Radiation—Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices

ISO 11607-1 Packaging for terminally sterilized medical devices Part 1: Requirements for materials, sterile barrier systems and packaging systems

ISO 11607-2 Packaging for terminally sterilized medical devices Part 2: Validation requirements for forming, sealing and assembly processes

ISO 11737-1 Sterilization of health care products—Microbiological methods—Part 1: Determination of a population of microorganisms on products

ISO 13019 Tissue-engineered medical products—Quantification of sulfated glycosaminoglycans (sGAG) for evaluation of chondrogenesis

ISO 13408-1 Aseptic processing of health care products—Part 1: General requirements

ISO 13408-2 Aseptic processing of health care products—Part 2: Sterilizing filtration

ISO 13408-3 Aseptic processing of health care products—Part 3: Lyophilization

ISO 13408-4 Aseptic processing of health care products—Part 4: Clean-in-place technologies

ISO 13408-5 Aseptic processing of health care products—Part 5: Sterilization in place

ISO 13408-6 Aseptic processing of health care products—Part 6: Isolator systems

ISO 13408-7 Aseptic processing of health care products—Part 7: Alternative processes for medical devices and combination products

ISO 13485 Medical devices—Quality management systems—Requirements for regulatory purposes

ISO 14644-1 Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration

ISO 14644-2 Cleanrooms and associated controlled environments—Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration

ISO 14644-3 Cleanrooms and associated controlled environments—Part 3: Test methods

ISO 14644-4 Cleanrooms and associated controlled environments—Part 4: Design, construction and start-up

ISO 14644-5 Cleanrooms and associated controlled environments—Part 5: Operations

ISO 14644-7 Cleanrooms and associated controlled environments—Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments)

ISO 14644-8 Cleanrooms and associated controlled environments—Part 8: Classification of air cleanliness by chemical concentration (ACC)

[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

ISO 14644-9 Cleanrooms and associated controlled environments—Part 9: Classification of surface cleanliness by particle concentration

ISO 14644-10 Cleanrooms and associated controlled environments—Part 10: Classification of surface cleanliness by chemical concentration

ISO 14644-13 Cleanrooms and associated controlled environments—Part 13: Cleaning of surfaces to achieve defined levels of cleanliness in terms of particle and chemical classifications

ISO 14644-14 Cleanrooms and associated controlled environments—Part 14: Assessment of suitability for use of equipment by airborne particle concentration

ISO 14644-15 Cleanrooms and associated controlled environments—Part 15: Assessment of suitability for use of equipment and materials by airborne chemical concentration

ISO 14698-1 Cleanrooms and associated controlled environments—Biocontamination control Part 1: General principles and methods

ISO 14698-2 Cleanrooms and associated controlled environments—Biocontamination control Part 2: Evaluation and interpretation of biocontamination data

ISO 14971 Medical devices—Application of risk management to medical devices

ISO 16379 Tissue-engineered medical products—Evaluation of anisotropic structure of articular cartilage using DT (Diffusion Tensor)-MR Imaging

ISO 19074 Leather—Physical and mechanical tests—Determination of water absorption by capillary action (wicking)

ISO 19090 Tissue-engineered medical products—Bioactive ceramics—Method to measure cell migration in porous materials

ISO 19997 Guidelines for good practices in zeta-potential measurement

ISO 20399-1 Biotechnology—Ancillary materials present during the production of cellular therapeutic products—Part 1: General requirements

ISO 20399-2 Biotechnology—Ancillary materials present during the production of cellular therapeutic products—Part 2: Best practice guidance for ancillary material suppliers

ISO 20399-3 Biotechnology—Ancillary materials present during the production of cellular therapeutic products—Part 3: Best practice guidance for ancillary material users

ISO 21560 General requirements of tissue-engineered medical products

2.3 *Other Documents:*

21 CFR 210 Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General[5]

21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceuticals[5]

21 CFR 820 Quality System Regulation[5]

21 CFR 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products[5]

21 CFR 1271.210 Human Cells, Tissues, and Cellular and Tissue-Based Products; Supplies and Reagents[5]

BS 3424-18 Testing Coated Fabrics—Part 18: Methods 21A and 21B: Methods for Determination of Resistance to Wicking and Lateral Leakage to Air[6]

FDA Guidance on 10993-1 Guidance for Industry and Food and Drug Administration Staff: Use of International Standard ISO 10993-1, "Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process" https://www.fda.gov/media/85865/download[7]

FDA Guidance on GMP for Combination Products Guidance for Industry and FDA Staff: Current Good Manufacturing Practice Requirements for Combination Products, https://www.fda.gov/media/90425/download[7]

FDA Guidance on Validation Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics, https://www.fda.gov/media/87801/download[7]

FDA Guidance on Surgical Meshes Guidance for Industry and/or for FDA Reviewers/Staff and/or Compliance: Guidance for the Preparation of a Premarket Notification Application for a Surgical Mesh, https://www.fda.gov/media/71828/download[7]

ICH Q2(R1) International Conference on Harmonisation, Validation of Analytical Procedures: Text and Methodology Q2(R1), https://www.ich.org/page/quality-guidelines

ICH Q7 International Conference on Harmonisation, Good Manufacturing Practice for Active Pharmaceutical Ingredients Q7, https://www.ich.org/page/quality-guidelines

NIST Special Publication 960-17 Porosity and Specific Surface Area Measurements for Solid Materials[8]

NIST SRM 1898 Titanium Dioxide Nanomaterial, Certificate of Analysis, https://www.nist.gov/srm[8]

NIST SRM 1900 Silicon Nitride Powder-Specific Surface Area Standard, Certificate of Analysis, https://www.nist.gov/srm[8]

NIST SRM 1917 Mercury Porosimetry Standard, https://www.nist.gov/srm[8]

NIST SRM 2206 Controlled Pore Glass—BET Specific Surface Area (Nominal Pore Diameter 300 nm), Certificate of Analysis, https://www.nist.gov/srm[8]

NIST SRM 2207 Controlled Pore Glass—BET Specific Surface Area (Nominal Pore Diameter 18 nm), Certificate of Analysis, https://www.nist.gov/srm[8]

NIST SRM 2696 Silica Fume (Powder Form), Certificate of Analysis, https://www.nist.gov/srm[8]

PDA Technical Report 13-2 Fundamentals of an Environmental Monitoring Program Annex 1: Environmental Monitoring of Facilities Manufacturing Low Bioburden

[5] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

[6] Available from British Standards Institution (BSI), 389 Chiswick High Rd., London W4 4AL, U.K., http://www.bsigroup.com.

