미토콘드리아 이식치료의 현황과 전망
-심근경색증을 중심으로

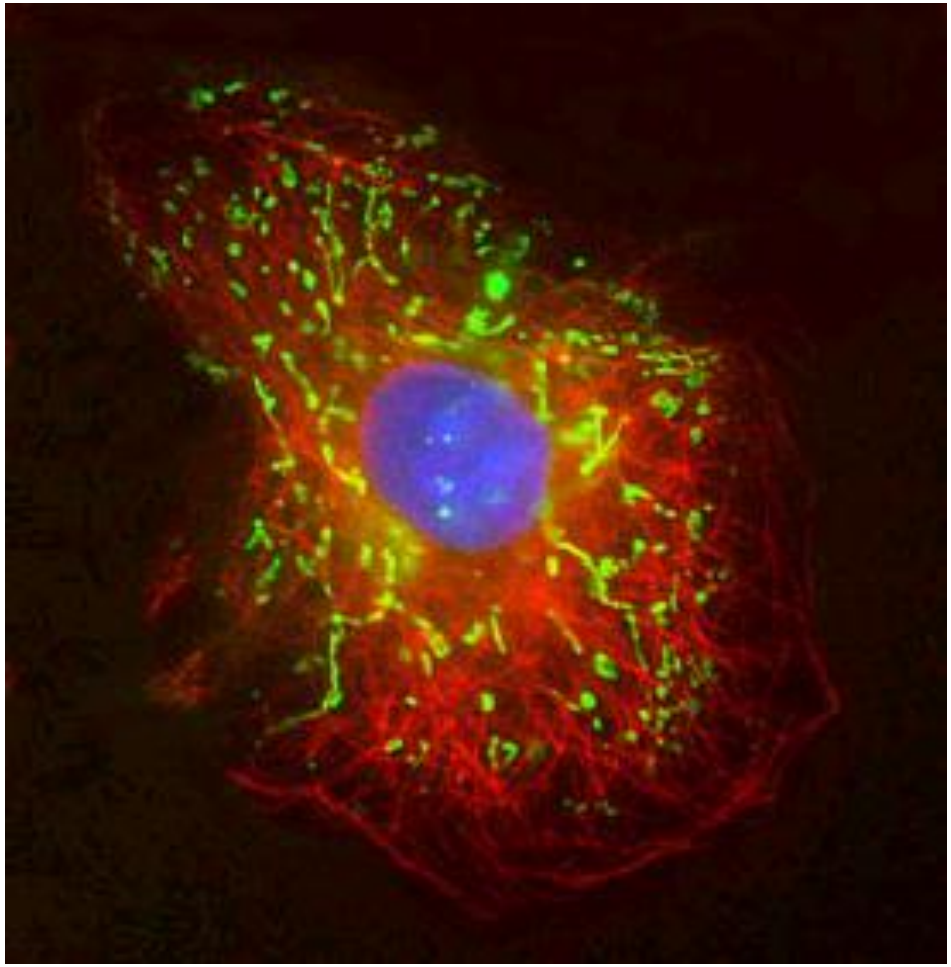
을지대학 을지병원

내과 이 홍규

2019. 12. 14
For Healing Heart Symposium, Busan

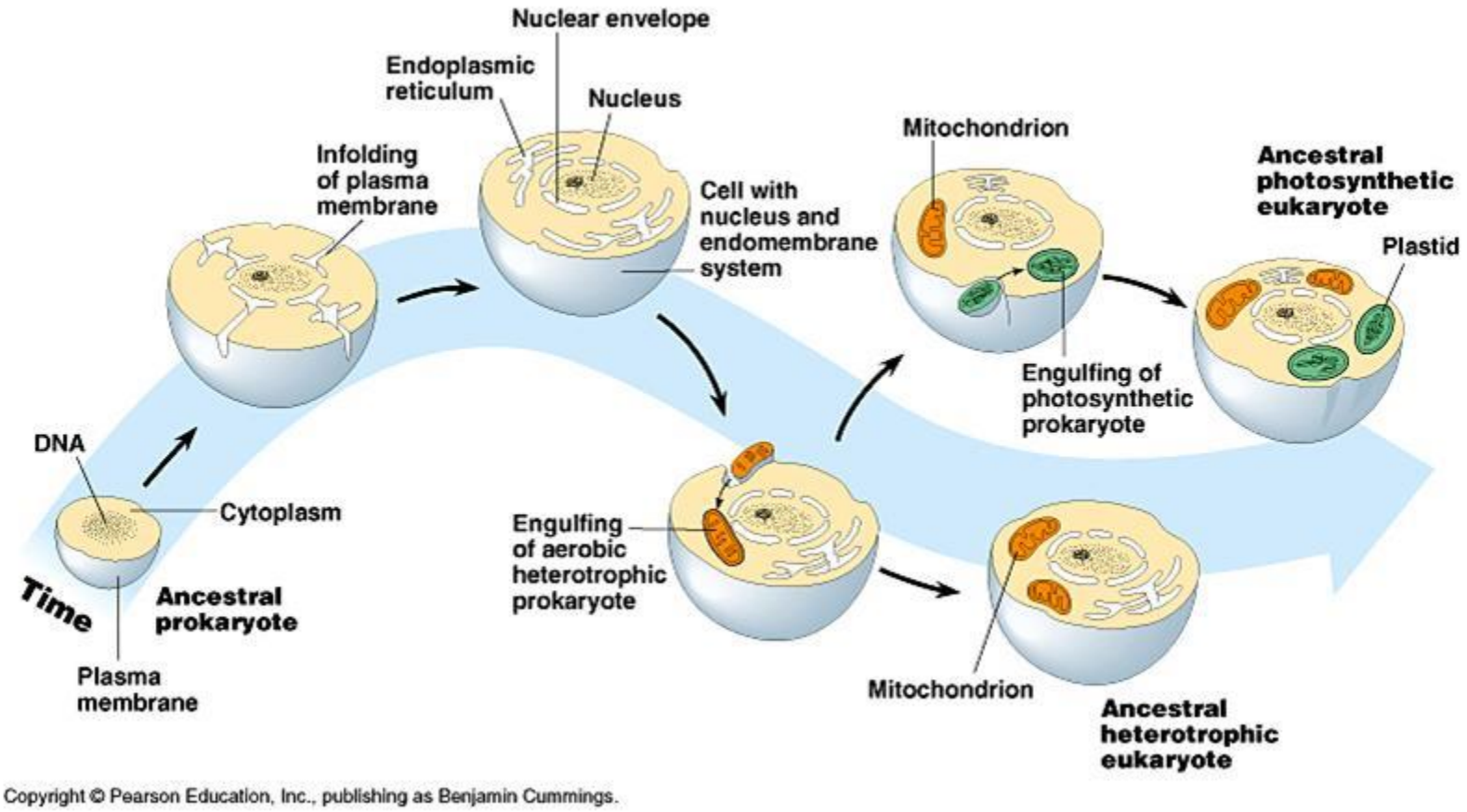
미토콘드리아

(사립체, 絲粒體, unit of 氣, chi, 氣體)



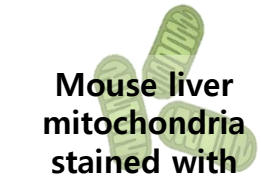
핵
(核,
核心)
Master

Mitochondria Symbiosis theory



http://www.sumanasinc.com/webcontent/animations/content/organelles.html

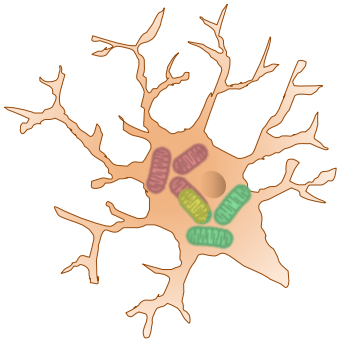
Transfer of mouse liver mitochondria (green) to DsRed-mito-SK-Hep1 cells (red)



Mouse liver mitochondria stained with Mitotracker Green

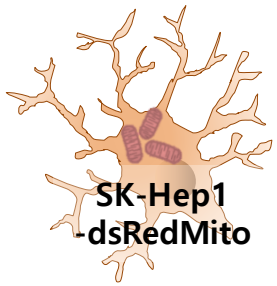
Resuspend in MIB buffer

- Mix together in Miro5 buffer
- Incubate at 37℃ for 1h while rotating at 150rpm



- Spin to harvest cells
- Seed on the culture plate
- Culture for 24h or 60h
- Confocal imaging

