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## Cardioporation Enhances Myocardial Gene Expression in Rat Heart

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### ABSTRACT

Damage from myocardial infarction (MI) and subsequent heart failure are serious public health concerns. Current clinical treatments and therapies to treat MI damage largely do not address the regeneration of cardiomyocytes. In a previous study, we established that it is possible to promote regeneration of cardiac muscle with vascular endothelial growth factor B gene delivery directly to the ischemic myocardium. In the current study we aim to optimize cardioporation parameters to increase expression efficiency by varying electrode configuration, applied voltage, pulse length, and plasmid vector size. By using a surface monopolar electrode, optimized pulsing conditions and reducing vector size, we were able to prevent ventricular fibrillation, increase survival, reduce tissue damage, and significantly increase gene expression levels.

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### 1. Introduction

An estimated of 92.1 million adults have at least one type of cardiovascular disease (CVD) in the United States. In 2013, the most common underlying reason of death was reported as CVD [1]. The first leading cause of death remained the same in the recent years (2015–2016) as heart disease and 17.6 million deaths were attributed to CVD globally [2], until Covid-19. Myocardial infarction (MI) occurs in about 790,000 people in the United States every year. 580,000 of these are a first time MI and 210,000 recorded from the cases who already had a first MI [1]. The 5-year survival rate remains low [1]. Unfortunately, the heart has insufficient regenerative capacity; cardiomyocytes do not return. Instead, cardiac fibroblast proliferation, infiltration and creation of collagenous, non-contractile scar tissue replaces cardiomyocytes, resulting in a thin myocardial wall unable to pump enough blood to meet the demand and leading to lower quality of life and heart failure [3]. Current clinical therapies do not regenerate cardiac myocardium [4,5], however cardiomyocyte proliferation is possible [6].

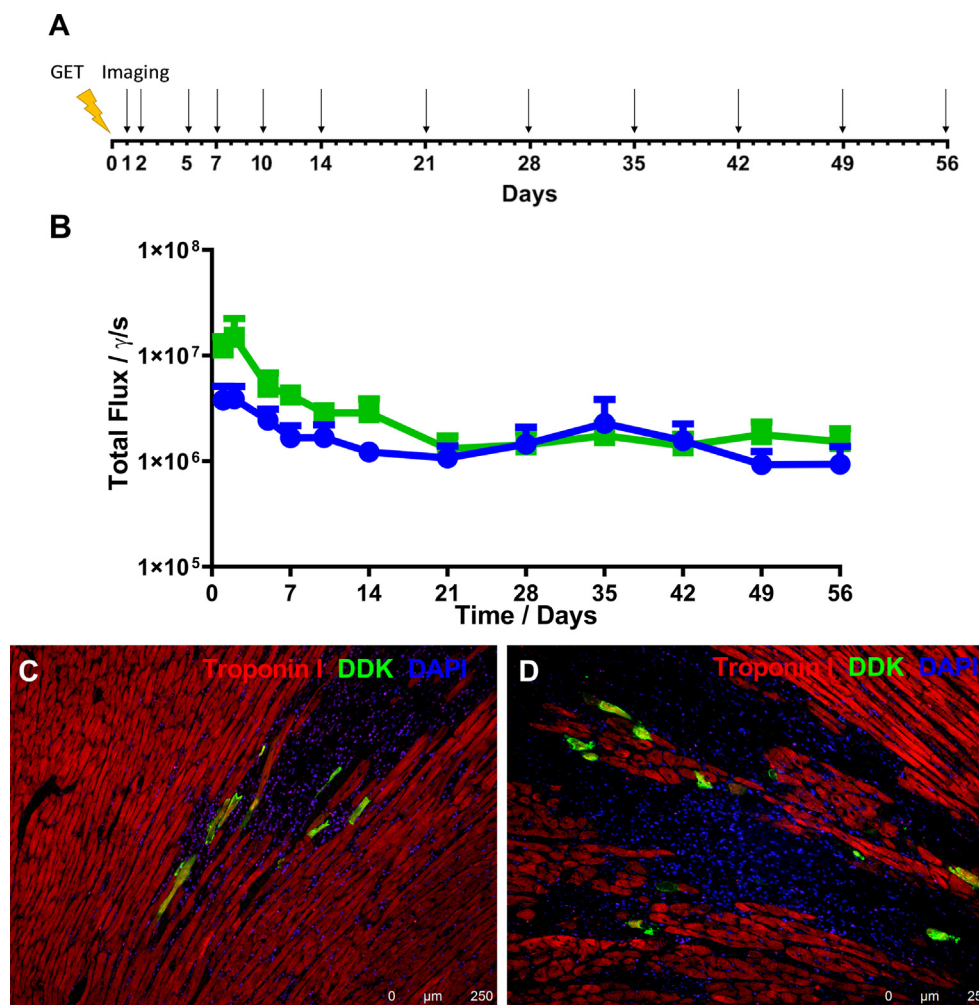
Current conventional therapies for acute MI includes pharmacological agents such as beta blockers, statins, and angiotensin-

converting-enzyme inhibitors. These are useful in mitigating MI symptoms and modestly improve long term prognosis but are unable to repair the infarcted tissue. Because the cardiac dysfunction remains an issue, there is a great scientific research effort being spent on finding new approaches focusing on stimulating angiogenesis, promoting myocardial regeneration, and further improving the clinical outcomes [4,5].

On-going research to regenerate cardiomyocytes includes many different methods, though cell and gene therapies are some of the most common methods. Cell therapies have been approved for clinical trials, however cell loss can occur within days and thus a large number of cells are required [5]. One of the most successful studies within this category required very high cell injection dose (100 million cells) and resulted in modest cardiac performance ( $\approx 4\%$  EF) [5]. While currently there are many clinical trials ongoing, cell therapies for treatment of MI are still being optimized, including the inclusion of biomaterials and/or proteins such as growth factors to aid the therapy [7,8].

Gene therapies (and more widely, growth factor therapies) are another commonly researched method to treat MI. Briefly, gene therapies for MI have not yet been approved [9]. Many target genes have been investigated, including VEGF-A, which was able to induce angiogenesis, however the resulting vasculature was not fully functional [9]. Recent studies, however, have showed

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**Fig. 1.** Intramyocardial injection of 100  $\mu L$  of plasmid DNA significantly damages myocardium. (A) Experimental timeline, with  $\bullet$  cardioporation or  $\blacksquare$  injection only on day 0, and imaging on days 1, 2, 5, 7, 10, 14, 21, 28, 35, 42, 49 and 56. (B) No significant differences in expression were observed over 56 days (note background subtraction was performed, thus there are detectable levels of expression through day 56). Immunofluorescence staining for DDK tag, and counterstaining for cardiac troponin I, show similar expression distribution, as well as notable damage from plasmid DNA injections of 100  $\mu L$  bolus intramyocardial injection.

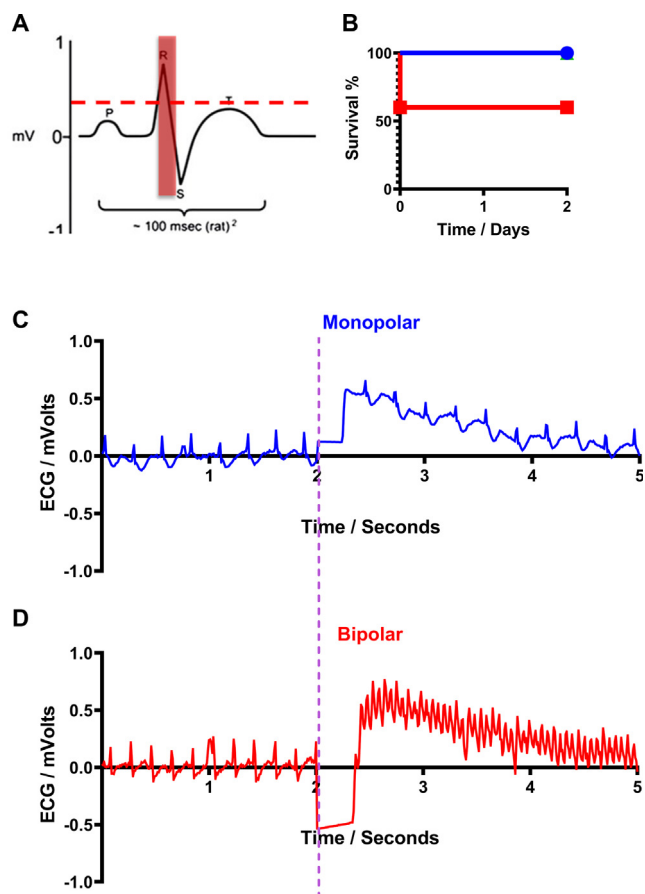
promising preliminary results using vascular endothelial growth factor B (VEGF-B), though more investigation in larger animal models is warranted [10,11]. Currently, the biggest challenge facing gene therapy for cardiac applications is seemingly the delivery of the transgenes [9]. Viral vectors have safety concerns and can induce unwanted effects such as inflammation [9]. Non-viral gene delivery methods, including naked plasmid delivery, tend to have gene expression levels too low to be considered clinically therapeutic [9]. In summary, delivery methods of gene therapy need to be improved and then tested in the appropriate models before gene therapy can be utilized for the treatment of MI [9].

In a previous study, we determined that gene electrotransfer to ischemic myocardium could deliver plasmid DNA encoding VEGF-B, which in turn induced cardiac myocyte proliferation and renewal at the site of myocardial ischemia [10]. One challenge that we discovered in our previous work, is that while cardiomyogenesis post ischemia can be induced in detectable quantities, these quantities are still very low, and not clinically useful. Therefore, the aim of the current work, is to improve exogenous gene expression levels in the heart.

Gene electrotransfer is an established physical method for gene delivery to various tissues of the body including the heart [10,12–14]. Typically, optimal gene expression parameters are determined empirically, and vary from tissue to tissue. Parameters that work

for gene delivery to the skin, may not work for gene delivery to the skeletal muscle and vice versa [14–19]. We introduce a new term – cardioporation – defined specifically as gene electrotransfer mediated delivery to the myocardium. Cardioporation was first attempted in a beating pig heart, and further studied in rat beating hearts [10,12,13,20,21]. Current study extensively explores different parameters that lead to improved protein expression levels of reporter genes, while minimizing tissue damage, ventricular fibrillation, and maximizing delivery efficiency.

