Sonodelivery in Skeletal Muscle

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Ultrasound-mediated gene delivery, also known as sonoporation or sonodelivery, is a technique that utilizes the ability of ultrasound to disrupt the cell membrane to allow for the delivery of genes, proteins, and other therapeutics into cells. One potential application of this technique is sonodelivery of therapeutic genes into skeletal muscle, which allows the muscle to act as a therapeutic "factory" for long-term gene therapy. There are some complications associated with this application and with sonoporation in general, but it still appears to be a promising method for gene delivery, particularly *in vivo*, due to its advantages.

Keywords: Sonoporation ; Sonodelivery ; Gene Therapy ; Skeletal Muscle ; Gene Delivery ; Ultrasound-Mediated Gene Transfer

1. introduction

Gene therapy shows increasing promise for the treatment of a wide range of diseases, from cancer to musculoskeletal and immunological disorders[1]. Although the basic premise of gene therapy is relatively simple on the surface—to introduce a gene or genes into a patient's cells/tissue that will allow the body to produce a therapeutic protein or facilitate a genetic modification—every step of the process holds its own challenges. One of the major challenges associated with making gene therapy widely (and safely) accessible is that of gene delivery *in vivo*. There are currently many methods for delivering DNA *in vitro* at high efficiencies, including electroporation, lipid-based transfection, and viral delivery. Each of these methods presents potential challenges for clinical translation, including the cost and time of production, the invasiveness of the technique, the efficiency of delivery, and/or safety concerns related to an immune response or unintended genomic integration of the therapeutic gene. The development of a gene delivery technique that is inexpensive, widely available, reusable, minimally invasive, and safe will catalyze progress in gene therapy. One promising technique that meets all of these criteria has been under investigation for many years but has not yet reached widespread use: ultrasound-mediated gene delivery, more frequently referred to as sonoporation or sonodelivery[2][3]. As its name suggests, this approach involves using ultrasound to deliver non-viral vectors encoding therapeutic proteins into skeletal muscle, allowing the body to then produce its own therapeutic.

2. Previous Work

Sonodelivery typically requires the addition of microbubbles (MBs) - common echo-contrast agents used in medical procedures in humans that typically consist of an inert gaseous bubble encapsulated by a biocompatible material[4][5][6][7][8][9][10][11]. The ultrasound waves cause the MBs to burst, leading to the formation of transient pores in the cell membrane. It has also been shown that MB-mediated sonoporation leads to disassembly of the cytoskeleton[12]. Sonoporation has been used to deliver a variety of cargos to a variety of cell types[8][13][14][15][16][17][18][19][20][21][22][23][24]. It has also been used *in vivo* to deliver cargo in a site-specific manner to multiple targets, including skeletal muscles, the abdomen, and the inner ear[4][6][25][26][27][28][29]. In all of these former studies, there was either no or minimal off-target delivery. Due to variations in sonoporation conditions, ranging from the instrument to the settings to the type of MBs and cargo, the results vary significantly between projects. Because of this, more work is needed before sonodelivery can become a mainstream treatment method.

3. Future Directions

The most pressing future direction for sonodelivery is that of standardization. With so many different sonoporators and sonoporation conditions, even for the same target tissue or cell type, and with such disparity in the results of the gene delivery, it is difficult to determine a standardized treatment method. Developing a standard unit of measure could help to expedite this process. Sonoporation conditions are typically reported by duty cycle, frequency, time, and intensity. Many reports also include probe size and some reports include other conditions, ranging from wave type to power output.

Unfortunately, with the differences between instruments, the conditions that are typically reported can lead to a variable power output, meaning that the delivery power is likely not the same, even if the conditions are very similar. Although more optimization is needed to find the average optimal conditions for delivering DNA into muscle using sonoporation, establishing a standard method for reporting sonoporation conditions, such as the inclusion of the power output, which can be calculated for each set of conditions, may help to ameliorate some of the disparities in results.

An interesting future direction for the sonodelivery field would include more work on combining sonoporation with other approaches that might increase the efficacy of gene delivery. Some work has already been done along this vein, including polymer-mediated sonodelivery[30][31][32]; adjustments to cell membrane permeability by adding enzymes, such as hyaluronidase[33], and by adding drugs such as lidocaine and adjusting temperature[14]; and electro-sonoporation[34][35][36]. Electro-sonoporation is a particularly interesting procedure in which electroporation and sonoporation are applied simultaneously to facilitate cell membrane pore formation. In a proof of concept investigation, Longsine-Parker et al. found that cell viability was 97.3% and membrane pore formation efficiency was 95.6% in mammalian cells after application[36]. These promising results were further explored with *in vitro* and *in vivo* studies that found that electro-sonoporation performed significantly better in both transfection efficiency and cell viability compared to electroporation alone[34][35]. While more work is needed to confirm these results and elaborate on the effectiveness of electro-sonoporation, it appears to be a highly promising technique. While all these possible adaptations would add more conditions to consider in the standardization procedure, they could greatly enhance the efficacy of sonodelivery for gene therapy.

Another direction of high importance and interest for intramuscular sonoporation will be the delivery of therapeutic genes, as opposed to reporter genes (i.e., luciferase). Although sonoporation has been used to deliver therapeutic genes intratumorally[10][37], the majority of the intramuscular sonodelivery completed to date has utilized reporter genes to test sonoporation conditions and efficiency. It could be argued that testing the delivery of therapeutic genes via sonoporation is of greater importance than standardization, as having a standardized method will only be clinically relevant when the conditions are optimized for therapeutic expression. To help mitigate the potential for non-standard conditions to be a confounding factor of a lack of therapeutic expression, this work could be conducted in tandem with the above-mentioned standardization work. Of particular interest would be the delivery of therapeutic proteins tagged or fused with a reporter gene, such as luciferase, which would allow for simultaneous testing of therapeutic expression and optimization of sonodelivery conditions.

Further improvements for intramuscular sonodelivery and gene therapy might include the incorporation of muscle-specific refinements of payload, such as microbubbles designed to target skeletal muscle or the extracellular matrix (ECM) or vectors containing skeletal muscle-specific promoters to increase the efficiency of transgene expression[38].

In light of all these considerations, it can easily be seen that there is additional optimization needed for these approaches and there would be great benefit to the scientific community in implementing more extensive testing of therapeutic delivery to skeletal muscle using sonoporation.

References

1. Misra, S. Human gene therapy: a brief overview of the genetic revolution. J Assoc Physicians India 2013, 61, 127–133.
2. Miller, D.L.; Pislaru, S.V.; Greenleaf, J.F. Sonoporation: Mechanical DNA Delivery by Ultrasonic Cavitation. Somat Cell Mol Genet 2002, 27, 115–134, doi:10.1023/A:1022983907223.
3. Newman, C.M.H.; Bettinger, T. Gene therapy progress and prospects: Ultrasound for gene transfer. Gene Therapy 2007, 14, 465–475, doi:10.1038/sj.gt.3302925.
4. Shapiro, G.; Wong, A.; Bez, M.; Yang, F.; Tam, S.; Even, L.; Sheyn, D.; Ben-David, S.; Tawackoli, W.; Pelled, G.; et al. Multiparameter evaluation of in vivo gene delivery using ultrasound-guided, microbubble-enhanced sonoporation. J Control Release 2016, 223, 157–164, doi:10.1016/j.jconrel.2015.12.001.
5. Bez, M.; Foiret, J.; Shapiro, G.; Pelled, G.; Ferrara, K.W.; Gazit, D. Nonviral ultrasound-mediated gene delivery in small and large animal models. Nat Protoc 2019, 14, 1015–1026, doi:10.1038/s41596-019-0125-y.
6. Lu, Q.L.; Liang, H.-D.; Partridge, T.; Blomley, M.J.K. Microbubble ultrasound improves the efficiency of gene transduction in skeletal muscle in vivo with reduced tissue damage. Gene Therapy 2003, 10, 396–405, doi:10.1038/sj.gt.3301913.
7. Wang, X.; Liang, H.-D.; Dong, B.; Lu, Q.-L.; Blomley, M.J.K. Gene Transfer with Microbubble Ultrasound and Plasmid DNA into Skeletal Muscle of Mice: Comparison between Commercially Available Microbubble Contrast Agents. Radiology 2005, 237, 224–229, doi:10.1148/radiol.2371040805.

