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Standard Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)¹

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INTRODUCTION

Collagen-based medical devices are becoming more prevalent especially in the area of soft tissue augmentation. The use of collagen in surgery dates back to the late 1800s, with the use of catgut sutures, human cadaveric skin, and fascia. More recently, collagen has been used in hemostatic sponges, dermal equivalents, injectables for soft tissue augmentation, as a matrix for cell-based products and as a vehicle for drug delivery. It is because of the versatility of collagen in medical applications that specific characterizations should be performed as a way to compare materials.

1. Scope

1.1 This guide for characterizing collagen-containing biomaterials is intended to provide characteristics, properties, and test methods for use by producers, manufacturers, and researchers to more clearly identify the specific collagen materials used. With greater than 20 types of collagen and the different properties of each, a single document would be cumbersome. This guide will focus on the characterization of Type I collagen, which is the most abundant collagen in mammals, especially in skin and bone. Collagen isolated from these sources may contain other types of collagen, for example, Type III and Type V. This guide does not provide specific parameters for any collagen product or mix of products or the acceptability of those products for the intended use. The collagen may be from any source, including, but not limited to animal or cadaveric sources, human cell culture, or recombinant sources. The biological, immunological, or toxicological properties of the collagen may vary depending on the source material. The properties of the collagen prepared from each of the above sources must be thoroughly investigated, as the changes in the collagen properties as a function of source materials is not thoroughly understood. This guide is intended to focus on purified Type I collagen as a starting material for surgical implants and substrates for Tissue Engineered Medical Products (TEMPs); some methods may not be applicable for gelatin nor for tissue implants. This guide may serve as a template for characterization of other types of collagen.

1.2 The biological response to collagen in soft tissue has been well documented by a history of clinical use (1,2)² and laboratory studies (3,4,5,21). Biocompatibility and appropriateness of use for a specific application(s) is the responsibility of the device manufacturer.

1.3 The following precautionary caveat pertains only to the test method portion, Section 5, 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 and health practices and determine the applicability of regulatory requirements prior to use.*

2. Referenced Documents

2.1 ASTM Standards:

- E 1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products³
- F 619 Practice for Extraction of Medical Plastics⁴
- F 720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test⁴
- F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices⁴
- F 749 Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit⁴
- F 756 Practice for Assessment of Hemolytic Properties of Materials⁴
- F 763 Practice for Short-Term Screening of Implant Materials⁴

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

³ *Annual Book of ASTM Standards*, Vol 11.05.

⁴ *Annual Book of ASTM Standards*, Vol 13.01.



F 813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices⁴

F 895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity⁴

F 981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone⁴

F 1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices⁴

F 1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials⁴

F 1903 Practice for Testing for Biological Responses to Particles *In Vitro*⁴

F 1904 Practice for Testing the Biological Responses to Particles *In Vivo*⁴

F 1905 Practice for Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity⁴

F 1906 Practice for Evaluation of Immune Responses in Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation and Cell Migration⁴

F 1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Application⁴

F 2148 Practice for Evaluation of Delayed Contact Hypersensitivity Using the Murine Local Lymph Node Assay (LLNA)⁴

2.2 ISO Standards:⁵

ISO 10993-1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing

ISO 10993-3—Part 3 Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

ISO 10993-9—Part 9 Framework for Identification and Quantification of Potential Degradation Products

ISO 10993-10 Biological Evaluation of Medical Devices—Part 10: Tests for Irritation and Delayed-Type Hypersensitivity

ISO 10993-17—Part 17 Methods for Establishment of Allowable Limits for Leachable Substances Using Health-Based Risk Assessment

ISO 13408-1: 1998 Aseptic Processing of Health Care Products—Part 1: General Requirements

2.3 EN (European Norm) Documents:⁶

EN 12442-1 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk

EN 12442-2—Part 2 Controls on Sourcing, Collection and Handling

EN 12442-3—Part 3 Validation of the Elimination and/or Inactivation of Virus and Transmissible Agents

