BTX Applications

Tech-Trends
Volume 3, Series 12

Optimization of electroporation-mediated transfection in DFCs

Improvement of electroporation to deliver plasmid DNA into dental follicle cells

Introduction

Electroporation-mediated gene delivery is a safe and reproducible physical transfection method widely used in research applications. Because each cell is unique, electroporation requires empirical optimization for each particular cell type or tissue to be transfected. In the recent publication "Improvement of electroporation to deliver plasmid into dental follicle cells," Yao, S. et al. demonstrated the effect of optimizing crucial electroporation variables on transfection efficiencies and viabilities in dental follicle cells (DFCs) using the ECM 830 Square Wave Electroporation system. In this research, several parameters were optimized including the field strength, the pulse duration, plasmid concentration, and the addition of serum into the electroporation medium.

Methods

To study the electric field strength effect, DFCs were electroporated at 50, 100, 150 and 200 V (equivalent to 125, 250, 375 and 500 V/cm respectively). To determine the optimal concentration of plasmid for transfection, cells were electroporated at 375 V/cm with buffer containing 0 (control), 0.02, 0.06, 0.1, 0.14, 0.18, 0.22, 0.26 and 0.3 µg/µl of plasmid. To determine the effect of FBS and BSA in transfection, heat inactivated FBS was added to the buffer at final concentrations of 0, 5, 10 or 20% and with 0.18 µg/µl plasmid. For BSA, the serum was added into the buffer at final concentrations of 0, 4, 6, 8 and 10 mg/ml. Electroporation was conducted at pulse settings of 375 V/cm.

To study the pulsing duration effect, cells were electroporated at 375 V/cm for durations of 0 (control), 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 ms in buffer containing 0.2 µg/µl plasmid and 6 mg/ml BSA.

Results

DFCs were subjected to Alamar blue test for viability assessment and X-gal staining to evaluate the transfection efficiencies. The optimal field strength was determined to be most effective at 375 V/cm, and with plasmid concentrations greater than 0.18 µg/µl. Furthermore, BSA or FBS in the pulsing buffer significantly improved cell survival and increased the number of transfected cells. Finally, the optimal pulsing duration was in the range of 45 to 120 milliseconds (ms) at 375 V/cm. Thus, an improved electroporation protocol was established by optimizing the above parameters. In turn, this electroporation protocol can be used to deliver DNA into dental follicle cells to study the roles of candidate genes in regulating tooth eruption.

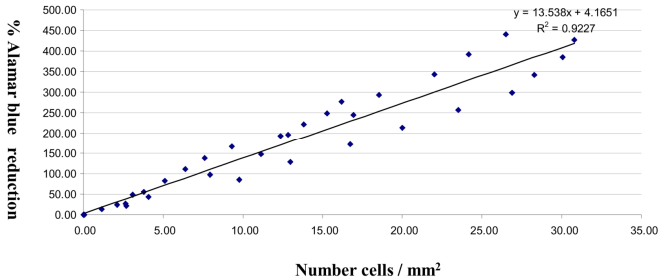

Fig. 1 A conventional standard curve showing that Alamar blue reduction (%) is linearly related to DFC density (cells/mm^2) grown in tissue culture wells. The regression line with the equation and R^2 are shown.

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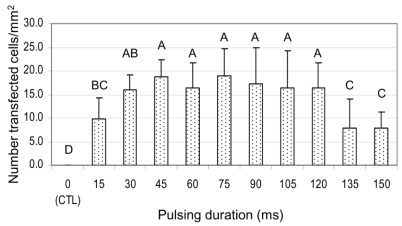
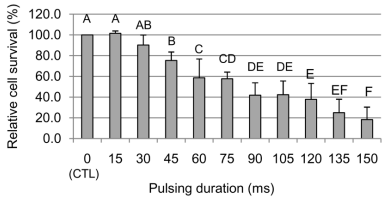
Effects of pulsing duration on transfection and survival of DCs



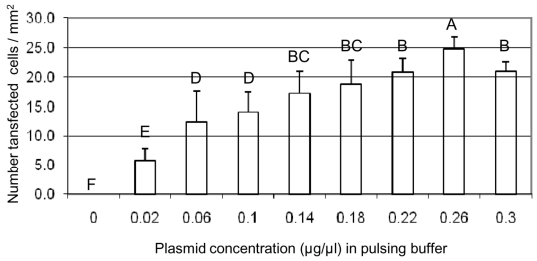
A



B

Fig. 5 Effect of pulsing duration on transfection and survival of DFCs. An optimal number of transfected cells per mm2 were achieved at a duration ranging from 30 to 120 ms (A), and the percentage of relative surviving cells were reduced as the pulsing duration increased (B). Bars labeled with the same letter are not significantly different (P ≤0.05). The error bars represent standard error of the mean for the number of transfected cells (A), and standard deviation for relative cell survival (B).

