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Standard Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products¹

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1. Scope

1.1 This guide is a resource of currently available test methods for the characterization of the compositional and structural aspects of biomaterial scaffolds used in the development and manufacture of regenerative medicine and tissue-engineered medical products (TEMPs).

1.2 The test methods contained herein guide characterization of the bulk physical, chemical, mechanical, and surface properties of a scaffold construct. Such properties may be important for the success of a TEMP, especially if the property affects cell retention, activity and organization, the delivery of bioactive agents, or the biocompatibility and bioactivity within the final product.

1.3 This guide may be used in the selection of appropriate test methods for the generation of an original equipment manufacturer (OEM) specification. This guide also may be used to characterize the scaffold component of a finished medical product.

1.4 This guide is intended to be used in conjunction with appropriate characterization(s) and evaluation(s) of any raw or starting material(s) used in the fabrication of the scaffold, such as described in Guide F2027.

1.5 This guide addresses natural, synthetic, or combination scaffold materials with or without bioactive agents or biological activity. This guide does not address the characterization or release profiles of any biomolecules, cells, drugs, or bioactive agents that are used in combination with the scaffold, but may be used to address the effects on other (e.g., structural) properties as a result of such release. A determination of the suitability of a particular starting material and/or finished scaffold structure to a specific cell type and/or tissue engineering application is essential, but will require additional *in vitro* and/or *in vivo* evaluations considered to be outside the scope of this guide.

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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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

- D412 Test Methods for Vulcanized Rubber and Thermoplastic Elastomers—Tension
- D570 Test Method for Water Absorption of Plastics
- 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
- D747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam (Withdrawn 2019)³
- 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
- D882 Test Method for Tensile Properties of Thin Plastic Sheeting
- D1042 Test Method for Linear Dimensional Changes of Plastics Caused by Exposure to Heat and Moisture
- D1238 Test Method for Melt Flow Rates of Thermoplastics by Extrusion Plastometer

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.

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

- 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
- D2857 Practice for Dilute Solution Viscosity of Polymers
- D2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics
- D3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships
- 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
- D4001 Test Method for Determination of Weight-Average Molecular Weight of Polymers By Light Scattering
- D4404 Test Method for Determination of Pore Volume and Pore Volume Distribution of Soil and Rock by Mercury Intrusion Porosimetry
- D4603 Test Method for Determining Inherent Viscosity of Poly(Ethylene Terephthalate) (PET) by Glass Capillary Viscometer
- D5226 Practice for Dissolving Polymer Materials
- D5296 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography
- D6420 Test Method for Determination of Gaseous Organic Compounds by Direct Interface Gas Chromatography-Mass Spectrometry
- D6474 Test Method for Determining Molecular Weight Distribution and Molecular Weight Averages of Polyolefins by High Temperature Gel Permeation Chromatography
- D6539 Test Method for Measurement of the Permeability of Unsaturated Porous Materials by Flowing Air
- D6579 Practice for Molecular Weight Averages and Molecular Weight Distribution of Hydrocarbon, Rosin and Terpene Resins by Size-Exclusion Chromatography
- E128 Test Method for Maximum Pore Diameter and Permeability of Rigid Porous Filters for Laboratory Use
- E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods
- E384 Test Method for Microindentation Hardness of Materials
- E456 Terminology Relating to Quality and Statistics
- E473 Terminology Relating to Thermal Analysis and Rheology
- E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
- E793 Test Method for Enthalpies of Fusion and Crystallization by Differential Scanning Calorimetry
- E794 Test Method for Melting And Crystallization Temperatures By Thermal Analysis
- E967 Test Method for Temperature Calibration of Differential Scanning Calorimeters and Differential Thermal Analyzers
- E968 Practice for Heat Flow Calibration of Differential Scanning Calorimeters
- E996 Practice for Reporting Data in Auger Electron Spectroscopy and X-ray Photoelectron Spectroscopy
- E1078 Guide for Specimen Preparation and Mounting in Surface Analysis
- E1142 Terminology Relating to Thermophysical Properties
- E1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products (Withdrawn 2014)³
- E1356 Test Method for Assignment of the Glass Transition Temperatures by Differential Scanning Calorimetry
- E1642 Practice for General Techniques of Gas Chromatography Infrared (GC/IR) Analysis
- E1829 Guide for Handling Specimens Prior to Surface Analysis
- E1994 Practice for Use of Process Oriented AOQL and LTPD Sampling Plans
- 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
- F1635 Test Method for *in vitro* Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants
- F1884 Test Methods for Determining Residual Solvents in Packaging Materials
- F1980 Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices
- F1983 Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications
- F2025 Practice for Gravimetric Measurement of Polymeric Components for Wear Assessment
- F2027 Guide for Characterization and Testing of Raw or Starting Materials for 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)
- F2312 Terminology Relating to Tissue Engineered Medical Products
- F2450 Guide for Assessing Microstructure of Polymeric Scaffolds for Use in Tissue-Engineered Medical Products
- F2603 Guide for Interpreting Images of Polymeric Tissue Scaffolds
- F2791 Guide for Assessment of Surface Texture of Non-Porous Biomaterials in Two Dimensions
- F2883 Guide for Characterization of Ceramic and Mineral Based Scaffolds used for Tissue-Engineered Medical Products (TEMPs) and as Device for Surgical Implant Applications
- F2900 Guide for Characterization of Hydrogels used in Regenerative Medicine
- F2902 Guide for Assessment of Absorbable Polymeric Implants

G120 Practice for Determination of Soluble Residual Contamination by Soxhlet Extraction

2.2 **AAMI Standards:**⁴

AAMI STBK-1 Sterilization—Part 1: Sterilization in Health Care Facilities

AAMI STBK-2 Sterilization—Part 2: Sterilization Equipment

AAMI STBK-3 Sterilization—Part 3: Industrial Process Control

2.3 **ANSI Standards:**⁵

ANSI/ISO/ASQ Q9000: Quality Management Systems—Fundamentals and Vocabulary

ANSI/ISO/ASQ Q9001: Quality Management Systems: Requirements

2.4 **ISO Standards:**⁶

ISO 1133-1 Determination of the Melt-Mass Flow Rate (MFR) and the Melt Volume-Flow Rate (MVR) of Thermoplastics

ISO 10993-1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing within a Risk Management Process

ISO 10993-9 Biological Evaluation of Medical Devices—Part 9: Degradation of Materials Related to Biological Testing

ISO 10993-13 Biological Evaluation of Medical Devices—Part 13: Identification and Quantification of Degradation Products from Polymers

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 Coated and Uncoated Metals and Alloys

ISO 10993-18 Biological Evaluation of Medical Devices—Part 18: Chemical Characterization of Medical Device Materials within a Risk Management Process

ISO 11357-1 Plastics—Differential Scanning Calorimetry (DSC)—Part 1: General Principles

ISO 11357-2 Plastics—Differential Scanning Calorimetry (DSC)—Part 2: Determination of Glass Transition Temperature and Glass Transition Step Height

ISO 13781 Implants for Surgery—Homopolymers, Copolymers and Blends of Poly(Lactide)—*In Vitro* Degradation Testing—Second Edition

ISO 22442-1 Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 1: Application of Risk Management—Second Edition

ISO 22442-2 Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 2: Controls on Sourcing, Collection and Handling—Second Edition

ISO 22442-3 Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 3: Validation of the Elimination and/or Inactivation of Viruses and Transmissible Spongiform Encephalopathy (TSE) Agents—First Edition

ISO TR 22442-4 Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 4: Principles for Elimination and/or Inactivation of Transmissible Spongiform Encephalopathy (TSE) Agents and Validation Assays for Those Processes—First Edition

ISO 80000-9 Quantities and Units—Part 9: Physical Chemistry and Molecular Physics

2.5 **U.S. Code of Federal Regulations:**⁷

21 CFR Part 58 Title 21—Food And Drug Administration, Part 58—Good Laboratory Practice For Nonclinical Laboratory Studies

