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Standard Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions¹

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INTRODUCTION

The collagen family of proteins represents the major structural and mechanical component of the *in-vivo* extracellular matrix of human tissues and organs. Type I collagen is the most abundant and as such, it is an ideal candidate for medical materials, tissue-engineered medical products, delivery of therapeutic cells/molecules, and *in-vitro* cell/tissue culture applications. Furthermore, it is now evident that specific collagen material properties, including microstructure, mechanical integrity (stiffness, strength), cell adhesion, immunogenicity, and resorption (degradation) are major determinants of the interfacial properties between cells and collagen-based materials, including guidance of fundamental cell behaviors that contribute to recapitulation and/or restoration of tissue structure and function. Advanced understanding of collagen self-assembly, as occurs *in vivo* and *in vitro*, is contributing to a rapid expansion of commercial and laboratory-produced purified collagen formulations that polymerize (self-assemble) or exhibit transitions from solution to semi-solid material (for example, gel, scaffold). Most recent developments have focused on polymerizable collagen formulations that support the rational design and custom fabrication of collagen polymeric materials for improved tissue integration, guidance of cell fate, and tissue response outcomes. Unfortunately, the term “collagen” is applied generally to describe various collagen types and formulations (soluble, insoluble, monomeric, gelatin/peptides, oligomeric, tropocollagen, atelocollagen) that vary significantly in their molecular compositions, polymerization capacity and properties, and ability to interact with cells. As such, the need exists for an expanded set of characterization and standardization strategies to facilitate comparison, safety and efficiency testing, and translation of the next generation polymerizable collagen formulations and associated collagen polymeric materials produced with these formulations.

1. Scope

1.1 This guide is intended to provide characteristics, properties, test methods, and standardization approaches for evaluation and identification of specific polymerizable collagen formulations and collagen polymeric materials produced with these formulations.

1.2 This guide focuses on characterization of purified polymerizable forms of type I collagen, which is the most abundant collagen in mammalian connective tissues and organs, including skin, bone, tendon, and blood vessels.

Polymerizable type I collagen may be derived from a variety of sources including, but not limited to, animal or cadaveric tissues, cell culture, recombinant cell culture, and chemical synthesis.

1.2.1 This guide covers evaluation of polymerizable collagens and collagen polymeric materials prepared from polymerizable collagens for use as a starting material for wound and hemostatic dressings, surgical implants, substrates for tissue-engineered medical products (TEMPs), delivery vehicles for therapeutic cells or molecules, and 3D *in-vitro* tissue systems for basic research, diagnostics, drug development, and toxicity testing. Most collagen products on the market today are regulated as devices since their primary intended purpose is not achieved through chemical action within or on the body. However, a medical product comprising polymerizable collagens or collagen polymeric materials may be regulated as a

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device, biologic, drug, or combination product depending on its intended use and primary mode of action.

1.2.2 Polymerizable collagen or collagen self-assembly implies that the collagen composition exhibits spontaneous macromolecular assembly from its components without the addition of exogenous factors such as cross-linking agents. Polymerizable collagens may include but are not limited to: (1) tissue-derived monomeric collagens, including tropocollagen or atelocollagen, and oligomeric collagens; (2) collagen proteins and peptides produced through *in vitro* cell culture, with or without using recombinant technology; and (3) chemically synthesized collagen mimetic peptides. It should be noted that the format of collagen polymeric material products also will vary and may include injectable solutions that polymerize *in situ* as well as preformed sheets, particles, spheres, fibers, sponges, matrices/gels, coatings, films, and other forms.

1.2.3 This guide may serve as a template for characterization and standardization of type I fibrillar collagen or other collagen types that demonstrate polymerization or self-assembly.

1.3 This guide does not provide a significant basis for assessing the biological safety (biocompatibility) of polymerizable collagens and collagen polymeric materials. While the ability of collagen polymeric materials to guide cellular responses through provision of cellular adhesion and proteolytic domains as well as physical constraints (for example, structural, cell-matrix traction force) has been well documented through extensive clinical and basic research studies (1-5),² users are directed to the ISO 10993 series for evaluating biological risks of medical devices. The biocompatibility and appropriateness of use for a specific application is the responsibility of the product manufacturer.

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

1.5 The following precautionary caveat pertains only to the test method portion, Sections 6 and 7, of this guide: *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.*

1.6 *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:³

² The boldface numbers in parentheses refer to a list of references at the end of this standard.

³ 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.

- E4 Practices for Force Calibration and Verification of Testing Machines
- F619 Practice for Extraction of Materials Used in Medical Devices
- F720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test
- F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices
- F749 Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit
- F756 Practice for Assessment of Hemolytic Properties of Materials
- F763 Practice for Short-Term Intramuscular Screening of Implantable Medical Device Materials
- F813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices
- F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity
- F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone
- F1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials
- F1903 Practice for Testing for Cellular Responses to Particles *in vitro*
- F1904 Practice for Testing the Biological Responses to Particles *in vivo*
- F1983 Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications
- F2914 Guide for Identification of Shelf-life Test Attributes for Endovascular Devices
- 2.2 ISO Standards:⁴
 - ISO 5725-1 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 1: General Principles and Definitions
 - ISO 5725-2 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 2: Basic Method for the Determination of Repeatability and Reproducibility of a Standard Measurement Method
 - ISO 5725-3 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 3: Intermediate Measures of the Precision of a Standard Measurement Method
 - ISO 5725-4 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 4: Basic Methods for the Determination of the Trueness of a Standard Measurement Method
 - ISO 5725-5 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 5: Alternative Methods for the Determination of the Precision of a Standard Measurement Method
 - ISO 5725-6 Accuracy (Trueness and Precision) of Measurement Methods and Results—Part 6: Use in Practice of Accuracy Values
 - ISO 10993-1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing with a Risk Management Process

⁴ Available from International Organization for Standardization (ISO), 1, ch. de la Voie-Creuse, CP 56, CH-1211 Geneva 20, Switzerland, <http://www.iso.org>.