CytoGarde® Verified for ProFusion™ Therapeutic Infusion Needle: Assessing Cytocompatibility

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Device Description:

The ProFusion™ Therapeutic Infusion Needle ("ProFusion") is intended for intramuscular or subdermal infusion of diagnostic and therapeutic agents.[1] ProFusion is a Luer lock-compatible stainless steel needle comprised of sideports arranged in a spiral pattern along the distal 1 cm length of the needle to facilitate the uniform delivery of diagnostic and therapeutic agents into the adjacent tissue. A trocar tip aids in precise placement for targeted agent delivery. ProFusion is available in needle gauges 19, 21, and 25 and needle lengths ranging from 2.5 cm to 15 cm.

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] ProFusion was tested per ASTM F3206-17 and assayed under clinically relevant conditions for cell recovery, viability, and functionality using two human cell types.

Methods:

For evaluation of ProFusion, comparator single lumen needles (spinal needles, Becton, Dickinson and Company, Franklin Lakes, NJ) of similar gauge and length were utilized. Large bore (19- or 21-gauge) 15-centimeter long ProFusion needles were tested and compared to 20-gauge 15-centimeter long spinal needles, and 25-gauge 7.5-centimeter ProFusion needles were tested and compared to 25-gauge 7.5-centimeter spinal needles.

CytoGarde Verified testing for ProFusion included assaying two clinically relevant cell types: human derived bone marrow mesenchymal stem cells (hBMMSC) and peripheral blood total nucleated cell (TNC) concentrate. Two lots of hBMMSC (RoosterBio, Inc., Frederick, MD) were obtained and cultured as per instructions. Prior to population doubling 18, hBMMSC were harvested and reconstituted at approximately 1 million cells per milliliter for testing. TNC concentrates were obtained from the peripheral blood of 6 healthy donors using the HemaTrate® Blood Filtration System (Cook Regentec LLC, Indianapolis, IN).[3]

Test injection rates were determined for each needle based on ISO 7864:2016 Section C3.2.[4] Briefly, the standard indicates that when possible, needles should be tested at an injection pressure of 825 mmHg, which simulates the upper pressure of the average user. Using a Nexus 6000 syringe pump (Chemyx Inc., Stafford, TX), pressure versus injection rates were determined for each needle. For the 25-gauge ProFusion and 25-gauge spinal needles, the target pressure was reached at 11.5 and 13.0 mL/min respectively. However, even under the fastest injection rates possible (>60 mL/min), the large bore needles did not approach the target injection pressure. As such, 30 mL/min (or 0.5 mL/sec) was utilized as injection rate for these needles, as that rate would be considered the upper limit for speed for an operator injecting cells at this injection volume in the clinical setting.

For the hBMMSC testing, 21-gauge 15-centimeter and 25-gauge 7.5-centimeter ProFusion needles were compared to 20-gauge 15-centimeter and 25-gauge 7.5-centimeter spinal needles. Cell suspensions were injected through the needles at the specific prescribed injection rates. For each cell lot, three 1 mL samples were injected per needle with three individual needles tested per needle type/size. For the 2 lots of cells, a total of 18 samples were collected for each needle type/size. Recovery was determined through two independent cell counters each performing two counts per sample using a hemocytometer. Viability was determined using Trypan Blue exclusion with the hemocytometer counts as above. Functionality was determined as proliferation—determined by plating 45,000 cells per well of a 6-well plate, culturing

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as directed for 7 days, determining cell counts as above and calculating the population doublings. ProFusion needles were considered cytocompatible as in reference to these cells if the resulting recovery and viability were non-inferior to the control spinal needles and if the cell proliferation was greater than 2 doublings in 7 days.

