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Standard Guide for Characterization of Ceramic and Mineral Based Scaffolds used for Tissue-Engineered Medical Products (TEMPs) and as Device for Surgical Implant Applications[1]

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1. Scope

1.1 This guidance document covers the chemical, physical, biological, and mechanical characterization requirements for biocompatible mineral- and ceramic-based scaffolds used solely as device or to manufacture tissue-engineered medical products (TEMPs). In this guide, the pure device or the TEMPs product will be referred to as scaffold.

1.2 The test methods contained herein provide guidance on the characterization of the bulk physical, chemical, mechanical, and surface properties of a scaffold construct. These properties may be important for the performance of the scaffold, especially if they affect cell behavior, adhesion, proliferation and differentiation. In addition, these properties may affect the delivery of bioactive agents, the biocompatibility and the bioactivity of the final product.

1.3 This document may be used as guidance in the selection of test methods for the comprehensive characterization of a raw materials, granules, pre-shaped blocks, or an original equipment manufacture (OEM) specification. This guide may also be used to characterize the scaffold component of a finished medical product.

1.4 While a variety of materials can be used to manufacture such scaffolds, the composition of the final scaffold shall contain mineral or ceramic components as its main ingredients.

1.5 This guide assumes that the scaffold is homogeneous in nature. Chemical or physical inhomogeneity or mechanical anisotropy of the scaffold shall be declared in the manufacturer's material and scaffold specification.

1.6 This guide addresses neither the biocompatibility of the scaffold, nor the characterization or release profiles of any biomolecules, cells, drugs, or bioactive agents that are used in combination with the scaffold.

1.7 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.8 *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 limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

C373 Test Method for Water Absorption, Bulk Density, Apparent Porosity, and Apparent Specific Gravity of Fired Whiteware Products

C693 Test Method for Density of Glass by Buoyancy

C729 Test Method for Density of Glass by the Sink-Float Comparator

C830 Test Methods for Apparent Porosity, Liquid Absorption, Apparent Specific Gravity, and Bulk Density of Refractory Shapes by Vacuum Pressure

C1198 Test Method for Dynamic Young's Modulus, Shear Modulus, and Poisson's Ratio for Advanced Ceramics by Sonic Resonance

C1274 Test Method for Advanced Ceramic Specific Surface Area by Physical Adsorption

C1424 Test Method for Monotonic Compressive Strength of Advanced Ceramics at Ambient Temperature

D695 Test Method for Compressive Properties of Rigid Plastics

D1621 Test Method for Compressive Properties of Rigid Cellular Plastics

D4404 Test Method for Determination of Pore Volume and Pore Volume Distribution of Soil and Rock by Mercury Intrusion Porosimetry

D6226 Test Method for Open Cell Content of Rigid Cellular Plastics

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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.

D6420 Test Method for Determination of Gaseous Organic Compounds by Direct Interface Gas Chromatography-Mass Spectrometry
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
E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
E996 Practice for Reporting Data in Auger Electron Spectroscopy and X-ray Photoelectron Spectroscopy
E1078 Guide for Specimen Preparation and Mounting in Surface Analysis
E1131 Test Method for Compositional Analysis by Thermogravimetry
E1252 Practice for General Techniques for Obtaining Infrared Spectra for Qualitative Analysis
E1269 Test Method for Determining Specific Heat Capacity by Differential Scanning Calorimetry
E1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products
E1504 Practice for Reporting Mass Spectral Data in Secondary Ion Mass Spectrometry (SIMS)
E1635 Practice for Reporting Imaging Data in Secondary Ion Mass Spectrometry (SIMS)
E1642 Practice for General Techniques of Gas Chromatography Infrared (GC/IR) Analysis
E1829 Guide for Handling Specimens Prior to Surface Analysis
E1876 Test Method for Dynamic Young's Modulus, Shear Modulus, and Poisson's Ratio by Impulse Excitation of Vibration
E2070 Test Method for Kinetic Parameters by Differential Scanning Calorimetry Using Isothermal Methods
E2253 Test Method for Temperature and Enthalpy Measurement Validation of Differential Scanning Calorimeters
F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices
F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone
F1088 Specification for Beta-Tricalcium Phosphate for Surgical Implantation
F1185 Specification for Composition of Hydroxylapatite for Surgical Implants
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
F1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Applications
F2024 Practice for X-ray Diffraction Determination of Phase Content of Plasma-Sprayed Hydroxyapatite Coatings
F2150 Guide for Characterization and Testing of Biomaterial Scaffolds Used in Tissue-Engineered Medical Products
F2450 Guide for Assessing Microstructure of Polymeric Scaffolds for Use in Tissue-Engineered Medical Products
F2809 Terminology Relating to Medical and Surgical Materials and Devices