21 CFR Part 820 Title 21—Food and Drugs Services, Part 820—Quality System Regulation

2.6 **U.S. Pharmacopeia (USP) Standards:**⁸

<51> Antimicrobial Effectiveness Testing

<71> Sterility Tests

<87> Biological Reactivity Tests, *in vitro*

<88> Biological Reactivity Tests, *in vivo*

<151> Pyrogen Test

<197> Spectrophotometric Identification Test

<232> Elemental Impurities—Limits

<233> Elemental Impurities—Procedures

<381> Elastomeric Closures for Injections

<616> Bulk Density and Tapped Density

<661> Containers—Plastics

<699> Density of Solids

<701> Disintegration

<731> Loss on Drying

<736> Mass Spectrometry

<741> Melting Range or Temperature

<761> Nuclear Magnetic Resonance

<776> Optical Microscopy

<786> Particle Size Distribution Estimation by Analytical Sieving

<846> Specific Surface Area

<851> Spectrophotometry and Light-Scattering

<881> Tensile Strength

<891> Thermal Analysis

<911> Viscosity

<921> Water Determination

<941> X-Ray Diffraction

<1031> Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants

<1045> Biotechnology-Derived Articles

<1050> Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

<1074> Excipient Biological Safety Evaluation Guidelines

⁴ Available from the Association for the Advancement of Medical Instrumentation, 901 N. Glebe Rd., Suite 300, Arlington, VA 22203, <http://www.aami.org>.

⁵ Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://www.ansi.org>.

⁶ Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, <http://www.iso.org>.

⁷ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, <http://www.access.gpo.gov>.

⁸ Available from U.S. Pharmacopeia, 12601 Twinbrook Pkwy., Rockville, MD 20852, or through <http://www.usp.org/products/USPNF/>. The standards are listed by appropriate USP citation number.

<1078> Good Manufacturing Practices for Bulk Pharmaceutical Excipients

<1181> Scanning Electron Microscopy

<1211> Sterilization and Sterility Assurance of Compendial Articles

<1225> Validation of Compendial Procedures

2.7 NIST Document:⁹

NIST SP811 Special Publication SP811: Guide for the Use of the International System of Units (SI)

2.8 ICH Guideline:¹⁰

ICH Q3D Guideline for Elemental Impurities

2.9 Other Documents/Web Sites:

U.S. Food and Drug Administration (FDA) Center for Devices and Radiologic Health (CDRH), Consensus Standards Database¹¹

FDA-CDRH Guidance Documents Database¹²

FDA-CDRH Premarket Approval (PMA) Database¹³

FDA-CDRH 510(k) (Premarket Notification) Database¹⁴

3. Terminology

3.1 Unless provided otherwise in 3.2, terminology shall be in conformance with the TEMPs terminology standard, Terminology **F2312**.

3.2 Definitions:

3.2.1 *bioactive agents, n*—any molecular component in, on, or within the interstices of a device that elicits a desired tissue or cell response. Growth factors, antibiotics, and antimicrobials are typical examples of bioactive agents. Device structural components or degradation byproducts that evoke limited localized bioactivity are not included.

3.2.2 *pores, n*—an inherent or induced network of channels and open spaces within an otherwise solid structure.

3.2.3 *porometry, n*—the determination of the distribution of pore diameters relative to the direction of fluid flow by the displacement of a wetting liquid as a function of pressure.

3.2.4 *porosimetry, n*—the determination of pore volume and pore size distribution through the use of a non-wetting liquid (typically mercury) intrusion into a porous material as a function of pressure.

3.2.5 *porosity, n*—property of a solid which contains an inherent or induced network of channels and open spaces. Porosity can be measured by the ratio of pore (void) volume to the apparent (total) volume of a porous material and is commonly expressed as a percentage.

⁹ Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, <http://www.nist.gov>.

¹⁰ Available from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Secretariat, 9, chemin des Mines, P.O. Box 195, 1211 Geneva 20, Switzerland, <http://www.ich.org>.

¹¹ Available from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>.

¹² Available from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpgp/search.cfm>.

¹³ Available from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm>.

¹⁴ Available from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm>

4. Summary of Guide

4.1 The physicochemical and three-dimensional characteristics of the scaffold material are expected to influence the properties of TEMPs. It is the intent of this guide to provide a compendium of materials characterization techniques for properties that may be related directly to the functionality of scaffolds for TEMPs.

4.2 Other characterizations for scaffolds used in TEMPs may include compositional identity, physical and chemical properties or characteristics, viable sterilization techniques, degradability/absorbability, and mechanical properties.

4.3 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 and characterization of a given scaffold material.

4.4 This guide does not suggest that all of the listed tests be conducted. The decision regarding applicability or suitability of any particular test method remains the responsibility of the supplier, user, or regulator of the scaffold material based on applicable regulations, characterizations, and preclinical/clinical testing.

5. Significance and Use

5.1 Scaffolds potentially may be metallic, ceramic, polymeric, natural, or composite materials. Scaffolds are usually porous to some degree, but may be solid. Scaffolds can range from mechanically rigid to gelatinous and can be either absorbable/degradable or non-absorbable/non-degradable. The scaffold may or may not have a surface treatment. Because of this large breadth of possible starting materials and scaffold constructions, this guide cannot be considered as exhaustive in its listing of potentially applicable tests. A voluntary guidance for the development of tissue-engineered products can be found in Omstead, et al (1).¹⁵ Guide **F2027** contains a listing of potentially applicable test methods specific to various starting materials. Guidance regarding the evaluation of absorbable polymeric materials and constructs can be found in Guide **F2902**. Guidance regarding the evaluation of collagen-based materials can be found in Guide **F2212**. Guidance regarding the evaluation of scaffolds composed of ceramic or mineral-based material is available in Guide **F2883**. Similarly, guidance for the assessment of unique aspects of scaffolds based on hydrogels (for example, gel kinetics, mechanical stability, and mass transport properties) may be found in Guide **F2900**.

5.2 Each TEMP scaffold product is unique and may require testing not within the scope of this guide or other guidance documents. Users of this guide are encouraged to examine the references listed herein and pertinent FDA or other regulatory guidelines or practices, and conduct a literature search to identify other procedures particularly pertinent for evaluation of their specific scaffold material (**2,3,4**). It is the ultimate responsibility of the TEMP scaffold designer to determine the appropriate testing, whether or not it is described in this guide.

¹⁵ The boldface numbers in parentheses refer to the list of references at the end of this standard.

5.3 A listing of potentially applicable tests for characterizing and analyzing the materials used to fabricate the scaffold may be found in Guide **F2027**. However, conformance of a raw material to this and/or any other compendial standard(s) does not, in itself, ensure that the selected material is suitable or that the provided quality is adequate to meet the needs of a particular application. Thus, other characterization procedures may also be relevant and not covered by this guide.

5.4 The following provides a listing of links to U.S. Food & Drug Administration (FDA)—Center for Devices & Radiologic Health (CDRH) web sites that may potentially contain additional guidance relevant to biomaterial scaffolds covered within this document.

5.4.1 *Recognized FDA-CDRH Consensus Standards Database:*

5.4.1.1 <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>

5.4.1.2 This database provides a resource for locating FDA-recognized consensus standards for medical products.

5.4.2 *FDA-CDRH Good Guidance Practice (GGP) Database:*

5.4.2.1 <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfggp/search.cfm>

5.4.2.2 This database provides a resource for locating non-binding FDA guidance documents intended for CDRH staff, regulated industry and the public that relate to the processing, content, and evaluation of regulatory submissions, the design, production, manufacturing, and testing of regulated products, and FDA inspection and enforcement procedures.

5.4.2.3 A document within this database possessing content that warrants particular consideration for its potential applicability for tissue-engineering scaffolds is *Guidance for the Preparation of a Premarket Notification Application for a Surgical Mesh; Final*.

5.4.3 *FDA-CDRH Premarket Approval (PMA) Database:*

5.4.3.1 <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm>

5.4.4 *FDA-CDRH 510(k) (Premarket Notification) Database:*

5.4.4.1 <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm>

6. Chemical Properties and Tests

NOTE 1—Chemical properties are the chemical composition characteristics of a compound. Chemical tests provide information about the identity or nature of the chemical components of a scaffold. Chemical tests include those that provide information about the nature or size of constituent molecules, the product's purity, and/or the chemical nature of the scaffold surface.

6.1 Identification of Impurities:

6.1.1 Chemical impurities are expected and unexpected materials that are not part of the intended design of the scaffold. Acceptable levels are a function of the nature of the impurity and the scaffold's intended *in vitro* or *in vivo* application, and may be evaluated by appropriate qualification studies. A more precise definition of both contaminants and impurities and guidance regarding their significance may be found in Guide **E1298**.

