

Differentiation-Protocol for the C2C12 cell line

Material:

- C2C12 cell line (DSMZ ACC 565)
- 6 well plate (Nunc #140675)
- DMEM high glucose (Gibco # 11995-065)
- L-glutamine (Gibco #25030081)
- Trypsin/EDTA (Gibco #25300-054)
- D-PBS without Mg^{2+}/Ca^{2+} (Gibco #14190-144)
- Fetal Bovine Serum (Sigma #F2442)
- Horse serum (Gibco #26050-088)

Maintenance medium

DMEM high glucose, 10% FBS, 2mM L-glutamine

Differentiation medium

DMEM high glucose, 2% horse serum, 2mM L-glutamine

Maintenance culture

- Culture the cell line in Maintenance medium
<https://www.dsmz.de/collection/catalogue/details/culture/ACC-565>
- Starting density: $1-2 \times 10^5$ in a T75 flask
- When cells reach 50-60% confluency (meaning that very few of them are physically touching each other), split 1:10 – 1:20 with Trypsin/EDTA.

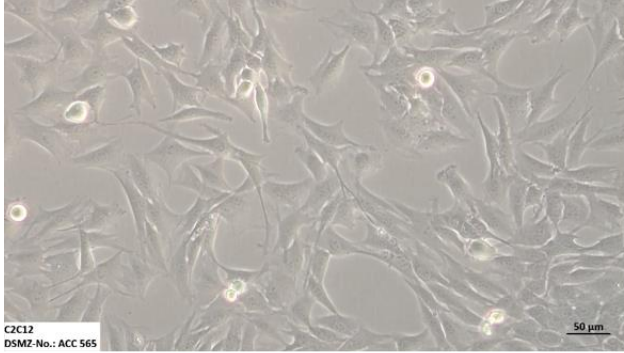
=> It is important to not let the cells become fully confluent because they can begin to fuse and partially differentiate upon cell-cell contact.

Differentiation protocol

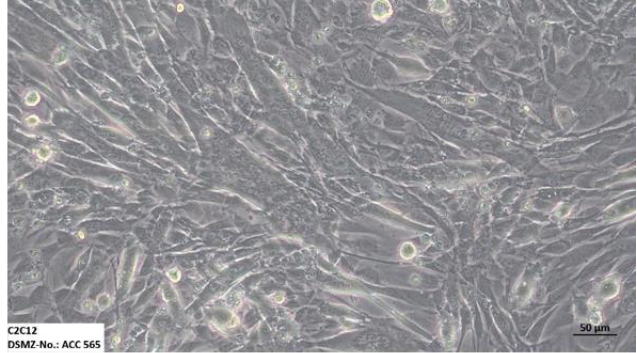
- Seed out $0.08 - 0.1 \times 10^5$ cells per well of a 6 well plate
- Cultivate in Maintenance medium until cells reach 60-80% confluency
- Change medium to Differentiation medium (= d0)
- Cultivate for the intended period of time (10 days or more) in Differentiation medium
- Every second day: wash cells once with D-PBS and perform full medium change with Differentiation medium

- The change in morphology is expected to occur from day 2.
- Around day 6 myotubes become clearly visible.

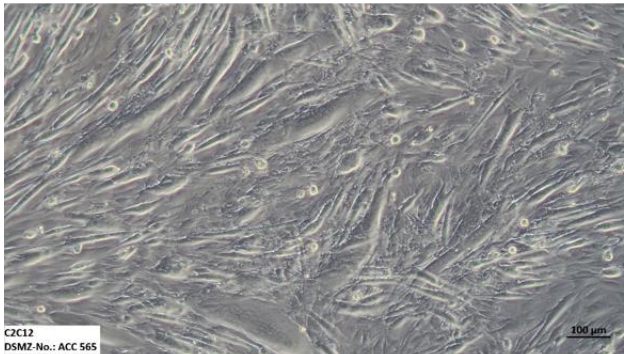
Phase contrast images during differentiation



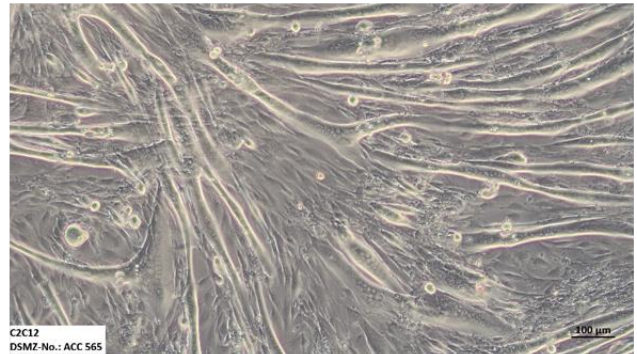
day 0 (60-80% confluent)



