ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΧΕΙΡΟΥΡΓΙΚΟΣ ΤΟΜΕΑΣ

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Η ΕΠΙΔΡΑΣΗ ΤΗΣ ΜΕΤΑΜΟΣΧΕΥΣΗΣ ΜΕΣΕΓΧΥΜΑΤΙΚΩΝ ΒΛΑΣΤΟΚΥΤΤΑΡΩΝ ΣΕ ΠΕΙΡΑΜΑΤΙΚΟ ΜΟΝΤΕΛΟ ΙΣΧΑΙΜΙΚΟΥ ΜΥΟΚΑΡΔΙΟΥ

ΑΝΤΩΝΕΛΛΑ ΚΟΥΤΕΛΑ

ΙΑΤΡΟΣ

ΕΙΔΙΚΟΤΗΤΑ ΧΕΙΡΟΥΡΓΙΚΗΣ ΘΩΡΑΚΟΣ-ΚΑΡΔΙΑΣ

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ: ΔΗΜΗΤΡΙΟΣ ΔΟΥΓΕΝΗΣ

ΜΕΛΟΣ ΤΡΙΜΕΛΟΥΣ ΕΠΙΤΡΟΠΗΣ: ΑΝΤΩΝΙΟΣ ΒΕΖΑΚΗΣ

ΜΕΛΟΣ ΤΡΙΜΕΛΟΥΣ ΕΠΙΤΡΟΠΗΣ: ΑΠΟΣΤΟΛΟΣ ΠΑΠΑΛΟΗΣ

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΑΘΗΝΑ 2024

NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

SCHOOL OF HEALTH SCIENCES

SCHOOL OF MEDICINE

SURGICAL SECTION

DEPARTMENT OF CARDIAC SURGERY

EXPERIMENTAL STUDY OF STEM CELLS TRANSPLANTATION IN A MODEL OF MYOCARDIAL ISCHEMIA

ANTONELLA KOUTELA

DOCTOR

CHIEF RESIDENT IN CARDIOTHORACIC SURGERY

SUPERVISOR: DIMITRIOS DOUGENIS

MEMBER: ANTONIOS VEZAKIS

MEMBER: APOSTOLOS PAPALOIS

PhD THESIS

ATHENS 2024

ΑΡΙΘΜΟΣ ΠΡΩΤΟΚΟΛΛΟΥ ΔΙΔΑΚΤΟΡΙΚΗΣ ΔΙΑΤΡΙΒΗΣ: 1718010682/ 29.11.2017
ΗΜΕΡΟΜΗΝΙΑ ΟΡΙΣΜΟΥ ΤΡΙΜΕΛΟΥΣ ΕΠΙΤΡΟΠΗΣ: 1718018532/ 13.02.2018
ΗΜΕΡΟΜΗΝΙΑ ΟΡΙΣΜΟΥ ΘΕΜΑΤΟΣ: 10.06.2018
ΑΡΙΘΜΟΣ ΠΡΩΤΟΚΟΛΛΟΥ ΑΔΕΙΑΣ ΠΕΙΡΑΜΑΤΙΣΜΟΥ: 1870/ 23.04.2018
ΗΜΕΡΟΜΗΝΙΑ ΟΡΙΣΜΟΥ ΓΛΩΣΣΑΣ ΣΥΓΓΡΑΦΗΣ «ΑΓΓΛΙΚΑ»: 131407/ 05.12.2022
ΗΜΕΡΟΜΗΝΙΑ ΚΑΤΑΘΕΣΗΣ ΔΙΔΑΚΤΟΡΙΚΗΣ ΔΙΑΤΡΙΒΗΣ: 38576/22.4.2024

ΣΥΣΤΑΣΗ ΤΡΙΜΕΛΟΥΣ ΣΥΜΒΟΥΛΕΥΤΙΚΗΣ ΕΠΙΤΡΟΠΗΣ :

1. **Δημήτριος Δουγένης**, Ομότιμος Καθηγητής Καρδιοχειρουργικής, τ. Διευθυντής Καρδιοχειρουργικής Κλινικής ΠΓΝ "ΑΤΤΙΚΟΝ", Ιατρική Σχολή ΕΚΠΑ (ΕΠΙΒΛΕΠΩΝ)
2. **Αντώνιος Βεζάκης,** Καθηγητής Χειρουργικής, Β' Χειρουργική Κλινική, ΓΝΑ "Αρεταίειο", Ιατρική Σχολή ΕΚΠΑ
3. **Απόστολος Παπαλόης**, Επισκέπτης Καθηγητής Ιατρικής Σχολής ΕΚΠΑ, Αναπληρωτής Διευθυντής Πειραματικού Χειρουργείου Β' Χειρουργικής Κλινικής Ιατρικής Σχολής ΕΚΠΑ, Πρόεδρος Επιστημονικής Επιτροπής ΕΜΒΙΕΕ Ιατρικής Σχολής ΑΠΘ

ΠΡΟΕΔΡΟΣ ΙΑΤΡΙΚΗΣ ΣΧΟΛΗΣ ΑΘΗΝΩΝ: Καθηγητής Νικόλαος Αρκαδόπουλος

ΕΠΤΑΜΕΛΗΣ ΕΞΕΤΑΣΤΙΚΗ ΕΠΙΤΡΟΠΗ :

1. Δημήτριος Δουγένης, Ομότιμος Καθηγητής Ιατρικής Σχολής ΕΚΠΑ
2. Ευστάθιος Ευσταθόπουλος, Αντιπρύτανης , Καθηγητής Ιατρικής Σχολής ΕΚΠΑ
3. Ιγνάτιος Οικονομίδης, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ
4. Αντώνιος Βεζάκης, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ
5. Δημήτριος Αγγουράς, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ
6. Απόστολος Παπαλόης, Επισκέπτης Καθηγητής Ιατρικής Σχολής ΕΚΠΑ
7. Δέσποινα Μυωτέρη, Επίκουρη Καθηγήτρια Ιατρικής Σχολής ΕΚΠΑ

ΒΑΘΜΟΣ ΔΙΔΑΚΤΟΡΙΚΗΣ ΔΙΑΤΡΙΒΗΣ: ΑΡΙΣΤΑ

DATE OF APPLICATION FOR PhD THESIS: 1718010682/ 29.11.2017
DATE OF SUPERVISING MEMBERS COMMITY SUBMISSION: 1718018532 / 13.02.2018
DATE OF PhD THESIS THEME CONFIRMATION: 10.06.2018
NUMBER OF EXPERIMENTAL LISENCE authorized by the National Animal Experiment Board of Greece: 1870 / 23.04.2018
DATE OF "ENGLISH" LANGUAGE WRITING APROVAL: 131407/ 05.12.2022
DATE OF PhD THESIS SUBMISSION: 38576/22.4.2024

MEMBERS OF SUPERVISING COMMITY:

1. **Dimitrios Dougenis**, Professor Emeritus of Cardiac Surgery, Chief of Cardiac Surgery Department "Attikon" Hospital, Teaching Medical School of Athens (Supervisor)
2. **Antonios Vezakis**, Professor General Surgery, B' Teaching Surgical Clinic "Aretaieion" Hospital, Teaching Medical School of Athens
3. **Apostolos Papalois**, Visiting Professor of Medical School of Athens - Associate Professor of Experimental Surgery of B' Clinic of Surgery "Aretaieion" Hospital, Teaching Medical School of Athens - Chairman of Scientific Committee BRESU, School of Medicine, Aristotle University

CHAIRMAN OF ATHENS MEDICAL SCHOOL: Professor Nikolaos Arkadopoulos

EXAMINING COMMITTEE:

1. Dimitrios Dougenis, Professor Emeritus , Medical School of Athens
2. Eustathios Eustathopoulos, Vice Dean, Professor, Medical School of Athens
3. Ignatios Oikonomides, Professor, Medical School of Athens
4. Antonios Vezakis, Professor, Medical School of Athens
5. Dimitrios Aggouras, Professor, Medical School of Athens
6. Apostolos Papalois, Visiting Professor, Medical School of Athens
7. Despoina Myoteri, Assistant Professor, Medical School of Athens

GRADE OF PhD THESIS EXAMINATION : EXCELLENT

Περιεχόμενα - Contents

ΒΙΟΓΡΑΦΙΚΟ ΣΗΜΕΙΩΜΑ

ΠΑΡΟΥΣΑ ΘΕΣΗ:

- Υποψήφια Διδάκτωρ Ιατρικής Σχολής Πανεπιστημίου Αθηνών
- Ειδικευόμενη ιατρός Χειρουργικής Καρδιάς - Θώρακος, Θωρακοχειρουργική κλινική ΓΝΑ Ερυθρός Σταυρός

ΠΡΟΗΓΟΥΜΕΝΕΣ ΘΕΣΕΙΣ ΕΡΓΑΣΙΑΣ:

- Α΄ Χειρουργική Κλινική 417 ΝΙΜΤΣ (2017 - 2020)
- Καρδιοχειρουργική Κλινική ΓΝΑ Ιπποκράτειο (2020 - 2022)

ΕΚΠΑΙΔΕΥΣΗ:

Εγκύκλιες Σπουδές:

1999-2005, 3ο Ενιαίο Γυμνάσιο - Λύκειο Καλαμάτας - Βαθμός Απολυτηρίου : Άριστα

Προπτυχιακή Εκπαίδευση:

2005-2010 Κτηνιατρική Σχολή, Πανεπιστήμιο Θεσσαλίας
Βαθμός Πτυχίου: Λίαν Καλώς (7,23)

2010 - 2016 Ιατρική Σχολή, (3η Ιατρική Σχολή) Πανεπιστήμιο του Καρόλου στην Πράγα (3rd Medical Faculty, Charles University of Prague)
Βαθμός Πτυχίου: Άριστα (9,03)

Μεταπτυχιακή εκπαίδευση:

FELASA accreditation (Federation of European Laboratory Animal Science Associations) Certificate ID:056/16_201008

Δ.Μ.Σ στις Ενδαγγειακές Τεχνικές - MSc in Endovascular Tachniques: Ιατρική Σχολή Πανεπιστημίου Αθηνών σε συνεργασία με το University Milano Bicocca

Άδεια Ασκήσεως Επαγγέλματος Ιατρού : 2016, Νομαρχία Αθηνών

Άδεια Ασκήσεως Επαγγέλματος Κτηνιάτρου: 2011, Γεωτεχνικό Επιμελητήριο Ελλάδος

ΘΕΜΑ ΔΙΔΑΚΤΟΡΙΚΗΣ ΔΙΑΤΡΙΒΗΣ:

Επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων σε πειραματικό μοντέλο ισχαιμικού μυοκαρδίου.

Τριμελής Επιτροπή: κ. Δουγένης Δημήτριος (επιβλέπων), κ. Ζωγράφος Γεώργιος, κ. Παπαλόης Απόστολος

ΔΙΔΑΚΤΙΚΟ ΕΡΓΟ:

2012-2016: Επικουρική διδασκαλία στο τμήμα Ανατομίας - Ιστολογίας και Εμβρυολογίας της 3ης Ιατρικής Σχολής του Πανεπιστημίου του Καρόλου στην Πράγα - (Αμοιβούμενη Διδασκαλία πρωτοετών και δευτεροετών φοιτητών του αγγλόφωνου τμήματος στα μαθήματα ανατομίας, ιστολογίας και εμβρυολογίας - 180 ώρες ανά εξάμηνο)

ΕΡΕΥΝΗΤΙΚΟ ΕΡΓΟ:

- Subcortical parcellation of white matter in Rhesus Monkey in DTI - 2013
- Neuroanatomical study of the nucleus accumbens in human brain - 2014 - 2016
- Experimental study of stem cells transplantation in an experimental model of myocardial ischemia - PhD thesis - since 2018

ΧΡΗΜΑΤΟΔΟΤΟΥΜΕΝΑ ΕΡΕΥΝΗΤΙΚΑ ΠΡΟΓΡΑΜΜΑΤΑ:

- **GAUK 1894214 - 2014** Neuroanatomická studie nucleus accumbens u člověka - **Charles University** of Prague
- **Υποτροφία Ερευνητικού - Πειραματικού Κέντρου ELPEN** Φαρμακευτικής Βιομηχανίας για την εκπόνηση της Διδακτορικής Διατριβής με θέμα: «Επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων σε πειραματικό μοντέλο ισχαιμικού μυοκαρδίου» (έναρξη Απρίλιος 2018)

ΑΔΕΙΕΣ ΕΠΑΓΓΕΛΜΑΤΟΣ:

Άδεια επαγγέλματος κτηνιάτρου στην Ε.Ε

Άδεια επαγγέλματος ιατρού στην Ελλάδα

Άδεια επαγγέλματος ιατρού στη Δημοκρατία της Τσεχίας

ΜΕΛΟΣ ΕΠΙΣΤΗΜΟΝΙΚΩΝ ΕΠΙΤΡΟΠΩΝ:

- Πανελλήνιος Κτηνιατρικός Σύλλογος (από 2010)
- Γεωτεχνικό Επιμελητήριο Ελλάδος (από 2010)
- Ιατρικός σύλλογος Αθηνών (από 2016)
- Ελληνική Χειρουργική Εταιρεία - Δόκιμο Μέλος (2018 - Δόκιμο μέλος)
- Ελληνική Εταιρεία Χειρουργικής Θώρακος Καρδιάς Αγγείων (2019 - Δόκιμο μέλος)
- Μέλος της Επιτροπής Αξιολόγησης Πρωτοκόλλων της Εγκατάστασης Χρήσης Ζώων Εργαστηρίου BIOEMTECH (Τεχνολογικό Πάρκο ΤΕΠΑ) ΕΚΕΦΕ ΔΗΜΟΚΡΙΤΟΣ (2020)

ΛΟΙΠΑ ΠΡΟΣΟΝΤΑ:

Ξένες γλώσσες:

- Ελληνικά (μητρική γλώσσα), Αγγλικά (άριστη γνώση), Γαλλικά (επαρκής γνώση), Τσέχικα (ικανοποιητική γνώση)
- Πιάνο (πτυχιακό επίπεδο στο κλασσικό ρεπερτόριο του Εθνικού Ωδείου Αθηνών)

ΑΚΑΔΗΜΑΪΚΕΣ ΥΠΟΤΡΟΦΙΕΣ:

- Υποτροφία για άριστη επίδοση σπουδών στην ιατρική σχολή (top 5%) κατά τα ακαδημαϊκά έτη 2011-12, 2012-13, 2013-14 και 2014-15.
- Υποτροφία Ερευνητικού - Πειραματικού Κέντρου ELPEN Φαρμακευτικής Βιομηχανίας για την εκπόνηση της Διδακτορικής Διατριβής με θέμα : «Επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων σε πειραματικό μοντέλο ισχαιμικού μυοκαρδίου» (έναρξη Απρίλιος 2018)

ΔΗΜΟΣΙΕΥΣΕΙΣ:

- Mrzílkova J, Koutela A, Kutová M, Patzelt M, Ibrahim I, Al-Kayssi D, Bartoš A, Řípová D, Čermáková P, Zach P. Hippocampal spatial position evaluation on MRI for research and clinical practice. PLoS One. **2014** Dec 12;9(12): e115174. doi: 10.1371/journal.pone.0115174. PMID: 25502906; PMCID: PMC4264873.

- Zach P, Vales K, Stuchlik A, Cermakova P, Mrzilkova J, Koutela A, Kutova M. Effect of stress on structural brain asymmetry. Neuro Endocrinol Lett. **2016** Sep;37(4):253-264. PMID: 27857040.
- Keramidaris D, Gourgiotis S, Koutela A, Mpairamidis E, Oikonomou C, Villias C, Rigas A. Rare Case of Hepatic Endometriosis as an Incidental Finding: Difficult Diagnosis of a Diagnostic Dilemma. Ann Hepatol. **2018** Aug 24;17(5):884-887. doi: 10.5604/01.3001.0012.3173. PMID: 30145568.
- Oikonomou C, Spathari N, Doumoulaki S, Koutela A, Stagkoglou C, Keramidaris D. Recurrent Struma Ovarii Presented with High Levels of Thyroglobulin. Case Rep Surg. **2021** Mar 22; 2021:8868095. doi: 10.1155/2021/8868095. PMID: 33824772; PMCID: PMC8007358.
- Koutela A, Loudos G, Rouchota M, Kletsas D, Karameris A, Vilaras G, Zografos GC, Grypari IM, Dougenis D, Papalois AE. A Novel Experimental Rat Model for the In Vivo Assessment of Myocardial Ischemia Based on Single Photon Emission Computed Tomography. In Vivo. **2023** Mar-Apr;37(2):649-654. doi: 10.21873/invivo.13124. PMID: 36881049; PMCID: PMC10026663.
- Koutela A, Loudos G, Rouchota M, Kletsas D, Karameris A, Vilaras G, Zografos GC, Myoteri D, Dougenis D, Papalois AE. Mesenchymal Stem Cell Transplantation Has a Regenerative Effect in Ischemic Myocardium: An Experimental Rat Model Evaluated by SPECT-CT Assessment. Diagnostics (Basel). **2024** Feb 12;14(4):401. doi: 10.3390/diagnostics14040401. PMID: 38396441; PMCID: PMC10888262.
- Zakszewski E, Adluru N, Tromp do PM, Kalin N, Alexander AL. A diffusion-tensor-based white matter atlas for rhesus macaques. PLoS One. **2014** Sep 9;9(9):e107398. doi: 10.1371/journal.pone.0107398. PMID: 25203614; PMCID: PMC4159318.

ΣΥΝΕΔΡΙΑ (ΕΝΕΡΓΗ ΣΥΜΜΕΤΟΧΗ):

- Εθνικό συνέδριο φοιτητών Ιατρικής της Τσεχίας - εκπροσωπώντας την 3η Ιατρική σχολή του Πανεπιστημίου του Καρόλου στην Πράγα - Πράγα (2013, 2014, 2015)

- 29ο Διεθνές Χειρουργικό Συνέδριο και Διεθνές Χειρουργικό Φόρουμ - Αθήνα 2014
- 31ο Διεθνές Χειρουργικό Συνέδριο και Διεθνές Χειρουργικό Φόρουμ - Αθήνα 2018
- 27ο Ιατρικό Συνέδριο Ενόπλων Δυνάμεων - Θεσσαλονίκη 2018
- 14ο Πανελλήνιο Συνέδριο Χειρουργικής Ογκολογίας - Αθήνα 2019
- 16th Congress of the Cell Transplant and Regenerative Medicine Society - Lesvos 2019
- 28ο Πανελλήνιο Πνευμονολογικό Συνέδριο - Αθήνα 2019
- 13ο Πανελλήνιο Συνέδριο της Ελληνικής Εταιρείας Χειρουργών Θώρακος-Καρδιάς- Αγγείων, Αθήνα 2020
- 21ο Πανελλήνιο Συνέδριο Μεταμοσχεύσεων - Αθήνα 2021
- 21st Congress of The European Society for Organ Transplantation, ESOT, Athens 2023
- 19th International Congress of Update in Cardiology and Cardiovascular Surgery, Istanbul 2023

POSTERS:

- Rupture of transverse colon post cesarean section due to uterine adhesions
- Rapture of jejunal diverticula in a rare case of acute abdomen
- Επείγουσα θυρεοειδεκτομή για οξεία αναπνευστική ανεπάρκεια λόγω αναπλαστικού καρκινώματος θυρεοειδούς
- Σπάνια περίπτωση Διροφιλαρίασης σε ενήλικα
- Λειομυοσάρκωμα οπισθοπεριτοναϊκού χώρου με σπάνια εντόπιση (κροταφίτης μυς)
- Επιτυχής αντιμετώπιση εμμένοντος δευτεροπαθούς πνευμοθώρακα με χρήση βαλβίδας Heimlich σε εξωτερικό ασθενή
- Η εφαρμογή μιας νέας θεραπείας τραύματος μεταβαλλόμενης διαλείπουσας αρνητικής πίεσης στην αντιμετώπιση του εμπυήματος μετά πνευμονεκτομή.
- Περιστατικό αντιμετώπισης υποτροπιάζοντος δευτεροπαθούς πνευμοθώρακα με χημική πλευροδεσία (πλευροδεσία με τάλκη)

- Η επιδιόρθωση της μιτροειδούς βαλβίδας με νέες χορδές και δακτύλιο: Η εμπειρία μας στο ΓΝΑ Ιπποκράτειο

ΠΑΡΟΥΣΙΑΣΕΙΣ ΣΕ ΕΛΛΗΝΙΚΑ ΚΑΙ ΔΙΕΘΝΗ ΣΥΝΕΔΡΙΑ

- Σπάνια περίπτωση ηπατικής ενδομητρίωσης ως τυχαίο ασυμπτωματικό εύρημα - Παρουσίαση περιστατικού και βιβλιογραφική αναφορά (27ο Ιατρικό Συνέδριο Ενόπλων Δυνάμεων)
- Οξεία σκωληκοειδίτιδα λόγω μετάστασης μικροκυτταρικού καρκινώματος πνεύμονα. Μια σπάνια κλινική περίπτωση και βιβλιογραφική αναφορά (31ο Πανελλήνιο Συνέδριο Χειρουργικής)
- Σειρά περιστατικών ανατασσόμενης βουβωνοκήλης με περιεχόμενο έκτοπο ή υπεράριθμο όρχι σε ενήλικες (27ο Ιατρικό Συνέδριο Ενόπλων Δυνάμεων)
- Παρουσίαση σπάνιας Περίπτωσης μετεγχειρητικής χολόρροιας (31ο Πανελλήνιο Συνέδριο Χειρουργικής)
- Η επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων σε πειραματικό μοντέλο ισχαιμικού μυοκαρδίου (13ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Χειρουργών Θώρακος-Καρδιάς-Αγγείων)
- The effect of transplantation of undifferentiated mesenchymal stem cells in experimental model of myocardial infarction : Preliminary Results (16th congress of the Cell transplant and Regenerative Medicine Society, CTRMS 2019)
- Ευεργετική επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων σε πειραματικό μοντέλο ισχαιμικού μυοκαρδίου (21ο Πανελλήνιο Συνέδριο Μεταμοσχεύσεων)
- Imaging and Functional Evaluation of the effect of transplantation of mesenchymal stem cells in experimental model of myocardial infarction (21st ESOT Congress)
- Imaging and Functional Evaluation of the Positive Effect of transplantation of Undifferentiated Mesenchymal Stem Cells in Experimental Model of Myocardial Infarction (19th UCCVS)

ΣΥΝΕΧΙΖΟΜΕΝΗ ΔΙΑ ΒΙΟΥ ΕΚΠΑΙΔΕΥΣΗ:

Laboratory Animal Science - Translational Research:

- Seminar of Experimental Research - Thessaloniki, 2006
- 1st Panhellenic Workshop on Experimental Biomedical Research, December 2007 - Experimental - Research Center ELPEN, Athens
- 2nd Panhellenic Workshop on Experimental Biomedical Research, December 2008 - KAT Hospital, Athens

Χειρουργική εκπαίδευση (Ελληνική Χειρουργική Εταιρεία):

- 4ο Σεμινάριο Βασικής Ανοιχτής Χειρουργικής
- 5ο Σεμινάριο Προηγμένης Ανοιχτής Χειρουργικής
- 9ο Σεμινάριο Λαπαροσκοπικής Χειρουργικής - Λαπαροσκοπικής συρραφής και αναστομώσεων

Ελληνική Εταιρεία ΚαρδιοΑναπνευστικής Αναζωογόνησης:

- Διαχείριση τραυματία: Από τον τόπο του ατυχήματος μέχρι την οριστική φροντίδα
- Διαλογή στο ΤΕΠ
- Διαχείριση Αεραγωγού
- Βασικές αρχές ΗΚΓ

Παρακολούθηση πολυάριθμων πανελληνίων συνεδρίων με χειρουργική θεματολογία

CURRICULUM VITAE

CURRENT POSITION:

- Chief Resident in CardioThoracic Surgery, Red Cross Hospital, Athens, Greece
- PhD Candidate of Athens Medical School , Kapodistriakon University, Greece
- Thesis: “Experimental study of stem cells transplantation in a model of myocardial ischemia”
- MSc student of Athens Medical School, Kapodistriakon University, Greece
- Subject “Endovascular techniques”

PREVIOUS WORKING POSITIONS:

- Chief Resident in CardioThoracic Surgery, Hippocration Hospital, Athens, Greece
- Resident in General Surgery , NIMTS Hospital, Athens, Greece

GENERAL EDUCATION:

- 2002-2005, 3rd General High School of Kalamata, Greece (Excellent)
- 2005-2010, Veterinary School, University of Thessaly, Greece (Very Good)
- 2010-2016, Medical school, 3rd Medical Faculty, Charles University of Prague, Czech Republic (Excellent)

POSTGRADUATE EDUCATION:

- FELASA accreditation (Federation of European Laboratory Animal Science Associations) - Certificate ID:056/16_201008
- MSc in Endovascular Techniques: Athens Medical School, Kapodistriakon University, Greece in cooperation with University Milano Bicocca

TEACHING POSITIONS:

2012-2016 Auxiliary teacher in the department of Anatomy-Histoly-Embryology of 3rd Medical Faculty, Charles University of Prague (180 hours per semester)

RESEARCH:

- Subcortical parcellation of white matter in Rhesus Monkey in DTI - 2013
- Neuroanatomical study of the nucleus accumbens in human brain - 2014 - 2016
- Experimental study of stem cells transplantation in an experimental model of myocardial ischaimia - PhD thesis - since 2018

GRANTED RESEARCH PROGRAMS:

- **GAUK 1894214 - 2014** Neuroanatomická studie nucleus accumbens u člověka - Charles University of Prague
- **Research scholarship of Experimental Research ELPEN** for the experimental project of the PhD thesis and the Experimental study of stem cells transplantation in an experimental model of myocardial ischaimia

LANGUAGES:

Greek (mother language),
English (excellent),
French (very good),
Czech (good)

PUBLICATIONS:

- Mrzílkova J, Koutela A, Kutová M, Patzelt M, Ibrahim I, Al-Kayssi D, Bartoš A, Řípová D, Čermáková P, Zach P. Hippocampal spatial position evaluation on MRI for research and clinical practice. PLoS One. **2014** Dec 12;9(12):e115174. doi: 10.1371/journal.pone.0115174. PMID: 25502906; PMCID: PMC4264873.
- Zach P, Vales K, Stuchlik A, Cermakova P, Mrzilkova J, Koutela A, Kutova M. Effect of stress on structural brain asymmetry. Neuro Endocrinol Lett. **2016** Sep;37(4):253-264. PMID: 27857040.
- Keramidaris D, Gourgiotis S, Koutela A, Mpairamidis E, Oikonomou C, Villias C, Rigas A. Rare Case of Hepatic Endometriosis as an Incidental Finding: Difficult Diagnosis of a Diagnostic Dilemma. Ann Hepatol. **2018** Aug 24;17(5):884-887. doi: 10.5604/01.3001.0012.3173. PMID: 30145568.
- Oikonomou C, Spathari N, Doumoulaki S, Koutela A, Stagkoglou C, Keramidaris D. Recurrent Struma Ovarii Presented with High Levels of Thyroglobulin. Case Rep Surg. **2021** Mar 22;2021:8868095. doi: 10.1155/2021/8868095. PMID: 33824772; PMCID: PMC8007358.
- Koutela A, Loudos G, Rouchota M, Kletsas D, Karameris A, Vilaras G, Zografos GC, Grypari IM, Dougenis D, Papalois AE. A Novel Experimental Rat Model for the In Vivo Assessment of Myocardial Ischemia Based on Single Photon Emission Computed Tomography. In Vivo. **2023** Mar-Apr;37(2):649-654. doi: 10.21873/invivo.13124. PMID: 36881049; PMCID: PMC10026663.
- Koutela A, Loudos G, Rouchota M, Kletsas D, Karameris A, Vilaras G, Zografos GC, Myoteri D, Dougenis D, Papalois AE. Mesenchymal Stem Cell Transplantation Has a Regenerative Effect in Ischemic Myocardium: An Experimental Rat Model Evaluated by SPECT-CT Assessment. Diagnostics (Basel). **2024** Feb 12;14(4):401. doi: 10.3390/diagnostics14040401. PMID: 38396441; PMCID: PMC10888262.
- Zakszewski E, Adluru N, Tromp do PM, Kalin N, Alexander AL. A diffusion-tensor-based white matter atlas for rhesus macaques. PLoS One. **2014** Sep 9;9(9):e107398. doi: 10.1371/journal.pone.0107398. PMID: 25203614; PMCID: PMC4159318.

Αφιερώνεται στα αστέρια της ζωής μου,
Στη μητέρα μου και την κόρη μου!!!

Dedicated to the stars of my life,
My mother and my daughter!!!

ΕΥΧΑΡΙΣΤΙΕΣ

Η ολοκλήρωση της διδακτορικής μου διατριβής είναι το αποτέλεσμα μιας μεγάλης προσπάθειας ετών που βασίστηκε στο όραμα και την ελπίδα για μια άρτια επιστημονική μελέτη.

Παραθέτω λοιπόν εδώ, με συγκίνηση, το ευχαριστήριο σημείωμά μου προς όλους όσους στάθηκαν στο πλάι μου όλα αυτά τα χρόνια και στους οποίους οφείλω πολλά. Στην πραγματικότητα, η σελίδα των ευχαριστιών προς όλους εκείνους που στήριξαν την προσπάθειά μου επί σειρά ετών έχει γραφτεί δεκάδες φορές στο μυαλό μου ως το αποτύπωμα μιας νοερής άσκησης ολοκλήρωσης αυτής της μακράς και επίπονης, δοκιμασίας εκπόνησης μιας διδακτορικής διατριβής. Χωρίς τη συνδρομή των ανθρώπων που στάθηκαν στωικά στο πλάι μου όλα αυτά τα χρόνια, θα ήταν εξαιρετικά δύσκολο να φτάσω ως εδώ. Το ευχαριστήριο αυτό σημείωμα εκφράζει ένα ελάχιστο μέρος της ευγνωμοσύνης που αισθάνομαι για εκείνους.

Το πρώτο ευχαριστώ δικαιωματικά το οφείλω στον επιβλέποντά μου, ομότιμο καθηγητή κ. Δημήτριο Δουγένη, για την επιστημονική και ηθική στήριξη που αφειδώς μου παρείχε κάθε φορά που τη χρειαζόμουν, για τον πολύτιμο χρόνο που μου αφιέρωσε, για την υπομονή και την κατανόηση με την οποία διαχειρίστηκε κάθε μου απορία ή σκέψη, για τη σχολαστικότητα και την επιμέλεια με την οποία έσκυψε πάνω από την ερευνητική μου εργασία. Το παρόν εγχείρημα δεν θα ήταν εφικτό χωρίς τις προτάσεις του και την καθοδήγησή του.

Θα ήθελα, επίσης, να ευχαριστήσω τον ομότιμο καθηγητή κ. Γεώργιο Ζωγράφο, που από την πρώτη στιγμή με υποδέχτηκε στην ακαδημαϊκή κοινότητα και στήριξε το κάθε μου βήμα σε αυτή την πολυετή προσπάθεια συμβάλλοντας με την κριτική του ματιά και τις επισημάνσεις του στη βελτίωση της μελέτης και την ολοκλήρωση αυτής της διδακτορικής διατριβής.

Ένα πολύ μεγάλο ευχαριστώ από καρδιάς στον αγαπητό κ. Απόστολο Παπαλόη για την αμέριστη στήριξη και την πολύπλευρη συμπαράστασή του σε κάθε στάδιο αυτού του εγχειρήματος. Τον ευχαριστώ θερμά για τη διαρκή επιστημονική καθοδήγηση από τα πρώτα χρόνια της ακαδημαϊκής μου ύπαρξης και για την ακέραια εμπιστοσύνη που μου δείχνει σε κάθε πτυχή της

πολυετούς πλέον συνεργασίας μας, για τις πολύτιμες ευκαιρίες που μου πρόσφερε στους τομείς της έρευνας, για την ελευθερία και το σεβασμό που επέδειξε στις ερευνητικές επιλογές μου και κυρίως για όλες τις φορές που, υπό τη διακριτική εποπτεία του, με πέταξε μεσοπέλαγα για να μου μάθει να κολυμπάω.

Επιπροσθέτως θα ήθελα να ευχαριστήσω με θέρμη τον αξιότιμο Καθηγητή Αντώνιο Βεζάκη ο οποίος με χαρά προστέθηκε στην τριμελή συμβουλευτική επιτροπή της διατριβής μου μετά την αφυπηρέτηση του κ. Ζωγράφου και με καθολική συμπαράσταση και πίστη στο έργο και την έρευνά μας προσέφερε πολύτιμη βοήθεια στην ολοκλήρωση και παρουσίαση της διδακτορικής μου διατριβής.

Η ολοκλήρωση της διδακτορικής μου διατριβής, βεβαίως, θα ήταν κυριολεκτικά αδύνατη χωρίς την υλική, ηθική και έμπρακτη συμπαράσταση της οικογένειάς μου. Οφείλω ένα τεράστιο ευχαριστώ στους γονείς μου, Χαράλαμπο και Στέλλα, για όλα όσα κάνουν για εμένα καθημερινά από τη μέρα που γεννήθηκα, και κυρίως για την αδιαπραγμάτευτη αγάπη και τη βαθιά πίστη τους σε μένα, βασικά συστατικά που, όπως αντιλαμβάνομαι μεγαλώνοντας, έχουν δημιουργήσει μέσα μου πολύ μεγάλα αποθέματα δύναμης και αντοχής. Είναι οι άνθρωποι που με έμαθαν να έχω όνειρα και να τα κυνηγάω με όλες μου τις δυνάμεις, να θέτω στόχους και να μένω προσηλωμένη σε αυτούς κάτω από οποιαδήποτε συνθήκη. Όπως χαρακτηριστικά μου λέει πάντα η μητέρα μου : “το στόχο σου”!!! Αυτή της η φράση και το χαμόγελό της σε κάθε στόχο της ζωής μου από τότε που θυμάμαι τον εαυτό μου είναι η κινητήριος δύναμη να συνεχίζω. Τους ευγνωμονώ καθημερινά για το σπάνιο αυτό δώρο που μου προσέφεραν, για τον τρόπο να σκέφτομαι. Επιπλέον ευχαριστώ θερμά το σύζυγό μου Γιώργο για τη διαρκή συμπαράσταση και κατανόηση στο δύσκολο έργο μου, για τη χαρά και τον ενθουσιασμό του σε κάθε μου επιτυχία, για το θαυμασμό του στο πρόσωπό μου και για την ακλόνητη πίστη του ότι “θα τα καταφέρουμε”.

Τέλος το πιο γλυκό και τρυφερό ευχαριστώ το οφείλω στην κόρη μου που ήρθε στο τέλος αυτής της διαδρομής για να με γεμίσει ελπίδα και νέα όνειρα, για να φωτίσει το μέλλον και να στολίσει τη ζωή μου με τα πιο υπέροχα χρώματα.

ΟΡΚΟΣ

ΟΜΝΥΜΙ ΑΠΟΛΛΩΝΑ ΙΗΤΡΟΝ ΚΑΙ ΑΣΚΛΗΠΙΟΝ ΚΑΙ ΥΓΕΙΑΝ ΚΑΙ ΠΑΝΑΚΕΙΑΝ ΚΑΙ ΘΕΟΥΣ ΠΑΝΤΑΣ ΤΕ ΚΑΙ ΠΑΣΑΣ, ΙΣΤΟΡΑΣ ΠΟΙΕΥΜΕΝΟΣ, ΕΠΙΤΕΛΕΑ ΠΟΙΗΣΕΙΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ ΟΡΚΟΝ ΤΟΝΔΕ ΚΑΙ ΣΥΓΓΡΑΦΗΝ ΤΗΝΔΕ· ΗΓΗΣΕΣΘΑΙ ΜΕΝ ΤΟΝ ΔΙΔΑΞΑΝΤΑ ΜΕ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ ΙΣΑ ΓΕΝΕΤΗΣΙΝ ΕΜΟΙΣΙ ΚΑΙ ΒΙΟΥ ΚΟΙΝΩΣΕΣΘΑΙ ΚΑΙ ΧΡΕΩΝ ΧΡΗΙΖΟΝΤΙ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ, ΚΑΙ ΓΕΝΟΣ ΤΟ ΕΞ ΕΩΥΤΟΥ ΑΔΕΛΦΟΙΣ ΙΣΟΝ ΕΠΙΚΡΙΝΕΕΙΝ ΑΡΡΕΣΙ ΚΑΙ ΔΙΔΑΞΕΙΝ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ, ΗΝ ΧΡΗΙΖΩΣΙ ΜΑΝΘΑΝΕΙΝ, ΑΝΕΥ ΜΙΣΘΟΥ ΚΑΙ ΣΥΓΓΡΑΦΗΣ, ΠΑΡΑΓΓΕΛΙΗΣ ΤΕ ΚΑΙ ΑΚΡΟΗΣΙΟΣ ΚΑΙ ΤΗΣ ΛΟΙΠΗΣ ΑΠΑΣΗΣ ΜΑΘΗΣΙΟΣ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ ΥΙΟΙΣΙ ΤΕ ΕΜΟΙΣ ΚΑΙ ΤΟΙΣ ΤΟΥ ΕΜΕ ΔΙΔΑΞΑΝΤΟΣ ΚΑΙ ΜΑΘΗΤΗΣΙ ΣΥΓΓΕΓΡΑΜΜΕΝΟΙΣΙ ΤΕ ΚΑΙ ΩΡΚΙΣΜΕΝΟΙΣ ΝΟΜΩ ΙΗΤΡΙΚΩ, ΑΛΛΩ ΔΕ ΟΥΔΕΝΙ. ΔΙΑΙΤΗΜΑΣΙ ΤΕ ΧΡΗΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ, ΕΠΙ ΔΗΛΗΣΕΙ ΔΕ ΚΑΙ ΑΔΙΚΙΗ, ΕΙΡΞΕΙΝ. ΟΥ ΔΩΣΩ ΔΕ ΟΥΔΕ ΦΑΡΜΑΚΟΝ ΟΥΔΕΝΙ ΑΙΤΗΘΕΙΣ ΘΑΝΑΣΙΜΟΝ ΟΥΔΕ ΥΦΗΓΗΣΟΜΑΙ ΣΥΜΒΟΥΛΙΗΝ ΤΟΙΗΝΔΕ· ΟΜΟΙΩΣ ΔΕ ΟΥΔΕ ΓΥΝΑΙΚΙ ΠΕΣΣΟΝ ΦΘΟΡΙΟΝ ΔΩΣΩ. ΑΓΝΩΣ ΔΕ ΚΑΙ ΟΣΙΩΣ ΔΙΑΤΗΡΗΣΩ ΒΙΟΝ ΤΟΝ ΕΜΟΝ ΚΑΙ ΤΕΧΝΗΝ ΤΗΝ ΕΜΗΝ. ΟΥ ΤΕΜΕΩ ΔΕ ΟΥΔΕ ΜΗΝ ΛΙΘΙΩΝΤΑΣ, ΕΚΧΩΡΗΣΩ ΔΕ ΕΡΓΑΤΗΣΙΝ ΑΝΔΡΑΣΙ ΠΡΗΞΙΟΣ ΤΗΣΔΕ. ΕΣ ΟΙΚΙΑΣ ΔΕ ΟΚΟΣΑΣ ΑΝ ΕΣΙΩ, ΕΣΕΛΕΥΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ, ΕΚΤΟΣ ΕΩΝ ΠΑΣΗΣ ΑΔΙΚΙΗΣ ΕΚΟΥΣΙΗΣ ΚΑΙ ΦΘΟΡΙΗΣ ΤΗΣ ΤΕ ΑΛΛΗΣ ΚΑΙ ΑΦΡΟΔΙΣΙΩΝ ΕΡΓΩΝ ΕΠΙ ΤΕ ΓΥΝΑΙΚΕΙΩΝ ΣΩΜΑΤΩΝ ΚΑΙ ΑΝΔΡΩΙΩΝ, ΕΛΕΥΘΕΡΩΝ ΤΕ ΚΑΙ ΔΟΥΛΩΝ. Α ΔΕ ΑΝ ΕΝ ΘΕΡΑΠΕΙΗ, Η ΙΔΩ Η ΑΚΟΥΣΩ, Η ΚΑΙ ΑΝΕΥ ΘΕΡΑΠΕΙΗΣ ΚΑΤΑ ΒΙΟΝ ΑΝΘΡΩΠΩΝ, Α ΜΗ ΧΡΗ ΠΟΤΕ ΕΚΛΑΛΕΙΣΘΑΙ ΕΞΩ, ΣΙΓΗΣΟΜΑΙ, ΑΡΡΗΤΑ ΗΓΕΥΜΕΝΟΣ ΕΙΝΑΙ ΤΑ ΤΟΙΑΥΤΑ. ΟΡΚΟΝ ΜΕΝ ΟΥΝ ΜΟΙ ΤΟΝΔΕ ΕΠΙΤΕΛΕΑ ΠΟΙΕΟΝΤΙ, ΚΑΙ ΜΗ ΣΥΓΧΕΟΝΤΙ, ΕΙΗ ΕΠΑΥΡΑΣΘΑΙ ΚΑΙ ΒΙΟΥ ΚΑΙ ΤΕΧΝΗΣ ΔΟΞΑΖΟΜΕΝΩ, ΠΑΡΑ ΠΑΣΙΝ ΑΝΘΡΩΠΟΙΣ ΕΙΣ ΤΟΝ ΑΙΕΙ ΧΡΟΝΟΝ· ΠΑΡΑΒΑΙΝΟΝΤΙ ΔΕ ΚΑΙ ΕΠΙΟΡΚΟΥΝΤΙ, ΤΑΝΑΝΤΙΑ ΤΟΥΤΕΩΝ.

ΙΠΠΟΚΡΑΤΗΣ

ΟΡΚΟΣ

ΟΡΚΙΖΟΜΑΙ ΕΙΣ ΤΟΝ ΑΠΟΛΛΩΝΑ ΤΟΝ ΙΑΤΡΟΝ ΚΑΙ ΕΙΣ ΤΟΝ ΑΣΚΛΗΠΙΟΝ ΚΑΙ ΕΙΣ ΤΗΝ ΥΓΕΙΑΝ ΚΑΙ ΕΙΣ ΤΗΝ ΠΑΝΑΚΕΙΑΝ ΚΑΙ ΕΙΣ ΟΛΟΥΣ ΤΟΥΣ ΘΕΟΥΣ ΚΑΙ ΕΙΣ ΟΛΑΣ ΤΑΣ ΘΕΑΣ, ΕΠΙΚΑΛΟΥΜΕΝΟΣ ΑΥΤΟΥΣ ΩΣ ΜΑΡΤΥΡΑΣ, ΟΤΙ ΘΑ ΤΗΡΗΣΩ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΑΤΑ ΤΗΝ ΚΡΙΣΙΝ ΜΟΥ ΑΥΤΟΝ ΕΔΩ ΤΟΝ ΟΡΚΟΝ ΚΑΙ ΑΥΤΗΝ ΕΔΩ ΤΗΝ ΕΓΓΡΑΦΟΝ ΣΥΜΦΩΝΙΑΝ. ΘΑ ΘΕΩΡΩ ΜΕΝ ΕΚΕΙΝΟΝ, Ο ΟΠΟΙΟΣ ΜΕ ΕΔΙΔΑΞΕ ΤΗΝ ΤΕΧΝΗΝ ΑΥΤΗΝ, ΙΣΟΝ ΠΡΟΣ ΤΟΥΣ ΓΟΝΕΙΣ ΜΟΥ ΚΑΙ ΘΑ ΤΟΝ ΚΑΜΩ ΚΟΙΝΩΝΟΝ ΤΟΥ ΒΙΟΥ ΜΟΥ ΚΑΙ ΘΑ ΤΟΝ ΒΟΗΘΩ ΟΤΑΝ ΕΥΡΙΣΚΕΤΑΙ ΕΙΣ ΟΙΚΟΝΟΜΙΚΗΝ ΑΝΑΓΚΗΝ, ΚΑΙ ΘΑ ΘΕΩΡΩ ΤΟΥΣ ΑΠΟΓΟΝΟΥΣ ΤΟΥ ΩΣ ΑΔΕΛΦΟΥΣ ΜΟΥ ΚΑΙ ΘΑ ΤΟΥΣ ΔΙΔΑΣΚΩ ΤΗΝ ΤΕΧΝΗΝ ΑΥΤΗΝ, ΕΑΝ ΘΕΛΟΥΝ ΝΑ ΤΗΝ ΜΑΘΟΥΝ, ΧΩΡΙΣ ΑΜΟΙΒΗΝ ΚΑΙ ΕΓΓΡΑΦΟΝ ΣΥΜΦΩΝΙΑΝ, ΘΑ ΠΑΡΕΧΩ ΔΕ ΣΥΜΒΟΥΛΑΣ, ΕΙΔΙΚΑ ΠΡΟΦΟΡΙΚΑ ΜΑΘΗΜΑΤΑ ΚΑΙ ΟΛΑΣ ΤΑΣ ΥΠΟΛΟΙΠΟΥΣ ΓΝΩΣΕΙΣ ΤΗΣ ΙΑΤΡΙΚΗΣ ΚΑΙ ΕΙΣ ΤΑ ΠΑΙΔΙΑ ΜΟΥ ΚΑΙ ΕΙΣ ΤΑ ΠΑΙΔΙΑ ΤΟΥ ΔΙΔΑΣΚΑΛΟΥ ΜΟΥ ΚΑΙ ΕΙΣ ΤΟΥΣ ΜΑΘΗΤΑΣ, ΟΙ ΟΠΟΙΟΙ ΚΑΙ ΕΓΓΕΓΡΑΜΜΕΝΟΙ ΕΙΝΑΙ ΚΑΙ ΕΧΟΥΝ ΔΩΣΕΙ ΟΡΚΟΝ ΣΥΜΦΩΝΟΣ ΠΡΟΣ ΤΟΝ ΙΑΤΡΙΚΟΝ ΝΟΜΟΝ, ΕΙΣ ΚΑΝΕΝΑ ΔΕ ΑΛΛΟΝ.

ΘΑ ΧΡΗΣΙΜΟΠΟΙΩ ΔΕ ΤΗΝ ΘΕΡΑΠΕΥΤΙΚΗΝ ΑΓΩΓΗΝ ΠΡΟΣ ΩΦΕΛΕΙΑΝ ΤΩΝ ΑΣΘΕΝΩΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΑΤΑ ΤΗΝ ΚΡΙΣΙΝ ΜΟΥ. ΘΑ ΠΡΟΛΑΜΒΑΝΩ ΔΕ ΒΛΑΒΗΝ ΥΓΕΙΑΣ ΚΑΙ ΑΔΙΚΙΑΝ. ΔΕΝ ΘΑ ΔΩΣΩ ΔΕ ΦΑΡΜΑΚΟΝ ΘΑΝΑΤΗΦΟΡΟΝ ΕΙΣ ΚΑΝΕΝΑ, ΟΥΤΕ ΚΑΙ ΕΑΝ ΜΟΥ ΖΗΤΗΘΗ, ΚΑΙ ΟΥΤΕ ΘΑ ΔΩΣΩ ΤΟΙΑΥΤΗΝ ΣΥΜΒΟΥΛΗΝ ΩΣΑΥΤΩΣ ΟΥΤΕ ΕΙΣ ΕΓΚΥΟΝ ΓΥΝΑΙΚΑ ΘΑ ΔΩΣΩ ΕΚΤΡΩΤΙΚΟΝ ΦΑΡΜΑΚΟΝ. ΘΑ ΔΙΑΤΗΡΩ ΔΕ ΤΟΝ ΒΙΟΝ ΜΟΥ ΚΑΙ ΤΗΝ ΤΕΧΝΗΝ ΜΟΥ ΚΑΤΑ ΤΡΟΠΟΝ ΑΓΝΟΝ ΚΑΙ ΣΥΜΦΩΝΟΝ ΠΡΟΣ ΤΟΝ ΘΕΙΟΝ ΝΟΜΟΝ. ΑΚΟΜΗ ΔΕ ΔΕΝ ΘΑ ΧΕΙΡΟΥΡΓΩ ΟΥΤΕ ΚΑΙ ΤΟΥΣ ΠΑΣΧΟΝΤΑΣ ΑΠΟ ΛΙΘΙΑΣΙΝ, ΑΛΛΑ ΘΑ ΤΟΥΣ ΠΑΡΑΠΕΜΠΩ ΕΙΣ ΑΝΔΡΑΣ ΧΕΙΡΟΥΡΓΟΥΣ, ΕΙΔΙΚΟΥΣ ΔΙΑ ΤΟ ΕΡΓΟΝ ΤΟΥΤΟ. ΕΙΣ ΟΣΑΣ ΔΕ ΟΙΚΙΑΣ ΜΕΤΑΒΑΙΝΩ, ΘΑ ΕΙΣΕΡΧΩΜΑΙ ΠΡΟΣ ΒΟΗΘΕΙΑΝ ΤΩΝ ΑΣΘΕΝΩΝ, ΑΠΕΧΩΝ ΠΑΣΗΣ ΕΚΟΥΣΙΑΣ ΑΔΙΚΙΑΣ ΚΑΙ ΑΛΛΗΣ ΔΙΑΦΘΟΡΑΣ ΚΑΙ ΣΑΡΚΙΚΩΝ ΣΧΕΣΕΩΝ ΜΕΤΑ ΓΥΝΑΙΚΩΝ Η ΑΝΔΡΩΝ ΕΛΕΥΘΕΡΩΝ ΚΑΙ ΔΟΥΛΩΝ.

ΟΣΑ ΔΕ ΙΔΩ Η ΑΚΟΥΣΩ ΚΑΤΑ ΤΗΝ ΘΕΡΑΠΕΙΑΝ Η ΚΑΤΑ ΤΗΝ ΕΠΙΚΟΙΝΩΝΙΑΝ ΜΟΥ ΜΕΤΑ ΤΩΝ ΑΛΛΩΝ ΑΝΘΡΩΠΩΝ ΕΙΣ ΤΗΝ ΚΑΘΗΜΕΡΙΝΗΝ ΤΟΥΣ ΖΩΗΝ, ΤΑ ΟΠΟΙΑ ΔΕΝ ΠΡΕΠΕΙ ΠΟΤΕ ΝΑ ΑΝΑΚΟΙΝΩΘΟΥΝ ΕΙΣ ΤΟΥΣ ΑΛΛΟΥΣ, ΘΑ ΚΡΑΤΩ ΜΥΣΤΙΚΑ, ΠΙΣΤΕΥΩΝ ΟΤΙ ΤΑ ΤΟΙΑΥΤΑ ΕΙΝΑΙ ΑΠΟΡΡΗΤΑ. ΕΦ' ΟΣΟΝ ΜΕΝ ΛΟΙΠΟΝ ΤΗΡΩ ΑΥΤΟΝ ΕΔΩ ΤΟΝ ΟΡΚΟΝ ΚΑΙ ΔΕΝ ΤΟΝ ΠΑΡΑΒΑΙΝΩ, ΕΙΘΕ ΝΑ ΑΠΟΛΑΥΩ ΚΑΙ ΤΗΣ ΖΩΗΣ ΚΑΙ ΤΗΣ ΤΕΧΝΗΣ, ΕΚΤΙΜΩΜΕΝΟΣ ΕΣΑΕΙ ΑΠΟ ΟΛΟΥΣ ΤΟΥΣ ΑΝΘΡΩΠΟΥΣ· ΕΑΝ ΟΜΩΣ ΠΑΡΑΒΩ ΑΥΤΟΝ ΚΑΙ ΓΙΝΩ ΕΠΙΟΡΚΟΣ, ΝΑ ΠΑΘΩ ΤΑ ΑΝΤΙΘΕΤΑ ΤΟΥΤΩΝ.

Hippocratic Oath

I swear by Apollo the physician, and Aesculapius and Health, and All-heal, and all the gods and goddesses, that, according to my ability and judgment, I will keep this Oath and this stipulation—to reckon him who taught me this Art equally dear to me as my parents, to share my substance with him, and relieve his necessities if required; to look upon his offspring in the same footing as my own brothers, and to teach them this Art, if they shall wish to learn it, without fee or stipulation; and that by precept, lecture, and every other mode of instruction, I will impart a knowledge of the Art to my own sons, and those of my teachers, and to disciples bound by a stipulation and oath according to the law of medicine, but to none others. I will follow that system of regimen which, according to my ability and judgment, I consider for the benefit of my patients, and abstain from whatever is deleterious and mischievous. I will give no deadly medicine to any one if asked, nor suggest any such counsel; and in like manner I will not give to a woman a pessary to produce abortion. With purity and with holiness I will pass my life and practice my Art. I will not cut persons laboring under the stone, but will leave this to be done by men who are practitioners of this work. Into whatever houses I enter, I will go into them for the benefit of the sick, and will abstain from every voluntary act of mischief and corruption; and, further from the seduction of females or males, of freemen and slaves. Whatever, in connection with my professional practice or not, in connection with it, I see or hear, in the life of men, which ought not to be spoken of abroad, I will not divulge, as reckoning that all such should be kept secret. While I continue to keep this Oath unviolated, may it be granted to me to enjoy life and the practice of the art, respected by all men, in all times! But should I trespass and violate this Oath, may the reverse be my lot!

Περίληψη

Οι ισχαιμικές μυοκαρδιοπάθειες παραμένουν ένα μέγιστο ιατρικό πρόβλημα παγκοσμίως με υψηλό ποσοστό θνητότητας. Παρά τις πολυάριθμες προσπάθειες ερευνών και τα πολυάριθμα πειραματικά μοντέλα που έχουν αναπτυχθεί στο πέρασμα των ετών, δεν έχει γίνει ακόμα απολύτως κατανοητό το πώς εγκαθίσταται το έμφραγμα του μυοκαρδίου οδηγώντας σε απώλεια μυοκαρδιακού ιστού.

Επιπλέον, η αναγέννηση του μυοκαρδίου και η βελτίωση της μυοκαρδιακής δραστηριότητας μετά την ισχαιμία είναι ένα από τα πιο σημαντικά πεδία έρευνας και ενδιαφέροντος στην παγκόσμια ιατρική (και ειδικά την καρδιαγγειακή) κοινότητα.

Διαπιστώθηκε η ευεργετική δράση της έγχυσης μεσεγχυματικών βλαστοκυττάρων σε ένα πρωτότυπο πειραματικό μοντέλο με ζωικό πρότυπο τον αρουραίο με χειρουργικά εγκατεστημένη ισχαιμία του μυοκαρδίου. Από κλινική σκοπιά αυτή η μελέτη υποστηρίζει τη χειρουργική εναπόθεση μεσεγχυματικών βλαστοκυττάρων στην ισχαιμική περιοχή κατά τη διάρκεια αορτοστεφανιαίας παράκαμψης.

Η αναγέννηση του μυοκαρδίου και η βελτίωση της μυοκαρδιακής δραστηριότητας μετά την ισχαιμία είναι από τα μέγιστα πεδία ενδιαφέροντος στην καρδιαγγειακή έρευνα.

Αναπτύχθηκε ένα νέο πειραματικό μοντέλο με χρήση αρουραίων και χρησιμοποιήθηκε για να αξιολογηθεί η δράση των μεσεγχυματικών βλαστοκυττάρων στη μυοκαρδιακή ισχαιμία με τη χρήση σπινθηρογραφήματος SPECT-CT και ανοσοϊστοχημείας. Σε ανοιχτή θωρακοτομή υποβλήθηκαν 40 θηλυκοί ενήλικες αρουραίοι εκ των οποίων οι 30 υπέστησαν ισχαιμική περίδεση του πρόσθιου κατιόντα κλάδου της στεφανιαίας αρτηρίας ενώ οι 10 όχι. Η βιωσιμότητα του μυοκαρδίου αξιολογήθηκε με σπινθηρογράφημα SPECT-CT επτά ημέρες πριν τη χειρουργική περίδεση καθώς και επτά και δεκατέσσερις ημέρες μετεγχειρητικά. Την ημέρα μηδέν (ημέρα χειρουργείου) τα 15 ζώα έλαβαν

μεσεγχυματικά βλαστοκύτταρα με άμεση έγχυση διεγχειρητικά στο ισχαιμικό πεδίο που είχε εγκατασταθεί στην αριστερή κοιλία της καρδιάς. Η ανάλυση του σπινθηρογραφήματος SPECT-CT έδειξε μειωμένη δραστηριότητα των μυοκαρδιακών κυττάρων στην αριστερή κοιλία μια εβδομάδα μετά την πρόκληση ισχαιμίας.

Η αναγέννηση του ισχαιμικού μυοκαρδίου δεκαπέντε ημέρες μετά το χειρουργείο διαπιστώθηκε μόνο στα ζώα που έλαβαν τα βλαστοκύτταρα.

Αυτά τα αποτελέσματα επιβεβαιώθηκαν με ιστολογική και ανοσοϊστοχημική ανάλυση με σημαντικά μεγαλύτερη έκφραση των GATA4 και Nkx2.5. Συμπερασματικά η θετική επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων στο ισχαιμικό μυοκάρδιο καταγράφηκε αδιαμφισβήτητα. Η χρήση σπινθηρογραφήματος SPECT-CT επέτρεψε μια ξεκάθαρη αξιολόγηση τόσο ποιοτική όσο και ποσοτική του ζωντανού μυοκαρδιακού ιστού μετά την πρόκληση ισχαιμίας οδηγώντας σε μια νέα προσέγγιση της έρευνας των καρδιαγγειακών παθήσεων. Από κλινικής πλευράς, η χρήση των μεσεγχυματικών βλαστοκυττάρων μπορεί να είναι ευεργετική όταν συνδυάζεται με τεχνικές επαναιμάτωσης του μυοκαρδίου.

Abstract

Ischemic heart disease remains a major medical problem with high mortality rates. Beside the great efforts devoted to research worldwide and the use of numerous experimental models, an absolute understanding of myocardial infarction and tissue loss has not yet been achieved. Furthermore, the regeneration of myocardial tissue and the improvement of myocardial activity after ischemia is one of the major areas of interest in the medical (and especially cardiovascular) community. In a novel experimental rat model, the beneficial effect of mesenchymal stem cell transplantation (MSCT) in a surgically induced ischemic myocardium was documented. From a clinical perspective, this work supports the surgical administration of MSCT in the infarcted area during coronary artery bypass surgery. The regeneration of myocardial tissue and the improvement of myocardial activity after ischemia is one of the major areas of interest in cardiovascular research. We developed a novel experimental rat model and used it to examine the effect of mesenchymal stem cell transplantation (MSCT) on myocardial ischemia evaluated by SPECT-CT and immunehistochemistry. Methods and results: An open thoracotomy took place for forty adult female Wistar rats with (n = 30) or without (n = 10) surgical ligation of the left anterior descending coronary artery (LAD) in order to cause myocardial ischemia. Myocardial viability was evaluated via SPECT/CT 7 days before surgery, as well as at 7 and 14 days post-surgery. At day 0, 15 animals received homologous stem cells injected at the ischemic myocardium area. A SPECT/CT evaluation showed decreased activity of the myocardial cells in the left ventricle one week post-infarction. Regeneration of the ischemic myocardium fifteen days post-infarction was recorded only in animals subjected to stem cell transplantation. These findings were also confirmed by histology and immunohistochemical analysis, with the significantly higher expression of GATA4 and Nkx2.5. Conclusions: The positive effect of mesenchymal stem cell transplantation in the ischemic myocardium was recorded. The application of SPECT-CT allowed a clear evaluation of both the quality and quantity of the living myocardium post-infarction, leading to a new approach in the research of cardiovascular diseases. From a clinical perspective, MSCT may be beneficial when accompanied by myocardial revascularization procedures.

General information
regarding this PhD Thesis

The study protocol was authorized by the National Animal ExperimentBoard of Greece and the Veterinary Association of Athens (License No.: 1870/23-04-2018) and was carried out in compliance with EU legislation conforming to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes relating to the conduct of animal experimentation.

The experimental procedures were performed in ELPEN Experimental Center, BIOMTECH Laboratories and Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, NCSR “Demokritos”. The contributions of the three centers are clearly described in the experimental protocol with the required licenses and specific working hours.

Funding: Partial funding with a scholarship by the Experimental, Educational and Research Center (EERC), ELPEN Pharmaceuticals, Athens, Greece.

Informed Consent Statement: Not applicable (The research is experimental using a rat model).

Data Availability Statement: Data are available upon request.

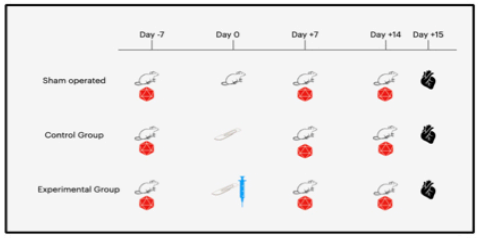
Publications
during and related to this PhD Thesis

1. Koutela, A.; Loudos, G.; Rouchota, M.; Kletsas, D.; Karameris, A.; Vilaras, G.; Zografos, G.C.; Myoteri, D.; Dougenis, D.; Papalois, A.E. Mesenchymal Stem Cell Transplantation Has a Regenerative Effect in Ischemic Myocardium: An Experimental Rat Model Evaluated by SPECT-CT Assessment. Diagnostics **2024**, 14, 401. https://doi.org/10.3390/diagnostics14040401
2. Koutela A, Loudos G, Rouchota M, Kletsas D, Karameris A, Vilaras G, Zografos GC, Grypari IM, Dougenis D, Papalois AE. A Novel Experimental Rat Model for the In Vivo Assessment of Myocardial Ischemia Based on Single Photon Emission Computed Tomography. In Vivo. **2023** Mar-Apr;37(2):649-654. doi: 10.21873/invivo.13124. PMID: 36881049; PMCID: PMC10026663.

Παρουσιάσεις αποτελεσμάτων Διδακτορικής Διατριβής σε Ελληνικά και Διεθνή Συνέδρια
Conferences

- Η επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων σε πειραματικό μοντέλο ισχαιμικού μυοκαρδίου (13ο Πανελλήνιο Συνέδριο Ελληνικής Εταιρείας Χειρουργών Θώρακος-Καρδιάς-Αγγείων - Αθήνα 2020)
- The effect of transplantation of undifferentiated mesenchymal stem cells in experimental model of myocardial infarction : Preliminary Results (16th congress of the Cell transplant and Regenerative Medicine Society, CTRMS 2019, Lesvos 2019)
- Ευεργετική επίδραση της μεταμόσχευσης μεσεγχυματικών βλαστοκυττάρων σε πειραματικό μοντέλο ισχαιμικού μυοκαρδίου (21ο Πανελλήνιο Συνέδριο Μεταμοσχεύσεων, Αθήνα 2021)
- Imaging and Functional Evaluation of the effect of transplantation of mesenchymal stem cells in experimental model of myocardial infarction (21st Congress of The European Society for Organ Transplantation , ESOT, Athens 2023)
- Imaging and Functional Evaluation of the Positive Effect of transplantation of Undifferentiated Mesenchymal Stem Cells in Experimental Model of Myocardial Infarction (19th International Congress of Update in Cardiology and Cardiovascular Surgery, UCCVS, Istanbul 2023)

Graphical Abstract

AIM OF THE STUDY **HYPOTHESIS**	Is mesenchymal stem cell (MSC) transplantation effective when administered in ischemic myocardium?
METHODS	 -Novel rat model with LAD ligation -Administration of MSC -Evaluation with SPECT-CT, Immunohistochemistry and LV/RV volume
RELULTS	**Regeneration of ischemic myocardium was documented**
CONCLUSION	MSC transplantation in the ischemic myocardium has a regenerative effect on cardiomyocytes. In clinical perspective, MSC may be used in combination with CABG

LAD left anterior descending artery,
MSC Mesenchymal stem cells,
SPECT/CT Single Positron Emission Computed Tomography /Computed Tomography,
CABG coronary artery bypass grafting

General Part

Introduction

Cardiovascular diseases such as ischemic heart disease remain a major cause of death worldwide. Despite extensive research on pharmaceutical treatments, the mortality rates remain high, and, therefore, further investigations are needed. [1,2] The first study of the experimental induction of myocardial ischemia was reported in 1862. Since then, among the various techniques of inducing myocardial infarction in experimental models, the ligation of the left anterior descending artery (LAD) has become the most prominent. [1-5]

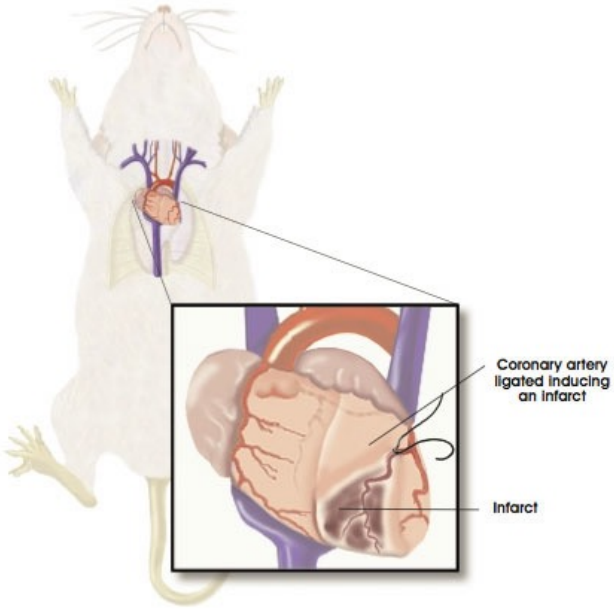
Mesenchymal stem cells are multipotent cells that were first recognized in the bone marrow of adults. They are characterized by neovascularization and the ability to differentiate to multiple cell lines, including adipocytes, osteoblasts, and chondroblasts. The discovery of these cells took place in 1960 and since then the research dynamically continues in order to isolate such cells from as many tissues as possible including adipose tissue, peripheral blood tissue, umbilical cord, skin, amniotic fluid. [2] Tha goal is the production of transplantable cells and the last years it has known great improvement.

Adipose tissue derived cells (ADSCs) are preferable and have replaced the bone marrow derived cells due to the easier way of selection and isolation, the satisfying quantity and the decreased danger of rejection. Therefore Adipose-derived stem cell (ADSCs) transplantation is nowadays considered the ideal tissue of choice. Their extensive ability for neovascularization explains their choice and the great interest for their use in regenerative medicine. Nowadays ADSCs are widely used in tissue factory and cellular therapies. Furthermore, it is known that they can be differentiated to cardiomyocytes and can lead to newly formed cardiac tissue. [1,2] Despite numerous current experimental trials, there are still many questions unanswered, such as the type of cells to be used, the number of cells necessary to be transplanted, the technique and the route of administration, and finally the confirmation of the improvement in the function of the target tissue, such as the myocardium.

The molecular markers used in our study to evaluate the action of ADSCs in the myocardium were GATA4, a zinc-finger transcription factor that is highly expressed in cardiomyocytes and is key to myocardial differentiation, and NKX2-5, a protein-coding gene that is involved in myocardial conduction and contractility. [1-2] In vitro results have shown an actively proliferating stem cell population 72 h after isolation, but it has been documented in immunochemical studies that the expression of stem cell markers needs approximately 7 days.

Ischemic Heart Experimental Model

A rat model was chosen for our experimental research. Rodents have been intensely used in pre-clinical trials as an accepted animal model. [3-7] Their use has been steadily increasing, especially in protocols related to cardiac activity. Myocardial ischemia is induced by variable methods either surgical or non-invasive, mainly as acute experiments leading to post-mortem evaluation. In vivo experiments in such protocols are highly challenging due to the increased mortality rates. Surgical techniques such as ligation of the left anterior descending coronary artery (LAD) has been widely used to establish a model of myocardial infarction. [5-9] There are some disadvantages in this technique including the high mortality rates after ligation and the variations in the infarct size. Therefore, improving the techniques causing ischemia is required, while non-invasive imaging methods in living animals post-infarction is extremely valuable for evaluating many aspects of the ischemic myocardium in vivo. [10-13] Myocardial ischemia and left ventricular defect causing heart failure can be associated with viable and non-viable myocardial tissue.



SPECT - CT Evaluation

Myocardial perfusion imaging with single-photon emission computed tomography (SPECT) is an important and widely used non-invasive imaging test for the diagnosis and semiquantification of myocardial ischemia. [14] SPECT data, acquired after a stress test and at rest on a gamma camera, show left ventricular tracer uptake during stress and at rest. The tracer distributions are proportionate to the relative, regional coronary-flow distributions, respectively. A stress-induced perfusion defect reflects myocardial ischemia, while a permanent defect, unchanged from stress to rest, indicates myocardial infarction. [15-17] Myocardial perfusion imaging (MPI) by single-photon emission computed tomography (SPECT) uses a radiopharmaceutical to show in gamma camera imaging studies the regional flow distribution of the left ventricle (LV) at stress and at rest, in order to depict ischemia and infarction.

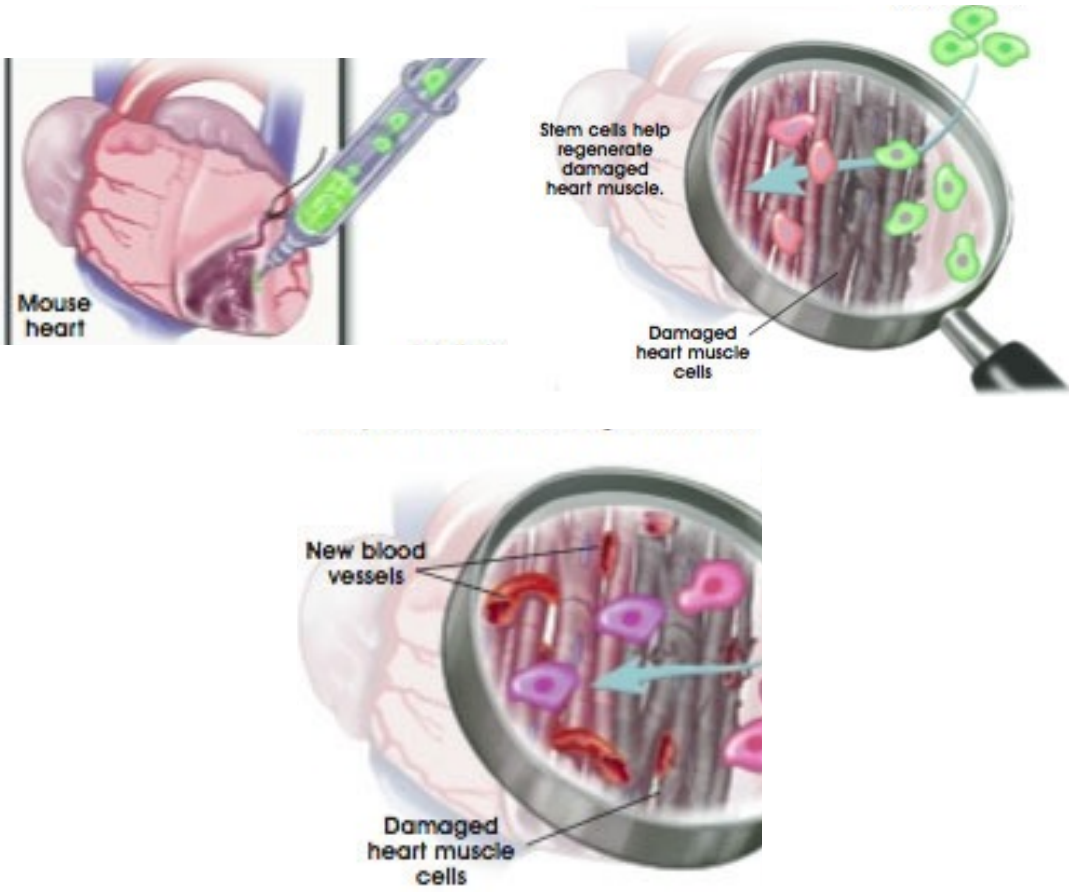
With ECG-triggering or “gating” information of global and regional left ventricular function is added. When available, the combination of SPECT and coronary computed tomography (CT) angiography (CCTA), so-called hybrid imaging, can provide detailed, exact anatomic and physiologic information in a one-step procedure. Different radiotracers, protocols, and imaging techniques are used in SPECT. [18] The uptake of the perfusion tracer is related to the

perfusion of the tissue. For perfusion imaging with SPECT, thallium-201 (or201Tl) and technetium-99m (or99 mTc) labeled radiopharmaceuticals are available. The technetium-99m labeled radiopharmaceuticals are now used more often. If a CT scanner is integrated with the gamma camera system information about coronary anatomy can be accurately overlaid the images of coronary perfusion. Image interpretation is mainly based on a visual inspection of the reconstructed images of the activity distribution in the LV. Short-axis, horizontal long-axis and vertical long-axis image planes are evaluated. [19-24]

During our protocol all animals underwent a SPECT-CT imaging prior to any further experimentation in order to analyze the functional myocardium of healthy individuals. In 2009, the American College of Cardiology Foundation published Appropriate Use Criteria on indications for SPECT. In 2013 and 2014, the ESC published indications for SPECT in their guidelines on management of stable CAD and on myocardial revascularization. [25] Therefore it is of major clinical significance to evaluate and analyze the myocardial function with the use of SPECT-CT in an experimental model in order to obtain in-vivo qualitative and quantitive results of the myocardial viability.

Single Photon Emission Computed Tomography (SPECT) and Computed Tomography (CT) are highly sensitive and specific methods for analyzing both physiological and anatomical aspects. Therefore, their use in preclinical trials is highly recommended. [26-27] We report here a novel reliable and reproducible rat model, in which myocardial ischemia and infarction were induced by ligation of the left anterior descending coronary artery, and SPECT/CT imaging, along with histology and biochemistry, were used for in vivo evaluation and assessment of the infarction in a qualitative and quantitative manner.

Adipose Derived Mesenchymal Stem Cells (ADSCs)



Adipose-derived stem cells (ASCs) are a subset of mesenchymal stem cells (MSCs) that can be obtained easily from adipose tissues and possess many of the same regenerative properties as other MSCs. ASCs easily adhere to plastic culture flasks, expand in vitro, and have the capacity to differentiate into multiple cell lineages, offering the potential to repair, maintain, or enhance various tissues. [28-32] Since human adipose tissue is ubiquitous and easily obtained in large quantities using a minimally invasive procedure, the use of autologous ASCs is promising for both regenerative medicine and organs damaged by injury and disease, leading to a rapidly increasing field of research.

ASCs are effective for the treatment of severe symptoms such as atrophy, fibrosis, retraction, and ulcers. Moreover, ASCs have been shown to be effective for pathological wound healing such as scar formation. [33-36]

Although many experimental procedures have been proposed, standardized harvesting protocols and processing techniques do not yet exist.

Mesenchymal Stem Cells (MSCs), originally identified in bone marrow, but it has been demonstrated that MSCs can be isolated from almost every tissue of the body including adipose tissue. In 2018, Mushahary et al. highlighted variations in the differentiation potential of MSCs from different tissue sources. [37-38] Adipose-derived stem cells (ASCs) have become one of the most promising stem cell populations identified so far because they are ubiquitous and can be relatively easily harvested in larger quantities with less donor-site morbidity. [39] Subcutaneous adipose tissue is the most clinically relevant source of ASCs among the several adipose tissue types, and can be isolated from subcutaneous adipose tissue of the abdomen, thigh, and arm. As adipose tissue is relatively abundant in the human body, ASCs can be obtained in higher numbers. [40] The multi-lineage capacity of ASCs offers the potential of cell therapy and tissue engineering.

ASCs harvested from superficial abdominal regions undergo significantly less apoptosis than do ASCs harvested from the upper arm, medial thigh, trochanteric, or superficial deep abdominal depots. Since Zuk et al. described a harvesting procedure for ASCs from adipose tissue in 2001, many modified experimental procedures have been proposed. However, there have been few studies comparing the efficacy of various methods, and therefore no standardized method has been defined. [41-42] In our study a standardized method of isolation of abdominal adipose tissue derived stem cells has been used as autologous cells from male Wistar rats.

Factors GATA-4 and Nkx-2.5

Recent studies identified that GATA-4 is a stress responsive transcription factor and can exert cell survival signaling in cardiac myocytes. GATA-4 is a member of the GATA family of zinc finger transcription factors that plays important roles in transducing nuclear events that modulate cell lineage differentiation during development. [43] Six GATA family members have been

identified and shown to alter transcription of target genes via binding to the consensus 5′-WGATAR-3′ sequence. Three members of this family, GATA-4/5/6, are expressed in the heart. Functionally relevant GATA-binding sites have been identified in numerous cardiac transcriptional regulatory regions, and recent data suggest that GATA-4 may play an important role in the development of cardiac hypertrophy. In addition to regulating cell growth and differentiation, there is increasing evidence that GATA factors also control cell survival. [44-47] The GATA-1-deficient erythroid precursors undergo apoptosis, and the induction of apoptosis by estrogen was dependent on the inhibition of GATA-1 in erythroleukemia cells. The bcl-x gene has two GATA consensus motifs in the 5′ promoter region, and GATA-1 induces the expression of the antiapoptotic protein Bcl-xL. Recently it was demonstrated that GATA-4 regulates the expression of Bcl-xL and exerts cell survival signaling in cardiac myocytes. [48] GATA elements are found in the promoters of other genes involved in antiapoptotic activities, such as nitric oxide synthases and antioxidant enzymes. We found that apoptosis of cardiac myocytes by anthracycline was associated with a decreased GATA-4 expression and that the restoration of GATA activity via adenovirus-mediated gene transfer attenuated the apoptosis, indicating that GATA-4 is involved in the mechanism of stress-induced apoptosis. These new findings that GATA-4 is involved in the regulation of cardiac muscle cell apoptosis and survival suggest that GATA-4 may respond to stress stimuli. [48-50] Transplanted mesenchymal stem cells (MSC) release soluble factors that contribute to cardiac repair and vascular regeneration.

GATA-4-treated animals showed significantly improved cardiac function as assessed by echocardiography. Furthermore, fluorescent microsphere and histological studies revealed increased blood flow and blood vessel density and reduced infarction size in GATA-4-treated animals. We conclude that GATA-4 overexpression in MSCs increased both MSC survival and angiogenic potential in ischemic myocardium and may therefore represent a novel and efficient therapeutic approach for postinfarct remodeling.

Cell-based therapeutics for heart disorders using autologous whole bone marrow (BM) and mesenchymal stem cells (MSCs) have been shown in both

experimental and clinical settings to improve heart function, attenuate infarct size expansion, and contribute to myocardial regeneration. Transdifferentiation into cardiomyocytes and into vascular lineage cells has been originally proposed as the principal mechanisms underlying their therapeutic action. [50-53] Recently, it has been reported that the functional benefits observed after stem cell administration in animal models of cardiac injury might be related to secretion of soluble factors that, acting in a paracrine fashion, protect the heart, attenuate pathological ventricular remodeling, induce neovascularization and promote regeneration.

We hypothesized that GATA-4 overexpression inhibits oxidative stress-induced injury to MSCs while also increasing MSC paracrine effects that promote postinfarction angiogenesis. Our results indicate that GATA-4 increases MSC survival and paracrine activity, which promotes neovascularization in the ischemic border zone and infarct area, thereby enhancing cardiac functional recovery.

NK-2 homeobox genes are a family of genes that encode for numerous transcription factors that go on to aid in the development of many structures including the thyroid, colon, and heart. Of the NK-2 genes, NKX2.5 transcription factor is mostly involved in cardiac development and defects with this gene can lead to congenital heart defects. [47]

NKX2.5 is expressed in precursor cardiac cells and this expression is necessary in order to lead to proper cardiac development. Nkx 2.5 is a gene that encodes a homeobox-containing transcription factor. This transcription factor functions in heart formation and development. Mutations in this gene cause atrial septal defect with atrioventricular conduction defect, and also tetralogy of Fallot, which are both heart malformation diseases. It is considered a transcription factor required for the development of the heart and the spleen. [54-55] During heart development, it acts as a transcriptional activator in cooperation with GATA4 (By similarity). Containing genes play critical roles in regulating tissue-specific gene expression essential for tissue differentiation. [47, 54-57] In some species it has been correlated to heart development and formation (ex. Drosophila). In humans, proper NKX2.5 expression is essential for the development of atrial, ventricular, and

conotruncal septation, atrioventricular (AV) valve formation, and maintenance of AV conduction. Mutations in expression are associated with congenital heart disease (CHD) and related ailments. Consistent with the direct transactivation of numerous cardiac genes reactivated in response to hypertrophic stimulation, cardiac transcription factors are profoundly involved in the generation of cardiac hypertrophy or in cardioprotection from cytotoxic stress in the adult heart. [58-60] The NKX2.5 transcription factor may help myocytes endure cytotoxic stress, however further exploration in this field is required. NKX2.5 is necessary for proper cardiac formatting as well as proper cardiac function after formatting as it is indicated by experimental discoveries. [60-62]

NKX2.5 has been shown to interact with GATA4 as it operates in a positive feedback loop with GATA transcription factors to regulate cardiomyocyte formation.

Until now these two transcription factors GATA4 and Nkx2.5 have been studied regarding cardiac regeneration in cell cultures and only a few protocols have been found in the literature worldwide regarding living heart interactions. [47] Therefore the research with the use of such factors in living animals and their evaluation in vivo post mortem in relation with the regeneration of cardiac tissue after ischaimia is of major importance.

The goal of this study was to determine whether ADSC transplantation in the ischemic myocardium could cause the regeneration of the myocardial cells and increase the viable contractile tissue. For this purpose, we used a previously described animal model with many prototype features. Furthermore, to test heart function, we utilized a SPECT-CT evaluation before and after surgically induced ischemia. As shown previously, this non-invasive method evaluates both anatomical and physiological changes, offers a serial quantitative approach to myocardial function and myocardial metabolism on a cellular level in the heart, and can be easily correlated with modern approaches to cardiovascular diseases, particularly when combined with molecular marker evaluations.

Transcription Factors

1. CTGF

CTGF is a major connective tissue mitoattractant secreted by vascular endothelial cells. It promotes proliferation and differentiation of chondrocytes, mediates heparin and divalent cation-dependent cell adhesion in many cell types including fibroblasts, myofibroblasts, endothelial and epithelial cells. It also enhances fibroblast growth factor-induced DNA synthesis. [63-64]] Is is secreted in extracellular space and matrix and therefore its localization is extracellular.

Antibody Anti-CTGF is a polyclonal antibody to CTGF which detects recombinant CTGF by Western Blotting suitable for immunohistochemistry reacting with rat tissue giving a positive signal in formaldehyde fixed cell lines. [65]

2. CD133

The role of CD133 in cell differentiation , proliferation and apoptosis is great as it binds with cholesterol in cholesterol-containing plasma membranes and may also play a role in the organization of the apical membrane in epithelial cells. [67]

Isoform 1 is selectively expressed on CD34 hematopoietic stem and progenitor cells in adult and fetal bone marrow, fetal liver, cord blood and adult peripheral blood.

Isoform 1 is not detected on other blood cells but it is expressed in a number of non-lymphoid tissues including retina, pancreas, placenta, kidney, liver, lung, brain and heart.

Isoform 2 is predominantly expressed in fetal liver, skeletal muscle, kidney and heart and is barely detectable in peripheral blood. Isoform 2 is expressed on hematopoietic stem cells and in epidermal basal cells at protein level.

CD133 belongs to the prominent family and both isoform 1 and 2 are glycosylated. It is located in apical cell membrane, cell projection and microvillous membrane. [68-70] Anti-CD133 is a stem cell marker suitable for immunohistochemistry use.

3. GATA4

GATA4 is a transcriptional activator that binds to the consensus sequence '5-AGATA-3' and plays a key role in cardiac development.

It is described to be involved in bone marrow morphogenic protein (BMP) and mediated induction of cardiac-specific gene expression. It can bind to BMP response element (BMPRE) DNA sequences within cardiac activating regions. [53] It promotes cardiac myocyte enlargement. Furthermore it acts as a transcriptional activator of ANF in cooperation with Nkx2,5. It may finally play a role in sphingolipid signaling by regulating the expression of sphingosine-1-phosphate degrading enzyme (lyase).

It is involved in diseases such atrial septal defect, ventricular septal defect, tetralogy of fallot, atrioventricular septal defect. [45-50] GATA 4 mutations can predispose to dilated cardiomyopathy resulting in congestive heart failure and arrhythmia leading such patients at risk of premature death.

4. Nkx 2.5

The function of Nkx2,5 is related to myocardium as it is implicated in commitment to and/or differentiation of the myocardial lineage. It acts as a transcriptional activator of ANF in cooperation with GATA4. [47] It is transcriptionally controlled by PBX1 and acts as a transcriptional repressor of CDKN2B. It also is required for spleen development.

The most important is the tissue specificity as it is only expressed in the heart.

It has involvement in diseases such as atrial septal defect with or without atrioventricular induction defects, tetralogy of Fallot, conotruncal heart malformations, hypothyroidism, ventricular septal defect, hypo plastic left heart syndrome and asplenia. [54-60]

It belongs to the NK-2 homeobox family and contains 1 homeobox DNA-binding domain and it is located in the nucleus.

Histology and Immunohistochemistry

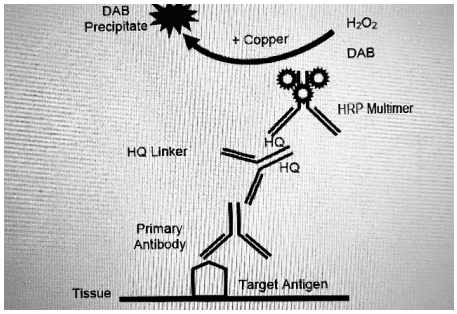
It is known that literature worldwide is full of debates among histopathological laboratories regarding the fixation methods especially about the ideal formaldehyde volume for the correct fixation of surgical samples and biopsies. Suggested ratio formaldehyde/tissue is between 0,5/1 to 200/1 depending on the tissue. Each laboratory uses their specific protocol according to subjective preferences without clear scientific data to support any protocol. [72-75] This is the reason why correct and modified procedure of fixation is of major importance.

Among the major examinations that a modern pathologoanatomical laboratory needs to perform are histological diagnosis and immunohistochemical analysis and their interpretation.

The necessity of quick and adequate fixation is high and actually it is a procedure of three stages happening at the same time and in different frequency depending more on the time and temperature than on the concentration of formaldehyde. [75-78] Extensive research showed that fixation influences to the maximum the absorption of liquid paraffin (paraffin wax infiltration) from the tissue, the microtome cutting of a paraffin cube with the fixed tissue and the histochemical and immunohistochemical analyses. [79-83]

Immunohistochemical staining allow the visualization of antigens through the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary and an enzymatic complex with colourform background after specific steps of clearance. [83]

Enzymatic activation results to the formation of a visible product of reaction at the place of the antigen. The sample can then be stained with opposite staining (ex. Hematoxylin) and be seen under the microscope.[84-86]



- STEP BY STEP PROCEDURE -

1. Remove the paraffin in xylene or similar . EZ Prep solution (EZ Preparation) is used for paraffin removal from tissue samples during immunohistochemistry
2. Wash the plates with reaction buffer
3. Uncover the antigen by Antigen retrieval Cell Conditioning (CC1)
4. Wash the plates with reaction buffer
5. Neutralize the endogenous hyperoxidase by using 0,5% v/v hydrogen peroxide for 10 minutes
6. Wash the plates with reaction buffer
7. Incubate the sections with the best seen primary antibody (specific details per antibody seen in a table in the end)
8. Wash the plates with reaction buffer
9. Incubate with Post Primary Block for 10 minutes [OptiView HQ Universal Linker contains a cocktail of HQ labeled (HQ is a proprietary hapten covalently attached to the goat antibodies) antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit)]
10. Wash the plates with reaction buffer
11. Incubate with Polymer OptiView HRP Multimer contains a mouse monoclonal anti HQ-labeled HRP tertiary antibody
12. Wash the plates with reaction buffer
13. Incubate the plates in DAB
14. Wash the plates with reaction buffer
15. Contrastaining with hematoxylin
16. Wash the plates with reaction buffer
17. Incubate with Bluing Reagent in order to change the hue of the hematoxylin to a blue color.
18. Wash the plates with reaction buffer
19. Dehydration and clearance of sections with bathing in increasing concentration of ethanol solutions and xylole for 5 minutes each.
20. Cover with glass

Reagents and consumables:

- **EZ Prep Concentrate** (10X) - EZ Prep Concentrate (10X) solution (EZ Prep) is used for paraffin removal from tissue samples during immunohistochemistry. This reagent is intended for in vitro diagnostic (IVD) use. Do the following to prepare diluted (1X) solution.

- **Cell Conditioning1 (CC1)** – Cell Conditioning Solution (CC1) is a prediluted solution used as a pretreatment step in the processing of tissue samples for IHC. CC1 is a tris based buffer which must not be diluted. Fixation of tissue by formalin results in the formation of covalent bonds between the aldehyde and amino groups present in the tissue. The formation of these bonds denatures protein and can result in the loss of antigenicity. In addition, the formaldehyde can form methylene bridges cross linking tissue proteins thus reducing the penetration of the tissue to large molecules such as antibodies. CC1 is a tris based buffer with a slightly basic pH, which, at elevated temperatures is capable of hydrolyzing the covalent bonds formed by formalin in tissue. Removing these bonds allows renaturation of protein molecules and increases antibody accessibility. Often these changes result in significant gains in antibody binding and improved signal to noise ratios. The automated slide stainer automatically heats the slide to the appropriate temperature and time as selected by the user.

- **Reaction Buffer** (10X) - Reaction Buffer Concentrate (10X) is a Tris based buffer Solution (pH 7.6 ± 0.2) used to rinse slides between staining steps and provide a stable aqueous environment for the immunohistochemistry (IHC). Ventana Reaction Buffer is used as a key component in maintaining a proper aqueous environment for many reactions to occur during IHC and ISH, such as general washing, antibody incubation and incubation of enzymes and other ancillaries when used on the automated slide stainer.
The IHC reaction involves a specific antibody that is localized by a biotin conjugated secondary antibody formulation that recognizes rabbit and mouse immunoglobins.

At the end of each incubation step, the Ventana automated slide stainer washes the sections with Reaction Buffer to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps.

- **OptiView DAB** IHC Detection Kit (OptiView) is an indirect, biotin-free system for detecting mouse IgG, mouse IgM and rabbit primary antibodies. The kit is intended to identify targets by immunohistochemistry (IHC) in sections of formalin-fixed, paraffin embedded and frozen tissue that are stained on the VENTANA automated slide stainers and visualized by light microscopy.
This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

- **(DAB) chromogen** hydrogen peroxide substrate and 3, 3'-diaminobenzidine tetrahydrochloride, which produces a brown precipitate that is readily observed by light microscopy.
Warning: Possible carcinogen. The International Agency for Research on Cancer (IARC) and the US National Toxicology
Program (NTP) have listed benzidine, a compound closely related to 3, 3'-diaminobenzidine tetrahydrochloride (DAB), as a known human carcinogen.

- **Hematoxylin Counter stain** - Hematoxylin counterstain reagent is a modified Gill's hematoxylin and is intended for staining cellular nuclei on slides containing cells from frozen tissue, formalin fixed and paraffin embedded tissue, or cytologic preparations.

- **Bluing Reagent** / Post counter stain - Bluing Reagent works through the combined effect of lithium ions and raising the pH of the wash buffer to blue the hematoxylin stained sections.
Bluing Reagent is applied after hematoxylin and changes the hue of the hematoxylin to a blue color.

Special Part

Introduction

Myocardial infarction is one of the leading causes of mortality creating the necessity of deep understanding of the mechanism underlying myocardial cells' damage, as well as evaluating their function in a qualitative and quantitative way. The understanding of pathophysiological mechanisms of the cardiovascular system is one of the greatest fields of modern medicine. [1] Therefore, basic and translational research is needed to better understand its underlying mechanisms and consequences for cardiac structure and function. Rodents have been intensely used in pre-clinical trials as an accepted animal model. Their use has been steadily increasing, especially in protocols related to cardiac activity. Myocardial ischemia is induced by variable methods either surgical or non-invasive, mainly as acute experiments leading to post-mortem evaluation. In vivo experiments in such protocols are highly challenging due to the increased mortality rate. Surgical techniques such as ligation of the left anterior descending coronary artery (LAD) has been widely used to establish a model of myocardial infarction. There are some disadvantages in this technique including the high mortality rates after ligation and the variations in the infarct size. [1-2] Therefore, improving the techniques causing ischemia is required, while non-invasive imaging methods in living animals post-infarction is extremely valuable for evaluating many aspects of the ischemic myocardium in vivo. Myocardial ischemia and left ventricular defect causing heart failure can be associated with viable and non-viable myocardial tissue. This ischemic myocardium that has lost viability can be evaluated using many approaches, such as in vitro histopathological measurements or in vivo imaging techniques, including ultrasound or magnetic resonance with different sensitivities and specificities. [2]

Single Photon Emission Computed Tomography (SPECT) and Computed Tomography (CT) are highly sensitive and specific methods for analyzing both physiological and anatomical aspects. Therefore, their use in preclinical trials is highly recommended. [1-2, 14-27]

Mesenchymal stem cells are multipotent cells that were first recognized in the bone marrow of adults. Adipose-derived stem cell (ADSC) transplantation is

nowadays considered the ideal tissue choice due to the decreased possibility of rejection. They are characterized by neovascularization and the ability to differentiate to multiple cell lines, including adipocytes, osteoblasts, and chondroblasts. Furthermore, it is known that they can be differentiated to cardiomyocytes and can lead to newly formed cardiac tissue. [1-2, 28-42]

Despite numerous current experimental trials, there are still many questions unanswered, such as the type of cells to be used, the number of cells necessary to be transplanted, the technique and route of administration, and the confirmation of the improvement in the function of the target tissue, such as the myocardium. The molecular markers used in our study to evaluate the action of ADSCs in the myocardium were GATA4, a zinc-finger transcription factor that is highly expressed in cardiomyocytes and is key to myocardial differentiation, and NKX2-5, a protein-coding gene that is involved in myocardial conduction and contractility. In vitro results showed an actively proliferating stem cell population 72 h after isolation, but it has been documented in immunochemical studies that the expression of stem cell markers needs approximately 7 days. [1-2, 43-62]

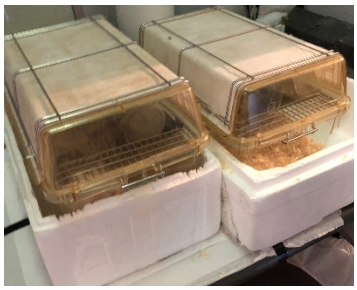
We report here a novel reliable and reproducible rat model, in which myocardial ischemia and infarction were induced by ligation of the left anterior descending coronary artery, and SPECT/CT imaging, along with histology and immunohistochemistry, were used for in vivo evaluation and assessment of the infarction in a qualitative and quantitative manner. The determination whether ADSC transplantation in the ischemic myocardium could cause the regeneration of the myocardial cells and increase the viable contractile tissue is the goal of our study. For this purpose, we used a previously described animal model with many prototype features. Furthermore, to test heart function, we utilized a SPECT-CT evaluation before and after surgically induced ischemia. As shown previously, this non-invasive method evaluates both anatomical and physiological changes, offers a serial quantitative approach to myocardial function and myocardial metabolism on a cellular level in the heart, and can be easily correlated with modern approaches to cardiovascular diseases, particularly when combined with molecular marker evaluations.

Animal model

The study protocol was approved and authorized by the Greek General Directorate of Veterinary Services (License No: 1870/23-04-2018) and was conducted in accordance with the Greek legislation regarding ethical and experimental procedures. Forty adult female Wistar rats with weight 280-350grams were used during our protocol. Our animal model of inducing myocardial ischemia, as well as the documentation and monitoring of heart function as detected by SPECT-CT, will be described in detail.

The animals were hospitalized in a certified laboratory with the necessary equipment and veterinarian superveillance . All animal experiments were approved and authorized by the national Animal Experiment Board of Greece and the Veterinary Association of Athens Greece and were carried out in compliance with EU legislation relating to the conduct of animal experimentation.

Following the 3Rs we kept the animals in special cages for adult rats , three animals per cage with free access to water and food.






The hygiene (waste, ventilations etc) and the pharmaceutical protocol were planned by the veterinarian committee as well as the biological waste according to the regulations of the authorized laboratory.

The animals underwent special transfer with certified and approved conditions from the laboratory to the SPECT-CT room and afterwards to the operation room and were examined by veterinarian before and after each procedure.

The ADSCs were retrieved from the abdominal adipose tissue of 6 male rats (donors), while for the experiment we used only female rats.

Forty female adult Wistar rats were randomly distributed in three groups :

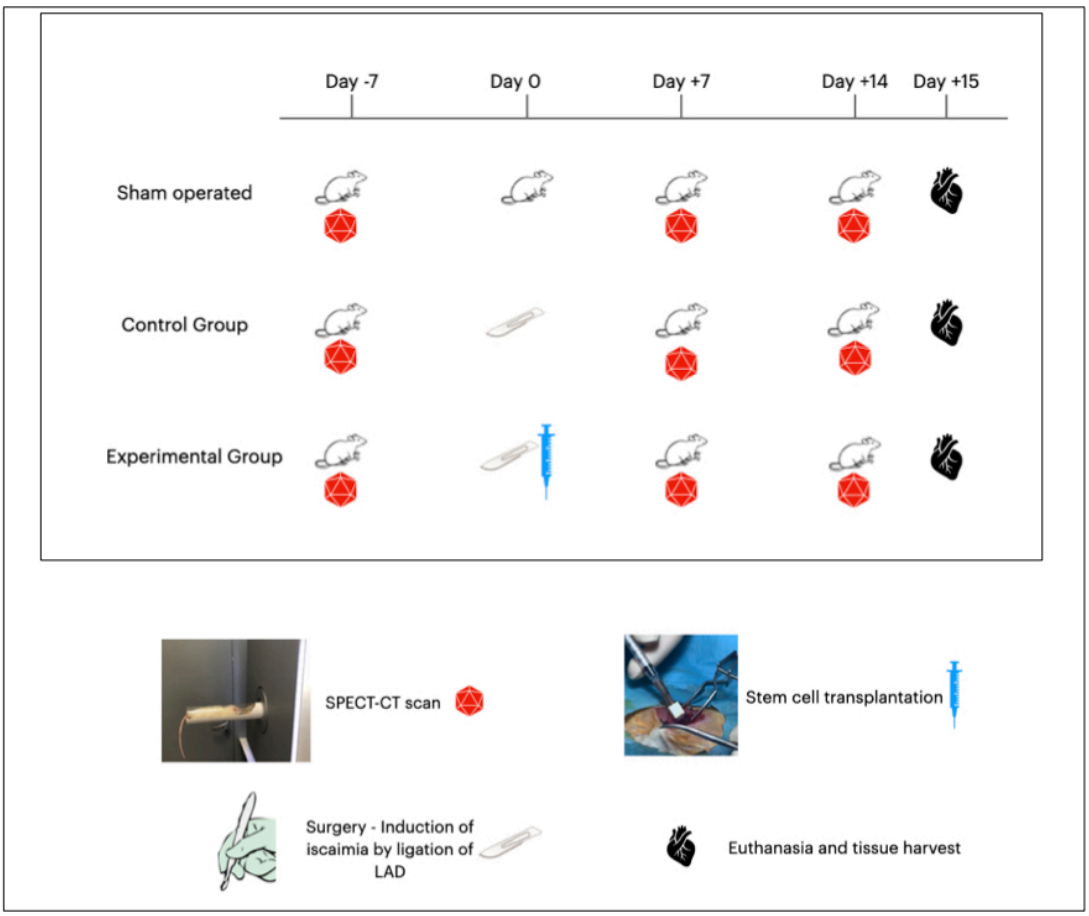
1. Control group
2. Sham operated group and
3. Experimental group.

All animals were weighing 280–350 grams.

These forty adult female Wistar rats underwent general anesthesia and mechanical ventilation. Tracheal intubation was achieved by a 17G tube and maintained with sevoflurane. A special temperature controlling mattress and a rat specific ECG monitoring combined with temperature and oxygen

measurement were applied throughout the procedure. Perioperative care was taken with subcutaneous adhesion of dolorex (0.06 ml in hour 0, 8 and 24), intramuscular adhesion of terramycin (0.03 ml), subcutaneous hydration with 3 ml of N/S 0.9% and eye protection with gel tobrex. Subsequently, under sterile conditions, a left mini thoracotomy between the 3rd and 4th intercostal space was undertaken and the pericardium was opened. A Prolene 6-0 stitch was used for handling the heart apex and a silk 4-0 for ligation of LAD in 20 animals, while 10 were used as sham operated (no-ligation group). Myocardial ischemic time was 45 minutes and ECG continuous recording for confirmation of ischemia was utilized. Afterwards, animals were extubated and all had an uneventful recovery and were able to undergo SPECT-CT imaging at 7 and at 14 days after surgery. On the 15th postoperative day, the rats were sacrificed and tissue and organ harvesting was performed. Euthanasia introduced by injecting KCL in the inferior vena cava (IVC).

After surgery, the animals were left to recover in cages alone or with other post-surgery animals in order to avoid cannibalism and other aggressive behaviors.



Timeline of the experimental study. All animals underwent SPECT-CT scan 7 days prior to surgery. Day 0 was the day of surgery when sham-operated animals underwent left thoracotomy without any further manipulation, control animals underwent left thoracotomy and surgically induced ischemia via ligation of LAD, and experimental animals underwent left thoracotomy and ligation of LAD followed by installation of stem cells at the point of ischemia in a cross pattern. All animals underwent SPECT-CT 7 days after surgery and 15 days after surgery. Finally, on the 15th day post-surgery, euthanasia took place, followed by

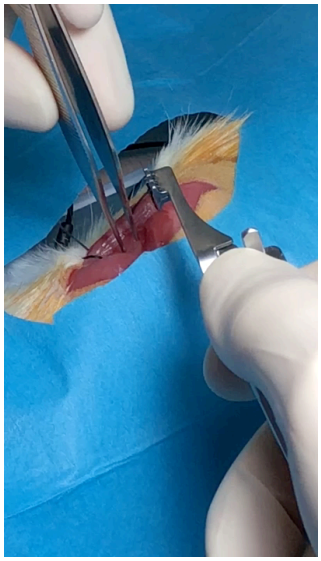
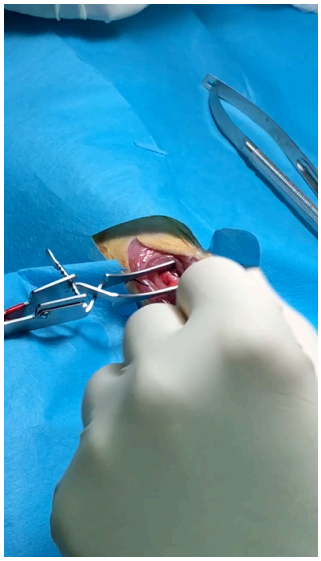
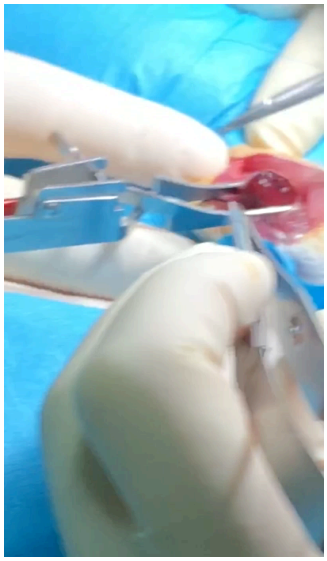
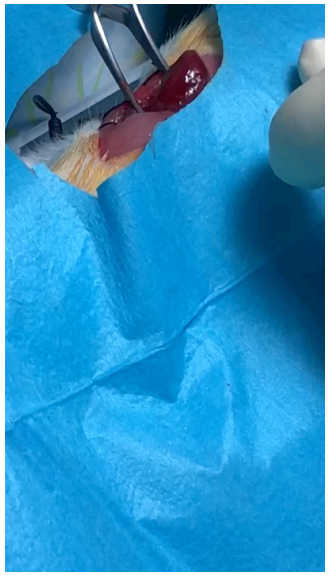
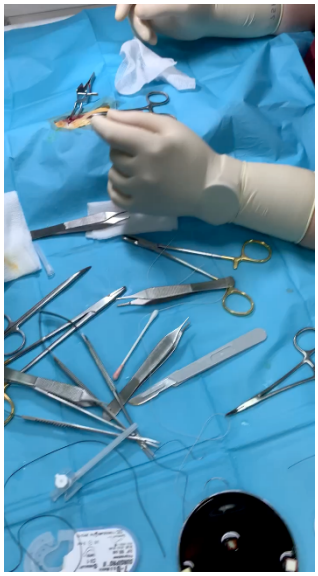
Surgical Procedures

Sterile conditions resembling those of an operating room in the surgical field were utilized. Accordingly, all instruments were sterilized in dry sterilization chambers. The surgical bed, a mattress specifically created for rodents and other small animals weighing below one kilogram, was connected to specialized equipment allowing continuous ECG recording, as well as an oximeter, thermometer, heater, and cooler.

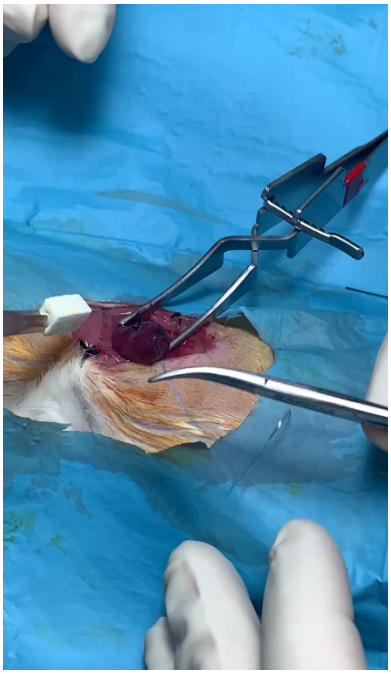
Nasopharyngeal intubation was achieved with a 17G tube connected to an inhalator with a mixture of sevoflurane 30–40%. Analgesia was achieved with the subcutaneous administration of Butorfanole (Dolorex) 10 mg/mL at the beginning of the procedure and every 3–5 h. Antibiotic coverage was achieved with the intramuscular administration of Oxytetracycline (Oxyvet 20%) 0.03 mL. A thermometer was placed in the rectal orifice of the animal, an oximeter was placed on the left foot, and the blood pressure was measured non-invasively by a cable placed on the left arm of the animal.

Surgical sterile conditions resembling those of an operating room in the surgical field were utilized

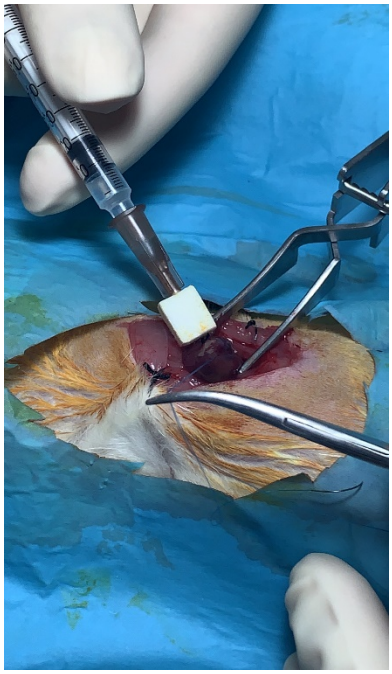
A small left thoracotomy was performed in the 4th intercostal space in order to enter the pericardium carefully. Except for the sham-operated group, all groups underwent an LAD ligation at its first third after the 1st diagonal branch using a 4-0 silk suture. After 45 min of ischemia documented in the ECG by ST elevation, the injection of 0.4 mL of liquid, containing one million stem cells, took place in a cross pattern (0.1 mL per spot) around the ischemic area.

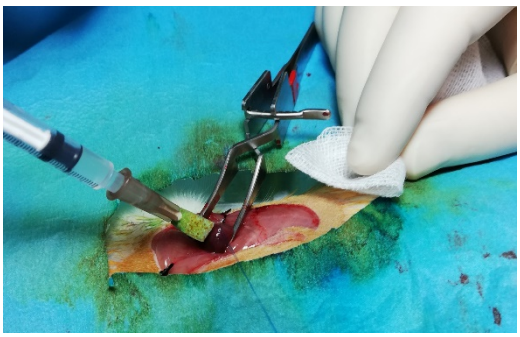






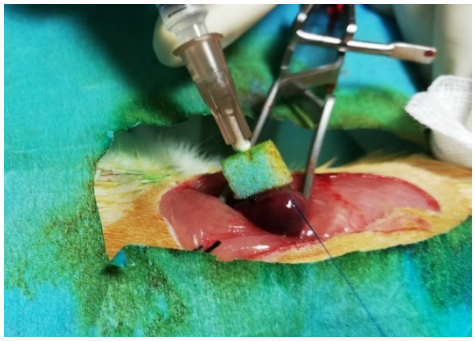
Pattern of ADSCs administration was in a cross-like injection around the ischemic area created by LAD ligation. The injection was carefully performed using a sterile sponge at the tip of the needle in order to avoid hemorrhage and leakage of the ADSCs from the spot.

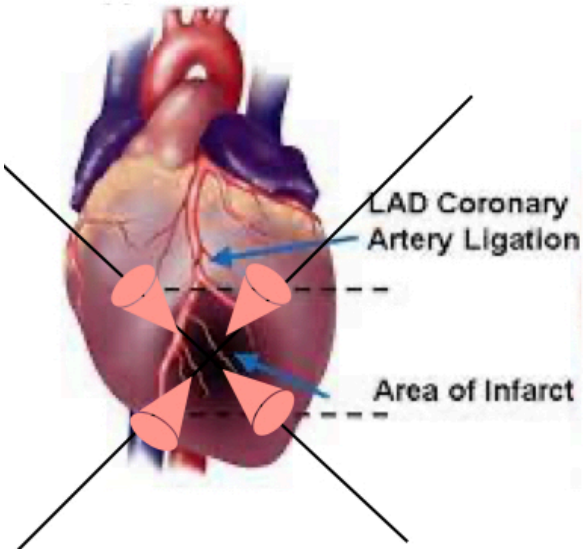


Control animals (n = 10) received a buffer solution with heart-friendly electrolytes, while experimental animals (n = 10) received previously prepared ADSCs.









0,4 ml of liquid containing 1.000.000 ADSCs was applied in 4 areas around the ischemia in a cross pattern (0,1ml at each point)

Afterwards, the thoracotomy was closed per layer. At the end of the experiment, via a small laparotomy, the inferior vena cava was used for blood sampling, followed by the administration of excessive KCl resulting in myocardial arrest.

Subsequently, the heart, lung, and liver were harvested and placed in formaldehyde. Cadavers were destroyed in biological waste material.

There were 9 deaths:

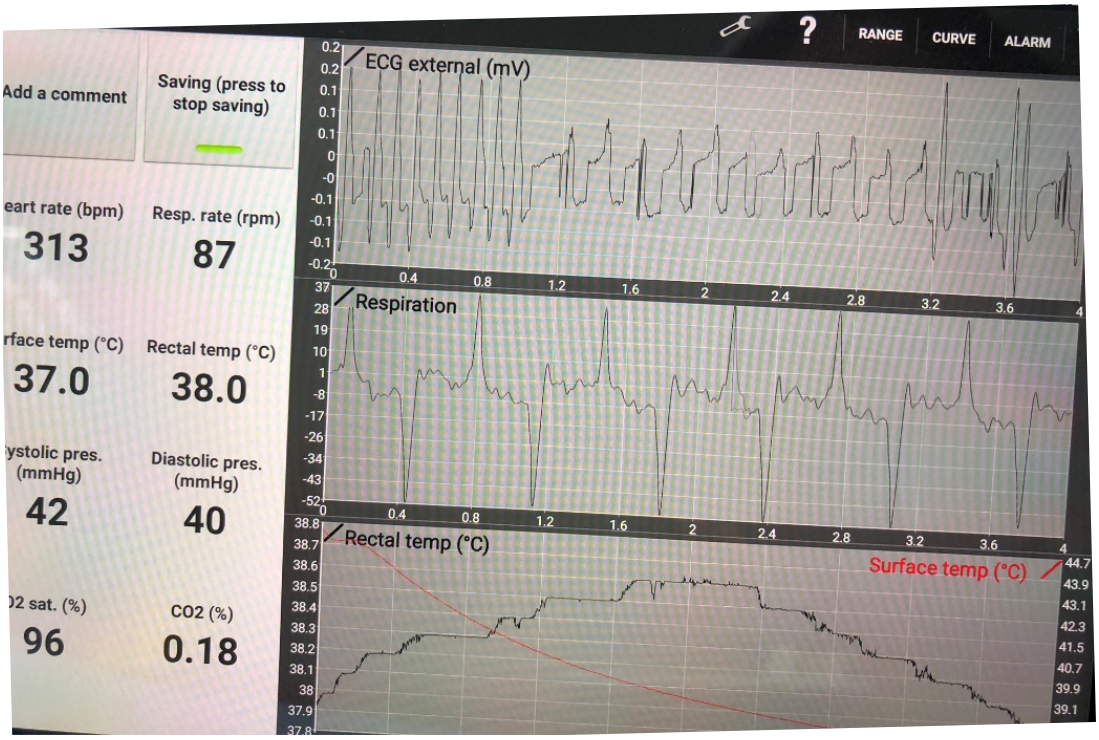
- 2 deaths at the closure of the thoracotomy of the animal due to lung injury and pneumothorax,
- 2 deaths due to cardiac arrhythmias leading to ventricular fibrillation,
- 1 death from hyperthermia due to accidental removal of the thermometer,
- 1 death due to dextrocardia and the numerous manipulations for heart immobilization,
- 2 deaths in the first 24 h PO, and
- 1 due to cannibalism in the PO cage.

Drugs administered during the surgical procedure: (1) Tobrex eyedrops for eye protection were administered locally to the eyes. (2) Natural saline 0.9% and dextrose 5% for hydration were administered subcutaneously (10–20 mg/kg) at the beginning. (3) Dolorex (Boutorfanole 10 mg/mL) for analgesia was administered subcutaneously (1–2 mg/kg) as a starting dose and repeated every 3–5 h. (4) Oxyvet 20% (Oxytetracycline 200 mg/mL) for antibiotic

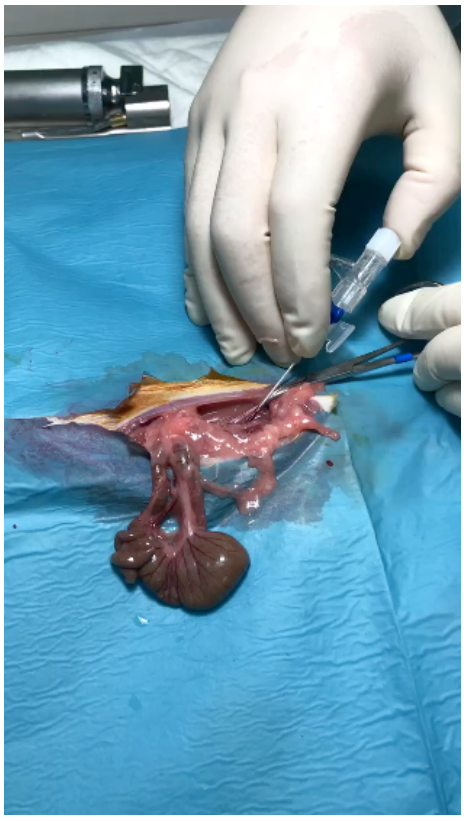
coverage was administered intramuscularly (20 mg/kg) at the beginning and 3 days post-surgery.

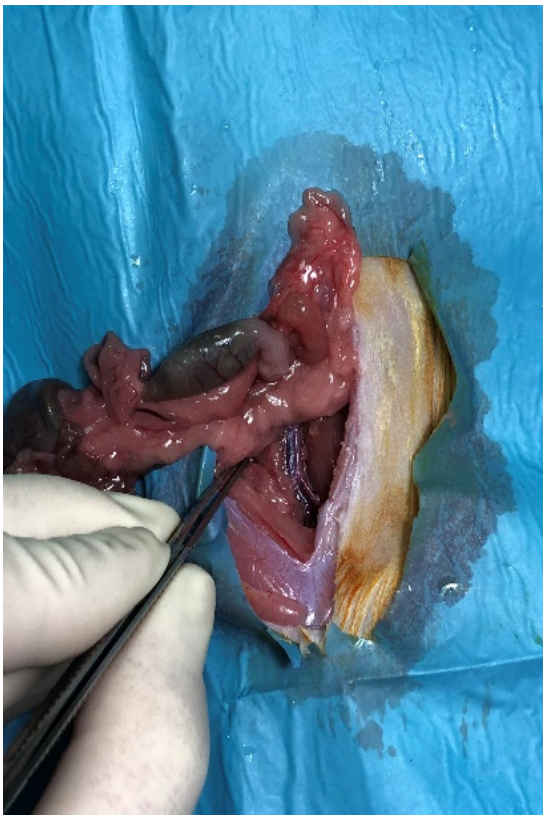
Type of drug	Name of drug	Time of administration	Way of administration and Dosage
Eye protection	Tobrex	At the beginning	locally
Solutions for hydration	N/S 0,9% or D/W 5%	(Preferably) At the beginning	Subcutaneously 10-20ml/kg
Analgesia	Boutorfanole - Dolorex 10mg/	At the beginning and every every 3-5 hours	Subcutaneously 1-2mg/kg
Antibiotics	Oxytetracycline - Oxyvet 20% (200mg/ml)	At the beginning and if necessary after 3 days	Intramascularly 20mg/kg

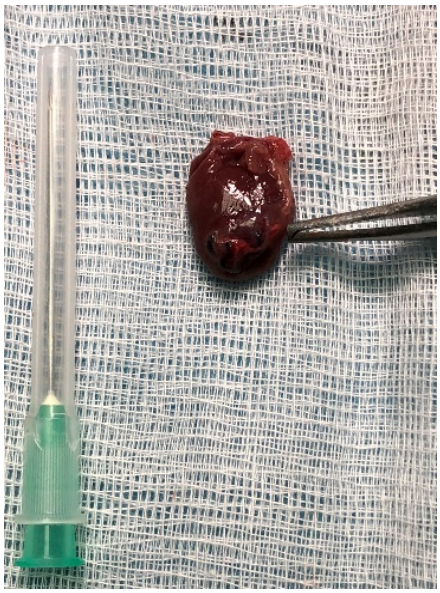
After surgical ligation of LAD ECG recording maintained continuously for 45 minutes in order to confirm and establish an infarct with characteristic ST elevation.

