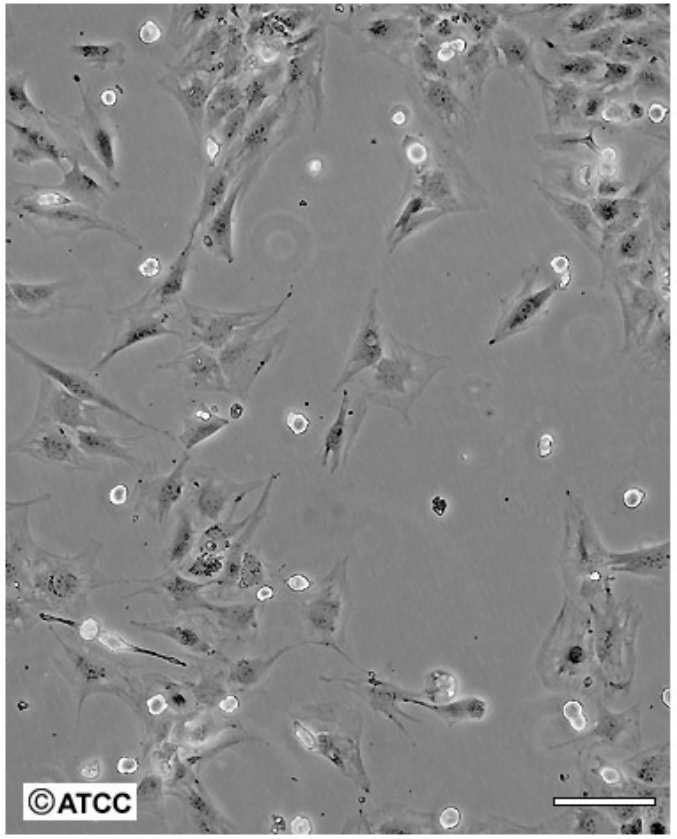
C2C12 Myoblast to Osteoblast

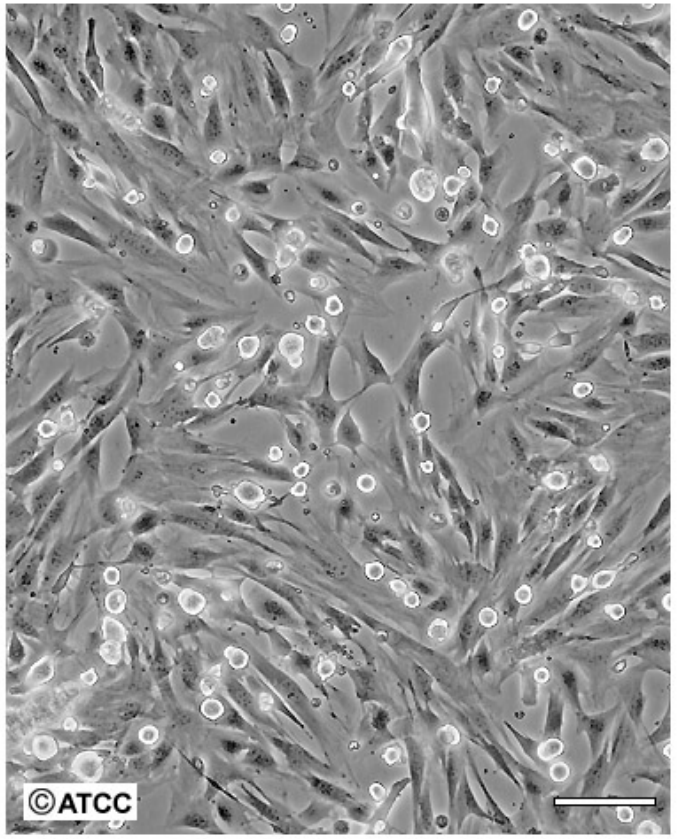
Maintain at 50 – 60% Confluency by Subculturing Frequently

The cells will remain multipotent at this density but will soon need to be subcultivated.

