Testing of METAFECTENE transfection reagent on C2C12 cells

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Protocol

C2C12 murine myoblasts were transfected with METAFECTENE and Lipid L in 6 well plates starting with 50000 cells /well resulting in 50 %confluency after one night incubation at 37ºC , 5%CO2.

2µg plasmid DNA (pCS2GFP*) encoding Green Fluorescent Protein gene under CMV promoter diluted in 100 µL OPTIMEM (serum-free medium from GIBCO) were mixed with METAFECTENE at different concentrations diluted in 100 µL OPTIMEM :

Tube	METAFECTENE used (µl /well)	DNA (pCS2GFP*)
1	2	2µg in 100µl OPTIMEM
2	4	
3	8	
4	16	
5	32	

The solutions were vortexed for 10 secs and incubated at RT for 20 mins to allow the complex formation, then diluted up to 1mL in OPTIMEM and spread over C2C12 murine myoblasts. 1 mL Dubelcoo's Modified Eagle Medium (DMEM) containing 10% Fetal Calf Serum (FCS) was added after 6 hours incubation at 37 °C, 5% CO_2. After 24 hours, cells were incubated another 24 hours with fresh DMEM+10% FCS to allow for GFP expression. A negative control (DNA only), and a positive control using liposome-based transfection reagent Lipid L were tested at the same time.

Discussion

We have shown that expression of GFP occurs following cell transfection using 2µg of plasmid and different amount of METAFECTENE . We have established that for these musce cells the optimum concentration is 8µl/well. This was less toxic to the cells and more efficient in terms of number of cells expressing GFP. More interestingly, when using Lipid L as a control on the same cells, we found less GFP expression.
The next step would consist of testing METAFECTENE *in-vitro* for expression of GFP in differenciated myotubes. Furthermore, we plan testing the expression of GFP *in-vivo* in mouse muscles.

Results

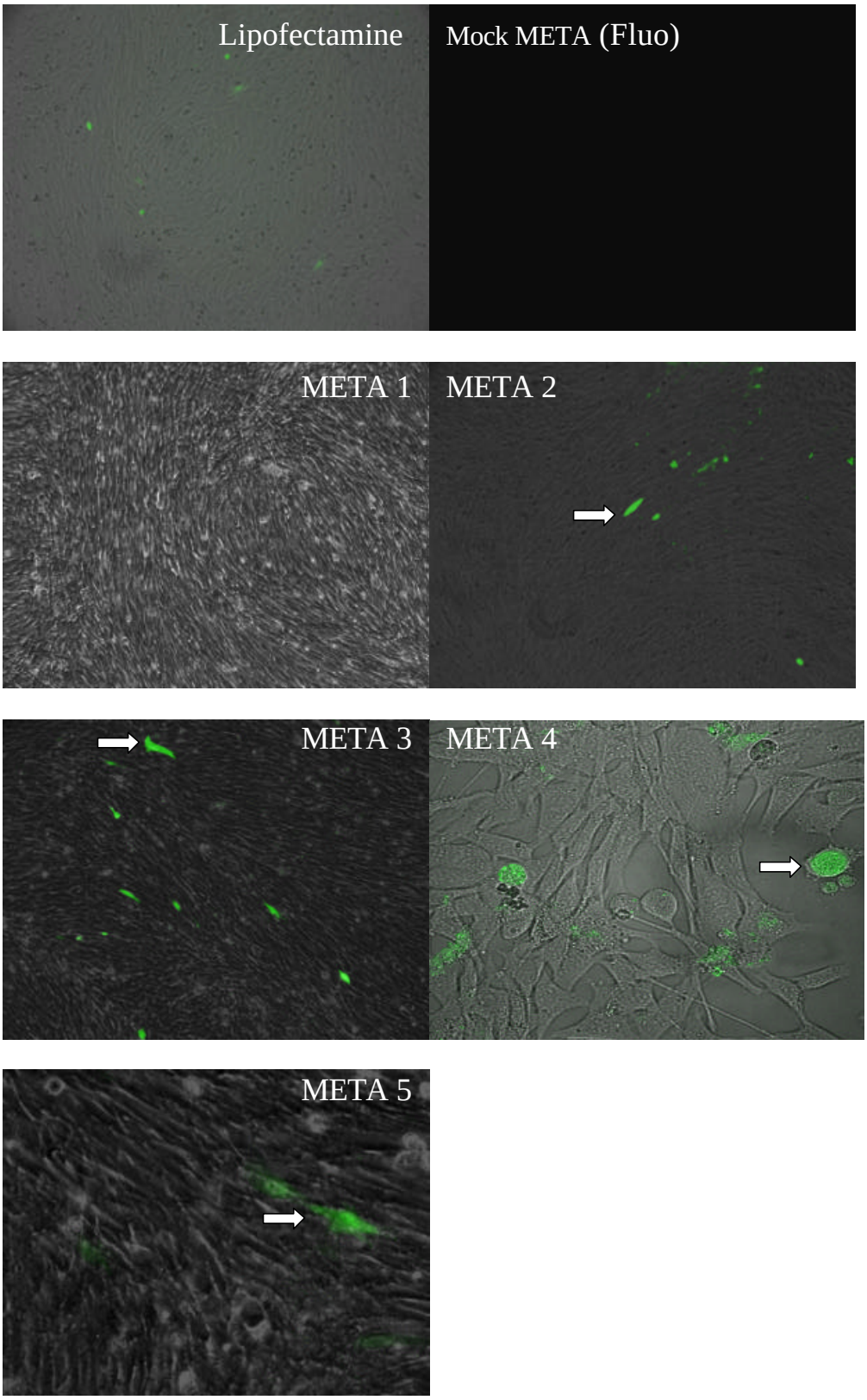

Figure 1: Confocal micrographs of C2C12 cells transfected with different concentrations of METAFECTENE and pCS2GFP* diluted in serum-free medium