BIOGRAPHICAL SKETCH

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NAME: Jaroszeski, Mark J.

eRA COMMONS USER NAME (credential, e.g., agency login): MARKJA

POSITION TITLE: Associate Professor of Chemical & Biomedical Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of South Florida College of Engineering, Tampa, FL	B.S.	05/1990	Chemical Engineering
University of South Florida College of Engineering, Tampa, FL	M.S.	05/1990	Chemical Engineering
University of South Florida College of Engineering, Tampa, FL	Ph.D.	12/1993	Engineering Science
University of South Florida College of Medicine	Postdoc	10/1996	Biomedical Sciences

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

The proposed research will optimize the use of a non-thermal direct current Helium plasma for the delivery of both DNA (plasmid) and a small molecule (bleomycin) to tumors *in vivo*. The plasma generating device essentially streams charge onto the surface of a tissue which imparts a measurable surface potential (voltage). This potential induces an electric field in the tissue which ultimately leads to increased cellular uptake of exogenous molecules. The physical nature of this molecular delivery method makes it broadly applicable as the basic electrical characteristics of tissues/cells are similar. This method is quite unique as an *in vivo* delivery method. My research group has been using surface charge for *in vivo* delivery since about 2002. There do not appear to be publications from other research groups relative to DNA delivery in vivo; however, several other groups have started working in this area based upon personal communications. This method was first investigated as a means to circumvent some of the shortcomings of traditional electroporation that are muscle stimulation (during pulsing), high current density at electrode tissue interfaces, tissue damage, and the need for anesthesia. The fact that no electrodes touch the tissue in the plasma method eliminates high current density. There does not appear to be any muscle stimulation and tissue damage in skin. This is likely due to the very low total electric current (microamps) as compared to electroporation (milliamps to amps).

Even though electroporation has shortcoming that appear to be addressable using plasma based delivery, electroporation has had success and is making its way into the clinic. My experience with this first electrical delivery technique began in 1992 in B16 murine melanomas established in C57Bl/6 mice. Electroporation *in vivo* relies upon electrodes with a geometry suited to the particular tumor/tissue. There were no commercially available electrodes in the early 1990's. By necessity, I have acquired extensive expertise at constructing these types of electrodes. I routinely make them for colleagues at USF and other Universities as well as for a handful of other investigators around the world. Currently, I am an inventor on 24 US patents that relate to electrodes and the methods to use them *in vivo*. Many of these have been licensed to industry for research and clinical work. Three additional issued US patents relate to surface charging devices for molecular delivery. The plasma generators used for surface charging are custom made in my lab as well. I also have substantial

experience at making custom pulse generation equipment for electroporation and systems to power plasma generators, because commercially available equipment has limited capability or is not available.

In addition to creating pulsing devices and tools for molecular delivery, I have considerable hands on experience in applying electroporation in animal models for both DNA and drug delivery. Some of these were tumor models for: melanoma, hepatocellular carcinoma, prostate cancer, rhabdomyosarcoma, renal cell carcinoma, and osteosarcoma. Many of these models required surgical access to implant tumors. Normal tissues that have been the target for delivery are: skin in mice, rats, guinea pigs; muscle in mice and rats; cardiac muscle in pigs; and liver in rats and mice. Most of the delivery experiments required follow up in the form of routine tumor measurements, histologic sampling, and blood analysis. This has resulted in 60 publications; most involve application of the technique in animal models as well as a clinical trial. The plasma method has been used primarily in murine skin for DNA delivery with limited work in murine melanoma. The procedures for plasma delivery are similar to electroporation. Therefore, I feel that I have more than adequate animal model experience to lead the experimental portion of this proposed SBIR Phase I study.

