Published in final edited form as:

Biotechnol J. 2009 October ; 4(10): 1488–1496. doi:10.1002/biot.200900039.

Improvement of electroporation to deliver plasmid DNA into dental follicle cells

Shaomian Yao, Samir Rana, Dawen Liu, and Gary E. Wise¹

Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA

Abstract

Electroporation DNA transfer is a simple and versatile approach to deliver genes. To develop an optimal electroporation protocol to deliver DNA into cells, we conducted square wave electroporation experiments with using rat dental follicle cells as follows: 1) the cells were electroporated at different electric field strengths with lac Z plasmid; 2) plasmid concentrations were tested to determine the optimal doses; 3) various concentrations of bovine serum albumin or fetal bovine serum were added to the pulsing buffer; and, 4) the pulsing durations were studied to determine the optimal duration. These experiments indicated that the optimal electroporation electric field strength was 375 V/cm, and that plasmid concentrations greater than 0.18 µg/µl were required to achieve high transfection efficiency. BSA or FBS in the pulsing buffer significantly improved cell survival and increased the number of transfected cells. 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.

Keywords

Electroporation; plasmid; tooth eruption; dental follicle cells

Introduction

Delivery of foreign DNA into eukaryotic cells has become an important tool in molecular biology and in gene therapy studies. Current DNA delivery techniques include electroporation, microinjection, particle bombardment, biological-based and chemical-based methods. Of those approaches, biological-based viral vectors are widely used in delivering foreign DNAs into mammalian cells. The disadvantages of biological methods include delivery of DNA molecules of only a limited length, biosafety concerns and complexity in preparing virus. Electroporation is a physical-based method for DNA delivery, and thus possesses some advantages over other methods. These advantages include 1) simplicity; 2) fewer side effects and biosafety concerns; 3) and capability in delivering DNA in vivo into a defined area [1]. Such local DNA delivery capability is especially valuable to study localized biological events, such as tooth eruption, which mainly involves a loose

¹Corresponding Author: Dr. Gary E. Wise, Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA; Tel: +1-225-578-9889, Fax: +1-225-578-9895; gwise@vetmed.lsu.edu.

Conflict of interest statement

The authors have declared no conflict of interest.

connective tissue sac, the dental follicle, of the given tooth. Because of these favorable features, electroporation gene transfer has been heavily investigated as an alternative strategy to viral methods in the development of gene therapy protocols [2].

Delivery of molecules by electroporation is based on the application of an electric field to cells or a tissue to produce transient pores in the plasma membrane of cells [3,4]. The mechanisms are not very clear; but, scientists generally agree that small molecules enter cells via simple diffusion through the pores while entry of DNA involves multiple-steps including electroinsertion of DNA to the de-stabilized membrane, translocation of DNA to the cytosol and intracellular trafficking to the nucleus for expression [5]. The major disadvantages of electroporation DNA transfer are the relatively low transfection efficiency and high cell death/damage [6]. For use of this technique, increase of transfection efficiency and improvement of cell survival are desired.

The dental follicle (DF) is a loose connective tissue sac surrounding the unerupted tooth, and the tooth does not erupt if the DF is removed prior to the onset of eruption [7]. The dental follicle is necessary for eruption because it regulates the expression of the molecules to promote alveolar bone resorption or bone formation in a timely basis. Bone resorption is required to form the eruption pathway in the coronal part of tooth crypt and bone formation is needed in the basal portion of the crypt to serve as the eruptive force [8,9]. Thus, development of protocols to introduce and to express eruption genes in the DF may lead to the development of non-surgical methods to induce the eruption of non-erupted teeth such as the impacted molars. In human, impacted 3rd molars (wisdom teeth) is a major dental care cost. In addition, delivering specific DNAs, such as expression vector containing siRNA, into dental follicle cells (DFCs) or the DF would allow us to elucidate the function of a given gene in eruption. In that vein, we have attempted to deliver plasmid vectors into DFCs as well as DF using electroporation. Although delivery of DNA into stem cells derived from dental follicles has recently been reported [10], no literature regarding transfection of fibroblast-like dental follicle cells (the non-stem cells that are the major component of the dental follicle cell population) are available. A highly efficient in vitro DFC transfection protocol is needed for development of procedures to induce the DFCs (non-stem cells) into stem cells by forcing expression of the defined factors [11,12] in the DFCs. Here, we report the effects of pulsing electric field strength, pulsing duration, plasmid concentration and addition of bovine serum albumin (BSA) or fetal bovine serum (FBS) in the pulsing buffer to improve in vitro electroporation of DFCs. Although the in vitro electroporation protocol cannot be directly extrapolated to in vivo electroporation of the DF, establishing an optimal electroporation delivery protocol for these cells will provide useful information in developing in vivo electroporation protocols for the DF. We are currently working on the in vivo electroporation protocols for transfection of the DF, which will be published separately.