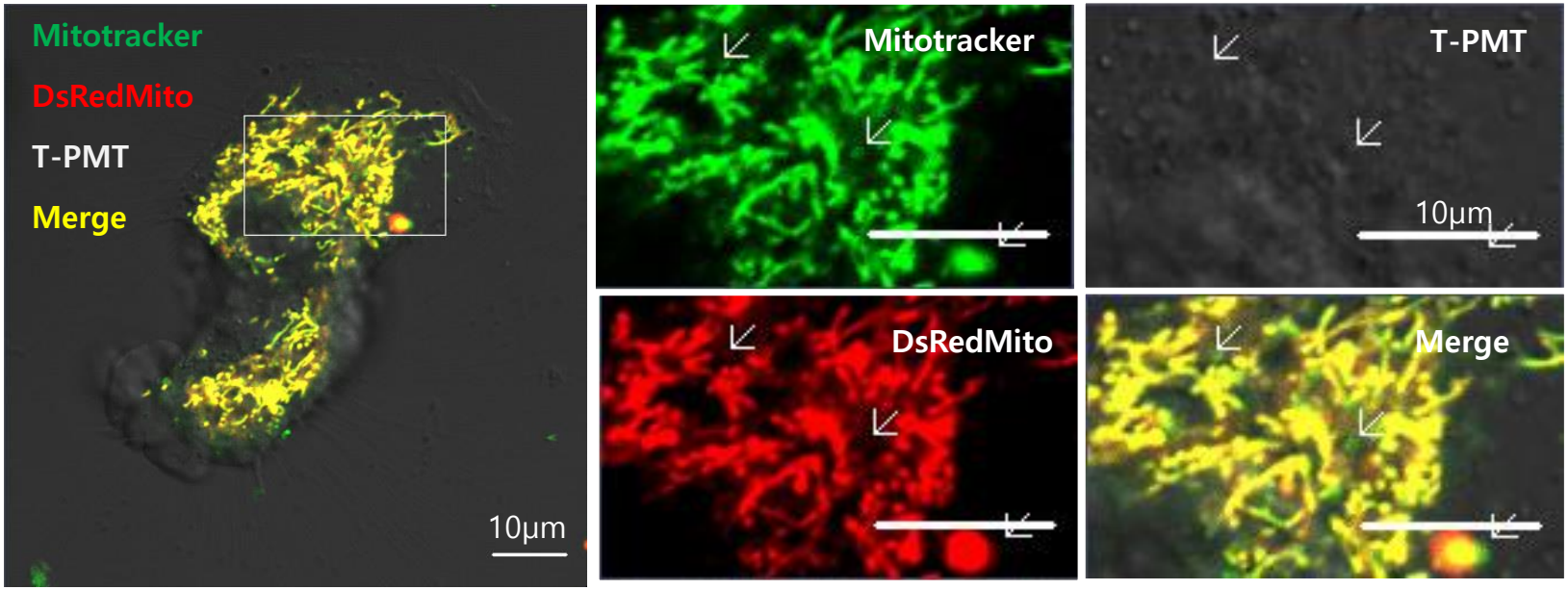
Harvest by trypsin digestion



SK-Hep1 -dsRedMito

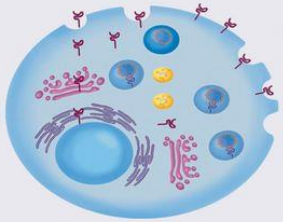
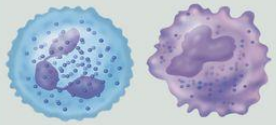
Transfer of mouse liver mitochondria (green) to dsRed-mito-SK-Hep1 cells (red)

elapsed time= 24 h



CENTRAL ILLUSTRATION: Multitarget Cardioprotective Strategies to Reduce Myocardial Infarction

Combination Strategies For Multi-Target Cardioprotection

Protective Modality	Time of Application	Cellular Target	Intracellular Target
1. Ischemic Conditioning 	1. During Ischemia	1. Cardiomyocytes 	1. Inhibition of Cell Death Pathways (Necrosis, Apoptosis, Pyroptosis, Necroptosis) 
2. Pharmacological Protection 	2. At Reperfusion	2. Non-Cardiomyocytes, Microvascular Obstruction 	2. Activation of Endogenous Pro-Survival Pathways (RISK, SAFE, PKG) 
3. Physical Intervention 	3. Late Into Reperfusion	3. Circulating Cells 	

Davidson, S.M. et al. J Am Coll Cardiol. 2019;73(1):89-99.

JACC

Myocardial rescue with autologous mitochondrial transplantation in a porcine model of ischemia/reperfusion

McCully group at Boston Children's Hospital

J Thorac Cardiovasc Surg, 2017

연구 목적

To demonstrate the clinical efficacy of autologous mitochondrial transplantation in preparation for translation to human application using an in vivo swine model.

선행연구들

- **Injection of isolated mitochondria during early reperfusion for cardioprotection. McCully JD et al. Am J Physiol Heart Circ Physiol 2009**
- **Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury. Masuzawa A et al. Am J Physiol Heart Circ Physiol. 2013**
- **Intracoronary Delivery of Mitochondria to the Ischemic Heart for Cardioprotection. Cowan DB et al. PLoS One, 2016**

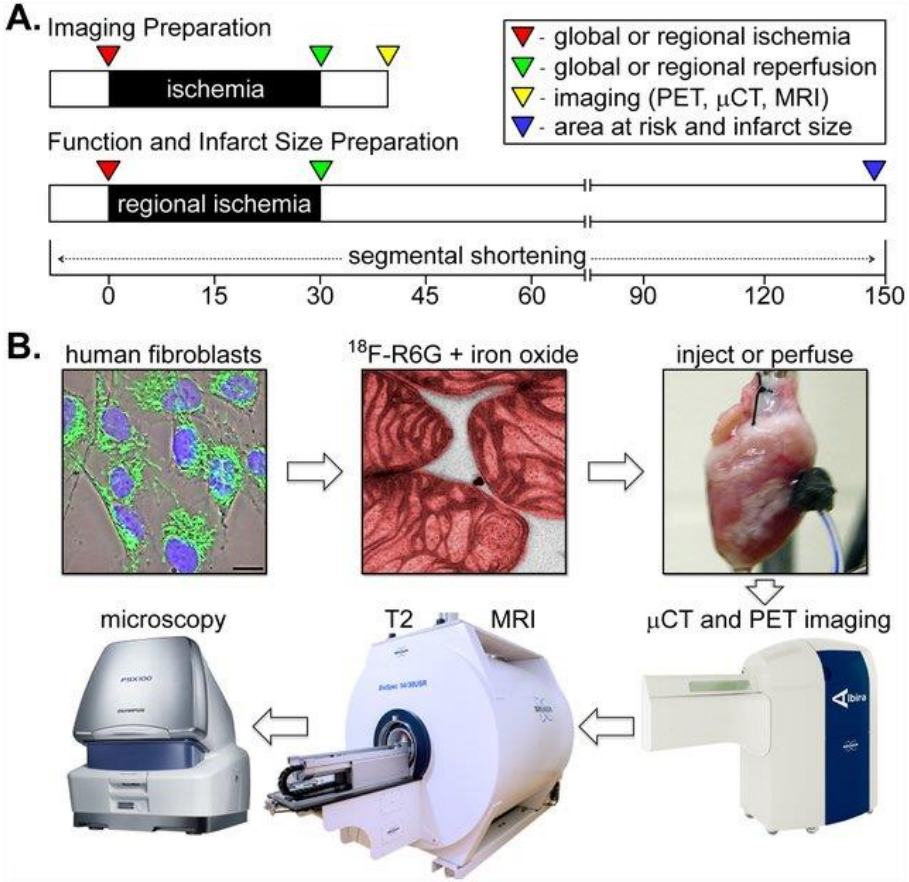


Fig 1. A schematic representation of the experimental procedures using **Langendorff heart**.

(A) For imaging of mitochondrial distribution, the ischemic interval was 30 minutes followed by 10 minutes of reperfusion (top). **Hearts used for functional and infarction measurements were subjected to 30 minutes of ischemia followed by 2 hours of reperfusion (bottom**). In both instances, mitochondria were delivered to the heart at the onset of reperfusion.

(B) Cultured human cardiac fibroblasts (fluorescently stained in this phase contrast image overlay with TOMM20 [green] to show mitochondria and DAPI [blue] to show nuclei), were used to isolate and label mitochondria with 18F-R6G (colorized as red in this transmission electron micrograph) and 30 nm iron oxide particles (black dots on the mitochondrial outer surface). **Dual-labeled mitochondria were injected or perfused into ischemic Langendorff-perfused isolated hearts**, which were imaged by PET and µCT followed with MRI. Hearts were then fixed, embedded, sectioned, and histologically stained for fluorescence and brightfield microscopy.

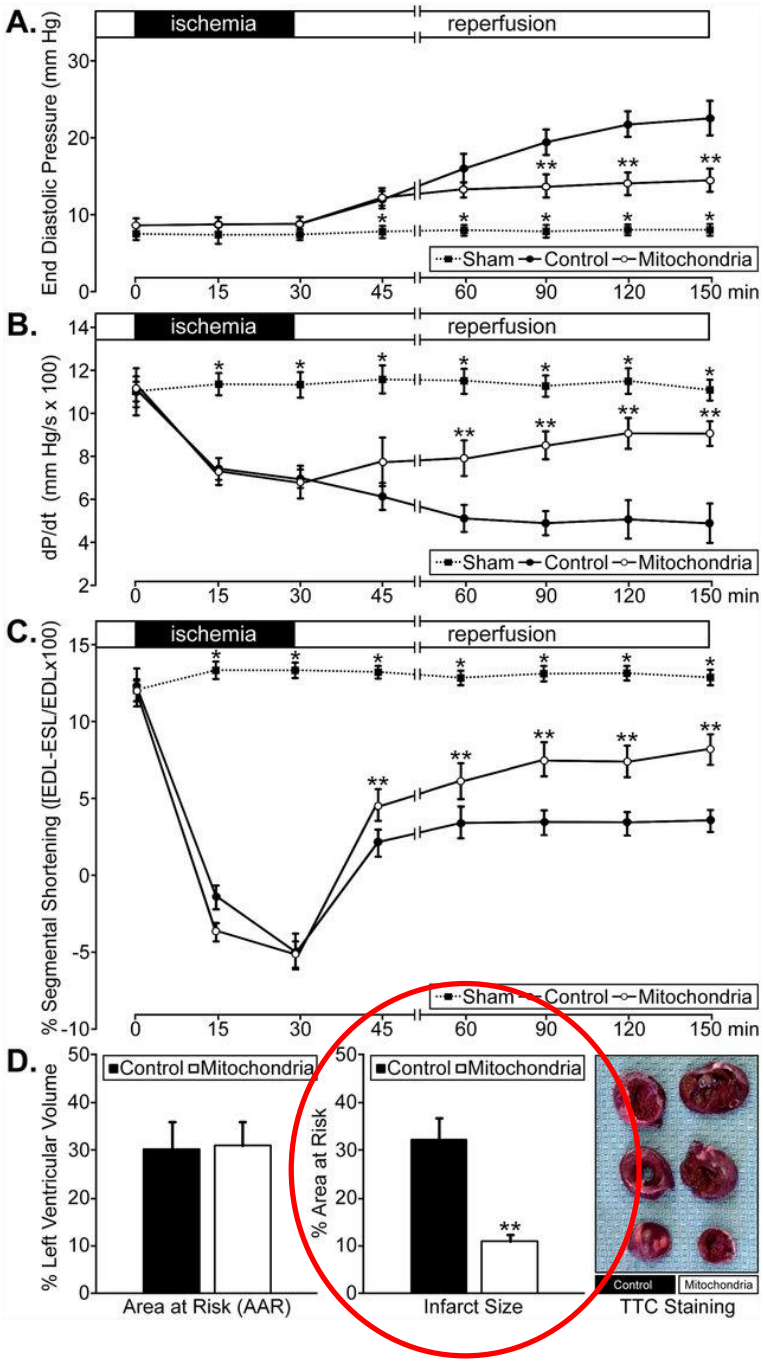


Fig 7. Myocardial function in regionally ischemic hearts perfused with autologous rabbit liver mitochondria with Langendorff rabbit hearts.

(A) End diastolic pressure (mm Hg);
(B) positive dP/dt (mm Hg/s x 100);
(C) % segmental shortening (end-diastolic length [EDL] minus end-systolic length [ESL] over end-diastolic length [EDL] x 100) in Control and Mitochondria heart groups, pre-ischemia, and during 30 minutes regional ischemia and 120 minutes of reperfusion. Sham groups were not subjected to ischemia and reperfusion or mitochondrial treatment.

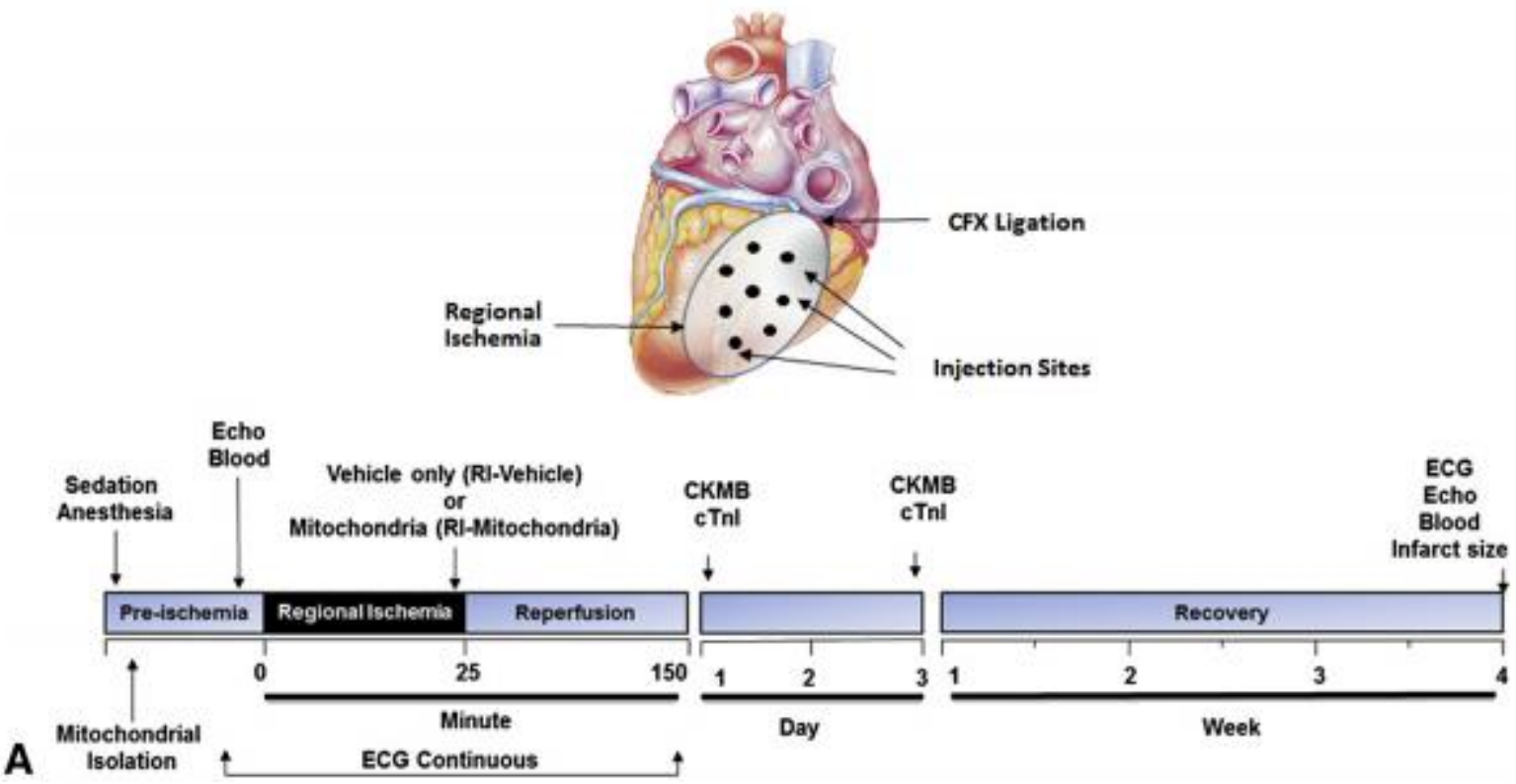
Myocardial rescue with autologous mitochondrial transplantation in a porcine model of ischemia/reperfusion