For the TNC concentrate testing, 19-gauge 15-centimeter and 25-gauge 7.5-centimeter ProFusion needles were compared to 20-gauge 15-centimeter and 25-gauge 7.5-centimeter spinal needles. TNC concentrates were injected through the needles at the specific prescribed injection rates. For each of the six TNC concentrate samples, three 0.5 mL samples were injected per needle type/size, resulting in a total of 18 samples collected for each needle type/size. TNC concentrate cell recovery was determined using a Sysmex XP-300 blood cell analyzer (Sysmex America, Inc., Mundelein, IL). TNC viability was determined using acridine orange and propidium iodide staining in conjunction with fluorescence automated cell counting using a Cellometer Auto 2000 (Nexcelom Bioscience, Lawrence, MA). Functionality for the TNC concentrate was assessed by calculating the change in lymphocyte % of the total recovered TNC pre- vs. post-injection through the needles. A greater change in % lymphocytes relative to the spinal comparator needle would suggest that the ProFusion needle is changing the nature of the injected TNC concentrate. Of note, the change in this ratio was calculated as the absolute value of the pre-injection lymphocyte % – post-injection lymphocyte %. ProFusion needles were considered cytocompatible as in reference to these cells if the resulting recovery and viability were non-inferior and the change in % lymphocytes was not significantly greater than the control spinal needles.

Results:

ProFusion needles passed the cytocompatibility acceptance criteria for each of the tests. ProFusion needles performed similarly to the control needles for both cell types under the conditions tested.

CELL RECOVERY

For hBMMSC, all needles demonstrated average cell recoveries greater than 85% (see Table 1, Figure 1). The results for the ProFusion needles were non-inferior to the comparator control needles. For the TNC concentrate, the average cell recoveries from all needles were equal to or greater than 99% (see Table 2, Figure 4). The results for the ProFusion needles were non-inferior to the comparator control needles.

CELL VIABILITY

For hBMMSC, all needles demonstrated average cell viability greater than 95% (see Table 1, Figure 2). The results for the ProFusion needles were non-inferior to the comparator control needles. For the TNC concentrate, the average cell viability from all needles were greater than 99.5% (see Table 2, Figure 5). The results for the ProFusion needles were non-inferior to the comparator control needles.

CELL FUNCTIONALITY

For hBMMSC, cell proliferation was not affected by injection through any of the needles with greater than 3.2 doublings for all needles tested (Table 1, Figure 3), As such, ProFusion needles met the criteria for maintaining cell functionality. For the TNC concentrate, the average change in the lymphocyte % of TNC for ProFusion needles was comparable and non-inferior to the comparator needles (1.4% vs 1.0% for 19g ProFusion vs. 20g spinal, 1.3% vs. 1.3% for 25g ProFusion vs. spinal). (see Table 2, Figure 6). The results for the ProFusion needles were non-inferior to the comparator control needles.

Table 1.

hBMMSC cytocompatibility testing of ProFusion versus spinal needles. Results are given as average (standard deviation).

Needle	Recovery	Viability	Functionality Population Doublings
ProFusion 21g x 15cm	90% (15%)	96% (2%)	3.4 (0.5)
Spinal 20g x 15cm	89% (13%)	97% (1%)	3.4 (0.4)
ProFusion 25g x 7.5cm	87% (20%)	95% (2%)	3.5 (0.3)
Spinal 25g x 7.5cm	90% (23%)	96% (2%)	3.3 (0.4)

Table 2.

TNC cytocompatibility testing of ProFusion versus spinal needles. Results are given as average (standard deviation).

Needle	Recovery	Viability	Functionality Δ % Lymphocyte
ProFusion 19g x 15cm	100% (3%)	99.7% (0.2%)	1.4% (1.4%)
Spinal 20g x 15cm	100% (2%)	99.8% (0.3%)	1.0% (1.1%)
ProFusion 25g x 7.5cm	99% (3%)	99.6% (0.4%)	1.3% (1.3%)
Spinal 25g x 7.5cm	99% (2%)	99.7% (0.2%)	1.3% (1.1%)

hBMMSC RESULTS

Recovery

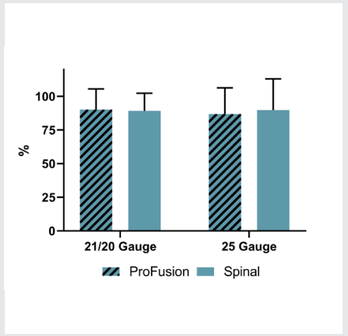

Figure 1.
hBMMSC recovery per needle type. Error bars represent standard deviation. (n=18 per group; p=0.86, 21/20G; p=0.68, 25G).