[7] Available from U.S. Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993, http://www.fda.gov.

[8] Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, http://www.nist.gov.

Products, https://webstore.ansi.org/standards/pda/pdatr132020[9]
USP <71> Sterility Tests[10]
USP <85> Bacterial Endotoxins Test[10]
USP <161> Medical Devices—Bacterial Endotoxin and Pyrogen Tests
USP <467> Residual Solvents[10]
USP <861> Sutures—Diameter[10]
USP <881> Tensile Strength[10]
USP <1043> Ancillary Materials for Cell, Gene, and Tissue-Engineered Products[10]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *fiber-based construct, n*—a construct composed of slender, elongated filaments intended for use in biological applications, such as a tissue-engineering scaffold.

3.1.2 *nonwoven fiber mat, n*—a textile structure held together by interlocking of fibers in a random web, accomplished by mechanical, chemical, thermal, or solvent means. (http://www.fabriclink.com/dictionaries/textile.cfm#N)

3.1.3 *yarn, n*—a continuous strand of textile fibers created when a cluster of individual fibers are twisted together. (http://www.fabriclink.com/dictionaries/textile.cfm#N)

4. Summary of Guide

4.1 The structural, mechanical, physical, chemical, and biological properties of the fiber-based constructs will influence their function in tissue-engineered medical products (TEMPs). It is the intent of this guide to provide a compendium of techniques for characterizing fiber-based constructs for use in TEMPs. Application of the test methods contained within this guide does not guarantee clinical success of a finished product but will help to ensure consistency in the properties, characterization of a given construct, and meaningful comparison between constructs using consistent test methodologies. This guide does not suggest that all of the listed tests be conducted. The decision regarding applicability of any particular test method is the responsibility of the developer and will depend on the intended use.

4.2 The reader should be aware of a guidance document issued by the U.S. Food and Drug Administration (FDA) for surgical meshes that may apply to some fiber-based constructs (FDA Guidance on Surgical Meshes).

5. Significance and Use

5.1 The test methods contained herein guide characterization of the structural, physical, chemical, mechanical, and biological properties of a fiber-based construct. Such properties may be important for the success of a TEMP, especially if they affect cell retention; activity and organization; tensile strength; the delivery of bioactive agents; or the biocompatibility and bioactivity of the construct.

5.2 Tests described herein may be used for quality control during manufacturing or to assess how the product may perform its intended clinical function.

5.3 Plans for product development, product characterization, and the regulatory pathway should be discussed with the appropriate regulatory body.

6. Structural Characterization

6.1 *General Considerations:*

6.1.1 Structure may be the most important attribute of fiber-based constructs. It is the fiber-based structure that makes these constructs attractive for biomedical applications. The fiber structure can mimic that of a native extracellular matrix that provides a supportive niche for cells. The gross geometry of a fiber-based construct is often planar, which makes them useful as barrier membranes and as scaffolds for epithelial tissues (guided tissue regeneration, dental, dura mater), tubular structures, or filamentous structures (urethra, bladder, esophagus, tendon, ligament). Fiber-based constructs typically have a significant void volume which makes them permeable to biological fluids, cell culture medium, nutrients, ions, small molecules, and proteins.

6.1.2 Many fiber-based constructs, such as those made by electrospinning, are nonwoven. The fibers may lay down upon one another creating a structure that resembles a bowl of noodles (Fig. 1(a)). Fiber-based constructs often have an irregularly shaped void volume that does not have a typical "pore" with a repeating structure. Nonwovens are often anisotropic, since the long axes of the fibers typically extend the in the X- and Y-direction with fibers stacking upon one another in the Z-direction.

6.1.3 Fiber-based constructs, such as electrospun mats, may have the consistency of fabrics, whereby they are thin and pliable.

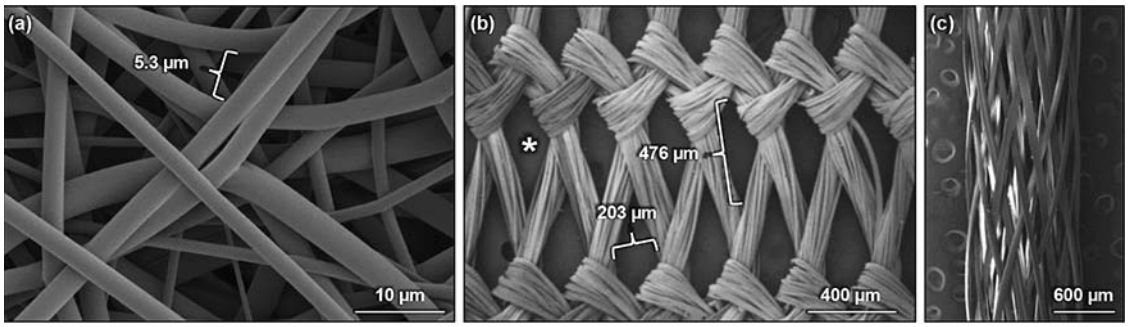
6.1.4 Fiber-based constructs must be handled carefully and consistently. The constructs are often delicate and their properties may be affected by their handling. They can be susceptible to perturbations by fingers or tweezers during simple tasks such as transfering from one container to another. This minute damage may manifest during imaging, structural measurements, or mechanical tests. Cells may respond to surface features caused by handling perturbations.

6.1.5 When fiber-based constructs are used for their intended clinical indication, they are likely to experience mechanical forces. This ought to be considered when planning how to characterize their structure. Porosity assessed under zero load may be higher than when a clinically relevant load is applied. It may be helpful to assess structural attributes when the construct is under a clinically relevant load. Application of a clinically relevant load could affect porosity and the ability of cells or solutes to penetrate the construct.

6.2 *Key Structural Attributes:*

6.2.1 *Porosity*—Porosity is the fraction of the total scaffold volume that is void space. It is defined as follows: Porosity = $(V_V / V_T) = [V_V / (V_V + V_F)]$. Porosity is calculated by dividing the void volume (V_V) by the total scaffold volume (V_T), where V_T is the sum of the void volume (V_V) and the volume of the fibers (V_F). Porosity is important for function since it will

[9] Available from Parenteral Drug Association (PDA), 4350 East West Highway, Suite 600, Bethesda, MD 20814, http://www.pda.org.
[10] Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.



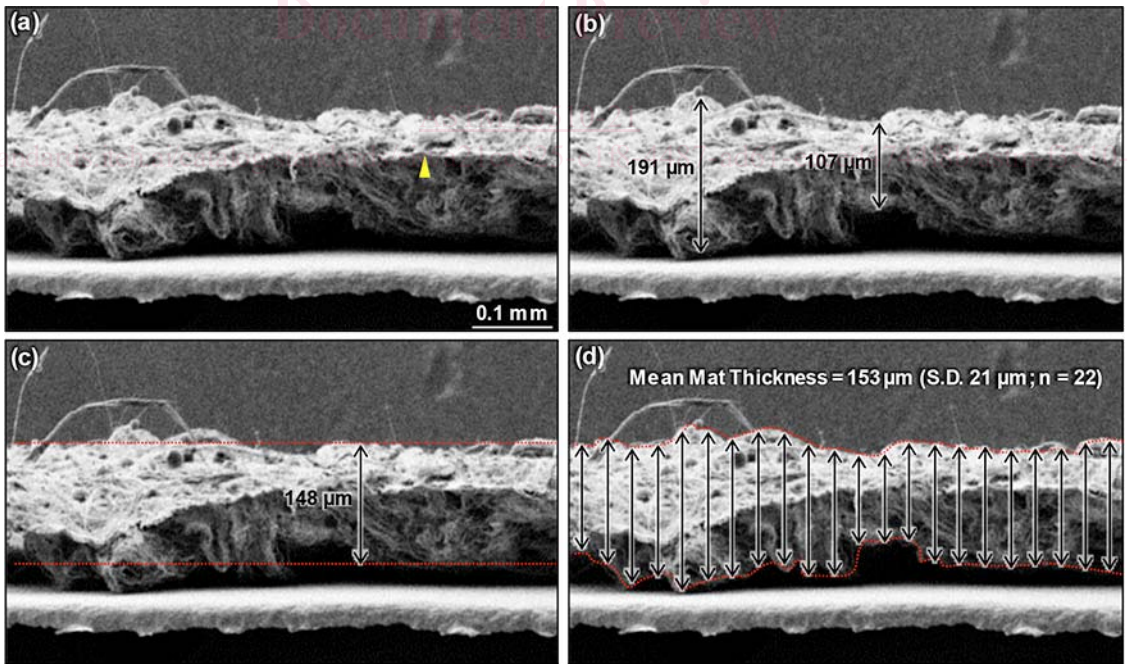
(a) SEM of a mat of electrospun polymer fibers (Photo Credit: Nathan Hotaling). There are no through-holes visible in the image. Determining the pore size by measuring the distance between two arbitrarily chosen fibers, as shown in the image, may not be meaningful. (b) SEM of a woven mesh of fiber bundles. The mesh has only a few layers of fibers and through-holes are present (indicated by asterisk). The dimensions of the through-holes (short axis 203 µm; long axis 476 µm) represent more meaningful measurements of a pore size. Image is used with permission from Xu and Simon (1). (c) SEM of a collagen braid. Collagen fibers were manufactured by microfluidic wet extrusion into a continuous yarn that was subsequently braided (Photo Credit: Michael Francis).

FIG. 1 Defining "Pore Size" for Fiber-Based Constructs

influence the flow of liquid and nutrients through the constructs, cell migration into the construct, and mechanical properties.

6.2.1.1 *Void Volume*—The void volume (V_V) or void fraction of a fiber-based construct is the empty regions within a construct that occupy the spaces between the fibers. For a nonwoven structure, the void volume is an irregular and continuous volume that is not broken into discrete pores.

6.2.1.2 *Defining Porosity*—For fiber-based constructs, the determined porosity value is dependent upon how the user defines porosity. The user must think carefully about how to define porosity for their construct. Since fiber mats are typically thin in the Z-direction (like a fabric), small deviations in defining the location of the top and bottom surfaces of the scaffold may have large effects on the volume calculation. This concept is illustrated in Fig. 2, which shows an example of a



The edge of the fiber mat was created by immersing in liquid nitrogen and slicing with a razor blade. The same micrograph is shown in all panels. (a) The yellow arrowhead indicates pinching of the edge of fiber mat that occurs during slicing. (b) The fiber mats do not have a consistent thickness. The measured thickness will depend upon the location at which the thickness is measured. The double-sided arrows show the thickness range. (c) The thickness could be approximated as indicated by the red lines. (d) The thickness could be determined at multiple positions and averaged. If image analysis routines were used for the analysis, then thickness determination would depend on the algorithm (Photo Credit: Wojtek Tutak).

