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

This standard is issued under the fixed designation F2150; 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 the compositional and structural aspects 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 in the selection of appropriate test methods for the generation of an 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 is intended to be utilized in conjunction with appropriate characterization(s) and evaluation(s) of any raw or starting material(s) utilized 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. 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.

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

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
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
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

[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.

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
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
E1294 Test Method for Pore Size Characteristics of Membrane Filters Using Automated Liquid Porosimeter (Withdrawn 2008)[3]
E1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products
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
F1634 Practice for *In-Vitro* Environmental Conditioning of Polymer Matrix Composite Materials and Implant Devices
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 Compatibility of Absorbable/Resorbable 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 Biomaterials 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
F2809 Terminology Relating to Medical and Surgical Materials and Devices
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

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

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 *British Standards Institute:*[5]
BSI BS EN 12441–1 British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk
BSI BS EN 12442–2 British Standard—Animal Tissues and Their Derivatives Utilized in the Manufacture of Medical Devices—Part 2: Controls on Sourcing, Collection, and Handling
BSI BS EN 12442–3 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
2.5 *ISO Standards:*[5]
ISO 1133–1 Determination of the Melt-Mass Flow Rate (MFR) and the Melt Volume-Flow Rate (MVR) of Thermoplastics
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 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 80000–9 Quantities and Units—Part 9: Physical Chemistry and Molecular Physics
2.6 *U.S. Code of Federal Regulations:*[6]
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
2.7 *U.S. Pharmacopeia (USP) Standards:*[7]
<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
<231> Heavy Metals
<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
<1045> Biotechnology Derived Articles
<1181> Scanning Electron Microscopy
<1211> Sterilization and Sterility Assurance of Compendial Articles
<1225> Validation of Compendial Procedures
2.8 *NIST Document:*[8]
NIST SP811 Special Publication SP811: Guide for the Use of the International System of Units (SI)
2.9 *Other Documents/Web Sites:*
U.S. Food & Drug Administration (FDA) Center for Devices & Radiologic Health (CDRH), Consensus Standards Database[9]
FDA-CDRH Guidance Documents Database[10]
FDA-CDRH Premarket Approval (PMA) Database[11]
FDA-CDRH 510(k) (Premarket Notification) Database[12]

[4] Available from the Association for the Advancement of Medical Instrumentation, 1110 N. Glebe Rd., Suite 220, Arlington, VA 22201-4795.
[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 U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.
[7] 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.
[8] Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 1070, Gaithersburg, MD 20899-1070, http://www.nist.gov.
[9] Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm.
[10] Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfggp/search.cfm.
[11] Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm.
[12] Available from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm

3. Terminology

3.1 Unless provided otherwise in 3.2, terminology shall be in conformance with Terminologies F2809 and 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 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.

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 utilized in TEMPs may include 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 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 nonresorbable/nondegradable. 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**).[13] 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.

5.3 A listing of potentially applicable tests for characterizing and analyzing the materials utilized 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*

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

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 immunonblot 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, ELISA 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 (µg/g;mg/kg), ppb (ng/g;µg/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 MethodF1884.

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.

TABLE 1 USP Chemical Tests

USP Test No.	Test Description
<197>	Spectrophotometric identification
<231>	Heavy metals
<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

6.2.3.4 *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 1133–1991.

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

$$= \frac{\text{Total intrusion (void) volume (in } cm^3/g)}{1 \,/\, [\text{bulk density (in } g/cm^3)]}$$

7.2.2 *Porometry*—Methodology suitable for the capillary flow measurement of pore size and its distribution include Test Methods E128, E1294, and F316.

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

7.2.3 *Pneumatic Permeability*—The methodology suitable for measurement of the pneumatic permeability of a scaffold structure includes Test Method D6539.

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

Average coefficient of pneumatic permeability—report in Darcy ($0.99\ \mu m^2$) or millidarcy ($0.000\ 99\ \mu m^2$)

NOTE 9—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.

NOTE 10—If evidence of collapse or distortion of the scaffold's porous structure is observed as a result of the application of analytic test pressures (that is, induced reversible or non-reversible distortions not reasonably expected under *in vivo* or *in vitro* service conditions), either method modifications (for example, use of an alternative fluid or reduced test pressure range) or alternative pore characterization methodologies should be employed. If significant distortion or other analytic interferences are suspected, utilization of one or more alternative characterization methods may be needed to either corroborate or discard the obtained results.

NOTE 11—If scaffold construction can be reasonably expected to possess either bimodal (for example, both macroporosity and microporosity) or multi-modal distribution of pore sizes, such characteristics should be both quantified and reported and, dependent on actual pore size, may require utilization of multiple pore characterization methodologies.

