Title
Reply: Improving In Vitro Cartilage Generation by Co-Culturing Adipose-Derived Stem Cells and Chondrocytes on an Allograft Adipose Matrix Framework

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content of allograft adipose matrix should be quantitatively analyzed in this article. Previous studies[3] have suggested the criterion for acellular matrix:DNA content ratio is less than 50 ng/mg dry weight. This standard may be one of the most important for the application of biological materials, because high concentrations of DNA can hamper the growth and differentiation of stem cells.

Finally, we are curious as to why the ratios of auricular chondrocytes to adipose-derived stem cells in this article were 1:5 and 1:9. Does a higher concentration of adipose-derived stem cells promote or inhibit the osteogenic potential of auricular chondrocytes? Maybe more experimental groups with different concentration gradients should have been reported in this article to enhance its seriousness.

In conclusion, the authors' efforts confirm that allograft adipose matrix, as a new biological scaffold material, could provide a good attachment point for cartilage osteogenic differentiation. We are looking forward to seeing the authors' further research to clarify the underlying mechanism of that differentiation function on allograft adipose matrix. The prospect of clinical application in the future is encouraging.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this communication.

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Reply: Improving In Vitro Cartilage Generation by Co-Culturing Adipose-Derived Stem Cells and Chondrocytes on an Allograft Adipose Matrix Framework

We would like to thank Dr. Hu et al. for their interest in our recent publication.[1] Here, we hope to address the questions raised by the authors and to also contribute to helping the readers better understand the implications of our study.

First, we acknowledge that full-thickness skin grafts are common in clinical practice and could be utilized to obtain allograft adipose matrix. However, the amount of allograft adipose matrix that would be obtained from this type of living tissue would be very limited. The use of cadaveric donor tissue overcomes this limitation. The cadaver tissue obtained in our study came from the back and upper leg regions and was separated by centrifugation, and about 40 percent of that was the adipose fraction. This fraction was separated out to continue through the decellularization process. On average, from a 5- to 7-kg starting weight of unprocessed adipose, the process yields 10.5 to 14.7 g of allograft adipose matrix. Thus, given these parameters, for allograft adipose matrix to be utilized for tissue engineering, cadaveric donors are the best option.

Second, the allograft adipose matrix used in our study was provided by MTF Biologics (Edison, N.J.) and has been characterized extensively after preparation.[2,3] We agree that properly prepared allograft adipose matrix should maintain the key protein components and allow for cell growth when used as a scaffold. Importantly, key adipose tissue collagens remain in the allograft adipose matrix after it has undergone the chemical treatment for delipidation, decellularization and microbial reduction, including collagens I, III, IV, and VI. Furthermore, the preparation of allograft adipose matrix also preserves the native growth factors needed to support adipose tissue growth. In addition, when allograft adipose matrix is seeded with adipose-derived stem cells, they undergo adipogenesis.[3] Moreover, the residual chemicals were previously quantified and found to be very low, and this processing method also passed the International Organization for Standardization 10993 biocompatibility test panel.[4] With regard to sterilization, the allograft adipose matrix was processed in an aseptic environment in International Organization for Standardization level 5 and level 4 cleanrooms. The allograft adipose matrix process also includes a validated microbial reduction step.[5] The sterility of the tissue was verified using the USP 71 Sterility Test guidelines (Nelson Laboratories, Salt Lake City, Utah). This tissue is considered "shelf-ready," and a version of the allograft adipose matrix similar to the one used in this study is currently commercially available (Renuva; MTF Biologics) and has been used in clinical studies.[3,6–8]

Third, it is absolutely essential to verify that the allograft adipose matrix has been properly decellularized. To ensure this, the efficacy of the decellularization

process was previously evaluated using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, Calif.) assay to quantify the residual DNA (threshold, <18 ng/mg of dry allograft adipose matrix).[9] Thus, we were assured that there was not enough residual DNA to hamper the growth of the adipose-derived stem cells or the chondrocytes when we seeded them on the allograft adipose matrix.

Finally, when examining the ratio of adipose-derived stem cells to chondrocytes, in our initial studies, we also examined 1:3 and 1:12 (chondrocytes:adipose-derived stem cells). However, it was only the 1:5 and 1:9 ratios that showed induction of chondrogenesis in this setting. Thus, we pursued these ratios when we conducted the subsequent experiments. As presented in the study, the 1:9 ratio proved to be better. In the future, we plan to improve on the study design by reformatting the allograft adipose matrix scaffold to better support the cells by refining the porosity via three-dimensional printing. At that time, we will determine whether the 1:9 ratio is still optimal.

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DISCLOSURE

Dr. Widgerow is chief medical officer of Alastin SkinCare, Inc., and receives royalties on technology from Skin Medica. Dr. Banyard is a paid consultant for Recros Medica, Inc. Neither author has any conflict of interest with regard to this study. The remaining authors have no financial interests to report.

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Hyperbaric Oxygen Therapy in Management of Diabetic Foot Ulcers: Indocyanine Green Angiography May Be Used as a Biomarker to Analyze Perfusion and Predict Response to Treatment

We read with great interest the article by Hajhosseini et al.[1] entitled "Hyperbaric Oxygen Therapy in Management of Diabetic Foot Ulcers: Indocyanine Green Angiography May Be Used as a Biomarker to Analyze Perfusion and Predict Response to Treatment," published in the January 2021 issue of the *Journal*. We highly appreciate the authors for such an innovative and novel idea of using indocyanine green angiography as a biomarker for analyzing perfusion and predicting the response to hyperbaric oxygen therapy. As the authors mention the controversies in the efficacy of hyperbaric oxygen therapy in the diabetic foot, we are sure that such promising results of hyperbaric oxygen therapy and the use of indocyanine green angiography will inspire and motivate many young research minds like ours on the same path.

As we read the article, we found that a better explanation is required for a few things in order for readers to have a better understanding.

First, the title mentions the application of hyperbaric oxygen therapy in the management of diabetic foot ulcers, although the inclusion criteria included soft-tissue radionecrosis wounds as well. The rationale