FIG. 2 Determining Thickness of a Mat of Airbrushed Poly(D,L-Lactic Acid) Fibers By Observing the Edge of the Mat By Scanning Electron Microscopy (SEM)

fiber mat and the variability in the different ways that the scaffold thickness could be defined.

6.2.2 *Pore Structure:*

6.2.2.1 The term "pore size" may be confusing when applied to fiber-based constructs. The term "pore size" implies that a construct has voids or pockets, with a characteristic and repeating size and shape. Constructs made by electrospinning are nonwovens and do not have pores in the traditional sense of the term, but instead have a continuous void volume which surrounds the fibers (Fig. 1(a)). The void volume of nonwoven fiber-based constructs is irregular and lacks a repeating structure. In addition, the void volume of fiber-based constructs is anisotropic whereby the distances between fibers in the X- and Y-directions are typically larger than the distances between fibers in the Z-direction. The term "void structure" might make more sense when discussing fiber-based constructs. However, the term "pore size" is embedded in the lexicon and is difficult to avoid, especially when discussing test methods for assessing structure.

6.2.2.2 If a fiber-based construct has only a few layers, then through-holes may be present. The through-holes may have a repeating shape with a characteristic size and may be more appropriately described as pores (Fig. 1(b)). Electrospun fiber mats may not have through-holes.

6.2.2.3 When reporting pore structure, it is critical to clearly describe how pore structure is being defined and how it is being measured. Many test methods report a pore size based on volume, pressure, or flow measurements for liquids and gases that are used to fill or flow through the voids of a construct. The user must consider what these "pore size" values mean for a fiber-based construct that has an ill-defined void structure that lacks pores with a repeating, uniform structure.

6.2.2.4 Pore structure and pore size are important since they affect diffusion of solutes in a construct and the ability of cells to penetrate a construct.

6.2.3 *Fiber Diameter*—Fiber diameter is the cross-sectional thickness of a fiber. This attribute of fiber-based constructs is probably the easiest to quantify and the most commonly quantified. It is important to measure the diameter of many fibers in a construct to provide an estimate of the fiber diameter distribution, since the diameter of fibers in fiber-based constructs often have a wide range of diameters. The fiber morphology must be considered when measuring fiber diameter. Fibers are typically cylindrical, but elliptical, ribbon-shaped, and irregularly shaped fibers have been fabricated. The consistency of the fiber diameter may be a measure of consistent manufacturing. Fiber diameter variation across batches may be an indicator that the raw materials are not homogeneous or that instabilities are present at the spinneret.

6.2.4 *Fiber Orientation*—Fibers can be randomly dispersed or aligned to varying degrees. Electrospun fibers are often deposited into a nonwoven mat in a random alignment. Electrospun fibers can be aligned through deposition onto a spinning mandrel during the electrospinning process. Melt-extruded fibers can be deposited into structured constructs through additive manufacturing mechanisms. Yarns of composite fibers may be plied or twisted together, while fibers and yarns may be braided with discrete fiber alignments. Fiber alignment can be advantageous for a given indication. For example, aligned fibers have anisotropic mechanical properties that may be useful for tendon, while randomly oriented fibers will have isotropic mechanical properties that might be suitable for planar tissues such as epithelium or bladder.

6.2.5 *Mat Thickness*—Fiber-based constructs are often planar. The mat thickness is the thickness along the short axis perpendicular to the plane of the mat. Consistent mat thickness is an indicator of consistent manufacturing. In addition, construct permeability, construct degradation, and cell infiltration into constructs may depend upon mat thickness. Mat thickness is also a key input value for determination of porosity by gravimetric methods. See Table 1.

6.2.6 *Attributes of Individual Fibers*—The properties of the individual fibers themselves may be important. There may be pores within the fibers, the fibers may have their own surface texture, and fibers may have a core sheath morphology, as can be obtained from coaxial electrospinning designs.

6.3 *Structural Measurements:*

6.3.1 *Scanning Electron Microscopy*—Scanning electron microscopy (SEM) is probably the most commonly used method to assess the structure of fiber-based constructs. Dry constructs may be sputter-coated with a thin layer of gold or other material to improve SEM contrast. SEM images can

TABLE 1 Common Measurement Methods for Assessing Key Structural Attributes of Fiber-Based Constructs[A,B,C]

Measurement Methods	Fiber Diameter (size range)	Fiber Orientation	Porosity	Pore Structure (size range)	Mat Thickness
Scanning electron microscopy	1 nm to 1 mm	yes	n/a[D]	n/a[D]	yes
Gravimetry	n/a	n/a	yes	n/a	n/a
Mercury intrusion porosimetry	n/a	n/a	yes	4 nm to 60 µm	n/a
Brunauer-Emmett-Teller (BET) gas adsorption	n/a	n/a	yes	2 nm to 300 nm	n/a
Liquid extrusion porosimetry	n/a	n/a	yes	1 µm to 1 mm	n/a
Porometry/bubble point test	n/a	n/a	n/a	100 nm to 100 µm	n/a
X-ray microcomputed tomography	5 µm to 1 mm	yes	yes	5 µm to 1 mm	yes
Confocal microscopy	1 µm to 1 mm	yes	yes	1 µm to 1 mm	yes
Atomic force microscopy	1 nm to 1 mm	yes	n/a	n/a	yes
X-ray microscopy	1 nm to 1 mm	yes	yes	1 nm to 1 mm	yes
Calipers	n/a	n/a	n/a	n/a	yes
Laser displacement profiler	n/a	n/a	n/a	n/a	yes

[A] The table lists common methods, but other suitable methods for measuring the given attributes may exist.
[B] Values given in the table are approximate and will depend on the characteristics of a given specimen.
[C] n/a = not applicable.
[D] Since SEM is a 2D imaging modality, porosity and pore structure measurements made with SEM are 2D approximations.

achieve several pixels per nm and provide the highest resolution 2D images of fibers.

