CBER Standards Recognition Program for Regenerative Medicine Therapies

Standard Recognition Summary (SRS)

Recognition Number: 030 **Date of Discussion:** 07/24/2023

SDO Name/Designation: ASTM F2997 **Year of Publication:** 2021

Title: Standard Practice for Quantification of Calcium Deposits in Osteogenic Culture of Progenitor Cells Using Fluorescent Image Analysis

Scope:
1.1 This practice defines a method for the estimation of calcium content at multiple time points in living cell cultures that have been cultured under conditions known to promote mineralization. The practice involves applying a fluorescent calcium-chelating dye that binds to the calcium phosphate mineral crystals present in the live cultures followed by image analysis of fluorescence microscopy images of the stained cell cultures. Quantification of the positively stained areas provides a relative measure of the calcium content in the cell culture plate. A precise correlation between the image analysis parameters and calcium content is beyond the scope of this practice.
1.2 Calcium deposition in a secreted matrix is one of several features that characterize bone formation (in vitro and in vivo), and is therefore a parameter that may indicate bone formation and osteoblast function (that is, osteoblastic differentiation). Calcium deposition may, however, be unrelated to osteoblast differentiation status if extensive cell death occurs in the cell cultures or if high amounts of osteogenic medium components that lead to artifactual calcium-based precipitates are used. Distinguishing between calcium deposition associated with osteoblast-produced mineralized matrix and that from pathological or artifactual deposition requires additional structural and chemical characterization of the mineralized matrix and biological characterization of the cell that is beyond the scope of this practice.
1.3 The parameters obtained by image analysis are expressed in relative fluorescence units or area percentage (area%), for example, fraction of coverage of the area analyzed.
1.4 Units—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.
1.5 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.
1.6 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee

Extent of Recognition: Complete Recognition

Rational for Recognition: The standard is applicable to tissue engineered products

Standard Development Organization: https://www.astm.org

Please note that this standard may also be recognized under the Center for Devices and Radiological Health's (CDRH) Recognized Consensus Standards Database for Medical Device, found here: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm