Differentiation-Protocol for the C2C12 cell line

Material:

- 6 well plate
- Differentiation medium
- Trypsin/EDTA
- DPBS
- FBS

Differentiation medium:

DMEM (high glutamine and glucose) + 2% HS

- **DMEM :** Gibco # 11995-065
- **Horse Serum:** Gibco 17005-042

6 well plate: Thermo Scientific Nunc 140675

Trypsin/EDTA: Gibco 25300-054

D-PBS: Gibco 14190-144

FBS: Sigma 20K284

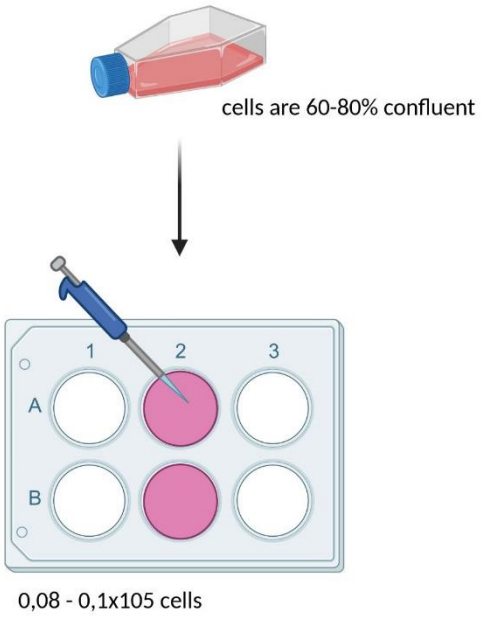
Differentiation treatment

1. culture the cell line in DMEM (high glutamine and glucose) with 10% h.i. FBS

   https://www.dsmz.de/collection/catalogue/details/culture/ACC-565

2. When cells are 50-60% confluent (meaning that very few of them are physically touching each other), split 1:10 – 1:20 with Trypsin/EDTA.

It is important to not let the cells become fully confluent because they can begin to fuse and partially differentiate upon cell-cell contact.



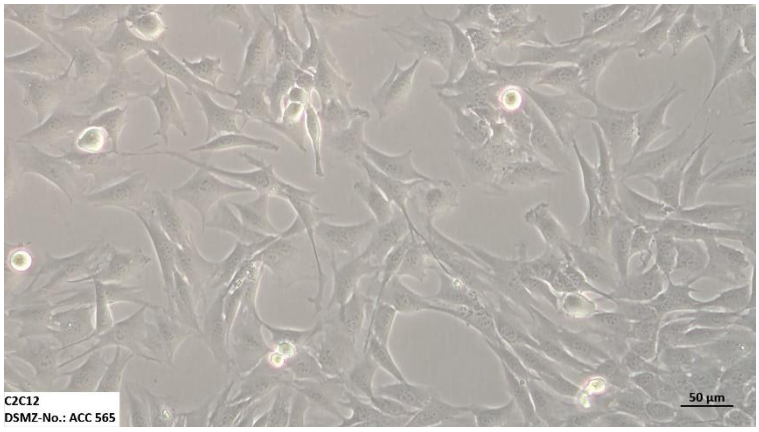
3. For differentiation, switch to differentiation medium when cells reach 60-80% confluency.

4. Seed out 0.08 – 0.1x10^5 cells in each well of a 6 well plate. The cells are ready in 3-4 days to start the differentiation.

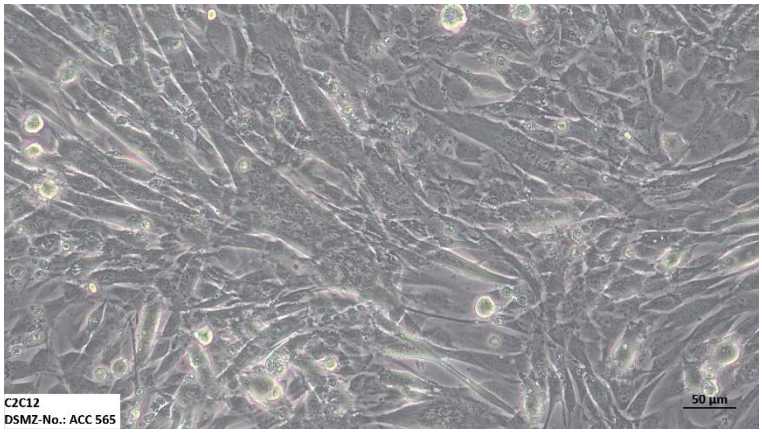
5. Differentiate for 1 to N (2-10 or more) days by washing cells once with D-PBS and adding fresh differentiation. Full Medium change every 2 days.