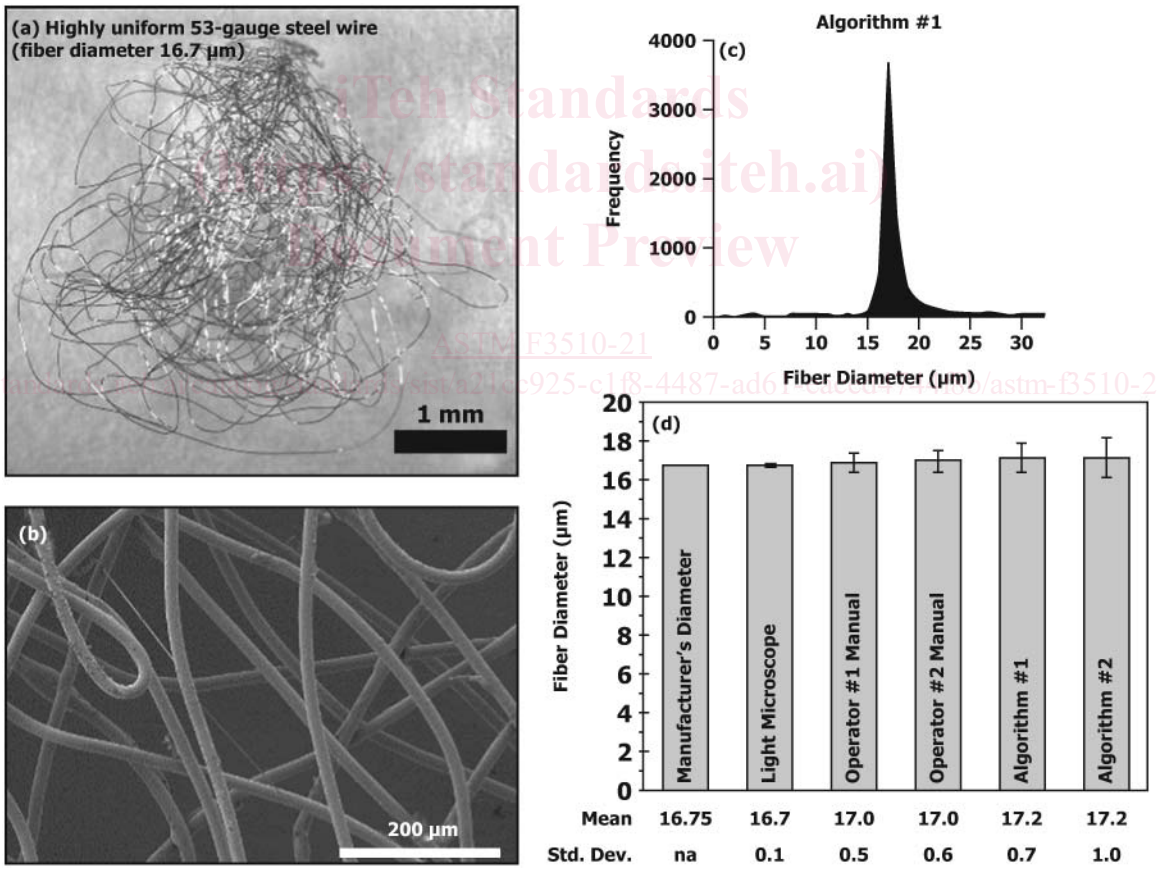
6.3.1.1 *SEM Measurement of Fiber Diameter*—SEM is well suited for determining fiber diameter. Image captures of fibers can be assessed manually using a line tool in an image analysis program. ImageJ and Fiji are open-source image analysis software that may be used (**2-4**).[11] Fiber diameter can also be determined with automated methods which are less biased and faster (**5, 6**). Automated methods can rapidly generate a lot of data so that histograms of fiber diameter that can be used to assess the uniformity of fiber diameter can be generated. A training website is available for one of the algorithms (**7**). In addition, reference images of highly uniform fibers with a known diameter are publicly available for users to ensure the performance of analytical routines (**8**). The reference images were generated by taking SEMs of steel wire that has a diameter of 17 µm, which is large enough to be verified by orthogonal methods, including calipers and light microscopy (Fig. 3).

6.3.1.2 The fiber samples can be tilted in the SEM to assess whether fibers have a cylindrical or elliptical morphology, contain a solid core, or are hollow and tube-like. This can be achieved by imaging the same field of view of fibers at 0° tilt and 60° tilt to see if there is a difference in fiber diameter.

6.3.1.3 *SEM Measurement of Porosity or Pore Structure*—SEM is often used to assess porosity and pore structure by measuring distances between fibers in micrographs. However, this approach may not be meaningful. SEM micrographs present a two-dimensional view of a 3D structure and may not be appropriate for determining 3D attributes such as porosity or pore structure.

6.3.1.4 When SEM is used to ascribe a pore size to a nonwoven mat of fibers, what is typically being measured is the interfiber spacing along the long axes of the fiber mat (X- and Y-direction). If the interfiber spacing is measured by

[11] The boldface numbers in parentheses refer to a list of references at the end of this standard.



(a) Optical image and (b) SEM image of reference wire with a fiber diameter of 16.7 µm. (c) Histogram of fiber diameter measurements determined using algorithm #1 to analyze SEM images of the reference wire. (d) Graph comparing measurements of the fiber diameter of the reference wire. "Manufacturer's Diameter" was reported by the manufacturer from caliper measurements. "Light Microscope" = human analysis of bright-field images of the reference wire by manually using a line tool in analysis software (ImageJ) (**2**). "Operator #1 Manual" and "Operator #2 Manual": Human manual analysis (by two different people) of SEM images of the reference wire using ImageJ line tool. "Algorithm #1" and "Algorithm #2": automated analysis of SEM images of the reference wire using two different image analysis algorithms. Data are adapted from Hotaling et al. (**6**). Image is used with permission from Garcia et al. (**9**).

