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Standard Guide for Bioinks Used in Bioprinting¹

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1. Scope

1.1 This guide is a resource for bioprinting tissue-engineered medical products (TEMPs) with bioinks and biomaterial inks. There are existing standards that cover biomaterials and scaffolds in a more general fashion (Guide F2150, Guide F2027, ISO 10993 series). This guide focuses specifically on extrusion bioprinting utilizing bioinks and biomaterial inks with inherent or inducible fluidic properties with or without encapsulated cells used to construct TEMPs. For the remainder of this guide, both bioinks and biomaterial inks will be collectively referred to as bioinks.

1.2 For the purposes of this guide, bioprinting is defined as the three-dimensional printing of materials (bioinks) to fabricate structured constructs for use in biological or medical applications.

1.3 TEMPs may be produced by many different bioprinting modalities, including but not limited to the following: electrospinning, electrospray, extrusion-based, droplet-based, inkjet-based, and laser-assisted bioprinting. Extrusion-based bioprinting is the primary focus of this document since it is currently the most well-understood modality used to construct TEMPs, but other bioprinting modalities are also addressed.

1.4 This guide will focus on bioinks and biomaterials used as inks with inherent or inducible fluidic properties. These inks may or may not contain encapsulated cells. Chemical properties of the inks and other factors that affect printability are addressed.

1.5 Pre-printing and printing considerations are the focus of this guide, but considerations regarding post-printing product stabilization are also addressed.

1.6 This guide will address assessments regarding the sterility and cytocompatibility of bioinks, including chemical and physical benchtop tests, as well as measures of post-printing cell viability.

1.7 *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.

1.8 *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

D7805 Terminology for Printing Ink Vehicles and Related Materials

F748 Practice for Selecting Generic Biological Test Methods for Materials and 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 Selected Tissue Effects of Absorbable Biomaterials for Implant Applications

F2027 Guide for Characterization and Testing of Raw or Starting Materials for Tissue-Engineered Medical Products

F2064 Guide for Characterization and Testing of Alginates as Starting Materials Intended for Use in Biomedical and Tissue Engineered Medical Product Applications

F2103 Guide for Characterization and Testing of Chitosan Salts as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Product Applications

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

F2212 Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)

F2315 Guide for Immobilization or Encapsulation of Living Cells or Tissue in Alginate Gels

F2347 Guide for Characterization and Testing of Hyaluronan as Starting Materials Intended for Use in Biomedical

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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.

and Tissue Engineered Medical Product Applications
F2450 Guide for Assessing Microstructure of Polymeric Scaffolds for Use in Tissue-Engineered Medical Products
F2475 Guide for Biocompatibility Evaluation of Medical Device Packaging Materials
F2603 Guide for Interpreting Images of Polymeric Tissue Scaffolds
F2739 Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds
F2902 Guide for Assessment of Absorbable Polymeric Implants
F2997 Practice for Quantification of Calcium Deposits in Osteogenic Culture of Progenitor Cells Using Fluorescent Image Analysis
F2998 Guide for Using Fluorescence Microscopy to Quantify the Spread Area of Fixed Cells (Withdrawn 2023)³
F3089 Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions
F3106 Guide for *in vitro* Osteoblast Differentiation Assays
F3224 Test Method for Evaluating Growth of Engineered Cartilage Tissue using Magnetic Resonance Imaging
F3259 Guide for Micro-computed Tomography of Tissue Engineered Scaffolds
F3354 Guide for Evaluating Extracellular Matrix Decellularization Processes
F3510 Guide for Characterizing Fiber-Based Constructs for Tissue-Engineered Medical Products
F3515 Guide for Characterization and Testing of Porcine Fibrinogen as a Starting Material for Use in Biomedical and Tissue-Engineered Medical Product Applications

2.2 ISO Standards:⁴

ISO/ASTM 52900 Additive manufacturing—General principles—Fundamentals and vocabulary
ISO 7198 Cardiovascular Implants and Extracorporeal Systems—Vascular Protheses—Tubular Vascular Grafts and Vascular Patches
ISO 9000 Quality management systems—Fundamentals and vocabulary
ISO 9001 Quality management systems—Requirements
ISO 10993-1 Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process
ISO 10993-2 Biological evaluation of medical devices—Part 2: Animal welfare requirements
ISO 10993-3 Biological evaluation of medical devices—Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity
ISO 10993-4 Biological evaluation of medical devices—Part 4: Selection of tests for interactions with blood
ISO 10993-5 Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity

ISO 10993-6 Biological evaluation of medical devices—Part 6: Tests for local effects after implementation
ISO 10993-7 Biological evaluation of medical devices—Part 7: Ethylene oxide sterilization residual
ISO 10993-9 Biological evaluation of medical devices—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 skin sensitization
ISO 10993-11 Biological evaluation of medical devices—Part 11: Tests for systemic toxicity
ISO 10993-12 Biological evaluation of medical devices—Part 12: Sample preparation and reference materials
ISO 10993-13 Biological evaluation of medical devices—Part 13: Identification and quantification of degradation products from polymeric medical devices
ISO 10993-14 Biological evaluation of medical devices—Part 14: Identification and quantification of degradation products from ceramics
ISO 10993-15 Biological evaluation of medical devices—Part 15: Identification and quantification of degradation products from metals and alloys
ISO 10993-17 Biological evaluation of medical devices—Part 17: Establishment of allowable limits for leachable substances
ISO 10993-18 Biological evaluation of medical devices—Part 18: Chemical characterization of materials
ISO 10993-19 Biological evaluation of medical devices—Part 19: Physico-chemical, morphological and topographical characterization of materials
ISO 10993-20 Biological evaluation of medical devices—Part 20: Principles and methods for immunotoxicology testing of medical devices
ISO 10993-22 Biological evaluation of medical devices—Part 22: Guidance on nanomaterials
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 11137-1 Sterilization of health care products—Radiation—Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices
ISO 11137-2 Sterilization of health care products—Radiation—Part 2: Establishing the sterilization dose
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 health care products—Microbiological methods—Part 1: Determination of a population of microorganisms on products
ISO 13019 Tissue-engineered medical products—Quantification of sulfated glycosaminoglycans (sGAG) for evaluation of chondrogenesis

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

⁴ Available from International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandinnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, <https://www.iso.org>.

- ISO 13408-1 Aseptic processing of health care products—Part 1: General requirements
 - ISO 13408-2 Aseptic processing of health care products—Part 2: Sterilizing filtration
 - ISO 13408-3 Aseptic processing of health care products—Part 3: Lyophilization
 - ISO 13408-4 Aseptic processing of health care products—Part 4: Clean-in-place technologies
 - ISO 13408-5 Aseptic processing of health care products—Part 5: Sterilization in place
 - ISO 13408-6 Aseptic processing of health care products—Part 6: Isolator systems
 - ISO 13408-7 Aseptic processing of health care products—Part 7: Alternative processes for medical devices and combination products
 - ISO 13485 Medical devices—Quality management systems—Requirements for regulatory purposes
 - ISO 14644-1 Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration
 - ISO 14644-2 Cleanrooms and associated controlled environments—Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration
 - ISO 14644-3 Cleanrooms and associated controlled environments—Part 3: Test methods
 - ISO 14644-4 Cleanrooms and associated controlled environments—Part 4: Design construction and start-up
 - ISO 14644-5 Cleanrooms and associated controlled environments—Part 5: Operations
 - ISO 14644-7 Cleanrooms and associated controlled environments—Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments)
 - ISO 14644-8 Cleanrooms and associated controlled environments—Part 8: Classification of air cleanliness by chemical concentration (ACC)
 - ISO 14644-9 Cleanrooms and associated controlled environments—Part 9: Classification of surface cleanliness by particle concentration
 - ISO 14644-10 Cleanrooms and associated controlled environments—Part 10: Classification of surface cleanliness by chemical concentration
 - ISO 14644-13 Cleanrooms and associated controlled environments—Part 13: Cleaning of surfaces to achieve defined levels of cleanliness in terms of particle and chemical classifications
 - ISO 14644-14 Cleanrooms and associated controlled environments—Part 14: Assessment of suitability for use of equipment and materials by airborne chemical concentration
 - ISO 14644-15 Cleanrooms and associated controlled environments—Part 15: Assessment of suitability for use of equipment and materials by airborne chemical concentration
 - ISO 14698-1 Cleanrooms and associated controlled environments—Biocontamination control Part 1: General principles and methods
 - ISO 14698-2 Cleanrooms and associated controlled environments—Biocontamination control Part 2: Evaluation and interpretation of biocontamination data
 - ISO 16379 Tissue-engineered medical products—Evaluation of Anisotropic Structure of Articular Cartilage Using DT (Diffusion Tensor)—MR Imaging
 - ISO 17668-1 Sterilization of health care products—Moist heat—Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices
 - ISO 20399-1 Biotechnology—Ancillary materials present during the production of cellular therapeutic products—Part 1: General requirements
 - ISO 20399-2 Biotechnology—Ancillary materials present during the production of cellular therapeutic products—Part 2: Best practice guidance for ancillary material suppliers
 - ISO 20399-3 Biotechnology—Ancillary materials present during the production of cellular therapeutic products—Part 3: Best practice guidance for ancillary material users
 - ISO 21560 General requirements of tissue-engineered medical products
 - ISO 21973 Biotechnology—General requirements for transportation of cells for therapeutic use
- 2.3 *USP Standards:*⁵
- USP <61> Microbiological Examination of Nonsterile Products
 - USP <71> Sterility Tests
 - USP <85> Bacterial Endotoxins Test
 - USP <161> Medical Devices—Bacterial Endotoxin and Pyrogen Tests
 - USP <1043> Ancillary Materials for Cell, Gene, and Tissue-Engineered Products
- 2.4 *Other Documents:*
- 21 CFR 210 Current Good Manufacturing Practice in Manufacturing, Processing, Packaging, or Holding of Drugs; General
 - 21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceuticals
 - 21 CFR 610.12 General Biological Products Standards—Sterility
 - 21 CFR 820 Quality System Regulation
 - 21 CFR 1271 Human Cells, Tissues and Cellular and Tissue Based Products
 - 21 CFR 1271.210 Human Cells, Tissues and Cellular and Tissue Based Products; Suppliers and Reagents
 - FDA Guidance on 10993-1 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” <https://www.fda.gov/media/85865/download>
 - FDA Guidance on GMP for Combination Products Guidance for Industry and FDA Staff Current Good Manufacturing