Previous studies focused on cardioporation feasibility, and therefore little optimization work was done. Myocardial damage assessment has not been reported. Cardioporation included using custom built 4-needle bipolar electrodes and injecting plasmid DNA and the electrode needles directly into the beating myocardium [10,12,13]. These bipolar electrodes work similarly to bipolar cauteries, where the active and return electrodes are placed closely so that the electric field is applied locally within or around the tissue of interest [22]. Similar to cauteries, monopolar electrode options exist for GET, where the active electrode is placed on the tissue of interest, and the return electrode is a plate placed somewhere else on the body [22]. In the current study, we have assessed less invasive options including a surface, monopolar electrode, pulsing parameters, plasmid DNA injection technique, and plasmid vector size. Resulting cardioporation method is efficient in reporter



**Fig. 2.** Monopolar electrode reduces ventricular fibrillation and associated survival. (A) Diagram showing a typical ECG pattern for rats. The shaded area indicates when the beginning of each pulse was delivered, synchronized to the absolute refractory period of the ECG. (B) Survival was significantly reduced for the ■ bipolar group when compared to the ● monopolar and ▲ injection only groups, due to ventricular fibrillation. (C) Representative ECG for — monopolar electrode group during a single pulse. (D) Representative ECG for — bipolar electrode group during a single pulse, the purple dashed line indicates the start of a pulse.

gene delivery to a beating heart in a rat model. Future work will focus on therapeutic gene delivery and cardiomyocyte regeneration.

## 2. Materials and methods

### 2.1. Animals

All rat studies followed an approved Old Dominion University Institutional Animal Care and Use Committee protocol, in accordance with the Guide for the Care and Use of Laboratory Animals at an AAALAC accredited facility. Animals were quarantined and acclimated for a 7-day period before any procedures were conducted and maintained in a specific pathogen-free condition in the host laboratory.

Sprague-Dawley male rats weighing an average of 500 g were obtained from Charles River Laboratories (Worcester, MA, USA).

### 2.2. Plasmid

Plasmid DNA containing a transgene encoding firefly luciferase and tagged with the myc and DDK tags was used. This transgene was purchased inserted into two different vectors: gWiz (Aldevron) and NTC9385R (Nature Technologies). Plasmid DNA was sus-

pended in sterile saline at 2 mg/mL. The endotoxin levels in both plasmids were  $<0.1$  EU/ $\mu$ g plasmid, confirmed by Aldevron and Nature Technologies.

### 2.3. Surgical procedure

Anesthesia was initiated with 3% isoflurane inhalation in the chamber and then animals were intubated with 16G IV catheter. Respiration was maintained with a volume-controlled ventilator at 70 breaths per minute. 2–3% isoflurane was used to maintain anesthesia. A heated surgery table was used throughout the procedure to support body temperature. Cardiac activity was monitored the entire time with a three-lead electrocardiogram (Accusync Medical Research Company, Milford, CT). Sterile technique was used throughout the surgery and a 14-day prophylactic antibiotic course of Trimethoprim Sulfa (SMZ-TMP) was given orally to prevent infection.

The left ventricle of heart was exposed with a left thoracotomy of the fifth intercostal space. After intramyocardial plasmid DNA injections and gene transfer, the thoracic cavity was closed with size 3-0 Monocryl suture with interrupted pattern. Any extra air might have remained in the thoracic cavity was pulled out with a 22G IV catheter to avoid pneumothorax. The muscle, skin and connective tissue layers were closed with 5-0 Monocryl sutures. Animals were then allowed to recover and continued to be monitored until they were ambulatory. Carprofen was administered before the surgery and given up to 48 h post operatively.