2.2 *ISO Documents:*[3]

ISO 5016 Shaped Insulating Refractory Products—Determination of Bulk Density and True Porosity
ISO 10993–1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing
ISO 10993–14 Biological Evaluation of Medical Devices—Part 14: Identification and Quantification of Degradation Products from Ceramics
ISO 11607–1 Packaging for Terminally Sterilized Medical Devices—Part 1: Requirements for Materials, Sterile Barrier Systems and Packaging Systems
ISO 11607–2 Packaging for Terminally Sterilized Medical Devices—Part 2: Validation Requirements for Forming, Sealing and Assembly Processes
ISO 11737–1 Sterilization of Medical Devices—Microbiological Methods—Part 1: Determination of a Population of Microorganisms on Products
ISO 12677 Chemical Analysis of Refractory Products by XRF-Fused Cast Bead Method
ISO 15901–2 Pore Size Distribution and Porosity of Solid Materials by Mercury Porosimetry and Gas Adsorption—Part 2: Analysis of Mesopores and Macropores by Gas Adsorption
ISO 9000 Quality Management Systems—Fundamentals and Vocabulary
ISO 9001 Quality Management Systems—Requirements

2.3 *United States Pharmacopeia (USP) Documents:*[4]

USP <211> Arsenic
USP <231> Heavy Metals Method 1
USP <251> Lead
USP <261> Mercury

2.4 *Association for the Advancement of Medical Instrumentation (AAMI) Documents:*[5]

AAMI ST72 Bacterial endotoxins—Test methodologies, routine monitoring, and alternatives to batch testing
AAMI STBK9–1 Sterilization-Part 1: Sterilization in Health Care Facilities
AAMI STBK9–2 Sterilization-Part 2: Sterilization Equipment
AAMI STBK9–3 Sterilization-Part 3: Industrial Process Control

2.5 *Other References:*

FDA Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.
[4] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.
[5] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

and Animal Parenteral Drugs, Biological Products, and Medical Device, 1987[6]

21 CFR United States Code of Federal Regulations, Title 21[7]

3. Terminology

3.1 Unless provided otherwise in 3.2, terminology shall be in conformance with Terminology F2809

3.2 *Definitions:*

3.2.1 *bioactive agent, n*—any molecular component in, on, or within the interstices of a scaffold that is intended to elicit a desired tissue or cell response. Growth factors, antibiotics, and antimicrobials are examples of bioactive agents. Scaffold structural components or degradation byproducts that evoke limited localized bioactivity are not considered bioactive agents.

3.2.2 *interconnectivity, n*—the degree of connections between pores via necks. The overall interconnectivity of a scaffold is expressed as the percentage of interconnected pores divided by the total number of pores (see also Guide F2450).

3.2.3 *macropore, n*—in life sciences, a pore with dimensions exceeding 100 micrometers. [Tissue Level]. (See also Guide F2450.)

3.2.4 *micropore, n*—in life sciences, a pore with dimensions between 100 nanometers and 100 micrometers. [Cell Level]. (See also Guide F2450.)

3.2.5 *nanopore, n*— in life sciences, a pore with dimensions between 2 and 100 nanometers. [Molecular Level]. (See also Guide F2450.)

