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Standard Guide for Assessing Medical Device Cytocompatibility with Delivered Cellular Therapies¹

This standard is issued under the fixed designation F3206; 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 outlines the parameters to consider when designing *in vitro* tests to assess the potential impact of a delivery device on a cellular product being dispensed. This guide does not provide specific protocols, but rather suggests what should be considered the minimum characterization necessary to assess device cytocompatibility. Topics discussed include selecting an appropriate cell line(s), cell physiology parameters to measure, and relevant test procedure variables. Only cells suspended in liquid and infused through a device are considered. Cell therapies paired with scaffolds, suspended in hydrogels, or administered via other methods (e.g., tissue grafting) are not included in the scope of this document. This document does not address physical characterization of delivery devices, such as mechanics, composition, or degradation.

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 and health 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

F813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices

F2394 Guide for Measuring Securement of Balloon Expandable Vascular Stent Mounted on Delivery System

F2739 Guide for Quantifying Cell Viability within Biomaterial Scaffolds

F2809 Terminology Relating to Medical and Surgical Materials and Devices

2.2 ISO Standard:³

ISO 10993-5 Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity

3. Terminology

3.1 Definitions:

3.1.1 *cell line, n*—a generic term that includes primary, stem, and immortalized cells.

3.1.2 *cytocompatible, adj*—referring to the lack of unacceptable impact on a cellular product from interaction with a medical device used for delivery or interaction with manufacturing components. For example, a cytocompatible device does not unacceptably impact the cells passing through it as to compromise the potency of the cell therapy product.

3.1.3 *immortalized cell, n*—a primary cell that has been transformed or otherwise altered to provide an extended replication capacity beyond that of the originating primary cell. An immortalized cell may be naturally isolated (e.g., cancer cell) or purposely transformed in the laboratory.

3.1.4 *primary cell, n*—a cell with a finite replication potential that has not been biologically altered to promote extended survival. A primary cell may be frozen or freshly isolated but the passage history must be known and display demonstrable senescence.

3.1.5 *senescence, n*—the property attributable to finite cell cultures; namely, their inability to grow beyond a finite number of population doublings. **F2809**

3.1.6 *stem cells, n*—progenitor cells capable of self-replication, proliferation, and differentiation. **F2809**

3.1.7 *viable cell, n*—a cell capable of sustaining metabolic activity that is structurally intact with a functioning cell membrane. **F2739**

3.2 Definitions of Terms Specific to This Standard:

¹ This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.43 on Cells and Tissue Engineered Constructs 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 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>.

3.2.1 *ancillary equipment, n*—equipment to be paired with the delivery device (e.g., fittings, syringes, etc.) to facilitate *in vitro* testing through which the cells will pass but are not part of the delivery device as used in the clinic.

3.2.2 *delivery device, n*—a medical device designed to deliver therapeutic cells into the body.

4. Significance and Use

4.1 This guide is designed to assist medical device manufacturers as they develop new devices or qualify existing devices (e.g., catheters, needles) for delivering clinical cell therapies. Cytocompatibility considers the impact of the delivery device on the cells passing through the device during the delivery procedure. The biological safety of the device (e.g., the device's cytotoxicity) should be addressed via other methods, such as ISO 10993-5. It is understood that this guide does not address testing of specific cellular products with specific delivery devices. Such testing may be required by regulatory authorities prior to clinical trial of cellular product or marketing applications. This guide outlines considerations to make the product qualification procedures more likely to succeed and more cost effective.

4.2 The key aspects of assessing device cytocompatibility include selecting a test cell line or cell lines and determining the cell physiology parameters that will be measured to make a determination of cytocompatibility. Acceptance criteria for designating a device as cytocompatible are not detailed here. It will be up to the delivery device end user to determine if the results of a cytocompatibility assessment are sufficient to consider that device cytocompatible. Delivery device lot to lot variability may impact cytocompatibility, therefore validated manufacturing processes should be considered when producing devices for cytocompatibility assessments.

5. Cell Selection

5.1 The cellular response to delivery device contact will comprise the readout for the cytocompatibility characterizations considered in this guide. Given this, selection of the test cell line to be used is critical. Selecting a cell line that represents the intended use of the delivery device is encouraged, but not required. A single cell line or a panel of lines, whether stem cells, primary cells, immortalized cells, or a mixture thereof, may be necessary to characterize the device. Regardless of the approach selected, the cell line(s) chosen must demonstrate sensitivity to one or more evaluation parameters being used to characterize cytocompatibility. The identity of selected cell lines should be authenticated by appropriate means. Be mindful that some cell lines have licensing fees or patent protection which must be addressed. Also consider the available supply and potential issues with obtaining additional cell stocks which may introduce unacceptable variability. Ideally, as the field develops further, a reference cell line or panel of lines may be established for the purpose of device cytocompatibility testing.