⁵ Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, <http://www.usp.org>.

Practice Requirements for Combination Products, <https://www.fda.gov/media/90425/download>

3. Terminology

3.1 Definitions:

3.1.1 *bioink*, *n*—a biologically active single or multicomponent formulation with inherent or inducible fluidic properties that contains one or more of the following: cells, biological components, and materials suitable for bioprinting.

3.1.1.1 *Discussion*—In this definition, the term “biologically active” is used to differentiate between an ink and a bioink.

3.1.2 *biomaterial*, *n*—a synthetic or natural substance or composite used for a biological or biomedical application.

3.1.3 *bioprinting*, *v*—three-dimensional printing of materials (bioinks) to fabricate structured constructs for use in biological or medical applications.

3.1.4 *crosslink*, *v*—union of high-polymer molecules by a system involving primary chemical bonds that is done either by addition of a chemical substance (crosslinking agent), exposing the mixture to heat, or by subjecting the polymer to high-energy radiation (UV or EB). **D7805**

3.1.5 *extrusion*, *n*—additive manufacturing process in which material is selectively dispensed through a nozzle or orifice.

ISO/ASTM 52900

3.1.6 *hydrogel*, *n*—a water-based open network of polymer chains that are crosslinked either chemically or through crystalline junctions or by specific ionic interactions. **F2603, F2450**

3.1.7 *photopolymerization*, *v*—additive manufacturing process in which liquid photopolymer in a vat is selectively cured by light-activated polymerization. **ISO/ASTM 52900**

3.1.8 *support material*, *n*—a process aide material that allows the bioprinting of an object with bioinks that can't hold a shape or dimensions (such as an overhang). The support medium is a liquid or liquid-like solid compatible with the bioink.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *bioprinter*, *n*—a machine that additively manufactures objects from materials (bioinks) that may or may not contain cells or biologically active components for use in biological and medical applications. Can also be referred as printer in this standard.

3.2.2 *cell sedimentation*, *v*—settling of the cells at the bottom of the printing syringe/cartridge during bioprinting.

3.2.3 *crosslinking agent*, *n*—a component added that facilitates the linking of polymer chains, resulting in changes to the chemical and physical properties of the bioink.

3.2.4 *embedded printing*, *v*—bioprinting within a support medium, such as a hydrogel or semisolid matrix, that is used to provide support during the printing of the object. This support medium may or may not be removed as a later step.

3.2.5 *fugitive bioink*, *n*—transient or temporary materials used in 3D printing and bioprinting that can be rapidly removed to form internal voids or channels within a printed construct. Examples of removal methods are leaching,

washing, temperature dissolution, chemical dissolution, and enzymatic processing.

