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Standard Guide for Characterization and Assessment of Vascular Graft Tissue Engineered Medical Products (TEMPs)[1]

This standard is issued under the fixed designation F3225; 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 intended as a resource for individuals and organizations involved in the development, production, delivery, and regulation of tissue engineered medical products (TEMPs) intended for use in the surgical repair, replacement, shunting, and/or bypass of blood vessels. This guide is intended for use related to the *in vitro* assessment of TEMP vascular grafts. *In vitro* cellular characterization and *in vivo* testing are not within scope for this standard guide.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.3 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F1635 Test Method for ***in vitro*** Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants

F2150 Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products

F2210 Guide for Processing Cells, Tissues, and Organs for Use in Tissue Engineered Medical Products (Withdrawn 2015)[3]

F2211 Classification for Tissue-Engineered Medical Products (TEMPs)

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

F2382 Test Method for Assessment of Circulating Blood-Contacting Medical Device Materials on Partial Thromboplastin Time (PTT)

F2739 Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds

STP 997-EB Compositional Analysis by Thermogravimetry

2.2 *US FDA Regulations and Guidance Documents:*[4]

21 CFR 610.12 General Biological Products Standards—Sterility

21 CFR 1270 Human Tissue Intended for Transplantation

21 CFR 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products

FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers

Guidance for Industry Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)

FDA Guidance for Industry Container and Closure System Integrity Testing *in Lieu* of Sterility Testing as a Component of the Stability Protocol for Sterile Products

Guidance for Industry and Food and Drug Administration Staff Use of International Standard ISO-10993-1, Biological Evaluation of Medical Devices—Part 1: Evaluation and testing within a risk management process

[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 F04.44 on Assessment 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.

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

[4] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

2.3 *ISO Standards:*[5]

ISO 7198 Cardiovascular implants and extracorporeal systems—Vascular prostheses—Tubular vascular grafts and vascular patches

ISO 10993 Biological evaluation of medical devices

ISO 11135 Sterilization of health-care products—Ethylene oxide—Requirements for the development, validation and routine control of a sterilization process for medical devices

ISO 11137 (Parts 1, 2 and 3) Sterilization of health care products – Radiation

ISO 11737-1 Sterilization of medical devices—Microbiological methods—Part 1: Determination of a population of microorganisms on products

ISO 11737-2 Sterilization of medical devices—Microbiological methods—Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

ISO 22442-1 Medical devices utilizing animal tissues and their derivatives—Part 1: Application of risk management

ISO 22442-3 Medical devices utilizing animal tissues and their derivatives—Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents

2.4 *Other Documents:*

United States Pharmacopeia XXVII <71> Sterility Tests

ICH Harmonized Tripartite Guideline Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, Q5A(R1)

American Association of Tissue Banks (AATB) AATB Standards for Tissue Banking

ANSI/AAMI ST72 Bacterial Endotoxins—Test Methods, Routine Monitoring, and Alternative to Batch Testing

3. Summary of Guide

3.1 It is the intent of this guide to provide a compendium of information that may be related to the functional characteristics of vascular graft TEMPs intended to surgically replace, bypass, or form shunts between sections of the vascular system. Examples of functional characteristics include vasoactivity and mechanical properties (e.g., burst pressure, tensile strength, creep) suitable for implantation. TEMPs may be composed of biological products (e.g., cells, organs, tissues, and processed biologics), biomaterials (e.g., substrates and scaffolds composed of polymers or extracellular matrix (ECM) components such as collagen), and/or biomolecules (e.g., recombinant proteins) (see Terminology F2312). Examples of TEMPs are listed in Classification F2211.

3.2 ISO 7198 provides basic requirements for sterile vascular prostheses and the methods of testing which will enable evaluation of vascular prostheses. The degree of sterility (sterility assurance level of 1×10^{-3} versus 1×10^{-6}) will be determined by the materials of construction and their ability to be sterilized without compromising their function once implanted.

3.3 Throughout this guide, the reader is referred to other documents that may provide specific information that can be applied in the manufacture and testing of TEMPs. Although many of these documents were not written with TEMPs in mind, parts are often applicable. Most of the potentially applicable position papers and guidance documents from many regions of the world can be accessed via the internet. New documents are continually produced, and existing documents are continually updated.

