Manuscript WJSC 46609

"Unmodified autologous stem cells at point of care for chronic myocardial infarction"

by A. Haenel, M. Ghosn, T. Karimi, J. Vykoukal, D. Shah, M. Valderrabano, D. Schulz, A. Raizner, C. Schmitz and E. Alt

Point-by-point reply to the comments and suggestions of the editor and the reviewers:

> **Editor (comments in file *46609-edited*):**
> *First comment on page 2 of our initial manuscript:*
> The approved grant application form(s) will be released online together with the manuscript in order for readers to obtain more information about the study and to increase the likelihood of subsequent citation. Our purpose of publishing the approved grant application form(s) is to promote efficient academic communication, accelerate scientific progress in the related field, and improve productive sharing of research ideas. In addition, a copy of the full approved grant application form(s), consisting of the information section and body section, should be provided to the BPG in PDF format.

The Alliance of Cardiovascular Researchers (1010 Common St #1810, New Orleans, LA 70112, USA) (henceforth: „Alliance") is a medical research organization (U.S. National Taxonomy of Exempt Entities Code H43 [Specific Organ Research: Heart and Circulatory]; https://www.guidestar.org/profile/72-1502598) that supports heart and circulatory research. The Alliance does not make use of grant application forms. Rather, it supports on a case-by-case basis medical research based on approved IACUC/IRB protocols that are brought forward to the Alliance by individual researchers.

In the case of this project IACUC protocol No. AUP-0910-0019 / IS00000596 titled „*Stem Cell Injection in a Myocardial Infarcted Pig Heart*" by A. Raizner, E. Alt, D. Amish, M. Valderrabano, D. Schulz, J. Vykoukal and D. Shaw (except of D. Amish all of these colleagues are co-authors of Manuscript WJSC 46609) was approved by The Methodist Hospital Research Institute (Houston, TX 77030, USA) on January 05, 2011 (see also below).

In the Fall of 2013, Mr. Alexander Haenel (at that time medical student at the University of Luebeck [Luebeck, Germany]; first author of Manuscript WJSC 46609) met Dr. Eckhard U. Alt (senior author of Manuscript WJSC 46609) at a scientific conference in Germany. During this meeting Mr. Haenel expressed his interest in heart and circulatory research to Dr. Alt, and Dr. Alt introduced the aforementioned research project (based on approved IACUC protocol No. AUP-0910-0019 / IS00000596) to Mr. Haenel. Mr. Haenel and Dr. Alt agreed that Mr. Haenel shall come in the framework of a foreign student exchange to Tulane University (New Orleans, LA 70112, USA) where certain parts of the aforementioned research project were carried out under the supervision of Dr. Alt (after all animal experiments had been peformed at The Methodist Hospital Research Institute (Houston, TX, USA)). Then, Dr. Alt brought forward the approved IACUC protocol No. AUP-0910-0019 / IS00000596 to the Alliance, the Alliance approved funding (of a monthly stipend to Mr. Haenel from March 15, 2014 to March 15, 2015) on December 28, 2013, and Mr. Haenel obtained his J1 visa on February 20, 2014.

We have uploaded the following documents to BPG when submitting our revised manuscript to the *World Journal of Stem Cells*:

- the aforementioned declaration, signed by Mr. Haenel and Dr. Alt,

- approved IACUC protocol No. *AUP-0910-0019 / IS00000596* titled „*Stem Cell Injection in a Myocardial Infarcted Pig Heart*“ by A. Raizner, E. Alt, D. Amish, M. Valderrabano, D. Schulz, J. Vykoukal and D. Shaw,
- the corresponding approval letter by James Davis, Ph.D., then IACUC Chair of The Methodist Hospital Research Institute (Houston, TX 77030, USA),
- a copy of Mr. Haenel's J1 visa, and
- the letter of funding by the Alliance of Cardiovascular Researchers.

> *Second comment on page 2 of our initial manuscript:*
> Please upload the primary version (PDF) of the Institutional Review Board's official approval, prepared in the official language of the authors' country to the system; for example, authors from China should upload the Chinese version of the document, authors from Italy should upload the Italian version of the document, authors from Germany should upload the Deutsch version of the document, and authors from the United States and the United Kingdom should upload the English version of the document, etc.

