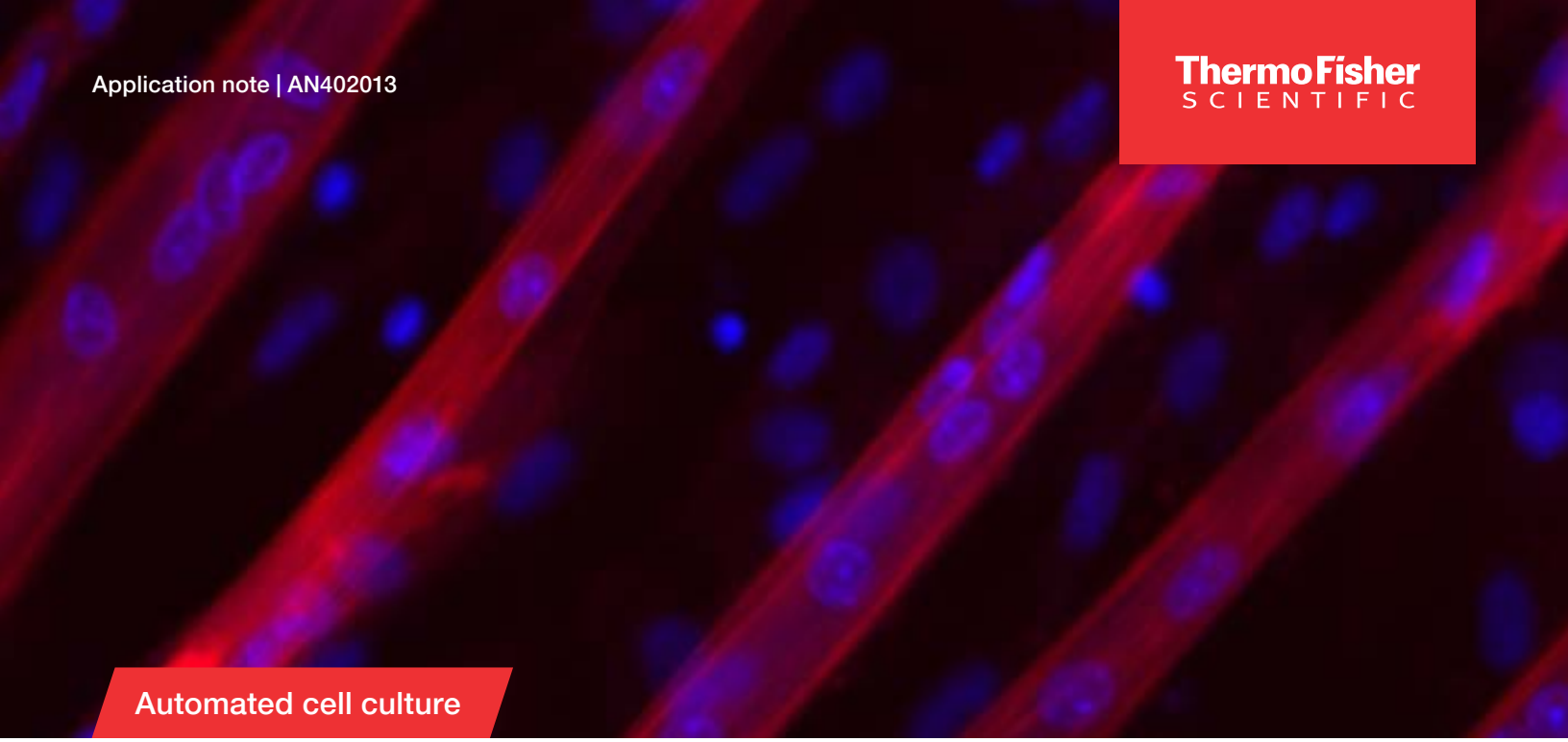
Application note | AN402013



Automated cell culture

Automated cell differentiation of myoblasts into skeletal muscle cells with the Tecan Fluent Automation Workstation