ATCC Number: **CRL-1772**
Designation: **C2C12**



Low Density — Scale Bar = 100µm



High Density — Scale Bar = 100µm

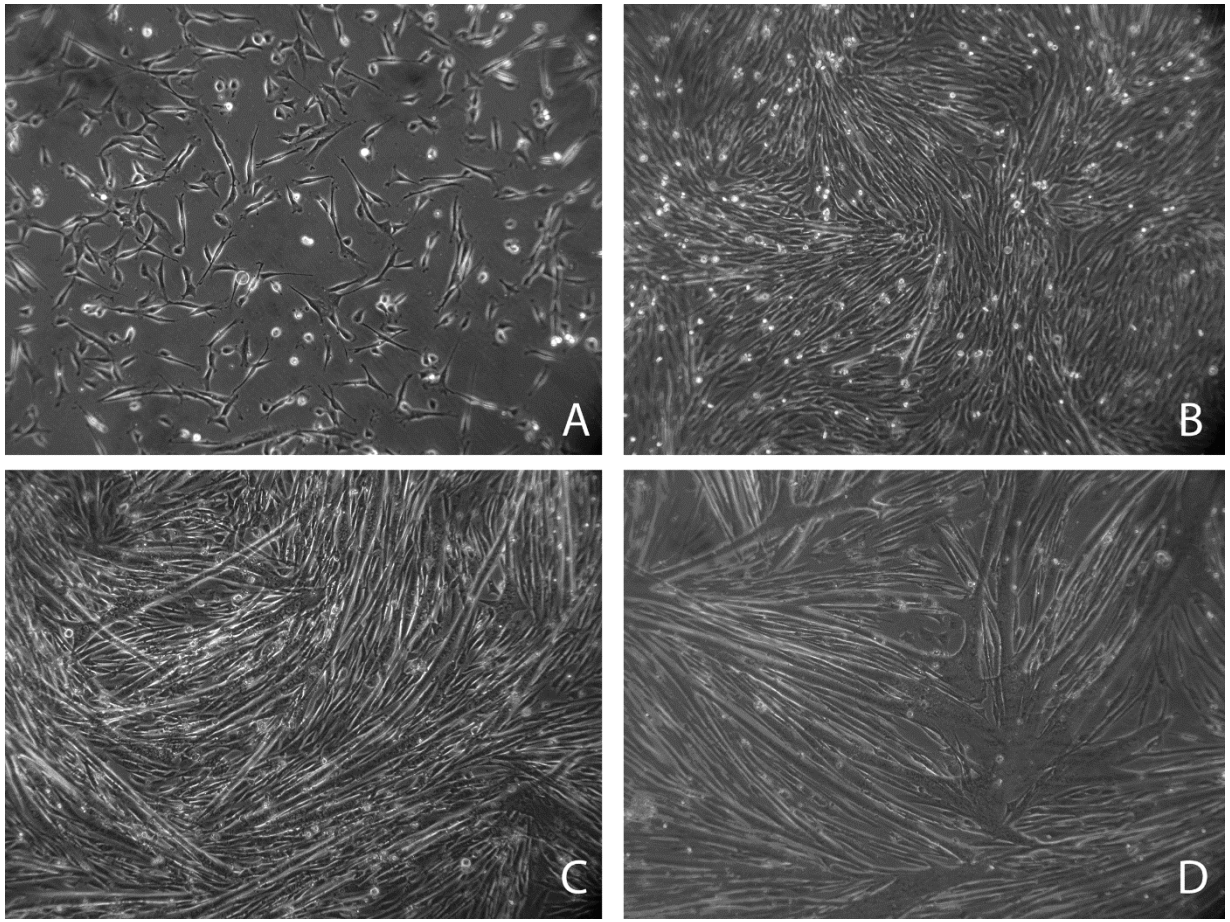
These cells have started to fuse into myotubes and will soon become the dominant feature of this culture.

If too dense will spontaneously form myotubes, a differentiated stage.

Panel A: Subcultivate to maintain multipotency

Panel B: Cells have lined up and fusion into myotubes has begun.

Pane C & D: Fusion progressing as time and density increase, leading to pronounced myotubes.



Direct differentiation into osteoblast using bone morphogenic protein 2 (BMP2).

An overview of the provided SOP

- Set up 2 sets of 3-wells each of ~5 X 10^3 C2C12 cells per well in 24-well plate
  - Growth media is DMEM-HG with 10% FBS
- 1 well of each set will be an untreated control which will be allowed to grow and develop into myotubes
- Treat 1 well of each set of 3-wells with 300 ng / mL BMP2
  - Follow the SOP for BMP2 treatment protocol