We have uploaded the following documents to BPG when submitting our revised manuscript to the *World Journal of Stem Cells*:

- approved IACUC protocol No. *AUP-0910-0019 / IS00000596* titled „*Stem Cell Injection in a Myocardial Infarcted Pig Heart*“ by A. Raizner, E. Alt, D. Amish, M. Valderrabano, D. Schulz, J. Vykoukal and D. Shaw, and
- the corresponding approval letter by James Davis, Ph.D., then IACUC Chair of The Methodist Hospital Research Institute (Houston, TX 77030, USA).

Note that the Institutional Animal Care and Use Committee (IACUC) of The Methodist Hospital Research Institute (Houston, TX, USA) is using a fully electronic documentation and information system (https://morti.tmhs.org). Accordingly, there are no signed documents. The documents uploaded to BPG are exact pdf copies of the Word files the PIs get. There is a chronological history in the IACUC website that shows the details of when the approval letter was sent out. However, access to the IACUC website requires a username and password.

> *Comments on pages 8, 17-19, 23-25 and 27 of our initial manuscript:*
> Similar, please rewrote. Thank you.

These comments by the editor refer to the CrossCheck report of our initial manuscript performed by BGP. Of note, our initial manuscript showed

- 13% similarity (1135 identical words) with a pre-print of an earlier version of this manuscript titled „*Unmodified, autologous adipose-derived regenerative cells improve cardiac function, structure and revascularization in a porcine model of chronic myocardial infarction*“ by A. Haenel, M. Ghosn, T. Karimi, J. Vykoukal, C. Kettlun, D. Shah, A. Dave, M. Valderrabano, D. Schulz, A. Azares, A. Raizner and E. Alt (https://doi.org/10.1101/286468; authors whose names are underlined are co-authors of Manuscript WJSC 46609; C. Kettlun and A. Dave provided data to this pre-print that are not part of Manuscript WJSC 46609);
- 3% similarity (278 identical words) with a pre-print of another manuscript authored by Dr. Alt (titled „*Isolation of adipose tissue derived regenerative cells from human subcutaneous tissue with or without the use of an enzymatic reagent*“ by N. Valenzuela, C. Alt, G. Winnier and E.U. Alt; https://doi.org/10.1101/485318); and

- <1% (between 44 and 20 identical words) with a total of four other publications (one of them could not be identified because the CrossCheck report only referred to www.wjgnet.com which is the website of the publisher of the *World Journal of Stem Cells*.

We have modified the wording of the corresponding text passages in our revised manuscript (the crizicized text passages are highlighted in gray in the track-record version of our revised manuscript).

> *Comment on page 15 of our initial manuscript:*
> Figure 4-10 should be listed in order, please check it.

We fully agree with the editor. In fact, the wording „*The photomicrographs shown in Figures 11-13 were produced by digital photography*…" does not refer to these figures. Rather, the corresponding paragraph in the *Materials* and *Methods* section of our manuscript refers to the creation of these figures, not to the figures themselves.

Accordingly, we have not modified our manuscript according to this comment by the editor.

> *Comment on page 28 of our initial manuscript:*
> The guidelines for writing and formatting Article Highlights are as follows:
> 1 Research background
> 2 Research motivation
> 3 Research objectives
> 4 Research methods
> 5 Research results
> 6 Research conclusions
> 7 Research perspectives

At first glance, we were quite surprised by this comment of the editor, because it may indicate that our initial submission was incomplete.

Therefore, we carefully re-examined the documents „*Guidelines for manuscript preparation, submission, and manuscript format: Basic study*" provided by the *World Journal of Stem Cells* (https://www.wjgnet.com/bpg/GerInfo/218). In fact, neither in file „*Format for Manuscript Submission: Basic Study*" nor in file „*Guidelines for Manuscript Preparation and Submission: Basic Study*" provided on this website a Section „*Article Highlights*" is mentioned. Accordingly, our initial manuscript did not come with a Section „*Article Highlights*" at the end of the main text because our initial manuscript was prepared fully in line with the aforementioned „*Guidelines for manuscript preparation, submission, and manuscript format: Basic study*" provided by the journal.

