Designation: F 2150 – 01

Standard Guide for Characterization and Testing of Biomaterial Scaffolds Used in Tissue-Engineered Medical Products[1]

This standard is issued under the fixed designation F 2150; 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 of currently available test methods for the characterization of biomaterial scaffolds used to develop and manufacture 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 they affect 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 as guidance in the selection of appropriate test methods for the generation of a raw material or original equipment manufacture (OEM) specification. This guide also may be used to characterize the scaffold component of a finished medical product.

1.4 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.

1.5 *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 and health practices and determine the applicability of regulatory requirements prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 412 Test Methods for Vulcanized Rubber and Thermoplastic Rubbers and Thermoplastic Elastomers—Tension[2]
D 570 Test Method for Water Absorption of Plastics[3]
D 638 Test Method for Tensile Properties of Plastics[3]
D 648 Test Method for Deflection Temperature of Plastics Under Flexural Load in the Edgewise Position[3]
D 671 Test Method for Flexural Fatigue of Plastics by Constant-Amplitude-of-Force[3]
D 695 Test Method for Compressive Properties of Rigid Plastics[3]
D 747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam[3]
D 790 Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials[3]
D 792 Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement[3]
D 882 Test Method for Tensile Properties of Thin Plastic Sheeting[3]
D 1042 Test Method for Linear Dimensional Changes of Plastics Under Accelerated Service Conditions[3]
D 1238 Test Method for Flow Rates of Thermoplastics by Extrusion Plastometer[3]
D 1388 Test Method for Stiffness of Fabrics[4]
D 1621 Test Method for Compressive Properties of Rigid Cellular Plastics[3]
D 1623 Test Method for Tensile and Tensile Adhesion Properties of Rigid Cellular Plastics[3]
D 1708 Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens[3]
D 1898 Practice for Sampling of Plastics[5]
D 2857 Practice for Dilute Solution Viscosity of Polymers[6]
D 2873 Test Method for Interior Porosity of Poly(Vinyl Chloride) (PVC) Resins by Mercury Intrusion Porosimetry[6]
D 2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics[6]
D 3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships[6]
D 3039/D 3039M Test Method for Tensile Properties of Polymer Matrix Composite Materials[7]
D 3417 Test Method for Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry (DSC)[6]
D 3418 Test Method for Transition Temperatures of Polymers by Differential Scanning Calorimetry[6]

[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 F04.43 on Tissue-Engineered Medical Products.
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[2] *Annual Book of ASTM Standards*, Vol 09.01.
[3] *Annual Book of ASTM Standards*, Vol 08.01.
[4] *Annual Book of ASTM Standards*, Vol 07.01.
[5] Discontinued 1998; see 1997 *Annual Book of ASTM Standards*, Vol 08.01.
[6] *Annual Book of ASTM Standards*, Vol 08.02.
[7] *Annual Book of ASTM Standards*, Vol 15.03.

