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Safe and Efficient siRNA Therapy using Nanosecond Laser and Electrical Pulses

Editorial

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RNA interference (RNAi) is a natural phenomenon existing in plants, insects, and many mammals, involved in gene silencing or the inhibition of gene expression. Small interfering RNAs (siRNAs) could be used as potential tools to implement this process that could lead to treatments in diseases, such as cancer. However, the delivery of siRNA to eukaryotic cells is a challenge due to its lower cellular uptake and easy degradation by enzymes in blood, tissues, and cells. Nanosecond duration laser and electrical pulses-based siRNA delivery is an upcoming therapeutic tool to deliver various siRNAs, both *in vitro* and *in vivo*.

siRNA consisting of 21+ nucleotides is gaining momentum as a potential therapeutic tool for treatment of a variety of diseases including cancer, as they can induce RNAi and inhibit the expression of target proteins, causing gene silencing or knock down [1, 2]. However, cellular uptake of siRNA is extremely low due to its poly ionic nature and it is easily degraded by enzymes in the blood, tissues, and cells, despite chemical modifications for improved stability. Thus, an effective delivery system is critical to induce RNAi at the site of action.

Non-viral, physical techniques, such as laser opto-injection using Laser Enabled Analysis and Processing (LEAP™) and and other devices, and electroporation (EP) are promising physical techniques to deliver siRNA. LEAP™ opto-injection is exciting because it provides highspeed selection and opto-injection of small molecules and genes into even difficult to transfect cells while maintaining viabilities at more than 90 percent. Opto-injection using LEAP™ has been demonstrated to have significant advantages over existing transfection methodologies [3, 4], using an array of molecules ranging from ions, small molecules, nucleic acids, proteins, and quantum dots. The various cells studied include NIH-3T3, HeLa, and 293T cells. NIH-3T3 cells were preloaded with Zn2+-sensitive dye RhodZin-1, 50µM $ZnCl_2$ buffer was added and cells were opto-injected. HeLa cells were counterstained with cell tracker Orange, Sytox Green, and Sytox Blue molecules. They were also opto-injected with dextran (3kDa) conjugated to tetramethylrhodamine. 293T cells were opto-injected with a protein consisting of the Cdc42 binding domain of WASP (55kDa), conjugated to an ISO dye (red). In addition, optoinjection of siRNA in a variety of cell types, demonstrating superior knock down of target genes compared to Lipofectamine was also explored.

In our lab, BHK, 4T1, HeLa and a number of other cell lines were studied using LEAP™. Nanosecond laser pulses of various intensities at different angles were applied. The number of pulses applied vary from 1 to 5 and there were 1-3 repeats of these pulses, and the optimal parameters were investigated using 10kDa, 100kDa and up to 500kDa Sytox Green molecules, uploaded into living human cancer cells, with efficiencies up to over 80%.

The findings indicate that it is relatively non-toxic with very high cell viability (70-90%) with no observed change in cell morphology or short- and long-term growth [3]. These attributes are highly desirable for siRNA delivery, inducing targeted gene silencing in challenging cell types with low toxicity.

EP is proven for its efficacy *in vitro* and *in vivo*, including clinical trials [5-8]. It is a safe, non-viral, efficient gene and other molecular (chemo drugs) delivery technique, wherein precisely controlled, high intensity, low duration electrical voltage pulses are applied to open up transient pores through semi-permeable membranes and tissues allowing targeted delivery of therapeutic molecules including genes, drugs, antibodies, and vaccines *in vitro* and *in vivo*, including clinical trials. EP has been shown to offer a 100-1000 fold improved therapeutic benefit compared to drug alone in electrochemotherapy. EP is also a useful tool to transfect siRNA *in vitro* into human cell lines that are refractive to current techniques. It was used to upload siRNA in human monocytes, dendritic cells (DC) [9], muscle, retina, arthritic joints, and tumors [1].

EP was found to be both a desirable and efficient method for siRNA delivery into monocyte-derived dendritic cells [10] while being able to resolve some of the cell line specific limitations

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associated with liposome-based transfection. It has further been demonstrated that EP can transfect siRNA into hard-to-transfect cells [11].

Jordan et al., [12] optimized the electroporation pulse conditions for a number of primary and other difficult-to-transfect cells. The various cells studied include human umbilical vein endothelial cells (HUVEC), human primary fibroblasts (HPF), human neuroblastoma cells (SK-N-SH), mouse neuroblastoma cells (Neuro-2A), Jurkat, MCF-7, and others. Both square wave and exponential decay pulses were used to upload various siRNAs and plasmid DNAs. The results indicate that under appropriate voltage and pulse durations, it is possible to silence genes. They obtained impressive transfection efficiencies of 75% for Neuro-2A, 93% for HPF, and 94% for HUVEC cells, using 250V, 20ms pulses. Similar results could also be obtained using nanosecond electroporation (nsEP). In nsEP, a number of 10-30kV/cm, 10-60ns pulses could be used to achieve the desired gene transfer [13-15]. Both the dosage and the pulse parameters could be tweaked to obtain the desired gene transfection rate.

EP was used to both efficiently and economically deliver DNMT1 siRNA into estrogen receptor (ER) negative breast cancer cell line, MDA-MB-468 to silence DNA methyl transferase mRNA [16]. The siRNA sense sequence was 5'-CGGUGCUCAUGCUUACAACTT-3' and the antisense sequence was 5'-GUUGUAAGCAUGAGCACCGTT-3'. 19 pulse conditions were studied to optimize the most viable condition for siRNA delivery. 16 exponential and 3 square wave pulse forms were used and 220V, 975µF exponential pulse condition was found suitable with 97% viability. 2, 5, and 10nM siRNA were delivered and their efficacy was studied using western blot analysis.

Golzio et al., uploaded the egfp22 siRNA (sense: 5'r (GCAAGCUGA CCC UGA AGU UCA U), ANTISENSE: 5'r (GAACUUCAG GGU CAG CUU GCC G) AND P76 SIrna (SENSE: 5'r(GCGGAGUGGCCU GCA GGU A)dTT, antisense: 5'r (UACCUG CAGGCC ACU CCG C)dTT into female C57Bl/6 mice [17]. Eight (4 each, positive and negative) 800V/cm, 5ms pulses were applied at 1Hz frequency in their study. The results indicated that electric field could be used an efficient method for siRNA delivery for gene silencing into cells of solid tumors *in vivo* using non-systemic approach.

SiRNA clinical trials indicate their applications as emerging biodrugs, due to their specific and potent RNAi triggering activity [18]. They are also applicable as therapeutic drugs due to their wide range of target proteins that can be used for gene silencing for treating diseases.

siRNA is a valuable tool for specific gene silencing for medical applications and basic research. However, enzymatic degradation, extremely low accumulation in the cells and difficult delivery, present challenges to *in vitro* and *in vivo* use of siRNAs. It is critical to use a delivery system that can achieve both stabilization and effective transfection. Highly promising, physical techniques, such as optoporation and electroporation has the potential for improved targeted delivery compared to existing methods to achieve successful gene silencing in target cells, *in vitro* and potentially, *in vivo*.

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