Application Note:

Content:

Title of Experiment:

Enhancement of cell transfection using pluronic response modifiers

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Introduction:

One of the major problems with gene therapy is the low efficacy of gene targeting and therefore low levels of expression. One of the most wide-spread non-viral transfection systems used in some gene therapy applications are the lipid-based macromolecular complexes. Although alone their gene transfer efficiency is lower than viral delivery systems they do have the advantages of being less immunogenic and toxic, are produced easily on a large scale and can transfect a reasonably high amount of DNA. Pluronic block copolymers are gaining increasing interest in the field of gene therapy owing to their potential use in enhancing transgene expression delivered *via* nonviral vectors. In this study we have analysed the enhancing potential of specific pluronic formulations.

Materials and methods:

Cos-7 cells; pDNA (pCS2-GFP expression casette); Lipofectamine 2000 or Metafectene PRO (Biontex Laboratories GmbH).

Experimental procedures / transfection protocol:

Cos-7 cells were plated in 6-well plates at 2.5 x 10^5 cells per well in 2ml DMEM and grown at 37^{o}C, 5% CO_2 for 24 hours. 4µg of pDNA (pCS2-GFP expression casette) was first diluted in 250µl Optimem as was 10µl Lipofectamine 2000 or 12µl Metafectene PRO (Biontex Laboratories GmbH) for five minutes at room temperature before being complexed together and incubated at room temperature for 20-30 minutes. DMEM was removed from cells, washed x2 with Optimem before being overlayed with 1.5 ml of medium. The pDNA complexes were added to the cells and the plates were rocked gently to ensure even distribution. After incubating for 4-6 hours at 37^{o}C, 5% CO_2 the transfection complex was removed and replaced with 2ml DMEM (allowing the cells to restart their growth) and incubated for 24 hours at 37^{o}C, 5% CO_2. DMEM was removed and cells were washed x2 with Optimem before being overlayed with 2ml optimem containing various concentrations of Pluronics. The cells were incubated at 37^{o}C, 5% CO_2 for 3 hours, Pluronic solution removed and replaced with 2ml DMEM and incubated for a further 24 hours. Transcriptional efficiency was studied *via* GFP expression (number of expressing cells and intensity

of expression) analysed using either flow cytometry or by confocal microscopy. All results were statistically analysed via Bonferroni's Multiple Comparison Test and Tukey's Multiple Comparison Test (see appendix 2 for full statistical analysis).

Results and discussion:

Conclusion / summary:

As the main aim of this study was to assess the enhancing effects of Pluronics, no specific comparisons between Lipofectamine 2000 and Metafectene PRO were made here. However, Metafectene PRO gave at least as good tranfection efficacy as Lipofectamine and it was used exclusively in the latter part of this experiment.

References:

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Appendix: Tables and/or figures:

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