FIG. 3 Use of Uniform 53-Gauge Steel Wire as a Reference Material for Validating Automated Measurements of Fiber Diameter in Scanning Electron Micrographs (SEMs)

scanning electron microscopy (SEM), then the fibers that are closer to the objective are more illuminated while illumination is decreased for fibers that are further from the objective (Fig. 1(a)). Eventually, the background in SEM images appears dark for the fibers that have no illumination. A pore size that is calculated from the well-illuminated fibers in the foreground of SEM micrographs arbitrarily depends upon the electron illumination depth for the given image and is not a true pore size.

6.3.1.5 *SEM Measurement of Mat Thickness*—The specimen can be immersed in liquid nitrogen to make it brittle and then cut with a razor to expose a cross section for examination by SEM. However, polymers often compress and pinch along the cut line, similar to cutting soft bread with a dull knife (Fig. 2). This makes it difficult to know if the fibers have been compressed by the cutting process. Analysis of the SEMs to determine mat thickness requires thought since the thickness of the mat may vary. The thickness of the fiber mat shown in Fig. 2(b) ranges from 107 µm to 191 µm. The top and bottom of the mat could be approximated (Fig. 2(c)) or multiple thickness measurements could be averaged (Fig. 2(d)). Note that different users may place the red dotted lines in Fig. 2(c) and (d) in different places. A consistent process for determining the top and bottom of the mat in SEM images is required to get a reproducible measurement. Fig. 2(b) shows that the thickness of fiber mats can vary locally, but thickness can also vary from region to region within a sample. Multiple regions in a sample should be examined for thickness to provide a more reliable fiber mat thickness measurement.

6.3.1.6 *SEM Measurement of Fiber Orientation*—Image analysis of SEM images of fibers can be used to determine the degree of fiber alignment. An open-source algorithm called "OrientationJ" **(10)**, which runs in ImageJ **(2-4)**, may be effective for this metric.

6.3.1.7 *Environmental Scanning Electron Microscopy (ESEM)*—ESEM allows imaging of fibers in a hydrated state. Hydrated imaging fibers may be helpful if the fibers are intended for use in a hydrated state, such as implantation into a patient. This would be particularly important if the fibers are expected to swell in an aqueous medium.

6.3.1.8 *SEM Data Capture*—A consistent method for sample preparation, SEM imaging, and image capture should be used for all analyses. All samples and image captures should use the same sample mounting procedure, sputter-coating process (if required), working distance, instrument settings (voltage), image size, and magnification. Image data should be saved in a noncompressed, lossless format such as "tif" (Guide F3294).

6.3.2 *Gravimetric Measurement of Porosity*—Gravimetric analysis uses the following relationship to determine porosity: $[(V_T - (M/D)] / V_T$; where M is mass of the construct, V_T is volume of the construct, and D is density of the material used to make the fibers. The fiber volume (V_F) equals mass divided by density (M/D). The density of the bulk material used to make the fibers can be obtained from the literature or measured (Test Methods D792 and D854). Electrospinning processes may affect polymer packing and crystallinity **(11, 12)** which may affect material density. Specimen mass can be determined on a balance. For a fiber mat that is a few hundred microns thick, a relatively large specimen should be used to reduce the contributions of errors in the mass and dimensional measurements. A 2 cm^2 specimen may be appropriate. The length and width (X- and Y-dimensions) of the specimen can be measured with a ruler. Since fiber mats are typically planar and thin (hundreds of micrometers), determining their volume is dependent upon a reliable measure of the thickness of the mat. However, measuring the thickness of a fiber mat is challenging and is discussed extensively in several sections below.

6.3.2.1 *Liquid Intrusion for Measuring Porosity*—Liquid intrusion is a variation on the gravimetric approach for measuring porosity of fiber-based constructs whereby a liquid (instead of air) fills the voids **(13)**. The mass of a dry specimen is determined by weighing on a balance and the specimen is immersed in a liquid that doesn't dissolve or swell the fibers. The specimens are sonicated to facilitate diffusion of the liquid into the void volume of the construct. The specimen is blotted on an absorbent material to remove external liquid, while much of the liquid within the void volume is retained. The specimen is weighed again. The mass of liquid absorbed by the specimen is determined by subtracting the dry mass of the specimen from the mass after liquid absorption. Knowing the mass of the absorbed liquid and density of the liquid allows the liquid volume (V_L) to be calculated. Knowing the mass of the dry specimen and the density of the material used to make the fibers allows the volume of the fibers (V_F) to be calculated. The following equation can then be used to calculate the percent porosity: $V_L / (V_L + V_F)$. The advantage of this approach is that it avoids having to determine the volume of the construct, which is dependent upon an accurate measurement of construct thickness. However, the variability in the blotting process may be hard to control, leading to variability in the determination of the liquid volume.

6.3.2.2 *Caliper Measurement of Mat Thickness*—Digital calipers can be used to measure the thickness of fiber mats. However, the accuracy and consistency of the results may not be reliable. Fiber mats may be soft and may compress when the calipers are closed upon the specimen. It may not be possible to reliably determine when the calipers are contacting the specimen. There may be significant variability between measurements due to differences in how much compression is applied to the specimen during measurements.

6.3.2.3 *Force Caliper Measurement of Mat Thickness*—Force calipers (also called "low-pressure calipers" or "spring-loaded micrometers") have a mechanism to ensure that a consistent amount of force is applied by the calipers to the specimen, which improves the repeatability of thickness measurements. A similar mechanism is used for measuring the thickness of textiles which employs a thickness gauge with weights to apply a constant force to the fabric using a weighted presser foot (Test Method D1777).

