Introduction
Currently, tissue engineering of skeletal muscle constructs fails to result in mature, functional muscle tissue. Previous research indicated that both biochemical and biophysical stimuli are important in achieving maturation of skeletal muscle cells. Since neuronal activity is crucial to myogenesis in vivo, we investigated the in vitro application of electrical stimulation (ES) on cultured muscle cells. Muscle differentiation and maturation were investigated at functional, morphological, and transcriptional levels.

Materials and methods
C2C12 skeletal myoblasts were induced to differentiate by serum deprivation and electrically stimulated according to different protocols (figure 1). After ES, cultures were examined for functional parameters (contractions), morphological characteristics of muscle maturation (immunocytochemistry: sarcomeric α-actinin, sarcomeric myosin, nuclei) and gene expression of differentiation and maturation markers (qPCR: myogenic regulatory factors and sarcomere proteins).

Results
Electrical stimulation protocols resulted in contracting myotubes (figure 2). Functionality of the cultured cells increased in time with ES.

Electrically stimulated cells developed cross striations after 48h of ES, that was started at day 1 (figure 3). Non-stimulated control cultures did not show these striations (data not shown).

Conclusions
Electrical stimulation accelerated the maturation process of skeletal muscle cells, based on development of cross striations and upregulated expression levels of genes related to late maturation. More specifically, a small window in time exists in which the effects of ES are beneficial to muscle maturation in vitro.

References