D 4001 Test Method for Determination of Weight-Average Molecular Weight of Polymers by Light Scattering[6]
D 4404 Test Method for Determination of Pore Volume and Pore Volume Distribution of Soil and Rock by Mercury Intrusion Porosimetry[8]
D 4603 Test Method for Determining Inherent Viscosity of Poly(Ethylene Terephthalate) (PET) by Glass Capillary Viscometer[9]
D 5226 Practice for Dissolving Polymer Materials[9]
D 5296 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography[9]
D 5732 Test Method for Stiffness of Nonwoven Fabrics Using the Cantilever Test[10]
D 6125 Test Method for Bending Resistance of Paper and Paperboard (Gurley Type Tester)[11]
D 6420 Test Method for Determination of Gaseous Organic Compounds by Direct Interface Gas Chromatography-Mass Spectrometry[12]
D 6474 Test Method for Determining Molecular Weight Distribution and Molecular Weight Averages of Polyolefins by High Temperature Gel Permeation Chromatography[9]
D 6539 Test Method for Measurement of Pneumatic Permeability of Partially Saturated Porous Materials by Flowing Air[13]
D 6579 Practice for Molecular Weight Averages and Molecular Weight Distribution of Hydrocarbon and Terpene Resins by Size-Exclusion Chromatography[14]
E 128 Test Method for Maximum Pore Diameter and Permeability of Rigid Porous Filters for Laboratory Use[15]
E 177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods[16]
E 456 Terminology for Relating to Quality and Statistics[16]
E 473 Terminology Relating to Thermal Analysis[16]
E 691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method[16]
E 793 Test Method for Enthalpies of Fusion and Crystallization by Differential Scanning Calorimetry[16]
E 794 Test Method for Melting and Crystallization Temperatures By Thermal Analysis[16]
E 967 Practice for Temperature Calibration of Differential Scanning Calorimeters and Differential Thermal Analyzers[16]
E 968 Practice for Heat Flow Calibration of Differential Scanning Calorimeters[16]
E 996 Practice for Reporting Data in Auger Electron Spectroscopy and X-Ray Photoelectron Spectroscopy[17]
E 1078 Guide for Procedures for Specimen Preparation and Mounting in Surface Analysis[17]
E 1142 Terminology Relating to Thermophysical Properties[16]
E 1294 Test Method for Pore Size Characteristics of Membrane Filters Using Automated Liquid Porosimeter[18]
E 1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products[18]
E 1356 Test Method for Assignment of the Glass Transition Temperatures by Differential Scanning Calorimetry or Differential Thermal Analysis[16]
E 1642 Practice for General Techniques of Gas Chromatography Infrared (GC/IR) Analysis[17]
E 1829 Guide for Handling Specimens Prior to Surface Analysis[17]
F 151 Test Method for Residual Solvents in Flexible Barrier Materials[11]
F 316 Test Method for Pore Size Characteristics of Membrane Filters by Bubble Point and Mean Flow Pore Test[19]
F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices[20]
F 1249 Test Method for Water Vapor Transmission Rate Through Plastic Film and Sheeting Using a Modulated Infrared Sensor[11]
F 1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices[20]
F 1634 Practice for In-Vitro Environmental Conditioning of Polymer Matrix Composite Materials and Implant Devices[20]
F 1635 Test Method for In Vitro Degradation Testing of Poly (L-lactic Acid) Resin and Fabricated Form for Surgical Implants[20]
F 1884 Test Method for Determining Residual Solvents in Packaging Materials[11]
F 1980 Guide for Accelerated Aging of Sterile Medical Device Packages[11]
F 1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Applications[11]
F 2025 Practice for Gravimetric Measurement of Polymeric Components for Wear Assessment[20]
F 2027 Guide for Characterization and Testing of Substrate Materials for Tissue-Engineered Medical Products[20]
G 120 Practice for Determination of Soluble Residual Contamination in Materials and Components by Soxhlet Extraction[15]

2.2 *AAMI Standards:*

AAMI STBK9-1 Sterilization—Part 1: Sterilization in Health Care Facilities[21]
AAMI STBK9-2 Sterilization—Part 2: Sterilization Equipment[21]
AAMI STBK9-3 Sterilization—Part 3: Industrial Process Control[21]

2.3 *ANSI Standards:*

[8] *Annual Book of ASTM Standards*, Vol 04.08.
[9] *Annual Book of ASTM Standards*, Vol 08.03.
[10] *Annual Book of ASTM Standards*, Vol 07.02.
[11] *Annual Book of ASTM Standards*, Vol 15.09.
[12] *Annual Book of ASTM Standards*, Vol 11.03.
[13] *Annual Book of ASTM Standards*, Vol 04.09.
[14] *Annual Book of ASTM Standards*, Vol 06.03.
[15] *Annual Book of ASTM Standards*, Vol 14.04.
[16] *Annual Book of ASTM Standards*, Vol 14.02.
[17] *Annual Book of ASTM Standards*, Vol 03.06.
[18] *Annual Book of ASTM Standards*, Vol 11.05.
[19] Discontinued 1995; see 1994 *Annual Book of ASTM Standards*, Vol 11.02.
[20] *Annual Book of ASTM Standards*, Vol 13.01.
[21] Available from the Association for the Advancement of Medical Instrumentation, 1110 N. Glebe Rd., Suite 220, Arlington, VA 22201-4795.

ANSI/ISO/ASQ Q9000-2000: Quality Management Systems—Fundamentals and Vocabulary[22]

ANSI/ISO/ASQ Q9001-2000: Quality Management Systems: Requirements[22]

2.4 *British Standards Institute:*

British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk (EN 12442-1)[22]

British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 2: Controls on Sourcing, Collection, and Handling (EN 12442-2)[22]

British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 3: Validation of the Elimination and/or Inactivation of Viruses and Transmissible Agents (EN 12442-3)[22]

2.5 *ISO Standards:*

ISO 1133-1991 Determination of the Melt-Mass Flow Rate (MFR) and the Melt Volume-Flow Rate (MVR) of Thermoplastics[22]

ISO 10993-9 Biological Evaluation of Medical Devices—Part 9: Degradation of Materials Related to Biological Testing[22]

ISO 10993-13 Biological Evaluation of Medical Devices—Part 13: Identification and Quantification of Degradation Products from Polymers[22]

ISO 10993-14 Biological Evaluation of Medical Devices—Part 14: Identification and Quantification of Degradation Products from Ceramics[22]

ISO 10993-15 Biological Evaluation of Medical Devices—Part 15: Identification and Quantification of Degradation Products from Coated and Uncoated Metals and Alloys[22]

ISO 11357-1 Plastics—Differential Scanning Calorimetry (DSC)—Part 1: General Principles[22]

ISO 11357-2 Plastics—Differential Scanning Calorimetry (DSC)—Part 2: Determination of Glass Transition Temperature[22]

2.6 *U.S. Code of Federal Regulations:*

Title 21—Food and Drugs Services, Part 820—Quality System Regulation (21 CFR Part 820)[23]

2.7 *U.S. Pharmacopeia (USP) Standards:*

Source: General Tests and Assays—USP24/NF19, Jan. 1, 2000[24]

3. Terminology

3.1 Unless provided otherwise in 3.2, terminology shall be in conformance with Terminology F 1251.

3.2 *Definitions:*

3.2.1 *bioactive agents*, *n*—any molecular component in, on, or with the interstices of a device that is intended to elicit 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 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 nonwetting 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.

3.2.6 *scaffold*, *n*—a support, delivery vehicle, or matrix for facilitating the migration, binding, or transport of cells or bioactive molecules used to replace, repair, or regenerate tissues.

3.2.6.1 *Discussion*—ASTM Committee F04 is continuing to refine definitions for the terms tissue engineering, tissue-engineered medical products (TEMPs), and scaffold. Final definitions will be from consideration of Committee F04 and other resources such as *The Williams Dictionary of Biomaterials* (**9**) and will be balloted at a later date.

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 Numerous general areas of characterization also should be considered when developing a scaffold for TEMPs. Among these are compositional identity, physical and chemical properties or characteristics, viable sterilization techniques, degradability/resorbability, and mechanical properties.

4.3 Application of the test methods contained within this guide do 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 may be solid or porous, mechanically rigid or gelatinous, absorbable/degradable, or nonresorbable/nondegradable. The scaffold may or may not have a surface treatment. Because of this large breadth of possible substrate materials and scaffold constructions, this guide cannot be considered as exhaustive in its

[22] Available from American National Standards Institute, 25 W. 43rd St., 4th Floor, New York, NY 10036.

[23] Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20593.

[24] Available from U.S. Pharmacopoeia, 12601 Twinbrook Pkwy., Rockville, MD 20852. The standards will be listed by appropriate USP citation number. Succeeding USP editions alternately may be referenced.

listing of potentially applicable tests. A voluntary guidance for the development of tissue-engineered products can be found in Omstead, et al **(1)**.[25]

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.

5.3 A listing of potentially applicable substrate specific tests may be found in Guide F 2027, with additional tests listed in X1.4 of this guide. Other unique characterization procedures may also be relevant and not covered by this guide.

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, or the chemical nature of the scaffold surface.

6.1 *Identification of Impurities*:

6.1.1 Chemical impurities are expected and unexpected contamination that is not part of the intended design of the scaffold. Acceptable levels of impurities are a function of the nature of the contamination and the scaffold's intended in vitro or in vivo application. A more precise definition of both contaminants and impurities and guidance regarding their significance may be found in Guide E 1298.

6.1.2 Expected impurities of potential biological significance should be monitored through appropriate analytic means. Such typical impurities may include, but are not limited to processing aids or solvents, unreacted cross-linking agents, residual monomers, endotoxins, sterilization residuals, and residual solutions used in the production of collagen, elastin, or other naturally derived products 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.

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, extraction in appropriate solvents becomes indicated.

6.1.4.1 *Solvation/Dissolution*—In the absence of known or established dissolution solvents for a particular substrate, Practice D 5226 may be referred to deliver added guidance in identifying suitable potential solvents for dissolving a scaffold material. Note that samples cannot be dissolved in analytic solvents that can also be considered as potential contaminants or create analytic interferences.

6.1.4.2 Extraction of residuals may be undertaken by utilization of methods such as Practice G 120. The extract then may 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 (µg/g;mg/kg), or ppb (ng/g;µg/kg) 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) is best used for the routine detection of volatile relatively low molecular weight impurities or contaminants. Some methods that may prove suitable include Test Methods F 151 and F 1884.

6.1.6.2 Gas chromatography can be coupled with both quantitative and qualitative analytic methods such as infrared spectrophotometry (IR) or mass spectroscopy (MS) to provide compositional identification while quantitatively detecting low molecular weight volatile impurities or contaminants. Some particular methods that may prove useful include Test Method D 6420 and Practice E 1642.

6.2 *Molecular Weight Determination*:

6.2.1 For polymeric materials (synthetic or natural), the molecular weight and molecular weight distribution may be determined through size exclusion chromatography (SEC) or gel permeation chromatography (GPC). Other procedures such as inherent or intrinsic viscosity, light scattering, or membrane osmometry may be used.

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 molecular weight test methods. In the absence of known or established dissolution solvents for a particular scaffold substrate, Practice D 5226 provides added guidance in identifying suitable potential solvents for dissolving a substrate material.

6.2.3 The following test methods may be applicable in the determining the molecular weight of the fabricated scaffold.

NOTE 2—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 D 5296 and D 6474 and Practices D 3016 and D 6579.

NOTE 3—The SEC solvent system and calibration standard polymer type should be specified with any obtained result.

6.2.3.2 *Inherent Viscosity*—See Practice D 2857 and Test Method D 4603.

NOTE 4—The test temperature, solvent system, and sample concentration should be included with any reported result.

6.2.3.3 *Light Scattering*—See Test Method D 4001.

NOTE 5—This test method is suitable for both linear and branched polymer systems.

6.2.3.4 *Melt Flow*—If a substrate is found insoluble after utilizing the guidance contained within Practice D 5226, melt

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

TABLE 1 USP Chemical Tests

USP 24-Test No.	Test Description	USP 24-Pages
<197>	Spectrophotometric identification	1855–1856
<231>	Heavy metals	1858–1859
<381>	Elastomeric closures for injections—physicochemical test procedures	1867–1868
<731>	Loss in drying (water content)	1954
<736>	Mass spectroscopy-purity or elemental analysis	1954–1958
<761>	Nuclear magnetic resonance-purity or component analysis (for example, copolymers)	1959–1965
<851>	Spectrophotometry and light scattering-(molecular weight information)	1992–1997
<891>	Thermal analysis (purity)	1999–2000
<911>	Viscosity (molecular weight)	2002–2005
<921>	Water determination	2003–2005

rheology (melt flow rate) may replace the measurements of solution properties to obtain an indication of the material's molecular weight and molecular weight distributions. Potentially useful methods include Test Method D 1238 and ISO 1133–1991.

6.3 *USP Chemical Tests*—See Table 1.

7. Physical Properties and Tests

NOTE 6—Physical properties are those of a compound that can change without involving a change in chemical composition.[26] 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 *Porosity Characterization*—The following test methodologies are recommended for consideration in the evaluation and characterization of the porosity of scaffolds to be used for TEMPs (see X1.2 of this guide for further discussion on the nature, significance, and potential applicability of these test methods):

7.1.1 *Porosimetry (Liquid Intrusion)*—Methodology suitable for the mercury intrusion measurement of porosity include Test Methods D 2873 and D 4404.

7.1.2 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 cm^3/g
Bulk density—in g/cm^3
Total percentage porosity

$$\frac{\text{Total intrusion (void) volume, } cm^3/g}{1/\text{ bulk density, } g/cm^3} \times 100 = \text{total \% porosity}$$

7.1.3 *Porometry*—Methodology suitable for the capillary flow measurement of porosity include Test Methods E 128, E 1294, and F 316.

7.1.4 The sample data recommended to be obtained and reported are maximum or bubble point pore diameter (in microns); mean flow pore diameter (in microns); and pore size range or distribution, or both, (in microns).

7.1.5 *Pneumatic Permeability*—The methodology suitable for measurement of the pneumatic permeability of a scaffold structure includes Test Method D 6539.

7.1.5.1 The sample data recommended to be obtained and reported is as follows:

Average coefficient of pneumatic permeability—report in darcy (0.99 μm^2) or millidarcy (0.000 99 μm^2)

NOTE 7—In each of the aforementioned porosity, porometry, and permeability tests, bulkier samples may require modification into a thinner profile to allow proper specimen placement into the apparatus (for example, microtome or other sectioning techniques). In such situations, the specimen thickness should be adjusted to be as thick as practical and the test thickness as tested reported with the result. If the sample is anisotropic in nature, separate porometry or permeability sampling profiles for each orientation is recommended.

7.2 Glass transition temperatures, melting temperatures, and crystallinity may have an effect on the mechanical properties of the scaffold. Measurement of these properties may be appropriate to ensure consistency in mechanical properties and to identify batch to batch variations of scaffold materials.

7.2.1 Methodology that may be suitable for DSC measurement of glass transition and melting temperatures, or crystallinity of scaffolds include Test Methods D 3417, D 3418, E 793, E 794, E 1356, Terminologies E 473 and E 1142, and Practices E 967 and E 968. Other potentially relevant standards include ISO 11357–1 and 11357–2.

NOTE 8—Crystallinity also may be measured by X-ray diffraction.

7.3 *USP Physical Tests*—See Table 2.

7.4 *Other Physical Tests*:

7.4.1 Water absorption characteristics may be evaluated using Test Method D 570.

7.4.2 Density may be assessed using Test Methods D 792 if not evaluated within a porosimetry method as described in 7.1.1.

7.4.3 *Surface Properties*—The extent of surface characterization of scaffold substrates will depend on the nature of the scaffold material and its particular use. Users are encouraged to consider Ratner, et al **(5,6)** for guidance into the methods of surface characterization of scaffold substrates. Other methods that may be pertinent include Guides E 1078 and E 1829, and Practice E 996.

7.4.4 *Vapor Permeability of Films*—In the event the scaffold is constructed in the form of a film, vapor permeability may be

TABLE 2 USP Physical Tests

USP 24-Test No.	Test Description	USP 24-Pages
<616>	Bulk density and tapped density	1913–1914
<661>	Containers—biological tests (PET, PE and Ophthalmic polymers)	1930–1936
<699>	Density of solids	1940
<701>	Disintegration	1941
<741>	Melting range or temperature	1958–1959
<776>	Optical microscopy	1965–1967
<786>	Particle size distribution by analytical sieving	1969-1970
<846>	Specific surface area	1990–1992
<941>	X-ray diffraction—crystallinity	2005–2007
<1045>	Biotechnology derived articles (may be useful for natural materials)	2011–2026
<1181>	Scanning electron microscopy (characterization of surfaces)	2125–2128

[26] S. P. Parker, Ed., *McGraw Hill Dictionary of Scientific and Technical Terms*, McGraw Hill Book Company, New York, third edition, 1984.

determined using Test Method F 1249. Reference **(7)** also contains methods potentially useful in determining film permeability.

8. Mechanical Properties and Tests

NOTE 9—Mechanical properties are those which involve a relationship between stress and strain or provide a reaction to an applied physical force.[26]

8.1 Mechanical evaluations should preferentially occur in an environment similar to the expected service condition or expected condition of use. Sample preconditioning may be needed and can be conducted as described in Practice F 1634. In vitro conditioning typically employs buffered saline solutions at 37°C as described in Test Method F 1635.

8.2 Special mounting of specimens may be necessary dependent on configuration of the scaffold and measurement equipment variety and dimensions.

8.3 *Compressive Properties*—Dependent on a scaffold's physical or dimensional characteristics, its compressive properties may be evaluated using methodology found in one or more of the following Test Methods: D 695 and D 1621.

8.4 *Tensile Properties*—Dependent on a scaffold's physical or dimensional characteristics, its tensile properties may be evaluated using methodology found in one or more of the following Test Methods: D 412, D 638, D 882, D 1623, D 1708, and D 3039/D 3039M.

8.5 *Flexural/Bending Properties*—Dependent on a scaffold's physical or dimensional characteristics, its flexural properties may be evaluated using methodology found in one or more of the following Test Methods: D 648, D 671, D 747, D 790, D 1388, D 5732, and D 6125.

8.6 *Creep Characteristics*—If a scaffold is to be used in applications in which it is expected to maintain its mechanical properties while under constant strain, methodology found in Test Methods D 2990 may be useful.

8.7 *USP Mechanical Tests*—See Table 3.

9. Biological Tests and Evaluations

9.1 For many biomaterials, the in vivo response has been thoroughly characterized by way of both clinical use and long-term evaluations in laboratory animals. When new applications of a biomaterial or modifications to the physical form of the biomaterial are being considered, then the recommendations and test methods described within the following Practices should be considered: F 748 and F 1983.

9.1.1 *ISO 10993*—This standard contains a series of parts, each of which can assist the user dependent on evaluation needs. Particularly relevant selections for consideration in the characterization of TEMP scaffolds include the following:

9.1.1.1 *Part 1*—Evaluation and testing;

9.1.1.2 *Part 3*—Tests for genotoxicity, carcinogenicity, and reproductive toxicity;

9.1.1.3 *Part 5*—Tests for cytotoxicity: in vitro methods;

TABLE 3 USP Mechanical Test

USP 24-Test No.	Test Description	USP 24-Pages
<881>	Tensile strengths (fibers or films)	1998–1999

9.1.1.4 *Part 6*—Tests for local effects after implantation;

9.1.1.5 *Part 9*—Framework for the identification and quantification of potential degradation products;

9.1.1.6 *Part 10*—Tests for irritation and sensitization;

9.1.1.7 *Part 11*—Tests for systemic toxicity;

9.1.1.8 *Part 12*—Sample preparation and reference materials;

9.1.1.9 *Part 13*—Identification and quantification of degradation products from polymeric medical devices; and

9.1.1.10 *Part 16*—Toxicokinetic study design for degradation products and leachables.

9.1.2 *USP-24: <1074> and <1078>*—These two references offer guidance for safety evaluation of and good manufacturing practices (GMP) for pharmaceutical excipients. These tests can be generally applied to medical materials used for TEMP scaffolds.

9.1.3 Further but more specific guidance may be indicated depending on the composition or intended use of the product. Examples of pertinent supplemental guidance are as follows:

9.1.3.1 *USP -24:<1045> to <1050>*—This series provides guidance for the proper characterization and assessment of biotechnology derived articles or products.

9.1.3.2 *British Standard—Animal Tissues and Their Derivatives Used in the Manufacture of Medical Devices, Parts 1 and 3*—This series addresses the special evaluation requirements of animal-derived products, for example, hyaluronic acid, collagen, gelatin, and ascites-derived monoclonal antibodies.

9.1.4 *Impurities*—A definition of biological contaminants and impurities and guidance regarding their detection and significance may be found in Guide E 1298. Additional guidance and tests regarding biological impurities include USP 24: <85>—Bacterial Endotoxin; Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices; and Interim Guidance for Human and Veterinarian Drug Products and Biologicals—Kinetic LAL Techniques.

9.2 *USP Biological and Microbiological Tests and Assays*—See Table 4.

9.3 *Histomorphometry*—Histomorphometric analytical methods of the scaffold material may be found in Von Recum **(2)**. Histomorphic features and parameters of particular interest to TEMP applications may be found in documents prepared by F04.42 Tissue Characterization and F04.41 Normal Biological Function Subcommittees.

TABLE 4 USP Biological and Microbiological Tests and Assays

USP 24-Test No.	Test Description	USP 24-Pages
<51>	Antimicrobial effectiveness	1809–1811
<71>	Sterility	1818–1823
<87>	Biological activity in vitro test which includes extractables from polymeric materials	1831–1832
<88>	Biological reactivity—in vivo	1833–1836
<151>	Pyrogen	1850–1851
<1045>	Biotechnology derived articles (may be useful for natural materials)	2011–2026
<1211>	Sterilization and sterility assurance of compended articles	2143–2148

10. Degradation Properties and Tests

10.1 Dependent on the substrate material and processing, many of the aforementioned chemical, physical, mechanical, or biological properties may change while the scaffold is degrading either in vivo or in cell culture conditions. A thorough characterization should be made of any property changes expected to occur under actual service conditions or expected conditions of use. Additionally, scaffold properties and their in vivo degradation profile may be affected by sterilization. Consequently, it is recommended that potentially affected scaffold properties be reevaluated for design compliance after sterilization processing.

10.2 Such degradation profiling can be conducted under specific controlled in vitro or in vivo conditions that model the intended application. When a material's degradation is primarily hydrolytic in nature, physiological conditions may be modeled in vitro at 37°C under controlled pH conditions as described in Test Method F 1635.

10.3 Besides the potentially appropriate chemical, physical, mechanical, and biological tests cited previously, other supplemental tests may become indicated to elicit pertinent scaffold property changes while under expected conditions of use. Some other tests to consider in such circumstances include Test Method D 1042 and Practice F 2025.

10.4 Additional guidance in the profiling of degradation and degradation products may be found in ISO 10993–9, ISO 10993–13, ISO 10993–14, and ISO 10993–15.

10.5 Acceleration of a scaffold's degradation profiling may be conducted. Guidance for such accelerated conditioning may be found in Practice F 1634 and Guide F 1980.

11. Sterilization

11.1 A summary of common sterilization methods, testing, and quality assurance can be found in USP <1211>. AAMI maintains a 3-volume set of sterilization standards and recommended practices containing 46 different standards: AAMI STBK9–1, AAMI STBK9–2, and AAMI STBK9–3. Additionally, a comprehensive discussion regarding radiation sterilization methods can be found in Burg, et al **(8)**.

12. Quality Assurance

12.1 *Test Validation*:

12.1.1 The precision and bias of each test method should be established. General guidelines for establishing precision and bias can be found in Practices E 177 and E 691 and Terminology E 456.

12.1.1.1 *USP <1225>*—See Table 5.

12.2 *Sampling*—It is suggested that the requirements shall be determined for each lot of the scaffold material by sampling sizes and procedures in accordance with Practice D 1898 or equivalent standard guidance.

12.3 *Packaging/Storage Conditions*:

12.3.1 *Maximum/Minimum Temperatures*—The maximum or minimum temperature to which the supplied product can be exposed safely without design compromise shall be marked plainly on the package.

12.3.2 *Storage Life*—The maximum time the supplied "as packaged" product can be safely stored at the maximum exposure temperature without adversely affecting product function or integrity shall be marked plainly on the package.

12.4 *Manufacturing Control Guidance*:

12.4.1 Acceptable levels of manufacturing control are highly desirable and likely to be required of commercially distributed TEMPs. General guidelines for achieving acceptable levels of manufacturing quality control may be found in the following standards:

12.4.1.1 United States Code of Federal Regulations, Title 21.

12.4.1.2 ANSI/ISO/ASQ Q9000-2000—Provides fundamentals for quality management systems as described in the ISO 9000 family (informative); and specifies quality management terms and their definitions (normative).

12.4.1.3 ANSI/ISO/ASQ Q9001-2000—Presents requirements for a quality management system. The application of this guide can be used by an organization to demonstrate its capability to meet customer requirements for products or services, and for assessment of that capability by internal and external parties.