3.4 The application of this guide does not guarantee clinical success of a finished product, but will help develop and characterize a given vascular graft TEMP developed for the purpose of surgically replacing, bypassing, or forming shunts between sections of the vascular system.

3.5 This guide does not suggest that all 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 material based on risk, applicable regulations, characterizations, and preclinical/clinical testing.

4. Significance and Use

4.1 A common therapy to mitigate the pathological effects of blood vessel occlusion or aneurysm-related vascular wall weakening is to reroute blood flow around the diseased vascular regions. Autologous and non-autologous grafts are often used as vascular substitutes surgically to achieve this therapeutic intervention. Vascular graft TEMPs may also be used for these purposes. They may also be used to create or revise arteriovenous shunts.

4.2 Coronary, carotid, renal, common iliac, external iliac, superficial femoral, and popliteal arteries are examples of vascular sites commonly requiring bypass surgery.

4.3 TEMPs may be composed of biological products (for example, cells, organs, and tissues), biomaterials (for example, substrates and scaffolds composed of polymers or collagen), biomolecules (for example, recombinant proteins, native/biological proteins, amino acids, peptides, fatty acids, sugars, and other macromolecules), and various combinations thereof (see Terminology F2312). Examples of TEMPs are listed in Classification F2211.

4.4 TEMPs may be used with the intent of facilitating the surgical outcome by improving the biological repair and/or reconstruction, by accommodating the mechanical loads at the repair site, or by a combination of these mechanisms.

4.5 Clinical evidence of improved surgical outcomes may include patency, reduced incidence of revision surgery, reduced rate of implant infection, and improved functionality after surgery.

5. Synthetic Biomaterials

5.1 *Polymer Types*—The biomaterial used may be formed from synthetic polymers, should elicit an acceptable biological response with minimal toxicity, and may be degradable or non-degradable. Examples of degradable polymers are glycolide, lactide, trimethylene carbonate, dioxanone,

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

caprolactone, ortho esters, and polymers and copolymers of some of these. Other examples of degradable polymers, in order of fast to slow degradation time, include polyglycolic acid, poly-lactic acid, and polycaprolactone. Non-absorbable materials include polypropylene, polyethylene, polyamide, polyalkylene terephthalate, polyvinylidene fluoride, polytetrafluoroethylene, and blends and copolymers of these. Non-synthetic polymers, such as silk, may also be used.

5.2 *Structure*—The biomaterial is typically manufactured into a structural material appropriate for a vascular TEMP such as a sheet or strip or tube, by weaving or knitting using a fiber as a base material, or a felt with random fiber orientation, as a membrane, or by using a different technique such as electrospinning. The structure of the material is selected based on its required function, for example to support cells and matrix deposition, to provide mechanical support for certain loading conditions, and/or to integrate into the surrounding tissues. For example, multi-layered biomaterials may be used to confer desired properties by using layers with distinct capabilities. Two-dimensional structures, such as sheets, may be rendered tubular by suturing, while three-dimensional structures may be manufactured in tubular form by casting. Geometries other than tubes may be appropriate depending on the implantation anatomic site. Additive manufacturing can be used to create more complex geometries.

5.3 *Degradation*—The biomaterial may be selected to be non-degradable, or to exhibit specific degradation characteristics (e.g., rapidly or slowly degrading) based on the time necessary for the TEMP to perform its desired function. If the function of the material is primarily to support cell attachment and matrix deposition, the biomaterial may be relatively rapidly degrading. If the function is to provide mechanical support *in vivo*, the biomaterial should degrade at a rate that allows natural repair to occur to a level that enables the new tissue ingrowth to accommodate the mechanical loading at the repair site.

