Designation: F3089 – 14

Standard Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions[1]

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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), cell adhesion, and biodegradation 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 collagen formulations that polymerize (self-assemble) or exhibit solution to gel (matrix) transition. Most recent developments have focused on collagen polymer formulations with tunable features to support the rational design of collagen materials for improved tissue integration and guidance of cell fate. Unfortunately, the term "collagen" is applied generally to describe various collagen types and formulations (soluble, insoluble, monomeric, atelocollagen) that vary significantly in their molecular compositions, self-assembly 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 collagen polymer formulations and associated self-assembled collagen-based materials produced with these formulations.

1. Scope

1.1 This guide for characterizing polymerizable collagens is intended to provide characteristics, properties, test methods, and standardization approaches for use by producers, manufacturers, and researchers to identify specific collagen polymer formulations and associated self-assembled collagen-based products produced with these formulations. This guide will focus on the characterization of polymer forms of Type I collagen, which is the most abundant collagen in mammalian connective tissues and organs, including skin, bone, tendon, and blood vessels. Type I collagen may be derived from a variety of sources including, but not limited to, animal or cadaveric tissues, cell culture, recombinant, and chemical synthesis. This guide is intended to focus on purified Type I collagen polymers 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, drug development, and toxicity testing. Polymerizable or self-assembly implies that the collagen composition exhibits spontaneous macromolecular assembly from its components in the absence of the addition of exogenous factors including cross-linking agents. Self-assembling collagen polymers may include, but are not limited to: *(1)* tissue-derived atelocollagens, monomers, and oligomers; *(2)* collagen proteins and peptides produced using recombinant technology; and *(3)* chemically synthesized collagen mimetic peptides. It should be noted that the format of associated self-assembled collagen-based 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. This guide may serve as a

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template for characterization and standardization of other fibrillar collagen types that demonstrate polymerization or self-assembly.

1.2 The ability of self-assembled collagen 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 (**1, 2**)[2] and basic research studies (**3, 4**). The biocompatibility and appropriateness of use for a specific application(s) is the responsibility of the product manufacturer.

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

1.4 **Warning—***Mercury has been designated by the Environmental Protection Agency (EPA) and many state agencies as a hazardous material that can cause central nervous system, kidney, and liver damage. Mercury, or its vapor, may be hazardous to health and corrosive to materials. Caution should be taken when handling mercury and mercury-containing products. See the applicable product Material Safety Data Sheet (MSDS) for details and the EPA website (http://www.epa.gov/mercury/faq.htm) for additional information. Users should be aware that selling mercury or mercury-containing products, or both, in your state may be prohibited by state law.*

1.5 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 limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products

F619 Practice for Extraction of Medical Plastics

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 Screening of Implant 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 Bone

F1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices (Withdrawn 2012)[4]

F1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials

F1903 Practice for Testing For Biological Responses to Particles ***In Vitro***

F1904 Practice for Testing the Biological Responses to Particles ***in vivo***

F1905 Practice For Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity (Withdrawn 2011)[4]

F1906 Practice for Evaluation of Immune Responses In Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration (Withdrawn 2011)[4]

F1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Applications

F2148 Practice for Evaluation of Delayed Contact Hypersensitivity Using the Murine Local Lymph Node Assay (LLNA)

2.2 *ISO Standards:*[5]

ISO 10993–1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing with a Risk Management Process

ISO 10993–3 Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

ISO 10993–9 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 Methods for Establishment of Allowable Limits for Leachable Substances Using Health-Based Risk Assessment

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

ISO 14971 Medical Devices—Application of Risk Management to Medical Devices

ISO 22442–1 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk

ISO 22442–2 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 2: Controls on Sourcing, Collection, and Handling

ISO 22442–3 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 3: Validation and the Elimination and/or Inactivation of Virus and Transmissable Agents

[2] The boldface numbers in parentheses refer to the 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.