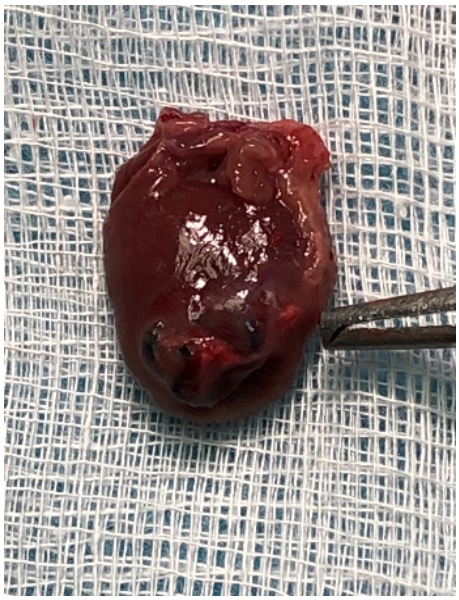


Euthanasia: On the day of euthanasia (day 15), the animals underwent the same procedure of anesthesia as on the day of surgery (described earlier), followed by laparotomy. The identification and isolation of the inferior vena cava was performed, where the administration of 3 mL of KCl was established. In this way, bradycardia was achieved and finally the heart stopped in the diastole phase. Afterwards, tissue harvesting was performed—specifically, the removal of the heart, lungs, and liver which were placed in 10% neutral formaldehyde kits. The waste products were discharged and handled according to European legislation by the authorized staff of the experimental laboratory.



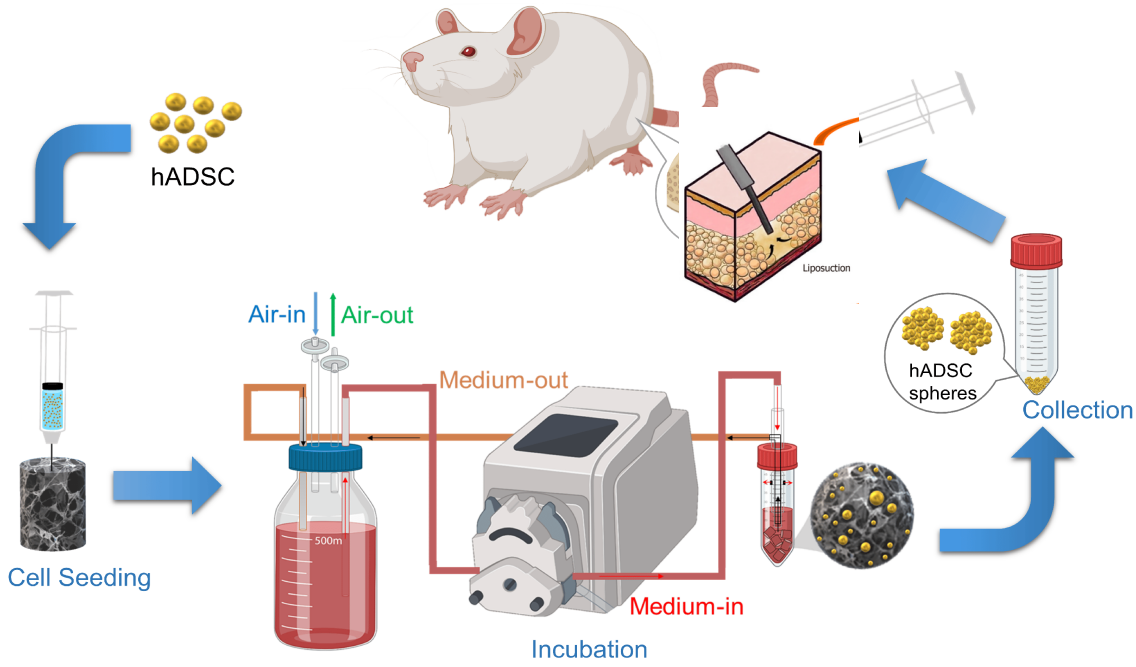






Isolation and Culture of ADSCs

ADSCs were isolated from 3-month-old male Wistar rats obtained from the experimental facility of the National Center for Scientific Research "Demokritos", Athens, Greece, with official coding for the breeding and provision of animals (EL 25 BIO 019 and EL 25 BIO 020, respectively). [28] The cells were collected from the subcutaneous layer of the adipose tissue of the abdominal wall and were washed with PBS, minced using two scalpels, and then digested in crude collagenase (1 mg/mL final concentration of collagenase; DMEM, Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 30 min at 37 °C. Later, the material was centrifuged (200× g for 5 min) at 37 °C to discard the supernatant, and the pellet was resuspended in DMEM, 10% FBS (Thermo Fisher Scientific, Inc.), and 1% penicillin/streptomycin and then transferred to a culture flask. Overnight incubition at 37 °C was followed by the change of the medium in order to remove the nonadherent cells, and the attached cells were further cultured in the same medium, under standard culture conditions. Novel DNA synthesis was performed with dual labeling with 5-bromo-2′-deoxyuridine (BrdU) and 4′,6-diamino-2-phenylindole (DAPI) dihydrochloride (Sigma, Kawasaki City, Japan). Furthermore, ADSCs' cell surface markers were examined, as described before. [28-34]



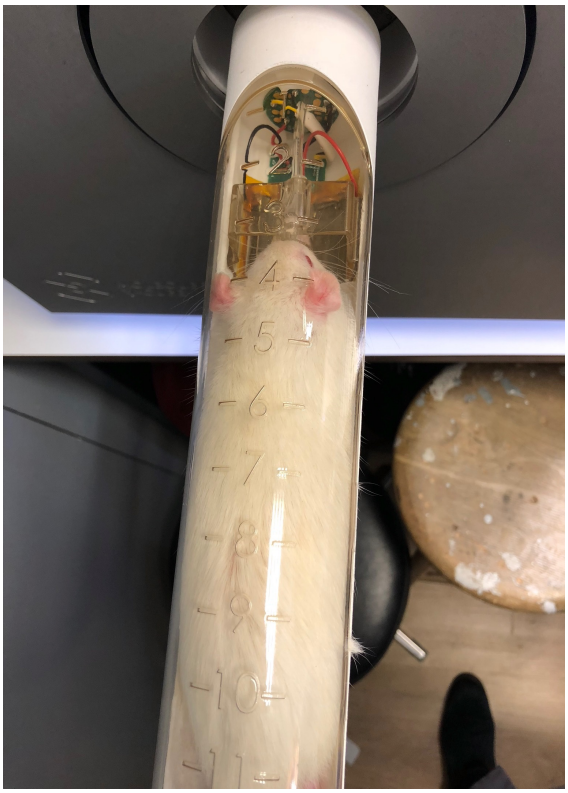
SPECT - CT Acquisition and Reconstruction

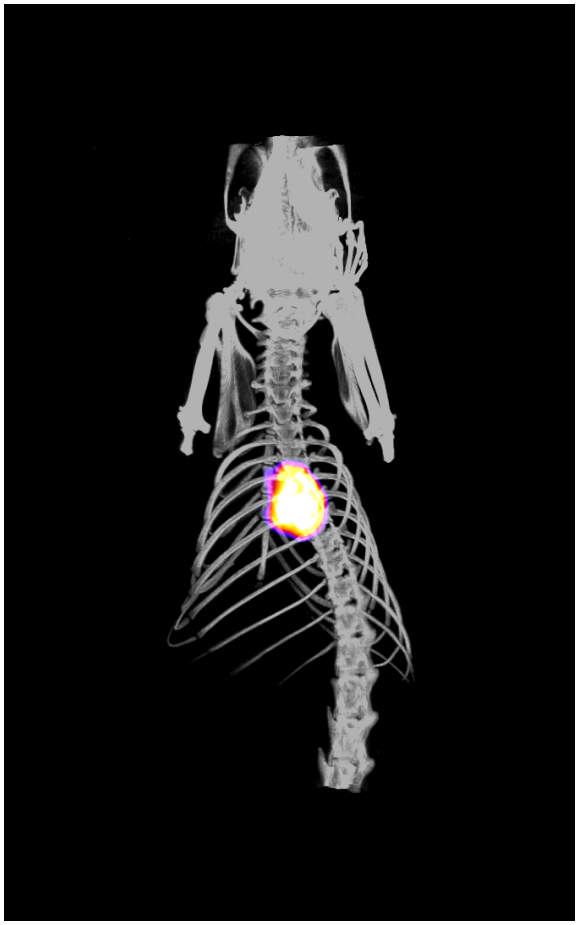
Our SPECT-CT imaging technique was especially created for small rodents and the use of a specialized chamber allowed us to focus on the thoracic cavity of the animal; details have been published before. Rats were injected with 200 μL of 1.5mCi-3mCi of 99mTc-Sestamibi, and imaging was performed at 20 min post-injection. SPECT-CT imaging was performed with the x-Cube and γ-Cube. CT acquisition and post-processing for the whole body was accomplished using a general-purpose protocol (50 kVp), performing an ISRA reconstruction with a 200 μm voxel size. All anatomical axes (short, long, and horizontal) were created for each animal, and 3D rendering with a color qualitative indication scale was used to display the myocardial activity at 7 and 14 days postoperatively. The measurement was performed in voxels from each cardiac chamber and the ratio of the left to right ventricle was evaluated in order to identify the changes in myocardial activity after establishing myocardial ischemia due to the surgical ligation of the LAD.

All animals underwent a preoperative SPECT-CT imaging prior to carrying out myocardial ischemia, in order to analyze the functional myocardium of the healthy heart. A novel technique for SPECT evaluation was developed especially for small rodents and the imaging chamber was formulated to focus on the thoracic cavity of the animal. 200 μl of 1.5mCi -3mCi of 99mTc-Sestamibi were injected in the rat via the inferior vena cava and subsequently, imaging was taking place at 20 minutes post injection. The SPECT-CT imaging was made with x-Cube and γ-Cube (Molecubes). SPECT acquisition was done with local scan spiral acquisition for 30 minutes and Maximum Likelihood Expectation Maximization (MLEM) reconstruction with 250μm voxel size and 500 iterations.



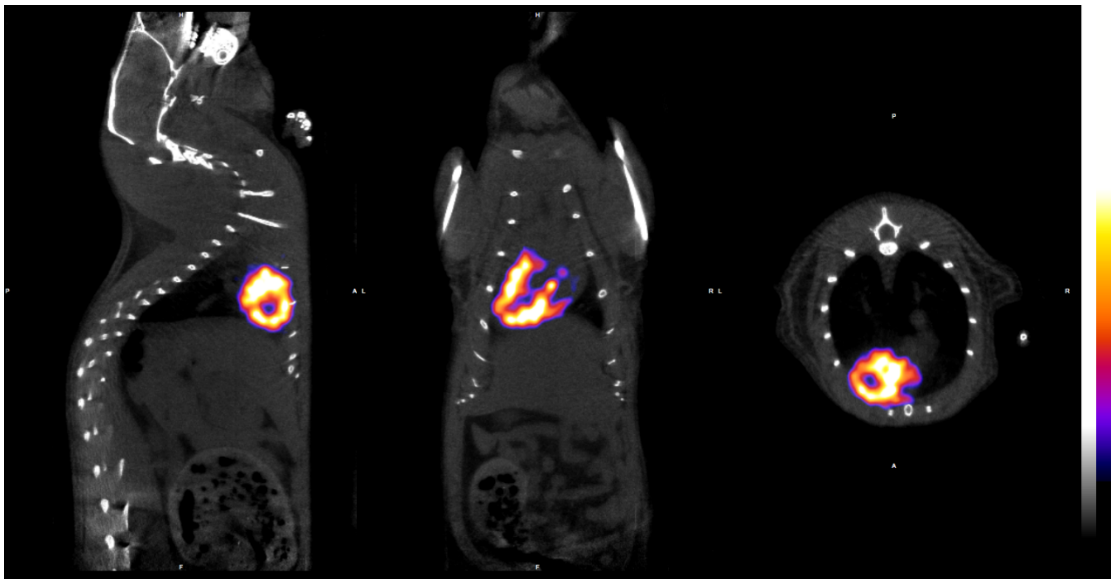

SPECT post-processing had to do with normalization of images on myocardium uptake [myocardium having the same maximum number of counts, removal of all other organs and smoothing median filter (1.8mm full width at half maximum (FWHM),





isotropic)]. Furthermore, CT acquisition and post processing for the whole body was performed using a general purpose protocol (50kVp), utilizing an ISRA reconstruction with 200μm voxel size. In all images, a scale was used for analyzing the absorption of the radiation particles from the myocardial cells, in order to evaluate the cellular activity in voxels.

Axial slices (short, long and horizontal) and 3D rendering with color indication scale was used to show the myocardial activity 7 and 14 days post-surgery, in order to analyze the changes in myocardial absorption of the radioactive particles after infarction. The post processing and the quantification of all images was performed with Vivo Quant 3.0 (Invicro, Boston, MA, USA). Results were measured in voxels from each cardiac chamber and the left to right ventricular ratio difference was used to identify the changes of myocardial activity after LAD ligation and the presence of ischemia in the left ventricle.



Histological and Immunohistochemical Analysis

On day 15 postoperatively, before euthanasia and under anesthesia, the heart was harvested and fixed in 10% neutral formaldehyde overnight and was routinely processed. Blood sampling was taken from the IVC and, from the same route of administration, excessive dose of KCl was administered for euthanasia. Hearts were rapidly excised and placed in 10% formaldehyde. [72] The samples were afterwards transferred to the histology laboratory, were fixated at room temperature using 10% formaldehyde in a ratio of 2:1 formaldehyde:tissue (v/v) for 48 hours and were then dehydrated and embedded in paraffin. In order to keep the long axis of the heart intact, we created slices in the perpendicular plane. [73-75] Hematoxylin and Eosin (H&E) stainining was used to identify the fibrotic areas of the myocardium. That way we documented histological proof of ischemia in the myocardium with a decrease in tissue fibers. Furthermore, in this study, immunohistochemical staining for the analysis of antigen expression was performed. The expression of antigens was additionally assessed in 4-μm-thick tissue. Immunohistochemical staining allowed the visualization of antigens through the specific sequence of antibody–antigen bonding, followed by the bonding of a secondary antibody to the primary one and the creation of an enzymatic complex that causes a chromogenic reaction. The anti-rat antibodies were used in this study after specific steps of clearance. The molecular markers used in our study to evaluate the action of ADSCs in the myocardium were directed against GATA4 and NKX2.5, CD133, and CTGF (Table 1). We used polyclonal antibodies from the following kits: (1) Origene TA354470 for CD133, (2) Origene TA323092 for CTGF, (3) Origene AP20302PU-N for GATA4, and (4) Abcam ab214296. Diaminobenzidine was used as a chrome agent and light hematoxylin as a counter stain. [83-89] Tissue sections, where the primary antibody was omitted, were used as negative controls.

Tissue harvest

On day 15 postoperatively the survived animals of our protocol were euthanized as described in order to harvest tissues and organs.

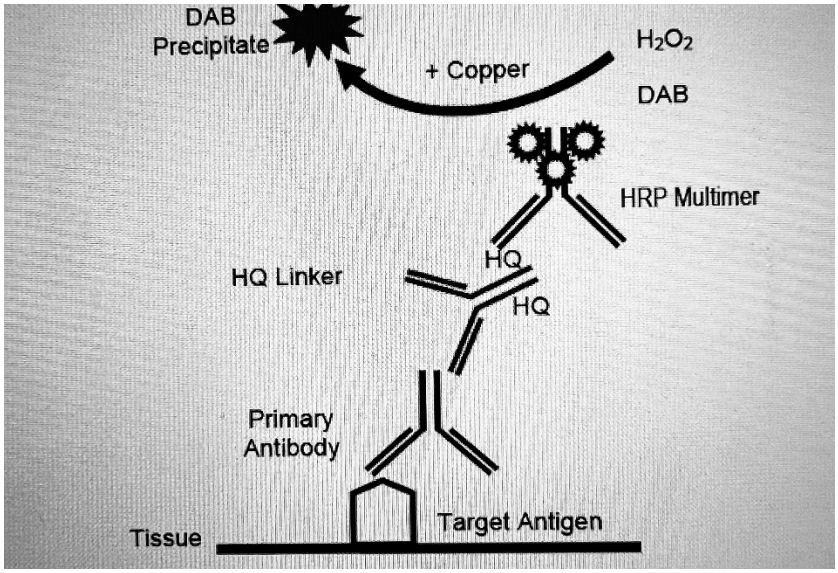
The procedure was done in the operation room with same anesthesiological conditions as in main operation. After full preparation of the animal and the surgical field we performed a laparotomy and prepared the intestines in order to reveal the great vessels of the abdomen. Puncture of the IVC (Inferior Vena Cava was done for blood sampling. Around 2ml were sucked per animal. From the same route of administration we administered KCl in order to keep the myocardium in diastole. A few minutes later the heart stopped beating and proceeded in heart, lung and liver harvest. [72-80]

The organs for maintainance were placed in formaldehyde. Afterwards we transferred the samples to the histology laboratory were they were dehydrated and embedded in paraffin. Fixation at 25 degrees celsius (room temperature in 1 atm pressure) using formaldehyde 10% in ratio 2:1 formaldehyde : tissue for 48 hours.

Immunohistochemistry

Immunohistochemical staining allow the visualization of antigens through the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary and an enzymatic complex with colourform background after specific steps of clearance. [83]

Enzymatic activation results to the formation of a visible product of reaction at the place of the antigen. The sample can then be stained with opposite staining (ex. Hematoxylin) and be seen under the microscope.



Reagents and consumables have been extensively described in the general part. The protocol followed in our experiment was the following:

Procedure	Method	Indications
Deparaffinization	EZ PREP	for 4 minutes at 72ºC
Antigen Unmasking	Cell Conditioning 1 (CC1)	Heating time at max. temp: 80 min Heating temp: 100ºC
Primary Antibody	Incubation antibody ready to use	Time / Tº:**DEPENDS** min Temp / 37ºC
Amplification / Detection	OptiView DAB IHC Detection Kit (POLYMER KIT)	Default-Incubation time linker:T°8 min/ Temp / 36ºC -Incubation time multimer: 8 min Temp / 36ºC
Counterstain	Hematoxylin II	Time/ T° 16 min
Post Counterstain	Bluing reagent	Time/ T° 16 min

All incubations took place in 37 degrees celsius and this is very characteristic for our procedure because it differs from all common protocols (generally used in laboratories worldwide).

Transcription Factors

1. CTGF

CTGF is a major connective tissue mitoattractant secreted by vascular endothelial cells. It promotes proliferation and differentiation of chondrocytes, mediates heparin and divalent cation-dependent cell adhesion in many cell types including fibroblasts, myofibroblasts, endothelial and epithelial cells. It also enhances fibroblast growth factor-induced DNA synthesis. Is is secreted in extracellular space and matrix and therefore its localization is extracellular. [63-66]

Antibody Anti-CTGF is a polyclonal antibody to CTGF which detects recombinant CTGF by Western Blotting suitable for immunohistochemistry reacting with rat tissue giving a positive signal in formaldehyde fixed cell lines.

Properties:

It is in liquid form stored in +4 degrees Celsius, preservative used 0.02% sodium azide and constituents used 1% BSA, PBS. pH 7,4. Immunogen affinity is purified, it is a polyclonal isotope of IgG. For positive control HepG2 is used. Heart tissue underwent fixation in formaldehyde 10%, heat mediated antigen retrieval in buffer and blocking (5 minutes/peroxidase block and then 10 minutes/protein block) for 15 minutes at 20 degrees celsius. ICC/IF Cells were 4% formaldehyde fixed for 10 minutes and then incubated in 1% BSA/ 10% normal goat serum /0.3M glycine in 0,1% PBS-Tween for one hour to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab5097 at 5μγ/ml overnight at 4 degrees celsius.

2. CD133

The role of CD133 in cell differentiation, proliferation and apoptosis is great as it binds with cholesterol in cholesterol-containing plasma membranes and may also play a role in the organization of the apical membrane in epithelial cells.

Isoform 1 is selectively expressed on CD34 hematopoietic stem and progenitor cells in adult and fetal bone marrow, fetal liver, cord blood and adult peripheral blood.

Isoform 1 is not detected on other blood cells but it is expressed in a number of non-lymphoid tissues including retina, pancreas, placenta, kidney, liver, lung, brain and heart.

Isoform 2 is predominantly expressed in fetal liver, skeletal muscle, kidney and heart and is barely detectable in peripheral blood. Isoform 2 is expressed on hematopoietic stem cells and in epidermal basal cells at protein level.

CD133 belongs to the prominent family and both isoform 1 and 2 are glycosylated. It is located in apical cell membrane, cell projection and microvillous membrane. Anti CD133 is a stem cell marker suitable for immunohistochemistry use. [67-71]

Properties:

Liquid form stored at +4 degrees celsius. Preservative 0,09% Sodium azide and constituent is PBS. It is a polyclonal gene isotyping IgG. As a positive control HepG2 is used.

3. GATA4

GATA4 is a transcriptional activator that binds to the consensus sequence ‘5-AGATA-3’ and plays a key role in cardiac development.

It is described to be involved in bone marrow morphogenic protein (BMP) and mediated induction of cardiac-specific gene expression. It can bind to BMP response element (BMPRE) DNA sequences within cardiac activating regions. It promotes cardiac myocyte enlargement. Furthermore, it acts as a transcriptional activator of ANF in cooperation with Nkx2,5. It may finally play a role in sphingolipid signaling by regulating the expression of sphingosine-1-phosphate degrading enzyme (lyase).

It is involved in diseases such atrial septal defect, ventricular septal defect, tetralogy of fallot, atrioventricular septal defect. GATA 4 mutations can predispose to dilated cardiomyopathy resulting in congestive heart failure and arrhythmia leading such patients at risk of premature death.

As a positive control HepG2 is used. Sequence contains two GATA-type zinc fingers and methylation at Lys-300 attenuates transcriptional activity. Its location is the cell nucleus. Heart tissue was formaldehyde fixed and prepared in paraffin embedded sections. Antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/50 in 10% goat serum) for 16 hours at 4 degrees celsius. [43-53]

Properties:

Liquid solution stored at +4 degrees Celsius with preservative 0.02% sodium azide and constituents 1% BSA, PBS. pH 7,4

4. Nkx 2.5

The function of Nkx2,5 is related to myocardium as it is implicated in commitment to and/or differentiation of the myocardial lineage. It acts as a transcriptional activator of ANF in cooperation with GATA4. It is transcriptionally controlled by PBX1 and acts as a transcriptional repressor of CDKN2B. It also is required for spleen development.

The most important is the tissue specificity as it is only expressed in the heart.

It has involvement in diseases such as atrial septal defect with or without atrioventricular induction defects, tetralogy of Fallot, conotruncal heart malformations, hypothyroidism, ventricular septal defect, hypo plastic left heart syndrome and asplenia.

It belongs to the NK-2 homeobox family and contains 1 homeobox DNA-binding domain.

It is located in the nucleus. [54-62]

The anti-Nkx2.5 antibody is a polyclonal antibody suitable for IHC-P and the immunogen is a synthetic peptide conjugated to keyhole limpet hemocyanin. Positive control is rat heart tissue.

