NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH Public Access
Author Manuscript

Methods Mol Biol. Author manuscript; available in PMC 2015 January 01.

Published in final edited form as:
Methods Mol Biol. 2014 ; 1121: 47–54. doi:10.1007/978-1-4614-9632-8_3.

The impact of non-electrical factors on electrical gene transfer

Jiemiao Hu[1,2], **Jeffry Cutrera**[2], and **Shulin Li**[2,#]

[1]Department of Musculoskeletal Oncology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China

[2]Department of Pediatric Research, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Abstract

Electrical pulses directly and effectively boost both *in vitro* and *in vivo* gene transfer, but this process is greatly affected by non-electrical factors that exist during electroporation. These factors include, but are not limited to, the types of cells or tissues used, the property of DNA, DNA formulation, and the expressed protein. In this mini-review, we only describe and discuss a summary of DNA properties and selected DNA formulations on gene transfer via electroporation. The properties of DNA were selected for review because a substantial amount of remarkable work has been performed during the past few years but has received less notice than other work, although DNA properties appear to be critical for boosting electroporation delivery. The selected formulations will be covered in this mini-review because we are only interested in the simple formulations that could be used for cell or gene therapy via electroporation. Plus, there was an extensive review of DNA formulations in the first edition of this book. The formulations discussed in this mini-review represent novel developments in recent years and may impact electroporation significantly. These advancements in DNA formulations could prove to be important for gene delivery and disease treatment.

1. Introduction

For electrical gene transfer, investigators often focus on how to define a set of electrical parameters that will maximize the DNA transfer, how to generate an electrode that will maximize the distribution of electricity for opening up the cell membrane, and how to safely use the electrical pulses (1–10). These questions were extensively examined for almost every application because the answers may hold the key for successful gene transfer in the targeted tissues. After these intensive efforts, though not totally agreed by every investigator, it seems multiple sets of electric parameters provide effective gene transfer. These sets could be summarized as high voltage (>1000 v/cm) with very short pulse duration (100 μs), low voltage (<100–200 v/cm) with longer pulse duration (20–50 ms), and medium voltage and pulse duration(1). Some studies have found that ultra-low voltage (10–30 v/cm) and longer pulse duration (around 50 ms) also work and that a combination of low and high voltage may work better than a single-set duration because the high voltage may benefit pore formation and the low voltage may benefit DNA migration to the

#Corresponding author; sli4@mdanderson.org.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

cells(11,12). These discoveries may reconcile the debate about whether high voltage or low voltage is better. For each specific tissue, cell line, cell type, and application, however, the determination of whether high or low voltage is optimal will continue because the benefits of high versus low voltage vary according to these factors(13).

In spite of the significance of these findings related to electrical parameters, non-electrical factors should be considered, which might be as important as the optimization of electrical conditions. The DNA formulation is the most obvious non-electrical factor that may regulate the efficiency of electroporation. Our earlier work has found that formulations containing glutamate acid may reduce the amount of DNA needed for gene transfer to muscles via electroporation(14). Other studies have provided specific data on formulations that significantly increase DNA transfer via electroporation into different tissues(8,15–19). Our recent work found that different types of cells favor different formulations but that some formulation additives, such as polyuronic acid, consistently achieve better results than other additives(16,20). Although the formulation is important for electroporation gene transfer, the focus of generating an effective formulation for electroporation should perhaps be on cell therapy, because the gene delivery could be completed *in vitro*. A complicated DNA formulation for *in vivo* use may limit future applications. In fact, half saline is one of the best formulations for DNA transfer via electroporation *in vivo*(21). Any DNA formulation for electroporation has to both be simple and outperform the saline formulation. In this regard, we will focus on reviewing simple DNA formulations that have high potential for *in vivo* gene delivery via electroporation and formulations that have potential for stem cell gene transfer via electroporation *in vitro*.

Another important non-electrical aspect of gene transfer via electroporation is the properties of the DNA itself, which include many features that may affect electroporation. The most commonly known property of DNA is its size: smaller DNA can enter cells via electroporation more easily than larger DNA. Many investigators could not repeat the gene transfer efficiency data from a nucleofection system because they use much larger DNA but not the company-provide GFP plasmid DNA. Other DNA properties that significantly affect the efficiency of gene transfer via electroporation include methylation, restriction enzyme sites, the drug selection gene, composition, and replication(22,23). Each of these will be reviewed here. Although these data were collected from different biological systems, investigators should take advantage of these discoveries to find benefit in their own targeted cells, tissues, or biological systems. A formulation that increases cell survival is critical for *in vitro* gene transfer via electroporation, but it will not be discussed in this mini-review.

2. Effects of DNA properties on DNA electroporation

2.1. Plasmid DNA generated from Escherichia Coli as the sole format for DNA electroporation to the targeted tissues

The advantage of this type of DNA is that it is stable and can be stored in a freezer or in dried pellets for years, for research laboratory or clinical application. The disadvantage of this product is that it is highly methylated. This methylated DNA inhibits the transformation efficiency via electroporation to lactic acid bacteria, the bacteria that was used to produce therapeutic recombinant proteins, by a factor of 1,000(23). Unfortunately, such a simple test

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

has not been found in either cell culture or *in vivo* tissues in a mammalian system but remains a project worthy of attention.

It has been shown that the mechanisms for enhanced transformation via electroporation in lactic acid bacteria were eliminating methylation-specific restriction enzyme activity and reducing DNA degradation(23). This explanation cannot exclude the possibility of an increased level of gene expression from the unmethylated DNA. This possibility may be especially true in the mammalian system because it is well known that methylation of the promoter inhibits tumor suppressor gene expression, one of the mechanisms of tumor development in the colon and, perhaps, many other types of cancer(24). In fact, not only methylation of the promoter but also methylation of the surrounding regions may yield more severe gene expression suppression in at least some cells(25). In support of this view, the introduction of the methylation site in a promoter via a polymerase chain reaction method reduces the level of gene expression(26). These encouraging data should direct investigators to study the application of demethylated DNA via electroporation. However, it is possible that demethylated DNA can be methylated quickly. Therefore, reducing the methylation sites may be the ultimate solution.

2.2. Other DNA properties that affect gene delivery via electroporation

Beside methylation, the size of the DNA and its restriction sites, bacterial backbone, viral sequence, resistance gene sequence, and CpG motifs also affect gene transfer efficiency. Although there has been no comprehensive study to consider all these factors at once, an isolated study definitively concluded that smaller plasmid DNA yield a higher level of gene expression and about a 2.6-fold increase in the plasmid DNA copy when using a mesenchymal stem cell system(22).

A vector system with only a few gene cloning sites, a kanamycin-resistant gene, a CMV promoter, and a human growth hormone polyadenylation tail consistently outperformed the transfection efficiency of vectors with multiple cloning sites, an ampcilin-resistant gene, and other types of promoters and polyadnylation sites via electroporation (unpublished data).

To address most of the problems associated with the use of the plasmid DNA, a mini-intronic plasmid (MIP) and a minicircle DNA have been generated(27–29). MIP DNA is generated by inserting an intron containing all essential elements for bacteria replication and selection into the intron to reduce the length of DNA flanking the transgene because the long piece of flanking DNA plays a major role in silencing gene expression(27). This design is brilliant because this MIP DNA does not require any change in the current manufacturing approach. However, the MIP DNA has not yet been tested in electroporation delivery. It will be very exciting to test this MIP for electroporation. The other minicircle DNA can also be manufactured using the current manufacturing method but requires a recombination step to remove the bacteria backbone and then a purification step to separate the minicircle DNA from the backbone(29). This minicircle DNA has been tested in electroporation and did increase the level of gene expression, though not necessarily the copy number. These two minisystems could really boost the interest in a non-viral plasmid DNA system. These mini-DNA systems, as well as the selection of a proper concentration, and perhaps volume of DNA may yield a high level of gene expression via electroporation. The volume and DNA

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

concentration are mentioned here because results suggest that electroporation interacts with these parameters to enhance the gene transfer efficiency and the level of gene expression(17).

