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Response to the letter to the editor: "Understanding the impact of preservation methods on the integrity and functionality of placental allografts"

Dear Editor,

We would like to thank you very much for the opportunity to respond to the critique letter from Dr Fetterolf and Dr Koob (MiMedx Group, Inc) of our article "Understanding the impact of preservation methods on the integrity and functionality of placental allografts" recently published in the *Annals of Plastic Surgery*. We also would like to thank Dr Fetterolf and Dr Koob for their interest in our study and for the initiation of a scientific discussion.

The key focus of our study was to address the scientific question regarding whether increased amounts of placental growth factors and extracellular matrix (ECM) proteins achieved by combining 2 devitalized membranes could compensate for the loss of viable endogenous cells during tissue dehydration. The selection of our test materials for this study was driven by the high interest of health care providers to answer this scientific question using commercial placental products. Therefore, both viable cryopreserved human amniotic membrane (vCHAM) and dehydrated human amnion/chorion membrane (dHACM) were "tools" to address the abovementioned scientific questions rather than subjects of the study. Our interpretation of the data agrees with the results of numerous studies published by other researchers. We believe that our extensive list of cited literature is adequate. It would have been outside the scope of the article to discuss 8 dHACM papers given that our study was not a review of dHACM but a side-by-side comparison of dHACM and vCHAM in experimental settings that differed from the experiments in the dHACM papers.

Conflicts of interest and sources of funding: Amy Johnson, Sandeep Dhall, and Alla Danilkovitch are full-time paid employees of Osiris Therapeutics, Inc. Alexandra Gyurdieva and Yi Duan-Arnold were full-time paid employees of Osiris Therapeutics, Inc. at the time this study was performed. This study was funded by Osiris Therapeutics, Inc., manufacturers of Grafix®.

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We were surprised that Dr Fetterolf and Dr Koob cannot see the differences between fresh placental matrix and the matrix in dHACM (Fig. 1). We have no difficulty visualizing the ECM changes. Moreover, the histological images of dHACM and the conclusion regarding alterations of structural matrix in dHACM are in line with other literature reports that show matrix degradation in placental tissues processed by different dehydration methods followed by radiation, including dHACM made by the PURION process.[1–3] In addition, multiple studies demonstrate the damaging effects of radiation on placental matrix.[3–5] In another study, authors stated that terminal sterilization by gamma and electron beam irradiation (a method employed in the PURION process) damages the basement membrane and elastin and collagen fibers and subsequently affects the quality of the graft's structure and integrity.[6] Paolin et al[5] confirm the detrimental effect of radiation and suggest using an aseptic process for placental tissue processing. The tissue layer underneath of the cytokeratin 18-positively stained chorionic trophoblast is maternal decidua.[7,8] This layer is clearly visible in both the dHACM and fresh placental tissue histological sections. It indicates the presence of maternal placental tissue in dHACM (Fig. 2).

The kinetics of vCHAM resorption and cell death in vCHAM after application to chronic wounds in preclinical models are in line with other published data.[9] This time frame of graft persistence in the wound is sufficient to provide benefits.[9] Also, our preclinical data are in line with our recommendation for weekly application of vCHAM clinically. Given that dHACM has no viable cells, it was not included in our cell persistence evaluation.

The excess of matrix metalloproteinases (MMPs) and inflammatory cytokines in chronic wounds is a well-documented fact.[10–12] Particularly, high levels of MMP9 are considered to be a predictive marker of poor healing.[13] The "dynamic reciprocity" between pro-inflammatory and anti-inflammatory factors that is a part of normal wound healing is impaired in chronic wounds.[14] According to Schultz et al,[14] "Following observations of elevated levels of various MMPs in chronic wound fluid, it was hypothesized that these enzymes could be causing excessive degradation of ECM proteins and chronic tissue turnover that prevented the wounds from healing." Therefore, the addition of exogenous MMPs either active or nonactive, which can be converted into active by endogenous wound MMPs, to chronic wounds could not be considered beneficial.[15]

Although randomized clinical trials are the criterion standard, it is well recognized that the results of such studies may not accurately reflect the effectiveness of therapies delivered in everyday practice. The International Society for Pharmacoeconomics and Outcomes Research supports comparative effectiveness research for

the purposes of assisting patients, clinicians, purchasers, and policy-makers in making informed health care decisions with respect to the real-world clinical effectiveness of medical treatments once broadly implemented in medical practice.[16] At the present time, there are 3 International Society for Pharmacoeconomics and Outcomes Research–guided comparative effectiveness studies that include dHACM: 1 study that compared the outcomes of vCHAM versus dHACM for wound management and 2 others that compared the effectiveness of 2 bioengineered living constructs to dHACM.[17–19] The results of all 3 studies showed better clinical outcomes for living constructs and viable placental tissue than for dHACM. We anticipate more comparative effectiveness studies in the future.

Lastly, our new "Prestige Lyotechnology" is outside of the subject this study. However, we would like to comment since Dr Fetterolf and Dr. Koob brought it up in their letter mistakenly equating this novel technology with traditional lyophilization methods. Unlike all current lyophilization methods, which are not suitable for cell preservation, Prestige Lyotechnology preserves the living cells as well as the tissue structure, allowing storage at room temperature. The Prestige Lyotechnology scientific data are a subject of future publications.

In summary, Osiris focused solely on addressing the scientific questions regarding methods of tissue preservation and made no claims regarding any products or their mechanisms of action. The article clearly states that additional studies need to be performed to understand the clinical significance of our described scientific findings. We are thankful to the editor and the reviewers for recognizing the importance of our research.

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Commentary to LTE Fetterolf and Koob

To the Editor:

An expanding aspect of contemporary political discourse consists of diverting discussion of information to criticism of the processes by which the information has come to light. The last sentence of the letter by Fetterolf and Koob introduces such a collateral criticism by expressing surprise that the article by Johnson et al "made it through the *Annals* review process."[1,2]

The original manuscript was received on May 10, 2016, and reviewed by me. I considered it worthy of detailed review, and forwarded it to an associate editor who then submitted it to 2 reviewers from the editorial board. These 3 reviewers generated 580 words of detailed critique. I received the reviews on August 24, 2016, and agreed with the reviewers' conditional acceptance. I forwarded the recommendations for revision and clarification to the authors.

The authors undertook a detailed response to the reviews and submitted a substantially rewritten manuscript on February 6, 2017. It went to press by electronic publication, to be followed by hardcopy publication upon assignment to an issue.

The article, therefore, underwent an initial close reading by 2 editors and 2 editorial board members. I read the revised manuscript, found the revisions appropriate to the reviewer's comments, and made the decision for acceptance—a decision by which I stand.

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