7.3 Glass transition temperatures, melting temperatures, and crystallinity may have an effect on the mechanical properties of polymer-based scaffolds. Measurement of these properties may be appropriate to ensure consistency in mechanical properties and to identify lot-to-lot variations of scaffold materials.

7.3.1 Methodologies that may be suitable for differential scanning calorimetry (DSC) measurement of glass transition and melting temperatures, or crystallinity of scaffolds include Test Methods D3418, E793, E794, E1356; Terminologies E473 and E1142; and Practices E967 and E968. Other potentially relevant standards include ISO 11357–1 and ISO 11357–2.

NOTE 12—Crystallinity also may be determined by X-ray diffraction.

7.4 *USP Physical Tests*—See Table 2.

7.5 *Other Physical Tests:*

7.5.1 Water absorption characteristics may be ascertained using Test Method D570.

7.5.2 Density may be assessed using Test Methods D792 if not evaluated within a porosimetry method as described in 7.2.1.

7.5.3 *Surface Properties*—The extent of surface characterization of a scaffold will depend on the nature of the scaffold material and its particular use. Users are encouraged to consider Ratner, et al **(6,7)** for guidance about the methods of surface characterization of scaffold starting materials, which includes determination of the surface free energy. A guide for the assessment of the surface texture of non-porous materials is available in Guide F2791. Other methods that may be pertinent include Guides E1078 and E1829, and Practice E996.

7.5.4 *Vapor Permeability of Films*—In the event the scaffold contains a film-like component, vapor permeability may be determined using Test Method F1249. Ref **(8)** also contains methods potentially useful in determining film permeability.

8. Mechanical Properties and Tests

NOTE 13—Mechanical properties are those which involve a relationship between stress and strain or provide a reaction to an applied physical force (**5**).

8.1 Where possible, mechanical evaluations should 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 F1634. *in vitro* conditioning typically employs buffered saline solutions at 37°C as described in Test Method F1635.

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

8.3 *Compressive Properties*—Depending 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: D695 and D1621.

8.4 *Tensile Properties*—Depending 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: D412, D638, D882, D1623, D1708, and D3039/D3039M.

8.5 *Flexural/Bending Properties*—Depending 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: D648, D747, D790, and D1388.

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 D2990 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:

9.1.1 Practice F748; and

9.1.2 Practice F1983.

9.1.3 *ISO 10993—Biological Evaluation of Medical Devices*—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:

9.1.3.1 *Part 1*—Evaluation and testing;

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

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

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

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

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

9.1.3.7 *Part 11*—Tests for systemic toxicity;

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

TABLE 2 USP Physical Tests

USP Test No.	Test Description
<616>	Bulk density and tapped density
<661>	Containers—biological tests (PET, PE and Ophthalmic polymers)
<699>	Density of solids
<701>	Disintegration
<741>	Melting range or temperature
<776>	Optical microscopy
<786>	Particle size distribution by analytical sieving
<846>	Specific surface area
<941>	X-ray diffraction—crystallinity
<1045>	Biotechnology derived articles (may be useful for natural materials)
<1181>	Scanning electron microscopy (characterization of surfaces)

TABLE 3 USP Mechanical Test

USP Test No.	Test Description
<881>	Tensile strengths (fibers or films)

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

9.1.3.10 *Part 16*—Toxicokinetic study design for degradation products and leachables;

9.1.3.11 *Part 17*—Establishment of allowable limits for leachable substances;

9.1.3.12 *Part 18*—Chemical characterization of materials;

9.1.3.13 *Part 19*—Physico-chemical, morphological and topographical characterization of materials; and

9.1.3.14 *Part 20*—Principles and methods for immunotoxicology testing of medical devices.

9.1.4 *USP: <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.

9.1.5 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:

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

9.1.5.2 *British Standard—Animal Tissues and Their Derivatives Used in the Manufacture of Medical Devices, Parts 1, 2, and 3 (BSI BS EN 12442–1, BSI BS EN 12442–2, and BSI BS EN 12442–3)*—This series addresses the special evaluation requirements of animal-derived products (for example, hyaluronic acid, collagen, gelatin, and ascites-derived monoclonal antibodies).

9.1.6 *Impurities*—A definition of biological contaminants and impurities and guidance regarding their detection and significance may be found in Guide E1298. Additional guidance and tests regarding biological impurities include USP: <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 *Good Laboratory Practice*—Non-clinical evaluations involving the use of biological test models to ascertain safety or biocompatibility of a scaffold product to a regulatory authority may need to be performed under Good Laboratory Practice (GLP) to assure the quality and integrity of the safety data. Specific details regarding GLP procedures and systems depend on the regulating authority. However, the most common citation for such practice may be found in: United States Code of Federal Regulations, Title 21, Chapter I, Subchapter A, Part 58—Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR Part 58—Food and Drug Administration).

TABLE 4 USP Biological and Microbiological Tests and Assays

USP Test No.	Test Description
<51>	Antimicrobial effectiveness
<71>	Sterility
<87>	Biological activity *in vitro* test which includes extractables from polymeric materials
<88>	Biological reactivity—*in vivo*
<151>	Pyrogen
<1045>	Biotechnology derived articles (may be useful for natural materials)
<1211>	Sterilization and sterility assurance of compended articles

9.4 *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.

10. Degradation Properties and Tests

10.1 Since a fundamental understanding of a scaffold's method of degradation is essential for its modeling, a brief description of the mechanism for any expected scaffold degradation, both *in vivo* and under *in vitro* culture conditions, shall be provided along with pertinent citations of select publications supporting that description. Examples of such description might contain wording such as "bond scission via ester group hydrolysis followed by renal excretion" or enzymatic cleavage.

10.2 Depending on the starting 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. For example, scaffold degradation products (for example, hyaluronic acid fragments or lactic acid from PLA) may deliver biological response properties quite different from the intact polymeric material. Thus, a thorough characterization and, if indicated, a suitable biological response assessment should be made of any property changes expected to occur under actual service conditions or expected conditions of use. For scaffolds fabricated from absorbable polymeric materials, additional assessment guidance can be found in Guide F2902. 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.

10.3 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 F1635. If scaffold degradation is dependent in whole or in part on enzymatic cleavage, enzymes or other reagents may be necessary for successful *in vitro* modeling of the material's *in vivo* performance. If *in vitro* evaluations are inadequate for modeling actual *in vivo* performance, direct *in vivo* evaluation of scaffold degradation properties may be necessary.

10.4 Besides the potentially appropriate chemical, physical, mechanical, and biological tests cited previously, other supplemental tests may be indicated to elicit pertinent scaffold property changes under expected conditions of use. Some other

tests to consider in such circumstances include Test Method D1042 and Practice F2025.

10.5 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.

10.6 Mechanical loading can impose stress that may affect the rate of scaffold degradation. Guidance regarding how to address the effect of mechanical loading and related concerns for creep and fatigue in absorbable polymeric constructs is discussed in Guide F2902.

10.7 Acceleration of a scaffold's degradation profiling may be conducted. Some guidance for such accelerated conditioning may be found in Practice F1634 and Guide F1980. However, the user is cautioned that the provided guidance is not necessarily complete for all situations and may not be applicable to many materials. (*1*) Appendix X2 provides a more comprehensive but non-exhaustive compilation of references that describe features common to an appropriate characterization of thermally accelerated degradation, some of which are specific to absorbable lactide/glycolide-based polymeric devices/specimens.

NOTE 14—It is essential that any accelerated study projections be validated with correlative real time aging data.

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 STBK, Parts 1, 2, and 3. Additionally, a comprehensive discussion regarding radiation sterilization methods can be found in Burg, et al (**9**).

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 E177 and E691 and Terminology E456.

12.1.1.1 *USP <1225>*—See Table 5.

TABLE 5 Precision and Bias

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

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 E1994 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 safely exposed 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 (CFR), Title 21, Part 820.

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 absorption; bioabsorption; biomaterials; biomedical material; bioresorption; cell seeding; matrix; porometry; porosimetry; porosity; scaffold; tissue engineering

APPENDIXES

(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 fabricated TEMP 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 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 that assess the characteristics of bulk and surface properties of these materials for medical applications are already published and are summarized in Guide F2027. 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 a 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 A preliminary observation of a scaffold structure is typically undertaken through light or scanning electron microscopy (SEM), with more sophisticated initial observations conducted with micro-CT. Very preliminary scaffold structure characterization can be undertaken by contrasting the density of the scaffold with the specific gravity of its solid form. However, none of these methods provide characterization of scaffold permeability. Guide F2450 contains additional discussion regarding the benefits and limitations of each of these methods, along with analytic alternatives.

X1.3.2 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 narrowest constriction within scaffold pore channels. Porosimetry, the measurement of liquid intrusion into open interstices, is not limited to penetrating porosity, treating both "blind holes" and "through pores" similarly. Neither method can detect closed pores that do not communicate to the outside of the scaffold structure and intrusion results are highly dependent on compositional surface free energy of both the scaffold and the non-wetting test liquid. Due to each method's respective difference in the principle of measurement, pore size results may differ as much as an order of magnitude 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 pore size data, whether derived from the permeability-based methods or by other means, should always be cited.

