Introduction
Muscle is the largest metabolic organ and the main storage site of proteins in the body. Moreover, animal muscle tissue is the main source of dietary protein intake in a large part of the world. Both properties make muscle an organ of interest for tissue engineers. We aim to produce high quality muscle tissue from muscle progenitor cells in a cost efficient, reproducible way.
Currently, our main concern is isolating a cell population from adult muscle that has a high proliferation capacity while preserving the ability to differentiate into muscle.

Methods for cell isolation
Preplating technique
Pig muscle tissue is minced, treated with 0.2% collagenase type I, 4 ug/ml prot. K and 0.1% trypsin consecutively, and cultured in gelatin-coated flasks in a series of preplates.

Single fibre isolation
Mouse Extensor Digitorum longus (EDL) is treated with 0.2% collagenase type I, triturated and cultured in 1mg/ml matrigel-coated wells.

Results
Muscle derived stem cells
The cells isolated with the preplating technique (figure 1) have a high proliferation capacity, showing no sign of slowing down after twelve passages. However, the population of cells present in preplate six is still a very heterogeneous population and has not yet been successfully differentiated to myotubes under low serum conditions.

Satellite cells
When single muscle fibres are isolated and cultured, satellite cell migrate out of the fibres and start proliferating (figure 2). The proliferation capacity of these cells has been shown to be quite poor in vitro compared to the in vivo situation, but they do spontaneously differentiate and fuse into myotubes.

Conclusion
MDSCs have a high proliferation capacity, but do not easily differentiate to myotubes. In contrast, isolated satellite cells spontaneously differentiate to myotubes, but have a low proliferation capacity. However, in vivo, the number of population doublings does not seem to be limited.

Future plans
We will focus on satellite cells isolated from single fibres, because their differentiation potential is much better compared to the MDSCs. We want to improve the proliferation capacity of these cells by mimicking their in vivo milieu making use of different coatings and additives to the culture medium.

References: