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Letter to the Editor

A recent publication of Johnson et al[1] in the Annals titled "Understanding the Impact of Preservation Methods on the Integrity and Functionality of Placental Allografts" recently came to our attention. This industry-sponsored research makes a number of observations and conclusions that we think warrant further comment. As the reported manufacturer of the dehydrated human amnion/chorion membrane (dHACM) used in the study, we would note the following.

The study was extensively referenced, with some 60 references, yet these do not include any of the substantial 8 existing, peer-reviewed articles in the scientific literature on the properties and attributes of dHACM, a material oversight in such a study, especially given that the article purports to study dHACM.[2–9]

Furthermore, claims made about the MiMedx dHACM material were often unsupported, such as the degradation of the extracellular matrix in this product (which cannot be determined at the staining and magnification level shown) or the suggestion that the material contains maternal components. Literature that is cited discussing the various effects of dehydration on amniotic membrane is not relevant to dHACM in the material used, because the proprietary PURION Process developed by MiMedx was created to expressly avoid these issues. The suggestion that all amniotic membranes researched present the same properties is well known to be an incorrect statement.

The discussion of the various attributes tested appeared to imply that these attributes materially contributed to a superior result in actual clinical patients, which was not demonstrated. If anything, the observation that preclinical models demonstrate 92% cell death in transplanted viable allografts would seem to negate the argument that Grafix would continue to produce growth factors well after implantation.

The discussion of the significance of matrix metalloproteinase-9 appears to be a sponsor-related statement on an isolated area of their research. Individual markers of inflammation are well known to be insufficient indicators (or treatments) of the wound healing process, which in fact requires the full milieu of both pro-inflammatory and anti-inflammatory growth factors and cytokines in what Schultz et al[10] describes as a process of "dynamic reciprocity." Furthermore, immunoreactivity testing only notes the presence of these factors without noting whether they are clinically active. One of the scientific articles ignored in this article relates directly to matrix metalloproteinases in dHACM, which shows that it is not active.[11]

Interestingly, this article notes that the materials were placed on in vivo chronic wounds in an animal model, but clinical results were not discussed in detail. The implication that dHACM somehow does not work or works less effectively for whatever reason in animals or patients remains unproven.

The only claim cited at the end of the article that Grafix is a superior product seems to be derived from a small retrospective study in a rural hospital that suffered from numerous methodological issues.[12] The study mentioned is far from a comparable standard. A level 3 nonrandomized, noncomparable, highly variable study groups created without a protocol can easily reflect any number of incorrect findings, good or bad. By definition, "comparative effectiveness research" seeks to truly compare equivalent groups for differences in effectiveness and/or cost. That discipline did not occur here. Clearly, it is inappropriate to use this study to counter multiple level 1 published randomized controlled trials using dHACM[13–16] and other evidence published in formally peer-reviewed, indexed, well-recognized medical journals including the *Annals of Plastic Surgery*.[17–23]

Most of the observations reported relate to various in vitro comparisons of the Osiris "living" tissue versus the "nonviable" dHACM tissue. It would seem rather obvious and a spurious study design to subject these very different tissue grafts to various stimuli that would potentially elicit a response of some sort from viable tissue and then conclude unsurprisingly that the nonviable tissue did not respond to stimuli as well. This conclusion could have been made without a study, and this challenges the relevance of the entire data set. Interestingly, Osiris[24] acknowledges the issues with live cell preservation to the extent it has introduced its own lyophilized product with its "Prestige Lyotechnology."

Whereas Grafix may be dependent on the presence of live cells to promote healing, the mechanism of action by which EpiFix promotes healing is independent of the need for viable cells. Upon implantation, EpiFix delivers a diverse array of vital bioactive factors into the wound, and as EpiFix matrices are remodeled within the wound environment, additional matrix bound growth factors are continually released from the tissue. These factors recruit endogenous cells including reparative stem cells into the wound environment and stimulate cells to proliferate and secrete factors to reset the wound healing response by endogenous cells.[8,9] Osiris, however, has not demonstrated these same bioactive mechanisms with Grafix, a single layer amnion product.

All in all, the article by Johnson et al[1] is disappointing in that it clearly presents an industry-sponsored bias, is devoid of appropriate literature references, and implies conclusions from the work done that are not supported in actual clinical trials.

We are surprised that the article made it through the *Annals* review process.

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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