Automated seeding, medium exchange, and incubation of the C2C12 myoblast cell line using a Tecan Fluent Automation Workstation and Cytomat automated incubator

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Keywords
Cell culture, cell differentiation, myoblasts, skeletal muscle cells, C2C12, automated cell seeding, media exchange, cell-based assays, lab automation, Fluent 780 Automation Workstation, microplate handling

Introduction

The murine myoblast cell line C2C12 is a well-documented and representative model of human skeletal muscle cells. It has been used extensively as a tool in pharmaceutical and biomedical research for studying metabolism, disease progression, muscle growth, and drug delivery. Under low serum conditions, C2C12 cells can be differentiated into functional skeletal muscle cells, which form physiologically and functionally relevant multinucleated myotubular structures.

This application note describes the successful automation of cell seeding and differentiation of the myoblast cell line C2C12 into functional muscle tissue using a Tecan® Fluent® 780 Automation Workstation with an integrated Thermo Scientific™ Cytomat™ 2 C450-LiN Automated Incubator to provide cell incubation in a highly stable environment.

Experimental

Automation workstation

Experiments were conducted on a Fluent 780 system, which includes an eight-channel Tecan Air Flexible Channel Arm (Air FCA), a Multiple Channel Arm (MCA) with an extended volume adapter for pipetting up to 500 µL with 96 tips in parallel, and a long Tecan Robotic Gripper Arm (RGA) that can reach below the Tecan Dynamic Deck (Figure 1A). A Cytomat 2 C450-LiN Automated Incubator with a water bath and equipped with two stackers—for 21 and 10 microplates, respectively—was integrated

with the Fluent 780 system, enabling the storage and incubation of cells in microplates throughout the entire muscle cell differentiation period (Figure 1B). The automated incubator's parameters were set at 37 °C and 5% CO_2 to provide an optimal, stable environment for the cells to grow and differentiate into skeletal muscle cells. The Cytomat 2 C450-LiN Automated Incubator also offers the ContraCon™ automated decontamination routine, simplifying cleans and eliminating variability in disinfection without harmful gases.

Figure 1. (A) A Fluent 780 Automation Workstation was integrated with a (B) Cytomat 2 C450-LiN Automated Incubator for the cell differentiation experiment.

To further ensure a clean environment for sterile liquid handling, the Fluent 780 system was equipped with a vertical laminar flow HEPA hood with UV light (Bigneat). Cell seeding was conducted with the Air FCA and MCA, using 1,000 µL and 150 µL filtered disposable tips, respectively. Sterile 50 mL Falcon™ tubes were placed into a tube runner for pipetting into sterile 96-well microplates (Greiner Bio-One™, Part No. 655090) with the Air FCA for the cell seeding step, whereas sterile 300 mL troughs (INTEGRA Biosciences™) were used with the MCA for the medium exchange steps. Individual scripts were developed using the Tecan® FluentControl® software for cell seeding and medium exchange with the Air FCA and MCA, as well as incubation in the Cytomat 2 C450-LiN Automated Incubator.

Cell thawing and expansion

C2C12 cells (ATCC™) were thawed at passage 7 following the manufacturer's protocol. Cells were seeded into T150 flasks at 300,000 cells per flask and expanded in Gibco™ DMEM, high glucose Gibco™ GlutaMAX™ DMEM, high glucose Supplement (Part No. 61965-026) supplied with 20% Gibco™ Fetal Bovine Serum (Part No. 10270-098). The cells were harvested manually with Gibco™ TrypLE™ Express Enzyme (1X), phenol red (Part No. 12605-010) four days later at 70% confluency.

Cell seeding, medium exchange, and plate incubation

C2C12 cells were seeded in each well of six black 96-well microplates (Greiner Bio-One™, Part No. 655090) at 3,000 cells per well in 150 µL volumes using the Air FCA, and then transferred to the humidified automated incubator at 37 °C, 5% CO_2, and stable relative humidity above 90%. Three microplates were placed in each of the two stackers—at the top, middle, and bottom—to assess consistent cell growth and differentiation, and to confirm that environmental conditions were stable regardless of the plate location inside the Cytomat incubator (Figure 2).

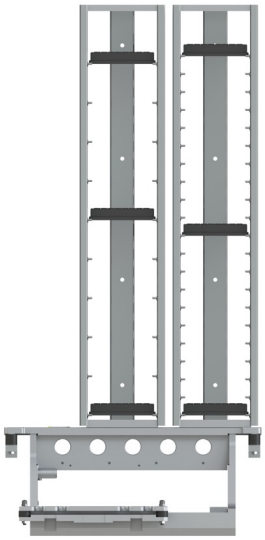

Figure 2. Plate locations within the Cytomat 2 C450-LiN Automated Incubator. Plates 1–3 were located in the 21-position stacker (right) at the bottom, middle, and top, respectively. Plates 4–6 were placed in the 10-position stacker (left) at the bottom, middle, and top, respectively.

All media exchanges were performed using the MCA, together with an extended volume adapter to enable pipetting of 150 µL with all 96 channels, simultaneously.

As shown in Figure 3, cells were grown to reach confluency for four days before the proliferation media was exchanged with 150 µL of DMEM, high glucose, GlutaMAX differentiation media (Part No. 61965-026) per well and supplied with 2% Gibco™ Horse Serum (Part No. 16050-130), thereby initiating the differentiation step. Media exchange was performed again every two to three days, assuring optimal growth conditions during cell differentiation.

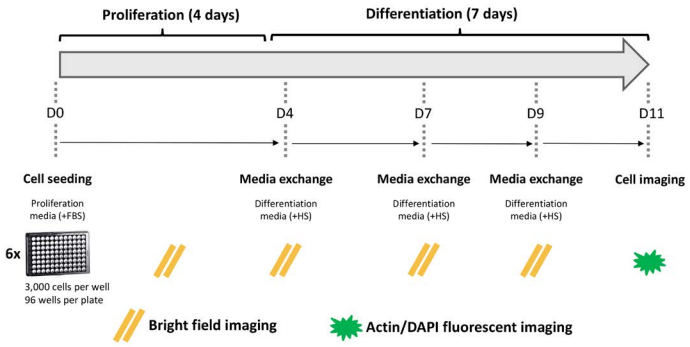

Figure 3. C2C12 cell differentiation protocol.

Immunofluorescent staining and cell imaging

Morphologically, skeletal muscle cell differentiation is accompanied by a fusion of myoblasts into multinucleated myotubes, accompanied by actin filament alignment. On each plate, the corner wells B2, B11, G2, and G11 were stained manually on day 11. Invitrogen™ Alexa Fluor™ 568 Phalloidin Labelling Probes (Part No. A12380) were used for staining actin filaments according to manufacturer's instructions. Cells were washed with phosphate-buffered saline (PBS) and fixed with 10% formalin solution (Sigma-Aldrich) for 10 minutes. The cells were then permeabilized with 0.1% Thermo Scientific™ Triton™ X-100 Surfact-Amps™ Detergent Solution for 10 minutes before 1% bovine serum albumin (BSA) blocking solution was applied for 30 minutes to mask unspecific binding sites. The samples were further incubated for 30 minutes at room temperature in the dark with the Alexa Fluor 568 Phalloidin Labelling Probe and washed three times for five minutes with PBS before imaging. For the nuclear staining, Invitrogen™ DAPI Stain (Part No. D1306) was included at 3 µg/mL in the first washing step. Imaging was performed using an Olympus IX81 automated microscope for bright field (20x) and immunofluorescent (4x and 40x) imaging.

Results and discussion

Fluorescent images taken on the last day of the experiment, together with bright field images taken at multiple times points, clearly show the formation and alignment of myotubular structures, indicating a successful differentiation process (Figures 4 and 5).