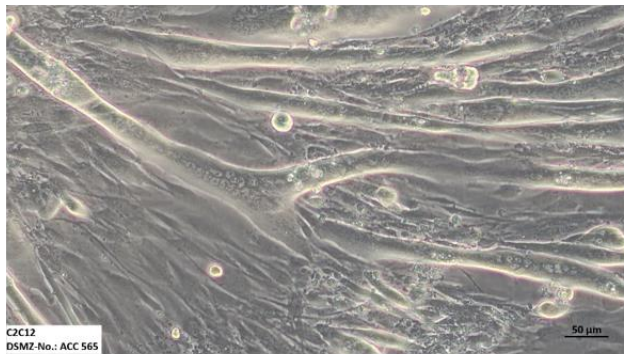
day 2



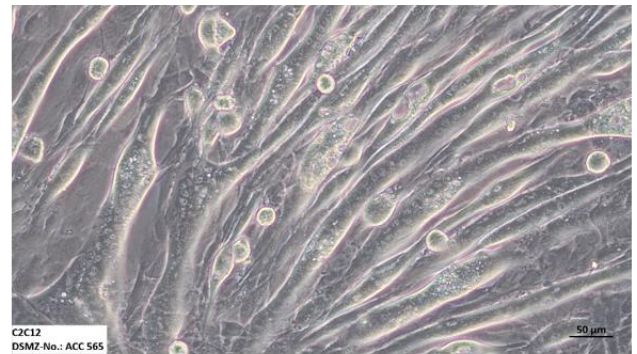
day 3



day 6

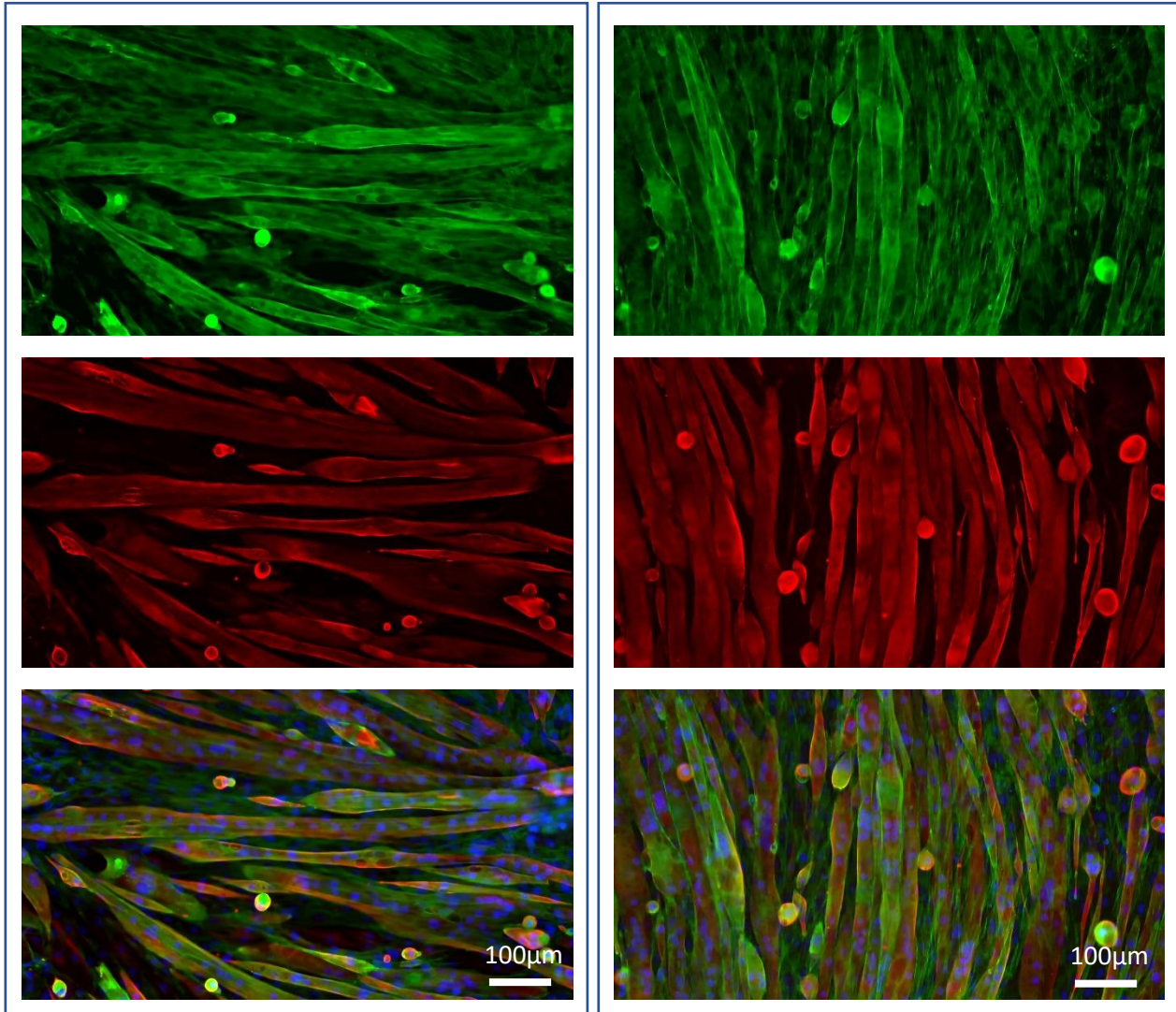


day 6 (higher magnification)



day 9

Immunostaining at day 9



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