Orbital Volume Augmentation After Injection of Human Orbital Adipose-Derived Stem Cells in Rabbits

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Purpose. This randomized, controlled animal study investigated the morphologic and histologic properties of rabbit orbital fat following injection of human orbital adipose-derived stem cells (hoADSCs).

Methods. The efficacy of hoADSCs was compared to that of hyaluronic acid gel (HAG) and human orbital stromal vascular fraction (hoSVF). A total of 30 orbits from 15 New Zealand white rabbits (25 weeks postnatal, 2500–3000 g) underwent injection with HAG (molecular weight [MW] 1,000,000, 0.5 mL, $n = 10$, HAG only), hoSVF (0.25 mL) mixed with HAG (0.25 mL, $n = 10$, HAG + hoSVF), or hoADSCs (0.25 mL) mixed with HAG (0.25 mL, $n = 10$, HAG + hoADSCs). The degree of proptosis, and the time course of changes were determined and compared among groups.

Results. The difference between the initial exophthalmometric value and that at 4 weeks after injection was 1.77 mm for HAG only and 2.01 mm for HAG + hoSVF. The difference between the initial value and that at 12 weeks decreased to 0.05 mm for HAG only and 0.24 mm for HAG + hoSVF. In contrast, injection of HAG + hoADSCs increased the exophthalmometric value by 2.43 mm at 4 weeks after injection, and this difference was maintained at 2.56 mm at 12 weeks. Histopathologic examination revealed specific inflammation around the injection materials at 4 weeks after injection, and inflammation subsided 8 weeks after injection in all three groups.

Conclusions. Thus, transplantation of hoADSCs with HAG is a safe and effective technique for orbital fat volume expansion. This is a new and promising method for orbital reconstruction and aesthetic orbital volume augmentation.

Keywords: adipose-derived stem cells, exophthalmos, stromal vascular fraction

Stem cell application has been suggested as an alternative tissue engineering technique for generating functional fatty tissue, because stem cells can self-renew and become a source of regenerating adipocytes. Although embryonic stem cells are capable of differentiating into all cell lineages, including fat cells, the use of embryonic stem cells for adipose tissue engineering presents a risk for teratoma development and difficulty in regulating the differentiation pathways with current methods.[1] Ideally, stem cells for regenerative medicinal applications should be available in abundant quantities (millions to billions of cells), and easily collected and harvested by a minimally invasive procedure. Moreover, it should be possible to transplant the stem cells safely and effectively to an autologous or allogeneic host.[2,3]

Postnatal or adult stem cells are not totipotent, but rather pluripotent. Although these cells retain a broad differentiation potential, their developmental potential is more restricted than that of embryonic stem cells. The differentiation capacity of adult stem cells initially was thought to be limited to their tissue of origin; however, recent studies have demonstrated that these stem cells have the capacity to differentiate into cells of mesodermal, endodermal, and ectodermal origins.[4–8]

Adult mesenchymal stem cells can be isolated from bone marrow and adipose tissue. With the increased incidence of orbital and eyelid surgery for cosmetic and functional reasons, orbital adipose tissue is abundant and readily obtained. Korn et al. demonstrated the successful differentiation of neuronal stem cells from orbital fat and identified a novel population of adult stem-like cells in human orbital fat. The orbital fat cell, thus, is thought to possess pluripotent capabilities in vitro.[9]

Here, we investigated whether adult orbital adipose stem cells could be used for adipose tissue engineering and whether they can be applied for orbital tissue expansion. The goal of our study was to assess the safety and tolerability of human orbital adipose-derived stem cells (hoADSCs) in rabbit orbits, including teratoma formation and immune reaction. Moreover, the potency and efficacy of hoADSCs and human orbital stromal vascular fraction (hoSVF) were evaluated, and compared to those of hyaluronic acid gel (HAG), which currently is the conventional treatment for volume expansion in human orbits.

Materials and Methods

Isolation and Culture of hoADSCs

Adipose tissue consists predominantly of adipocytes, adipose-derived stem cells (ADSCs), vascular endothelial cells, peri-

FIGURE 1. Stromal vascular fraction (SVF) isolation method. Orbital adipose tissue was obtained during blepharoplasty procedures, and was digested with collagenase to release individual adipocytes and associated cell types. The cell suspension was filtered, and the SVF was collected after centrifugation.

cytes, fibroblasts, macrophages, and extracellular matrix.[10,11] ADSCs were isolated from adipose tissue by a previously described method.[12] The study was conducted in accordance with the tenets of the Declaration of Helsinki. In brief, orbital adipose tissue obtained by a fat debulking procedure during an upper or lower blepharoplasty operation was microdissected into small fat pearls, which were washed in PBS to remove erythrocytes, digested with type I collagenase, filtered, and suspended. The stromal vascular fraction, which represents a heterogeneous cell population that includes ADSCs,[13] was separated from mature lipid-laden adipocytes by centrifugation (Fig. 1). A large number of ADSCs can be harvested in this manner, with yields of approximately 250,000 cells per gram of tissue.[14]

It generally is recognized that there are no specific markers readily available to identify nonhematopoietic stem cells, and ADSCs do not exhibit hematopoietic stem cell markers, such as CD45 and CD14. However, ADSCs do display a marker surface profile similar to that of marrow-derived mesenchymal stem cells.[15,16] ADSCs express the stromal markers CD9, CD10, CD29, CD44, CD73, CD90, and CD166. With an increasing number of passages, the expression of these markers is elevated, whereas the presence of hematopoietic markers declines.[17,18] CD34 is a cell-surface marker expressed by ADSCs and endothelial cells, but ADSCs do not express CD31. Kishi et al. discovered that in human cadavers, fat tissues harvested from the thoracic back and lower abdomen had the highest concentration of CD34(+)/CD31(–) ADSCs.[1,19] In our study, the cell markers CD31, CD34, CD45, CD73, CD90, CD105, and CD146 were used to identify ADSCs.

The hoADSCs were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C in an incubator with 5% CO_2. The cultured cells were analyzed by fluorescence-activated cell sorting (FACScalibur flow cytometer; BD Biosciences, San Jose, CA), and assays for cell adherence and self-assembly were performed (Fig. 2).

FIGURE 3. Retrobulbar injection was performed under intramuscular anesthesia.

Stem Cell Injection

In this randomized, controlled animal study, 30 orbits of 15 New Zealand white rabbits (25 weeks postnatal, 2500–3000 g) were injected with HAG (cross linked, 1,000 μm/1 mL, 20 mg/mL; 0.5 mL, $n = 10$, HAG only), hoSVF (0.25 mL) mixed with HAG (0.25 mL, $n = 10$, HAG + hoSVF), or hoADSCs (0.25 mL) mixed with HAG (0.25 mL, $n = 10$, HAG + hoADSCs, Fig. 3). The study was conducted in accordance with compliance with the ARVO Animal Statement for the Use of Animals in Ophthalmic and Vision Research. We hypothesized HAG absorbed at regular rate at all rabbits. We didn't think HAG affects the survival of the stem cells. HAG was used for controls. Retrobulbar injections were performed in rabbits anesthetized with an intramuscular injection of tiletamine with zolazepam (Zoletil, 12 mg/100 g; Virbac, Carros, France) and xylazine HCl (Rumpun, 3.7 mg/100 g; Bayer, Seoul, Korea). Immediately after injection of HAG, HAG + hoSVF, or HAG + hoADSCs, gentamicin (Gentamycin, 2.5 mg/kg; Daesung, Seoul, Korea) was injected once intramuscularly for prevention

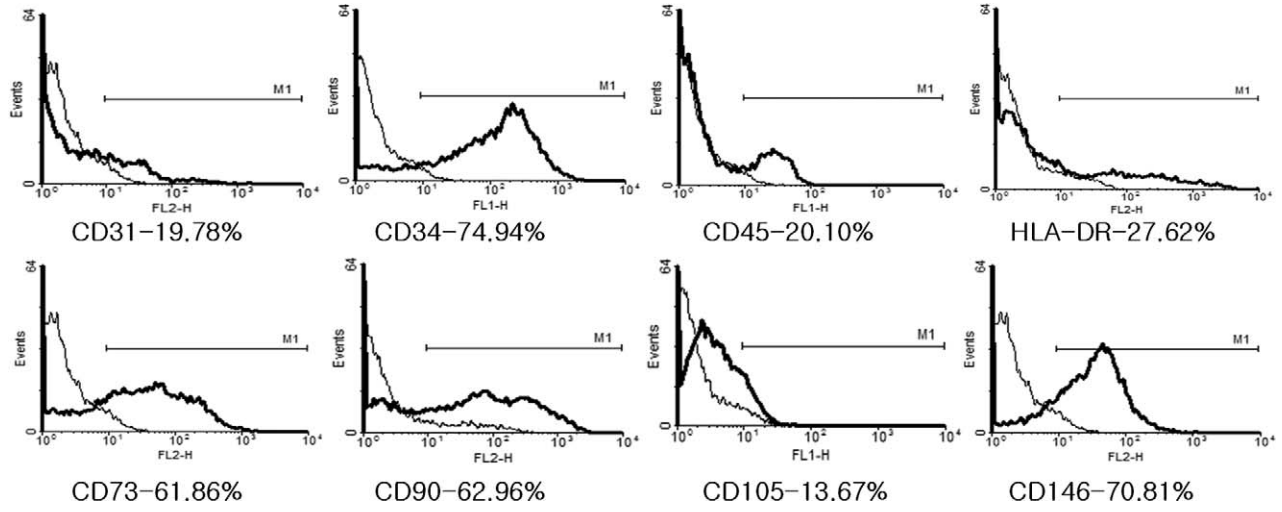

FIGURE 2. Adipose-derived stem cells were analyzed by fluorescence-activated cell sorting (FACS).

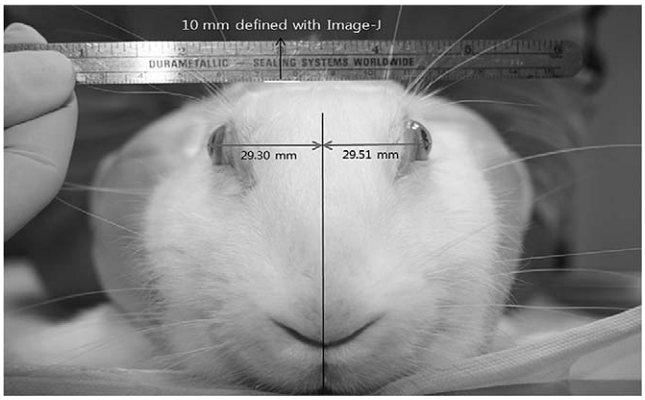

FIGURE 4. The degree of exophthalmos was evaluated. The exophthalmometric value was measured as the distance of the corneal vertex from the midline of the front of the head, using the Image J program.

of bacterial infection. Moreover, an immunosuppression regimen of intramuscularly injected cyclosporine A (Cipol, 6 mg/kg/day; Chongkundang, Seoul, Korea), methylprednisolone (Salon, 1.5 mg/kg/day; Hanlim, Seoul, Korea), and cyclophosphamide (Endoxan, 20 mg/kg/day; Baxter, Munich, Germany) was started, and it was continued until the animals were sacrificed, except that after 1 week, methylprednisolone was tapered to half dose. To prevent viral infection, ganciclovir (Cymevene, 2.5 mg/kg/day; Roche, Seoul, Korea) was administered by intramuscular injection after the procedure and was continued for 1 week because immunocompromised hosts are vulnerable to viral infection.

Measurement of Exophthalmometric Value

At 2, 4, 8, and 12 weeks after injection, pictures were taken of coronal, sagittal, axial, and lateral views of the rabbit eyes, using a Canon EOS 400D camera with a zoom lens (EF-S18-55 mm, f/3.5–5.6 SI II, Ø58 mm; Canon, Tokyo, Japan). The degree of exophthalmos was determined from the digital images. The distance of the corneal vertex from the midline of the front of the head was measured and evaluated using the Image J program (NIH, Bethesda, MD, Fig. 4).

