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331 OPTIMIZATION OF BRANCHED 25 kDa POLYETHYLENIMINE FOR EFFICIENT GENE DELIVERY IN BOVINE FETAL FIBROBLASTS

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Abstract

Cost-effective, highly efficient, and noncytotoxic transfection of bovine fetal fibroblasts (BFF) has proven difficult to achieve by regular chemical and physical methods. The aims of this study were to evaluate transient transfection efficiency and toxicity of commercially available branched 25 kDa polyethylenimine (25 kDa PEI, Sigma-Aldrich, St. Louis, MO, USA) and to optimize the transfection conditions leading to high percentages of PEI-transfected fibroblasts with minimum cytotoxic effects. Bovine fetal fibroblast (BFF) cells were seeded a day before transfection in 24-well plates at a density of 3×10^4 cells per well in DMEM with antibiotics and 10% SFB. When 70 to 90% confluence was reached, cells were washed with PBS and incubated in DMEM without antibiotics or SFB. For the transfection-mix preparation, increasing amounts of plasmidic DNA (pZsGreen1; 2 to 6 µg) were added to 50 µL of DMEM without antibiotics or SFB, incubated for 5 min at room temperature, and complexed with 0.5 to 4 µg of PEI (from 1 mg mL^{-1} solution) in 50 µL of PBS for 10 min. This transfection mix was added to the cell cultures and, 2 h later, 500 µL of DMEM with antibiotics and 10% SFB was added to each well. Detection of green fluorescent protein (GFP) expression by flow cytometry (reported as percentage of green fluorescent cells) was performed 48 h after transfection. Results were analysed by ANOVA and Tukey test and expressed as mean ± SEM ($P < 0.05$). We found no significant difference between the percentage of GFP-positive cells transfected with 1 or 2 µg of 25 kDa PEI at 2 µg of DNA/well (15.2 ± 1.3 *v.* 16.9 ± 0.9%, respectively; $P > 0.05$), whereas cells transfected with 1 or 2 µg of low-molecular-weight PEI (2 kDa) showed extremely low transfection efficiencies. Increasing the DNA load up to 6 µg significantly enhanced cell transfection (3.5- and 6-fold comparing 2 µg *v.* 4 µg and 6 µg of DNA, respectively; $P < 0.05$) at 1 and 2 µg of 25 kDa PEI/well. In order to evaluate the cytotoxic effect of PEI, cell viability was determined using the MTT assay in 96-well plates (cells/well), with each condition scaled down to replicate the effect of 2 kDa or 25 kDa PEI in a 24-well plate. The MTT results (expressed as % of the control) indicated that PEI became cytotoxic at concentrations equivalent to 2 and 4 µg/well (54.7 ± 3.4 and 18.5 ± 5.7, respectively), whereas 1 µg/well produced a slight detrimental effect on cell viability (90.0 ± 2.6). No evidence of cytotoxicity was observed when the BFF were incubated with 0.5 µg/well of 25 kDa PEI and 1 or 2 µg/well of 2 kDa PEI. To study if a combination of low- and high-molecular-weight PEI could improve transfection efficiency and reduce toxicity, we tested a mixture (1 : 1) of 2 kDa and 25 kDa PEI. Even though the 1 : 1 mixture was less cytotoxic, the efficiency of gene delivery was not improved. We conclude that, under our experimental conditions, the highest percentage of GFP-expressing cells with good viability was obtained when 1 µg of 25 kDa PEI was added per well. Therefore, branched 25 kDa PEI transfection represents an efficient, simple, and cost-effective alternative for gene delivery in bovine fibroblast cells in culture.