We then checked a number of recent articles published in the *World Journal of Stem Cells* and found a Section „*Article Highlights*" in all of these articles.

Accordingly, our revised manuscript contains the requested Section „*Article Highlights*".

We would like to recommend to the editors of the *World Journal of Stem Cells* to describe the need for providing a Section „*Article Highlights*" in the documents „Guidelines for manuscript preparation, submission, and manuscript format: Basic study" provided by the journal (https://www.wjgnet.com/bpg/GerInfo/218).

Reviewer #1:

The manuscript submitted by Haenel A et al. report delivery of fresh, uncultured, unmodified, autologous adipose-derived regenerative cells (UA-ADRCs) in chronic myocardial infarction. According to the authors, the treatment is effective, producing a significant increase in cardiac output without adverse effects. The study is very good, with a lot of methods and interesting results.

We are grateful for this statement by the reviewer.

The manuscript has a little unusual situation. It is found online in the form of .pdf under the title "Unmodified, autologous adipose-derived regenerative cells improve cardiac function, structure and revascularization in a porcine model of chronic myocardial infarction (https://www.biorxiv.org/content/biorxiv/early/2018/03/21/286468.full.pdf) and even has a citation in WJSC Feb 2019 (https: /www.ncbi.nlm.nih.gov/pmc/articles/PMC6397807/), reference 95. However, it does not appear to be published in a journal.

We would like to disagree with the reviewer here.

Actually the pre-print cited by the reviewer (https://www.biorxiv.org/content/biorxiv/early/2018/03/21/286468.full.pdf) is a pre-print of an earlier version of this manuscript, titled „*Unmodified, autologous adipose-derived regenerative cells improve cardiac function, structure and revascularization in a porcine model of chronic myocardial infarction*“ by A. Haenel, M. Ghosn, T. Karimi, J. Vykoukal, C. Kettlun, D. Shah, A. Dave, M. Valderrabano, D. Schulz, A. Azares, A. Raizner and E. Alt (authors whose names are underlined are co-authors of our Manuscript WJSC 46609; C. Kettlun and A. Dave provided data to this pre-print that are not part of our Manuscript WJSC 46609).

In fact, the CrossCheck report of our initial Manuscript WJSC 46609 performed by BGP showed only 13% similarity (1135 identical words) with the pre-print cited by the reviewer. In particular, the *Introduction* and *Discussion* sections of our initial Manuscript WJSC 46609 fundamentally differed from the *Introduction* and *Discussion* sections of the pre-print cited by the reviewer.

In any case, it is the policy of the *World Journal of Stem Cells* that authors can archive pre-prints of manuscripts submitted to this journal on preprint servers such as BioRxiv.

Accordingly, we have not modified our manuscript according to this comment by the reviewer.

Minor suggestions:

Introduction: It is unusual that in the Introduction section, with the aim and hypothesis of the study, the results to be also presented: “Thus, it was the aim of the present feasibility study to test in a porcine model for the study of CMI the following hypotheses: 1) occlusion of the left anterior descending……. statistically significant ($p<0.05$) improvement of the LVEF by at least 15%..... and 2) the same animal model shows statistically significant improvements….”

We are grateful for this statement by the reviewer, because it indicates that the *Introduction* section of our initial manuscript was slightly misleading. In fact, no results of our study were presented in the *Introduction* section. The text passages cited by the reviewer („…*statistically significant (p<0.05) improvement of the LVEF by at least 15%*...“) were part of our initial hypothesis and considered a benchmark for success.

To prevent misinterpretation we have modified the corresponding paragraph of the *Introduction* section of our revised manuscript as follows:

„*Thus, it was the aim of the present feasibility study to test in a porcine model for the study of CMI the following hypotheses: 1) occlusion of the left anterior descending (LAD) coronary artery for three hours results in a clinically relevant reduction of the LVEF to less than 40% on average four weeks post-MI (demonstrating significance of the used animal model); 2) delivery of UA-ADRCs into the LAD vein four weeks post-MI in this model leads to improved LVEF by more than 15% (relative change) on average ten weeks post-MI (primary objective of this study); and 3) the same animal model shows improvements in cardiac structure six weeks after delivery of UA-ADRCs (i.e., ten weeks post-MI) (secondary objective of this study)*."

> Results: Characterization of UA-ADRCs: The phenotype of ADRC cells is unclear. Population seems incompletely characterized by flow cytometry. Important stem cell markers such as CD90 or CD105 are missing.

We assume that the reviewer is referring here to a joined position statement published by the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) in 2013 regarding the stromal vascular fraction (SVF) and culture-expanded adipose derived stem cells (ASCs) (Bourin et al., Cytotherapy 2013; 15: 641-648). In this joined position statement it was stated that primary stable positive surface markers for stromal cells are CD13, CD29, CD34 (>20%), CD44, CD73 and CD90 (>40%), whereas primary negative surface markers for stromal cells are CD31 (<20%) and CD45 (<50%) (Bourin et al., 2013). Furthermore, at least 20% of the SVF would contain a stromal cell population that is immunopositive for the surface marker CD34 and immunonegative for the surface markers CD31, CD45 and CD235a (i.e., CD31-/CD34+/CD45-/CD235a- cells) (Bourin et al., 2013). This statement was based on an earlier position statement published by ISCT in 2006 that described the following minimal criteria for defining multipotent mesenchymal stromal cells (MSCs): being adherent to plastic, expressing the surface markers CD73, CD90 and CD105, and having the ability to differentiate into osteoblasts, adipocytes and chondrocytes (Dominici et al., Cytotherapy 2006; 8: 315e7).

It should be pointed out that a major shortcoming of this definition of multipotent MSCs is the fact that, for example, fibroblasts are also adherent to plastic and express the surface markers CD73, CD90 and CD105, without having the ability to transdifferentiate into other lineages or being MSCs (Alt et al., Biol Cell 2011; 103: 197-208; Reference no. 56 in our initial/revised manuscript). Furthermore, the true pluripotent stem cells do not yet express CD73, CD90 and CD105 (Alt et al. (2019) DOI: 10.20944/preprints201904.0200.v1). Rather, expression of cell surface markers is a dynamic process. For example, when cultured in fetal bovine serum or platelet lysate culture media, MSCs can turn on new surface markers (Alt et al., 2019). Alternatively, MSCs in culture can lose their surface marker expression, such as for example the loss of the previously expressed progenitor marker CD34 or the endothelial progenitor marker CD31 (Alt et al., 2019).

Nevertheless, Tables 1 and 2 (on the following page) summarize the relative amount of adipose-derived regenerative cells (ADRCs) expressing the surface markers CD13, CD29, CD34, CD44, CD73, CD90, CD31 and CD45 as reported in all studies describing enzymatic and non-enzymatic methods for isolating ADRCs that were published so far (note that in some studies surface markers were investigated but relative amounts of ADRCs expressing a certain surface marker or a combination of surface markers were not provided).

Table 1 Relative amount of ADRCs expressing certain surface markers as reported in studies describing non-enzymatic methods for isolating ADRCs (Note: the references cited in this table [Column „R"] can be found in a study by Winnier et al. [DOI: https://doi.org/10.1101/485318] from which these data were taken)

R	Y	CY	CD13	CD29	CD34	CD44	CD73	CD90	CD31	CD45
[39]	2015	0.0	--	--	--	--	--	--	--	--
[40]	2013	0.07	--	--	28[(a)]	--	--	--	[(a)]	[(a)]
[40]	2013	0.35	--	--	26[(a)]	--	--	--	[(a)]	[(a)]
[41]	2014	0.72	--	--	--	--	--	--	--	--
[42]	2015	1.00	--	--	20[(b)]	--	--	--	4[(c)]	--
[43]	2016	1.01	--	--	11[(a)]	--	--	--	[(a)]	[(a)]
[42]	2015	1.03	--	--	25[(b)]	--	--	--	5[(c)]	--
[40]	2013	1.07	--	--	22[(a)]	--	--	--	[(a)]	[(a)]
[42]	2015	1.47	--	--	--	--	--	--	--	--
[44]	2012	1.60	--	--	42[(b)]	--	--	--	--	36
[45]	2014	2.30	--	--	--	--	60[(d)]	[(d)]	--	[(d)]
[40]	2013	2.41	--	--	44[(a)]	--	--	--	[(a)]	[(a)]
[39]	2015	2.55	--	--	--	--	--	--	--	--
[44]	2012	2.60	--	--	41[(b)]	--	--	--	--	34
[43]	2016	2.85	--	--	9[(a)]	--	--	--	[(a)]	[(a)]
[46]	2019	2.93	33	76	60	60	48	68	45	23
[47]	2015	2.79	--	--	36[(e)]	--	--	[(e)]	--	5[(f)]
[48]	2008	2.95	--	--	0.8[(g)]	--	--	--	8[(h)]	--
[49]	2005	3.00	--	--	--	--	--	--	--	--
[50]	2006	3.08	37	47	60	64	25	55	22	--
[51]	2014	3.60	--	--	35	--	--	--	8[(i)]	50
[52]	2014	3.60	--	--	--	--	--	--	--	--
[53]	2014	3.60	--	--	--	--	--	--	--	--
[54]	2014	3.68	--	--	--	--	--	--	--	--
[23]	2004	4.04	--	--	--	--	--	--	--	--
[55]	2013	4.07	--	--	--	--	--	--	--	--
[56]	2013	4.80	--	90	81	6	37	81	--	28
[57]	2015	5.34	--	--	20[(a)]	--	--	--	--	6
[46]	2019	5.35	48	89	67	68	59	76	55	31
[43]	2016	5.36	--	--	9[(a)]	--	--	--	[(a)]	[(a)]
[43]	2016	6.25	--	--	7[(a)]	--	--	--	[(a)]	[(a)]
[58]	2013	7.01	--	--	18[(b)]	--	--	--	27[(i)]	--
[58]	2013	7.02	--	--	43[(b)]	--	--	--	25[(i)]	--
[59]	2017	9.06	--	--	--	--	--	--	--	--
[57]	2015	12.1	--	--	19[(a)]	--	--	--	--	13
[60]	2006	13.1	--	--	28[(j)]	--	--	--	4[(k)]	30[(l)]
[61]	2001	13.3	--	--	--	--	--	--	--	--
[62]	2016	35.0	--	--	--	--	--	--	--	--
[63]	2016	387	--	--	--	--	--	22[(m)]	[(m)]	--

Surface marker data are given in percent. Abbreviations: R, reference; Y, year of publication; CY, cell yield [$\times 10^5$/ml lipoaspirate]; --, data not provided. (a), $CD34^+/CD31^-/CD45^-$ cells; (b), $CD34^+/CD31^-$ cells; (c), $CD34^-/CD31^+$ cells; (d), $CD45^-/CD73^+/CD90^+$ cells; (e), $CD34^+/CD90^+/CD45^-/CD146^-$ cells; (f), $CD34^-/CD45^+/CD14^+$ cells; (g), $CD34^+/CD90^-/CD31^-/CD45^-/CD105^-/CD146^+$ cells; (h), $CD34^+/CD90^+/CD31^+/CD45^-/CD105^-/CD146^+$ cells; (i), $CD34^+/CD31^+$ cells; (j) $CD34^+/CD90^+/CD31^-/CD45^-/CD105^-/CD146^-$ cells; (k), $CD34^+/CD90^+/CD31^+/CD45^-/CD105^-/CD146^+$ cells; (l), $CD34^-/CD31^-/CD45^+/CD105^-/CD14^-/CD15^+$ cells; (m), $CD90^+/CD105^+/CD31^-$ cells.

