# CytoGarde® Verified for Compass® CT and Compass® CT Port Disposable Pressure Transducers: Assessing Cytocompatibility

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## Device Description:

Compass® CT ("Compass CT") is a self-calibrating disposable pressure transducer with integrated digital display powered by an internal battery. Compass® CT Port ("Compass CT Port") is distinguished from the standard transducer model by the inclusion of an integrated sealed port through which wire guides or other ancillary devices up to 0.038 inch (0.97 mm) in diameter can be inserted. Compass CT and Compass CT Port are intended for direct measurement and monitoring of physiologic pressure, including during the infusion of fluids and therapeutic and diagnostic agents. The pressure transducers have a pressure range of -199 to 999 mm Hg, an accuracy of +/- 2 mm Hg for pressure readings below 50 mm Hg and +/- 3% for pressure readings above 50 mm Hg, and allow for multiple readings throughout a single procedure.[1]

## Purpose:

CytoGarde® Verified is a designation ascribed to a device that has been evaluated in accordance with ASTM F3206-17: Standard Guide for Assessing Medical Device Cytocompatibility with Delivered Cellular Therapies.[2] Compass CT and Compass CT Port were tested per ASTM F3206-17 and assayed under clinically relevant conditions for cell recovery, viability, and proliferation population doublings as a measure of functionality using primary human T-lymphocytes (T-cells) and human bone marrow mesenchymal stem cells (hBMMSC).

Cytocompatibility testing requires careful selection of representative and clinically meaningful cell types. T-cells were chosen because they are a non-adherent suspension cell population and are being currently evaluated in several clinical investigations and applications, particularly in the area of immune-oncology, where localized delivery may be of paramount interest. Similarly, hBMMSC were chosen because they are an adhesion-dependent cell population that have been studied in an extensive number of clinical trials evaluating their clinical potential for mediating tissue healing and regeneration. Therefore, the selection of these two cell types ensures wide applicability of the cytocompatibility testing results.

## Methods:

For CytoGarde evaluation of Compass CT and Compass CT Port (Centurion Medical Products, product nos. CCT001 and CCTP001, respectively),[3] the pressure transducers were attached via integrated Luer lock connectors to Cantata® Microcatheters (Cook Medical Incorporated, product no. G54532, MCS-2.5-NT-150-15-HP). The purpose of connecting the microcatheter to the outlet of the pressure transducer was to generate a clinically meaningful pressure gradient through which the cells would flow during infusion through the pressure transducer. For all tests, infused T-cells or hBMMSC were recovered at the catheter tip and assayed for a) percent recovery, b) percent viability, and c) proliferation population doublings as a measure of cell functionality. Test controls consisted of cells either held in static conditions or infused through Cantata Microcatheters without pressure transducers attached.

Two lots of primary human peripheral blood mononuclear cells (PBMC) (StemCell Technologies Inc., product no. 70025, lot nos. 190181302C and 1902190208) were activated and cultured for 8 days to generate T-cells. Cells were verified to be T-cells by flow cytometry using cells stained with (a) anti-CD3 antibody, (b) isotype-matched control antibody, or (c) unstained to serve as background controls. Flow cytometry confirmed that the PBMC had

expanded into a primary T-cell population (donor cell lot #1: 97.4% CD3+ with 0.5% isotype positive; donor cell lot #2: 97.7% CD3+ with 1.2% isotype positive). Separately, two lots of primary hBMMSC (Rooster Biosciences, MSC lot nos. 55 and 71) were plated and expanded in culture per the manufacturer's instructions to generate sufficient numbers of hBMMSC for CytoGarde testing.

The injection rate for infusing cells through the test devices was based on ISO 7864:2016 Section C3.2.[4] Briefly, the standard indicates that an injection pressure of 825 mmHg simulates the upper pressure for the average syringe user. Therefore, a Nexus 6000 syringe pump (Chemyx Inc.) was calibrated to deliver an injection pressure of approximately 825 mmHg through the Compass CT and/or Cantata Microcatheter by setting the infusion flow rate at 11 mL/min.

The CytoGarde testing protocol evaluated the following device test configurations: (a) Compass CT + Cantata Microcatheter, (b) Compass CT Port + Cantata Microcatheter, and (c) Cantata Microcatheter (control). For each device test configuration run, three 1 mL cell suspension samples (T-cells: $2x10^6$ cells/mL or hBMMSC: $0.6\text{-}1.0x10^6$ cells/mL) were infused for each cell type lot. Therefore, for each cell type, the combination of the two lots of cells yielded a total of 18 unique samples collected and pooled for analyses for each of the three device test configurations (Figure 1).