8. Taniyama, Y.; Tachibana, K.; Hiraoka, K.; Aoki, M.; Yamamoto, S.; Matsumoto, K.; Nakamura, T.; Ogihara, T.; Kaneda, Y.; Morishita, R. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. Gene Therapy 2002, 9, 372–380, doi:10.1038/sj.gt.3301678.
9. Burke, C.W.; Suk, J.S.; Kim, A.J.; Hsiang, Y.-H.J.; Klibanov, A.L.; Hanes, J.; Price, R.J. Markedly enhanced skeletal muscle transfection achieved by the ultrasound-targeted delivery of non-viral gene nanocarriers with microbubbles. Journal of Controlled Release 2012, 162, 414–421, doi:10.1016/j.jconrel.2012.07.005.
10. Zolochevska, O.; Xia, X.; Williams, B.J.; Ramsay, A.; Li, S.; Figueiredo, M.L. Sonoporation Delivery of Interleukin-27 Gene Therapy Efficiently Reduces Prostate Tumor Cell Growth In Vivo. Hum Gene Ther 2011, 22, 1537–1550, doi:10.1089/hum.2011.076.
11. Upadhyay, A.; Dalvi, S.V. Microbubble Formulations: Synthesis, Stability, Modeling and Biomedical Applications. Ultrasound in Medicine & Biology 2019, 45, 301–343, doi:10.1016/j.ultrasmedbio.2018.09.022.
12. Wang, M.; Zhang, Y.; Cai, C.; Tu, J.; Guo, X.; Zhang, D. Sonoporation-induced cell membrane permeabilization and cytoskeleton disassembly at varied acoustic and microbubble-cell parameters. Scientific Reports 2018, 8, 3885, doi:10.1038/s41598-018-22056-8.
13. Feril, L.B. Ultrasound-Mediated Gene Transfection. In Gene Therapy of Cancer: Methods and Protocols; Walther, W., Stein, U.S., Eds.; Methods in Molecular BiologyTM; Humana Press: Totowa, NJ, 2009; pp. 179–194 ISBN 978-1-59745-561-9.
14. Nozaki, T.; Ogawa, R.; Feril, L.B.; Kagiya, G.; Fuse, H.; Kondo, T. Enhancement of ultrasound-mediated gene transfection by membrane modification. The Journal of Gene Medicine 2003, 5, 1046–1055, doi:10.1002/jgm.445.
15. Li, Y.S.; Davidson, E.; Reid, C.N.; McHale, A.P. Optimising ultrasound-mediated gene transfer (sonoporation) in vitro and prolonged expression of a transgene in vivo: Potential applications for gene therapy of cancer. Cancer Letters 2009, 273, 62–69, doi:10.1016/j.canlet.2008.07.030.
16. Suzuki, R.; Oda, Y.; Namai, E.; Takizawa, T.; Negeshi, Y.; Utoguchi, N.; Tachibana, K.; Maruyama, K. Development of Site Specific Gene Delivery System with Sonoporation. The Pharmaceutical Society of Japan 2008, 128, 187–192.
17. Mehier-Humbert, S.; Bettinger, T.; Yan, F.; Guy, R.H. Ultrasound-mediated gene delivery: Kinetics of plasmid internalization and gene expression. Journal of Controlled Release 2005, 104, 203–211, doi:10.1016/j.jconrel.2005.01.011.