McCully group at Boston Children's Hospital

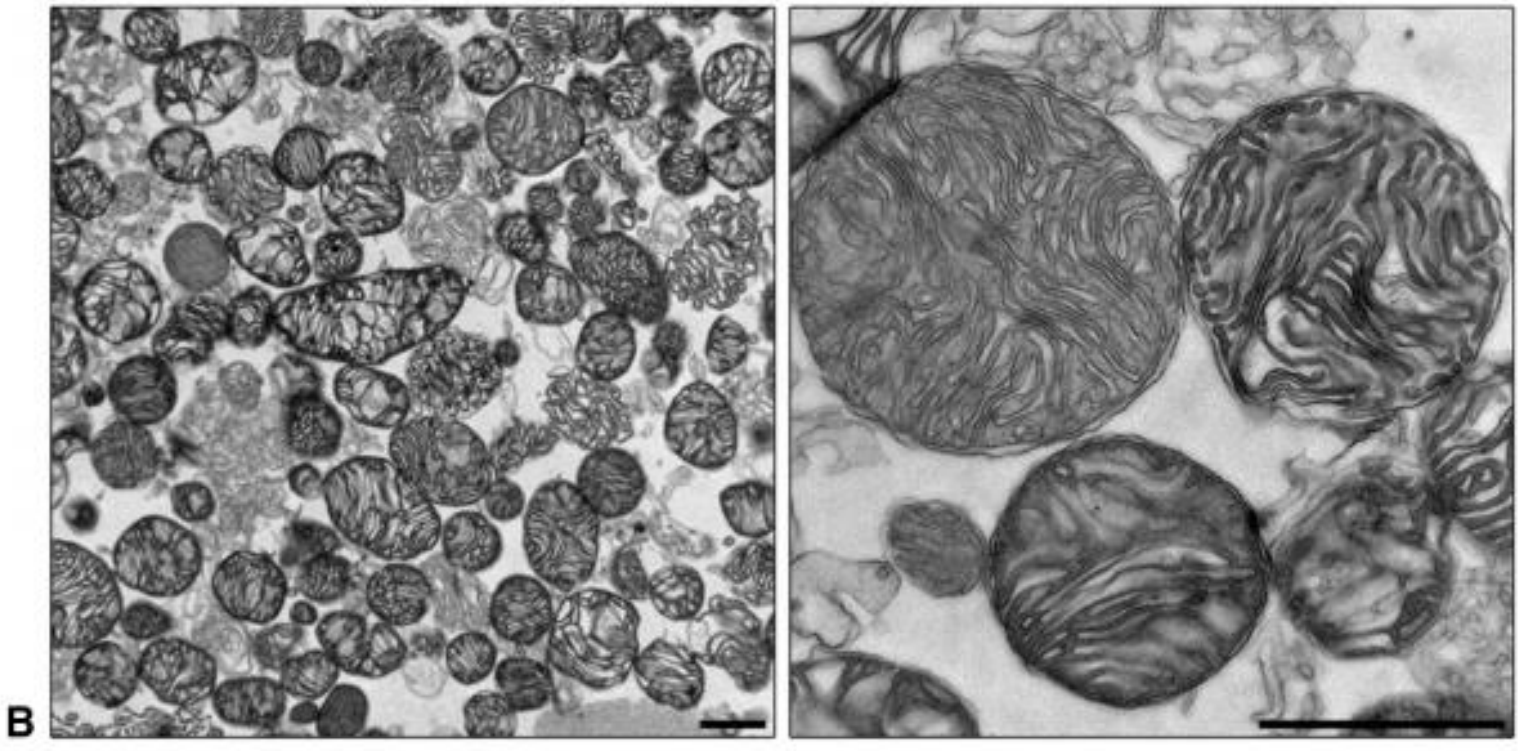
J Thorac Cardiovasc Surg, 2017

방법

- A left mini-thoracotomy was performed on Yorkshire pigs.
- The pectoralis major was dissected, and skeletal muscle tissue was removed and used for the isolation of autologous mitochondria.
- The heart was subjected to regional ischemia (RI) by temporarily snaring the circumflex artery.
- After 24 minutes of RI, hearts received 8 x 0.1 mL injections of vehicle (vehicle-only group; n= 6) or vehicle containing mitochondria (mitochondria group; n= 6) into the area at risk (AAR), and the snare was released.
- The thoracotomy was closed, and the pigs were allowed to recover for 4 weeks.



A. Experimental protocol. Injection sites in the area at risk are indicated.



B, Representative electron micrographs of isolated pig skeletal muscle mitochondria. The isolated mitochondria were free from cellular contamination and were electron dense and had preserved morphology and shape. (Scale bars: 1 mm.)

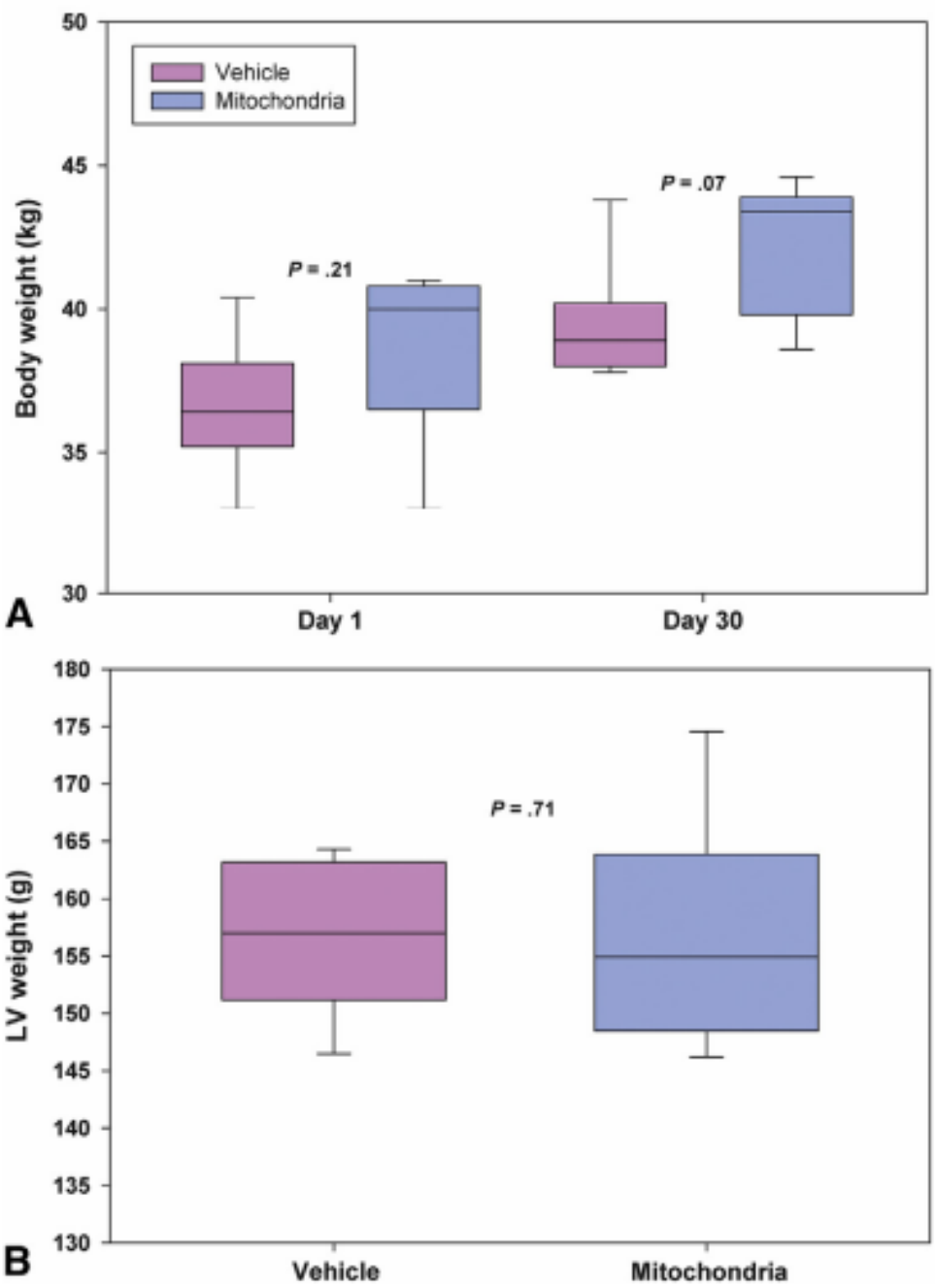


FIGURE 2. **Body weight (A) and heart weight (B)**.
There was no significant difference in body weight between groups either at the time of initial or terminal surgery.
The mean change in body weight over 4 weeks (d) is shown in (A).
There was no significant difference in left ventricular weight at the time of initial or terminal surgery (B). LV, Left ventricular.

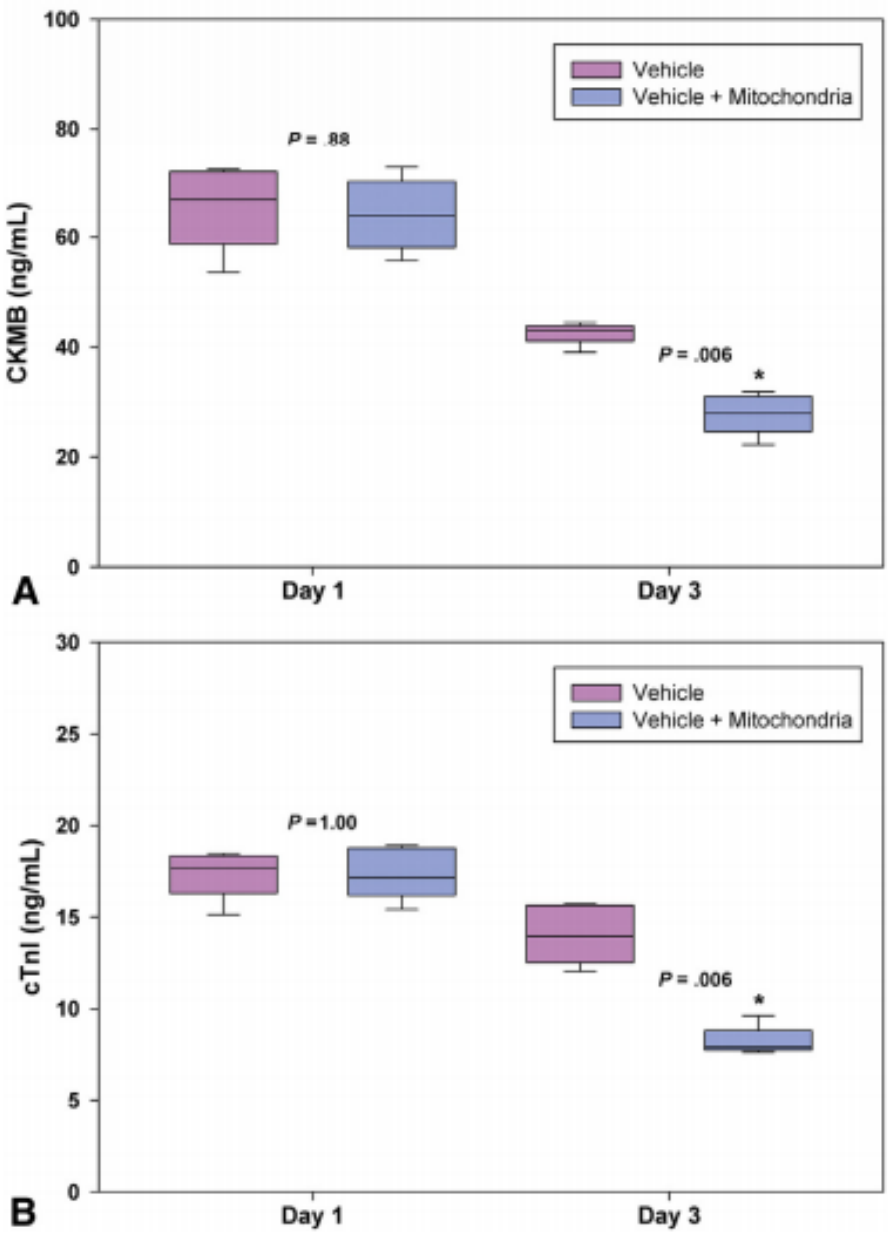


FIGURE 3. **Creatine kinase-MB isoenzyme (CK-MB) (A) and cardiac troponin I (cTnI) (B)** at day 1 and day 3 after surgery in the vehicle-only and mitochondria groups. Both CK-MB and cTnI were significantly decreased (P<.001) in mitochondria hearts compared with vehicle hearts at day 3.

There was no significant difference between the 2 groups at day 1. Results are shown for n= 6 in each group.
Significant differences compared with vehicle are shown.
*Significant differences compared with vehicle at P=.006

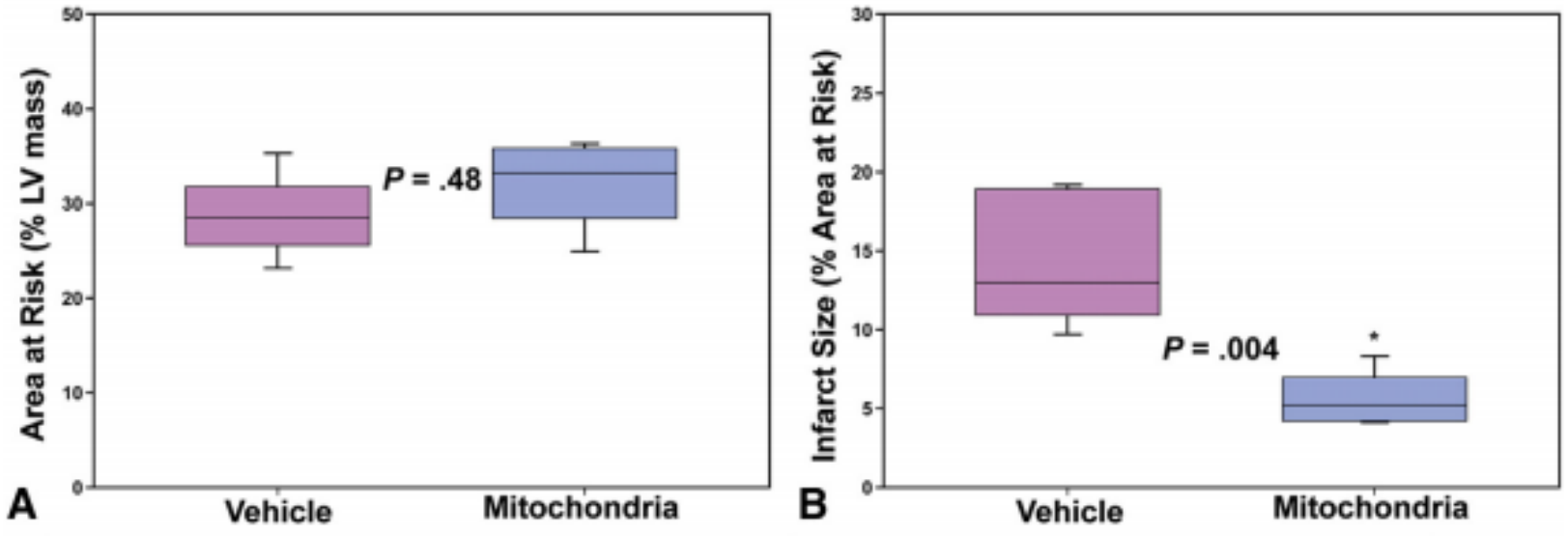
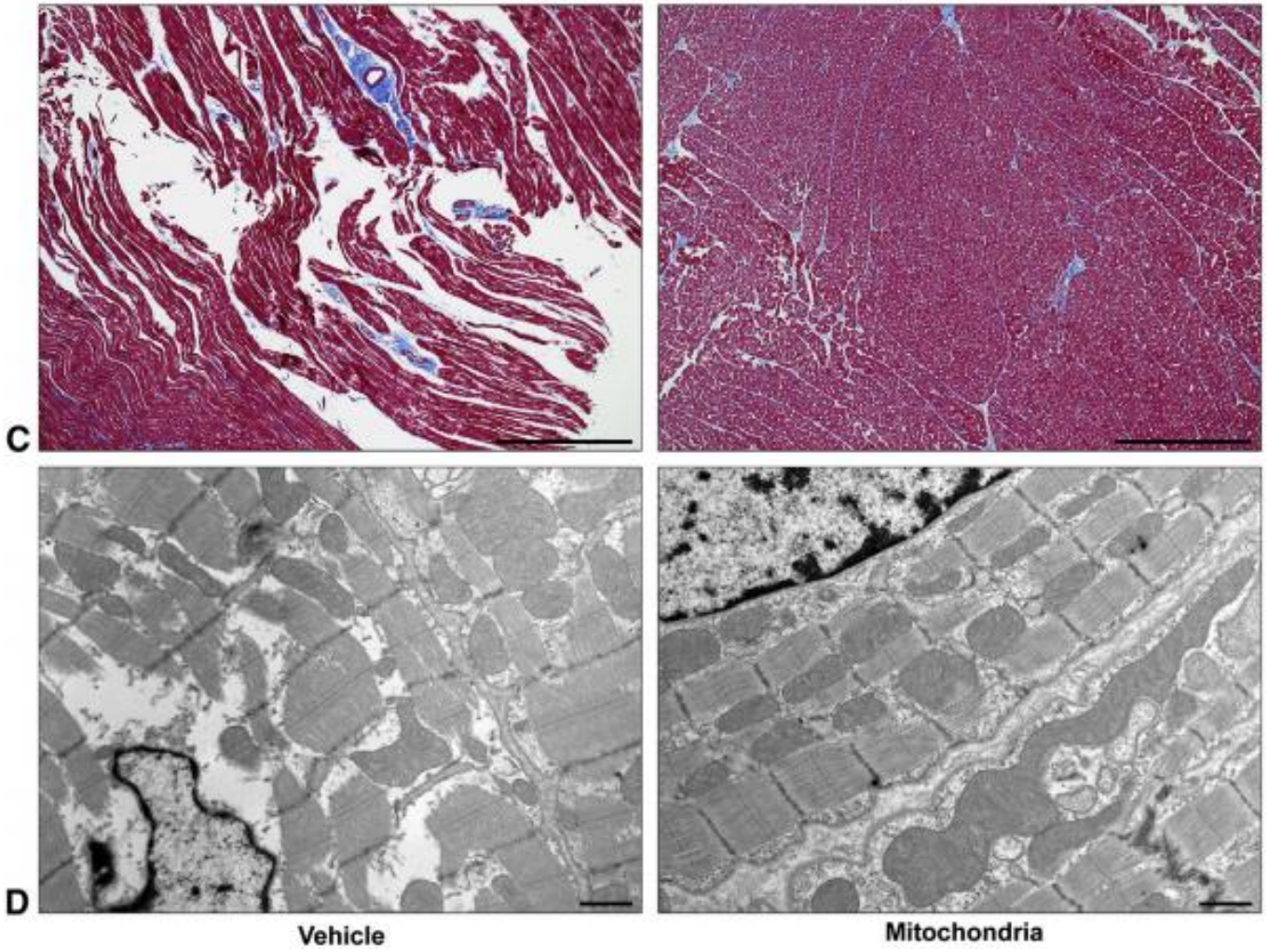


FIGURE 4. A; Area at risk and B; infarct size (%AAR) for vehicle and mitochondria. Results are shown as the mean standard deviation for n= 6 in each group.