Cell recovery was measured through comparison of cell counts prior to and after infusion by two independent cell counters each performing two counts per sample using a hemocytometer. Similarly, cell viability was assessed by trypan blue exclusion for cell counts prior to and after infusion using hemocytometer counting as described above. Cell functionality was assessed through quantification of cell proliferation population doublings after infusion through the respective test devices. Post-infused T-cells were seeded at $1x10^6$ T-cells/well into 12-well tissue culture plates and post-infused hBMMSC cells were plated at $4.5x10^4$ hBMMSC cells/well into 6-well tissue culture plates pre-coated with collagen type-I (Becton Dickinson, product no. 35-4400, lot no. 2074328). T-cells were cultured for 6 days and hBMMSC were cultured for 7 days, after which the cell population doublings were calculated by hemocytometer counting as described above. Concurrent with the device testing assessments, static, non-infused cell control groups were assayed for cell viability and functionality (6- or 7-day population doublings) as an additional comparator and verification for the cytocompatibility of the tested devices. Compass CT and Compass CT Port were deemed cytocompatible if cell recovery, viability, and functionality assessments were not statistically different than the Cantata Microcatheter controls and static cell controls, where statistical difference was defined as $p<0.05$ using a two-tailed, unequal variance student's T-test.

## CYTOGARDE TESTING PROTOCOL

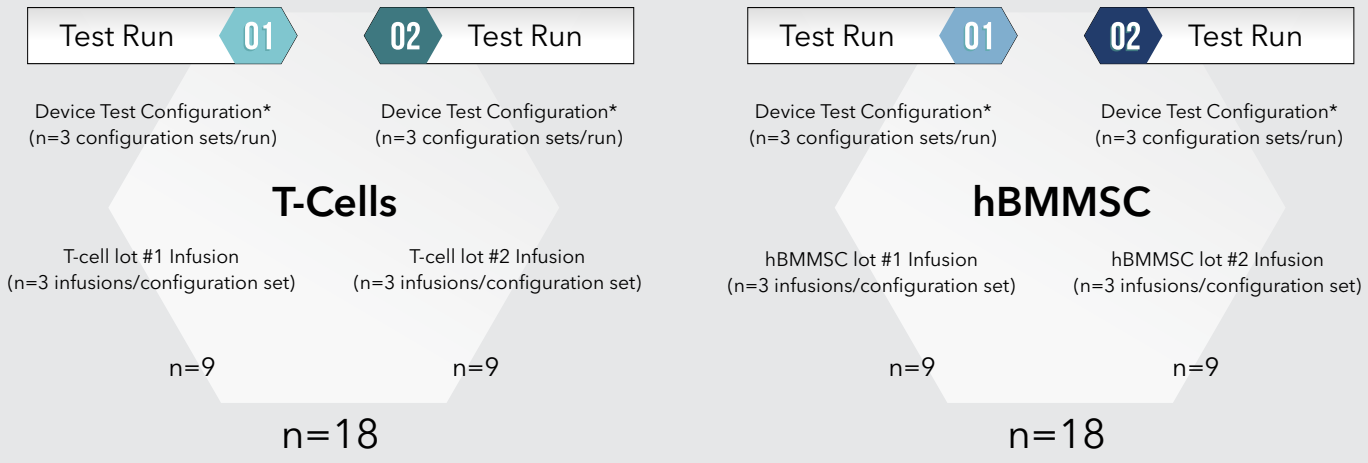



**Figure 1.**
CytoGarde Testing Protocol. A total of 18 samples per test device configuration per cell type were collected and pooled to assess for cell recovery, viability, and proliferation (*Note: device test configuration: Compass CT + Cantata, Compass CT Port + Cantata, or Cantata control).

## Results:

Compass CT and Compass CT Port passed the cytocompatibility acceptance criteria for each of the tests by performing similarly to the Cantata Microcatheter and static cell controls under the conditions tested.