The change in morphology is expected to occur from day 2.

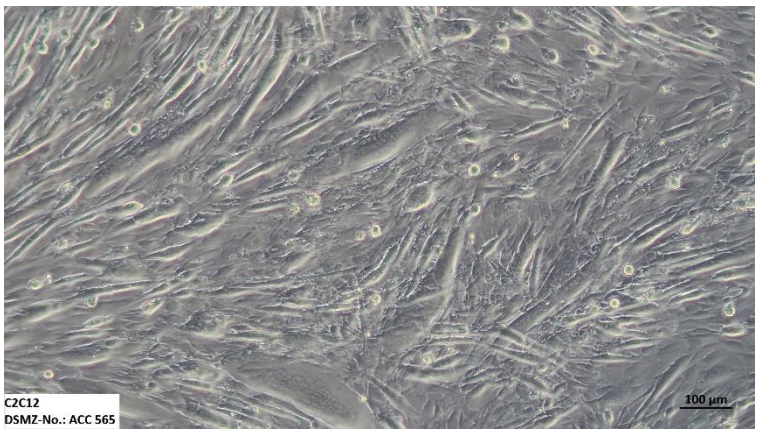
Around day 6 myotubes become clearly visible.



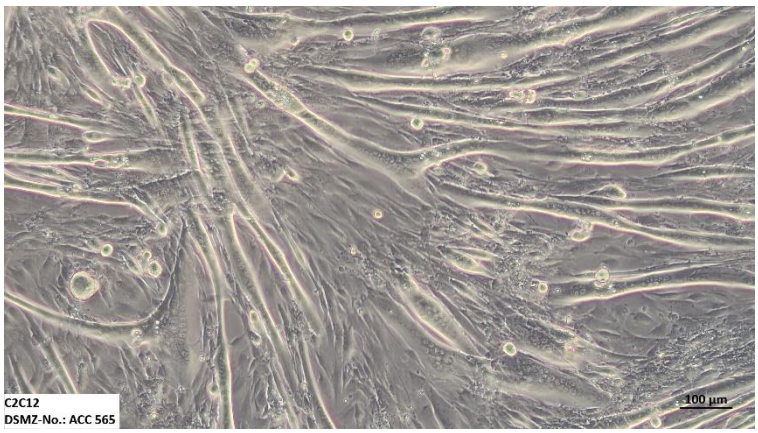
Day 0



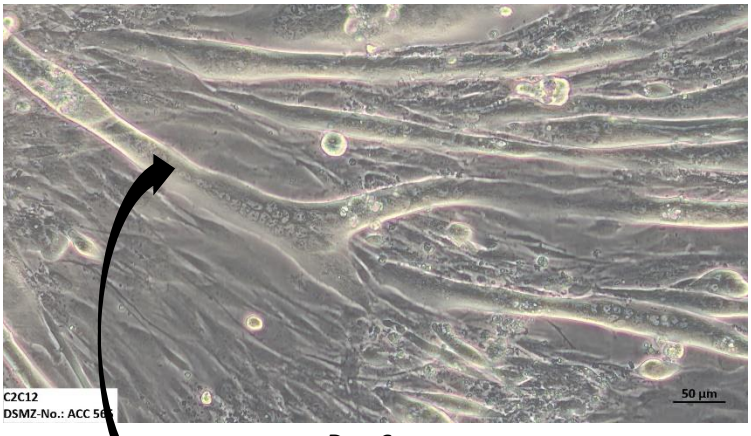
Day 2



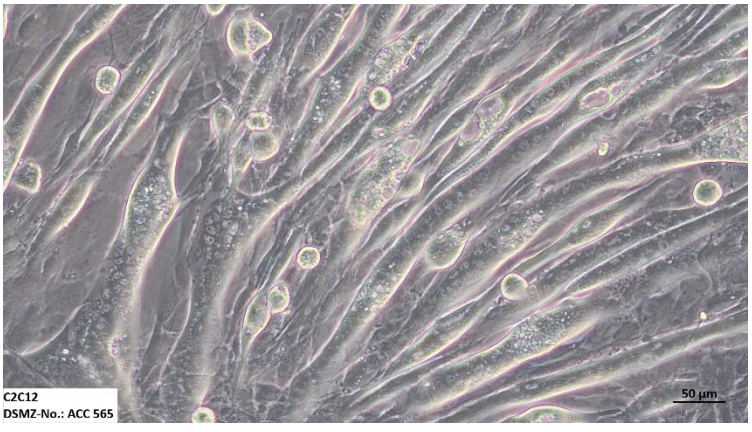
Day 3



Day 6



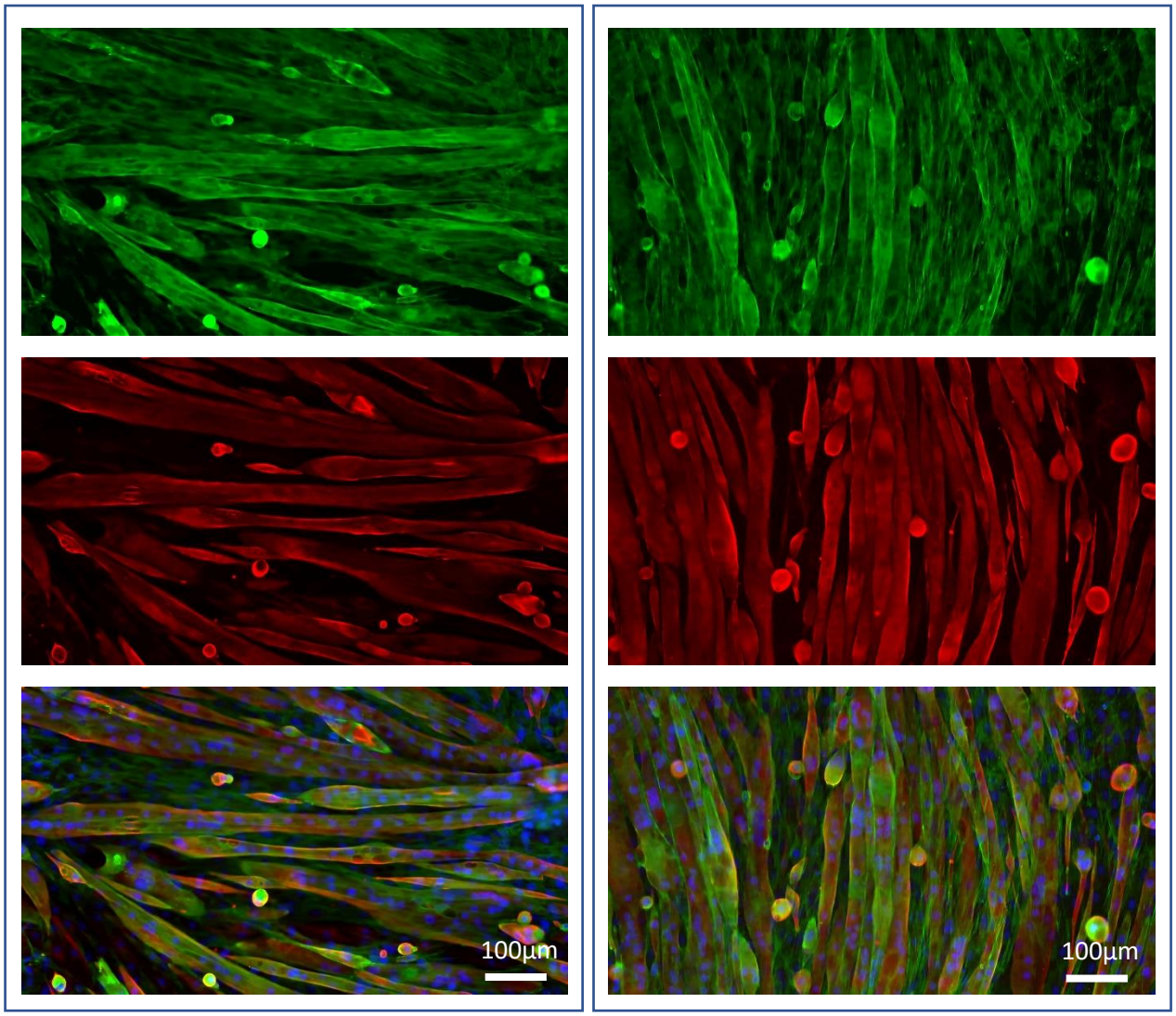
Day 6



Day 9

Myotubes have several nuclei.

IF_staining



Staining with Desmin (DAKO M0760, 1:200, red), Phalloidin Alexa Fluor 488 (Cell Signaling 8878S, 1:200, green). Nuclei were stained with DAPI (blue).