6. Extracellular Matrices

6.1 *Native Matrices*—Human- and animal-derived tissues may be used to provide an ECM with an appropriate form. Tissues that may be appropriate include, but are not limited to, skin, submucosa, arteries, and veins. The tissue should be processed to ensure that cell debris and other immunogenic processing reagents are minimized. For human-derived tissues, aseptic processing techniques should be employed as described by Freshney (**1**)[6] to prevent the introduction or transmission of communicable diseases (also see 21 CFR 1271). For animal-derived tissues or ECM, herd information should be provided and testing should be conducted to confirm that there are no potential transmissible diseases. Human- or animal-derived proteins and glycoproteins such as collagen, elastin, proteoglycans, and hyaluronan may be used. Collagen may be purified from native tissues (for example, skin, tendon, or ligament). The collagen should be made into a biomaterial with a specific shape (for example, tube), and may be cross-linked to maintain its shape and to reduce degradation rates.

6.2 Plant-derived biomaterials such as starch and cellulose may also be used.

6.3 *Cell Culture-Derived Components*—Cells may be maintained in culture to synthesize ECM components that are secreted into the tissue culture media. These components may include collagen, elastin, proteoglycans, hyaluronan and other proteins, and glycoproteins. These components may then be isolated, purified if necessary, and used to develop a biomaterial with a specific shape (for example, tube), and may be cross-linked to maintain its shape and to modulate degradation rates. Alternatively, cells may be seeded onto a biomaterial, cultured *in vitro* to synthesize an ECM, and then be decellularized.

6.4 Biomaterials fabricated from combinations of native matrices, plant-derived matrices, and cell culture-derived proteins may be considered. An adverse immunologic response may occur due to the presence of cells or cellular debris.

7. Biomaterial Characterization

7.1 Biomaterials may be synthetic, non-synthetic, or ECM. While biomaterial characterization is beyond the scope of this document, there are several applicable reference documents, including the following: Guide F2210, Guide F2150, and Guide F2212 (also see 21 CFR 1270).

8. Cells

8.1 *Cell Types*—The cell population used may be of one or multiple cell types such as *(a)* arterial, venous, or dermal fibroblasts; *(b)* arterial or venous smooth muscle cells; *(c)* arterial or venous endothelial cells; *(d)* stromal cells; or *(e)* other progenitor cells and may be derived from various tissue sources. These cells are likely to have undergone expansion prior to being seeded into the TEMP, and the cell karyotype and phenotype should be characterized and compared to a population of freshly isolated or early passage cells prior to use in the TEMP. Characteristics of the final product should match its intended use.

8.2 *Cell Performance Requirements*—In formation of the TEMPs *in vitro* or *in situ*, the cells may be combined with the biomaterial and/or ECM, and must be able to attach to the biomaterial and/or ECM of the TEMPs. For some TEMPs, the cells should be able to proliferate and secrete a functional ECM *in vitro*. When implanted, the cells may be required to synthesize an ECM *in vivo* or function in biologic repair but the cells should not induce an inflammatory or immune response that may have a negative effect on repair. Distribution of seeded cells in biomaterials should be evaluated for intended use.

8.3 Cellular characterization guidelines are beyond the scope of this document. Refer to FDA Guidance documents for more information under Tissue Guidances and Cellular & Gene Therapy Guidances as well as to references Guide F2210; Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps); and ICH Harmonized Tripartite Guideline—Viral

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

Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, Q5A(R1).

9. Attachment of the TEMPs *in vivo*

9.1 *Attachment in vivo*—The TEMPs should be able to be attached firmly to the adjacent vessels without leaks such that they can function as patent fluid-tight vessels capable of withstanding the intravascular pressures and hemodynamic shear stresses *in vivo*. If the TEMP is to be secured *in vivo* using sutures, then it should be able to retain them in the manner that is appropriate for the surgical implantation site and forces. Once implanted, the TEMP should be retained in place for the time required for it to complete its functional requirements and should remain patent and free from thrombosis.