Table 2 Relative amount of ADRCs expressing certain surface markers as reported in studies describing non-enzymatic methods for isolating ADRCs (Note: the references cited in this table [Column „R"] can be found in a study by Winnier et al. [DOI: https://doi.org/10.1101/485318] from which these data were taken)

R	Y	CY	CD13	CD29	CD34	CD44	CD73	CD90	CD31	CD45
[39]	2015	0.07	--	--	--	--	--	--	--	--
[53]	2014	0.11	--	--	--	--	--	--	--	--
[45]	2014	0.12	--	--	--	--	60[(a)]	[(a)]	--	[(a)]
[45]	2014	0.23	--	--	--	--	60[(a)]	[(a)]	--	[(a)]
[56]	2013	0.25	--	48	24	5	9	23	--	82
[39]	2015	0.30	--	--	--	--	--	--	--	--
[64]	2014	1.25	--	--	--	--	--	--	--	--
[54]	2014	1.39	--	--	--	--	--	--	--	--
[65]	2009	2.40	--	--	--	--	--	--	--	--
[57]	2015	4.44	--	--	25[(a)]	--	--	--	--	8

Surface marker data are given in percent. Abbreviations: R, reference; Y, year of publication; CY, cell yield [$\times 10^5$/ml lipoaspirate]; --, data not provided. (a) $CD45^-/CD73^+/CD90^+$ cells; (b), $CD34^+/CD31^-/CD45^-$ cells.

The data summarized in Tables 1 and 2 demonstrate the following (note that the references cited in the following paragraph can be found in a study by Winnier et al. [DOI: https://doi.org/10.1101/485318] from which these data were taken):

- for only very few methods[46,50,51 56] the relative amount of $CD34^+$ ADRCs was determined, with substantial variation among methods (range, 35% - 81%);
- for most methods CD34 was determined together with at least one other surface marker, resulting in a range of published data between 0.8% ($CD34^+/CD90^-/CD31^-/CD45^-/CD105^-/CD146^+$ cells[48]) and 44% ($CD34^+/CD31^-/CD45^-$ cells[40]);
- the relative amount of $CD45^+$ ADRCs varied between 6%[57] and 50%[51] for enzymatic methods, and between 8%[57] and 82%[56] for non-enzymatic methods;
- for only few methods the relative amounts of $CD13^+$ cells, $CD29^+$ cells, $CD44^+$ cells, $CD73^+$ cells, $CD90^+$ cells and $CD31^+$ cells were determined; and
- for no any method the relative amount of $CD31^-/CD34^+/CD45^-/CD235a^-$ cells (as proposed by Bourin et al., 2013) was determined.

Collectively, the data summarized in Tables 1 and 2 demonstrate that the vast majority of methods for isolating ADRCs were not characterized according to the position statements published by IFATS and ISCT (Dominici et al., 2006; Bourin et al., 2013).

Considering the available data summarized in Tables 1 and 2 and the general concerns about characterizing ADRCs and MSCs by surface markers outlined above it appears reasonable to hypothesize that determining surface markers of ADRCs is in principle not suitable for characterizing a method for isolating ADRCs from adipose tissue. This was the reason why no such characterization was performed in this study.

The information provided here was taken from a manuscript that is under consideration at another journal (a pre-print of this manuscript was cited as Reference no. 20 in our initial manuscript). This is the reason why we have not included these data in our revised manuscript. Rather, we have added the following paragraph in the *Discussion* section of our revised manuscript (pages 26-27) (the references provided in the following paragraph refer to the references in our revised manuscript):

"It should be mentioned that characterization of the cells delivered in the present study did not follow recommendations published in a joined position statement by the International Federation