13. Keywords

13.1 biomaterials; biomedical material; bioresorption; cell seeding; matrix; porometry; porosimetry; porosity; scaffold

TABLE 5 Precision and Bias

USP 24-Test No.	Test Description	USP 24-Pages
<1225>	Validation of compended methods (accuracy, precision, detection limit, quantitation limit, linearity range for new assay methods)	2149–2152

APPENDIX

(Nonmandatory Information)

X1. STANDARD METHODS FOR TESTING MATERIALS THAT WILL BE USED AS SCAFFOLDS

X1.1 As tissue engineered medical products (TEMPs) are being developed, there will be need to define standard methods for testing materials that will be used as scaffolds. The assumed primary purpose of these scaffolds is the support and delivery of biomolecules or living cells until the functional aspect of the TEMP is achieved. Thus, the purpose of this guide is to outline known test methods that help ensure safe functionality of the TEMP substrate material and fabricated scaffold. As the technology associated with TEMPs evolves, new and appropriate functional test methods for particular tissue or organ constructs will need to be developed.

X1.2 *References to Test Procedures*—This guide was written with the intention of providing a framework to assess materials that may be used as scaffolds. It was intended to encompass both absorbable and nonabsorbable materials, so it includes metals, ceramics, polymers, and composites. Many ASTM, ISO, and USP test methods are already published that assess the characteristics of bulk and surface properties of these materials for medical applications, therefore efforts were made to include these test methods in this guide. As the number of potential materials for application in TEMPs is great, no exclusion/inclusion criteria were used to select these test methods. Also, no attempts were made to outline all the safety concerns for a scaffold, as it will be the ultimate responsibility of the user to establish the safety of scaffold for a particular application.

X1.3 *Significance and Characterization of Scaffold Porosity*—The nature and extent of a scaffold's porous structure will inevitably affect the potential for cell and tissue ingrowth within its interstices. The permeability of a scaffold can potentially affect the transport and distribution of cells, cell nutrients, and waste products across its structure. Tissue response factors, such as oxygen tension and microvascularization, may be influenced by both the size of an implanted scaffold's pores, as well as the scope of their interconnectivity; thus, permeation techniques that additionally assess the size and extent of connectivity constrictions within a fully integrated scaffold structure provide superiority in both scope and objectivity of porous characterization when compared to simple sectioning techniques. Consequently, permeation techniques deliver a deeper understanding of the nature of a scaffold's interstitial void spaces and their related potential for cellular and tissue penetration.

X1.3.1 There are two fundamental methods for measuring the permeation characteristics of scaffold pores engineered for tissue ingrowth: flow and intrusion. The measurement of flow, known as porometry, generally uses the flow of gases or liquids, or both, completely across a porous structure to elucidate the characteristics of the substrate's pores; however, porosimetry, the measurement of liquid intrusion into open interstices, is not limited to penetrating porosity completely, treating both "blind holes" and "through pores" similarly. As a result, porosity data may differ dramatically between these two test methods dependent on the design features of the scaffold. Often, the combination of information derived from both test methods will elicit significant insight regarding the presence or absence of blind holes that may potentially affect oxygen tension and microvascularization within the implant. Consequently, the specific test method used to develop porosity data should always be cited.

X1.3.2 Flow porometry test methods restrict themselves to the measurement of "through pores" that allow fluid transport to penetrate through a structure completely. Since complete passage of the test gas or liquid is essential, porometry characterizes the nature of a pore at its narrowest restriction. Results are typically reported as mean pore size and pore size distribution. Since porometry measures points of greatest restriction, the test method does not provide information regarding the total pore volume that encompasses the porous structure on either side of the flow restriction. Additionally, porometry does not measure the size or dimension of closed "blind hole" or "dead end" pores that do not fully penetrate the structure. Porometry results determine the effectiveness of a sample as a barrier to particulates. Typically, such porometry test methods can measure pore sizes ranging from 0.013 to 500 µm, depending on the quality of the equipment and nature of the material. Porometry is a nondestructive, nontoxic test method.

X1.3.3 Intrusion test methods measure pores that are open to the outside of the material and can be permeated by a liquid, typically mercury. As pressure is increased, increasingly smaller pores are penetrated by the intruding liquid and the volume displacement measured. Such penetration does not differentiate between "blind holes" and "through pores," treating each similarly. Additionally, such a penetration pattern restricts measurement of the volume of internal spatial voids that communicate to the outside only through smaller pore structures. Also, since the liquid volume penetrating the interstices is measured, the test method can yield the total pore volume exposed to the outside of a structure, as well as the substrate's interstitial surface area and apparent/bulk density. Intrusion test methods can typically measure pore sizes ranging from 0.0035 to 500 µm, depending on the quality of the equipment and nature of the test substrate.