Materials and methods

Dental follicle cells

Dental follicles (DFs) were surgically isolated from the first mandibular molars of postnatal rat pups at day 5 to day 7. DFs were minced and then digested in trypsin solution to obtain a suspension of cells. After centrifugation, the cell pellet was collected and resuspended into regular growth medium consisting of MEM, 10% newborn calf serum, sodium pyruvate and antibiotics and placed in T-75 flasks to establish primary cultures as described previously [13]. At confluence, cells were passaged into new flasks until passage 5. Cells at passages 5 to 9 were detached from the culture surface by trypsin and then used for experiments.

Cell viability test

To determine the relationship between Alamar blue reduction and DFC number, DFC suspensions were serially diluted and seeded into 12 well plates. After 24 hours of incubation, the medium was removed and 0.5 ml of fresh medium consisting of α -MEM (without phenol-red), 10% FBS and 10% Alamar blue (Invitrogen Corp, Carlsbad, CA, USA) was added into each well. After 2 hours of incubation, 100 μ l medium was loaded into each well of a 96-well plate in triplicate pattern and read with a micro-plate reader at 570 nm and 595 nm. The percentage of Alamar blue reduction was calculated according to the manufacturer's instructions. The number of cells seeded in the 12-well plate was determined by counting 8 random spots (1.42 mm²/spot) under an inverted microscope. A regression equation describing cell number and Alamar blue reduction was established accordingly.

Plasmid and lac Z gene

The plasmid was a generous gift from Dr. Shulin Li's lab at Louisiana State University. This plasmid (6558bp) contains NptII gene for Kanamycin resistance as a selectable marker, bacterial origin of replication and a reporter gene, Lac Z, driven by a CMV promoter. The plasmid was hosted in E coli cells. The plasmid was isolated from the E coli cells using Sigma (Sigma-Aldrich, St. Louis, MO, USA) or Invitrogen (Invitrogen Corp, Carlsbad, CA, USA) Max prep kits for transfection experiments.

Electroporation experiments

One day before the transfection experiments, DFCs were cultured in fresh growth medium to maximize their viability. For the electroporation experiments, the cells were collected by centrifugation at 1000 rpm (210 g) for 5 min after detachment by trypsinization. The cells were suspended with a pulsing buffer to a density of approximately 10⁶ cells/ml in a tube. The pulsing buffer (pH 5.5) contains KCl 125mM, NaCl 15mM, glucose 3mM HEPES 25 mM, MgCl₂ 1.2 mM [14] and designated concentrations of plasmid DNA (see below). 100 μ l of the buffer mixture was transferred into each 4-mm gap cuvette and a single-pulse square wave electroporation was conducted at designated electric field strengths for 75 milliseconds (ms) unless otherwise stated using a BTX ECM850 Electro Square Porator (Harvard Apparatus, Holliston, MA, USA). The electroporation protocol was optimized regarding pulse field strength, plasmid concentration, addition of FBS and BSA in the pulsing buffer, and pulse duration, as described below.

To study the electric field strength effect, DFCs were suspended in the pulsing buffer containing approximately 10⁶ cells/ml and 0.05 μ g/ μ l plasmid, and cells 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 μ g/ μ l 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 μ g/ μ l plasmid. For BSA, BSA (Sigma-Aldrich, St. Louis, MO, USA, Product # A6003) 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 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 μ g/ μ l plasmid and 6 mg/ml BSA. For all studies, cells not subjected to electroporation were included as a reference control.