Properties:

liquid solution stored at 4 degrees Celsius. Preservative is 0,09% sodium azide and constituents are 50% glycerol and 1% BSA. It is a polyclonal with IgG isotope.

Molecular Factors	Brand Kit Used	Properties	Dilution
GATA-4	Origene AP2030PU-N	Key role in cardiac development	Supplier instruction: IHC-P 1/50-1/200 Used dilution: IHC-P 1/75
Nkx2.5	Abcam Ab214296	Differentiation of myocardial lineage	Supplier instruction: IHC-P 1/100-1/500 Used dilution: IHC-P 1/300
CD133	Origene TA354470	Cell differentiation, proliferation, and apoptosis	Supplier instruction: IHC 2-10 µg/mL Used dilution: IHC 6 µg/ml
CTGF	Origene TA323092	Connective tissue mitoattractant secreted by vascular endothelial	Supplier instruction: IHC-Fr 1/200 Used dilution: IHC-Fr

Statistical Analysis

For quantitative variables, the selected data were expressed as the mean ± standard deviation (SD), while, for qualitative variables, they were expressed as frequencies and percentages. In order to analyze the normality of the quantitative variables, the Kolmogorov–Smirnov test was used. In order to compare the variables, both qualitative and quantitative pairwise comparisons between experimental groups were performed using one-way ANOVA with Bonferroni correction and the Chi-square test with Bonferroni correction, respectively. All tests were two-sided. A statistically significant difference was defined by a p-value < 0.05. Statistical analysis was performed using the statistical package SPSS version 21.00 (IBM Corporation, Somers, NY, USA).

Results

In this experiment, all animals had comparable imaging results and none, neither perioperatively nor during the imaging procedures, was lost indicating a very reliable in-vivo experiment. [1-2] The initial images were evaluated after removal of all other organs and by performing a whole body spiral acquisition in a high resolution protocol. The territories between normally functioning myocardial tissue and ischemic parts were easily recognizable after the axial and 3D reconstruction leading to a physiological and anatomical map of the rat's heart. [14-17] The standardization of the procedure by scanning all animals preoperatively helped to visualize the distribution of the radioactive tracer after the establishment of the ischemia and the changes occurring after 7 and 14 days respectively. [28-35]

Accumulated activity was recognized in highly metabolic myocardial cells while decreased or no activity was shown in ischemic areas.

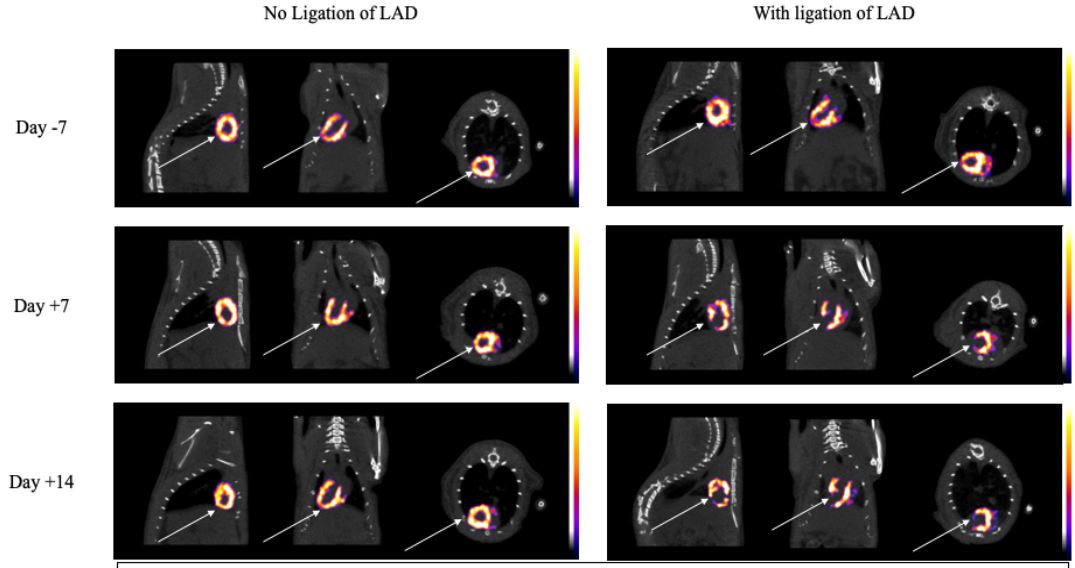
The distribution of the radioactive particle was measured via the differentiation of the left ventricular to right ventricular area (LV/RV) which is a mathematical ratio without being influenced by any other parameters of the experimental method, such as animal weight, dosage or radioactive substance, absolute number of voxels. [14-27] Specifically, in healthy individuals the ratio LV/RV 7 days prior to surgery is about 8.7±0.3 (Day -7) and remains stable in the animals which underwent only thoracotomy without ligation. The group of 20 animals with ischemia established by ligation of LAD, showed same values preoperatively but had significant decrease of the active myocardium with values of LV/RV ratio 7.5±0.2 post surgically. Confirmation of the ischemic areas was further completed by histological and immunohistochemical analysis.

SPECT - CT Evaluation

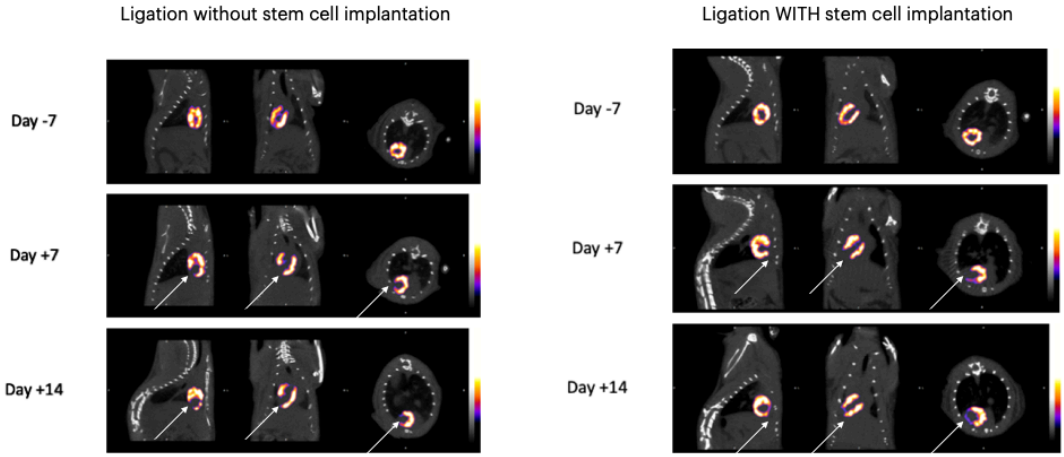
The initial images were evaluated by performing a whole body spiral acquisition in a high-resolution protocol. The visualization was firstly standardized by scanning all the animals before surgery, and the distribution of the radioactive tracer at 7 and 14 days after the surgical ligation of the LAD proved the changes related to ischemia. The differences between the healthy and ischemic myocardial tissue were easily recognized after the axial and 3D reconstruction, leading to the creation of a physiological and anatomical map of the rat's heart. [5-7,25-27,30-35] In the healthy myocardial cells, the radioactive absorbance was high as the cells were highly metabolic, while, in the ischemic cells, the absorbance was decreased or absent. [14-20] It is notable that, in the animals receiving stem cell transplantation, the SPECT-CT evaluation revealed definite signs of regeneration and an increase in myocardial activity.

The creation of the ratio of the left ventricular area to the right ventricular area (LV/RV), which is purely mathematical and not influenced by any other parameters of the experimental method, such as the animal weight, the dosage of the radioactive substance, or the absolute number of voxels, led to a clear analysis of the distribution of the radioactive particle. Due to the ligation of the LAD, the induced ischemia causes a decreased left ventricular area; therefore, the LV/RV ratio changes pre- and postoperatively. In more detail, healthy individuals presented an LV/RV ratio of about 8.7 ± 0.3 (day −7) 7 days prior to surgery, and this remained stable in the animals that underwent only thoracotomy without ligation. However, there was a statistically significant difference between groups with respect to the ratio of variables at 7 days ($p < 0.005$), and pairwise comparisons showed that the sham-operated animal group presented higher values compared to the control group ($p < 0.005$). The animals that belonged to the control group had also a significant decrease in the activity of the myocardium, with an LV/RV ratio of 7.5 ± 0.2 post-surgically. The animals in the sham-operated group showed no increase in myocardial activity fourteen days post-surgery, while the animals in the experimental group that received the mesenchymal stem cells showed

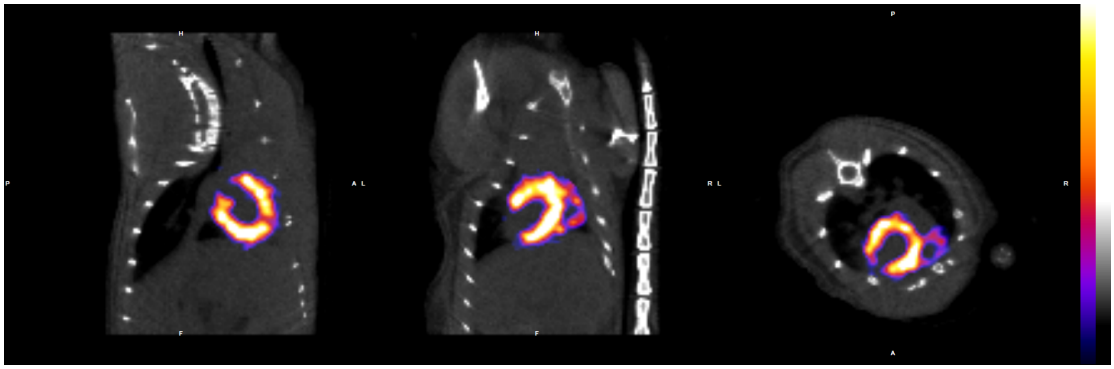
an increase in functioning myocardial cells and specifically the values of the LV/RV ratio were 8.00 ± 0.2, demonstrating thus the beneficial effect of stem cell transplantation.



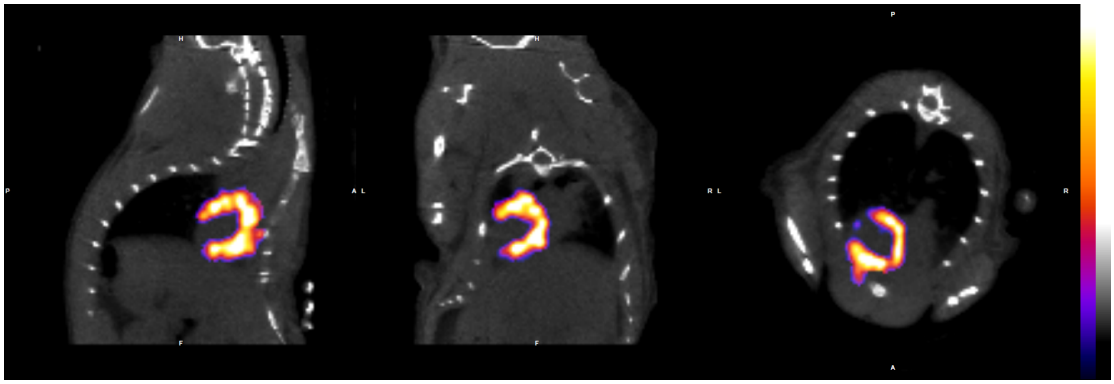
SPECT - CT The color bar indicates the difference in accumulated activity (deep blue being the lowest and white the highest). All tomography views, showing the myocardium 7 days before surgery (Day -7) and 7 and 14 days after surgery (Day +7 and Day +14). The white arrows show the area supplied by LAD - heart apex . The difference between the animals without ligation (left column) and the animals with LAD ligation (right column) is pointed on days +7 and +14 where there is no absorbance of radioactive particles from the ischemic myocardium (no cellular activity).

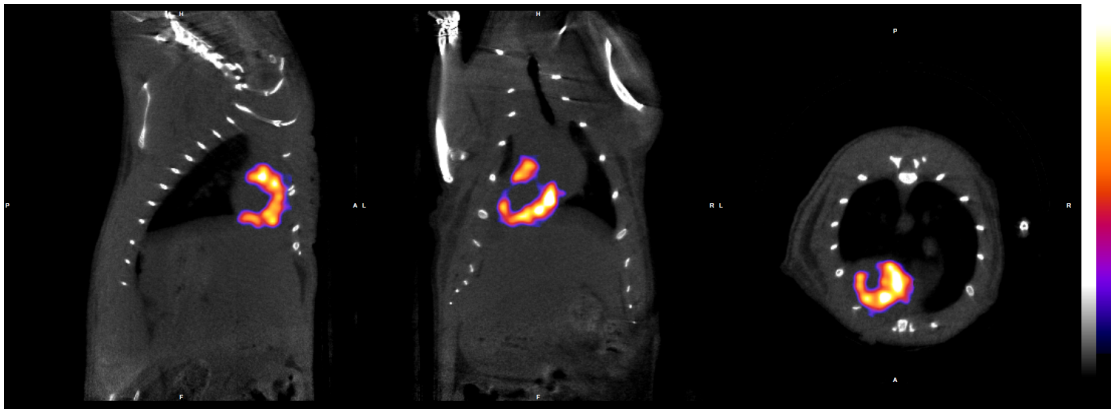


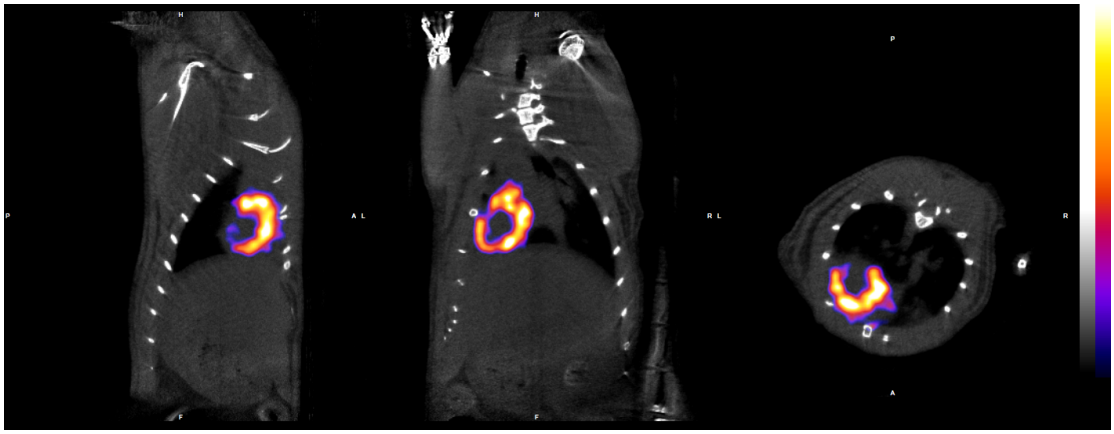
SPECT - CT The color bar indicates the difference in accumulated activity (deep blue being the lowest and white the highest). All tomography views, showing the myocardium 7 days before surgery (Day -7) and 7 and 14 days after surgery (Day +7 and Day +14). The white arrows show the area supplied by LAD - heart apex . The difference between the animals without ligation (left column) and the animals with LAD ligation (right column) is pointed on days +7 and +14 where there is no absorbance of radioactive particles from the ischemic myocardium (no cellular activity).



Sham Operated animals show no change of myocardial ischaimia 7 and 14 days post infarction.
The ischemic area remains unchanged with complete absence of cellular activity and no radioactive substance absorption.







Experimental group animals myocardial activity 7 (above) and 14 days (below) after stem cell administration in post-infarcted area. The color bar indicates the different levels of the accumulated radiopharmaceutical, directly linked to the level of blood circulation (lowest values shown by deep blue and high values by white). All results were measured in voxels from each cardiac chamber. Photo above shows the decreased absorption of radioactive particles in the apex of the heart supplied by LAD due to surgically induced ischaimia. (Day +7) Photo below shows increased absorption of radioactive substance 14 days post stem cell administration proving the presence of myocardial regeneration in prior ischemic areas of the heart apex. These animals with stem cell implantation belong to the experimental group (n = 10) and received 1,000,000 cells.

Left ventricle to right ventricle ratio (LV/RV) changes among sham, control, and experimental groups of animals during the period of experimentation. During the period of experimentation, the ratio of the myocardial volume of the left to right ventricles changed according to the presence and the degree of ischemia. In order to compare the variables, both qualitative and quantitative pairwise comparisons between experimental groups were performed using one-way ANOVA with Bonferroni correction and the Chi-square test with Bonferroni correction, respectively. All tests were two-sided. Statistically significant differences were defined by a p-value < 0.05. Black dots represent the sham-operated group, where no change was seen during this time—the LV/RV ratio was about 8.7 0.3 ± at every measurement (days −7, +7, +14).

Variable	Group			p-Value
	Sham	Control	Stem Cells	
RATIO LV/RV (−7d)	8.72 ± 0.36	8.77 ± 0.20	8.86 ± 0.22	0.480
RATIO LV/RV (7d)	8.69 ± 0.37	7.50 ± 0.24 *	7.50 ± 0.29 *	<0.005
RATIO LV/RV (14d)	8.71 ± 0.34	7.59 ± 0.28 *	8.00 ± 0.27 * †	<0.005

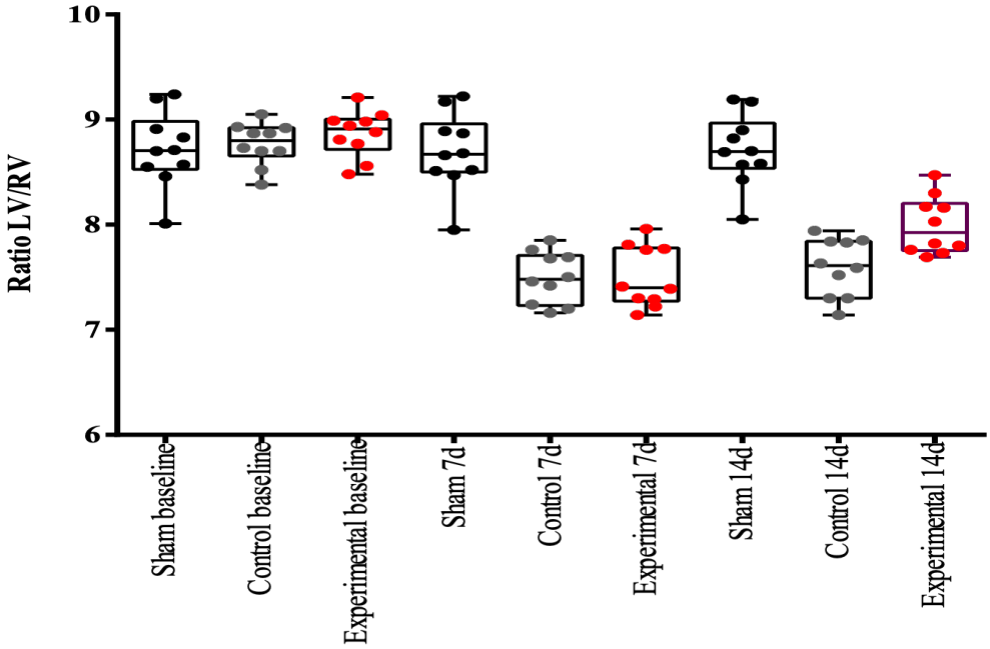
* *p < 0.005 vs. sham,* † *p < 0.05 vs. control.*

Comparison of LV/RV area ratio between the animal groups at the specific time points of experimentation.
In healthy individuals, the LV/RV ratio 7 days prior to surgery is about 8.7 ± 0.3 (day −7) and remains stable in the sham-operated animal group.
There is a statistically significant difference between groups with respect to the ratio of variables at day 7.

Variable	Group			p-Value
	Sham	Control	Stem Cells	
CTGF negative/positive; n (%)	6 (60)/4 (40) †	11 (100)/0 (0) **	4 (40)/6 (60)	0.011
CD133 negative/positive; n (%)	10 (100)/0 (0) **	11 (100)/0 (0) **	2 (20)/8 (80)	<0.005
GATA4 negative/positive; n (%)	9 (90)/1 (10) **	11 (100)/0 (0) **	0 (0)/10 (100)	<0.005
Nkx2.5 negative/positive; n (%)	8 (80)/2 (20) *	11 (100)/0 (0) **	2 (20)/8 (80)	<0.005

** $p < 0.05$ vs. stem cells, ** $p < 0.005$ vs. stem cells, † $p < 0.05$ vs. control.*

Statistical analysis of four stem-cell-related factors between the experimental groups. There was a statistically significant difference between groups with respect to percentage of positive results for the CD133, GATA4, and Nkx2.5 variables ($p < 0.005$). Pairwise comparisons showed that the experimental group presented higher percentages of positive results for the CD133, GATA4, and Nkx2.5 variables compared to the sham ($p = 0.045$) and control ($p < 0.005$) groups, respectively. Pairwise comparisons between groups were performed using one-way ANOVA with Bonferroni correction.



Grey dots represent the control group, where the LV/RV ratio decreased after the ligation of the LAD and remained low during the period after LAD ligation and establishment of ischemia—the LV/RV ratio was about 7.5 ± 0.27 (days +7, +14). Red dots represent the experimental group, where the LV/RV ratio decreased after ligation of the LAD—LV/RV ratio was about 7.59 ± 0.28 (day +7). It tended to return to normal on day +14 following stem cell administration—LV/RV ratio was about 8 ± 0.27 (Day +14).