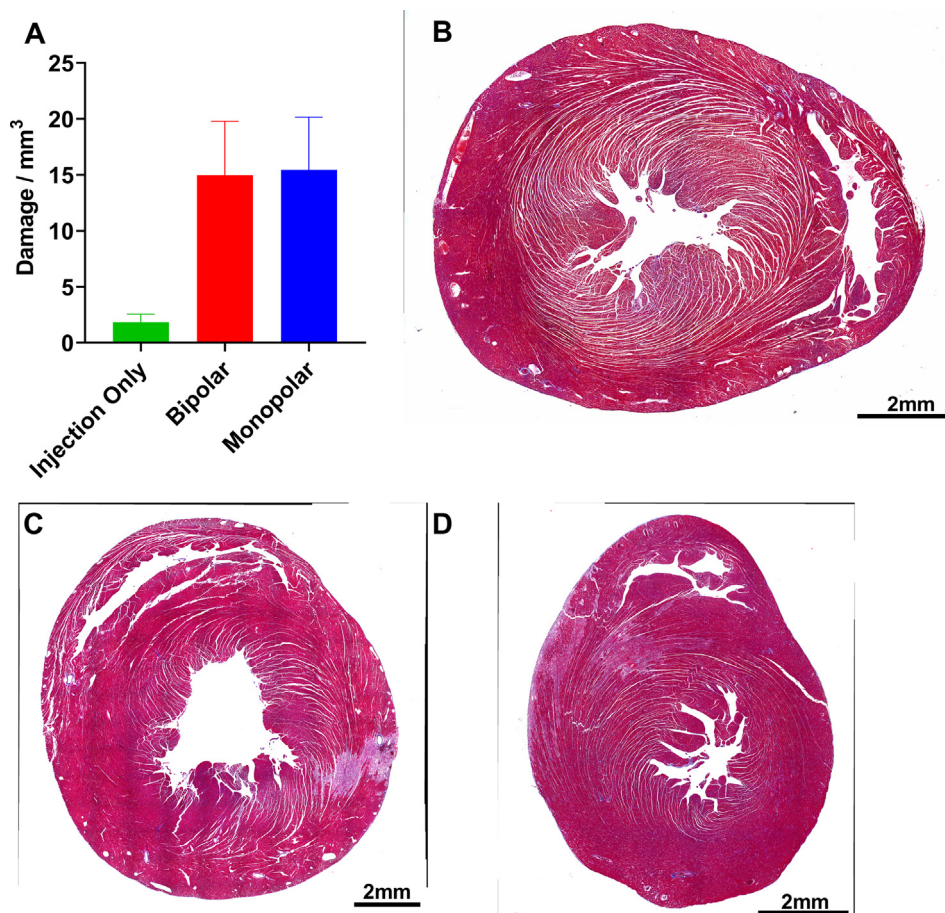
### 2.4. Gene electrotransfer

Luciferase encoding plasmid DNA was used to determine success and quantity of gene expression. Animals were randomly assigned to the groups for either pDNA injection + Gene Electro-Transfer (GET) or pDNA injection only (IO). For the initial stages of the study, the gWizLucMycDDK plasmid was used. In the Nanoplasmid™ portion of the study NTC9385R-Luc-Myc-DDK was used.

Initially (Fig. 1), we used the same injection protocol as in our previous studies injecting 100  $\mu$ L plasmid DNA in one bolus into the left ventricular myocardial wall, using a 30G needle on a 0.5 mL Monoject™ syringe. After the observations reported in Fig. 1, we reduced the injection volume to 3 injections of 30  $\mu$ L, thus eliminating damage to the myocardium from the injection.

Cardioporation was performed using either a traditional bipolar electrode with 4 needles that is inserted into the left ventricular myocardium, or a 1 cm<sup>2</sup> square platinum flat surface non-invasive monopolar electrode, which requires a large grounding plate, to be placed on the opposite flank (Supplemental Fig. 1). The monopolar and bipolar groups described in this paper are differentiated by the use of either electrode. Further information regarding these electrodes is provided in another previously published paper [23].

For GET groups using the bipolar 4-needle electrode, the needles were inserted into the myocardium of the ventricle. For GET groups using the monopolar electrode, the electrode was then placed onto the myocardial wall over the injection site. All pulses were synchronized with the rise of the R-wave of the ECG. The IO group received no electric pulses. For the bipolar needle electrode group, 4 pulses were delivered at 60 V with a 20 ms pulse length. For the monopolar groups, pulsing parameters varied. The starting parameters (shown in Fig. 1), the applied voltage was 200 V, for 20 ms pulse length, with 4 pulses, as to mimic the previously established parameters for bipolar electrodes [10]. Following damage and delivery assessments, monopolar condition was altered to 4 pulses with 120 V applied voltage, and 100 ms pulse length (shown in Figs. 3 and 4). Finally, further analysis led to



**Fig. 3.** Cardioporation induces minimal damage to myocardium. (A) Damage assessment shows no significant difference between all three groups. Example trichrome staining for IO (B), Bipolar electrode (C) and Monopolar electrode (D).

monopolar cardioporation pulsing parameters of 90 V applied, 100 ms pulse length with 4 pulses and Nanoplasmid™ backbone (Fig. 5).

### 2.5. Bioluminescence imaging

Animals were imaged for luciferase expression with the In-Vivo Imaging System (IVIS) at 1, 2, 5, 7, 10, 14, 21, 28, 35, 42, 49, and 56 days after surgery (Fig. 1). For subsequent experiments, imaging was performed on days 1 and 2, when peak expression is expected. Animals were anesthetized with 3% isoflurane and 150 mg/kg D-Luciferin, Potassium Salt (GoldBio, Cat#: LUCK) was applied subcutaneously. Animals were imaged every 5 min until the peak luciferase-luciferin reaction was achieved and then returned to the cages and observed until they were fully recovered from the anesthesia.

### 2.6. Immunofluorescence imaging

Hearts were harvested 48 h after surgery following the rats being humanely euthanized, washed with saline and fixed in 4% paraformaldehyde. After appropriate time of fixation, ventricular portions of the hearts were sliced in 1.5 mm thickness and sent to Idexx BioResearch (Columbia, MO) for paraffin embedding, cutting, hematoxylin & eosin and Masson's Trichrome staining.