6.1.2 Expected impurities of potential biological significance should be monitored through appropriate analytic means. Impurities can occur in both synthetic and natural based materials (for example, proteins, such as collagen and elastin; polysaccharides, such as cellulose, alginate, hyaluronan, and chitin based derivatives) and may include, but are not limited to, processing aids or solvents, unreacted cross-linking agents, residual monomers, endotoxins, sterilization residuals, and residual solutions that, by their chemical nature or relative concentrations, carry potential for influencing cell or tissue response.

6.1.3 Impurities may be identified or quantitatively determined by infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), combined gas chromatography/mass spectrometry (GC/MS), or other analytic methods as appropriate. Polyacrylamide gel electrophoresis is a possible method for assessing the presence of impurities in biologically derived scaffold materials (for example, collagen, hyaluronic acid). Impurities separated within such gels can be detected with Coomassie Blue (as a general protein stain) or silver (as a general protein and carbohydrate stain), and characterized further by immunoblot analysis and/or protein sequencing to identify specific impurities that may possess critical biological activities (for example, elastin immunogenicity, cytokines and growth factors). Once characterized, such impurities can be assessed by other robust and sensitive methods well suited to a manufacturing environment (for example, the enzyme-linked immunosorbent assay (ELISA) test for specific substances identified by immunoblot analysis or protein sequencing.)

6.1.4 Generally, impurities are isolated more readily when the scaffold in its entirety can be solvated along with possible contaminants. If the scaffold cannot be dissolved, exhaustive extraction with one or more solvents appropriate to the suspected impurity is necessary.

6.1.4.1 *Solvation/Dissolution*—In the absence of known or established dissolution solvents for a particular material, Practice **D5226** may provide added guidance in identifying suitable potential solvents for dissolving a scaffold material. Samples should not be dissolved in analytic solvents that can be considered as potential contaminants or create analytic interferences.

6.1.4.2 Extraction of residuals may be undertaken by methods such as Practice **G120**. The extract may then be concentrated and analyzed by appropriate chromatographic analysis.

6.1.5 The amount of any expected impurity should be quantified and the analytic detection limit reported. Both solvated and extracted samples should provide results that specify the amount of expected impurity per mass of test sample in either percentage, ppm ($\mu\text{g/g}$; mg/kg), ppb (ng/g ; $\mu\text{g/kg}$), or other appropriate units.

6.1.6 The following analytic methods may be applicable in the determination and quantification of potential impurities:

6.1.6.1 Gas chromatography (GC) may be used for the routine detection of volatile relatively low molecular mass (formerly known as molecular weight) impurities or contaminants. Some methods that may prove suitable include Test Method **F1884**.

6.1.6.2 Gas chromatography can be coupled with both quantitative and qualitative analytic methods such as IR or MS to provide compositional identification while quantitatively detecting low molecular mass volatile impurities or contaminants. Some particular methods that may prove useful include Test Method [D6420](#) and Practice [E1642](#).

6.2 Molar Mass Determination:

NOTE 2—The term molecular weight (abbreviated MW) is obsolete and should be replaced by the SI (Système Internationale) equivalent of either relative molecular mass (Mr), which reflects the dimensionless ratio of the mass of a single molecule to an atomic mass unit (see ISO 80000–9), or molar mass (M), which refers to the mass of a mole of a substance and is typically expressed as grams/mole. For polymers and other macromolecules, use of the symbols Mw, Mn, and Mz continue, referring to mass-average molar mass, number-average molar mass, and z-average molar mass, respectively. For more information regarding proper utilization of SI units, see NIST SP811.

6.2.1 For polymeric materials (synthetic or natural), the molar mass and molar mass distribution may be determined through size exclusion chromatography (SEC) or gel permeation chromatography (GPC). Other procedures such as inherent or intrinsic viscosity (both abbreviated with the acronym “IV”), light scattering, or membrane osmometry may be used. For protein impurities, SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) has proven robust and generally applicable. In specific instances, mass spectrometry can provide highly accurate mass determinations as well.