3.2.6 *permeability, n*—measure of fluid, particle, or gas flow through an open pore structure.

3.2.7 *pores, n*—an inherent or induced network of channels and open spaces within an otherwise solid structure. Pores may be open (interconnected), blind-end (open at one end) or closed (blind).

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

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

3.2.10 *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 or bulk) volume and is commonly expressed as a percentage.

3.2.11 *pure device, n*—A scaffold with no additional cells, genes, proteins or other biological agents that may cause antigenicity.

3.2.12 *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.

3.2.13 *specific surface area,, n*—the sum of external and internal accessible surfaces of voids, cracks, open porosity and fissures of a solid or powder in relation to its mass.

4. Summary of Guide

4.1 The physicochemical characteristics and three-dimensional structure of scaffolds influence the biological response of cells. The intent of this guide is to provide a selection of test methods that are required for comprehensive characterization of chemical, physical, and mechanical properties of a scaffold influencing consequently the biological performance.

4.2 A portfolio of characteristics should be considered when developing a mineral- or ceramic-based scaffold for TEMPs or as bone void filler for surgical implantation. Among these are identification of the following characteristics: scaffold composition, physical, chemical, and mechanical properties; viable sterilization techniques; and degradation/resorption behavior.

4.3 Application of the test methods contained within this guide does not guarantee clinical or regulatory success of a finished scaffold or product but will help to ensure consistency in the properties of a given scaffold material.

5. Significance and Use

5.1 Scaffolds may be composed of purely mineral or ceramic materials, or they may be composed of a composite material with its main phase being a mineral or ceramic. Scaffolds may be porous or non-porous, mechanically rigid or compliant, and degradable or non-degradable. The scaffold may or may not have undergone a surface treatment.

6. Chemical Properties and Tests

NOTE 1—Chemical properties are the chemical composition characteristics of a bulk 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 the chemical nature of the scaffold surface. If possible, all constituents used for manufacturing the mineral scaffolds should be pharmaceutical or medical grade or the final product should comply with the requirements for medical scaffolds. If the constituents are not of medical grade, the manufacturer of the device or TEMP shall demonstrate their quality and biocompatibility using appropriate standard test methods.

6.1 *Chemical Composition:*

6.1.1 There are several methods that can be used to determine the elemental composition of the material including, but not limited to, X-ray fluorescence analysis (XRF), atomic absorption analysis (AAS), and infrared spectrometry (IR). Guidelines are found in ISO 12677 or in Practice E1252. X-ray diffraction (XRD) can also be used to determine the chemical composition as an indirect method. Its use requires special care as it is an indirect method that requires identification of the crystal lattice. It does not produce accurate information when isoforms occur or amorphous and organic fractions are present.

[6] Available from Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993-0002, http://www.fda.gov.

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

6.1.2 The elemental composition of the bulk material shall be determined with a standard uncertainty of at least 0.5 %.

6.2 *Determination of the Total Organic Fraction:*

6.2.1 The total organic fraction includes synthetic and natural organic compounds. The total organic volumetric fraction is assumed to be smaller than the inorganic volumetric fraction in ceramic- or mineral-based scaffolds.

6.2.2 Several methods may be used to quantify the total organic fraction. Thermogravimetry is a versatile technique that allows for the quantification of highly volatile matter, medium volatile matter, combustible material, and the ash content of compounds. Note that surface water and chemically-bound water will also contribute to mass loss registered by thermogravimetry. A detailed description and guideline is found in Test Method E1131.

6.2.3 Organic fractions shall be identified by appropriate techniques. Identification of low molecular mass constituents may be obtained by gas chromatography (GC) or liquid chromatography (LC) coupled to highly sensitive detectors, including mass spectrometry (MS) or time-of-flight mass spectrometry (TOF). High molecular mass constituents can be analyzed with techniques including gel permeation chromatography and electrophoresis. A washing or extraction procedure with an appropriate organic solvent might be necessary as a primary step (see also 6.4.4).