Unstained serial sections were then processed for immunofluorescence (IF) staining for DDK and cardiac-Troponin I counterstaining. Briefly, slides were deparaffinized with Citrisolv clearing agent (FisherScientific, Cat: 22-143-975) and rehydrated in EtOH

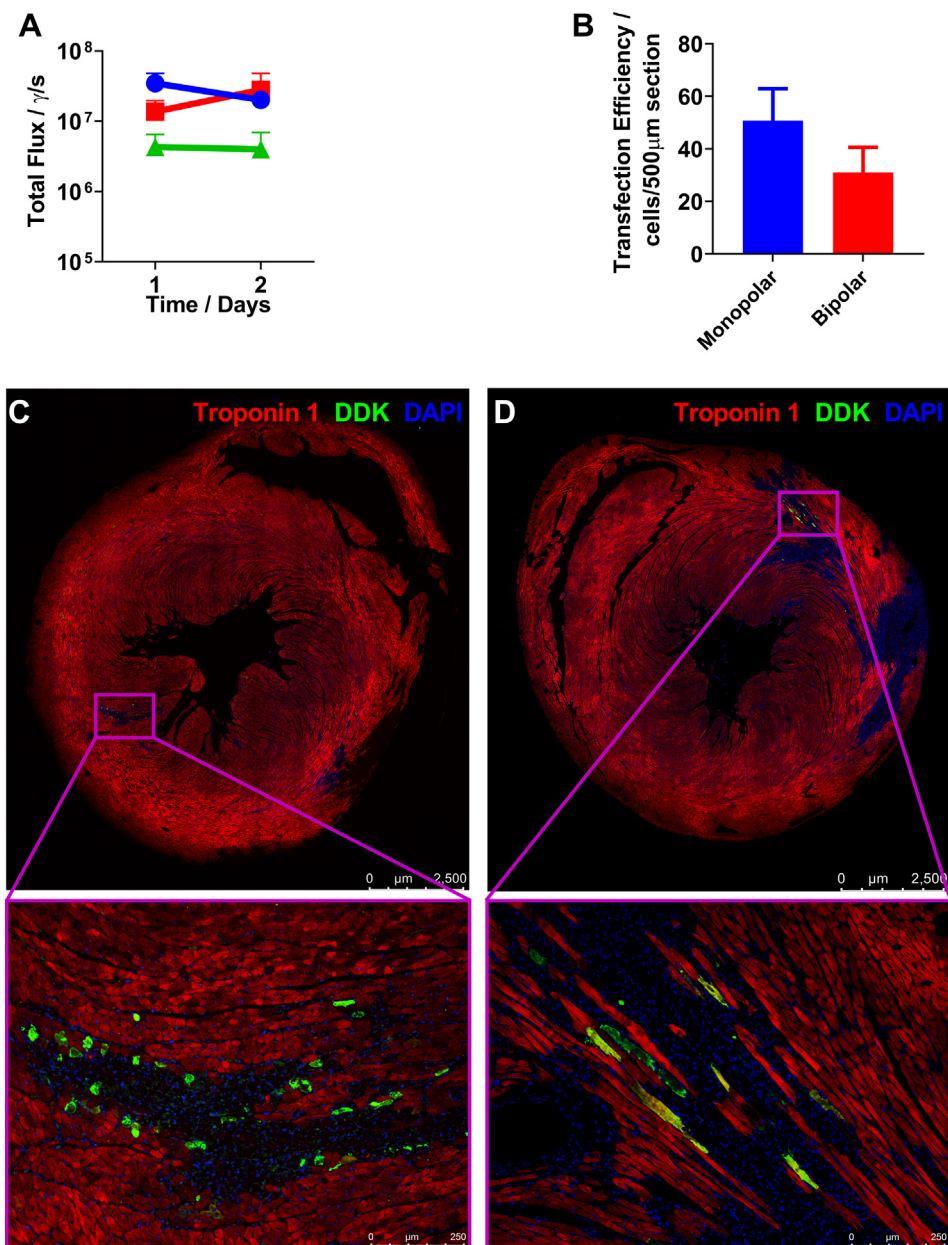
gradient series. Slides then buffered in PBST (BioWorld, Cat: 41620020-1), permeabilized with 0.25 %Triton-X and boiled with AR6 Buffer (Perkin Elmer, Cat: AR6) for antigen retrieval. Then the samples were blocked with 4% bovine serum albumin (Fisher Scientific, Cat: BP1605100) and incubated with rabbit polyclonal primary antibody against DDK (OriGene Technologies, Cat: TA150078) and mouse monoclonal antibody against rat Cardiac Troponin I (Abcam, Cat: ab10231) overnight at a 1:200 dilution.

Slides were washed the following day with PBST and blocked again in 4% BSA. Secondary antibodies of highly cross adsorbed Alexa Fluor 488 conjugated, goat anti-Rabbit IgG (Invitrogen, Cat: A-11034) for DDK tag, and highly cross adsorbed Alexa Fluor 546 conjugated, Goat anti-Mouse IgG (Invitrogen, Cat: A-11030) for Troponin I were used in addition to Spectral-DAPI (Perkin Elmer, Cat: FP1490). Only secondary antibody was applied to the negative control samples, without primary antibody. After 2 h of incubation at ambient temperature, slides were then washed with PBST and mounted with Vectashield hardset antifade mounting medium (Vector Laboratories, Cat: H-1400).