B. Positions and Honors

Positions

Associate Professor (2002-present), Department of Chemical & Biomedical Engineering, University of South Florida, Tampa, FL
Research Assistant Professor (1996-2002), Department of Surgery, Div. of Surgical Research, Univ. of South Florida College of Medicine, Tampa, FL

Honors

1987 Lifetime Member of the Tau Beta Pi Engineering Honor Society
1993 Recipient of the University of South Florida Outstanding Dissertation of the Year Award for “Mechanically Facilitated Cell-Cell Electrofusion”
2006 University of South Florida USF Outstanding Undergraduate Teaching Award for the 2005-2006 academic Year.
2015 University of South Florida Dept. of Chemical & Biomedical Engineering Biomedical Engineering Professor of the Year

C. Contribution to Science

In Vivo Delivery of Chemotherapeutic Agents to Tumors using Electroporation. I was part of a small handful of researchers worldwide that were performing *in vivo* electroporation during the early 1990’s because the collective view was that there was much potential for such a generic physical molecular delivery tool. Most of my work in the 1990’s was conducted jointly with 2 colleagues at USF and was focused on developing basic electrodes and pulsing protocols (dose of DC electricity) to deliver chemotherapeutic agents (principally bleomycin) to tumors in animal models. Other groups in the world (2 in France and 1 in Slovenia) were performing similar studies at about the same time. The resulting proof of principal studies showed that electroporation could be safely conducted *in vivo* in animal models and could result in very strong antitumor effects. My involvement in these first studies covered the full spectrum of experimental activities. I made electrodes and modified instrumentation because absolutely none were available specifically for *in vivo* use at that time. I also grew tumor cells, harvested them, and induced tumors. These were primarily B16F10 murine melanoma cells used to produce subcutaneous melanomas in C57BL/6 mice. In subsequent experiments, other tumor types were induced to provide *in vivo* tumor models. In these studies I was also actively involved in the treatment process by either inserting/applying electrodes to tumors after injecting a chemotherapeutic agent or by applying pulses while a colleague injected and positioned the electrode. I also made follow-up tumor measurements on several thousand animals. These first drug delivery studies were translated into clinical trials for the delivery of bleomycin to melanomas and basal cell carcinomas at USF. These were the first *in vivo* electroporation clinical trials in the US (second published in the world). My role in these was to make sure that the electrodes, developed at USF, and instrumentation were suitable for the tumors treated for each patient. I also operated the pulse generating equipment during all patient treatment and recorded electrical data during the process. I participated in the follow up of each tumor with a USF colleague and clinical staff. There was considerable initial skepticism from the scientific and medical communities when this work was ongoing. This skepticism gradually diminished due to work done at USF, the studies performed by a

few other key groups in the world, and a growing number of researchers in the field by the late 1990's. It is now reasonably well accepted.

a. Heller, R., Jaroszeski, M. J., Leo-Messina, J., Perrott, R., Van Voorhis, N., Reintgen, D., and Gilbert, R. (1995) Treatment of B16 mouse melanoma with the combination of electropermeabilization and chemotherapy. Bioelectrochemistry and Bioenergetics 36:83-87.

b. Heller, R., Jaroszeski, M., Glass, F., Messina, J., Rapaport, D., DeConti, R., Fenske, N., Gilbert, R., Mir, L. M., and Reintgen, D. (1996) Phase I/II trial of cutaneous and subcutaneous malignancies using electrochemotherapy. Cancer 77(5):964-971. PubMed PMID: 8608491

c. Glass, L. F., Fenske, N. A., Jaroszeski, M., Perrott, R., Harvey, D. T., Reintgen, D. S., and Heller, R. (1996) Bleomycin-mediated electrochemotherapy of basal cell carcinoma. Journal of the American Academy of Dermatology 34(1):82-6. PubMed PMID: 8543699

d. Jaroszeski, M. J., Messina, J., Perrott, R., Van Voorhis, N., Gilbert, R., and Heller, R. (1996) Enhanced effects of treating B16 melanoma with multiple treatment electrochemotherapy. Melanoma Research 6:427-433. PubMed PMID: 8915314

<u>In Vivo Delivery of DNA to Tumors and Normal Tissues Using Electroporation</u>. *In vivo* DNA delivery was initiated during the drug delivery work, mentioned above, because it was clear that there are many more applications for gene delivery relative to drug delivery. The first paper from this work was published in 1996 and focused on the delivery of reporter genes to normal rat liver. My role in this study included creating the electrode, performing surgery for liver access, delivering DNA, and harvesting tissue for analysis. This was the second known paper that employed *in vivo* electroporation for DNA delivery. Work on *in vivo* electroporation for gene delivery continued with applications to many animal models. Some were tumor bearing and others focused on normal tissues (such as liver, heart in swine, skin). It was clear from multiple animal models and tissue types that the electric pulse parameters for DNA delivery were highly dependent on tissue type which was contrary to drug delivery.