6.3.2.4 *Impedance Caliper Measurement of Mat Thickness*—Home-built systems using digital calipers that have been equipped with impedance-sensing hardware can be used to assess the thickness of fiber mats. When the surfaces of the caliper contact the specimen, a change in capacitance is detected by the sensor to make possible a repeatable measurement of mat thickness.

6.3.2.5 *Non-Contact Optical Measurements of Mat Thickness*—Non-contact optical systems (also called optical calipers, laser micrometers, laser displacement sensors, or laser profilers) can be used to assess the thickness of nonwoven electrospun fiber mats, which are often thin and pliable, are prone to surface undulations, and may not lay flat. Light sources, typically lasers, are directed at the top and bottom of the specimen and surface reflections are detected by sensing elements that determine the distance to the surface using triangulation or interferometry. By determining the positions of the top surface and bottom surface at the same X-Y positions, the specimen thickness is determined. These optical systems can have a spot size as low as tens of nm and a Z-axis repeatability as low as 1 µm. Gauge blocks of relevant dimensions can be used to calibrate the optical systems. For fibers that are translucent, the beam may penetrate into the sample before reflecting, resulting in a measurement that results in a low estimate of mat thickness. It may be possible to overcome this issue by adjusting how the reflection pattern coming back from translucent fibers is interpreted. The repeatability of these systems makes them useful for assessing consistency in batches of manufactured fiber-based constructs.

6.3.2.6 Non-contact optical measurements using single laser systems that determine the position of one side of the fiber mats may be adequate for samples that lay flat and do not wrinkle. If the sample does not lay flat, then a single measurement of the top of the specimen may not account for the space between the specimen and the stage and will yield an inaccurate thickness measurement. A weighted disc may be put on top of the sample to enable consistent measurements, although this may compress the mat (**14**).

6.3.2.7 Automated stages can be used to translate the specimen in the X- and Y-directions so that the thickness can be mapped across the entire specimen. In this manner, millions of positional data points can be collected from both the top and bottom surfaces of a specimen. This approach can be the most reliable way of performing accurate mat thickness measurements for many systems.

6.3.2.8 *Ultrasonic Measurement of Mat Thickness*—Ultrasonic testers measure thickness from one side of a device. A probe sends a sound wave into one side of the device which reflects off the other side of the device. A detector determines thickness by measuring the time delay between the initial sound wave and the echo. A thickness accuracy of 50 µm can be achieved for samples that have flat surfaces. Samples with uneven surfaces cause scattering, which compromises the measurement. Ultrasonic measurement also requires the material to be solid to accurately measure the pulse travel time. For these reasons, ultrasonic measurements may not be appropriate for measuring thickness of fiber-based constructs.

6.3.2.9 *Light Scattering Measurement of Mat Thickness*—Light scattering techniques work by measuring the intensity of transmitted light through a specimen. Fiber-based constructs are often opaque to light, which prevents transmission. For specimens that do transmit light, the inhomogeneous nature of fiber-based constructs makes it difficult to interpret light scattering results. For these reasons, light scattering measurements may not be appropriate for measuring the thickness of fiber-based constructs.

6.3.3 *Fluorescence Confocal Microscopy*—Fluorescence confocal microscopy may be used to image fiber-based constructs, but a fluorophore is required. The fluorophore could be spiked into the bulk material during fabrication or adsorbed onto the surface of fibers to render them fluorescent. Some materials may be autofluorescent, such as some collagen constructs. Lateral resolution is approximately 500 nm (Guide F2603), which is suitable for imaging fibers with diameters of approximately 5 µm and greater (Guide F3259). An image of 5 µm diameter fibers that has 500 nm square pixels yields ten pixels per fiber diameter with a theoretical minimum error of 10 % (assuming an error of ±1 pixel out of ten pixels per fiber diameter). Confocal images are similar to SEM, whereby a top-down view of the constructs that is useful for measurements of fiber diameter is captured. One advantage of confocal is that the positional information of the top surface of the fibers is more quantitative than the SEM. However, as with SEM images, assessment of porosity and pore structure may not be reliable. The positional data is only reliable for the top layer of fibers and image quality of fibers that are underneath of other fibers may be compromised. Confocal is not commonly employed for analyzing fiber-based constructs since SEM is typically faster and has higher resolution. Confocal microscopy may be useful for analyzing fibers that swell in liquid since confocal does not require dry samples as does SEM.

6.3.4 *Reflection-Mode Confocal Microscopy*—Reflection-mode confocal microscopy may be used to image fiber-based constructs in a label-free manner. The surface position of the fibers in mats can be determined and may be useful for assessing fiber diameter, fiber spacing, or surface roughness of fiber mats. Reflection-mode laser scanning confocal microscopes can be equipped with analytical routines that identify the point of strongest reflected light intensity which may achieve 20 nm resolution in the Z-direction (500 nm resolution in X-Y direction).

6.3.5 *X-Ray Microcomputed Tomography*—X-ray microcomputed tomography (µCT) uses scattered X-ray attenuation to generate 3D images that can be used to image constructs (Guide F3259). Most µCT systems have minimum cubic voxel diameters of a few microns, which are only useful for imaging fibers with a diameter of tens of micrometers. X-ray nanoCT systems attain a minimum cubic voxel diameter of a few hundred nm, which may be useful for imaging fibers with diameters of a few microns (**15**). High-intensity X-ray sources such as from synchrotrons may achieve higher resolution 3D imaging (**16**). Ceramics and metals generate high X-ray contrast, but polymeric fibers may not have sufficient radiopacity to generate the X-ray contrast that is necessary for robust 3D images. It may be necessary to spike constructs with radiocontrast agents to improve image quality (**17**).

6.3.6 *X-Ray Microscopy*—X-ray microscopes can achieve cubic voxels with diameters of tens of nm, which is suitable for imaging constructs with fibers with diameters of hundreds of nm (**18**, **19**). X-ray microscopes can be configured with phase contrast, which may eliminate the need for radiocontrast agents.