5.2 It is critical that the chosen cells are well characterized. Cell and culture condition variables such as growth rate, handling protocols, media requirements, culture vessel coating, dissociation methods (if adherent), typical morphologies, sta-

bility during passaging, acceptable passage number, and similar parameters should all be well established. Delivered cells may be assayed for viability or function as part of the cytocompatibility evaluation. It is vital that the baseline viability and functionality of the cells is established and tracked over time in order to detect any adverse device impacts.

5.3 Animal cell lines may be a possibility for use in cytocompatibility testing; however, human cell data is preferable. Animal cells offer an advantage given that the *in vitro* testing can be supplemented by delivering cells directly into established animal models for further characterization; however, that is outside the scope of this guide.

6. Test Method Design

6.1 Determining the device impact on delivered cells necessitates careful characterization of the chosen cell line(s). The appropriate cell morphology and harvest density for use in an assay must be established. The potential impact of the dissociation method if using adherent cells (e.g., trypsin versus non-enzymatic dissociation) may also need to be considered. It is assumed that cells in culture will be used for purposes of expediency and throughput, but using freshly thawed cell aliquots to mimic a clinical application is also suitable. If this method is selected, the baseline physiology of the cells after thawing must be established. Consideration should be given to how the liquid chosen for suspension of the cells, or other suspension liquids, may interact with the delivery device materials (e.g., degrade the materials, extract leachables) and impact cytocompatibility.

6.2 Positive and negative assay controls will be included whenever possible. The negative control will be cells harvested and assayed having never touched the delivery device. Negative control cells should be prepared as if being passed through the device (e.g., at the set density, added to any ancillary equipment required). The positive control will be cells that show diminished viability and/or function. Producing a positive control can be accomplished by different approaches. A specific delivery device known to impair cells by impacting cell viability or function would be an ideal positive control. Alternatively, chemical or mechanical treatments known to impair cell line viability and/or function will suffice to demonstrate that the cell line is sensitive enough to report these aspects of physiology. Processing agents used in device manufacture or known to be present in the manufacturing environment may make suitable positive controls. If using chemical or mechanical treatments, a dose-response relationship should be evident to demonstrate the sensitivity of the chosen cell to impairment. Different treatments may be used on separate positive control samples in the same assay to perturb specific functions (i.e., multiple positive controls are acceptable).

6.3 A key aspect of testing will be selecting device delivery flow rates that approximate clinical use rates. At a minimum, delivery should be assessed at both a minimum and a maximum approximated clinical use flow rate. Syringe pumps or similar calibrated equipment should be used to ensure delivery consistency. Since the delivery flow rate will determine intra-device forces and the impact on delivered cells, when possible,

the ancillary equipment required to use the device being tested (e.g., syringes, adapter fittings, needles, etc.) should be kept constant for all testing. Different device sizes may necessitate multiple groups of ancillary equipment. The chosen cell line(s) and intended clinical use of the delivery device may dictate that additional delivery rates besides the minimum and maximum should be part of the assessment.

6.4 Cell density in the test sample delivered through the device may also be important as different cell lines show density effects. As with delivery rates, careful characterization of the chosen cell line during assay development will determine if a range of densities should be part of the protocol or if a single standard density is sufficient. The volume of liquid suspension to deliver should be sufficient to account for any dead space that the device will hold and still yield enough sample volume for all subsequent characterization. The methods used to determine recovery and viability may dictate an acceptable cell density range to use in order to provide confidence with the selected method.

6.5 Delivery devices should be evaluated in their finished form as they would be offered for clinical use (i.e., not modified to facilitate cytocompatibility testing). If testing a range of devices (e.g., an infusion catheter available in a range of French sizes and lengths), it is acceptable to select the largest and smallest for each parameter as representative of the range as long as the delivery rates, cell densities, and any required ancillary equipment does not change. Smaller devices designed to hold and deliver minimal volumes may require the delivered cell suspension from multiple devices be pooled to acquire sufficient volume for the cell physiology assays. The age of delivery devices should be considered when selecting devices to evaluate so that newly manufactured devices and those at the end of their shelf life are tested separately.

6.6 The time interval from preparation of the cell suspension for testing to actually performing the physiology assays should be part of the experimental design. Some cell lines may provide for a longer working time before adverse effects manifest which may impact assay throughput and protocol ordering.

6.7 Cell contact time with the device must be considered. Cells allowed to sit in a device in anticipation of delivery may clump, lose viability, or undergo other undesired physiological changes. A maximum dwell time at ambient temperatures should be established for the test cell line in order to standardize this aspect of testing. Extended dwell time may leach compounds from the delivery device depending on the cell suspension medium used.

6.8 Delivery device configuration during clinical use should be considered to account for changes in geometry in flexible devices that may impact cells. For example, if the test device is a catheter, consider whether the most demanding configuration (e.g., maximum shear forces, most challenging geometry) for infusion is with the catheter in a straight configuration or with some degree of tortuosity. Guide [F2394](#) and similar documents may be instructive when designing methods to approximate a clinically relevant infusion model. Also consider if the cells will be in the device during deployment as this may

impact cytocompatibility. If so, simulated deployment prior to delivery should be included in the test method.