3.2.6 *printability*, *n*—the ability of a material to be processed into three-dimensional structures with predefined geometries by a (bio)printer.

4. Significance and Use

4.1 *Standard Structure*—This standard has been organized according to a typical bioprinting operational workflow. In this way the standard has three main sections: pre-printing considerations, printing considerations, and post-printing considerations. There is also a section on bioprinting modalities and additional considerations of product release, containers, and transport. Certain processes will appear across multiple sections, for example cytocompatibility or crosslinking, as these issues have considerations that take place prior to printing, during the printing process, and following the printing process. Contents of main sections are listed below.

Scope	Section 1
Referenced Documents	Section 2
Terminology	Section 3
Significance and Use	Section 4
Bioprinting Modalities	Section 5
Pre-Printing Considerations	Section 6
Printing Considerations	Section 7
Post-Printing Considerations	Section 8
Additional Considerations	Section 9
Keywords	Section 10
References	

4.1.1 *Pre-Printing Considerations*—Pre-printing considerations include: bioink common applications, support material, and bioink selection considerations. Bioink selection considerations include: formulation of bioinks, bioink properties, changes in properties resulting from formulations, sterility, cellular component, and fugitive element considerations. Within the bioink properties there are considerations related to the viscoelastic properties, chemical properties, structure of polymer and functional groups, purity of material, mechanism of crosslinking, and degradation considerations. Contents of the section on pre-printing considerations are listed below.

Bioink Common Applications	6.2
Support Material Considerations	6.3
Bioink Selection	6.4
Formulation of Bioinks	6.4.1
Concentration of Components	6.4.1.1
Function of Each Component	6.4.1.2
Bioink Properties	6.4.2
Viscoelastic Properties	6.4.2.1
Chemical Properties	6.4.2.2
Structure of Polymer and Functional Groups	6.4.2.3
Purity of Material	6.4.2.4
Mechanism of Crosslinking	6.4.2.5
Degradation of Bioink	6.4.2.6
Changes in Properties Resulting from Formulations	6.4.3
Formulation Modification to Influence Biological Response	6.4.3.1
Formulation Modification to Influence Rheology	6.4.3.2
Formulation Modification to Influence Mechanical Properties	6.4.3.3
Sterility	6.4.4
Sterilization Approach	6.4.4.1
Assessments	6.4.4.2
Cellular Component Considerations	6.4.5
Cytocompatibility	6.4.5.1
Cell Sedimentation	6.4.5.2
Fugitive Element Considerations	6.4.5.3



Documents

Document Sharing Hub

4.1.2 Printing Considerations—Printing considerations include: printability, specifically bioink considerations, cellular component, support material, stabilization, and aseptic printing considerations. Within the cellular component section considerations cover cell viability, temperature, cell shearing, cell distribution, sedimentation, and concentration in the bioink. Contents of the section on printing considerations are listed below.

Printability	7.2
Bioink Considerations	7.2.1
Effect of Cells on Printability	7.2.1.1
Effect of Biomaterials on Printability	7.2.1.2
Temperature	7.2.2
Assessments	7.2.3
Cellular Component Considerations	7.3
Viability of Cells During the Printing Process	7.3.1
Temperature	7.3.2
Cell Shearing	7.3.3
Cell Distribution in the Bioink	7.3.4
Cell Sedimentation	7.3.4.1
Cell Concentration	7.3.5
Support Material Considerations	7.4
Printed Support Material	7.4.1
Support Material Used as an Embedding Medium	7.4.2
Stabilization	7.5
Cytocompatibility	7.5.1
Crosslinking	7.5.2
Temperature	7.5.3
Aseptic Printing Considerations	7.6

4.1.3 Post-Printing Considerations—Post-printing considerations include: post-print bioink, stabilization, and considerations for removal of provisional components and materials. Post-printing stabilization focuses on the modalities (such as crosslinking, temperature-induced self-assembly, and evaporation) as well as stabilization effects on the cell health, function, and modification of properties. The considerations for removal of provisional components and materials include the types of components, timing and method of removal, and the removal effects. Contents of the section on post-printing considerations are listed below.

