March 25, 2020

Dear Editor:

We are grateful for the time you and the reviewers spent reviewing our work entitled “Evaluation of the function and recovery of adipose-derived stromal cells in breast cancer patients after exposure to neoadjuvant chemotherapy”. We have thoroughly considered all the comments of the reviewers and the major revised portions are 1) changed the title to “Isolation and identification of adipose-derived stromal/stem cells from breast cancer patients after exposure neoadjuvant chemotherapy” and reworded the manuscript keywords, 2) we have made the suggested changes added the undifferentiated ASC control photos into the figure 4 and have made the figure legends details to clear for figure 5 & 6, and 3) we have reviewed the entire manuscript carefully to improve the language with regard to grammar and typos clarity. We believe that we have addressed each of your concerns and have made the appropriate changes. We remain enthusiastic about this report and hope that you agree that this is now worthy of publication in your esteemed journal.

Sincerely,

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Reponses to reviewers' comments:

Reviewer #1:

Manuscript needs language revision.

We thank the reviewer for this comment. We have reviewed the entire manuscript carefully to improve the language with regard to grammar and typos clarity.

1. Statements “Compared to the no receiving NAC patients, the numbers of ASCs yield were not altered in patients after received NAC treatment. The proliferation rates of ASCs from received NAC patients were also not effected upon cultured in vitro” can be rephrased to “The yield of ASCs didn’t alter much after NAC treatment of patients. Moreover, the proliferation rates of ASCs derived from patients didn’t differ much before and after NACs upon in vitro culture “

Response: We appreciate the reviewer for these suggestions; we have made the suggested changes.

2. Phrase in entire manuscript “NAC patients or no-receiving NAC patients” can be changed to “patients receiving NAC or not-receiving NAC”

Response: We thank the reviewer for these insightful comments. We have made the suggested changes within the manuscript.

Reviewer: #2

1. Title: The title is too specific and unnecessarily clinical.
   Response: We appreciate the reviewer’s viewpoint here and have provided a new title.

2. Abstract: Yes, the abstract summarize and reflect the work described in the manuscript.

3. Keywords: Too cumbersome
   Response: Thank you for pointing this out; we have reworded the keywords.

4. Background: No. There is two much assumption about the study's clinical value.
   Response: We appreciate the reviewer’s viewpoint here and have revised the background of the manuscript.

5. Methods: Yes, the manuscript describe methods is in adequate detail.

6. Results: The study is mainly experiental. The results do not lead to clinical application.
   Response: We agree with the reviewer that the results do not lead to clinical application. The purpose of this experiment was to determine in patients if receiving chemotherapy would adversely affect the functional capacity of ASCs. Our results provided important clinical information that ASCs may have recovery potential after post-chemotherapy such as in breast cancer patients. We have been clarified this point within the manuscript.

7. Discussion: The study was focused on breast related fat cells under a divergent influences, viz with cancer, without cancer/ with different chemotherapy at unknown dosages. The clinical value is doubtful.
   Response: We appreciate this point brought up by the reviewer. In this study, we isolated ASC from cancer patient breast adipose tissue specimens to evaluate the recovery potential of the functional capacity of ASCs after received chemotherapy in patients. We would note that the clinical value is that our results of the study provided the clinically relevant information as ex vivo examination of ASC from patients and promote further understand the use of autologous ASCs for fat grafting and

reconstruction in cancer patients undergoing treatment. We have further discussed this important point within the manuscript.

8. Illustrations and tables: Good
9. Biostatistics: /
10. Units: /
11. References: Good
12. Quality of manuscript organization and presentation: Needs language improvement.
    Response: We thank the reviewer for this comment. We have reviewed the entire manuscript carefully to improve the language concerning grammar and typos clarity.

13. Research methods and reporting: The report refers to laboratory work only.
    Response: We appreciate this point brought up by the reviewer and agree that this study examining ASC function capacities by laboratory work; however, our works at the experiments, which analyze ASCs isolated from the clinical patient's tissues, are ex-vivo studies, not in vitro only. We ask that this is acceptable to the reviewer.

14. Ethics statements: Acceptable Specific Comments to Authors: First: Original finding are different types of fat cells related to the breast with or without cancer. Observations are real. Implications are very limited. Second: There should be no assumption of real clinical value.
    Response: We appreciate the practicality of these comments. Our previous human in vitro data demonstrates that direct exposure to chemotherapeutic reagents modulated ASC cellular functions. In this study, we chose to clinically and determined the function recovery of ASCs outcomes in patients after post-exposure to chemotherapy. We would note that for clinical translation to patients that have undergone chemotherapy, is necessary to understand the effects of these cancer therapies on the ASC ability to improve fat graft survival in clinical practice. Our study further promotes to better understand the use of autologous ASCs for fat grafting and reconstruction in breast cancer patients undergoing treatment. We ask that this is acceptable to the reviewer.

