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Electroporation Formulation for Cell Therapy

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Abstract

Cell transfection efficiency often determines the success of cell-based gene therapy. Cell transfection via Nucleofector technology yields high transfection efficiency and low cytotoxicity. However, owing to trade secrecy, the components in each buffer are unknown, which not only increases the cost of electroporation studies but also limits the application of Nucleofector in clinical cell-based gene therapies. Thus, we developed a three-step method to determine the optimal conditions, including buffer, program and additional polymer, in electroporation for multiple cancers and stem cell lines. This method could reduce the cost, allow researchers to find the optimal electroporation conditions for their cell lines of interest, and greatly boost the application potential of electroporation in clinical cell-based gene therapies.

1. Introduction

Cell-based gene therapy has been applied in the clinical setting to boost antitumor immunity, treat graft-versus-host disease (1) and enhance immunity against other diseases(2). Stem cells, which have the feature of differentiation, have great potential in gene therapy for cancers(3,4) and damaged tissues(5). Recently, adipose-derived stem cells (ASCs) were isolated (6) and characterized (7) as candidate cells for use in gene therapy. For such use, ASCs have great advantages over other cell types (8), such as easy access, various cell lineages(7) and cancer cell-specific targeting(9).

Many methods have been applied to modify cells for therapeutic use. One of the most commonly used cell modification methods is through viral transduction. The advantages of viral transduction are its high efficiency and broad options for use with different types of viruses (10–12). However, viral vectors often trigger an immune response in the host that attenuates the therapeutic efficacy (13). Certain viral vectors were even found to be tumorigenic, which aroused safety concerns of using viral transduction(14).

Therefore, highly efficient non-viral methods of transfection are in great demand, but most of the current methods have drawbacks. Cationic polymer-based transfection reagents such as polyethyleneimine (PEI) disrupts cell membrane and mitochondrial membrane to cause

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extreme cytotoxicity(15). Lipid-based transfection reagents, including lipofectamine 2000, induce endogenous interferon (16), which limits these reagents' applications in many studies.

A non-viral transfection method that has shown some potential is electroporation, the use of an electric field to polarize cells and in turn permeabilize cells with hydrophilic molecules(17). The exact mechanism of electroporation is not well known, but investigators found that the microseconds of cell polarization in an electric field opens small pores on the cell surface, which allows large molecules to enter the cell cytosol(18). Electroporation reduces cytotoxicity, but in order to apply this method in cell-based gene therapy, the transfection efficiency in some cell types needs to be improved.

Such improvement could be achieved by using Amaxa Nucleofector technology, which comes with a variety of special buffers and programs for electroporation in difficult challenging cell types, including primary cells, immune cells, non-dividing cells and even stem cells. The advantages of using this device along with its buffers are high efficiency, high cell viability, and low risk of cellular modifications(19), but the limitations are also obvious. First, using the optimized buffers purchased from the manufacturer is quite costly. Second, as a trade secret, the components in each buffer are unknown, which is inconvenient when researchers try to transfect a new cell line for which the manufacturer has given no protocol. Third, since information on the components of all solutions is lacking, for reason of safety, this method is unlikely to be applied in clinical cell-based gene therapies.

Herein, we report our method to select the transfection solution for the Amaxa Nucleofector (20). Our solution is nearly as effective as the commercial buffers are in many easy-to-transfect or difficult-to-transfect cell lines. Moreover, with the recipe of known chemicals, our solution is quite affordable and safe for bench and clinical studies.

2. Materials

2.1 Plasmid DNA

1. The luciferase gene construct used for this study was obtained from Valantis, Inc. (Burlingame, CA) and contains DNA fragments encoding firefly luciferase. The luciferase encoding gene is driven by a cytomegalovirus (CMV) promoter and terminated by an independent bovine growth hormone polyadenylation signal.
2. The green fluorescent protein (GFP) encoding gene is also driven by a CMV promoter and terminated by an independent bovine growth hormone polyadenylation signal.

2.2 Cells for culture

1. HeLa, RM1, C2C12, 4T1 cells, SCCVII, B16F10, MCF7, MDA-MB-231, HT29, EC40, NCTC 1469, K7M3 and D1 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM)/F-12 medium containing 10% fetal bovine serum. MPC11 cells and MC3T3E1 cells were maintained in alpha-MEM without ascorbic acid and supplemented with 10% fetal bovine serum. SL12.4, Jurkat, mouse primary

bone marrow and 3LL cells were grown in RPMI supplanted with 10% fetal bovine serum and 1% Penicillin/Streptomycin solution.

2. The murine ASCs were culture expanded in DMEM/F12 Ham's Medium, 10% fetal bovine serum and 1% antibiotic/antimycotic. The hASCs were cultured in DMEM/F12 Ham's Medium, 10% FBS and 1% antibiotic/antimycotic.
3. 0.5% Trypsin-EDTA solution
4. Gibco Trypan Blue Stain Hemacytometer

2.3 Buffers and polymers

1. OptiMEM (Buffer O)
2. Pulsing buffer (Buffer P), consisting of 125mM KCl, 15mM NaCl, 3mM Glucose, 25mM HEPES and 1.2mM MgCl₂ (pH 7.4), is made from a 10× homemade stock solution, filtered and stored at 4 °C.
3. LMA1 stock was made to a 10% (wt/vol) of Poly-L glutamic acid (mw 15–50 kDa; Sigma-Aldrich, St Louis, MO) in milliQ-H₂O.
4. Polymers LME1 (polyethylene glycol, mw 8 kDa; Amresco, Solon, OH), LMP8 (Poloxamer 188, mw 8 kDa; Spectrum Chemicals, Gardena, CA), LMP1 (Poloxamer 181, mw 2 kDa; Spectrum), LMP7 (Poloxamer 407, mw 12 kDa; Spectrum) and LMV1 (Polyvinylpyrrolidone, mw 40 kDa; Fisher, Pittsburgh, PA) stocks were all made to 20% (wt/vol) in Milli Q-H₂O.
5. LMP3 (Pop313) was made to a stock concentration of 0.01% (wt/vol) in MQ-H₂O.
6. LMC1 (Crown-5) was provided at a stock concentration of 10%. All stock buffers were stored at 4 °C and were kept for 1 month.

2.4 Electroporation

1. Amaxa nucleofector
2. Amaxa nucleofection cuvette

2.5 Transfection efficiency

1. 1.0% formaldehyde solution
2. Flow cytometer
3. cell lysis kit (Promega, Madison, WI; part #E4030)
4. Packard LumiCount (Perkin-Elmer, Boston, MA)
5. BCA protein assay kit (Thermo Scientific, Rockford, IL; Product #23227.)
6. Packard SpectraCount (Perkin-Elmer, Boston, MA)

3 Methods

3.1 Determine the optimal buffer and program for electroporation

1. Warm buffer O and buffer P to room temperature (Note 1).
2. Cells are pelleted and resuspended in 100 μ l transfection buffer (O or P) at a concentration of 1.0×10^6 cells per 100 μ l (Note 2).
3. Cells are then transferred to sterile 3-mm Amaxa nucleofection cuvette and incubated with 2 μ g reporter gene-encoding plasmid DNA (Note 3).
4. Cells are electroporated using one of the nucleofection programs: A-20, T-20, T-30, X-01, X-05, L-29, and D-23.
5. Nucleofected cells are then rinsed with 500 μ l sterile culture medium and transferred to the well of a sterile 12-well plate.
6. Cells are incubated at 37°C for 24 h before analysis.
7. Cells are harvested by trypsinization and counted using trypan blue cell staining.
8. Cell viability is determined by the following equation:

$$(\text{number of non-blue cells}) / (\text{total number of cells}) \times 100\%$$
9. Cells are rinsed and resuspended in 1 ml phosphate-buffered saline, and 500 μ l of cell suspension is used for flow cytometry analysis.
10. The percentage of GFP positive cells is determined using flow cytometry.
11. The remaining cells are spun down and lysed with 200 μ l lysis reagent.
12. The total protein amount is determined using protein assay (Note 4).
13. 20 μ l of cell lysates was transferred to a 96-well plate. Luciferase activity was measured using a Packard LumiCount (Note 5).
14. All luciferase activity is normalized to protein assay data using the following equation:

$$((\text{luciferase assay data in relative luminescence units}) \times 200) / (\text{protein assay data in mg})$$
15. Optimal combination of transfection buffer and program is selected based on the percentage of GFP-positive cells and the normalized luciferase activity.

3.2 Select the proper polymer to assist the optimal buffer in electroporation

1. Make and filter polymers LMA1, LME1, LMV1, LMP3 and LMP8 (see Materials).

¹Cells in good condition are ideal for electroporation. Cells that are just pulled out from liquid nitrogen or are undergoing overgrowth could reduce transfection efficiency.

²Buffer P is made from 10 $\times$ stock buffer.

³The percentage of GFP-positive cells represents the number of cells that express the transfected gene. The levels of luciferase represent the amount of proteins that the transfected cells produce.

⁴BCA protein assay was performed to determine the amount of total proteins.

⁵An equal volume of cell lysates is needed to evaluate relative luciferase intensity. For this purpose, we used a multi-channel pipette to transfer cell lysates to 96-well plates.

2. Combine each polymer with the optimized condition (See 3.1) for electroporation.
3. Compare transfection efficiency with the absence or presence of each polymer and select the best polymer to assist transfection in each cell line.

3.3 Compare our buffer with Amaxa commercial buffer in electroporation

1. Transfect the same cell lines with luciferase and GFP-encoding plasmid DNAs using buffers and programs recommended by Amaxa.
2. Determine the percentage of GFP-positive cells using flow cytometry assay.
3. Determine the levels of luciferase production using luciferase assay.
4. Compare the cell viability and transfection efficiency between our buffers and commercial buffers (Note 6).

References

1. Paczesny S, Choi SW, Ferrara JL. Acute graft-versus-host disease: new treatment strategies. *Curr Opin Hematol*. 2009; 16:427–436. [PubMed: 19812490]
2. Rappa G, Anzanello F, Alexeyev M, Fodstad O, Lorico A. Gamma-glutamylcysteine synthetase-based selection strategy for gene therapy of chronic granulomatous disease and graft-vs.-host disease. *Eur J Haematol*. 2007; 78:440–448. [PubMed: 17331133]
3. Lee ST, Jang JH, Cheong JW, Kim JS, Maeng 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]
4. 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]
5. Dupont KM, Sharma K, Stevens HY, Boerckel JD, Garcia AJ, Guldberg RE. Human stem cell delivery for treatment of large segmental bone defects. *Proc Natl Acad Sci U S A*. 2010; 107:3305–3310. [PubMed: 20133731]
6. 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]
7. Gimble J, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy*. 2003; 5:362–369. [PubMed: 14578098]
8. 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]
9. 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]
10. Saraf A, Mikos AG. Gene delivery strategies for cartilage tissue engineering. *Adv Drug Deliv Rev*. 2006; 58:592–603. [PubMed: 16766079]
11. Hendrie PC, Russell DW. Gene targeting with viral vectors. *Mol Ther*. 2005; 12:9–17. [PubMed: 15932801]

⁶For murine ASC electroporation, buffer O plus 0.5% LMP8 and program X-01 yielded the highest transfection efficiency but still failed to match Amaxa buffer. For human ASC electroporation, buffer O plus 0.001% LMP5 and program X-01 successfully surpassed Amaxa buffer.

12. Schaffer DV, Koerber JT, Lim KI. Molecular engineering of viral gene delivery vehicles. *Annu Rev Biomed Eng.* 2008; 10:169–194. [PubMed: 18647114]
13. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, Kay MA. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med.* 2006; 12:342–347. [PubMed: 16474400]
14. Strauss BE, Costanzi-Strauss E. Combating oncogene activation associated with retrovirus-mediated gene therapy of X-linked severe combined immunodeficiency. *Braz J Med Biol Res.* 2007; 40:601–613. [PubMed: 17464421]
15. Godbey WT, Wu KK, Mikos AG. Poly(ethylenimine)-mediated gene delivery affects endothelial cell function and viability. *Biomaterials.* 2001; 22:471–480. [PubMed: 11214758]
16. Li XL, Boyanapalli M, Weihua X, Kalvakolanu DV, Hassel BA. Induction of interferon synthesis and activation of interferon-stimulated genes by liposomal transfection reagents. *J Interferon Cytokine Res.* 1998; 18:947–952. [PubMed: 9858316]
17. Cukjati D, Batiuskaite D, Andre F, Miklavcic D, Mir LM. Real time electroporation control for accurate and safe in vivo non-viral gene therapy. *Bioelectrochemistry.* 2007; 70:501–507. [PubMed: 17258942]
18. Mir LM. Nucleic acids electrotransfer-based gene therapy (electrogenotherapy): past, current, and future. *Mol Biotechnol.* 2009; 43:167–176. [PubMed: 19562526]
19. Jacobsen F, Mertens-Rill J, Beller J, Hirsch T, Daigeler A, Langer S, Lehnhardt M, Steinau HU, Steinstraesser L. Nucleofection: a new method for cutaneous gene transfer? *J Biomed Biotechnol.* 2006; 2006:26060. [PubMed: 17489014]
20. 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]