Post-Print Bioink Considerations	8.2
Structural Fidelity	8.2.1
Viability of Cells	8.2.2
Biomaterial Properties	8.2.3
Cell Sedimentation	8.2.4
Cell Morphology	8.2.5
Stabilization Considerations	8.3
Stabilization Modalities	8.3.1
Crosslinking	8.3.1.1
Temperature-Induced Self-Assembly	8.3.1.2
Evaporation	8.3.1.3
Stabilization Effects	8.3.2
Cell Health and Function	8.3.2.1
Modification of Mechanical Properties	8.3.2.2
Considerations for Removal of Provisional Components and Materials	8.4
Types of Components	8.4.1
Support Components	8.4.1.1
Bioink Elements	8.4.1.2
Support Bath Materials	8.4.1.3
Timing of Removal	8.4.2
Method of Removal	8.4.3
Chemical Removal	8.4.3.1
Temperature-Based Removal	8.4.3.2
Physical Removal	8.4.3.3
Removal Effects	8.4.4
Structural Fidelity	8.4.4.1
Cell Health and Function	8.4.4.2
Modification of Properties	8.4.4.3

4.1.4 Additional Considerations—Additional considerations include: discussion on product release, container, and transport considerations. Container considerations focus mainly on the storage stability of bioinks, transfer of bioinks to the print cartridge, as well as container closing integrity and assessing the quality of the container. Contents of the section on additional considerations are listed below.

Product Release Considerations	9.2
Bioink-Specific Release Considerations	9.2.1
Sterility Assurance	9.2.2
Adventitious Agent and Pyrogen Testing	9.2.3
Functional Testing	9.2.4
Particle Testing	9.2.5
Container Considerations	9.3
Storage Stability of Bioinks	9.3.1
Transfer of Bioinks to the Print Cartridge	9.3.2
Preparing the Working Station	9.3.2.1
Maintenance of Sterility	9.3.2.2
Physical Challenges	9.3.2.3
Additional Container Considerations	9.3.3
Container Closure Integrity	9.3.3.1
Extractables and Leachables	9.3.3.2
Particulates	9.3.3.3
Container Opacity	9.3.3.4
Transport	9.4

5. Bioprinting Modalities

5.1 Several factors drive the selection of bioprinting technologies, including but not limited to cost, ease of use, material capabilities, resolution, and fabrication speed. The operation of any of the bioprinting platforms needs bioinks with a particular set of properties. Specific parameters must be considered when designing a bioink for a particular bioprinting method, for example, extrusion bioprinting needs shear-thinning materials. Generally, within these modalities the resolution and speed are primarily governed by the bioink properties (for example, photoinitiator concentration, optical density) and processing parameters (for example, laser intensity, energy, exposure duration). Although general characteristics of typical bioprinting modalities are described herein, processes and capabilities may vary across specific implementations.

5.2 Extrusion-Based Bioprinting—Extrusion-based direct-write 3D printing (EDP) techniques, including bioplotting and microextrusion printing, consist of robotically controlled deposition of a viscous material as continuous filaments (**1**).⁶ The material is stored in a reservoir and is dispensed through a nozzle onto a substrate as either a self-supporting construct or, in the case of embedded printing, within a bath of another supporting material. The driving mechanism for material extrusion is generally either pneumatic or mechanical. The 3D construct is created layer-by-layer by depositing two-dimensional layers by moving the nozzle and substrate relative to one another. The subsequent layers are printed by moving the stage or the nozzle in the z-direction, with the deposited layer serving as the foundation for the next layer. The 3D construct can also be created freeform by depositing bioinks through some combination of x-, y-, and z-direction motion,

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

for example during embedded printing. The 3D construct is stabilized by various processes including material phase transition, physical, and chemical crosslinking mechanisms. A few systems use multiple print heads to facilitate serial deposition of several materials. EDP processes are compatible with a wide range of fluid properties and can create constructs with varying resolution, sizes, and cell densities.

5.3 Droplet-Based Bioprinting—Droplet-based bioprinting techniques, including inkjet printing, acoustic jet printing, and micro-valve printing, involve creating 3D constructs by layer-on-layer deposition of controlled volumes of fluid droplets in targeted spatial locations. The droplets can be produced in two modes, a continuous mode where the ongoing generation of droplets produces a jet, or in a droplet-on-demand mode where the droplets are generated only when needed for printing. Thermal or acoustic forces are used to eject droplets of the fluid through a print head with a valve. The print head is heated electrically or contains a piezoelectric crystal that produces acoustic waves to produce pulses of pressure that force the droplets from the valve at regular intervals. The print heads can be combined in an adjustable array format to facilitate simultaneous printing of multiple cells and materials. 3D constructs may be created by printing binder droplets onto a powder bed (called binder jetting) or a photopolymer/monomer/oligomer which is subsequently crosslinked (called material jetting). These techniques enable high-throughput printing, high resolution, inexpensiveness, and high reproducibility, but suffer from limited material availability due to the requirement for the biological material to be in liquid form. Droplet-based approaches can also be sensitive to the density or volume fraction of suspended particles (including cells), which can affect the droplet formation process.