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

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

2.3 *U.S. and European Pharmacopeia Documents:*[6]

United States Pharmacopeia (USP), Edition XXX (30)

USP 30/NF 19 Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

European Pharmacopeia 5.0

2.4 *Code of Federal Regulations:*[7]

21 CFR 312 Investigational New Drug Application

21 CFR Part 820 Quality System Regulation

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

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

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

Federal Register, Vol. 72 No. 8, Jan. 12, 2007, pp. 1581–1619, Proposed Rule: Use of Materials Derived from Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants

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

2.5 *ICH Documents:*[8]

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

ICH S2A Guideline for Industry S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals 61 FR 18199 (1996)

ICH S2B Guidance for Industry S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals 62 FR 62472 (1997)

ICH S5A Guideline for Industry S5A Detection of Toxicity to Reproduction for Medicinal Products 59 FR 48746 (1994)

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

ICH S1A Guideline for Industry S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals 61 FR 8153 (1996)

ICH S1B Guidance for Industry S1B Testing for Carcinogenicity of Pharmaceuticals 63 FR 8983 (1998)

ICH S1C Guideline for Industry S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals 60 FR 11278 (1995)

ICH 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 (1997)

ICH Q1A ICH Harmonized Tripartite Guidance for Stability Testing of New Drug Substances and Products (September 23, 1994)

2.6 *FDA Documents:*[9]

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

FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers, DHHS, June 2012

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

2.7 *AAMI Documents:*[10]

ANSI/AAMI/ISO 11737-1: 2006 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

[6] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

[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.

[8] Available from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Secretariat, c/o IFPMA, 15 ch. Louis-Dunant, P.O. Box 195, 1211 Geneva 20, Switzerland, http://www.ich.org.

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

[10] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

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"

2.8 *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[11]

Council Directive 93/42/EEC, with Respect to Medical Devices Using Tissues of Animal Origin[12]

Commission Directive 2003/32/EC, with Respect to Medical Devices Manufactured Using Tissues of Animal Origin[12]

EMEA/410/01-rev.2, Committee for Proprietary Medical Products, Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medical Products[13]

The European Agency for the Evaluation of Medicinal Products, (EMA), Committee for Proprietary Medicinal Products (CPMP) Guidance Document for Decision Trees for the Selection of Sterilization Methods (CPMP/QWP/054/98 corr 2000) and Annex to Note for Guidance on Development Pharmaceutics (CPMP/QWP/155/96)[14]

3. Terminology

3.1 *Definitions:*

3.1.1 *adventitious agents, n*—an unintentionally introduced microbiological or other infectious contaminant.

3.1.2 *atelocollagen, n*—triple helical molecule in which the telopeptide regions have been partially or completely removed from tropocollagen (see Fig. 1). Such preparations are typically

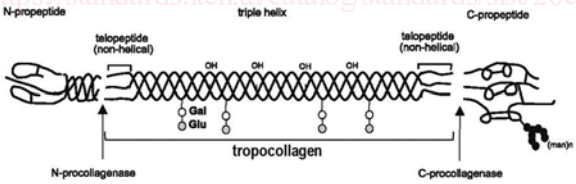

FIG. 1 Schematic of Procollagen Molecule and Associated Propeptide, Telopeptide, and Triple Helical Regions. Enzymatic Removal of Amino- and Carboxy-terminal Propeptide Ends of Procollagen Molecule by Procollagenases Yields Tropocollagen.

the outcome of enzyme-based (for example, pepsin) collagen extraction procedures from tissues.

[11] Available from the FDA, 5600 Fishers Ln., Rockville, MD 20857. http://www.fda.gov/cdrh/ode/oderp850.html.

[12] Available from Office for Official Publications of the European Communities—European Law, 2, rue Mercier, L-2985, Luxembourg, http://eur-lex.europa.eu/en/'index.htm.

[13] Available from European Medicines Agency (EMEA), 7 Westferry Circus, Canary Wharf, London E14 4HB, U.K., http://www.eudora.org/emea.html, and http://www.emea.europa.eu/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf.

[14] Available from European Medicines Agency (EMEA), 7 Westferry Circus, Canary Wharf, London E14 4HB, U.K., http://www.eudora.org/emea.html, and http://www.emea.europa.eu/pdfs/human/qwp/005498en.pdf.