X1.3.3 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 pore characteristics outside that area. Additionally, porometry does not measure the sizes or dimensions of closed "blind hole" or "dead end" pores that do not penetrate the structure sufficiently to allow flow. 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.

X1.3.4 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 scaffold'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 test material composition.

X2. ACCELERATED DEGRADATION REFERENCES

X2.1 The following provide selected references perceived to be relevant to the development of evaluations involving the accelerated degradation of hydrolysable materials:

X2.1.1 Nelson, Wayne. "Accelerated Testing Statistical Models, Test Plans, and Data Analyses," John Wiley and Sons, New York, 1999.

X2.1.1.1 Comprehensive overview of accelerated aging factors and techniques.

X2.1.2 AAMI TIR17 — Compatibility of Materials Subject to Sterilization (see Section 6, Annex G, and Annex G references)

X2.1.2.1 Accelerated aging and combination device product stability programs.

X2.1.3 Guide F1980 — Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices.

X2.1.3.1 General planning for accelerated aging of packaging.

X2.1.4 M. Deng, J. Zhou, Chen, D. Burkley, Y. Xu, D. Jamiolkowski, T. Barbolt. "Effect of Load and Temperature on In Vitro Degradation of Poly(glycolide-co-l-lactide) Multifilament Braids," *Biomaterials*, Vol. 26, Issue 20, July 2005, pp. 4327–4336.

X2.1.4.1 Effect of loading during degradation; effect of degradation temperatures; reaction order determination; Arrhenius relationship.

X2.1.5 Meng Deng, Gavin Chen, Daniel Burkley, Jack Zhou, Dennis Jamiolkowski, Yunmei Xu, Robert Vetrecin. "A Study on In Vitro Degradation Behavior of a Poly(glycolide-co-l-lactide) Monofilament, *Acta Biomaterialia*, Vol. 4, Issue 5, September 2008, pp. 1382–1391.

X2.1.5.1 Molecular weight and mechanical property loss over time; effect of degradation temperatures; reaction order determination; Arrhenius relationship; activation energy; morphological observations.

X2.1.6 Hideto Tsuji, Tomonori Tsuruno, "Accelerated Hydrolytic Degradation of Poly(l-lactide)/Poly(d-lactide) Stereocomplex up to Late Stage," *Polymer Degradation and Stability*, Vol. 95, Issue 4, April 2010, pp. 477–484.

X2.1.6.1 Impact of hydrolytic degradation (accelerated) on crystallinity and molecular weight; Arrhenius relationship.

X2.1.7 Xiaoxiao Han, Jingzhe Pan, Fraser Buchanan, Neill Weir, David Farrar, "Analysis of Degradation Data of Poly(l-lactice-co-l, d-lactide) and Poly(l-lactide) Obtained at Elevated and Physiological Temperatures Using Mathematical Models," *Acta Biomaterialia*, Vol. 6, Issue 10, October 2010, pp. 3882–3889.

X2.1.7.1 Detailed summary and analysis of degradation models; Arrhenius relationship.

X2.1.8 William S. Pietrzak, Mukesh Kumar, Barry L. Eppley. "The Influence of Temperature on the Degradation Rate of LactoSorbCopolymer," *Journal of Craniofacial Surgery*," Vol. 14, Issue 2, March 2003, pp. 176–183.

X2.1.8.1 Thermal sensitivity of hydrolysis; Arrhenius relationship; temperature variation in animal models.

X2.1.9 Suming Li. "Hydrolytic Degradation Characteristics of Aliphatic Polyesters Derrived from Lactic and Glycolic Acids," *Journal of Biomedical Materials Research (Applied Biomaterials)*," Volume 48, 1999, pp. 342–353.

X2.1.9.1 Heterogeneous degradation; differences in degradation rate dependent on composition; crystallinity.

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(2) Von Recum, A. F., Ed., *Handbook of Biomaterials Evaluation Scientific, Technical, and Clinical Testing of Implant Materials*, Macmillan Publishing Co., New York, second edition, 1986.

(3) Shoichet, M. S. and Hubbell, J. A., Eds., *Polymers for Tissue Engineering*, VSP, Utrecht, The Netherlands, 1998.

(4) Lanza, R. P., Langer, R., and Chick, W. L., Eds., *Principles of Tissue Engineering*, Academic Press Inc., R. G. Landes Company, Austin, TX, 1997.

(5) S. P. Parker, Ed., *McGraw Hill Dictionary of Scientific and Technical Terms*, McGraw Hill Book Company, New York, third edition, 1984 .

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(9) Burg, K. J. L., and Shalaby S. W., "Radiation Sterilization of Medical and Pharmaceutical Devices," in *Radiation Effects of Polymers: Chemical and Technological Aspects*, ACS, Washington, DC, 1996, pp. 240–245.