Viability

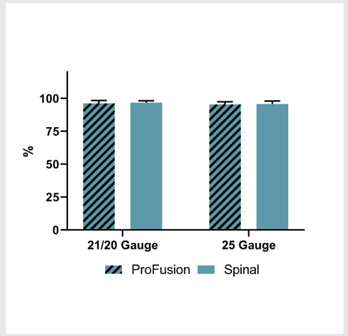

Figure 2.
hBMMSC viability per needle type. Error bars represent standard deviation. (n=18 per group; *p*=0.49, 21/20G; *p*=0.68, 25G).

Functionality

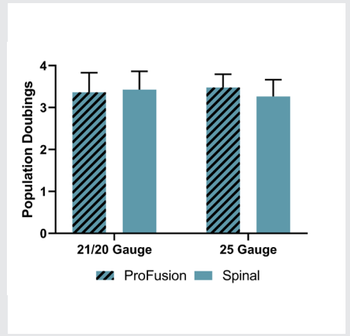

Figure 3.
hBMMSC functionality as measured by 7-day proliferation per needle type. Error bars represent standard deviation. (n=18 per group; *p*=67, 21/20G; *p*=0.09, 25G).

TNC RESULTS

Recovery

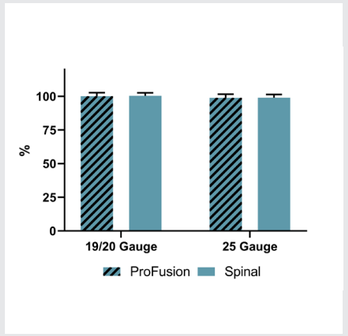

Figure 4.
TNC recovery per needle type. Error bars represent standard deviation. (n=18 per group; *p*=0.76, 19/20G; *p*=0.89, 25G).

Viability

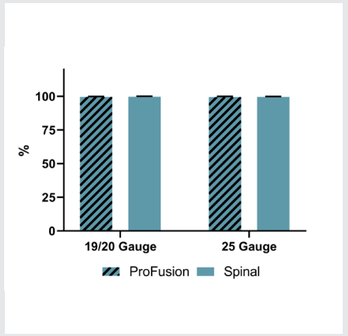

Figure 5.
TNC viability per needle type. Error bars represent standard deviation. (n=18 per group; *p*=0.16, 19/20G; *p*=0.35, 25G).

Functionality

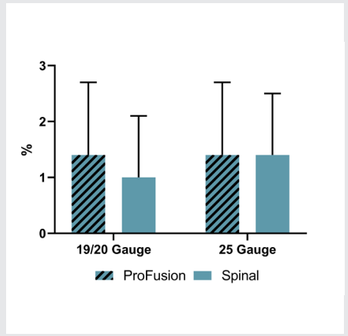

Figure 6.
TNC functionality as measured by change in % lymphocyte pre- vs. post-injection per needle type. Error bars represent standard deviation. (n=18 per group; *p*=0.40, 19/20G; *p*=0.96, 25G).

Conclusions:

ProFusion needles passed the CytoGarde Verified cytocompatibility tests for two clinically relevant cell types, hBMMSC and TNC concentrate. These results suggest that the ProFusion needles are compatible with the injection of cells for therapeutic purposes and therefore display the CytoGarde Verified insignia.

References:

1. ProFusion™ Therapeutic Infusion Needle product information: https://www.cookregentec.com/products/therapeutic-delivery/profusion/
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. The HemaTrate® Blood Filtration System is manufactured by Pall Medical (Fribourg, Switzerland) and is 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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