6.3.7 *3D Imaging Sampling and Analysis*—The high-resolution 3D imaging methods described above often have relatively small imaging volumes which may represent <1 % of the total specimen volume. Thus, several volumes of interest should be imaged for each specimen to account for positional variability in fiber structure. Analysis of 3D image data is also challenging, especially 3D image segmentation. User-friendly tools for 3D image analysis are not yet widely available, and programming and image analysis expertise may be required. There are many computational algorithms for analyzing 3D structural data sets. Examples include a calculation of the maximum sphere size that can pass through a porous structure, or a calculation of the number of spheres of a given diameter that can be placed within a porous structure.

6.3.8 *Atomic Force Microscopy*—Atomic force microscopy (AFM) uses a stylus to quantitatively map surface topography. The data are appropriate for determining surface roughness of fiber-based constructs (Guide F2791). AFM data may also be suitable for determining attributes such as fiber diameter and fiber orientation, although SEM is more commonly used for these attributes. AFM can also be used for assessing mat thickness by running the stylus over an edge of a specimen and onto the supporting substrate. This approach will only be reliable if the specimen lays flat on the substrate without substantial wrinkling or undulations. AFM can also be used to assess roughness of individual fibers.

6.3.9 *Cryomilling*—Cryomilling is a process for breaking a specimen into smaller pieces by immersing it in a cryogenic liquid and then crushing it repeatedly for hours with a steel ball that is mechanically agitated (rotating vessel, oscillating magnets). If the fibers are made of materials that become brittle in the cryogen, then cryomilling can be used to break fiber-based constructs into pieces that expose intact cross sections. The cross sections can be viewed with SEM to assess mat thickness or fiber morphology. This approach may enable an end-on view of fibers to assess whether fibers are cylindrical, elliptical, or ribbon-like. This approach may determine if the fibers have a lumen, pores within the fibers, or a core-sheath morphology as may result from a coaxial electrospinning configuration.

6.3.10 *Wetting, Intrusion, and Flow Measurements Using Liquids or Gases:*

6.3.10.1 *Liquid Pycnometry for Porosity Measurements*—Liquid pycnometers enable a specimen's density to be determined in reference to a working fluid such as water or ethanol (Test Methods D854). First, the device is weighed when it is filled with the working fluid. Next, the specimen is placed in the working fluid, where it will displace some of the working fluid, and the device is weighed again. With knowledge of the density of the working fluid, density of the material used to make the fibers, and the volume of the specimen, the void volume can be determined. This method has not been widely used for assessing fiber-based constructs. It requires that the specimen displace enough of the working fluid to provide a significant change in mass; very small changes in mass will be prone to erroneous results. Determining the volume of a fiber mat can be challenging, since it is difficult to determine fiber mat thickness (discussed above). The penetration of the working fluid into the void volume is also uncertain. The construct may be exposed to changes in pressure to help wet the voids in fiber-based constructs.

6.3.10.2 *Measurement of Specific Surface Area (SSA) by Brunauer, Emmett, and Teller (BET) Gas Adsorption Method*—The BET method can be used to measure the SSA of fiber-based constructs **(20)**. It measures gas adsorption to the surface of a specimen while pressure is applied (NIST Special Publication 960-17, ISO 9277). Nitrogen gas is commonly used. As the gas adsorbs to the specimen through weak interactions such as Van der Waals, the amount of gas in vapor phase decreases and causes a measurable drop in pressure. In addition, the mass of the specimen increases, which can be detected by a microbalance. Voids smaller than 2 nm may not be detected since the gas molecules may be too large to penetrate voids of this size. The BET method may be able to quantify the amount of porosity due to voids in the size range of 2 nm to 300 nm if certain assumptions can be met (NIST Special Publication 960-17). This may be useful for detecting voids within a single fiber. Reference materials for calibrating BET measurements are available (ISO 9277, NIST SRM 1898, NIST SRM 1900, NIST SRM 2206, NIST SRM 2207, and NIST SRM 2696).

6.3.10.3 *Mercury Intrusion Porosimetry*—Mercury intrusion porosimetry (MIP) uses pressure to force mercury, which is a non-wetting liquid, into the voids of a porous material (Test Method D4404). Charting the volume of intruded mercury against pressure provides information about the voids, such as porosity and void size **(20)**. The pressure and weight of the mercury may deform fiber-based constructs **(15, 21, 22)**. Another concern is that the fiber sample may have to be folded or deformed to fit inside the penetrometer, which is the measuring chamber. Deforming an electrospun fiber mat would change its porosity and affect the results. A reference material consisting of alumina beads with nominal 70 % porosity and 10 nm pore diameter is available for assessing MIP measurements (NIST SRM 1917). MIP is useful for assessing void sizes in the range of approximately 4 nm to 60 µm (NIST Special Publication 960-17) and can detect both through-pores and blind pores (dead-end pores). **Warning—**Appropriate safety precautions should be followed when working with mercury because mercury liquid is toxic and liquid mercury releases toxic fumes **(23)**.

6.3.10.4 *Liquid Extrusion Porosimetry for Assessing Porosity and Void Structure*—For liquid extrusion porosimetry (LEP), a porous specimen is placed on a membrane whose pores are smaller than the specimen's pores. The pores of both the specimen and membrane are filled with a wetting liquid. Pressure is applied to the specimen using a gas which forces the liquid out of the specimen's pores and into and through the pores of the membrane. However, the pressure does not get high enough to force the liquid out of the pores of the membrane. The change in pressure and volume of extruded liquid are measured and used to provide information about the porosity and void structure. LEP uses pressures that are approximately ten times lower than MIP and may be less likely to deform delicate fiber specimens **(15, 21, 22)**. LEP may be useful for assessing void sizes in the range of approximately 1 µm to 1 mm.