7. Cell Physiology Assessment Post Device Delivery

7.1 A multitude of characteristics could be considered in evaluating the cytocompatibility of the delivery device with the test cell. For the simplest assessment, three key measures of cytocompatibility will be discussed and should be the minimum required in any report on device cytocompatibility. Additional parameters may be added at the discretion of the user. The technical methods used to quantify the three key measures should be well characterized in their use with the chosen test cell line.

7.2 *Total Cell Recovery*—One component of a device cytocompatibility assessment is determining recovery of cells passed through a device relative to the input population. Cells may adhere to the delivery device or become trapped in device geometry and be lost to the recoverable population. Cells may also clump leading to clogging of the device or force the delivered cells into unintended aggregates. Cell recovery can be determined by counting the number of intact input cells that pass through the delivery device. Methods to determine recovery should be able to distinguish intact individual cells from debris and aggregates. Manual or automated cell counting methods are both acceptable; provided the error associated with a given method is known. In addition, visual inspection of the used delivery device may help identify potential recovery failure points.

7.3 *Cell Viability*—A cytocompatible delivery device should not unacceptably reduce the viability of delivered cells when compared to the negative control cells. Shear or other physical forces exerted by the device on the cells during delivery may damage cell membranes or initiate apoptotic responses that will reduce the viable delivered population. The same is true for chemical residues or leachates from the device. The extent of viability in a delivered sample should be assessed using relevant techniques such as dye exclusion, apoptotic marker identification, etc. The viability of the delivered cells should be reported for comparison to the viability of the positive and negative controls.

7.4 *Cell Function*—A cytocompatible delivery device should not alter the intended functionality or identity of the delivered cells. Defining cell function is wholly dependent upon the cell line selected and its characterization level. Multiple methods to assess impacts on functionality may be required, depending on the selected cell line and the intended clinical use of the delivery device. Parameters to consider may include growth rate, morphology, target protein production, and gene expression profiling. The functional profile of delivered cells compared to that of the control cells should be reported. The simplicity and robustness of functional characterization will be a key consideration when selecting the cell line. The suggested minimum acceptable cell function assessment will consider only the growth rate of delivered cells relative to the negative control to gauge adverse device associated impacts; however, using other parameters is encouraged.

8. Additional Considerations

8.1 In addition to the previously discussed aspects of device cytocompatibility testing, there are additional factors to consider when designing test methods. The testing of a delivery device in isolation may or may not be appropriate depending on the device and its clinical applications. In cases where a medical device is intended to be paired with additional devices (e.g., an infusion catheter as the delivery device in question paired with a syringe containing cells during clinical use), the test device should be paired with the appropriate ancillary equipment to simulate testing under clinical delivery conditions. The assembled unit will provide the required information on cytocompatibility relative to device materials and delivery forces. It may be necessary to prequalify this ancillary equipment by performing cytocompatibility testing on each in isolation. Also, if a device itself has multiple separate components regardless of being paired with ancillary equipment, each component may require additional cytocompatibility testing if the assembled device does not provide acceptable performance.

8.2 In some instances it may be desirable to evaluate extracts from delivery devices to assess potential impacts on delivered cell therapies. This alternative approach presents additional testing design challenges and is beyond the scope of this document. If desired, the cell function and viability testing described here could be conducted using cell delivery fluid in contact with just the delivery surfaces of the delivery device for a specified duration. The test cell line could then be exposed to any leachates and impacts assessed.

9. Cell Delivery Device Development Process

9.1 Developing a cytocompatible cell delivery medical device must start early, not just with engineering challenges but also manufacturing and process material considerations. Materials that have been determined to be not cytotoxic by alternative test methods (e.g., Practice F813 or ISO 10993-5) may have unacceptable impacts on a cell therapy product. While building a library of cytocompatible materials relative to select cell lines is of value, the manufacturing process at each stage must be closely monitored. This will ensure that the pre-qualified material is not degraded or adversely impacted thus changing the final cytocompatibility evaluation of a completed device. It may also be desirable to assess the physical condition of the devices post-delivery to confirm that the cell suspension solution does not negatively impact the delivery device materials (e.g., adhesives); however, that is outside the scope of this document.

9.2 Computational engineering analyses (e.g., computational fluid dynamics) may be very helpful in elucidating the fluid dynamic stresses (e.g., shear, compression) that cells may experience and thereby identify the worst-case device and simulated use conditions that may adversely affect cells. Adequate validation of the computational analytical method with experimental data is important to ensure credible utilization in cytocompatibility assessments.

10. Keywords

10.1 catheter; cell compatibility; cell therapy; cytocompatibility; medical device; stem cells

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