18. Liang, H.-D.; Lu, Q.L.; Xue, S.-A.; Halliwell, M.; Kodama, T.; Cosgrove, D.O.; Stauss, H.J.; Partridge, T.A.; Blomley, M.J.K. Optimisation of ultrasound-mediated gene transfer (sonoporation) in skeletal muscle cells. Ultrasound in Medicine & Biology 2004, 30, 1523–1529, doi:10.1016/j.ultrasmedbio.2004.08.021.
19. Duvshani-Eshet, M.; Machluf, M. Therapeutic ultrasound optimization for gene delivery: A key factor achieving nuclear DNA localization. Journal of Controlled Release 2005, 108, 513–528, doi:10.1016/j.jconrel.2005.08.025.
20. Unger, E.C.; Hersh, E.; Vannan, M.; Matsunaga, T.O.; McCreery, T. Local drug and gene delivery through microbubbles. Progress in Cardiovascular Diseases 2001, 44, 45–54, doi:10.1053/pcad.2001.26443.
21. Sloand, J.N.; Nguyen, T.T.; Zinck, S.A.; Cook, E.C.; Zimudzi, T.J.; Showalter, S.A.; Glick, A.B.; Simon, J.C.; Medina, S.H. Ultrasound-Guided Cytosolic Protein Delivery via Transient Fluorous Masks. ACS Nano 2020, 14, 4061–4073, doi:10.1021/acsnano.9b08745.
22. De Carlo, F.; Thomas, L.; Brooke, B.; Varney, E.T.; Nande, R.; Boskovic, O.; Marshall, G.D.; Claudio, P.P.; Howard, C.M. Microbubble-mediated delivery of human adenoviruses does not elicit innate and adaptive immunity response in an immunocompetent mouse model of prostate cancer. J Transl Med 2019, 17, 19, doi:10.1186/s12967-019-1771-0.
23. Negishi, Y.; Endo, Y.; Fukuyama, T.; Suzuki, R.; Takizawa, T.; Omata, D.; Maruyama, K.; Aramaki, Y. Delivery of siRNA into the cytoplasm by liposomal bubbles and ultrasound. Journal of Controlled Release 2008, 132, 124–130, doi:10.1016/j.jconrel.2008.08.019.
24. Sarisozen, C.; Salzano, G.; Torchilin, V.P. Recent advances in siRNA delivery. Biomolecular Concepts 2015, 6, 321–341, doi:10.1515/bmc-2015-0019.
25. Liao, Z.-K.; Tsai, K.-C.; Wang, H.-T.; Tseng, S.-H.; Deng, W.-P.; Chen, W.-S.; Hwang, L.-H. Sonoporation-mediated anti-angiogenic gene transfer into muscle effectively regresses distant orthotopic tumors. Cancer Gene Therapy 2012, 19, 171–180, doi:10.1038/cgt.2011.73.
26. Shih, C.-P.; Chen, H.-C.; Lin, Y.-C.; Chen, H.-K.; Wang, H.; Kuo, C.-Y.; Lin, Y.-Y.; Wang, C.-H. Middle-ear dexamethasone delivery via ultrasound microbubbles attenuates noise-induced hearing loss. The Laryngoscope 2019, 129, 1907–1914, doi:10.1002/lary.27713.