Follow-Up and Euthanasia

At 4, 6, 8, and 12 weeks after injection, two rabbits were humanely euthanized. Orbital exenteration was performed for pathologic examination of the orbital tissue. Coronal slices (5 μm) were cut and fixed in 4% paraformaldehyde. Paraffin sections were stained with hematoxylin and eosin to define the general morphology and composition of the periorbital tissue. Immunohistochemical staining was performed on 5 μm sections cut from formalin-fixed, paraffin-embedded tissue using anti-human nuclei and chromosomes, histone H1 protein antibody (mouse monoclonal, clone 1415-1, 1:200 dilutions; Milipore, Billerica, MA). The visualization system used was the BenchMark XT (Ventana, Tucson, AZ) with heat-induced epitope retrieval (CC1 solution; Ventana) and the ultraView Universal DAB detection kit (Ventana).

Statistical Analysis

Values are expressed as means ± SEM. Differences between groups were evaluated using the Kruskal-Wallis test. A value of $P < 0.05$ was considered to indicate statistical significance. All statistical analyses were performed using the SPSS Statistics 17.0 package (SPSS, Inc., Chicago, IL).

RESULTS

Retrobulbar injections of HAG + hoADSCs, HAG + hoSVF, and HAG only were performed safely and without any complications, such as retrobulbar hemorrhage or gross ocular injury. Two of the 30 rabbits died of unknown causes after 2 weeks. Stress during the procedure or an infection were potential causes of the deaths. The remaining 28 rabbits completed the study without any infection, or orbital or systemic complications.

The preinjection baseline exophthalmometric values were 24.87 ± 1.25, 24.79 ± 1.22, and 25.67 ± 1.59 mm in the HAG only, HAG + hoSVF, and HAG + hoADSCs groups, respectively, and those were not significantly different ($P > 0.05$ for each pairwise comparison). At 4 weeks after injection, the exophthalmometric values showed increases of 1.77 ± 0.53 and 2.01 ± 0.66 mm in the HAG only and HAG + hoSVF groups, respectively, compared to baseline values. However, at 12 weeks after injection, the difference in the exophthalmo-

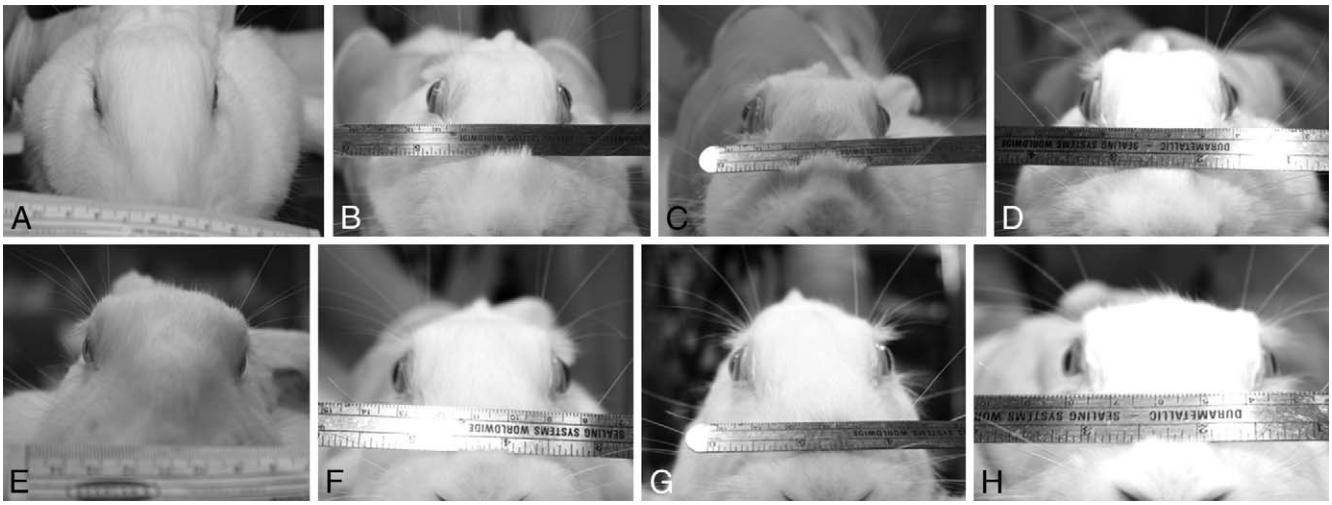

FIGURE 5. Ocular injection of HAG, hoSVF, or hoADSCs in New Zealand white rabbits. (**A–D**) HAG (0.5 mL) in the right eye and hoSVF (0.25 mL) mixed with HAG (0.25 mL) in the left eye. (**E–H**) HAG (0.5 mL) in the right eye and hoADSCs (0.25 mL) mixed with HAG (0.25 mL) in the left eye. (**A**, **E**) Before injection. (**B**, **F**) 2 weeks after injection. (**C**, **G**) 4 weeks after injection. (**D**, **H**) 12 weeks after injection.

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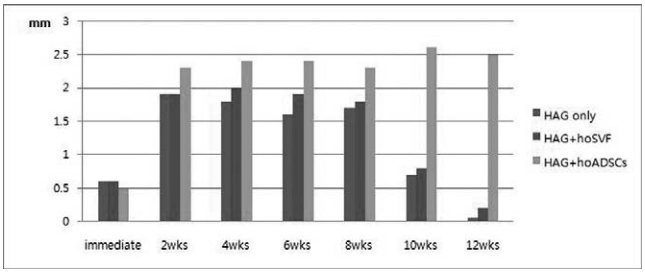

Figure 6. Change in the exophthalmometric value in rabbits after injection with HAG only, HAG + hoSVF, or HAG + hoADSCs. The exophthalmometric values increased during the first 4 weeks after injection in all groups, but increased progressively and remained elevated only in the HAG + hoADSCs group.

metric value compared to baseline had decreased to 0.05 mm in the HAG only group and to 0.24 mm in the HAG + hoSVF group. In contrast, in the HAG + hoADSCs group, the difference in the exophthalmometric value was 2.43 ± 0.75 mm at 4 weeks after injection compared to the baseline value, and it increased gradually to 2.51 ± 0.76 mm by 12 weeks. Proptosis after injection was observed in all three groups, with the greatest increase seen in the HAG + hoADSCs group (Figs. 5, 6).

The histologic changes after injection were examined. At 4 weeks after injection, moderate inflammation was observed around the injection site in the HAG only, HAG + hoADSCs, and HAG + hoSVF groups (Fig. 7). However, this inflammation had subsided by 8 weeks after injection in all three groups. Intraorbital inflammation had subsided, and fibrosis with collagen formation was observed at 6 and 8 weeks after injection in the HAG + hoSVF and HAG + hoADSCs groups, respectively. The inflammation had disappeared and mature fibrosis was noted at 12 weeks after injection. Thus, volume augmentation was achieved, and no teratoma formation was observed in the rabbit orbits.

At 12 weeks after injection, we could confirm immunohistologically the presence of the survived human adipose stem cells using anti-human nuclei and chromosomes, histone H1 protein antibody in the HAG + hoADSCs and HAG + hoSVF groups. Strong stained dark brown nuclei in contrast to the rabbit fibroblasts with negative stained blue nuclei was manifested. There was persistence of these cells at 12 weeks after injection in the HAG + hoSVF and HAG + hoADSCs groups regarding the tracking of human origin adipose-derived stem cells (Fig. 8).

Discussion

Orbital volume deficiency most often is characterized by the sunken appearance of an ocular prosthesis and a deepened upper eyelid sulcus. The recommended treatment for anophthalmic sockets has been the insertion of alloplastic implants into the orbit. Further orbital volume deficiency can be corrected by replacing the existing implant with a larger orbital implant or by using various other alloplastic materials (e.g., porous polyethylene, hydroxyapatite, or silicone blocks/gels) or autogenous materials (e.g., fat).[20] As all of these options for enophthalmic patients involve surgery, soft tissue fillers, such as hyaluronic acid, were developed and introduced to provide a nonsurgical option for facial rejuvenation.