### CELL RECOVERY

Cell recovery was measured as the cell concentration recovered after infusion divided by the average of the control cell concentrations for the corresponding cell lot. The T-cell recovery means for the Cantata Microcatheter alone, Compass CT + Cantata Microcatheter, and Compass CT Port + Cantata Microcatheter all were slightly greater than 100%. There were no statistically significant differences for cell recovery between the Compass CT + Cantata or Compass CT Port + Cantata versus the Cantata Microcatheter alone. T-cell recovery means and statistical comparisons are provided in Table 1 and shown in Figure 2. The hBMMSC recovery means for the Cantata Compass CT + Cantata Microcatheter and Compass CT Port + Cantata Microcatheter were 99% and 93%, respectively, neither of which was statistically different than the 96% mean cell recovery for the Cantata Microcatheter alone. The hBMMSC recovery means and statistical comparisons are provided in Table 2 and shown in Figure 3.

### CELL VIABILITY

Cell viability was measured as the live cell concentration divided by the total cell concentration (live + dead) for each sample following infusion. The T-cell viability means for the static cell controls, Cantata Microcatheter alone, Compass CT + Cantata Microcatheter, and Compass CT Port + Cantata Microcatheter all were 97%. There were no statistically significant differences between the Compass CT or Compass CT Port + Cantata Microcatheter versus Cantata Microcatheter alone or static cell controls. T-cell viability means and statistical comparisons are provided in Table 1 and shown in Figure 4. The hBMMSC viability means for the Cantata Compass CT + Cantata Microcatheter and Compass CT Port + Cantata Microcatheter were 86% and 85%, respectively, neither of which was statistically different from the 87% mean cell viabilities obtained each for the Cantata Microcatheter alone or static cell control. The hBMMSC viability means and statistical comparisons are provided in Table 2 and shown in Figure 5.

### CELL PROLIFERATION

Cell functionality was measured as the 6-day proliferation (T-cells) or 7-day proliferation (hBMMSC) for each sample after infusion through the devices. Proliferation was determined as final cell number divided by initial cell number and reported as number of population doublings. The T-cell 6-day proliferation means for the static cell controls, Cantata Microcatheter alone, Compass CT + Cantata Microcatheter, and Compass CT Port + Cantata Microcatheter were between 2.1 and 2.3 doublings. There were no statistically significant differences between the Compass CT or Compass CT Port + Cantata Microcatheter versus Cantata Microcatheter alone or static cell controls. T-cell population doubling means and statistical comparisons are provided in Table 1 and shown in Figure 6. The hBMMSC 7-day proliferation means for the static cell controls, Cantata Microcatheter alone, Compass CT + Cantata Microcatheter, and Compass CT Port + Cantata Microcatheter were between 3.7 and 4.0 doublings. Similarly, there were no statistically significant differences between the Compass CT or Compass CT Port + Cantata Microcatheter versus Cantata Microcatheter alone or static cell controls. The hBMMSC population doubling means and statistical comparisons are provided in Table 2 and shown in Figure 7.

## Table 1.

T-cell CytoGarde testing of Compass CT and Compass CT Port Disposable Pressure Transducers. Results are given as mean ± standard deviation.

| Device/Condition | Cell Recovery* | Cell Viability | Cell Proliferation Population Doublings |
|---|---|---|---|
| **Compass CT + Cantata Microcatheter** | 103 ± 11% | 97 ± 1% | 2.1 ± 0.3 |
| **Compass CT Port + Cantata Microcatheter** | 102 ± 12% | 97 ± 1% | 2.2 ± 0.2 |
| **Cantata Microcatheter (control)** | 101 ± 12% | 97 ± 1% | 2.1 ± 0.3 |
| **non-infused, static cells (control)** | NA | 97 ± 2% | 2.3 ± 0.3 |

*Recoveries >100% within the expected error of the hemocytometer counting method.

## Table 2.

hBMMSC CytoGarde testing of Compass CT and Compass CT Port Disposable Pressure Transducers. Results are given as mean ± standard deviation.

| Device/Condition | Cell Recovery | Cell Viability | Cell Proliferation Population Doublings |
|---|---|---|---|
| **Compass CT + Cantata Microcatheter** | 99 ± 13% | 86 ± 4% | 3.9 ± 0.5 |
| **Compass CT Port + Cantata Microcatheter** | 93 ± 15% | 85 ± 4% | 4.0 ± 0.5 |
| **Cantata Microcatheter (control)** | 96 ± 18% | 87 ± 4% | 3.7 ± 0.5 |
| **non-infused, static cells (control)** | NA | 87 ± 2% | 3.7 ± 0.5 |