Reviewer: #3

Current study has focused on evaluating in vitro, the cellular functions of Adipose stem cells (ASC) after exposure to three commonly utilized clinical chemotherapeutic agents: 5-fluorouracil (5-FU), Doxorubicin (DXR), and cyclophosphamide (Cytoxan), Authors address important issue to determine whether the number and function of ASCs viability was affected in the presence of these agents commonly used in neoadjuvant chemotherapies, and also studied patterns of recovery after withdrawal of these chemotherapeutic drugs in

culture. Findings are important for those in relevant field. Introduction, results and discussion sections are well written. However, manuscript needs major language revision. Along with ASC, it would be interesting to evaluate comparative sensitivity of breast cancer cells from tumor tissue to chemotherapeutic drugs 5-FU, DXR and Cytoxan. If authors have studied any immunomodulatory activity of ASC may be provided to strengthen the data.

Response: We appreciate the reviewer for these insightful comments and suggestions. Our previous research has demonstrated that the chemotherapeutic drug of Paclitaxel treatment was significantly more toxic for breast cancer cells than for ASCs (Marcotte et al. *Ann Plast Surg.* 2018; 81(4):482-486; Koko et al. *Ann Plast Surg*. 2017; 78(6):728-735). We, therefore, chose simply to confirm that the presence of these clinical chemotherapeutic drugs affects the ASC viability.

We agree with the reviewer that the determination of the immunomodulatory properties of ASC would help to select the source of ASCs with better potential for therapeutic application in future clinical studies. Again, in this pilot study, our goal here was simply to demonstrate that whether the ASCs have the potential for recovery of the cellular function after exposure to the NAC treatment in cancer patients. However, this may represent a limitation to the current study and an opportunity for further research. We ask that this is acceptable to the reviewer.

Few errors as shown below need to be addressed.

1. Pg10. Word Rerults to be rectified to 'Results'.
   Response: We thank the reviewer for this comment which has revealed our mistake in labeling "results" of our manuscript. We have corrected this in the manuscript.

2. Pg 28 Table 1 and pg 29 Figure 1 show discrepancy in patient number before neoadjuvant chemotherapy (NAC) and after NAC.
   Response: We appreciate this viewpoint; we have corrected the labeling in figure 1.

3. Pg 32, Figure 4: Microscopic image of undifferentiated ASC belongs to -NAC or + NAC group in both top and lower panel. Details need to be clarified and undifferentiated ASC controls need to be for each differentiated image. Scale bar or magnification needs to be mentioned.
   Response: We appreciate the reviewer's suggestion. We have added the undifferentiated ASC control photos into figure 4 and have made the figure legends details to clarify.

4. Pg 32, Figure 4: Lipoprotein lipase seems to be overexpressed (copy ratio up to 10000) in ASC before after NAC therapy. Appropriate justification may be included in discussion.
   Response: We thank the reviewer for these insightful comments. In this study the mRNA expression for adipocyte-specific markers by Real-time RT-PCR, the data analysis has referred to the control group by using the relative fold change that has compared with each patient's control group undifferentiated ASC and normalized to GAPDH. Regarding the Lipoprotein lipase, two patient's differentiated ASCs showed a very higher expression of the LPL when compared with their control group cells (undifferentiated), and that both from the tumor side of tissue with received the chemo-treatment. We acknowledge that we did not collect the control data from before starting the treatment in the same patients and failed to provide a negative control (normal person's ASC); we cannot definitively comment upon LPL expression differences that may exist with this population.
   In addition, at this time, the purpose of this experiment was to demonstrate whether the ASCs have the potential for recovery of the differentiation potential after exposure to the NAC treatment in cancer patients. The most important property to demonstrate will be the ASC can retain the differentiation capacity to acquire adipocyte traits. Again, this was a pilot study consisting of small numbers of patients; a larger randomized study of patient samples is needed to confirm our findings. We have, however, further discussed this point within the manuscript. We ask that this is acceptable to the reviewer.

5. Pg 33 and 34 Figures 5 and 6: It is not clear ASC were obtained from -NAC or +NAC breast tissue. Appropriate details need to be mentioned. All suggested corrections need to be addressed before manuscript is accepted for publication.
   Response: We appreciate this point brought up by the reviewer. ASCs were isolated from female patients during reconstructive procedures as described in the methods section. We have also corrected the figure legends details to clarify.