After the electroporation, 1 ml of culture medium was pipetted into the cuvette to suspend the cells, and then transferred into a well of 12-well plates. Next, the cuvette was rinsed with another 0.5 ml medium and the medium was pooled to the same well to maximize cell recovery. The cells were cultured at 37°C, 5% CO₂ for overnight to allow the surviving cells

to adhere. Dead cells were removed by replacing the wells with fresh medium, such that the surviving cells remained in the well. The cells were subjected to Alamar blue test and X-gal staining to evaluate the effects of the treatments as detailed below.

For assessment of cell survival in electroporation experiments, at 24 hours post-electroporation, the DFCs were incubated in the medium containing 10% Alamar blue for 2 hours. The reduction of Alamar blue was acquired as stated above, and the reduction was converted into cell number with the regression equation established from the standard curve experiment (see cell viability test section).

X-gal staining and transfection assessment

To detect transfected cells, cells were fixed with solution containing 2% formaldehyde and 0.2% glutaraldehyde in PBS buffer for 5 min. After washing with PBS buffer, the cells were stained with X-gal solution [1mg/ml X-gal, 5 mM potassium ferricyanide, 5mM potassium ferrocyanide, 2 mM magnesium chloride and 1% DMSO (DMSO was used to dissolve the X-gal)] for 6 hours to develop blue staining. Some cultures were also counterstained with eosin. Cells were viewed and photographed with an Axiovert 25 inverted microscope (Carl Zeiss, Inc., Thornwood, NY, USA), and the number of blue transfected cells were counted in 5 random areas for assessing the treatment effects.

To confirm that lac Z was indeed transferred and expressed in DFCs, 24 hours after electroporation DFCs were collected into TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) and total RNA was extracted. The RNA samples were treated with DNase I to remove any DNA contamination from genomic DNA as well as from the plasmid. Total RNA of 1 µg from each sample was reverse transcribed into cDNA using random primer and reverse transcriptase, followed by PCR to determine gene expression using lac Z specific primers (Forward: GACGTCTCGTTGCTGCATAA; Reverse: CAGCAGCAGACCATTTTCAA). RNA from non-transfected DFCs was used as the negative control and plasmid DNA was used as the positive control.

Statistical analysis

Experiments were repeated at least 4 times to achieve sufficient replication for statistical analysis. ANOVA was used to evaluate treatment effects, and least significant difference (LSD) was used to separate the differences of the means at $P \leq 0.05$.

Results

The relationship between Alamar blue reduction and DFC number/mm² is shown in Figure 1. A regression equation, $Y=13.534x+4.1651$ was established based on the data to describe the relationship between the Alamar blue reduction (x %) and the cell number (Y cells/mm²). The regression equation has an R^2 of 0.9227, indicating that more than 92 % of the variation was explained/described by the equation as shown in Figure 1. Thus, this equation was used to calculate the DFC number based on the Alamar blue reduction acquired in some experiments of this study. In such experiments, the Alamar blue reduction was converted into cell numbers using this equation to assess the effects of treatments on cell survival rates.

Cell survival and transfection efficiency were greatly affected by pulsing field strengths (Figure 2). Electroporation at 125 V/cm did not cause significant cell death as compared to the control not subjected to electroporation, whereas 500 V/cm resulted in death of the majority of the cells (Figure 2). Regarding the transfection efficiency, no transfected cells were seen without electroporation although plasmids were present. Thus, DFCs were not able to uptake plasmid DNA spontaneously. Transfected cells were first seen in 125 V/cm electroporation, and increased number of transfected cells were seen at 250 and 375 V/cm

with the maximal number transfected seen at 375 V/cm (Figure 2A). Electroporation of DFCs at 500 V/cm resulted in very few transfected cells and high cell death (Figure 2A). RT-PCR confirmed that the lac Z gene was delivered and expressed in the electroporated DFCs (Figure 2B). Lack of a band in normal DFCs indicates that native DFCs do not have or do not express the native lac Z gene.

Regarding the concentration effect of plasmid on transfection of DFCs, very few transfected cells were seen at low plasmid concentration of 0.02 $\mu\text{g}/\mu\text{l}$. The number of transfected cells progressively increased as plasmid concentrations rose from 0.02 to 0.26 $\mu\text{g}/\mu\text{l}$. The optimal concentration appeared to be at 0.18 to 0.26 $\mu\text{g}/\mu\text{l}$ because transfection did not continue to increase when higher doses of plasmid (0.3 $\mu\text{g}/\mu\text{l}$) were used (Figure 3A). We also noticed that cell survival was adversely affected by high plasmid concentrations. As plasmid concentrations were increased, the percentage of Alamar blue reduction declined, indicating reduction of cell survival (Figure 3B).

When BSA or FBS was added to the pulsing buffer, both the cell survival and number of transfected cells per mm^2 were significantly improved as compared to the treatment without BSA or FBS (Figure 4A–D). For BSA, the maximal number of transfected cells was approximately 25 cells per mm^2 which was seen at 6 mg/ml of BSA. However, all concentrations tested significantly improved the cell survival from electroporation as compared to the control without BSA or FBS. No statistically significant difference was seen for different concentrations of BSA. For FBS, the maximal transfected cells were achieved using a 5% concentration of FBS. Significant reduction in transfected cell numbers was seen when FBS concentrations were increased to 10% or 20%. Comparing BSA and FBS, Using BSA resulted in better transfection efficiency than FBS, but FBS resulted in better survival rate at high doses (Figure 4D) whereas high doses of BSA had no effect on cell survival (Figure 4B). For example, after electroporation, 56 % of the cells survived at the optimal dose of BSA (6mg/ml) whereas 62 % survived at the optimal dose of FBS (Figures 4B and 4D).

In the pulsing duration study, a maximal number of cells appeared to be transfected in the durations ranging from 30 to 120 ms at 375 V/cm. Durations shorter than 30 ms or longer than 120 ms significantly reduced the transfection as compared to the optimal durations (Figure 5A). However, the cell survival rate was significantly higher at shorter durations. As the pulsing duration increased, cell survival was reduced (Figure 5B). For example, more than 75% of the cells survived at 45 ms whereas only approximately 60% and 20% of the cells survived at 75 ms and 150 ms, respectively. Although no significant cell death was seen at pulsing durations of 30 ms or less, lower transfection was seen (Figures 5A and B).

Discussion

Efficiency of electroporation depends on cellular, physicochemical factors and electrical parameters [15]. We have attempted to optimize some of the factors and parameters for electroporation of the DFCs. Ideally, good electroporation protocols should produce a maximum number of transfected cells with minimal cell damage or death when a given number of cells are electroporated. In this regard, the effects of the treatments in this study were evaluated by measuring the number of transfected cells in a given area and cell viability after electroporation. Although one may argue with this (i.e., number of transfected cells/number of surviving cells in the given area), we believe that the number of transfected cell/ mm^2 is appropriate because electroporation affects both the number of transfected cells and the number of viable cells. Thus, the treatments with a significantly lower number of transfected cells could have a significantly higher transfection rate if the number of viable cells were also low. For example, if treatment A had 3 transfected cells/ mm^2 and 5 viable

cells/mm², the transfection rate would be 60% (3/5). In contrast, treatment B might have 20 transfected cells and 110 surviving cells, and the transfection rate would be 18% (20/110). The results (60% vs 18%) would lead to the conclusion that treatment A is superior to treatment B, but that would not take into account cell viability. In many cases, obtaining a maximal number of transfected cells is desirable for a given pool of cells.

In this study, we have employed Alamar blue to assess the treatment effects on cell survival/viability. The Alamar blue assay is based on the ability of living cells to uptake the dye and oxidize it to the pink, reduced form. The degree of reduction is proportional to the number of living cells. We have previously used this assay to evaluate the growth of osteoclast precursors [16]. Current study suggests that Alamar blue reduction and DFC density fit a linear regression, in a positive correlation manner. We used the assay to obtain percentage of Alamar blue reduction and then converted the reduction to the number of surviving DFCs after electroporation with the well-fit regression equation derived from DFC density standard curve (Figure 1). However, for other cell types, the equation would have to be modified to that culture system.

Pulsing electric field strength is one of the most important factors in electroporation to deliver DNA [17]. This study suggests that at pulsing duration of 75 ms, 375 V/cm achieved the optimal transfection for DFCs (Figure 2A). In contrast, lower electric field strengths (250 V/cm and 125 V/cm) resulted in a lower transfection (Figure 2A) perhaps, because fewer pores were created in the membrane. Very few cells survived when pulsing strength reached 500 V/cm, suggesting that too many pores were created such that the cells ruptured. The optimal field strength probably is dependent on cell types because in transfection of sperm cells, the optimal strength is 1000 V/cm [17] and for an immortalized human eosinophilic cell (Eol-1) line, optimal field strength is 687 V/cm [18]. DFCs appear to have a lower optimal field strength range for electroporation to deliver DNA molecules.

In addition to electric field strength, pulsing duration plays a critical role in affecting electroporation. Duration likely affects the pore formation processes, but increased duration can cause thermal stress to the cells and result in cell damage [19]. A simulation study of pore creation by electroporation suggests that the effect of pulsing duration on the number of pores depended on the pulsing strength; i.e., the number of pores increased with an increase of duration under low voltage electroporation, but increase of the duration did not change the pore number at high voltage electroporation [20]. In the current study, the threshold duration appeared to be at 30–45 ms at pulsing strength of 375 V/cm. The maximum number of transfected cells was seen at pulsing durations between 30–120 ms and durations in this range did not show a significant difference in the number of transfected cells produced, suggesting that the maximal number of pores were created. Significant reduction of transfection was seen at durations longer than 120 ms (Figure 5A), perhaps because the prolonged duration significantly decreased cell survival (Figure 5B). These results compare favorably with those of Yamashita et al. [21] who found that a pulsing duration of 50 ms was optimal for delivery plasmid DNA into tumors. A thermal effect resulting from prolonged pulsing duration is likely the cause of the cell death. Others also have reported that prolonged pulsing duration caused cell shrinkage, but no membrane ruptures [20].

DNA concentration is another important factor in cell transfection. DNA concentration not only affects transfection efficiency, but also cell survival [22,23]. It has been reported that electroporation requires smaller amounts of DNA than other methods for gene transfer [24]. We noticed that when less than 0.26 µg/µl plasmid DNA was in the buffer, the number of transfected cells was positively related to increased plasmid dosage. The transfection efficiency did not continue to increase in the treatments with plasmid dosages higher than 0.26 µg/µl at a pulsing strength of 375 V/cm for 75 ms (Figure 3A), suggesting that the

amount of plasmid may be saturated. The amount of plasmid required seems to be higher for DFC transfection than for transfection of some other cell types. For example, optimal plasmid concentration is 0.1 $\mu\text{g}/\mu\text{l}$ and 0.08 $\mu\text{g}/\mu\text{l}$ to transfect sperm cells [17] and Eo1-1 cells [18], respectively. Using a plasmid dosage as low as possible is desired in an electroporation protocol because high doses of plasmid reduce cell viability (Figure 3B) [22].

One of the major disadvantages of electroporation is that it can cause cell damage and death. Most likely, this is because electroporation is based on an electrical pulse creating temporary holes on the cell membrane for molecules to enter a cell. If the holes fail to close, the cell is damaged or ruptured [6]. This study suggests that addition of BSA or FBS into the pulsing buffer can dramatically improve the cell survival post-pulsing. Most likely, this is because BSA or FBS helps to reseal the holes or helps to prevent the disrupted membrane from rupturing. BSA or FBS may also reduce thermal damage, perhaps by stabilizing either the cell membrane lipid bilayer [25] or the trans- membrane proteins [26]. Albumin and other plasma components have been reported to interact with lipid bilayers to stabilize them [25]. BSA can also increase the structural stability of a transmembrane protein, LH/ICG receptor [26]. In cell culture experiments, albumin was observed to have antioxidant capabilities and to protect cells against the toxic effects of oxidative stress [27]. BSA is the major component of FBS, and thus the protective function of FBS seen in this study may be due to the existing BSA.

Regarding the transfection, BSA in pulsing buffer gave a better result than FBS, suggesting that other components in FBS may somehow prevent the ability of electrical pulse to create holes on the cell membrane; i.e., they stabilize the membrane.