RESULTS

## Mean T-cell percent recovery

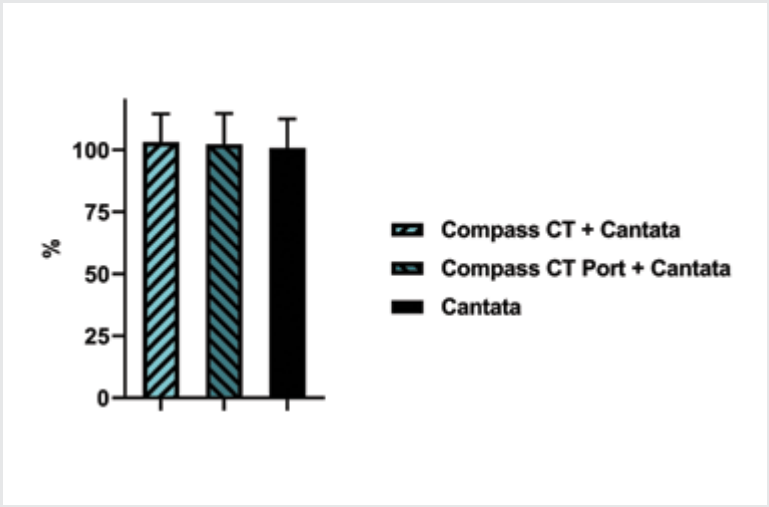



**Figure 2.**
Mean T-cell percent recovery. Error bars represent standard deviation. Device groups, n=18; Compass CT + Cantata vs. Cantata: p=0.52; Compass CT Port + Cantata vs. Cantata: p=0.70.

## Mean hBMMSC percent recovery

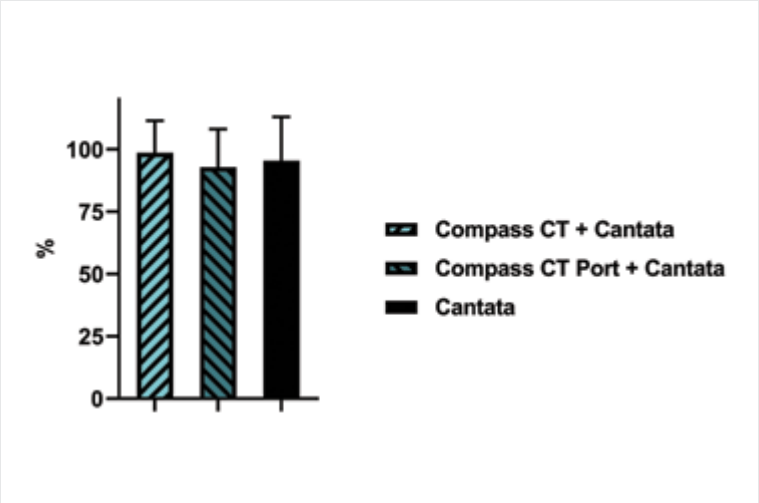



**Figure 3.**
Mean hBMMSC percent recovery. Error bars represent standard deviation. Device groups, n=18; Compass CT + Cantata vs. Cantata: p=0.57; Compass CT Port + Cantata vs. Cantata: p=0.62.

## Mean T-cell percent viability

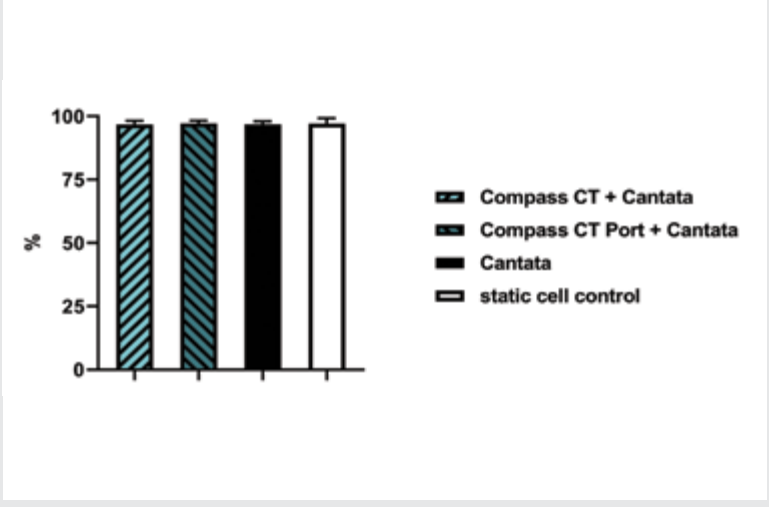
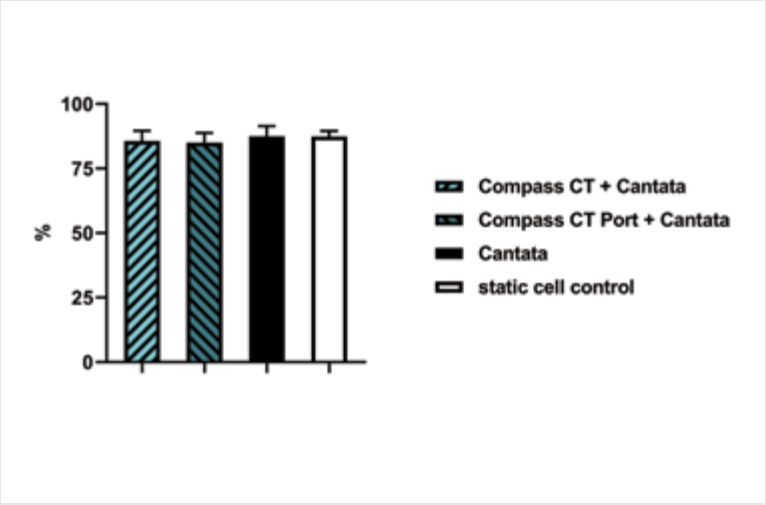



**Figure 4 .**
Mean T-cell percent viability. Error bars represent standard deviation. Device groups, n=18; static cell control group, n=6; Compass CT + Cantata vs. Cantata: p=0.82; Compass CT + Cantata vs. static cell control: p=0.63; Compass CT Port + Cantata vs. Cantata: p=0.48; Compass CT Port + Cantata vs. static cell control: p=0.90; Cantata vs. static cell control: p=0.70.

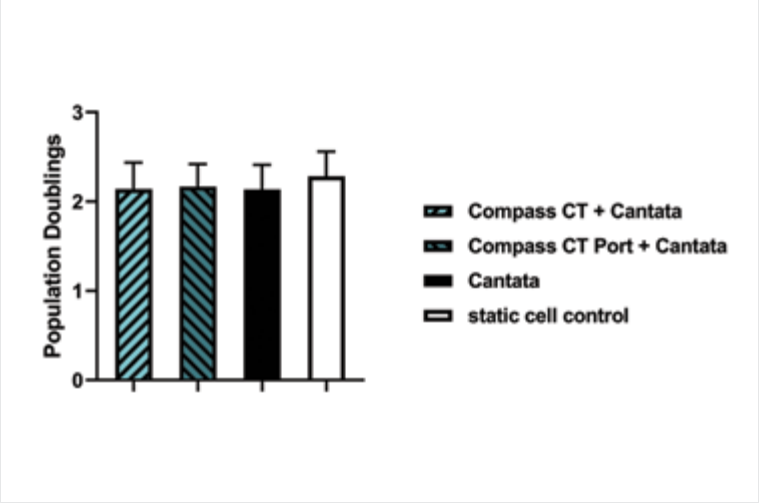
## Mean hBMMSC percent viability




### Figure 5.

Mean hBMMSC percent viability. Error bars represent standard deviation. Device groups, n=18; static cell control group, n=9; Compass CT + Cantata vs. Cantata: p=0.20; Compass CT + Cantata vs. static cell control: p=0.21; Compass CT Port + Cantata vs. Cantata: p=0.06; Compass CT Port + Cantata vs. static cell control: p=0.05; Cantata vs. static cell control: p=0.86.

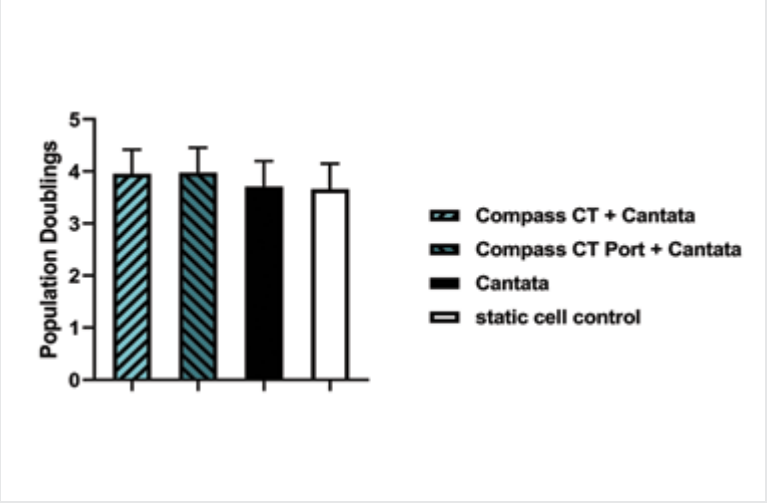
## Mean T-cell proliferation




### Figure 6.

Mean T-cell proliferation in 6-day population doublings. Error bars represent standard deviation. Device groups, n=18; static cell control group, n=6; Compass CT + Cantata vs. Cantata: p=0.88; Compass CT + Cantata vs. static cell control: p=0.40; Compass CT Port + Cantata vs. Cantata: p=0.65; Compass CT Port + Cantata vs. static cell control: p=0.47; Cantata vs. static cell control: p=0.33.

## Mean hBMMSC proliferation




### Figure 7.

Mean hBMMSC proliferation in 7-day population doublings. Error bars represent standard deviation. Device groups, n=18; static cell control group, n=9; Compass CT + Cantata vs. Cantata: p=0.14; Compass CT + Cantata vs. static cell control: p=0.17; Compass CT Port + Cantata vs. Cantata: p=0.11; Compass CT Port + Cantata vs. static cell control: p=0.14; Cantata vs. static cell control: p=0.82.

## Conclusions:

Cell recovery, viability, and functionality as measured by proliferation population doublings were not statistically different among primary T-cells or hBMMSC infused through Compass CT + Cantata Microcatheters or Compass CT Port + Cantata Microcatheters versus counterpart cells infused through Cantata Microcatheters alone or non-infused, static control cells. The results of this study support the cytocompatibility of human T-cells and hBMMSC in contact with the Compass CT and Compass CT Port pressure transducers. While this CytoGarde testing was specific to the cell types tested, the general applicability to a wide variety of primary and transformed cell populations is supported by the choice of models for suspension (T-cells) and adherent (hBMMSC) primary cell types. Cytocompatibility for any specific cell type, for research or clinical purposes, would still need to be verified. In summary, these results indicate that the Compass CT and Compass CT Port Disposable Pressure Transducers are compatible with the injection of cells for therapeutic purposes and can display the CytoGarde® insignia.

## References:


1. Compass® CT and Compass® CT Port product information: https://www.cookregentec.com/products/compass-ct/

2. ASTM F3206-17, Standard Guide for Assessing Medical Device Cytocompatibility with Delivered Cellular Therapies, ASTM International, West Conshohocken, PA, 2017; DOI: 10.1520/F3206-17; http://www.astm.org/cgi-bin/resolver.cgi?F3206

3. Compass® CT and Compass® CT Port are manufactured by Centurion Medical Products (Williamston, MI) and are distributed by Cook Regentec LLC (Indianapolis, IN).

4. ISO 7864:2016, Sterile hypodermic needles for single use – Requirements and test methods, International Organization for Standardization, Geneva, Switzerland, 2016; https://www.iso.org/standard/60481.html



AUTHOR

## Chad Johnson, Ph.D.

Chad joined Cook Biotech Incorporated as a research engineer in December 2003. As a member of the Research Department, he was in charge of biomaterial assessment and improvement efforts. In 2007, Chad was promoted to research manager and lead a team in the discovery, identification and feasibility testing of new biomaterials along with responsibilities for biocompatibility testing, scientific presentations to doctors, and authoring information for regulatory submissions. In September 2015, Chad transitioned to Cook Regentec as a senior research scientist where, as part of a larger team of engineers and scientists, he is focused on development and commercialization of device-based regenerative medicine therapies.

AUTHOR

## Eric Rodenberg, Ph.D.

Eric began his association with Cook companies in 2010. As a member of the Research Department biomaterials team, Eric was responsible for leading and coordinating pre-clinical small animal studies; evaluating external technologies; conducting early-phase biomaterials characterizations and base material improvement experimentation; and serving as a scientific CME presenter. In September 2015, Eric transitioned to Cook Regentec as a research scientist where, as part of a larger team of engineers and scientists, Eric focused on the development and commercialization of device-based regenerative medicine therapies. In 2019, Eric accepted an opportunity to become a scientific communications medical writer wherein he is tasked with coordinating scientific writing and messaging across the different functional teams of Cook Regentec.



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