10. Sterilization

10.1 TEMPs must be provided sterile to the clinical field. Acellular products may be sterilized after manufacture by ethylene oxide, liquid chemical sterilization, gamma irradiation, or other sterilization techniques if there is no negative impact on functionality. If the TEMP is cellular, it must be manufactured using standard aseptic techniques (**1**) and/or a closed culture system. Some TEMP properties, including but not limited to degradation profiles, could be affected by sterilization. Consequently, it is recommended that potentially affected properties be evaluated for design compliance after sterilization processing. Sterilization is beyond the scope of this document; consult reference documents such as FDA Guidance for Industry: Container and Closure System Integrity Testing *in Lieu* of Sterility Testing as a Component of the Stability Protocol for Sterile Product; ISO 11737-1 and ISO 11737-2; ISO 11135; ISO 11137; and USP Sterility Tests for more information (also see 21 CFR 610.12).

11. Packaging

11.1 The TEMP shall be packaged so that it can be stored and transported to the clinical site while remaining sterile and functional. The packaging shall be validated to ensure sterility over the shelf life of the product. The stability of the TEMP should also be validated in parallel with the packaging using specific measurable performance testing, e.g., pressure integrity.

12. Biochemical Composition and Tests

12.1 *ECM Composition*—The ECM of vascular TEMPs is usually a collagen-based (such as Types I and III) material which may also contain other collagens as well as non-collagenous ECM components including, but not limited to, elastin, proteoglycans (such as versican, decorin, biglycan, lumican, and perlecan), and other glycosaminoglycans (such as hyaluronan) and glycoproteins (such as laminin, fibronectin, thrombospondin, and tenascin). These components can be quantified, and usually their amounts are expressed per wet weight or dry weight. These components can be measured through histological analysis, immunoassays, gel electrophoresis, or biochemical assays. Total collagen content may be measured with the hydroxyproline assay (**2, 3**), elastin through the ninhydrin or Fastin™ elastin assays, and sulfated glycosaminoglycans through the dimethylmethylene blue spectrophotometric assay (**4**). There are many other commercial assay kits available for ECM quantification that may be used.

12.2 *DNA*—For grafts that contain live cells, DNA is typically used to measure cell number by converting DNA content per given cell type into the number of cells. The Hoechst assay, which stains cell nuclei with Hoechst dye 33258, is often used (**5, 6**). Briefly, vascular grafts are frozen at –20 °C, lyophilized, and the construct dry weight is measured. ECM is digested by overnight incubation with proteinase K solution at 55 °C. Digested samples are stored at –20 °C until used for biochemical assays. A small aliquot sample of the digested sample is then mixed with the Hoechst dye solution, placed in a 96-well plate, and its optical density is measured in a microplate spectrophotometer at excitation/emission 365/460 wavelengths. DNA content is calibrated using standards such as calf thymus DNA. Cell number is calculated from DNA content by assuming a given DNA content per cell type of interest (e.g., 7.6 pg of DNA per smooth muscle cell) (**5**). Alternatively, a DNA standard curve may be created using DNA extracted from a known number of cells used in the graft fabrication. Other assays may also be used such as rapid UV detection of DNA at 260/280nm (**7**).

12.2.1 To obtain the DNA content of cellular tissue the above method can be utilized; however, a standard addition approach should be taken to account for matrix interference with the assay. Standard addition involves spiking with known quantities of DNA into the sample and back calculating the DNA content of the sample.

12.2.2 Other DNA intercalating dyes such as SYBR Green 1 (**8**) or PicoGreen (**9**) may be used for DNA quantification instead of Hoechst 33258.

12.3 *Water Content*—Water content can be measured by taking sample weights before and after repeated drying cycles. For products where the TEMP is freeze-dried and provided separately from the excipient or as a freeze dried product, residual moisture should be quantified, and targets should be justified. For example, the American Association of Tissue Banks has established that residual moisture should be maintained at or below 6 % for freeze-dried products for the shelf life of the product. A validated accurate and precise method should be employed to ascertain the residual moisture for each lot of TEMPs and prove that acceptable residual moisture is maintained for the shelf life of the product. Several methods are available including, but not limited to, Karl Fischer titration, gravimetric loss upon drying, and thermogravimetric (STP 997-EB) methods. Additionally, there should be a validated sampling plan that addresses how many samples and from how many locations samples must be obtained and assessed to statistically ensure that the entire lot of TEMPs meets the manufacturer's residual moisture specification over time.

12.4 *Metabolic Activity*—For grafts that contain live cells, metabolic activity is often measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or