C and D, Histochemical and electron microscopy analysis of myocardial tissue at 4 weeks of recovery. Representative Masson's trichrome-stained (C) and transmission electron (D) micrographs from vehicle and mitochondria hearts are shown. Longitudinal and transverse interfibrillar separation was observed in vehicle hearts but not in mitochondria hearts (C), but there was no difference in collagen between the 2 groups (C). Electron microscopy analysis (D) shows mitochondrial damage and contraction bands in vehicle hearts that are not present in mitochondria hearts. (Scale bars: 500 mm.).

Then they studied the efficacy of delayed mitochondrial transplantation via intracoronary administration in a model of regional IRI as a strategy for cardio-protection.

Delayed Transplantation of Autologous Mitochondria for Cardioprotection in a Porcine Model.

Blitzer D. et al. Ann Thorac Surg. 2019 Aug

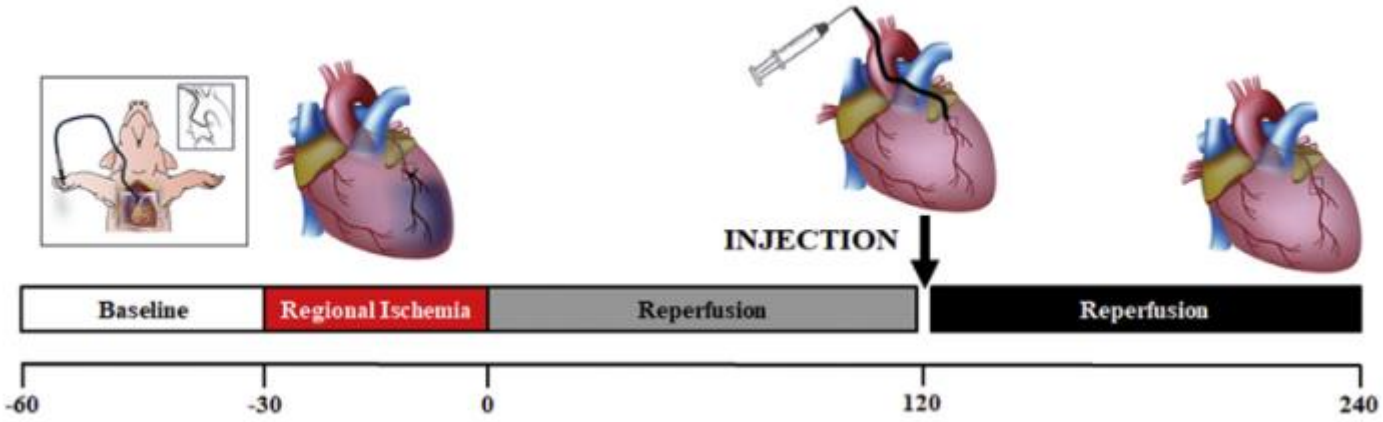


Figure 1. Description of experimental model. Female and male Yorkshire pigs (40-50 kg; n¼16) were sedated and intubated. A sternotomy was performed, and the pectoralis major was located and dissected, two small pieces of which were to be excised with the use of a 6-mm biopsy punch for mitochondrial isolation. A 3-0 Prolene suture (Ethicon, Somerville, NJ) was then passed around the left anterior descending artery and snared down to create a period of temporary ischemia that lasted 30 minutes. The snare was then released, and the heart was allowed 120 minutes of reperfusion. After 120 minutes of reperfusion the hearts received either vehicle alone or mitochondria (1109) in vehicle. Vehicle or mitochondriain vehicle was delivered as a 5-mL bolus, antegrade to the left coronary artery under fluoroscopic guidance with the use of a 5F JR angiography catheter. After injection, the heart was then allowed a further 120 minutes of reperfusion (240 minutes of total reperfusion). Global and regional function were determined. All pigs were humanely euthanized under deep anesthesia after 240 minutes of reperfusion.

방법

- Female Yorkshire pigs (40-50 kg; n=16) underwent 30 minutes of ischemia by snaring of the left anterior descending artery (LAD) and the hearts were then reperfused for 120 minutes.

- At that point, Vehicle only or Autologous Mitochondria ($1x10^9$ in 5 mL vehicle) were delivered as a bolus to the left coronary ostium followed by a further 120-minute reperfusion.