6.3 *Endothermic/Exothermic Behavior:*

6.3.1 Differential Scanning Calorimetry (DSC) or Isothermal Calorimetry (IC) may be used to assess the thermal behavior of the scaffold, the interaction between different phases, and changes in kinetic properties as a function of temperature. Experiments shall be carried out according to current standards, for example Test Method E1269 and E2253.

6.3.2 Isothermal calorimetry test methods are applicable to exothermic and endothermic reactions where the thermal curves do not exhibit shoulders, discontinuities, or shifts in the baseline. A detailed description of isothermal methods is found in Test Method E2070.

6.4 *Identification of Impurities, Residues, and Contamination:*

6.4.1 Chemical impurities and residues are components that are not part of the intended scaffold composition. They are contaminants that may be either expected or unexpected based on knowledge of the manufacturing process. Acceptable levels of impurities depend on the nature of the contamination and the scaffold's intended application. A more precise definition of both contaminants and impurities and guidance regarding their significance may be found in Guides E1298 and Guide F2150.

6.4.2 Expected impurities of potential biological significance should be monitored through appropriate analytical means. Typical impurities may include, but are not limited to, processing aids, solvents, unreacted reagents, endotoxins, trace elements, and metals.

6.4.3 Inductively coupled plasma/mass spectroscopy (ICP/MS), atomic absorption spectroscopy (AAS), or the methods listed in the USP <211>, USP <231>, USP <251>, and USP <261> shall be used for determination of inorganic residues. The maximum allowable limit for all impurities and trace metals shall not exceed the amounts as defined in Specification F1088, Specification F1185, or in the United States Pharmacopeia (USP). Amounts exceeding that limit shall be indicated and included in the material specification.

6.4.4 Extraction with appropriate organic solvents shall be used to determine or detect the presence of organic impurities. The extract shall be analyzed quantitatively and identified using gas chromatography (GC) coupled to high sensitivity detectors, such as a mass spectrometer (MS), diode array (DA), or a time-of flight mass spectrometer (TOF), to provide compositional identification and to quantitatively detect low molecular mass volatile impurities or contaminants. Document Test Method D6420 and Practice E1642 describe the standard Test Method for GC-MS/GC-IR (see 6.2.3 for acronym definitions).

6.5 *Determination of pH:*

6.5.1 The pH of the solution surrounding the scaffold is important, since the pH affects the metabolism of surrounding cells and tissues. A saturated solution shall be made by incubating a defined mass and volume of a scaffold in a covered vial containing a known volume of pure water (distilled or deionized and degassed by boiling and cooling) under constant stirring for 18 and 24 h at 37°C or until a constant pH is reached (that is, the system has reached equilibrium). Subsequently, the pH shall be measured with a calibrated pH meter after removing the scaffold and waiting at least 2 h. The pH of a control sample (no scaffold) shall be determined in parallel. Special care shall be taken to ensure that enough material is added, so that the amount of each sample component is large enough to reach saturation. This may be achieved and tested by using variable mass-to-volume ratios.

6.5.2 Special care shall be taken or procedures modified (for example, adding filtration of the solution) when using materials that disintegrate in aqueous media.

6.5.3 The test gives indications on locally occurring changes in pH in the tissue after implantation. The *in vitro* pH measurement does not fully reflect the *in vivo* situation; however, it can provide a good indicator of pH changes that might occur locally (for example, upon implantation or in cell culture). This is especially true for large changes in pH in combination with low ionic strength.

6.6 *Chemical Characterization of the Scaffold Surface:*

6.6.1 The chemical surface composition may be different from the bulk properties of the scaffold due to diffusion processes, chemical treatment of solid materials, deposition of coatings, loading with pharmaceuticals, or simply due to contamination.

6.6.2 The surface composition shall be validated with an appropriate technique such as X-ray photoelectron spectroscopy (XPS) or time-of-flight secondary ion mass spectrometry (TOF-SIMS). Analytical data shall be reported according to the Practice E996, Practice E1504, or Practice E1635.

6.6.3 The handling and preparation of specimens for surface analysis requires special care. A guide on proper handling is found in Guide E1829 and in Guide E1078.

7. Physical Properties and Tests

NOTE 2—The terms macropore, micropore, and nanopore as defined by ASTM Committee F04 in Guide F2450 consider the size scale relevant in biological applications. The terms differ from the International Union of Pure and Applied Chemistry (IUPAC) definitions and in many referenced documents, but it is strongly recommended that the definitions in Guide F2450 be used.

7.1 *Density:*

7.1.1 The density of the bulk material shall be determined in order to calculate the porosity of the scaffolds. The densities of ceramic and mineral scaffold materials are dependent on the material composition and the process history.

7.1.2 The density can be measured using test methods suggested in Test Method C373, Test Method C729, or ISO 5016. Fast dissolving or disintegrating materials may require special consideration.

7.1.3 The theoretical density may also be calculated from the known chemical composition and components.

7.2 *Porosity:*

7.2.1 The apparent porosity or open porosity is defined as the volume of open pores expressed as a percentage of the total volume or bulk volume of the sample. Fractions of macropores, micropores, or nanopores shall be identified according to ASTM definitions as applied in life sciences. Deviation thereof shall be indicated.

7.2.1.1 Nanopore [Molecular Level] — 0.002 to 0.1 μm.

7.2.1.2 Micropore [Cellular Level] — 0.1 to 100 μm.

7.2.1.3 Macropore [Tissue Level] — >100 μm.

NOTE 3—The above pore size ranges are adopted from Guide F2450. See subsection X2.1 of Guide F2450 for detailed information regarding pore size classifications and nomenclature.

7.2.2 The total porosity includes all pores (that is, open, blind-ended, and blind), whereas the permeability and interconnectivity as described in 7.5 is mainly affected by open, interconnected pores.

7.2.3 The apparent porosity or open porosity can be determined using test methods suggested in Test Methods C373, C830, D6226, or by adapting buoyancy methods in Test Methods C693 or C729.

7.3 *Characterization of Pores:*

7.3.1 The average pore size and pore size distribution of the scaffold shall be determined. Unless otherwise noted, an isotropic distribution within the scaffold is assumed. Anisotropic distribution of pores shall be indicated, including the axis of anisotropy.

7.3.2 Optical microscopy (light or electron microscopy techniques) or micro-computer tomography shall be applied in the central parts of the scaffolds to analyze the macro- and micropores.

7.3.3 Guide F2450 provides an overview on current methods suitable for pore characterization with respect to pore size and shape.

7.4 *Determination of Specific Surface Area:*

7.4.1 The specific surface area (SSA) of the scaffold shall be determined. Measurements using Mercury Intrusion Porosimetry (MIP) or by gas adsorption methods (BET—Brunauer, Emmett, and Teller) are suggested, though BET is preferred since the calculated values of the MIP method may underestimate the surface area due to the presence of ink-bottle pores (blocking the mercury) in many interconnected networks. Neither technique, however, will include closed pores. The SSA is expressed as surface area per unit mass of sample (m^2/g).

7.4.2 BET analysis is a general method for determination of the SSA. A minimum surface area of 0.5 m^2 is recommended for exact analysis when using nitrogen. Higher sensitivities may be achieved by using krypton gas. A general procedure is described in Test Method C1274 or in ISO 15901–2.

7.4.3 Mercury intrusion porosimetry (MIP) is a useful method for measuring small pores open to the outside of a porous scaffold. It will not give the volume of any pores completely enclosed by surrounding solids. MIP is typically applied for apparent pore entrance diameters between approximately 100 μm and 2.5 nm. Larger pores must be measured by another method. A general standard test method is found in Test Method D4404.

7.5 *Permeability:*

7.5.1 The permeability, also called the interconnectivity, of the scaffold shall be determined. Mineral or ceramic scaffolds may be anisotropic and may exhibit axis-dependent permeability. Therefore, the orientation (for example, axis of insertion) of the measure of permeability shall be indicated. The permeability shall be expressed as volumetric flow rate, Q, through the scaffold.