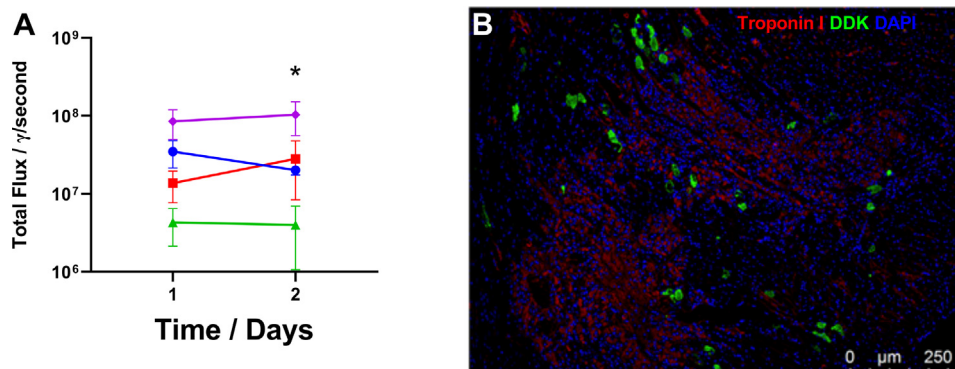
Following IF staining, slides were imaged and analyzed with a Leica DMI8 microscope.

## 3. Results

Initially, a 100  $\mu$ L bolus injection into the ventricular myocardium was followed by 4 pulses of conditions of 200 V, and 20 ms long, administered via the monopolar electrode. These conditions were based on interpolation of previous data achieved with a 4-needle bipolar electrode [10]. There was no significant difference



**Fig. 4.** Gene expression levels, transfection efficiency, and distribution measured by bioluminescence imaging and IF show minimal difference between monopolar and bipolar cardioporation. Gene expression levels per group (● monopolar, ■ bipolar, and ▲ injection only) measured by bioluminescence imaging (A). Transfection efficiency measured by cell counts from IF data,  $p < 0.026$  (B). IF staining within the myocardium for the monopolar (C) and 4-needle bipolar electrodes (D).



**Fig. 5.** Reduction of vector backbone size significantly improved gene expression levels. Gene expression levels as measured by bioluminescence (◆ Nanoplasmid™, ● monopolar, ■ bipolar, and ▲ injection only groups, A). IF of the myocardium from the Nanoplasmid™ group (B).

in gene expression levels between monopolar cardioporation and injection only groups (Fig. 1). There was considerable damage to the myocardium 2 days after gene delivery, observed in trichrome images, which occupied nearly one half of the ventricular wall (data not shown). The damage was present in both IO and Monopolar cardioporation groups, thus was attributed to plasmid DNA injection.

For the next set of experiments, the plasmid DNA injection was adjusted to 3 injections of 30  $\mu$ L, which resulted in no significant damage to the myocardium (Fig. 3A-B). We directly compared the bipolar 4-needle electrode condition (60 V, 20 ms, 4 pulses) to the monopolar electrode pulsing conditions of 120 V applied, 100 ms pulse length with 4 pulses. The pulses of the bipolar and monopolar electrode groups were synchronized with the rise of the R-wave of the ECG (Fig. 2). The monopolar electrode and IO groups had 100% survival rates when compared to the bipolar electrode group. This was largely due to ventricular fibrillation during pulsing that was observed only the bipolar electrode group. Ventricular fibrillation was not observed at all for any of the monopolar conditions used for this study. Fig. 2B-C shows a typical ECG signal following one pulse administration with the bipolar and monopolar electrodes, with ventricular fibrillation observed for the bipolar group.

Damage assessment from tiled trichrome images of heart sections harvested on day 2 showed no significant difference in terms of tissue damage between the monopolar, the bipolar and the IO groups (Fig. 3), based on one-way ANOVA. There was a significant difference in gene expression levels measured by bioluminescence between the monopolar conditions, bipolar conditions or injection only conditions (Fig. 4A). However, in terms of transfection efficiency, measured by the number of DDK positive cardiomyocytes (Fig. 4B-D), the monopolar group had significantly higher transfection efficiency.

A 2-fold smaller plasmid vector (Nanoplasmid™) that was engineered to provide high expression, particularly when compared to the gWiz vector [24]. When measured by bioluminescence, the Nanoplasmid™ vector delivered by monopolar cardioporation (90 V, 100 ms pulses, 4 pulses) had significantly higher levels of gene expression than IO, gWiz vector delivering the same insert with a monopolar configuration (Fig. 5).

#### 4. Discussion

We would like to report that while it is common to report a 100  $\mu$ L injection into rat ventricular myocardium, according to our findings, there injections cause significant damage to the myocardium, may skew results and should be avoided. Fig. 1 indicates that while naked plasmid DNA injection leads to some expression in ventricular myocardium, the damage caused by the large volume of the injection, masked any potential benefit from cardioporation for efficient gene delivery. Once damage from the injection itself was mitigated, monopolar cardioporation optimization could readily be evaluated.

Our results suggest that the monopolar electrode configuration was able to address the issue of ventricular fibrillation that was present when using the bipolar 4-needle electrode configuration. This resulted in 100% survival in the monopolar electrode group, while the bipolar electrode resulted in a drop in survival due to induction of ventricular fibrillation (Fig. 2). Damage from bipolar and monopolar cardioporation was minimal as can be seen in representative trichrome images (Fig. 3B-D) and not significantly higher than IO (Fig. 3A) as evaluated by a one-way ANOVA.

