



Standard Guide for Characterization of Ceramic and Mineral Based Scaffolds used for Tissue-Engineered Medical Products (TEMPs) and as Device for Surgical Implant Applications¹

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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, 1987⁶

21 CFR United States Code of Federal Regulations, Title 21⁷

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 materials 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>.