2015

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Anna Bulysheva
Old Dominion University, abulyshe@odu.edu

Amy Donate
Old Dominion University

Chelsea Edelblute
Old Dominion University, cedelblu@odu.edu

Derrick Jung
Old Dominion University

Karl Schoenbach
Old Dominion University, kschoenb@odu.edu

See next page for additional authors

Original Publication Citation

Bulysheva, A., Donate, A., Edelblute, C., Jung, D., Muhammad, M., Karl, S., & Richard, H. (2015). Increased tissue temperature improves electro-transfer mediated gene delivery to skin. *Molecular Therapy, 23*, S145. doi:10.1016/s1525-0016(16)33974-0

Authors
Anna Bulysheva, Amy Donate, Chelsea Edelblute, Derrick Jung, Karl Schoenbach, and Richard Heller

365. Increased Tissue Temperature Improves Electro-Transfer Mediated Gene Delivery to Skin

Anna Bulysheva,[1] Amy Donate,[1,3] Chelsea Edelblute,[1] Derrick Jung,[1] Malik Muhammad,[1] Shoenbach Karl,[1] Heller Richard.[1,2]
[1]Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA; [2]School of Medical Diagnostics and Translational Sciences, Old Dominion University, Norfolk, VA; [3]Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL.

Introduction: Developing an optimal gene electro-transfer system for delivery of plasmid DNA to the skin in vivo has been challenging with expression often confined in the epithelium requiring high voltage electric fields, which can cause cellular and tissue damage. Minimizing such damage, while optimizing gene expression profiles, are highly desirable for therapeutic applications of gene delivery to the skin. Cell membrane fluidity is temperature dependent, thus moderate temperature elevation can increase membrane fluidity. Utilizing this concept, we developed a novel gene electro-transfer electrode for in vivo applications with ability to heat tissue with a laser integrated into a four pin multi-electrode array, to improve gene delivery efficiency.

Methods: We tested a range of gene electro-transfer conditions in vivo in guinea pigs with and without elevating skin temperature to 43°C immediately prior to application of electric pulses. Firefly luciferase encoding plasmid DNA was delivered and expression levels were measured by luminescence (IVIS Spectrum) for quantifying expression profiles in photons/second. Green florescence protein (GFP) encoding plasmid DNA was also delivered for assessing gene expression location.

Results Our results indicated that increasing the temperature resulted in significantly higher gene expression compared to controls without applied heat in luciferase groups. GFP immunohistochemistry showed gene expression was localized in the epidermal layers of the skin.

Significance: It is therefore possible to use lower applied electric fields and achieve comparable gene expression to higher electric fields if the tissue temperature is slightly elevated above normal body temperature. Lower applied electric fields, result in reduced cellular and tissue damage compared to higher applied electric field, thus such conditions are more efficient for therapeutic applications. In summary, we developed a safer and a more efficient delivery system for in vivo gene transfer to the skin by moderately elevating tissue temperature during gene-electro transfer.

366. Development of Engineered Magnetic Nanoparticles Coupled With Lentiviral Vectors for Targeted Cancer Therapy and Hyperthermia

Ester Borroni,[1] Marta Miola,[2] Andrea Cochis,[1] Sara Ferraris,[2] Enrico Catalano,[1] Sasha Novak,[3] Maria Prat,[1] Lia Rimondini,[1] Enrica Verne',[2] Antonia Follenzi.[1]
[1]Dept of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Novara, Italy; [2]Applied Science and Technology Department, Politecnico di Torino, Torino, Italy; [3]Department for Nanostructured Materials, Jožef Stefan Institute, Ljubljana, Slovenia.

Background

In recent years, considerable efforts have been spent to develop magnetic nanoparticles (MNPs) and to improve their applicability in several areas including hyperthermia and target cancer gene therapies.

The aim of the present study was to synthesize and characterize Fe3O4, magnetite core-silica shell and magnetite core-silica shell doped with calcium ions nanoparticles (NPs) in combination with lentiviral vectors to deliver therapeutic genes in vivo.

Materials and Methods

Magnetite NPs were prepared by co-precipitation method, the silica shell was obtained by wet chemistry on the magnetic core stabilized with citric acid. Calcium ions were added to the silica shell modulating the NPs surface reactivity.

The NPs were characterized with X-Ray diffraction, transmission electron microscopy, Vibrating Sample Magnetometer and zeta potential.

Cytocompatibility tests were performed using both direct and not-direct contact models with murine endothelial cells (MS1) both in static and dynamic conditions using MNP coupled with/out LV.

MNP and MNP-LV were tail vein injected intravenously in C57/Bl6 mice, biodistribution and expression studies were performed by histology and immunofluorescence using GFP as a marker gene.

Results

Spherical magnetite nanoparticles of about 15 nm in diameter were obtained with good dispersion in water. Addition of silica and calcium allowed obtaining a thin and amorphous silica or Ca-enriched silica shell, maintaining good dispersion in water. All the MNPs displayed a superparamagnetic behaviour.

The MNP+/-LV used demonstrated to be cytocompatible in both static traditional and dynamic cytocompatibility models. Moreover when MNP-LV injected in mice we detected GFP expression mainly in the liver and spleen with biodistribution differences based on the MNPs-LV combination used.

Conclusion

These results suggest these NPs as promising for in vivo applications. Biodistribution studies in vivo of Fe3O4 NPs in mice models were performed and accumulation of NPs into vital organs was minimal with no toxicity in mice up to 1 month later and sustained GFP expression detected with no inflammatory responses. The present studies can significantly improve the cancer therapy effectiveness by means of a selective and localized delivery of transgenes together with the opportunity to conjugate hyperthermic and genetic approaches using therapeutic transgenes.

367. *In Vitro* Electrotransfer of Small and Large Plasmids in Mesenchymal Stem Cells: Calcium, Temperature and PH Impact

Hugues O. Gontier,[1] Léa L. Lesueur,[1] Lluis M. Mir,[1] Franck M. Andre.[1]
[1]Laboratoire de Vectorologie et Thérapeutiques Anticancéreuses, UMR 8203, CNRS, Gustave Roussy, Univ Paris-Sud, Villejuif, France.

Nucleic acids electrotransfer has been shown to be a safe and efficient non-viral technique in a wide variety of cells (including primary cells) even with large nucleic acids. Using a small 3.5 kbp GFP reporter plasmid we previously obtained electrotransfer efficiency of up to 90%, with around 70% cell viability, in Mesenchymal Stem Cells (MSCs). However with larger plasmids (about 15 kbp), we observed highly decreased cell viability and electrotransfer efficacy in MSCs. Our results suggested that this could be the consequence of the increased time necessary for larger plasmids to cross the plasma membrane.

Here we further studied the mechanisms of small and large plasmid electrotransfer. We investigated the roles of calcium, temperature and pH.

Our results shed light on some of the mechanisms involved in gene electrotransfer as well as provide means to enhance electrotransfer efficacy and cell viability for small and large plasmids.