Electroporation of DNA into DFCs gives us an alternative tool to study gene function in tooth eruption. Although this was an in vitro study, the results from this study provide us useful information about the response of DFCs to electroporation, which also would help us to design and improve the in vivo experiments to deliver genes into the intact dental follicle. Regarding in vivo DNA delivery, there are recent reports that electroporation has been successfully used to transfer plasmid based RNAi into a specific region of an organ, such as a defined region of mouse brain or muscle [1,28]. This feature is particularly important in studying tooth eruption because eruption of a tooth is locally regulated by the DF. Precise delivery of DNA into the target tissue is desired for studying gene function in eruption and for developing a gene therapy technique to induce the eruption of impacted teeth. Delivery of plasmid DNA into intact rat dental follicles by electroporation recently has been achieved in our laboratory, and the results will be published separately.

In conclusion, improvement of electroporation for delivery of DNA into DFCs was achieved by optimizing the plasmid concentrations, the electric field strength and pulsing duration. Addition of BSA or FBS into the electroporation buffer also significantly improved cell survival and increased transfected cell number. Although, these studies were conducted using fibroblast-like dental follicle cells, the above four parameters should be considered for optimal electroporation delivery of DNA into other cell types, as well. Certainly, the results reported here can provide useful information for achieving the best delivery of plasmid DNA into dental cells utilizing electroporation.

Acknowledgments

This research was supported by NIDCR RO1 grant DEO08911-17 to G.E.W and S.Y. The authors would like to thank Dr. Shulin Li for providing the plasmid and for giving us valuable suggestions. We also want to thank Ms. Xueqing Xia and Dr. Boyu Zhang for their help in setting up the electroporation experiments.

Abbreviations

DF	Dental follicles
DFCs	Dental follicle cells
FBS	Fetal bovine serum
α-MEM	Alpha-Minimal essential medium
ANOVA	Analysis of variance
LSD	Least significant difference

References

1. Akaneya Y, Jiang B, Tsumoto T. RNAi-induced gene silencing by local electroporation in targeting brain region. *J Neurophysiol.* 2005; 93:594–602. [PubMed: 15604463]
2. Li, S. Delivery of DNA into tum ors. In: Li, editor. *Electroporation Protocols. Methods in Molecular Biology.* Humana Press; Totowa, NJ: 2008. p. 311-318.
3. Weaver JC, Chizmadzhev YA. Theory of electroporation: A review. *Biochemistry and Bioenergetics.* 1996; 41:135–160.
4. Melikov KC, Frolov VA, Shcherbakov A, Samsonov AV, et al. Voltage-induced nonconductive prepores and metastable single pores in unmodified planar lipid bilayer. *Biophys J.* 2001; 80:1829–1836. [PubMed: 11259296]
5. Rols, MP. Mechanism by which electroporation mediates DNA migration and entry into cells and targeted tissues. In: Li, editor. *Electroporation Protocols. Methods in Molecular Biology.* Humana Press; Totowa, NJ: 2008. p. 19-33.
6. Weaver JC. Electroporation theory. Concepts and mechanisms. *Methods Mol Biol.* 1995; 47:1–26. [PubMed: 7550723]
7. Cahill DR, Marks SC Jr. Tooth eruption: evidence for the central role of the dental follicle. *J Oral Pathol.* 1980; 9:189–200. [PubMed: 6777476]
8. Wise GE, Yao S, Henk WG. Bone formation as a potential motive force of tooth eruption in the rat molar. *Clin Anat.* 2007; 20:632–639. [PubMed: 17415742]
9. Wise GE, King GJ. Mechanisms of tooth eruption and orthodontic tooth movement. *J Dent Res.* 2008; 87:414–434. [PubMed: 18434571]
10. Yalvac ME, Ramazanoglu M, Gumru OZ, Sahin F, et al. Comparison and Optimisation of Transfection of Human Dental Follicle Cells, a Novel Source of Stem Cells, with Different Chemical Methods and Electro-poration. *Neurochem Res.* 2009; 34:1272–1277. [PubMed: 19169817]
11. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006; 126:663–676. [PubMed: 16904174]
12. Yu J, Vodyanik M, Smuga-Otto K, Antosiewicz-Bourget J, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 2007; 318:1917–1920. [PubMed: 18029452]
13. Wise GE, Lin F, Fan W. Culture and characterization of dental follicle cells from rat molars. *Cell Tissue Res.* 1992; 267:483–492. [PubMed: 1571962]
14. Li L, Shivakumar H, Feller RS, Allen C, et al. Highly efficient, large volume flow electroporation. *Technol Cancer Res Treat.* 2002; 1:341–350. [PubMed: 12625759]
15. Prasanna GL, Panda T. Electroporation: basic principles, practical considerations and applications in molecular biology. *Bioprocess and Biosystems Engineering.* 1997; 16:261–264.
16. Yao S, Liu D, Pan F, Wise GE. Effect of vascular endothelial growth factor on RANK gene expression in osteoclast precursors and on osteoclastogenesis. *Arch Oral Biol.* 2006; 51:596–602. [PubMed: 16443190]
17. Sin FYT, Mukherjee UK, MCKenzie JC, Sin IL. Electroporation of abalone sperm enhances sperm-DNA association. *Journal of Fish Biol.* 2006; 47:20–28.