It became evident about a decade after the first *in vivo* gene electroporation papers were published, that electroporative gene delivery could be tuned to any tissue. It was also clear that real clinical applications would emerge as the number of clinical trials was increasing year by year (currently there are about 100 clinical trials for *in vivo* electroporation completed and ongoing). However, different pulsing parameters appear to be optimal for different tissue types (for gene delivery). Furthermore, animal study results from nearly all researchers show considerable variation when the same pulsing parameters were used in the same tissue type. Pulsing parameters have historically been empirically determined to arrive at conditions that result in the best mean expression results. Such data distributions mean that some animals do not receive adequate delivery. There could be many factors that influence this; however, making sure that each animal receives optimal electrical treatment would reduce variability. There has been no attempt, to the best of our knowledge, to adjust pulsing parameters to compensate for variation that occurs from animal to animal (ultimately patient to patient). A measurable parameter that can be related to the degree of electroporation could be used to customized pulse delivery to improve results. It appears that impedance spectroscopy can be used to measure the degree of electroporation and to change pulsing parameters in real time so that a desired level of electroporation is achieved. One paper has been published, below, that reflects our early work. Data from this paper and other preliminary data resulted in a funded R21 study 1R21AR061136 (Jaroszeski ,PI) that was completed in 2014. The first publications have been submitted from this work; one has been accepted (below). It is clear that in murine skin, changing pulsing parameters in real time during electroporation can improve the biological response (DNA expression levels) compared to using fixed pulsing parameters for all animals. Thus, I have been involved in in vivo gene delivery by electrical methods from the inception of field. The future will likely include some sort of real time electrical treatment customization in real time.

a. Heller, R., Jaroszeski, M., Atkin, A., Moradpour, D., Gilbert, R., Wands, J., and Nicolau, C. (1996) In vivo gene electroinjection and expression in rat liver. Federation of European Biochemical Societies (FEBS) Letters 389:225-228. PubMed PMID: 8766704

b. Heller, R., Cruz, Y., Heller, L., Gilbert, R., and Jaroszseski, M.J., (2010) Electrically Mediated Delivery of Plasmid DNA to the Skin, using a Multielectrode Array. Human Gene Therapy 21:357-362

c. Marshall, W.G., Boone, B.A., Burgos, J.D., Gografe, S.I., Baldwin, M.K., Danielson, M.L., Larson, M.J., Caretto, D.R., Cruz, Y., Ferraro, B., Heller, L.C., Ugen, K.E., Jaroszeski, M.J., and Heller, R. (2009)

Electroporation-Mediated Delivery of a naked DNA plasmid expressing VEGF to the porcine heart enhances protein expression. Gene Theapy Epub ahead of print PMID 19956270.

d. Connolly, R.J., Rey, J. I., Jaroszeski, M.J., Hoff, A.M., Lewelllyn, J.A., and Gilbert, R. (2009). Effectiveness of non-penetrating electroporation applicators to function as impedance spectroscopy electrodes. IEEE Transactions on Dielectrics and Electrical Insulation16(5): 1348-1355.

e. Atkins, R.M., Fawcett, T.J., Gilbert, R.A., Hoff, A.M., Connolly, R.C., Brown, D. W., Jaroszeski, M.J. (2016) Impedance spectroscopy as an indicator for successful in vivo electric field mediated gene delivery in a murine model. Bioelectrochemistry (Accepted for Publication)