Histological Results and Evaluation of Immunohistochemistry

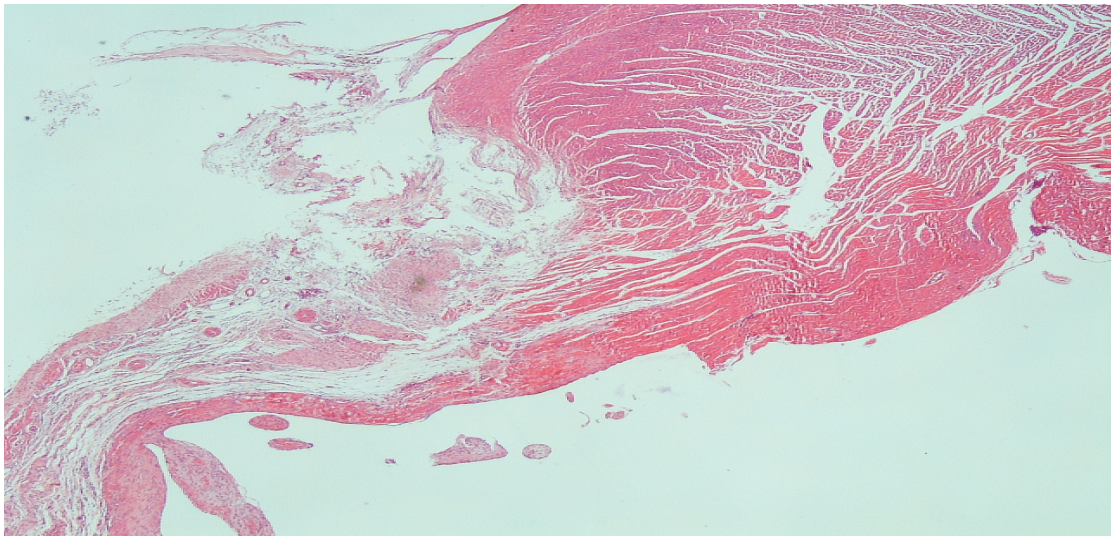
Histological images in this protocol help in the confirmation of ischemic areas around the LAD ligation showing clearly the loos of myocardial tissue due to infarction. Histological changes take 6 to 12 hours to develop with the first apparent change being coagulative necrosis. After the necrosis, neutrophilic influx is seen in around 12 to 24 hours. [72-75] Loss of nuclei occurs on days 1 to 3, phagocytosis by macrophages on days 3 to 7, and granulation tissue formation at the margins. Myocardial infarction or heart attack is the death of cardiac muscle cells caused by loss of blood supply (ischemia) to the cardiac muscle tissue. [72-78]

Acute myocardial infarction shows wavy cardiac muscle cells that represent dead or necrotic cells. The histologic parameters of tissue damage and repair overtime in myocardial infarction are the stretched or wavy fibers which occur hours after infarction, the coagulative necrosis characterized by hypereosinophilia and nuclear changes which develop as early as hours post infarction and remains in full development for days, the interstitial oedema, polymorphonuclear infiltration as well as the presence of macrophages and lymphocytes. Dense fibrosis occurs weeks after infarction and remains for months in the cardiac tissue. [79-82]

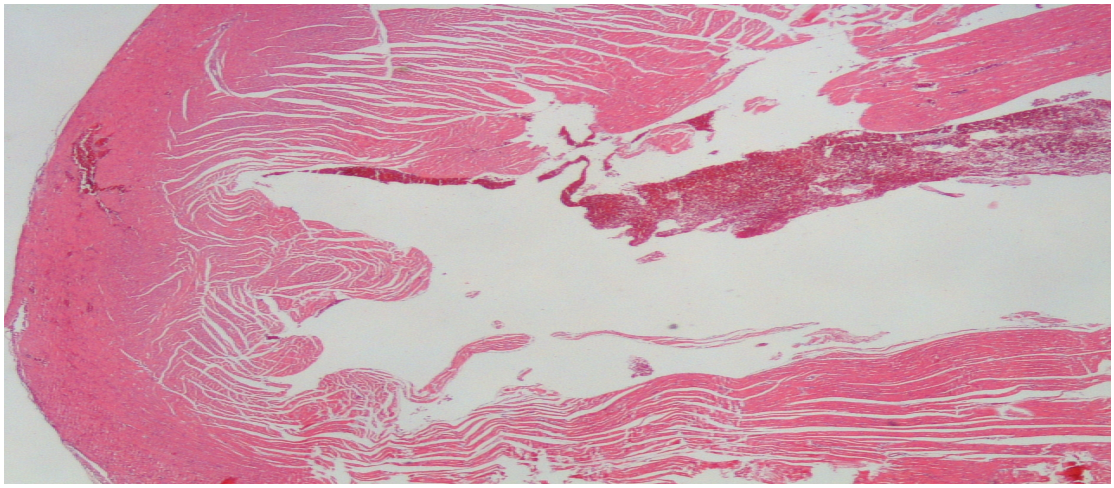
The images of the myocardium under the microscope revealed the characteristic pattern of the infarction histologically in all animals. Histological analysis took place post euthanasia in the 16th postoperational day and in the animals belonging in the experimental group which received the stem cells the ischemic tissue was less abundant.

In our protocol histological evaluation with simple Hematoxylin-Eosin staining was used in order to confirm the successful infarction protocol.

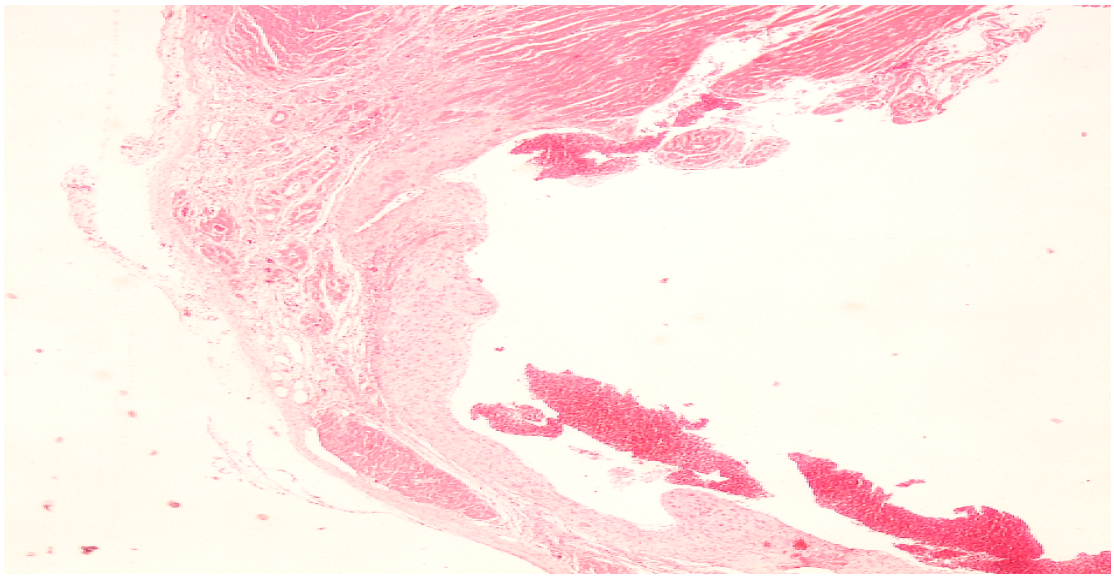
Hematoxylin- eosin 2x magnification - ischemic changes in myocardium with loss of cardiac muscle fibers (control and experimental animal group)



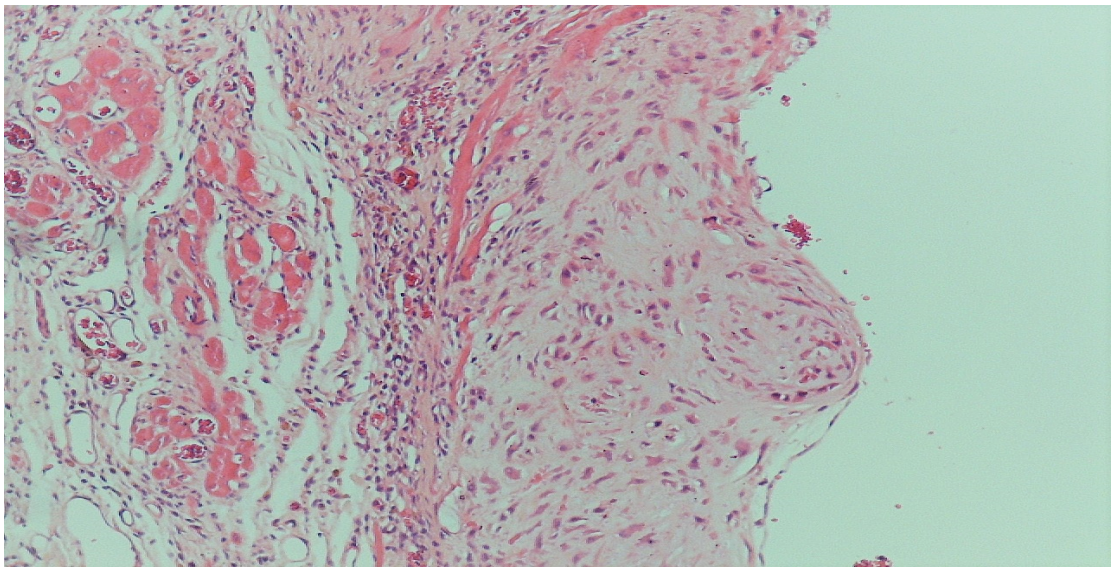
Hematoxylin - eosin 2x magnification - normal rat myocardium (sham operated group)



Hematoxylin - eosin 2x magnification - regenerative tissue over ischemia in myocardial tissue



Hematoxylin - eosin 10x magnification - regenerative tissue over ischemia in myocardial tissue



Strong immunostaining in myocardial cells was considered positive. There was a statistically significant difference between groups regarding the percentage of positive results for the CTGF variable ($p = 0.011$), as well as with the CD133 variable. Pairwise comparisons showed that the experimental group that received the stem cells presented a higher percentage of positive results for the CD133 variable compared to the sham-operated ($p < 0.005$) and control groups ($p < 0.005$), respectively. There was also a statistically significant difference between groups with respect to the percentage of positive results for the GATA4 variable ($p < 0.005$). Pairwise comparisons showed that the experimental group (stem cells) presented a higher percentage of positive results for the GATA4 variable compared to the sham-operated ($p < 0.005$) and control groups ($p < 0.005$), respectively. There was a statistically significant difference between groups with respect to the percentage of positive results for the Nkx2.5 variable ($p < 0.005$). Pairwise comparisons showed that the experimental group presented a higher percentage of positive results for the Nkx2.5 variable compared to the sham-operated ($p = 0.045$) and control groups ($p < 0.005$), respectively. These favorable findings indicate the beneficial effect of SCT in this rat model, in terms of the regeneration of the ischemic myocardium.

Immunohistochemical analysis of myocardium gave a clear appearance of the type of myocardial cells in each animal group.

In sham operated animal group the myocardial cells appeared normal without any presence of ischemia or antigens. Therefore all CD133, CTGF, GATA4 and Nkx2,5 were negatively stained.

In the control animal group were ischemia was established via ligation of LAD but there was no administration of stem cells there was a midly positive staining of CTGF as a general regenerative factor while the other three factors CD133, GATA4 and Nkx2,5 were negatively stained.

Following stem cell administration, immunohistochemical analysis of four factors—antigens in myocardium showed a clearly positive staining confirming the action and presence of stem cells in the harvested myocardial tissue. Therefore all CD133, CTGF, GATA4 and Nkx2,5 were positively stained in the animals of the experimental group.

Immunohistochemical analysis of myocardium without stem cell administration in control animal groups.

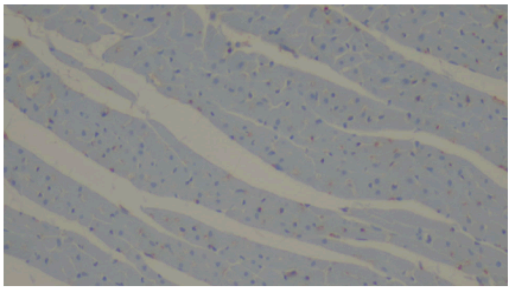
All photos were taken with 10× magnification.

Top left: CD133 antigen negatively stained.

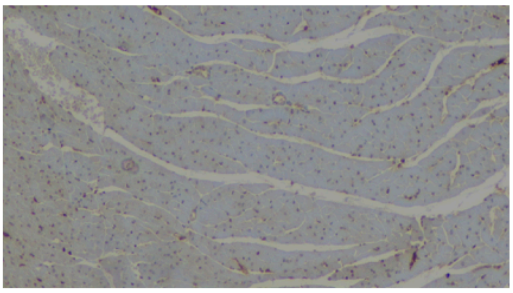
Top right: CTGF antigen mildly stained positively.

Bottom left: GATA4 antigen negatively stained.

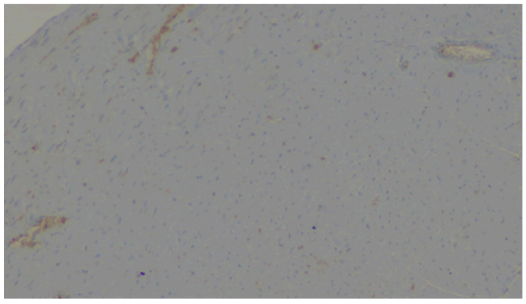
Bottom right: Nkx2.5 antigen negatively stained.



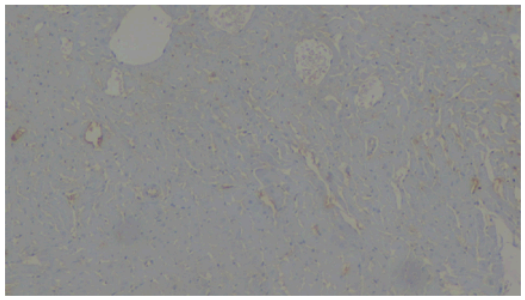
CD133 NEGATIVE 10X



CTGF MIDLY POSITIVE 10X



GATA4 NEGATIVE 10X



Nkx2,5 NEGATIVE 10X

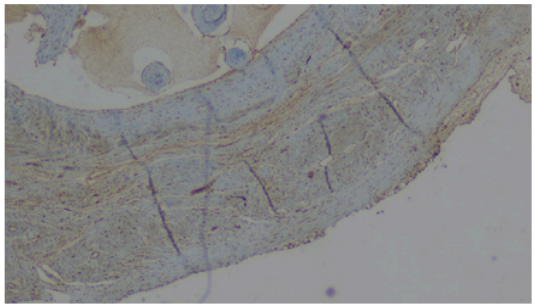
Immunohistochemical analysis of four factors—antigens in myocardium after stem cell administration, experimental animal group.

Top left CD133 antigen positively stained,

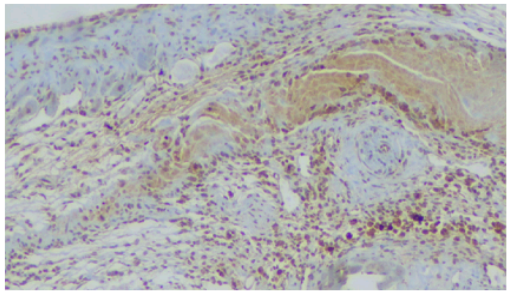
Top right CTGF antigen positively stained,

Middle left (2,5x) and right (10x) GATA4 antigen positively stained,

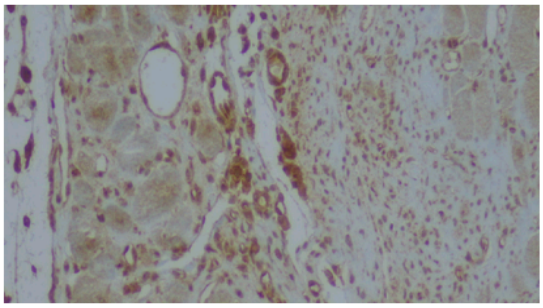
Bottom left (2,5x) and right (10x) Nkx2.5 antigen positively stained.



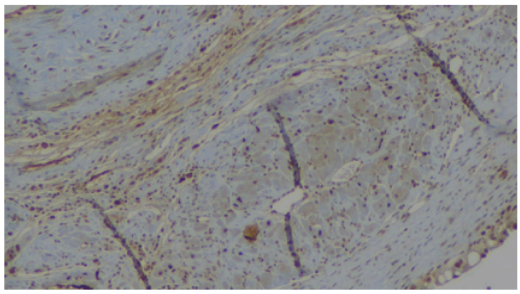
GATA POSITIVE 2,5X



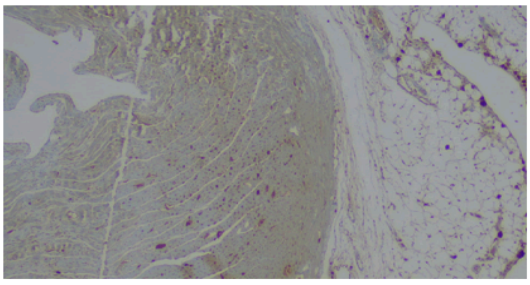
CTGF POSITIVE 2,5x



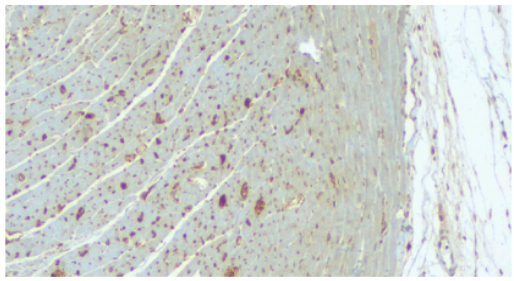
CD133 POSITIVE 2,5X



GATA4 POSITIVE 10X



Nkx2,5 positive 2,5x



Nkx 2,5 positive 10x

Discusion

Myocardial infarction is a leading cause of death worldwide. Therefore development of new therapies and diagnostic approaches are urgently needed. This has led to the creation of numerous experimental protocols, as well as guidelines for experimental models, in order to establish more reliable pre-clinical trials.

The aim of experimental models of myocardial ischemia is to offer better mechanistic understanding that cannot be gained from a clinical situation and may have a low direct applicability to the clinical practice. [3-8] Furthermore, an experimental model could provide mechanistic insight from an experimental study for translation to the clinical situation, and for this purpose models must imitate the clinical situation, as close as possible. [9-12] We consider our model to be in this latest category.

The possibility of having trustworthy results in living animals in comparison to post mortem analysis is of major importance. Until now, most experimental models were based on histological analysis of heart tissue and identification of fibrotic areas, and only few experimental protocols have been developed for in vivo analysis of the myocardial activity.

Rodents are highly comparable animals to human physiology and anatomy concerning the heart. Therefore, the analysis of rat heart tissue post infarction can lead to a solid knowledge which can easily be repeated in laboratories worldwide for the in-vivo evaluation of many novel surgical, pharmaceutical or other treatments. [3-13] In this particular model, a well-established experimental animal model was used to produce myocardial ischemia and infarction by ligating the LAD. This procedure is associated with an increase mortality rate, above 50%, due to the great stress for the animals. Consequently, most of the reported experimental rat models are “acute” experiments that permit only in vitro myocardial evaluation of the infarction postmortem. In contrary, we managed not only to decrease the overall mortality rates to below 20% by a meticulous and standardizing technique for LAD ligation via a left thoracotomy, but also to perform in vivo imaging analysis with a special developed for small animals SPECT/CT, as all animals

survived until the 15th post-operational day of final evaluation, after which euthanasia followed. [1-2] Until now, such in vivo experimental studies often use an intravascular or pharmaceutical method to achieve myocardial infarction in order to minimize the losses associated with open surgical interventions.

The presence of ischemia was well recognized and documented in the left ventricle and, specifically in the area supplied by LAD, with loss of the ability to absorb radioactive particles. The areas and the territories between healthy and ischemic tissue are greatly seen in every axis of the heart and the 3D reconstruction in a combination of SPECT and CT imaging lead to a full anatomical and physiological map of the rat's heart. [14-17] The ability to combine SPECT/CT imaging with x-cube and γ-cube creating a high-resolution outcome, although considered as a great challenge, in our model, it was completed very smoothly and effectively.

This new infarction model was developed keeping in mind the increasing awareness of the need for rigor and reproducibility in designing and performing scientific research to ensure validation of results, as postulated by the reported guidelines.

Based on the results illustrated, the validity of our model was established in every single animal used with a clear imaging of heart chambers and visualization of myocardial activity by SPECT-CT, which is able to evaluate the functional and the non-functional areas and transform the qualitative results into quantitative so that precise myocardial changes can be evaluated. [18-25]

We conclude that our model of an in-vivo evaluation of myocardial ischemia is highly reliable and our evaluation protocol can be used with high accuracy in various trials in cardiovascular experimental research.

Ischemic heart disease is a leading cause of death worldwide; thus, quick and accurate diagnosis and effective therapeutic protocols are needed. Numerous experimental protocols have been created in order to understand the mechanism of myocardial regeneration and develop guidelines for pre-clinical and clinical trials. The overall goal of such experimentation is the understanding of the ischemic mechanism in such a way that the approach of

regeneration is directed via the fastest and most effective pathway. The experimental model used in our study sought to translate the clinical symptoms of myocardial infarction into a qualitative and quantitative presentation, while the therapy with stem cells served to ensure long-term regeneration, clearly recognizable in vivo and postmortem. Therefore, we strongly believe that our findings can be a helpful tool in the hands of researchers worldwide in order to understand, evaluate, present, and cure the ischemic conditions of the myocardium.This study is important for two major reasons. The first is the ability to evaluate experimentally the degree of ischemia in living animals, instead of the commonly used method of postmortem analysis.

Secondly, the use of specific heart-oriented mesenchymal stem cells is leading to a new era of experimentation that is organ-target-specific and can take stem cell transplantation towards a new level of therapeutical goals. The experimental model (rodents) was chosen due to its high compatibility with the human physiology and anatomy concerning the heart. Therefore, the knowledge gained can be compared and replicated worldwide with great accuracy for in vivo evaluation. The establishment of myocardial ischemia and infarction by ligation of the LAD is highly demanding due to increased mortality rates resulting from the stress of animals and the waste products of the ischemic cells in circulation. [3-13] Through the standardization of the technique via a left thoracotomy, we managed to decrease the mortality rate to less than 20%, making it attractive for in vivo experimentation and real-time results, in contrast to the use of pharmaceutical causes of infarction, which only resemble an actual heart attack. [1,2]

Furthermore, the SPECT-CT imaging of the living animals pre- and post-infarction allowed us to understand and present the changes that occur in a living myocardium both physiologically and anatomically, with measurements that are of high qualitative and quantitative value. The use of SPECT creates a very recognizable pattern of living cells absorbing the radioactive substances, which can be measured and evaluated. The CT gives the exact anatomical structure of the heart and the changes in the left ventricle because

of the infarction. [14-27] Furthermore, the regeneration and the increase in contractility of the heart tissue in the animals that received the stem cell therapy was undeniable. Mesenchymal stem cells have been intensively studied in the past few years, in an effort to find new therapeutical methods for numerous diseases. Their use in myocardial tissue is not yet popular since there have not been any particular cells addressed to this tissue with comparable results. [28-42] Our goal was to isolate myocardial-specific cells, specifically GATA4 and Nkx2.5, and to study their specific action in the post-infarcted myocardium. [43-48, 54-58] Most researches have been using cell cultures with positive results, but it is challenging to study their efficacy in living animals. In this study, the administration of the cells was performed directly in the infarcted area in the beating heart and the positive regenerative results were confirmed via the immunohistochemical analysis of the heart tissue. It was confirmed that CD133 was present only in the animals of the experimental group as a general stem cell marker, while CTGF was present in both the experimental and control groups as a general regenerative marker, but not in the sham-operated group, where no ischemia was induced. [63-66, 67-71]

It was proven that the animals that received the stem cells had an increased volume of the left ventricle and better contractility at 15 days postoperatively. Only in the experimental group of animals, the stem cells were isolated and recognized postmortem. It is notable that, using this model and methodology, the great challenges of such an experiment were addressed in a smooth and effective manner.

Limitations of the study

Among the limitations of this study are as follows. (1) Only female rats were used as stem cell receivers and only male rats were used as stem cell donors. (2) The period of survival and monitoring until euthanasia was short. This was necessary for reasons related to the obtained license for this experiment. (3) The weight of the animals was not the same for all, but was within a range corresponding to adult rats of the same age; similarly, the weight of their hearts was within an acceptable range.

Conclusion

In conclusion, this novel, valid, and highly reproducible infraction model and the methodology used gave a transparent vision of the myocardial activity, with both qualitative and quantitative parameters. Furthermore, verification by immunohistochemical analysis demonstrated the therapeutic potential of ADSC transplantation. From a clinical perspective, therefore, concurrent ADSC administration may be applied in combination with coronary artery bypass grafting. However, additional studies are required to further clarify the regenerative effect of stem cell transplantation in the ischemic myocardium.

Abbreviations

ADSCs	adipose-derived stem cells
ARRIVE	Animal Research Reporting of In Vivo Experiments
BrdU	5-bromo-2-deoxyuritidine
CD45, CD105, CD73, CD44, CD29, CD133	cell markers—cluster of differentiation (surface markers that are very useful for the identification and characterization of leukocytes and subpopulations of leukocytes)
CTGF	cell marker—connective tissue growth factor
DAPI	6-diamino-2-phenylindole
DNA	deoxyribonucleic acid
DMEM	Dulbecco's modified eagle medium
ECG	electrocardiogram
FBS	fetal bovine serum
GATA4	mesenchymal factor—transcription factor located in nucleus
IVC	inferior vena cava
ISRA	Image Space Reconstruction Algorithm
KCl	potassium chloride
LAD	left anterior descending coronary artery
LCA	left coronary artery
LV/RV	left ventricle/right ventricle (volume)
MSCT	mesenchymal stem cell transplantation
Nkx2.5	mesenchymal factor—transcription factor located in cytoplasm
PBS	phosphate-buffered saline
PO	postoperatively
SCT	stem cell transplantation
SPECT/CT	single positron emission computed tomography/ computed tomography

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