for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) in 2013 regarding the SVF and ASCs[30]. In this joined position statement it was stated that primary stable positive surface markers for stromal cells would be CD13, CD29, CD34 (>20%), CD44, CD73 and CD90 (>40%), whereas primary negative surface markers for stromal cells would be CD31 (<20%) and CD45 (<50%)[30]. Furthermore, at least 20% of the SVF would contain a stromal cell population that is immunopositive for the surface marker CD34 and immunonegative for the surface markers CD31, CD45 and CD235a (i.e., $CD31^-/CD34^+/CD45^-/CD235a^-$ cells)[30]. This statement was based on an earlier position statement published by ISCT in 2006 that described "being adherent to plastic, expressing the surface markers CD73, CD90 and CD105, and having the ability to differentiate into osteoblasts, adipocytes and chondrocytes"[68] as minimal criteria for defining MSCs. However, it should be pointed out that a major shortcoming of this definition of multipotent MSCs is the fact that, for example, fibroblasts also adhere to plastic and express the surface markers CD73, CD90 and CD105, without having the ability to transdifferentiate into other lineages or being MSCs[64]. Furthermore, the true pluripotent stem cells do not yet express CD73, CD90 and CD105[24]. Besides this, a recent study compiled the relative amount of ADRCs expressing the surface markers CD13, CD29, CD34, CD44, CD73, CD90, CD31 and CD45 as reported in all studies describing enzymatic and non-enzymatic methods for isolating ADRCs that were published so far[24]. In very brief, it was found that 1) the relative amount of $CD34^+$ cells was determined for only very few methods, with substantial variation among methods (ranging between 35% and 81%); 2) the relative amount of $CD45^+$ cells varied between 6% and 82% among published studies; 3) relative amounts of $CD13^+$ cells, $CD29^+$ cells, $CD44^+$ cells, $CD73^+$ cells, $CD90^+$ cells and $CD31^+$ cells were only determined for few methods; and 4) the relative amount of $CD31^-/CD34^+/CD45^-/CD235a^-$ cells (as proposed in[30]) was not reported for any method[24]. Collectively, these data and the general concerns about characterizing ADRCs and MSCs by surface markers outlined above render determination of surface markers of ADRCs as proposed by IFATS and ISCT[30,68] in principle not suitable. This was the reason why no such characterization as proposed by IFATS and ISCT[30,68] was performed in this study."

> **Reviewer #2:**
> The authors report results derived from a feasibility study on pigs whose left anterior descending (LAD) artery was occluded for 180 min. Four weeks later, the fresh, uncultured, unmodified, autologous adipose-derived regenerative cells (UA-ADRCs) were retrogradely delivered into the balloon blocked LAD vein (control: delivery of saline). Another six weeks later, mean left ventricular mass (+29%) and cardiac output (+37%) had increased ($p<0.01$) after delivery of cells. The combination of the procedure used for isolating stem cells and the novel cell delivery route applied in the present study potentially opens new horizons for clinical therapy for chronic myocardial infarction. The manuscript provided some new idea to the readers.

We are grateful for this statement by the reviewer.

> However, there're still two issues which should be addressed. 1. The characterization of adipose-derived regenerative cells is not enough. The multidirectional differentiation induction experiments should be conducted.

To address this comment by the reviewer we added the following sentences at the end of Subsection „Isolation of adipose-derived regenerative cells" in the Materials and Methods section (page 9f):

„Note that UA-ADRCs that were isolated from adipose tissue with the Transpose RT system and the enzymatic Matrase Reagent were comprehensively characterized in a number of studies[19-21], including demonstration of expression of regenerative cell-associated genes Oct4, Klf4 and Hes3[21] as well as their differentiative potential into adipogenic, osteogenic, hepatogenic and neurogenic cell lines[21]. On this basis, UA-ADRCs isolated from adipose tissue with the Transpose RT system and the enzymatic Matrase Reagent were used in a number of clinical pilot trials[22-24] and are currently under investigation in a number of Investigational Device Exemption (IDE) studies approved by the U.S. Food and Drug Administration[25-29]."

2. In the discussion part, a review of previous studies should be added in.

It is critical to note that our study addressed treatment of chronic MI with UA-ADRCs. All studies on treatment of chronic MI with stem cells on humans and large animal models (pigs, sheep) that have ever been reported in the literature are cited and discussed in the *Discussion* section of our manuscript. Studies on mice and rats are not comparable because cardiac function and structure cannot be evaluated with MRI on these models.