Effects of plasmid concentration on transfection efficiency



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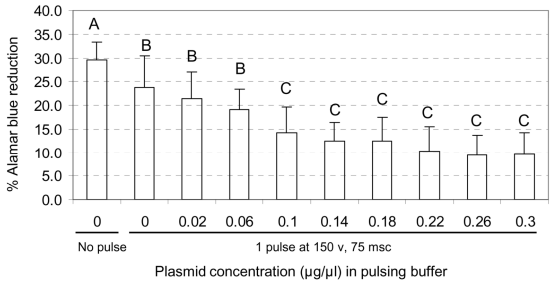

B

Fig 3. Comparison of the effect of plasmid concentrations on transfection efficiency (number transfected cells/mm^2) and cell survival (as reflected by % Alamar blue reduction). Note that the plasmid concentrations significantly affect the transfection efficiency; i.e., the number of transfected cells/mm^2 increased as plasmid concentration increased up to 0.26 µg/µl (A). Plasmid concentrations also significantly affect cell survival in an adverse manner (B). The means were separated with least significant difference (LSD) at P ≥0.05. Bars labeled with the same letter are not significantly different. The error bars represent standard error of the mean for the number of transfected cells (A), and standard deviation for % Alamar blue reduction (reflecting cell survival) (B).

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Effects of pulsing electric field strengths on transfection efficiency

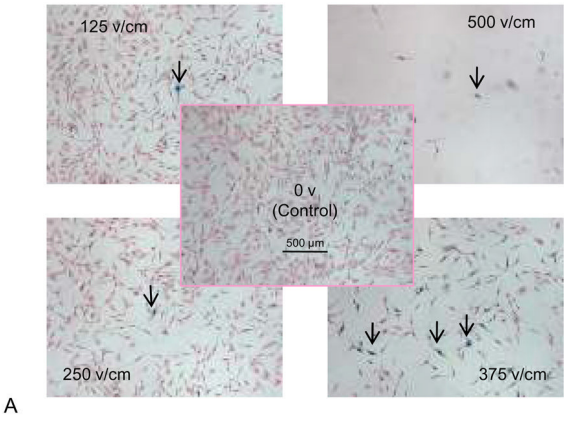

Fig. 2 Transfection of DFCs with plasmid containing lac Z reporter gene using electroporation. (A) Effect of pulsing electric field strengths on transfection efficiency. The cells stained blue indicate the transfection (arrows). Note that increased transfection efficiency was seen in the treatment using 375 V/cm. Cell survival was greatly reduced in the treatment of 500 V/cm. (B) Delivery and expression of the Lac Z gene into DFCs was confirmed by RT-PCR in a separate electroporation experiment.

Source:
Improvement of electroporation to deliver plasmid DNA into dental follicle cells
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Effects of adding BSA and FBS in the pulsing buffer

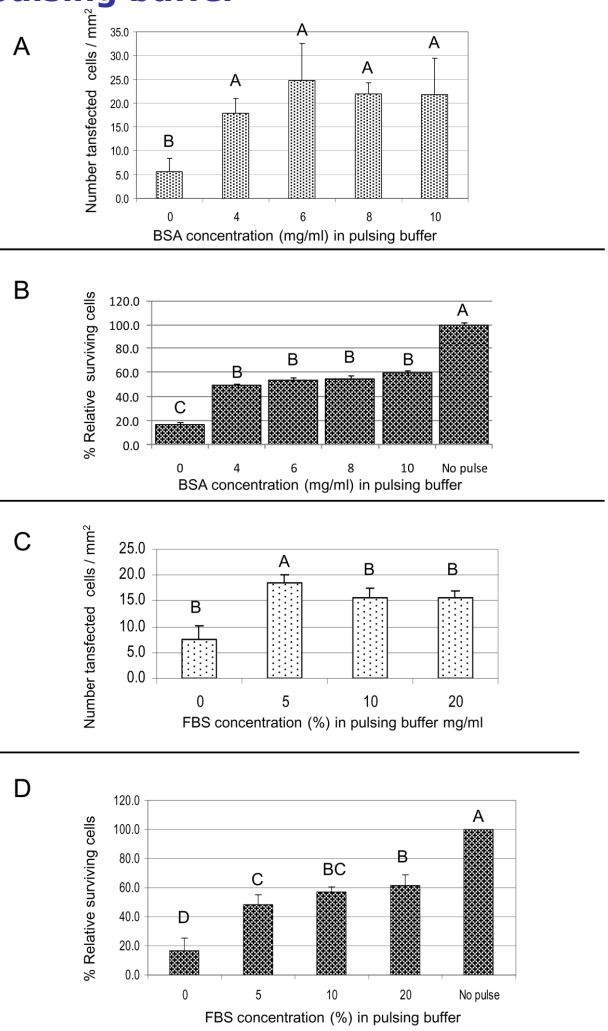

Fig. 4 Effect of additional of BSA and FBS in the pulsing buffer on DFC transfection and cell survival. Number transfected cells per mm^2 and percentage of relative surviving cells were significantly improved by adding BSA (A, B) and FBS (C, D) into the buffer. Bars labeled with the same letter are not significantly different ($P \leq 0.05$). The error bars represent standard error of the mean for the number of transfected cells, and standard deviation for relative cell survival.