18. Ohyama H, McBride J, Wong DT. Optimized conditions for gene transfection into the human eosinophilic cell line EoL-1 by electroporation. *J Immunol Methods*. 1998; 215:105–111. [PubMed: 9744752]
19. Powell KT, Morgenthaler AW, Weaver JC. Tissue electroporation. Observation of reversible electrical breakdown in viable frog skin. *Biophys J*. 1989; 56:1163–1171. [PubMed: 2611330]
20. Smith KC, Neu JC, Krassowska W. Model of creation and evolution of stable electropores for DNA delivery. *Biophys J*. 2004; 86:2813–2826. [PubMed: 15111399]
21. Yamashita YI, Shimada M, Hasegawa H, Minagawa R, et al. Electroporation-mediated interleukin-12 gene therapy for hepatocellular carcinoma in the mice model. *Cancer Res*. 2001; 61:1005–1012. [PubMed: 11221826]
22. Rols MP, Coulet D, Teissie J. Highly efficient transfection of mammalian cells by electric field pulses. Application to large volumes of cell culture by using a flow system. *Eur J Biochem*. 1992; 206:115–121. [PubMed: 1316837]
23. Oldak T, Kruszewski M, Machaj EK, Gajkowska A, et al. Optimisation of transfection conditions of CD34+ hematopoietic cells derived from human umbilical cord blood. *Acta Biochim Pol*. 2002; 49:625–632. [PubMed: 12422232]
24. Withers HL. Direct plasmid transfer between bacterial species and electrocuring. *Methods Mol Biol*. 1995; 47:47–54. [PubMed: 7550753]
25. Liu DX, Huang L. Small, but not large, unilamellar liposomes composed of dioleoylphosphatidylethanolamine and oleic acid can be stabilized by human plasma. *Biochemistry*. 1989; 28:7700–7707. [PubMed: 2611208]
26. Kolena J, Jezova M, Vranova J, Scsukova S. Structure-stabilizing effect of albumin on rat ovarian LH/hCG receptors. *Biochim Biophys Acta*. 1999; 1416:208–216. [PubMed: 9889368]
27. Halliwell B. Albumin - An important extracellular antioxidant? *Biochem Pharmacol*. 1988; 37:569–571. [PubMed: 3277637]
28. Eefting D, Grimbergen JM, de Vries MR, van Weel V, et al. Prolonged in vivo gene silencing by electroporation-mediated plasmid delivery of small interfering RNA. *Human Gene Ther*. 2007; 18:861–869. [PubMed: 17850190]

Figure 1.

A conventional standard curve showing that Alamar blue reduction (%) is linearly related to DFC density (cells/mm²) grown in tissue culture wells. The regression line with the equation and R² are shown.

Figure 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.

A

B

Figure 3. Comparison of the effect of plasmid concentrations on transfection efficiency (number transfected cells/mm²) 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/mm² 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 $P \geq 0.05$. 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).

Figure 4.

Effect of additional of BSA and FBS in the pulsing buffer on DFC transfection and cell survival. Number transfected cells per mm² 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.

A

B

Figure 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 ($P \leq 0.05$). The error bars represent standard error of the mean for the number of transfected cells (A), and standard deviation for relative cell survival (B).