7.5.2 Test Method E128 describes a method that can be applied for measuring the interconnectivity of rigid porous materials. It is applicable to scaffolds made of sintered glass, ceramic, metal, or plastic. This test method establishes a uniform designation for maximum pore diameter and also provides a means of detecting and measuring changes which occur through continued use.

7.6 *Crystallinity*—The crystallinity of the scaffold shall be determined. X-ray diffraction (XRD) analysis is recommended for characterization of the crystalline phases. In addition, the ratio between crystalline and amorphous phases can be calculated. The XRD technique determines the mass fractions of individual phases in a mixture by comparing the integrated intensity of one or more peaks from the phase(s) of interest to an external standard tested under identical instrumental conditions. The mass absorption coefficients of the sample and standard must be known to obtain accurate results. The use of XRD analysis and its applications in mineral and ceramic scaffolds is described in Practice F2024.

8. Biological Properties

8.1 *Biocompatibility*—The biocompatibility of scaffold shall be established. Test methods for biocompatibility are found in Practice F748 or in ISO 10993–1.

8.2 *Absorbability/Resorbability:*

8.2.1 As appropriate, the biological absorbability of the scaffold shall be determined *in vivo* and reported. Test methods similar to the ones described in ISO 10993–14 or Practice F1983 should be used. The *in vivo* model shall be relevant with regard to the targeted application of the scaffold to location and material volume.

8.2.2 The *in vivo* lifespan of the scaffold shall be indicated as a function of scaffold size. Histological sections and microscopy should be used to identify residual material. The resorption time values may be calculated, extrapolated, or estimated from discrete time points in *in vivo* experiments.

8.2.3 If the residence time of a scaffold is longer than three years, it should be considered as non-degradable (see Practice F1983). A scaffold is considered fully resorbed when more than 95 % of its materials have been metabolized.

8.2.4 Residual material shall be identified and quantified with appropriate methods, for example, by histomorphometry or histochemistry.

8.3 *Bioburden*—The total number of viable microbes in a scaffold shall be determined prior to sterilization. It shall be measured with an extraction method as described in standard ISO 11737–1.

8.4 *Endotoxin Analysis:*

8.4.1 The level of bacterial endotoxins shall be measured. It is suggested that a Limulus Amebocyte Lysate (LAL) assay or its biotechnological analogue-based test for endotoxin determination is used. Guidelines are found in the standard AAMI ST72 or in the FDA Guideline on Validation of the Limulus Amebocyte Lysate Test.

8.4.2 The analysis of cytokine expression as a response to endotoxin may be used as an alternative cell-based method. The profile of expressed cytokines varies with the cell source. The method requires analysis of more than one highly responsive cytokine, and its amount should be normalized to reporter-molecules and compared to negative and positive controls.

8.4.3 The extraction of endotoxin from the scaffold may be very difficult, since endotoxins exhibit a high affinity for certain material surfaces. Furthermore, test results may be confounded by extracted components of the scaffold (attenuation or amplification of enzyme response). Therefore, the extraction process should be validated by deliberately adding known amounts of endotoxin to the sample (soiling).

9. Mechanical Properties and Tests

NOTE 4—Mechanical evaluations should preferentially be performed in dry state and in wet equilibrium state. Sample pre-conditioning may be needed and can be conducted as described in Practice F1634. *In vitro* conditioning typically uses buffered saline solutions at 37°C as described in Test Method F1635.

NOTE 5— All mechanical tests of a series should be preformed with specimens of the same shape, size, and lot. The number of samples used shall be in accordance with the standard Practice E177. For compression testing and elastic modulus assessment, specimens with an aspect ratio of 2:1 and 4:1, respectively, are recommended for strength and modulus as described in Test Method D695. Each sample shall be used only once. It shall be indicated whether the reported values were acquired in ambient or physiological conditions and the time in solution shall be reported. All mechanical tests shall be performed on samples of the same geometry and isotropic orientation if possible. A cylindrical shape with a diameter of 10 mm and a length of 20 mm is suggested.