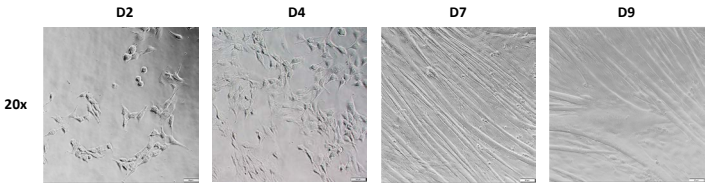

Figure 4. Bright field images of cultured C2C12 cells. Taken on days 2, 4, 7, and 9 post-cell seeding using a 20x magnification. Differentiation was initiated four days after cell seeding.

Spontaneous contractions of muscle fibers could be observed as early as five days after initiating the differentiation process (data not shown), demonstrating physiologically and functionally relevant composite structures. Densely populated wells with typically aggregated clusters of C2C12 muscle cells, partially overgrowing each other, became evident when looking at stitched 4x overview fluorescent images (Figure 5).

Robust muscle fiber buildup and alignment could be confirmed using 40x magnification. Confluent layers of muscle fibers present in all wells analyzed, both within and between the six plates, suggest homogenous and robust environmental conditions irrespective of plate location within the incubator.

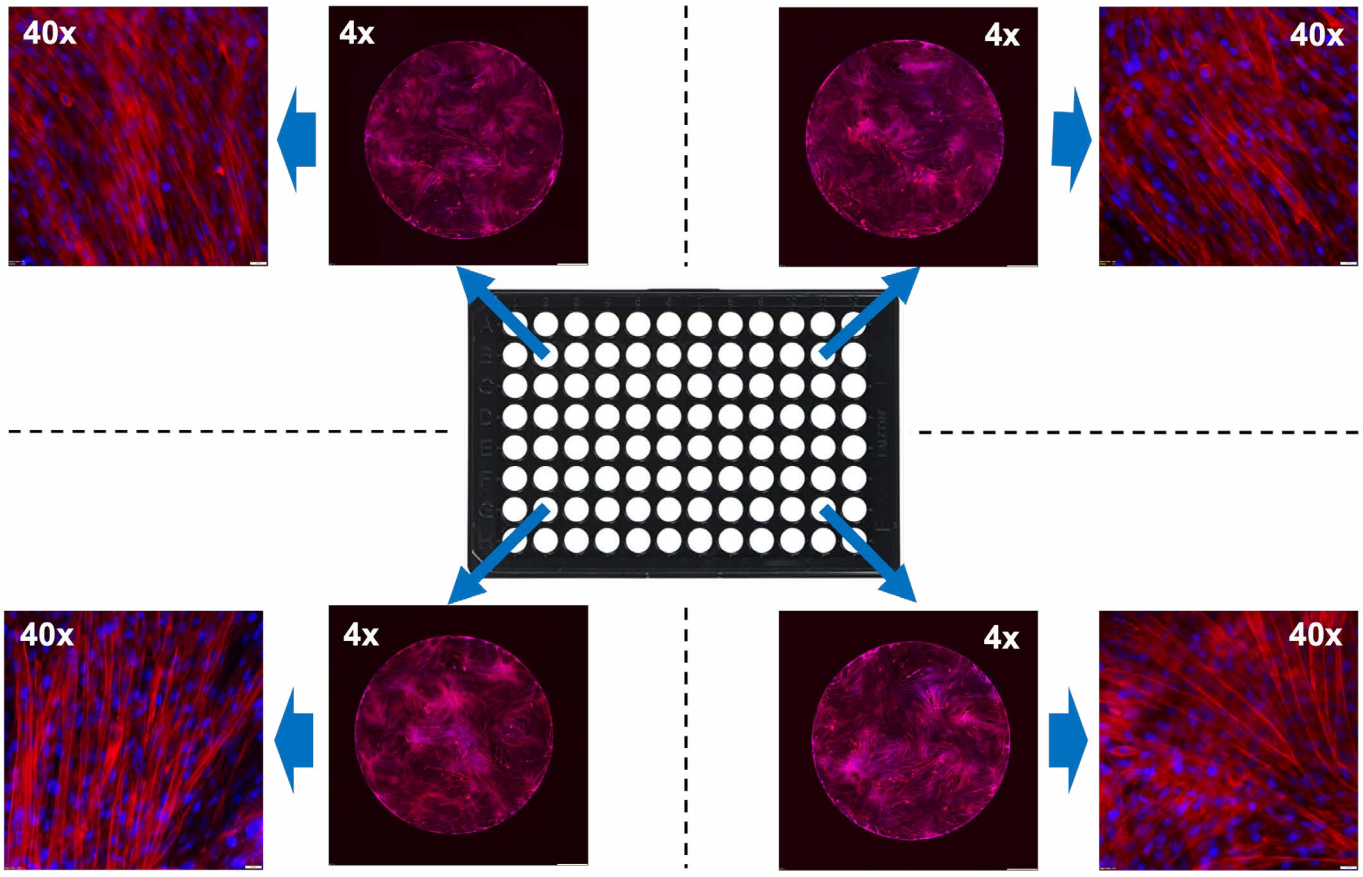

Figure 5. Immunofluorescent staining of actin (red) and DAPI (blue) on the seventh day of differentiation (D11) for four corner wells B2, B11, G2, and G11 across plate 2.

The environmental conditions—temperature and CO_2 concentration—inside the Cytomat incubator were monitored with 10-minute gaps between data points, demonstrating highly stable incubation conditions with maximal temperature fluctuations of 0.5 °C and CO_2 concentrations between 0.2% throughout the whole experimental period (Figure 6A). The opening of the main door (Figure 6B) for 10 seconds resulted in a drop of 1 °C and 1.1% CO_2, but preset values were restored within a few minutes after the intervention. The microplate transfer through the small side gate (Figure 6C) is a fast process, with a mean access time of only 12 seconds. The opening of the side gate for the microplate transfers did not significantly influence the environmental conditions, which remained stable even after multiple plate handling (open–close) cycles.

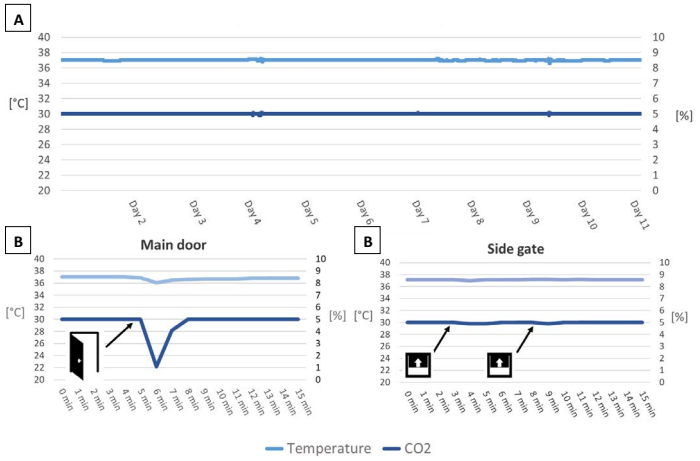

Figure 6. Temperature and CO_2 concentration changes over the (A) whole experiment and before and after opening the (B) main door or (C) side gate.

Conclusions

This application note shows that the Fluent 780 Automation Workstation is well suited to automating cell seeding, media exchange, microplate handling, and storage processes. The Cytomat 2 C450-LiN Automated Incubator provides highly stable and safe environmental conditions during the cell growth and differentiation process irrespective of the plate location, as evidenced by the robust differentiation process observed in all microplates and wells. The combination of consistent liquid and microplate handling with solid storage possibilities contributes to reducing human interventions and increasing productivity and reproducibility. No contamination issues were observed during the experiment, demonstrating that the system ensures a clean and sterile environment for cell culture maintenance applications.

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