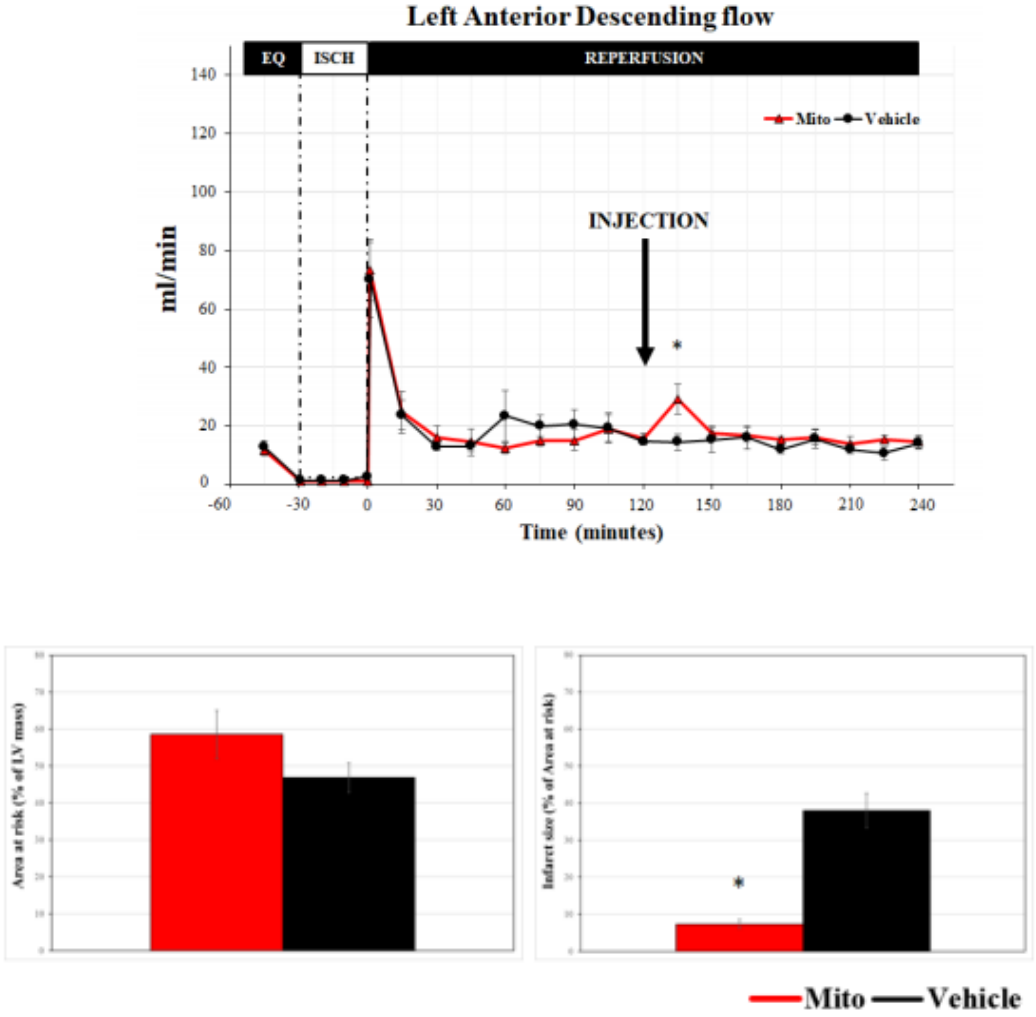
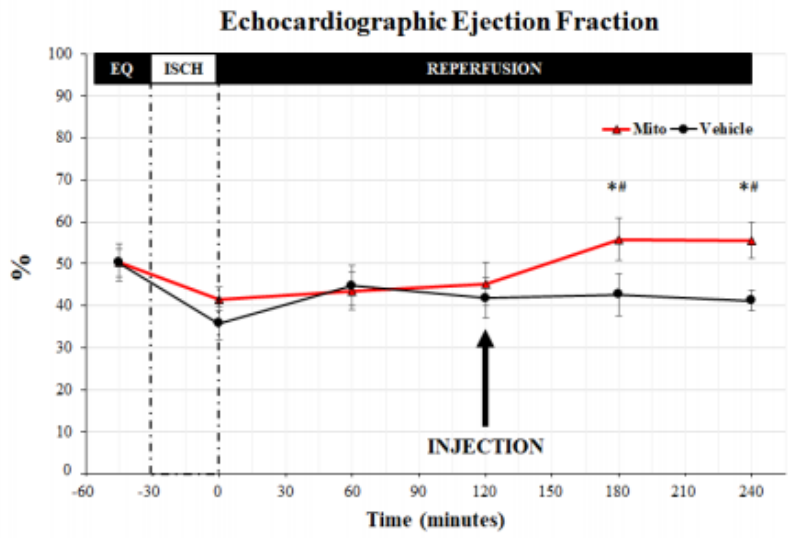
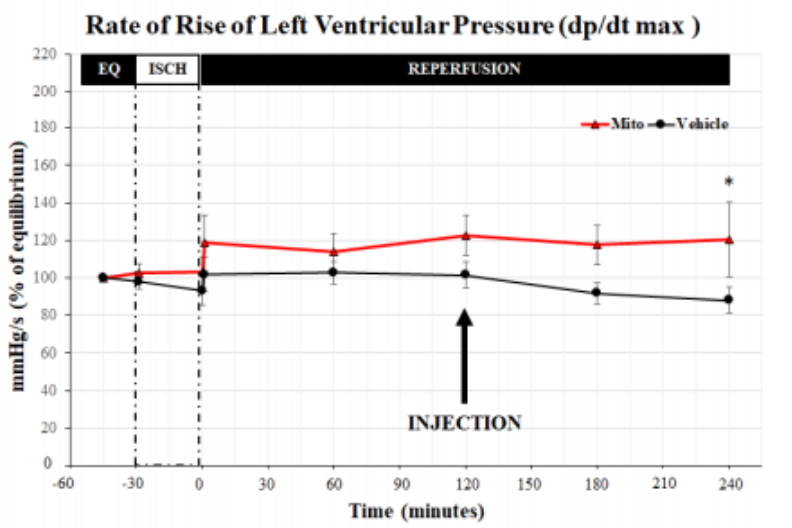
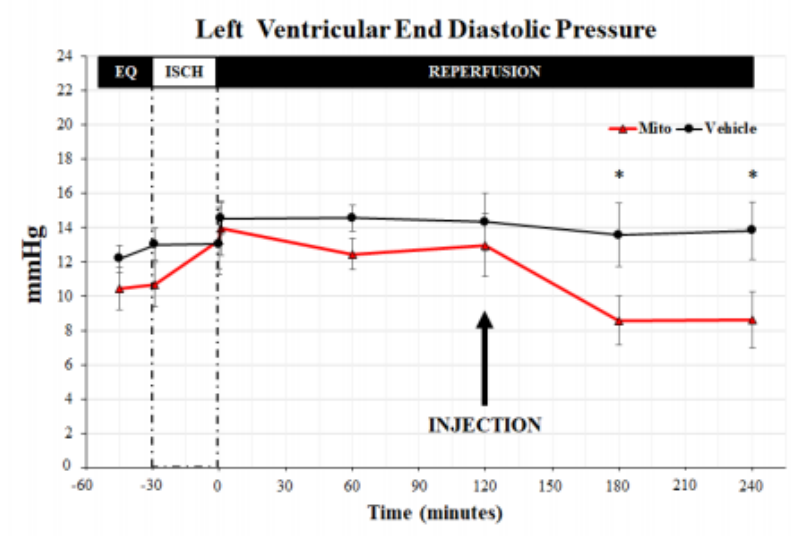
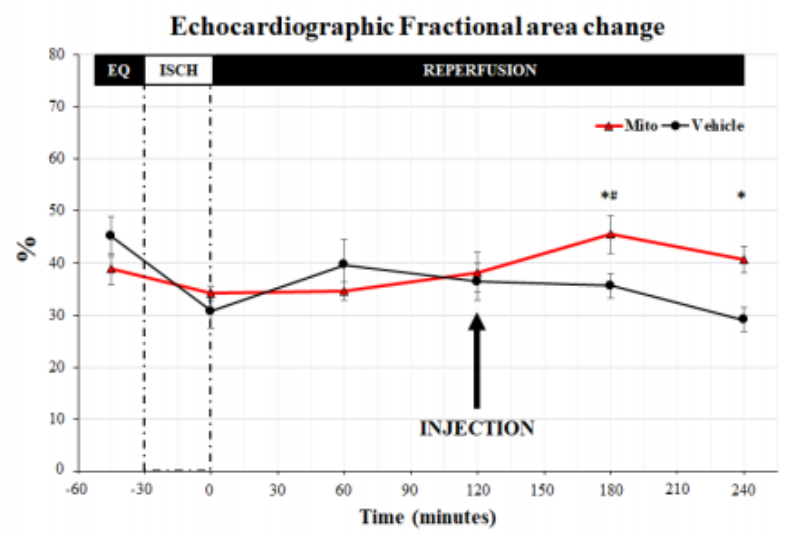


Figure 9. Measurement of Area at Risk and Infarct Size of the Left Ventricular Myocardium. There was no significant difference in the AAR between Mitochondria and Vehicle hearts. Infarct size (%AAR) was significantly decreased in Mitochondria as compared to Vehicle hearts. All results are shown as mean +/-SEM for n=8 each group. *p<0.05; Mitochondria vs Vehicle.


Echocardiographic Ejection Fraction
EQ
ISCH
REPERFUSION
Mito
Vehicle
*#
*#
INJECTION
%
Time (minutes)


Rate of Rise of Left Ventricular Pressure (dp/dt max)
EQ
ISCH
REPERFUSION
Mito
Vehicle
*
INJECTION
mmHg/s (% of equilibrium)
Time (minutes)


Left Ventricular End Diastolic Pressure
EQ
ISCH
REPERFUSION
Mito
Vehicle
*
*
INJECTION
mmHg
Time (minutes)


Echocardiographic Fractional area change
EQ
ISCH
REPERFUSION
Mito
Vehicle
*#
*
INJECTION
%
Time (minutes)

Take Home Message

Autologous mitochondrial transplantation provides a novel technique to significantly enhance myocardial cell viability following ischemia and reperfusion at least in pig.

최근 연구동향

- Mitochondrial transplantation ameliorates acute limb ischemia. Orfany A. et al. J Vasc Surg. 2019, Jul.
- Mitochondrial transplantation prolongs cold ischemia time in murine heart transplantation. Moskowitzova K, et al. J Heart Lung Transplant. 2019, Jan.
- Mitochondrial transplantation enhances murine lung viability and recovery after ischemia reperfusion injury. Moskowitzova K. et al. Am J Physiol Lung Cell Mol Physiol. 2019 Nov

Mitochondrial transplantation:
applications for pediatric patients with congenital heart disease.
Emani SM, McCully JD. Transl Pediatr. 2018 Apr.

Kate Bowen with her infant, Georgia, in the intensive care unit at Boston Children's Hospital. Doctors tried to revive the baby's heart with an infusion of one billion mitochondria.