The gene expression results (Fig. 4) results with the gWizLuc-MycDDK plasmid show no significant difference between the GET and IO groups in gene expression levels. The transfection efficiency

is significantly higher with the monopolar electrode use. To address marginal differences in gene expression, we utilized a plasmid DNA backbone engineered to provide higher gene expression levels, by reducing the size of the vector [24]. The Nanoplasmid™ was able to achieve significantly higher gene expression levels at day 2 despite the lower voltage used when compared to the gWizLucMycDDK group (Fig. 5). This is beneficial, as lowering voltage during GET has been shown to reduce tissue damage [25]. For further research using therapeutic genes, we plan to use the same Nanoplasmid™ vector to deliver VEGF-B. By utilizing both the Nanoplasmid™ and monopolar electrode, we were able to optimize cardioporation in terms of safety from ventricular fibrillation and tissue damage, as well as achieving enhanced levels of gene expression.

#### 5. Conclusion

We have improved many parameters of our cardioporation method for efficient gene delivery to the myocardium. The monopolar cardioporation protocol was shown safer than previously optimized protocol utilizing a bipolar 4-needle electrode, with no observed ventricular fibrillation occurring. Monopolar cardioporation further showed comparable expression levels and minimal tissue damage. The monopolar configuration is, therefore, advantageous over the traditional bipolar approach and may expand clinical utility in the heart. Additionally, by reducing the plasmid vector size, we were able to further improve gene expression levels, while further lowering the applied voltage. Monopolar cardioporation of nanoplasmid DNA is thus a more efficient, safer and potentially more effective method for gene electrotransfer to the heart, which presents opportunity for effector gene delivery to induce therapeutic cardiac regeneration in the future.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioelechem.2021.107892>.

#### References

- [1] E.J. Benjamin, M.J. Blaha, S.E. Chiuve, M. Cushman, S.R. Das, R. Deo, S.D. de Ferranti, J. Floyd, M. Fornage, C. Gillespie, C.R. Isasi, M.C. Jimenez, L.C. Jordan, S. E. Judd, D. Lackland, J.H. Lichtman, L. Lisabeth, S. Liu, C.T. Longenecker, R.H. Mackey, K. Matsushita, D. Mozaffarian, M.E. Mussolino, K. Nasir, R.W. Neumar, L. Palaniappan, D.K. Pandey, R.R. Thiagarajan, M.J. Reeves, M. Ritchey, C.J. Rodriguez, G.A. Roth, W.D. Rosamond, C. Sasson, A. Towfighi, C.W. Tsao, M.B. Turner, S.S. Virani, J.H. Voeks, J.Z. Willey, J.T. Wilkins, J.H. Wu, H.M. Alger, S.S. Wong, P. Muntner, C. American Heart Association Statistics, S. Stroke Statistics, Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association, *Circulation* 135(10) (2017) e146–e603.
- [2] E.J. Benjamin, P. Muntner, A. Alonso, M.S. Bittencourt, C.W. Callaway, A.P. Carson, A.M. Chamberlain, A.R. Chang, S. Cheng, S.R. Das, F.N. Delling, L. Djousse, M.S.V. Elkind, J.F. Ferguson, M. Fornage, L.C. Jordan, S.S. Khan, B.M. Kissela, K.L. Knutson, T.W. Kwan, D.T. Lackland, T.T. Lewis, J.H. Lichtman, C.T. Longenecker, M.S. Loop, P.L. Lutsey, S.S. Martin, K. Matsushita, A.E. Moran, M.E. Mussolino, M. O'Flaherty, A. Pandey, A.M. Perak, W.D. Rosamond, G.A. Roth, U.