In Vivo Application of Surface Charge for Molecular Delivery. The use a non-thermal Helium plasma was employed as a non-contact method for electrically treating tissues. It was investigated as a means to avoid the insertion of electrodes into tissue (needles) or the placement of electrodes on the target tissue surface; thus, it reduced invasiveness relative to electroporation. It did not result in muscle contractions during electrical treatment like traditional electroporation often does. Based on animal model work, it does not appear that local anesthesia will be required as with standard electroporation. Tissue damage was undetectable. It was successfully used to deliver reporter genes to skin and was also capable of inducing cellular and humoral responses when DNA encoding an antigen was delivered. These responses were higher than those resulting from optimized electroporation. Plasma has also been used to deliver plasmid DNA encoding murine erythropoietin to murine skin and resulted in increased levels of the molecule in the blood as well as increased hemoglobin levels (a surrogate for hematocrit). One preliminary study focused on delivering plasmid encoding IL-28 to established B16.F10 tumors in C57Bl/6 mice. Unoptimized plasma mediated delivery resulted in antitumor effects similar to those from optimized electroporation. Surface charge based systems have been the focus of 3 separate R21 grants for skin (Jaroszeski, PI); None have focused on tumor delivery.

a. Connolly, R.J., Lopez, G.A., Hoff, A.M., Jaroszeski, M.J. (2009) Plasma facilitated delivery of DNA to skin. Biotechnology and Bioengineering 104(5): 1034-1040 PubMed PMID: 19557830

b.Connolly, R. J., Rey, J. I., Lambert, V. M., Wegerif, G., Jaroszeski, M. J., and Ugen, K. E., (2011) Enhancement of antigen specific humoral immune responses after delivery of a DNA plasmid based vaccine through a contact-independent helium plasma. Vaccine, 29:6781-6784. PMID: 21195804.

c. Connolly, R. J., Chapman, T., Hoff, A. M., Kutzler, M. A., Jaroszeski, M. J., Ugen, K. E. (2012) Non-contact helium-based plasma for delivery of DNA vaccines: Enhancement of humoral and cellular immune responses. Human Vaccines and Immunotherapeutics, 8 (11). PMID: 22894954

d. Connolly RJ, Hoff AM, Gilbert R, Jaroszeski MJ. Optimization of a plasma facilitated DNA delivery method. Bioelectrochemistry. 2015 Jun;103:15-21. doi: 10.1016/j.bioelechem.2014.09.003. Epub 2014 Oct 13.PMID:25455213

e. Shah K, Connolly RJ, Chapman T, Jaroszeski MJ, Ugen KE. Electrogenetherapy of B16.F10 murine melanoma tumors with an interleukin-28 expressing DNA plasmid. Hum Vaccin Immunother. 2012 Nov 1;8(11):1722-8. PMID:23151446

f. Connolly, R.J., Hoff, A.M., Gilbert, R., and Jaroszeski, M.J. (2015) Optimization of a plasma facilitated DNA delivery method. Bioelectrochemistry 13;103:15-21 Epub Oct. 13, 2014. PMID:25455213 PMCID:PMC4346600.

The public URL to a full list of my published work is: http://www.ncbi.nlm.nih.gov/sites/myncbi/mark j.jaroszeski.1/bibliograpahy/40928163/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support
None

Completed

1R21AR061136 (Jaroszeski ,PI) 02/17/11-6/30/14
NIH/NIAMS
Impedance Changes as an indicator of Successful
Skin Electroporative DNA Delivery
The focus of this study is to identify tissue impedance changes that correlate with successful electroporation and use those measureable changes to improve the electroporation process in skin.

1R21AI090561-01 (Jaroszeski, PI) 07/05/10-06/30/13
NIH/NIAID
Topical Charge Driven DNA Delivery to the Skin
This grant will investigate a combination of transdermal delivery and intradermal delivery in one delivery process driven by charge deposited on the surface of a small volume of DNA solution located on the skin surface

1R21AI079706-01A2 (Jaroszeski, PI) 08/05/10-07/31/13
NIH/NIAID
Development of Streamed Ion Deposition for Efficient Plasmid DNA Delivery
This grant will investigate the feasibility of a novel method for delivering DNA to the skin. The method involves a traditional intradermal injection (standard hypodermic needle) of DNA into the skin followed by the application of plasma charge to the surface of the injected skin as a means for delivering DNA.