3.1.3 *collagen, n*—a family of at least 20 genetically different secreted proteins that serve a predominantly structural function and possess a unique triple helical structure configuration of three polypeptide units known as alpha chains.

3.1.4 *collagen mimetic peptides, n*—specific amino acid sequences representing the triple helical portion of collagen, often $–(Pro–Hyp–Gly)_x–$, forms a triple helix conformation that resembles that found in natural collagens.

3.1.5 *collagen polymer, n*—purified Type I collagen formulation that demonstrates the capacity to self-assemble or polymerize into higher order structures (macromolecular assemblies) in absence of exogenous agents such as cross-linkers.

3.1.6 *diffusion, n*—the random thermal motion of atoms, molecules, clusters of atoms, etc., in gases, liquids, and some solids.

3.1.7 *endotoxin, n*—pyrogenic high molar mass lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria.

3.1.7.1 *Discussion*—Although endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a Limulus Amebocyte Lysate (LAL) test (USP<85> Bacterial Endotoxin Tests).

3.1.8 *fibrillogenesis, n*—the process of tropocollagen monomers assembling into mature fibrils and associated fibril-network structures.

3.1.9 *gel, n*—the three-dimensional network structure arising from intermolecular polymer chain interactions.

3.1.9.1 *Discussion*—Such chain interactions may be covalent, ionic, hydrogen bond, or hydrophobic in nature.

3.1.10 *mechanotransduction, n*—process by which cells convert mechanical stimuli into a chemical response.

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

3.1.12 *permeability, n*—a measure of the ability of porous materials to transmit fluids; the rate of flow of a liquid through a porous material.

3.1.13 *procollagen, n*—collagen molecule comprising three hydroxylated prototcollagen (alpha) chains; amino- and carboxy-terminal propeptide ends are intact (Fig. 1).

3.1.14 *propeptides, n*—amino- and carboxy-terminal nontriple-helical domains of individual collagen protocollagen (alpha) chains that direct triple-helix folding and formation of procollagen molecules (Fig. 1); propeptide removal is required for collagen fibrillogenesis and self-assembly.

3.1.15 *protocollagen, n*—single collagen alpha polypeptide chain as produced by ribosomes.

3.1.16 *recombinant collagen protein/peptide, n*—collagen or collagen-like polypeptide produced by recombinant methods, such as by expression of a nucleotide sequence encoding the protein or peptide in a microorganism, insect, plant, or animal host. Such compositions often comprise Gly-X-Y triplets where Gly is the amino acid glycine and X

and Y can be the same or different, are often proline or hydroxyproline, but can be any known amino acid.

3.1.17 *self-assembly, n*—the process by which a complex macromolecule (as collagen) or a supramolecular system (as a virus) spontaneously assembles itself from its components.

3.1.18 *solution, n*—a type of homogenous mixture in which atoms, ions, or molecules (the solute) are distributed uniformly throughout another substance (the solvent) and which does not separate upon standing.

3.1.19 *sterilization, n*—the destruction or removal of all microorganisms in or about an object (for example, by chemical agents, electron beam, gamma irradiation, or filtration).

3.1.20 *stiffness, n*—a general term describing the extent to which a material resists deformation in response to an applied force; specific measures of stiffness depend upon the material loading format (for example, tension, compression, shear, bending).

3.1.21 *suspension, n*—the dispersion of a solid through a liquid with a particle size large enough to be detected by purely optical means and which separates or settles upon standing.

3.1.22 *telopeptide, n*—amino- and carboxy-terminal nontriple-helical domains of tropocollagen strands known to be important to fibrillogenesis and intermolecular cross-link formation (Fig. 1).

3.1.23 *tropocollagen, n*—collagen molecule comprising three alpha chains with amino- and carboxy-terminal propeptide ends removed (Fig. 1); carboxy- and amino-terminal non-helical telopeptide ends are intact; able to undergo self-assembly into fibrillar matrix.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *adhesion, n*—steady or firm attachment; in the context of collagen, adhesion refers to the ability of cells to physically attach or bind to collagen molecules and macromolecular assemblies of collagen via cell surface proteins like integrins.