- K.A. Sampson, G.M. Satou, E.B. Schroeder, S.H. Shah, N.L. Spartano, A. Stokes, D. L. Tirschwell, C.W. Tsao, M.P. Turakhia, L.B. VanWagner, J.T. Wilkins, S.S. Wong, S.S. Virani, E. American Heart Association Council on, C. Prevention Statistics, S. Stroke Statistics, Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association, *Circulation* 139(10) (2019) e56–e528.
- [3] C.L. Hastings, E.T. Roche, E. Ruiz-Hernandez, K. Schenke-Layland, C.J. Walsh, G. P. Duffy, Drug and cell delivery for cardiac regeneration, *Adv. Drug Deliv. Rev.* 84 (2015) 85–106.
- [4] S. Pascual-Gil, E. Garbayo, P. Díaz-Herráez, F. Prosper, M.J. Blanco-Prieto, Heart regeneration after myocardial infarction using synthetic biomaterials, *J. Control. Release* 203 (2015) 23–38.
- [5] K.M. Fish, K. Ishikawa, R.J. Hajjar, Stem cell therapy for acute myocardial infarction: on the horizon or still a dream?, *Coron Artery Dis.* 29 (2) (2018) 89–91.
- [6] O. Bergmann, R.D. Bhardwaj, S. Bernard, S. Zdunek, F. Barnabe-Heider, S. Walsh, J. Zupicich, K. Alkass, B.A. Buchholz, H. Druid, S. Jovinge, J. Frisen, Evidence for cardiomyocyte renewal in humans, *Science (New York, N.Y.)* 324 (5923) (2009) 98–102.
- [7] M. Rheault-Henry, I. White, D. Grover, R. Atoui, Stem cell therapy for heart failure: medical breakthrough, or dead end?, *World J Stem Cells* 13 (4) (2021) 236–259.
- [8] M. Mazzola, E. Di Pasquale, Toward cardiac regeneration: combination of pluripotent stem cell-based therapies and bioengineering strategies, *Front. Bioeng. Biotechnol.* 8 (2020) 455, 455.
- [9] A. Cannatà, H. Ali, G. Sinagra, M. Giacca, Gene therapy for the heart lessons learned and future perspectives, *Circ. Res.* 126 (10) (2020) 1394–1414.
- [10] A.A. Bulysheva, N. Burcus, C.G. Lundberg, M.P. Francis, R. Heller, VEGF-B electrotransfer mediated gene therapy induces cardiomyogenesis in a rat model of cardiac ischemia, *Bioelectrochemistry* 124 (2018) 105–111.
- [11] M. Räsänen, I. Sultan, J. Paech, K.A. Hemanthakumar, W. Yu, L. He, J. Tang, Y. Sun, R. Hlushchuk, X. Huan, E. Armstrong, O.-Z. Khoma, E. Mervaala, V. Djonov, C. Betsholtz, B. Zhou, R. Kiveliä, K. Alitalo, VEGF-B promotes endocardium-derived coronary vessel development and cardiac regeneration, *Circulation* 143 (1) (2021) 65–77.
- [12] A.A. Bulysheva, B. Hargrave, N. Burcus, C.G. Lundberg, L. Murray, R. Heller, Vascular endothelial growth factor-A gene electrotransfer promotes angiogenesis in a porcine model of cardiac ischemia, *Gene Ther.* 23 (8-9) (2016) 649–656.
- [13] B. Hargrave, R. Strange, S. Navare, M. Stratton, N. Burcus, L. Murray, C. Lundberg, A. Bulysheva, F. Li, R. Heller, X.-L. Ma, Gene electro transfer of plasmid encoding vascular endothelial growth factor for enhanced expression and perfusion in the ischemic swine heart, *PLoS One* 9 (12) (2014) e115235.
- [14] R. Heller, M. Jaroszeski, A. Atkin, D. Moradpour, R. Gilbert, J. Wands, C. Nicolau, In vivo gene electroinjection and expression in rat liver, *FEBS Lett.* 389 (3) (1996) 225–228.
- [15] L.C. Heller, M.J. Jaroszeski, D. Coppola, A.N. McCray, J. Hickey, R. Heller, Optimization of cutaneous electrically mediated plasmid DNA delivery using novel electrode, *Gene Ther.* 14 (3) (2007) 275–280.
- [16] R.A. Gilbert, M.J. Jaroszeski, R. Heller, Novel electrode designs for electrochemotherapy, *Biochim. Biophys. Acta* 1334 (1) (1997) 9–14.
- [17] M.J. Jaroszeski, R. Gilbert, C. Nicolau, R. Heller, Delivery of genes in vivo using pulsed electric fields, *Methods Mol. Med.* 37 (2000) 173–186.
- [18] M.L. Lucas, L. Heller, D. Coppola, R. Heller, IL-12 plasmid delivery by in vivo electroporation for the successful treatment of established subcutaneous B16. F10 melanoma, *Mol. Ther.: J. Am. Soc. Gene Ther.* 5 (6) (2002) 668–675.
- [19] B. Ferraro, Y.L. Cruz, D. Coppola, R. Heller, Intradermal delivery of plasmid VEGF(165) by electroporation promotes wound healing, *Mol. Ther.: J. Am. Soc. Gene Ther.* 17 (4) (2009) 651–657.
- [20] W.G. Marshall, B.A. Boone, J.D. Burgos, S.I. Gografe, M.K. Baldwin, M.L. Danielson, M.J. Larson, D.R. Caretto, Y. Cruz, B. Ferraro, L.C. Heller, K.E. Ugen, M.J. Jaroszeski, R. Heller, Electroporation-mediated delivery of a naked DNA plasmid expressing VEGF to the porcine heart enhances protein expression, *Gene Ther.* 17 (3) (2010) 419–423.
- [21] B. Hargrave, H. Downey, R. Strange, L. Murray, C. Cinnamon, C. Lundberg, A. Israel, Y.-J. Chen, W. Marshall, R. Heller, Electroporation-mediated gene transfer directly to the swine heart, *Gene Ther.* 20 (2) (2013) 151–157.
- [22] I. Cordero, Electro-surgical units - how they work and how to use them safely, *Commun. Eye Health* 28 (89) (2015) 15–16.
- [23] A. Bulysheva, L. Heller, M. Francis, F. Varghese, C. Boye, R. Heller, Monopolar gene electrotransfer enhances plasmid DNA delivery to skin, *Bioelectrochemistry* 140 (2021) 107814.
- [24] J.A. Williams, Vector design for improved DNA vaccine efficacy, safety and production, *Vaccines (Basel)* 1 (3) (2013) 225–249.
- [25] J.D. Schertzer, D.R. Plant, G.S. Lynch, Optimizing plasmid-based gene transfer for investigating skeletal muscle structure and function, *Mol. Ther.* 13 (4) (2006) 795–803.