5.4 Vat Photopolymerization-Based Bioprinting—Vat photopolymerization-based bioprinting processes including stereolithography (SLA), digital light processing (DLP), and two-photon polymerization (2PP) involve selective crosslinking of photosensitive bioinks using an appropriate mode and wavelength of irradiation. The bioink is contained in a vat, and photopolymerization is achieved by layerwise scanning of a laser beam (SLA), layerwise projection of images using a digital micromirror device (DLP), or by direct writing using ultrashort laser pulses (2PP). Vat photopolymerization-based processes can typically offer a higher resolution and faster speed than many other bioprinting processes. Bioink properties (for example, rheology, thermal conductivity), ribbon properties (for example, optical transparency, thermal conductivity, thickness of bioink coating), and processing parameters (for example, laser intensity, energy, and exposure duration) impact the resolution. Due to the requirement of photosensitivity, the current library of bioinks suitable for vat photopolymerization is limited compared to that of other bioprinting processes.

5.5 Electrostatic Direct Writing (EDW) Bioprinting—Electrostatic direct writing (EDW) techniques including melt electrowriting, electrohydrodynamic printing, and near-field electrospinning use an electric field for the generation of a continuous, stable fluid jet that is collected onto a computer-controlled translating collector. The fluid (for example, melt, solution, gel) extruded at a low flow rate through a nozzle

experiences a force due to the applied voltage between the nozzle and the collector. Once this force overcomes the fluid surface tension, a continuous jet is drawn towards the collector. Appropriate levels of fluid flow rate, voltage, and collector distance are used in EDW processes to prevent the whipping instability in the jet, typically associated with traditional electrospinning, as it is collected onto the collector. The relative motion between the nozzle and collector is governed by a computer-aided toolpath characteristic of additive manufacturing. Depending on the nature of the material, solidification of the fluid jet can be achieved via cooling, solvent evaporation, or chemical crosslinking using coagulation baths. Bioink properties (for example, rheology, thermal conductivity), ribbon properties (for example, optical transparency, thermal conductivity, thickness of bioink coating), and processing parameters (for example, laser intensity, energy, and exposure duration) impact the resolution. It should be noted that EDW processes are not typically suitable for processing bioinks containing living cells due to high solution temperature, solution volatility, and/or process-induced shear stresses.

5.6 Laser-Assisted Bioprinting—Laser-assisted bioprinting refers to a set of direct writing processes including laser-induced forward transfer (LIFT), biological laser processing (BioLP), and matrix-assisted pulsed laser evaporation direct writing (MAPLE DW) that utilize a pulsed laser to deposit bioinks onto a substrate. Central to the process is a glass or quartz ribbon that is coated with the bioink. The nanoseconds laser with UV or near-UV wavelength scans the ribbon causing rapid volatilization of the bioink and ejection of droplets or a plume which transfers material onto a receiving substrate. The receiving substrate may be coated with a supportive material to enable cellular adhesion and sustained growth after cell transfer from the ribbon. Laser-assisted bioprinting processes can typically enable single cell deposition and achieve picoscale to microscale resolution. Bioink properties (for example, rheology, thermal conductivity), ribbon properties (for example, optical transparency, thermal conductivity, thickness of bioink coating), and processing parameters (for example, laser intensity, energy, and exposure duration) impact the resolution.

6. Pre-Printing Considerations

6.1 Prior to performing a bioprint, there are important pre-print considerations to take into account. For example:

6.1.1 Establish if the bioink will include live cells. If so, is the same bioink that includes the cells being used to provide the scaffold structural portion of the print, or will a separate bioink be used for structural printing and the cells subsequently deposited within the scaffold either during the print (perhaps on a layer-by-layer basis or via a dual-head printer)?

6.1.2 Establish if a bioink with cells will provide the desired structural properties needed for the print.

6.1.3 Establish if the bioink with cells will provide the appropriate cell density required for the given application or if subsequent cell culture and proliferation will be required.

6.1.4 Establish the effects of the printing process on cell viability.