3.2.2 *degradation, n*—change in chemical, physical, or molecular structure or appearance (that is, gross morphology) of material; degradation of collagen under physiologic conditions involves site-specific cleavage within the central triple helical region by proteolytic enzymes known as collagenases. Collagenases are members of the larger family of proteases known as matrix metalloproteases.

3.2.3 *matrix, n*—loose meshwork within which cells are embedded or arrangement of connected things. In the context of collagen, matrix refers to a composite material comprised of an insoluble collagen-fibril network or amorphous nanostructure surrounded by an interstitial fluid phase.

3.2.4 *monomer, n*—individual tropocollagen molecule (Fig. 1).

3.2.5 *oligomer, n*—two or more tropocollagen molecules covalently attached by a naturally occurring intermolecular cross-link.

3.2.6 *solubility, n*—a measure of the extent to which a material can be dissolved; in the context of collagen polymers, solubility refers to collagen molecules (partial, full, or multiples) or peptides in a solution; further qualification of solubility may include “acid-soluble” and “neutral salt-soluble” which describes compositions that are soluble in dilute acids and neutral salt solutions, respectively.

4. Significance and Use

4.1 The objective of this document is to provide guidance in the production, characterization, testing, and standardization of: *(a)* collagen polymers as a starting material for surgical implants, substrates for tissue-engineered medical products (TEMPs), vehicles for therapeutic cells and molecules, and 3D *in-vitro* tissue systems for basic research, drug development, and toxicity testing; and *(b)* self-assembled collagen-based materials produced with collagen polymer formulations. This guide can be used as an aid in the selection, characterization, and standardization of the appropriate collagen polymer starting material as well as associated self-assembled collagen-based products for a specific use. Not all tests or parameters are applicable to all uses of collagen.

4.2 The collagen covered by this guide may be used in a broad range of applications, forms, or medical products, for example (but not limited to) wound and hemostatic dressings, surgical implants or injectables, hybrid medical devices, tissue-engineered medical products (TEMPs), injectable or implantable delivery vehicles for therapeutic cells, molecules, and drugs, and 3D *in-vitro* tissue systems or models for basic research, drug development, and toxicity testing. The practical application of the collagen polymers and associated self-assembled collagen-based materials should be based, among other factors, on biocompatibility, application-specific performance measures, as well as chemical, physical, and biological test data. Recommendations in this guide should not be interpreted as a guarantee of success for any research or medical application.

4.3 The following general areas should be considered when determining if the collagen supplied satisfies requirements for use in the above mentioned medical and research applications: source of collagen polymer, impurities profile, and comprehensive chemical, physical, and biological characterization and testing.

4.4 The following documents or other relevant guidances from appropriate regulatory bodies relating to the production, regulation, and regulatory approval of devices, biologics, drugs, and combination products should be considered when determining if the collagen supplied satisfies requirements for use in medical and research products, including TEMPs, therapeutic delivery vehicles, and 3D *in-vitro* tissue systems:

FDA CFR:
21 CFR 3: Product Jurisdiction:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=3
21 CFR 58: Good Laboratory Practice for Nonclinical Laboratory Studies:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58

FDA/CDRH CFR and Guidances:
21 CFR Part 803: Medical Device Reporting:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=803
21 CFR 812: Investigational Device Exemptions:

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=812
21 CFR 814: Premarket Approval of Medical Devices:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=814
21 CFR 820: Quality System Regulation:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=820
Design Control Guidance for Medical Device Manufacturers:
http://www.fda.gov/cdrh/comp/designgd.pdf
Preproduction Quality Assurance Planning Recommendations for Medical Device Manufacturers (FDA 90-4236):
http://www.fda.gov/cdrh/manual/appende.html
The Review and Inspection of Premarket Approval Applications under the Bioresearch Monitoring Program—Draft Guidance for Industry and FDA Staff:
http://www.fda.gov/cdrh/comp/guidance/1602.pdf

FDA/CDRH Search Engines:
CDRH Guidance Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfggp/search.cfm
CDRH Premarket Approval (PMA) Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm
CDRH 510(k) Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm
CDRH Recognized STANDARDS Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