6.2.2 In any of the preceding tests, the solvent solubility characteristics of the scaffold will be highly significant in allowing determination of suitable molar mass test methods. In the absence of known or established dissolution solvents for a particular scaffold construct, Practice [D5226](#) provides added guidance in identifying suitable potential solvents for dissolving a particular material.

6.2.3 The following test methods may be applicable in the determining the molar mass of the fabricated scaffold.

NOTE 3—The following GPC/SEC and IV methods are considered to be suitable for use on linear polymer systems only. Branched polymer systems should use light-scattering techniques.

6.2.3.1 Gel Permeation Chromatography (GPC), Also Known as Size Exclusion Chromatography (SEC)—See Test Methods [D5296](#) and [D6474](#) and Practices [D3016](#) and [D6579](#).

NOTE 4—The SEC solvent system and calibration standard polymer type should be specified with any obtained result.

6.2.3.2 Inherent Viscosity—See Practice [D2857](#) and Test Method [D4603](#).

NOTE 5—The test temperature, solvent system, and sample concentration should be included with any reported result.

6.2.3.3 Light Scattering—See Test Method [D4001](#).

NOTE 6—This test method is suitable for both linear and branched polymer systems.

6.2.3.4 Melt Flow—If a scaffold or starting material is found to be insoluble after utilizing the guidance contained within Practice [D5226](#), melt rheology (melt flow rate) may replace the measurements of solution properties to obtain an indication of the material’s molar mass and molar mass distributions. Potentially useful methods include Test Method [D1238](#) and ISO 1133–1.

TABLE 1 USP Chemical Tests

USP Test No.	Test Description
<197>	Spectrophotometric identification
<232>	Elemental Impurities—Limits
<233>	Elemental Impurities—Procedures
<381>	Elastomeric closures for injections—physicochemical test procedures
<731>	Loss in drying (water content)
<736>	Mass spectroscopy-purity or elemental analysis
<761>	Nuclear magnetic resonance-purity or component analysis (for example, copolymers)
<851>	Spectrophotometry and light scattering (molar mass information)
<891>	Thermal analysis (purity)
<911>	Viscosity (molar mass)
<921>	Water determination

6.3 USP Chemical Tests—See [Table 1](#).

7. Physical Properties and Tests

NOTE 7—Physical properties are those of a compound that can change without involving a change in chemical composition (5). Physical testing determines the physical properties of materials based on observation and measurement. Such tests include those that provide information about the porosity, density, crystallinity, or physical surface properties of a scaffold material.

7.1 Visual Image Interpretation—Guide [F2603](#) covers considerations needed when interpreting visual images of three-dimensional polymeric (including collagen-based) and hydrogel structures.

7.2 Porosity Characterization—The porous macrostructure and microstructure of a scaffold exert a strong influence on both the elicited cell response and the tissue-engineered result. Guide [F2450](#) provides an overview of available pore characterization methods and their respective range of applicability with respect to pore sizes and material characteristics. While Guide [F2450](#) may indicate more suitable method(s) for a specific scaffold structure, the following test methodologies are recommended for consideration in the evaluation and characterization of the porosity of scaffolds possessing the 50 to 500 μm pore sizes most typical for the encouragement of cell growth within TEMPs (see [X1.2](#) of this guide for further discussion on the nature, significance, and potential applicability of these test methods):

7.2.1 Porosimetry (Liquid Intrusion)—Methodologies suitable for the mercury intrusion measurement of porosity include Test Method [D4404](#).

NOTE 8—An alternative porosimetry suitable non-wetting liquid may be used instead of mercury, provided that the resulting maximum pore size limitation is acceptable based on scaffold design and both recognized and accounted for within the results interpretation.

7.2.1.1 The sample data recommended to be obtained and reported are as follows:

- Median pore diameter and standard deviation (based on volume)—in μm
- Pore diameter range or distribution—in μm
- Total intrusion (void) volume—in cm³/g
- Bulk density—in g/cm³
- Total percentage porosity
- Total intrusion (void) volume (in cm³/g)
- = $\frac{\text{Total intrusion (void) volume (in cm}^3\text{/g)}}{1 / [\text{bulk density (in g/cm}^3\text{)]}}$