By Gina Kolata July 10, 2018, **The New York Times**

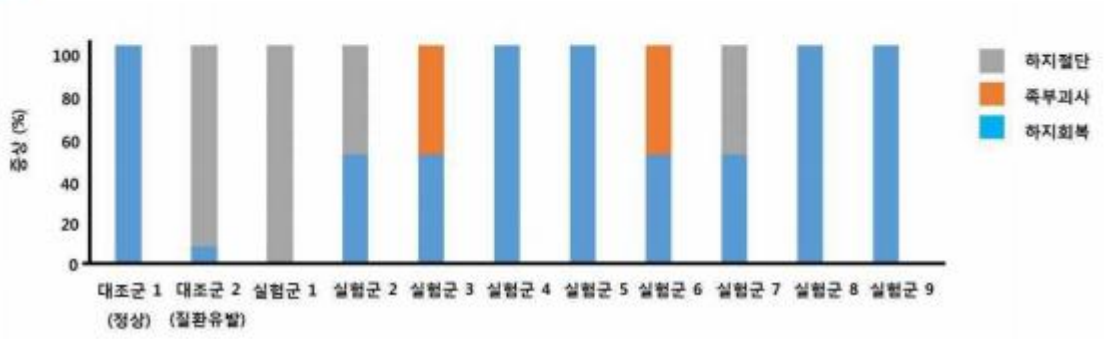
	MIRACLE Session I Mitochondrial transfer	Organizers: Hong Kyu Lee, Chin-San Liu Chairs: Hong Kyu Lee, Chin-San Liu Panels: Chae Hun Leem, Seung-Kyu Cha
12:40-12:55	Mitochondrial Transplantation: Applications and Potential	James D. McCully, Harvard University
12:55-13:10	Application of Artificial Mitochondrial Therapy in Parkinson Disease	Chin-San Liu, Changhua Christian Hospital
13:10-13:25	Transfer of isolated mitochondria: uptake mechanism and therapeutic	Youngmi Kim Pak, Kyung Hee University
13:25-13:40	Reactivation of dihydroorotate dehydrogenase by respiration restores tumor growth of mitochondrial DNA-depleted cancer cells	Jiri Neuzil, Griffith University
13:40-13:55	Mitochondrial transfer in the brain in a tumour model lacking mitochondrial DNA and in brain cell co-cultures	Michael V. Berridge, Malaghan Institute
13:55-14:10	Role of astrocytic mitochondria for neuroprotection and neuroplasticity after stroke	Kazuhide Hayakawa, Massachusetts General Hospital

(54) 발명의 명칭 **미토콘드리아를 포함하는 허혈성 질환 예방 또는 치료용 조성물**

(57) 요 약

본 발명은 허혈성 질환 예방 또는 치료용 조성물에 관한 것이며, 보다 상세하게는 미토콘드리아를 유효성분으로 포함하는 허혈성 질환 예방 또는 치료용 조성물에 관한 것이다. 이를 통해, 본 발명에 따른 조성물은 허혈성 질환이 발생한 환부에 직접적으로 정상적인 활성을 갖는 외래 미토콘드리아를 공급할 수 있어, 미토콘드리아 기능이 저하된 세포의 활성을 증가시키거나 미토콘드리아 기능 이상 세포 재생에 유용하며, 미토콘드리아 이상 허혈성 질환의 치료 또는 예방에 이용될 수 있다.

대 표 도



시리즈A에 한국투자파트너스, 하나벤처스, 타임폴리오자산운용 등 참여.. 줄기세포 유래 미토콘드리아 분리 및 전달기술 확보.. 근염·자가면역 파이프라인 개발

PAEAN
BIOTECHNOLOGY

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MEETING ABSTRACTS

2019 ACR/ARP Annual Meeting

ABSTRACT NUMBER: 119

Mitochondrial Transplantation Suppressed Muscle Inflammation and Improved the Mitochondrial Dysfunction in C Protein-induced Myositis Model

Jeong Yeon Kim[1], Seon Uk Kim [2], Ji soo Park [1], Ji Hye Lee [3], Jae Hwan Shin [4], Do Wan Hwang [5], Yun Sang Lee [4], Jin Chul Paeng [4], Yong Soo Choi [6], Jung Wook hwang [7], Kyuboem Han [8], Chun Hyung Kim [8], Mi Jin Kim [8], Yeong-Wook Song [9] and Eun Young Lee [10], [1]Division of Rheumatology, Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea., Seoul, Republic of Korea, [2]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, and College of Medicine, Seoul National University, Seoul, Republic of Korea,

8 PAEAN Biotechnology Inc., Daejeon, Korea, Daejeon, Republic of Korea.

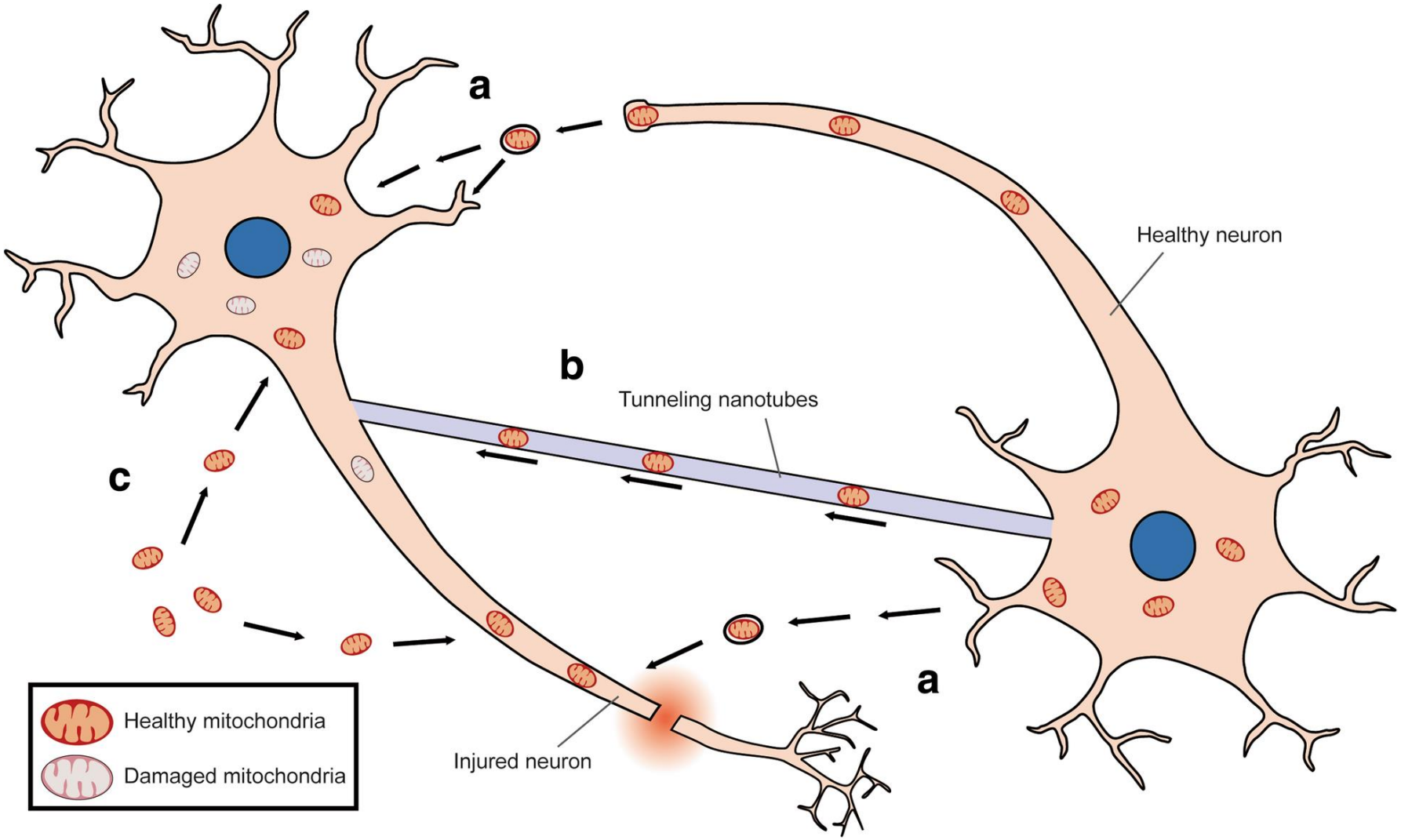
을지대학 등과 공동 연구를
기획 중입니다.

Table 2 Registered interventional studies for mitochondrial transplantation on ClinicalTrials.gov

Chang et al. Translational Neurodegeneration (2019) 8:17

Conditions/ Diseases	Status	Phase	Intervention	Mitochondria donor	NCT number
Age-related deterioration of oocyte quality	With-drawn	1 & 2	Injection of autologous mitochondria to the oocytes	Autologous granulosa cells	NCT01631578
Infertility	Complet ed	NA	Autologous micro-injection of mitochondria into the oocytes during ICSI	Autologous ovarian stem cells	NCT02586298
Mitochondrial diseases: Pearson Syndrome	Not yet recruit-ing	Early 1	Mitochondria augmentation therapy: transplantation of autologous stem cell enriched with MNV-BLDa	Autologous peripheral hematopoietic stem cells	NCT03384420
Extracorporeal membrane oxygenation complication	Recruit-ing		Autologous mitochondria injected or infused into the ischemic myocardium	Autologous skeletal muscle cells	NCT02851758

NA not applicable, ICSI intracytoplasmic sperm injection, a MNV-BLD refers to blood-derived mitochondria.



Chang et al. Translational Neurodegeneration (2019) 8:17
https://doi.org/10.1186/s40035-019-0158-8