2.4 U. S. Pharmacopeia Documents:⁷

United States Pharmacopeia (USP), Edition XXIV (24)

USP 24/NF 19 Viral Safety Evaluation of Biotechnology

Products Derived from Cell Lines of Human or Animal Origin

2.5 Code of Federal Regulations:⁸

Code of Federal Regulations, Title 21, Part 820

Federal Register Vol. 43, No. 141, Friday, July 21, 1978

Human Cells, Tissues and Cellular and Tissue-Based Products, Establishment Registration and Listing. 21 CFR Parts 207, 807, and 1271

Federal Register/Vol. 66, No. 13, Jan 19, 2001/Rules and Regulations, page 5447

Suitability Determination for Donors of Human Cell and Tissue-based Products, 21 CFR 1271 Part C, Proposed Rule

Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products, Inspection and Enforcement. Proposed Rule. Federal Register/Vol. 66, No. 5/January 8, 2001/Proposed Rules, pages 1552-1559

Guidance for Screening and Testing of Donors of Human Tissue Intended for Transplantation, Availability. Federal Register/Vol. 62, No. 145/July 29, 1997/Notices/Draft Guidance for Preclinical and Clinical Investigations of Urethral Bulking Agents used in the Treatment of Urinary Incontinence. November 29, 1995. (ODE/DRARD/ULDB), Document No. 850

Guidance for Industry and for FDA Reviewers, Medical Devices Containing Materials Derived from Animal Sources (Except for *In Vitro* Diagnostic Devices), November 6, 1998, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health

CFR 610.13(b), Rabbit Pyrogen Assay

2.6 ICH Documents:⁹

International Conference on Harmonization (1997) Guidance for Industry M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals 62 FR 62922

International Conference on Harmonization (1996) Guideline for Industry S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. 61 FR 18199

International Conference on Harmonization (1997) Guidance for Industry S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals 62 FR 62472

International Conference on Harmonization (1994) Guideline for Industry S5A Detection of Toxicity to Reproduction for Medicinal Products. 59 FR 48746

International Conference on Harmonization (1996) Guidance for Industry S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility. 61 FR 15360

International Conference on Harmonization (1996) Guideline for Industry S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals. 61 FR 8153

International Conference on Harmonization (1998) Guidance for Industry S1B Testing for Carcinogenicity of

⁵ Available from International Organization for Standardization (ISO), 1 rue de Varembe, Case postale 56, CH-1211, Geneva 20, Switzerland.

⁶ Available from European Committee for Standardization, CEN Management Centre 36, rue de Stassart B-1050 Brussels, Belgium.

⁷ Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852.

⁸ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.

⁹ ICH Secretariat, c/o IFPMA, 30 rue de St-Jean, P.O. Box 758, 1211 Geneva 13, Switzerland.





- Pharmaceuticals. 63 FR 8983
- International Conference on Harmonization (1995) Guideline for Industry S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals. 60 FR 11278
- International Conference on Harmonization (1997) S1C(R) Guidance for Industry Addendum to Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limit Dose and Related Notes. 62 FR 64259
- International Conference on Harmonization (ICH) Q1A ICH Harmonized Tripartite Guidance for Stability Testing of New Drug Substances and Products (September 23, 1994)
- U.S. Food and Drug Administration (FDA and Committee for Proprietary Medicinal Products (CPMP), 1998 International Conference on Harmonization (ICH), Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, Consensus Guideline ICH Viral Safety Document: Step 5
- 2.7 *FDA Documents:*¹⁰
- FDA Guideline on Validation of the Limulus Amebocyte Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Healthcare Products, DHHS, December 1987
- U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1993 Points to Consider in the Characterization of Cell Lines Used to produce Biologicals
- U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1997 Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, 94D-0259
- FDA Interim Guidance for Human and Veterinary Drug Products and Biologicals, Kinetic LAL techniques, DHHS, July 15, 1991
- 2.8 *AAMI Documents:*¹¹
- ANSI/AAMI/ISO 11737-1: 1995 Sterilization of Medical Devices—Microbiological Methods—Part 1: Estimation of Bioburden on Product
- ANSI/AAMI/ISO 11737-2: 1998 Sterilization of Medical Devices—Microbiological Methods—Part 2: Tests of Sterility Performed in the Validation of a Sterilization Process
- AAMI TIR No. 19-1998 Guidance for ANSI/AAMI/ISO 10993-7: 1995, Biological Evaluation of Medical Devices—Part 7: Ethylene Oxide Sterilization Residuals
- AAMI/ISO 14160-1998 Sterilization of Single-Use Medical Devices Incorporating Materials of Animal Origin—Validation and Routine Control of Sterilization by Liquid Chemical Sterilants
- AAMI ST67/CDV-2: 1999 Sterilization of Medical Devices—Requirements for Products Labeled “Sterile”
- 2.9 *Other References:*
- Draft Guidance for Preclinical and Clinical Investigations of