27. Tlaxca, J.L.; Rychak, J.J.; Ernst, P.B.; Konkalmatt, P.R.; Shevchenko, T.I.; Pizzaro, T.T.; Rivera-Nieves, J.; Klibanov, A.L.; Lawrence, M.B. Ultrasound-based molecular imaging and specific gene delivery to mesenteric vasculature by endothelial adhesion molecule targeted microbubbles in a mouse model of Crohn's disease. Journal of Controlled Release 2013, 165, 216–225, doi:10.1016/j.jconrel.2012.10.021.

28. Tomizawa, M.; Shinozaki, F.; Motoyoshi, Y.; Sugiyama, T.; Yamamoto, S.; Sueishi, M. Sonoporation: Gene transfer using ultrasound. World J Methodol 2013, 3, 39–44, doi:10.5662/wjm.v3.i4.39.

29. Xu, W.; Zhang, X.; Hu, X.; Zhiyi, C.; Huang, P. Translational Prospects of ultrasound-mediated tumor immunotherapy: Preclinical advances and safety considerations. Cancer Letters 2019, 460, 86–95, doi:10.1016/j.canlet.2019.06.017.

30. Parelkar, S.S.; Letteri, R.; Chan-Seng, D.; Zolochevska, O.; Ellis, J.; Figueiredo, M.; Emrick, T. Polymer–Peptide Delivery Platforms: Effect of Oligopeptide Orientation on Polymer-Based DNA Delivery. Biomacromolecules 2014, 15, 1328–1336, doi:10.1021/bm401878p.

31. Chen, Y.-C.; Jiang, L.-P.; Liu, N.-X.; Wang, Z.-H.; Hong, K.; Zhang, Q.-P. P85, Optison microbubbles and ultrasound cooperate in mediating plasmid DNA transfection in mouse skeletal muscles in vivo. Ultrasonics Sonochemistry 2011, 18, 513–519, doi:10.1016/j.ultsonch.2010.08.013.

32. Figueiredo Neto, M.; Letteri, R.; Chan-Seng, D.; Emrick, T.; Figueiredo, M.L. Sonodelivery Facilitates Sustained Luciferase Expression from an Episomal Vector in Skeletal Muscle. Materials (Basel) 2015, 8, 4608–4617, doi:10.3390/ma8074608.

33. Danialou, G.; Comtois, A.S.; Dudley, R.W.R.; Nalbantoglu, J.; Gilbert, R.; Karpati, G.; Jones, D.H.; Petrof, B.J. Ultrasound Increases Plasmid-Mediated Gene Transfer to Dystrophic Muscles without Collateral Damage. Molecular Therapy 2002, 6, 687–693, doi:10.1006/mthe.2002.0723.

34. Yamashita, Y.-I.; Shimada, M.; Tachibana, K.; Harimoto, N.; Tsujita, E.; Shirabe, K.; Miyazaki, J.-I.; Sugimachi, K. In Vivo Gene Transfer into Muscle via Electro-Sonoporation. Human Gene Therapy 2002, 13, 2079–2084, doi:10.1089/10430340260395929.

35. Meschersky, M.E.; Kaaba, S.I.; Sokolovsky, A.A.; Moskovtsev, A.A.; BLOKHIN, D.Yu.; ANDRIYANOV, Yu.V.; Kubatiev, A.A. A new method of combined electroporation and sonoporation with a reduced stress-inducing effect on cells. Pathogenesis 2012, 10, 59–67.

36. Longsine-Parker, W.; Wang, H.; Koo, C.; Kim, J.; Kim, B.; Jayaraman, A.; Han, A. Microfluidic electro-sonoporation: a multi-modal cell poration methodology through simultaneous application of electric field and ultrasonic wave. Lab Chip 2013, 13, 2144–2152, doi:10.1039/C3LC40877A.

37. Zolochevska, O.; Ellis, J.; Parelkar, S.; Chan-Seng, D.; Emrick, T.; Wei, J.; Patrikeev, I.; Motamedi, M.; Figueiredo, M.L. Interleukin-27 Gene Delivery for Modifying Malignant Interactions Between Prostate Tumor and Bone. Hum Gene Ther 2013, 24, 970–981, doi:10.1089/hum.2013.091.

38. Piekarowicz, K.; Bertrand, A.T.; Azibani, F.; Beuvin, M.; Julien, L.; Machowska, M.; Bonne, G.; Rzepecki, R. A Muscle Hybrid Promoter as a Novel Tool for Gene Therapy. Molecular Therapy - Methods & Clinical Development 2019, 15, 157–169, doi:10.1016/j.omtm.2019.09.001.