X1.4 *Supplemental Substrate Specific Test Methods*—Each individual biomaterial has unique features and properties that may require characterizations that are highly specific and beyond the scope of more the general tests presented in Table X1.1. Examples of specifications containing such specialized characterizations are presented as follows. Dependent on the manufacturing, and application of the scaffold material, other specifications not listed herein may be pertinent for the proper

TABLE X1.1 General Tests

Biomaterial Category	Specification or Test Description
Allographic or xenographic materials	F 1581 Specification for Composition of Anorganic Bone for Surgical Implants[A]
	Guide for Characterization of Collagen for Surgical Implants and Substrates for Tissue-Engineered Medical Products (TEMPs) (TBD F04.43.03 document currently under development in TEMP Division IV)
Ceramics	F 603 Specification for High-Purity Dense Aluminum Oxide for Surgical Implant Applications[A]
	F 1088 Specification for Beta-Tricalcium Phosphate for Surgical Implantation[A]
	F 1185 Specification for Composition of Ceramic Hydroxyapatite for Surgical Implants[A]
	F 1538 Specification for Glass and Glass Ceramic Biomaterials for Implantation[A]
	F 1873 Specification for High-Purity Dense Yttria Tetragonal Zirconium Oxide Polycrystal (Y-TZP) for Surgical Implant Applications[A]
	F 1926 Test Method for Evaluation of the Environmental Stability of Calcium Phosphate Coatings[A]
	ISO 6474:1994 Implants for Surgery—Ceramic Materials Based on Alumina[B]
Metals	F 67 Specification for Unalloyed Titanium for Surgical Implant Applications (UNS R50250, UNS R50400, UNS R50550, UNS R50700)[A]
	F 75 Specification for Cobalt-28, Chromium-6 Molybdenum Casting Alloy and Cast Products for Surgical Implant Applications[A]
	F 90 Specification for Wrought Cobalt-20 Chromium-15 Tungsten-10 Nickel Alloy for Surgical Implant Applications[A]
	F 136 Specification for Wrought Titanium 6Aluminum-4Vanadium ELI Alloy for Surgical Implant Applications[A]
	F 138 Specification for Wrought 18 Chromium-14 Nickel-2.5 Molybdenum Stainless Steel Bar and Wire for Surgical Implants (UNS S31673)[A]
	F 139 Specification for Wrought 18 Chromium-14 Nickel-2.5 Molybdenum Stainless Steel Sheet and Strip for Surgical Implants (UNS S31673)[A]
	F 562 Specification for Wrought Cobalt-35 Nickel-20 Chromium-10 Molybdenum Alloy for Surgical Implant Applications[A]
	F 563 Specification for Wrought Cobalt-20 Nickel-20 Chromium-3.5 Molybdenum-3.5 Tungsten-5 Iron Alloy for Surgical Implant Applications (UNS R30563)[A]
	F 1713 Specification for Wrought Titanium-13, Niobium-13 Zirconium Alloy for Surgical Implant Applications[A]
Polymers	F 451 Specification for Acrylic Bone Cement[A]
	F 602 Criteria for Implantable Thermoset Epoxy Plastics[A]
	F 604 Specification for Silicone Elastomers Used in Medical Applications[A]
	F 619 Practice for Extraction of Medical Plastics[A]
	F 624 Guide for Evaluation of Thermoplastic Polyurethane Solids and Solutions for Biomedical Applications[A]
	F 639 Specification for Polyethylene Plastics for Medical Applications[A]
	F 648 Specification for Ultra-High-Molecular Weight Polyethylene Powder and Fabricated Form for Surgical Implants[A]
	F 665 Classification for Vinyl Chloride Plastics Used in Biomedical Applications[A]
	F 754 Specification for Implantable Polytetrafluorethylene (PTFE) Polymer Fabricated in Sheet, Tube, and Rod Shapes[A]
	F 1635 Test Method for In Vitro Degradation Testing of Poly (L-lactic Acid) Resin and Fabricated Form for Surgical Implants[A]
	F 1925 Specification for Virgin Poly (L-Lactic Acid) Resin for Surgical Implants[A]
	F 2064 Guide for Characterization and Testing of Alginates as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Products Application[A]
	F 2103 Guide for Characterization and Testing of Chitosan Salts as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Product Applications[A]
	USP-24, NF-19; Jan. 1, 2000[C]
	Methacrylate acid copolymer: pp. 2477–2479
	Polyoxamer: pp. 2492–2493
	Polyethylene glycol: pp. 2493–2500
	Polysorbate 40,60,80: pp. 2501–2502
	Polyvinyl acetate phthalate: pp. 2502–2503
	Propylene glycol-alginate: pp. 2506–2507
	Shellac: pp. 2512–2513
	Sodium alginate: p. 2515
	Xanthum gum: pp. 2537–2538

[A] *Annual Book of ASTM Standards*, Vol 13.01.
[B] Available from American National Standards Institute, 25 W. 43rd St., 4th Floor, New York, NY 10036.
[C] Available from U.S. Pharmacopoeia, 12601 Twinbrook Pkwy., Rockville, MD 20852.

evaluation of the biomaterial. An appropriate quality standard should be adopted regardless of the published standard's specificity to implantable applications.

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