NOTE 6—Mineral or ceramic scaffolds may not be isotropic; therefore, the results of mechanical testing must include information on the orientation of the force axis with respect to that of the sample geometry. Testing of the samples in more than one axis is recommended if they exhibit anisotropic properties.

9.1 *Compressive Strength*—The mechanical test for compressive strength shall be performed under monotonic uniaxial loading. Monotonic loading refers to a test conducted at a constant rate with no direction reversal from test initiation to final fracture. The compressive strength shall be evaluated using either the Test Method C1424 or Test Method D1621.

9.2 *Elastic Moduli*—Elastic moduli of scaffolds containing organic fractions can be determined by quasi-static compression testing. However, the resultant stress-strain curve may not be linear due to the viscoelastic contribution of the organic fraction. In this case, it may be more appropriate to change the compression rate or to use dynamic mechanical compression. If dynamic mechanical analysis (DMA) is used, it should be carried out at more than one frequency. It is suggested to perform test according to Test Method E1876 or Test Method C1198.

10. Sterilization and Storage

10.1 *Sterilization*—The methods and conditions which can be used for sterilization of the scaffolds shall be reported. A summary of current sterilization methods can be found in AAMI STBK9–1, AAMI STBK9–2, and AAMI STBK9–3 or ISO 11737–1.

10.2 *Storage*—The maximum and minimum temperatures to which the supplied product can be exposed safely shall be reported.

10.3 *Shelf-Life*—The maximum time period shall be reported as the length of time during which the “as packaged and sterilized” product can be safely stored at recommended storage conditions without adversely affecting product function or package integrity. Details for testing are found in ISO 11607–1 and ISO 11607–2.

11. Quality Assurance

11.1 *Testing*—Unless otherwise noted, all tests shall be performed on samples that were produced, packaged, and sterilized under the usual manufacturing conditions. Packaging and sterilization processes may change or interfere with specific properties.

11.2 *Test Validation*—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.

11.3 *Sampling*—It is suggested that the requirements be determined for each lot by sampling sizes and procedures in accordance with standard guidance.

11.4 *Manufacturing Control Guidance:*

11.4.1 Acceptable levels of manufacturing control are essential if regulatory approval (for example, FDA or CE mark) for commercially distributed products is to be sought.

11.4.2 Corresponding documents may be found in 21 CFR, ISO 9000, or ISO 9001.

12. Keywords

12.1 biological; calcium phosphate; calcium sulfate; ceramic; characterization; chemical; hydroxyapatite; implant; mechanical; mineral; physical; scaffold; TCP

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 This guide is needed to meet the high quality requirements of tissue-engineered materials and material used in medical scaffold applications. The chemical, physical, biological, and mechanical characterization methods serve as criteria for a high consistency of the product. The methods and requirements described above do not replace specific material specifications for biocompatible grades of raw materials for use in the physiological environments.

X1.2 It is recognized that separate performance standards may be necessary for each end-use product, that is, to address *in vivo* degradation and tissue-specific responses.

X2. BIOCOMPATIBILITY

X2.1 This guide addresses neither the biocompatibility of the scaffold, nor the characterization or release profiles of any biomolecules, cells, drugs, or bioactive agents that are used in combination with the scaffold.

X2.2 This guide is needed to ensure a high quality material for use in biological applications. The biological response of the mineral and ceramic scaffolds needs to be tested according to Practices F981 and F748 or equivalent. The comprehensive characterization of chemical, physical, biological and mechanical properties contained in this specification serve as criteria for high quality and consistent products that can be implanted in the body. The suitability of the material from a human implant perspective is dependent on the specific application. The biological test appropriate for the specific site, such as recommended in Practice F748 or F1983 should be used as a guideline. Further testing of specific properties may be required for specific applications.