2.3. “Stuff DNA” and gene expression-promoting peptide

Two other aspects that should be mentioned are stuff DNA(30) and in frame foreign minipeptide in the transgene(31). These two concepts have not drawn any significant attention but are simple and significant for improving electroporation-mediated gene transfer and the level of transgene expression. Stuff DNA is short, non-coding DNA that is “stuffed into,” or mixed with, plasmid DNA to enhance electrical gene transfer. This enhancement of DNA transfer via electroporation is highly dependent on the size of the stuff DNA fragment; a maximum of enhancement was found with a size of 300 bp (up to a 21-fold increase)(30). This stuff DNA-mediated increase in gene transfer via electroporation may be partially due to the inhibition of plasmid DNA degradation, but there might be other mechanisms, because neither a large nor a small stuff DNA fragment provided the benefit of increased gene transfer in this study(30). It is very possible that the large DNA may compete with plasmid DNA to enter the electroporation pore, while the smaller DNA cannot bind the DNA nuclease efficiently. Regardless of the mechanism, the benefit of adding stuff DNA may suggest that separation of the DNA bacteria backbone in the minicircle DNA preparation, as discussed above, may not be necessary. That separation procedure perhaps should be changed to a bacteria backbone fragmentation procedure by inserting specific restriction sites every 300 bp.

There are many ways to further improve the level of gene of interest expression after transfer via electroporation, such as the inclusion of a nuclear localization signal(1,32). One simple approach without changing the plasmid DNA backbone, formulation, or electroporation parameters is to insert a minipeptide encoding the DNA fragment in the same reading frame as the gene of interest. It has not been tested in therapeutic genes, yet, but testing in the reporter gene *SEAP* showed that this approach may work on other genes because the insertion of many peptides into the *SEAP* gene increased the level of SEAP activity by a factor of 10 *in vitro* and by a factor of 5 *in vivo*(31). If it occurs in a therapeutic gene, the total amount of DNA administration can be reduced by 80–90% of the current level.

3. Simple DNA formulation for enhancing DNA transfer via electroporation

The DNA formulation is the solution in which plasmid DNA is suspended and administered via syringe, followed by electroporation. The formulation has a great impact on DNA stability, polarity, and migration potential. As indicated in the Introduction, this review only focuses on simple formulations for improving electroporation gene transfer of stem cells and tissues *in vivo* because a more complicated formulation was published in the first edition of this book. These simple formulations will include sodium, the simple cell culture medium OptiMem, and an additive for this cell culture medium.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

3.1. Sodium

Sodium chloride (DNA in 150 mM saline) is the most commonly used formulation for *in vivo* gene electroporation(1). This simple formulation works superbly for electroporation-mediated gene transfer, increasing the gene expression by a factor of 1,000(2,3,21). Compared to other complicated formulations, saline is extreme and has been used in human clinical treatment. A detailed comparison of different sodium chloride concentrations found that a half-saline solution yields better gene transfer efficiency (a 3-fold increase in gene expression) via electroporation than a full-saline solution(21). However, further reduction of the salt concentration induces muscle injury due to the induction of hyperosmotic stress and electrical injury from low conductivity.

3.2. Formulation for stem cells

Cell-based gene therapy has great potential for treating both incurable diseases and genetic diseases. Stem cells are the most widely used cell-based gene delivery among many different types of cell therapies. Stem cells have been tested for the treatment of autoimmune diseases and cancer and for tissue repair and regrowth(33–35). Among stem cells, adipose-derived stem cells are an excellent source for cell therapy because they are present in relatively large amounts in the body, can be harvested and isolated more easily than most other stem cell lineages, and give rise to a variety of different cell lineages, including adipocytes, chondrocytes, myoblasts, and endothelial cells(36–38). More importantly, adipose-derived stem cells possess a homing ability for some cancer cell lines(39), making them excellent potential candidates for anti-tumor cell therapy.

The current formulation for cell transfer is primarily controlled by Amaxa and is not only expensive but also secretive. With the use of pluronic-block copolymers in combination with the cell culture medium OptiMem, a transfection efficiency of up to 40% can be easily achieved; this combination successfully outperformed the Amaxa buffer, which achieved an efficiency of 32% (20). The OptiMem buffer alone is also effective in DNA transfer to stem cells via electroporation. Although the specific composition for OptiMem is not clear, this formulation is much less expensive and has a long shelf life than the Amaxa buffer. OptiMem likely contains regular cell culture medium and L-glutamate, because others have found that glutamate boosts electroporation gene transfer (8,15). Therefore, when using OptiMem, L-glutamate should be added when the medium is kept longer than 6 months, to maximize the DNA transfer efficiency via electroporation.

Although several formulations work *in vitro*, the pluronic-block copolymer family comprising an internal polyoxypropylene (hydrophobic) chain bordered by external polyoxyethylene (hydrophilic) chains shows the most consistent promise of boosting transfection efficiency via electroporation. This statement also applies stem cells although a slightly different pluronic-blocker polymer should be used to maximize the transfection efficiency(20).

4. Summary

Electroporation gene transfer was initiated in 1982 *in vitro*(20). Thirty years later, this technology has become one of the major vehicles for gene transfer for both *in vitro* and *in*

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

vivo cases and for both preclinical and clinical non-viral DNA transfer(40). This achievement is partially due to its simplicity in real practice. With the plasmid DNA vector improvement discussed in the first section of this chapter, a higher level of gene expression will be achieved for many therapeutic genes, which will make electroporation useful for more therapeutic genes that require a high therapeutic threshold.

The primary DNA formulation for *in vivo* electroporation is physiological saline, and the primary DNA formulation for *in vitro* cell culture is the Amaxa buffer. As our brief discussion of DNA formulation, stuff DNA, and DNA properties indicates, the current primary formulation may be changed. For example, a half-saline solution could replace the full-saline solution for *in vivo* electroporation. The proposed combination of OptiMem buffer and pluronic polymer is also achievable for *in vitro* stem cell transfer and provides a significant benefit in cost reduction over the Amaxa buffer. Therefore, these simple and practical ideas should be considered when generating GMP materials.

References

1. Li S. Electroporation gene therapy: new developments in vivo and in vitro. Curr Gene Ther. 2004; 4:309–316. [PubMed: 15384944]
2. Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D. High-efficiency gene transfer into skeletal muscle mediated by electric pulses. Proc Natl Acad Sci U S A. 1999; 96:4262–4267. [PubMed: 10200250]
3. Li S, Zhang X, Xia X, Zhou L, Breau R, Suen J, Hanna E. Intramuscular electroporation delivery of IFN-alpha gene therapy for inhibition of tumor growth located at a distant site. Gene Ther. 2001; 8:400–407. [PubMed: 11313817]
4. Aihara H, Miyazaki J. Gene transfer into muscle by electroporation in vivo. Nat Biotechnol. 1998; 16:867–870. [PubMed: 9743122]
5. Heller LC, Coppola D. Electrically mediated delivery of vector plasmid DNA elicits an antitumor effect. Gene Ther. 2002; 9:1321–1325. [PubMed: 12224015]
6. Maruyama H, Ataka K, Higuchi N, Sakamoto F, Gejyo F, Miyazaki J. Skin-targeted gene transfer using in vivo electroporation. Gene Ther. 2001; 8:1808–1812. [PubMed: 11803401]
7. Nishi T, Yoshizato K, Yamashiro S, Takeshima H, Sato K, Hamada K, Kitamura I, Yoshimura T, Saya H, Kuratsu J, Ushio Y. High-efficiency in vivo gene transfer using intraarterial plasmid DNA injection following in vivo electroporation. Cancer Res. 1996; 56:1050–1055. [PubMed: 8640760]
8. Draghia-Akli R, Khan AS, Cummings KK, Parghi D, Carpenter RH, Brown PA. Electrical enhancement of formulated plasmid delivery in animals. Technol Cancer Res Treat. 2002; 1:365–372. [PubMed: 12625762]
9. Vierra DA, Irvine SQ. Optimized conditions for transgenesis of the ascidian Ciona using square wave electroporation. Dev Genes Evol. 2012; 222:55–61. [PubMed: 22246069]
10. Vry JD, Martinez-Martinez P, Losen M, Bode GH, Temel Y, Steckler T, Steinbusch HW, Baets MD, Prickaerts J. Low Current-driven Micro-electroporation Allows Efficient In Vivo Delivery of Nonviral DNA into the Adult Mouse Brain. Mol Ther. 2010
11. Pavlin M, Flisar K, Kanduser M. The role of electrophoresis in gene electrotransfer. J Membr Biol. 2010; 236:75–79. [PubMed: 20640850]
12. Bureau MF, Gehl J, Deleuze V, Mir LM, Scherman D. Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. Biochimica et biophysica acta. 2000; 1474:353–359. [PubMed: 10779687]
13. Andre FM, Gehl J, Sersa G, Preat V, Hojman P, Eriksen J, Golzio M, Cemazar M, Pavselj N, Rols MP, Miklavcic D, Neumann E, Teissie J, Mir LM. Efficiency of high- and low-voltage pulse combinations for gene electrotransfer in muscle, liver, tumor, and skin. Hum Gene Ther. 2008; 19:1261–1271. [PubMed: 19866490]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

14. Nicol F, Wong M, MacLaughlin FC, Perrard J, Wilson E, Nordstrom JL, Smith LC. Poly-L-glutamate, an anionic polymer, enhances transgene expression for plasmids delivered by intramuscular injection with in vivo electroporation. Gene Ther. 2002; 9:1351–1358. [PubMed: 12365000]
15. Fewell JG, MacLaughlin F, Mehta V, Gondo M, Nicol F, Wilson E, Smith LC. Gene therapy for the treatment of hemophilia B using PINC-formulated plasmid delivered to muscle with electroporation. Mol Ther. 2001; 3:574–583. [PubMed: 11319920]
16. Flanagan M, Gimble JM, Yu G, Wu X, Xia X, Hu J, Yao S, Li S. Competitive electroporation formulation for cell therapy. Cancer Gene Ther. 2011; 18:579–586. [PubMed: 21660061]
17. Barbon CM, Baker L, Lajoie C, Ramstedt U, Hedley ML, Luby TM. In vivo electroporation enhances the potency of poly-lactide co-glycolide (PLG) plasmid DNA immunization. Vaccine. 2010; 28:7852–7864. [PubMed: 20943208]
18. Gopalakrishnan AM, Kundu AK, Mandal TK, Kumar N. Novel Nanosomes for Gene Delivery to Plasmodium falciparum-infected Red Blood Cells. Sci Rep. 2013; 3:1534. [PubMed: 23525038]
19. Mignet N, Vandermeulen G, Pembouong G, Largeau C, Thompson B, Spanedda MV, Wasungu L, Rols MP, Bessodes M, Bureau MF, Preat V, Scherman D. Cationic and anionic lipoplexes inhibit gene transfection by electroporation in vivo. J Gene Med. 2010; 12:491–500. [PubMed: 20527042]
20. Flanagan M, Gimble JM, Yu G, Xia X, Bunnell BA, Li S. Competitive DNA transfection formulation via electroporation for human adipose stem cells and mesenchymal stem cells. Biol Proced Online. 2012; 14:7. [PubMed: 22512891]
21. Lee MJ, Cho SS, Jang HS, Lim YS, You JR, Park J, Suh H, Kim JA, Park JS, Kim DK. Optimal salt concentration of vehicle for plasmid DNA enhances gene transfer mediated by electroporation. Exp Mol Med. 2002; 34:265–272. [PubMed: 12515391]
22. Ribeiro S, Mairhofer J, Madeira C, Diogo MM, Lobato da Silva C, Monteiro G, Grabherr R, Cabral JM. Plasmid DNA size does affect nonviral gene delivery efficiency in stem cells. Cell Reprogram. 2012; 14:130–137. [PubMed: 22339198]
23. Spath K, Heinl S, Grabherr R. Direct cloning in Lactobacillus plantarum: electroporation with non-methylated plasmid DNA enhances transformation efficiency and makes shuttle vectors obsolete. Microb Cell Fact. 2012; 11:141. [PubMed: 23098256]
24. Lin SY, Yeh KT, Chen WT, Chen HC, Chen ST, Chiou HY, Chang JG. Promoter CpG methylation of tumor suppressor genes in colorectal cancer and its relationship to clinical features. Oncology reports. 2004; 11:341–348. [PubMed: 14719065]
25. Chen Y, Dhupelia A, Schoenherr CJ. The Igf2/H19 imprinting control region exhibits sequence-specific and cell-type-dependent DNA methylation-mediated repression. Nucleic acids research. 2009; 37:793–803. [PubMed: 19074953]
26. Arakawa T, Ohta T, Abiko Y, Okayama M, Mizoguchi I, Takuma T. A polymerase chain reaction-based method for constructing a linear vector with site-specific DNA methylation. Analytical biochemistry. 2011; 416:211–217. [PubMed: 21669180]
27. Lu J, Zhang F, Kay MA. A Mini-intronic Plasmid (MIP): A Novel Robust Transgene Expression Vector In Vivo and In Vitro. Mol Ther. 2013
28. Gao L, Xie L, Long X, Wang Z, He CY, Chen ZY, Zhang L, Nan X, Lei H, Liu X, Liu G, Lu J, Qiu B. Efficacy of MRI visible iron oxide nanoparticles in delivering minicircle DNA into liver via intrabiliary infusion. Biomaterials. 2013; 34:3688–3696. [PubMed: 23419644]
29. Kobelt D, Schleef M, Schmeer M, Aumann J, Schlag PM, Walther W. Performance of high quality minicircle DNA for in vitro and in vivo gene transfer. Mol Biotechnol. 2013; 53:80–89. [PubMed: 22467123]
30. Peng J, Zhao Y, Mai J, Guo W, Xu Y. Short noncoding DNA fragment improve efficiencies of in vivo electroporation-mediated gene transfer. J Gene Med. 2012; 14:563–569. [PubMed: 22930438]
31. Cutrera J, Dibra D, Xia X, Li S. Enhancement of reporter gene detection sensitivity by insertion of specific mini-peptide-coding sequences. Cancer Gene Ther. 2010; 17:131–140. [PubMed: 19713998]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

32. Miller AM, Dean DA. Tissue-specific and transcription factor-mediated nuclear entry of DNA. Advanced drug delivery reviews. 2009; 61:603–613. [PubMed: 19393704]
33. Lee ST, Jang JH, Cheong JW, Kim JS, Maemg HY, Hahn JS, Ko YW, Min YH. Treatment of high-risk acute myelogenous leukaemia by myeloablative chemoradiotherapy followed by co-infusion of T cell-depleted haematopoietic stem cells and culture-expanded marrow mesenchymal stem cells from a related donor with one fully mismatched human leucocyte antigen haplotype. British journal of haematology. 2002; 118:1128–1131. [PubMed: 12199796]
34. de Prata KL, Orellana MD, De Santis GC, Kashima S, Fontes AM, de Carrara RC, Palma PV, Neder L, Covas DT. Effects of high-dose chemotherapy on bone marrow multipotent mesenchymal stromal cells isolated from lymphoma patients. Experimental hematology. 2010; 38:292–300. e294. [PubMed: 20138957]
35. Wild JM, Krutzfeldt NO. Neocortical-like organization of avian auditory 'cortex'. Commentary on Wang Y, Brzozowska-Prechtl A, Karten HJ (2010): Laminar and columnar auditory cortex in avian brain. Proc Natl Acad Sci USA. 2010; 107:12676–12681. [PubMed: 20616034] Brain, behavior and evolution. 76:89–92.
36. Katz AJ, Llull R, Hedrick MH, Futrell JW. Emerging approaches to the tissue engineering of fat. Clinics in plastic surgery. 1999; 26:587–603. viii. [PubMed: 10553215]
37. Gimble J, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy. 2003; 5:362–369. [PubMed: 14578098]
38. Morizono K, De Ugarte DA, Zhu M, Zuk P, Elbarbary A, Ashjian P, Benhaim P, Chen IS, Hedrick MH. Multilineage cells from adipose tissue as gene delivery vehicles. Hum Gene Ther. 2003; 14:59–66. [PubMed: 12573059]
39. Josiah DT, Zhu D, Dreher F, Olson J, McFadden G, Caldas H. Adipose-derived stem cells as therapeutic delivery vehicles of an oncolytic virus for glioblastoma. Mol Ther. 2010; 18:377–385. [PubMed: 19904233]
40. Li S. Electroporation gene therapy. Preface. Methods in molecular biology. 2008; 423:v–vii. [PubMed: 18453074]