FDA/CBER CFR and Guidances:
21 CFR 312: Investigational New Drug Application:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=312
21 CFR 314: Applications for FDA Approval to Market a New Drug:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=31
21 CFR 610: General Biological Products Standards:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=610
21 CFR 1271: Human Cells, Tissues and Cellular and Tissue-Based Products:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271
Cellular & Gene Therapy Guidances and Other Publications:
http://www.fda.gov/cber/genetherapy/gtpubs.htm
Human Tissue Guidances and Other Publications:
http://www.fda.gov/cber/tissue/docs.htm
CBER Product Approval Information:
http://www.fda.gov/cber/efoi/approve.htm
21 CFR 600, 601 BLA Regulations:
http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfrv7_07.html
21 CFR 210, 211 GMP Regulations:
http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfr210_07.html

5. Chemical, Physical, and Biological Characterizations of Collagen Polymers and Associated Self-assembled Collagen-based Products

5.1 *General Comments*—These methods represent suggested chemical, physical, and biological assays or analyses; however, other validated assays and analyses may be used (**5**). Method selection will vary, depending on the formulation and source of the collagen (for example, tissue-derived molecular collagen or collagen peptides produced synthetically). The user should ensure that the method selected is reliable and commonly accepted for protein, polymer, biological, and biomaterial analyses. In addition, the test should have appropriate dynamic range, detection limits, and sensitivity.

5.2 *Collagen Concentration*—Collagen concentration should be expressed in mass/volume or mass/mass. Calibrated colorometric assays for collagen, such as Sirius red, or amino acid analysis for hydroxyproline, are commonly used methods to measure collagen content.

5.3 *Viscosity*—Viscosity of collagen polymer formulations depends on a number of factors which may include, but are not limited to, the following: solution or dispersion/suspension, concentration, molecular composition, molecular size, temperature, operating condition, and so forth. Viscosity measurements are routinely performed with a viscometer or rheometer. The user must clearly state the conditions of the test. Determinations of intrinsic viscosity can be used in calculation of average polymer molecular weight.

5.4 *Purity of Collagen Polymer Formulations*—Collagen polymer formulations should be highly purified solutions with impurity levels lower than 2 % by mass. Formulations can be analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), either on the collagen polymer directly or after specific enzymatic (bacterial collagenase, trypsin) or chemical (cyanogen bromide (CNBr)) cleavage reactions to analyze cleavage products. The following represents a non-inclusive list of chemical analyses available: SDS-PAGE, peptide mapping, and amino-terminal sequencing. Assay methods for specific non-collagenous impurities such as hexosamine (that is, detection of glycoproteins), lipid, total sugar, desmosine (that is, elastin), and amino acid composition (that is, collagen composition profile; non-collagenous amino acids) may also be included.

5.5 *Collagen Type Composition*—Tissues commonly used to isolate Type I collagen typically contain other collagen types since co-assemblies of different collagen types are commonplace. Collagen Type I is the predominant collagen type found in the majority of connective tissues and organs, including skin, bone, tendon, cornea, and the interstitial extracellular matrix. Type II collagen is found primarily in cartilage, while Type IV collagen is a major component of basement membranes. The collagen type composition is an important determinant of the polymerization capacity and properties of collagen formulations. Since it is well established that other collagens, such as Type III and Type V, affect Type I self-assembly kinetics and products, the levels of these should be evaluated and controlled for manufacturing consistency. Collagen type composition is usually determined via western blot or ELISA analysis and requires the use of type-specific antibodies. Validation of antibody specificity, as well as the test procedure, using suitable standards, should be conducted prior to analysis. A risk assessment should be performed on the potential for other collagens in the product. If the presence of other collagens is likely, an assessment should be completed for collagens that have the potential to generate undesired responses. The extent of analysis required will depend upon the risk of other collagen types being present as impurities in a particular collagen product.

5.6 *Elastin Assay*—Elastin can be a component of the impurities in tissue-derived collagen polymer preparations. One method to assay for elastin involves the detection of desmosine (**6**). Western blot, enzyme-linked immunosorbent assay (ELISA), and other types of analyses also may be used.