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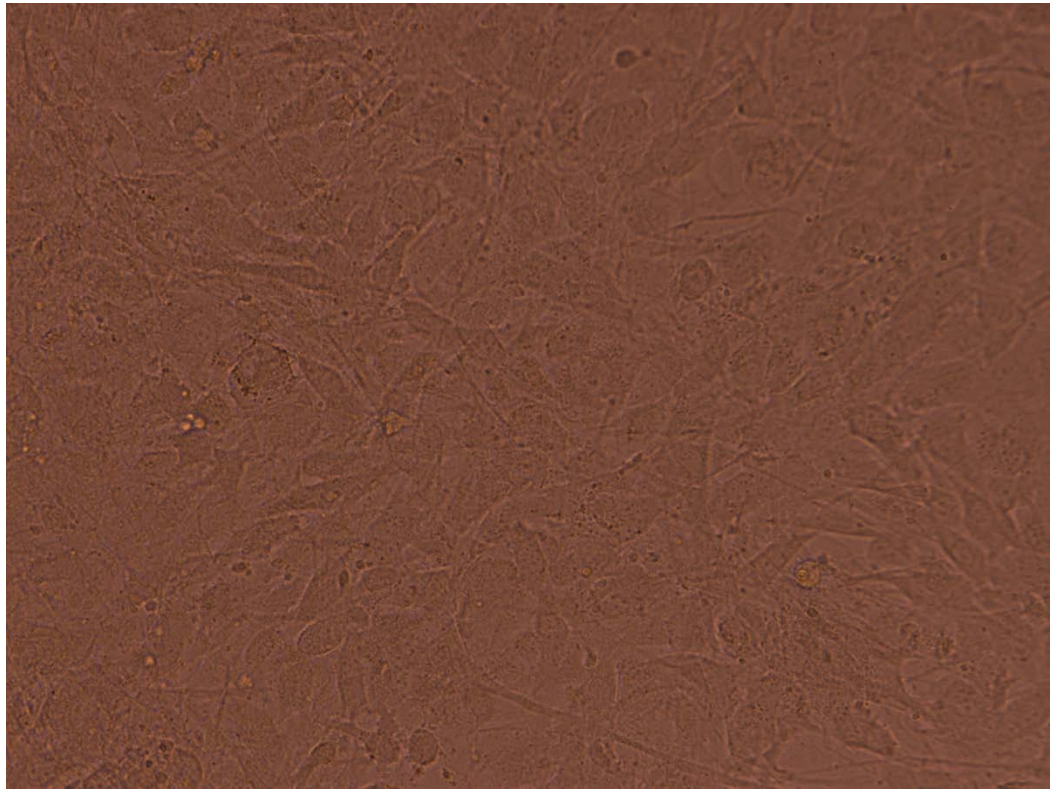
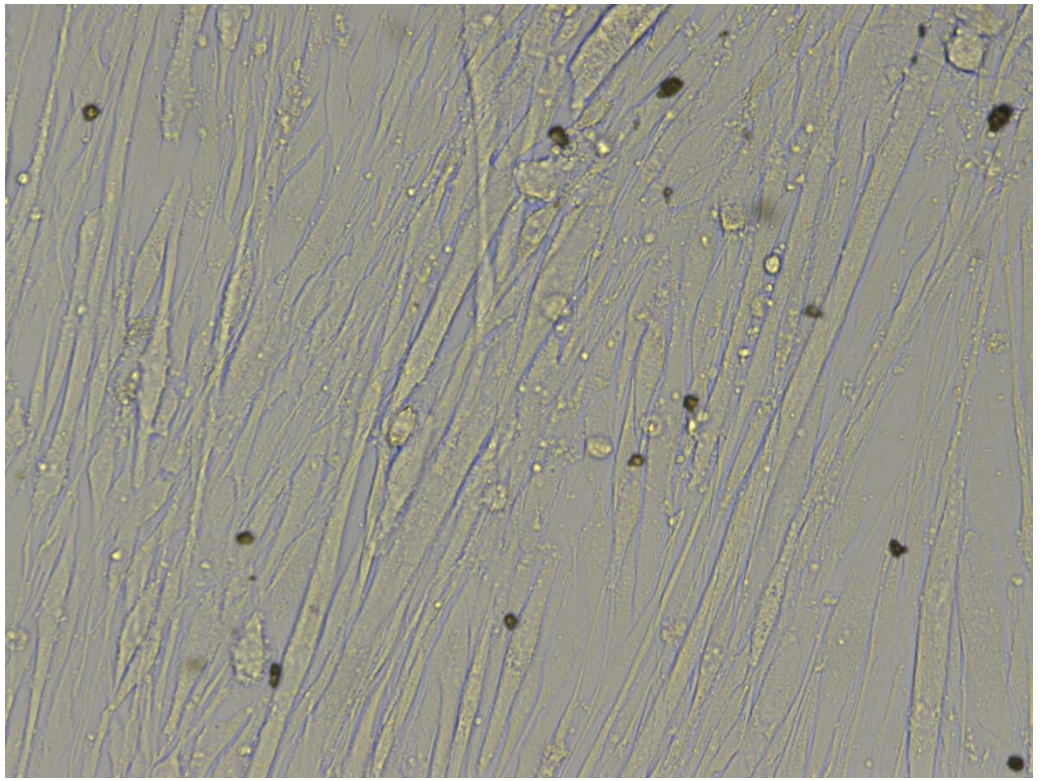
- On day 3 or 4, change the media in the 3rd well to DMEM-HG + 2% Horse Serum
  - Optionally, you can use 0.25% FBS
  - The change in serum type or concentration will reduce the amount of growth signals while still supporting good cell proliferation
- Analyze wells on day 6 or 7 (from BMP2 treatment) for myotube formation and differentiation into osteoblasts

Analysis of differentiation on day 6

- Compare morphology of treated to untreated controls



BMP-2 treated, Day 6



DMEM-HG+ 10% FBS, Day 6

Direct differentiation into osteoblast using bone morphogenic protein 2 (BMP2).

- The next step in analysis is a determination of the relative activity of alkaline phosphatase (AP)in the BMP-2 treated and untreated wells
  - AP activity is significantly higher in osteoblasts than in myoblasts and this simple, quick test will show the difference by the formation of a blue-to-black precipitate in the well

Functional assay to test for Alkaline Phosphatase Activity

- High AP activity in osteoblast, low to none in myoblast / myotubes
- Compound used is BCIP/NBT (follow protocol)