We assume that this reviewer refers to the many studies on treatment of acute MI with stem cells that were reported in the literature. However, it is critical to note that in treatments of acute MI, stem cells act via different molecular and cellular mechanisms of action than in treatments of chronic MI. In case of acute MI the predominant effect of application of stem cells may be the prevention of apoptotis of cardiomyocytes (c.f. Liu et al., Cell Death Discovery 2019; 5: 79). However, in the present study delivery of UA-ADRCs into the LAD vein took place four weeks post-MI. A study on a rat model for the study of MI demonstrated high numbers of active caspase-3 immunopositive (i.e., apoptotic) cells in cardiomyocytes and nonmyocytes at 7-10 days post-MI but not at 28 days post-MI (Rafatian et al., Am J Physiol Regul Integr Comp Physiol 2014;307: R879-R887). Accordingly, in case of chronic MI the predominant effect of application of stem cells may be enhancement of the neovascularization response after MI (demonstrated in Fig. 12 in our manuscript) and the formation of new cardiomyocytes. With regard to the latter it was a recent key finding that endogenous cardiomyocyte generation can be activated by exercise in the normal and injured adult mouse heart (Vujic et al., Nat Commun 2018; 9: 1659).

On this basis it would not make sense to provide a review of previous studies on treatment of acute MI with stem cells in the *Discussion* section of our revised manuscript. Rather, because of different molecular and cellular mechanisms of action treatment of acute MI with stem cells should be considered a separate topic, and should be addressed in separate publications.

In order to highlight this important difference between treatment of acute and chronic MI with stem cells we have modified the third-to-the-last paragraph in the *Discussion* section of our revised manuscript as follows:

„In our study, the delivery of UA-ADRCs in CMI resulted in an increased mean mass of the left ventricle and a reduced mean relative amount of scar volume of the left ventricular wall (Figures 8-10). In this regard it is critical to note that in treatments of acute MI, stem cells act via different molecular and cellular mechanisms of action than in treatments of chronic MI. In case of acute MI the predominant effect of application of stem cells may be the prevention of apoptotis of cardiomyocytes[73]. However, in our study delivery of UA-ADRCs into the LAD vein took place four weeks post-MI. A study on a rat model for the study of MI demonstrated high numbers of active caspase-3 immunopositive (i.e., apoptotic) cells in cardiomyocytes and nonmyocytes at 7-10 days post-MI but not at 28 days post-MI[8]. Accordingly, in case of chronic MI the predominant effect of application of stem cells may be enhancement of the neovascularization response after MI (demonstrated in Figure 12 as well as in[38,40,41]) and the formation of new cardiomyocytes. With

regard to the latter it was a recent key finding that endogenous cardiomyocyte generation can be activated by exercise in the normal and injured adult mouse heart[74]. Other mechanisms described in the literature (including inhibited cardiac fibroblast growth, reduced collagen expression, beneficial effects on the ratio between matrix metalloproteinases and tissue inhibitors of metalloproteinases, as well as limited local inflammation[75-78]) may equally apply for the results obtained in the present study.

> Furthermore, the creative points of this study should be clearly discussed in comparison with previous studies. So, major revision should be recommended.

This is addressed in detail in our answer to the previous comment by this reviewer.

> **Reviewer #3:**
> This an interesting novel basic study evaluating the use of unmodified autologous stem cells at point of care for treatment of chronic myocardial infarction. It has a clear hypothesis and a sound design and the results have an internal and external validity.

We are grateful for this statement by the reviewer.

> However, the following minor corrections are needed
> 1. According to the Journal style, No section for the background in the abstract

We have modified the Abstract in our revised manuscript accordingly.

> 2. Please be consistent in putting the value of significance to be = or > or <

We have changed all information about *P* values to „*P* = 0.XXX“ throughout our revised manuscript, with the exception of „*P* < 0.001“ and the description of the asterisks used in Figure 5 (B, C, D, E), Figure 6, Figure 7 (A, B) and Figure 10 to indicate statistical significance.

> 3. I wonder why the authors use (e. g.) in citing the references in the text

We have removed all „e.g.“ when citing the references in our revised manuscript.