of inguinal fat and fat injection. The authors injected fat particles with a 19-gauge needle. We usually use a 16-gauge rather than a 19-gauge needle based on the following considerations: on the one hand, the fine needle produces high shear force, which impairs graft viability. On the other hand, the fat particles need to be cut smaller to pass through the fine needle, leading to long extracorporeal ischemia time.

**DISCLOSURE**

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

**REFERENCES**


**Reply: Supplementation with Extracellular Vesicles Derived from Adipose-Derived Stem Cells Increases Fat Graft Survival and Browning in Mice: A Cell-Free Approach to Construct Beige Fat from White Fat Grafting**

**Sir:**

We thank Drs. Zhao and Sun for their insightful comments on our article entitled “Supplementation with Extracellular Vesicles Derived from Adipose-Derived Stem Cells Increases Fat Graft Survival and Browning in Mice: A Cell-Free Approach to Construct Beige Fat from White Fat Grafting.”1 Zhao and Sun ask for detailed information about this study as follows: (1) How are the dose and frequency of extracellular vesicle injection determined? (2) Why use extracellular vesicle–derived human lipoaspirates rather than adipose tissue from the mice?

As described in our article, the protein concentration of adipose-derived stem cell–derived extracellular vesicle suspension was measured by the BCA Protein Assay Kit (Sigma, San Jose, Calif.). We co-grafted free fat and adipose-derived stem cell–derived extracellular vesicles at doses of 50, 100, 150 μg/ml in the preexperiment. However, the survival of fat graft showed a dose-dependent increase between 50 and 100 μg/ml and no statistical difference between 100 and 150 μg/ml. Therefore, the suspension of extracellular vesicles at the dose of 100 μg/ml was prepared for this study. In addition, we believe that it is limited to screen the optimal dose of adipose-derived stem cell–derived extracellular vesicles by means of co-culture with a single type of cell in vitro as a reference for in vivo experiments, because the uptake rate of extracellular vesicles by target cells may be decreased because of the multicellular microenvironment in vivo.

We co-grafted PKH26-labeled extracellular vesicles and free fat, then observed that the expression of red fluorescence gradually decreased from postoperative day 3, and almost disappeared on postoperative day 5. Of note, extracellular vesicles are not a type of nanoparticle composed entirely of proteins; they carry a large amount of genetic material, such as noncoding RNAs, these RNAs may be involved in cell activity by interfering with signal pathways. The action time of these genetic materials should be confirmed by a series of complex in vitro experiments, and cannot be evaluated just by fluorescence tracing. The supplement of adipose-derived stem cell–derived extracellular vesicles once per week is a design for preliminary exploration. The specific action time of adipose-derived stem cell–derived extracellular vesicles should be closely related to which type of cells they target, the microenvironment in which the cells are located, and which biological activities they regulate.

The murine model in our study is not an animal model with immunosuppression. One hundred fifty milligrams of inguinal fat was weighed and then cut it into injectable pieces and autologously grafted into the scalp. The adipose-derived stem cell–derived extracellular vesicles we used in this study were derived from human lipoaspirates. The study of human adipose-derived stem cell–derived extracellular vesicles is of more clinical significance than that of mice. Extracellular vesicles have been demonstrated as a low-immunogenic cell secretion that can be allogeneic or xenogeneic. There are several studies that use human extracellular vesicles for xenogeneic application.2,5,6 So far, there have been studies that explore the effect of human liposuction-derived adipose-derived stem cell–derived extracellular vesicles on fat grafting in nude mice.5–8 Thus, we first studied the effect of xenogeneic application of human liposuction-derived adipose-derived stem cell–derived extracellular vesicles on fat...
grafting in a mouse model with immunosuppression. After repeated adipose-derived stem cell–derived extracellular vesicle supplementation, we did not observe the death of mice or any events that were not conducive to graft survival. We preliminarily consider xenogeneic local injection of adipose-derived stem cell–derived extracellular vesicles as a safe method in a mouse model with immunosuppression, and propose the possibility of allogeneic application of extracellular vesicles in the clinic. Compared with stem cell therapy, the main advantage of clinical application of stem cell–derived extracellular vesicles is its lower immunogenicity (no nucleus) that is more suitable to develop into an allogeneic biological agent, in addition to easier cryopreservation and transportation.

We agree with the view of Zhao and Sun that the grafted fat pad is damaged more severely when cut into smaller pieces to pass through a fine needle. In the follow-up experiments, we will consider grafting free fat with a 16-gauge needle in the murine model. In addition, according to a recent study performed by Wang et al., more macrophages will be recruited in the donor site than in the recipient site after autologous fat grafting. Therefore, in the mouse model of autologous inguinal fat grafting we used in this study, tissue repair at the donor site may lead to decreased infiltration of macrophages in the grafted area and ultimately decrease the survival rate of the grafts, compared with human fat xenografting in the nude mouse model.

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REFERENCES

Supplementation with Extracellular Vesicles Derived from Adipose-Derived Stem Cells Increases Fat Graft Survival and Browning in Mice: A Cell-Free Approach to Construct Beige Fat from White Fat Grafting

Sir:

We have read with great interest the study entitled “Supplementation with Extracellular Vesicles Derived from Adipose-Derived Stem Cells Increases Fat Graft Survival and Browning in Mice: A Cell-Free Approach to Construct Beige Fat from White Fat Grafting.” In this article, Zhu et al. seek to demonstrate the value of microvesicles derived from adipose-derived stromal cells. They conclude that these microvesicles increase the survival rate of fat grafting and promote the regeneration of beige adipose tissue.

As the authors make very clear, the main disadvantage of lipofilling is the fat resorption rate, which can reach 20 to 70 percent of the initial transfer. Many current methods have been examined to try to lower this rate with varying degrees of success, including addition of adipose-derived stromal cells (cell-assisted lipotransfer), platelet-rich-plasma, and various growth factors (e.g., vascular endothelial growth factor, erythropoietin). The effectiveness of adipose-derived stromal cells lies essentially in their paracrine mode of action through a variety of pathways: cell-cell