Urethral Bulking Agents Used in the Treatment of Urinary Incontinence, November 29, 1995. (ODE/DRARD/ULDB), Document No. 850.¹²

3. Terminology

3.1 *Definitions:*

3.1.1 *adventitious agents*—an unintentionally introduced microbiological or other infectious contaminant. In the production of TEMP, these agents may be unintentionally introduced into the process stream or the final product, or both.

3.1.2 *biocompatibility*—a material may be considered biocompatible if the materials perform with an appropriate host response in a specific application (22).

3.1.3 *collagen*—Type I collagen is a member of a family of structural proteins found in animals. Type I collagen is part of the fibrillar group of collagens. It derives from the COL1A1 and COL1A2 genes, which express the alpha chains of the collagen. All collagens have a unique triple helical structure configuration of three polypeptide units known as alpha-chains. Proper alignment of the alpha chains of the collagen molecule requires a highly complex enzymatic and chemical interaction *in vivo*. As such, preparation of the collagen by alternate methods may result in improperly aligned alpha chains and, putatively, increase the immunogenicity of the collagen. Collagen is high in glycine, L-alanine, L-proline, and 4-hydroxyproline, low in sulfur, and contains no L-tryptophan. Natural, fibrillar Type I collagen is normally soluble in dilute acids and alkalis. When heated (for example, above approximately 40°C), collagen is denatured to single alpha chains (gelatin). At each end of the chains are short non-helical domains called telopeptides, which are removed in some collagen preparations. Through non-covalent interactions with sites on adjacent helices, fibrillogenesis is achieved. Subsequently, non-reducible cross-links are formed. Type I collagen can be associated with Type III and Type V collagen and also with the other non-collagenous proteins like elastin and other structural molecules like glycosaminoglycans and complex lipoproteins and glycoproteins.

3.1.4 *degradation*—change in chemical, physical, or molecular structure or appearance (that is, gross morphology) of material.

3.1.5 *endotoxin*—a high molecular weight lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria that is pyrogenic in humans. Though endotoxins are pyrogens, not all pyrogens are endotoxins.

3.1.6 *microorganism*—bacteria, fungi, yeast, mold, viruses, and other infectious agents. However, it should be noted that not all microorganisms are infectious or pathogenic.

3.1.7 *solubility*—a measure of the extent to which the material can be dissolved. In the context of collagen, refers to the dissociation of the fibrillar aggregates of collagen molecules into a solution. Native Type I collagen which is soluble in dilute acids, but not soluble in neutral pH conditions is termed “insoluble” or “acid soluble,” while simple aggregates of non-fibrillar collagen soluble in neutral salt solutions are

¹⁰ Available from the Food and Drug Administration, 5600 Fishers Ln., Rockville, MD 20857.

¹¹ Association for the Advancement of Medical Instrumentation, 1110 N. Glebe Rd., Suite 220, Arlington, VA 22201-4795.

¹² Available from the FDA, 5600 Fishers Ln., Rockville, MD 20857. <http://www.fda.gov/cdrh/ode/odep850.html>.