Zamani et al. reviewed the clinical, photographic and radiologic records of 16 patients with anophthalmic or enophthalmic orbits who underwent volume augmentation by HAG injection into the muscle cone and extraconal posterior orbit. All patients had marked volume replacement on initial examination, most were satisfied with the initial results, and more than 80% had retained most of their volume enhancement after 6 months. It is important to note that none of these patients had added risks for visual loss, ocular motility disturbance, or other problems with the injections associated with a seeing eye.[21] Although the effect of the HAG filler was not sustained for long after its absorption, its advantages

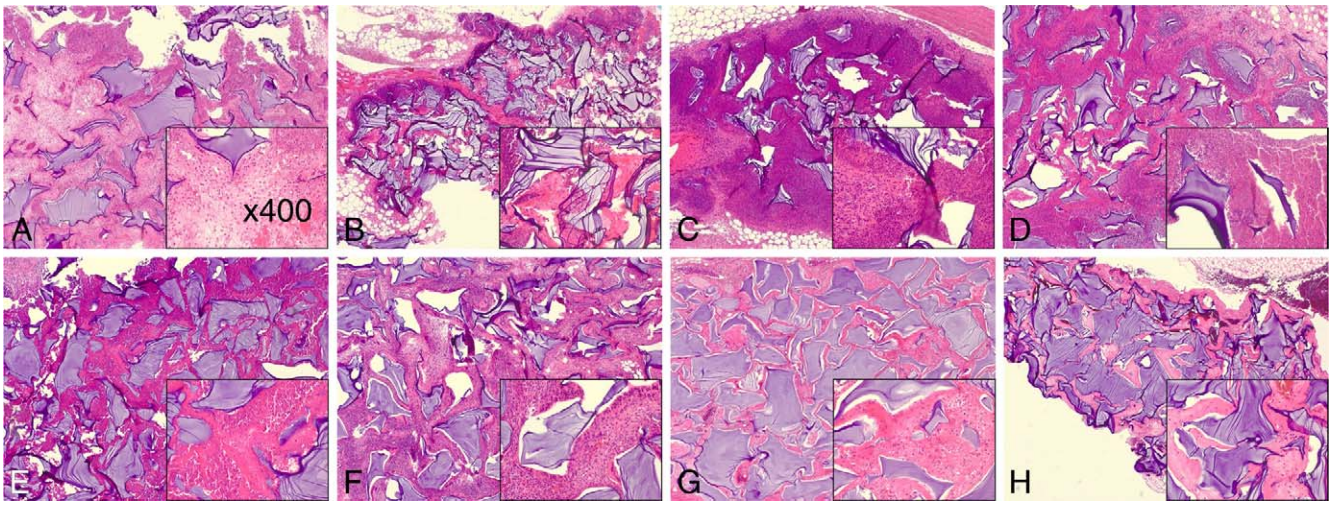

Figure 7. Sequential histologic findings in adipose tissue after injection with HAG only, HAG + hoSVF, or HAG + hoADSCs using hematoxylin and eosin staining. (**A**) Four weeks after HAG only injection, subacute severe inflammation is seen in the rabbit orbital fat. Injected HAG is seen as a basophilic molecule. (**B**) At 4 weeks after HAG + hoSVF injection, subacute moderate inflammation is seen. (**C**) At 4 weeks after HAG + hoADSCs injection, severe inflammation is seen in (**A**) and (**B**). HAG molecules are visible between the orbital adipose tissue of the rabbit. (**D**) At 6 weeks after HAG only injection, inflammation is moderate compared to that at 4 weeks (**A**). (**E**) At 6 weeks after HAG + hoSVF injection, fibrosis with collagen formation is seen, and inflammation is less than at 4 weeks (**B**). (**F**) At 8 weeks after HAG + hoADSCs injection, the findings are similar to those in (**E**). (**G**) At 12 weeks after HAG + hoSVF injection, inflammation has disappeared, and mature fibrosis is observed. (**H**) At 12 weeks after HAG + hoADSCs injection, inflammation has disappeared, and fibrosis is observed. Thus, volume augmentation was achieved, and no tumor formation was observed in the rabbit orbits. All images are ×100 magnification.

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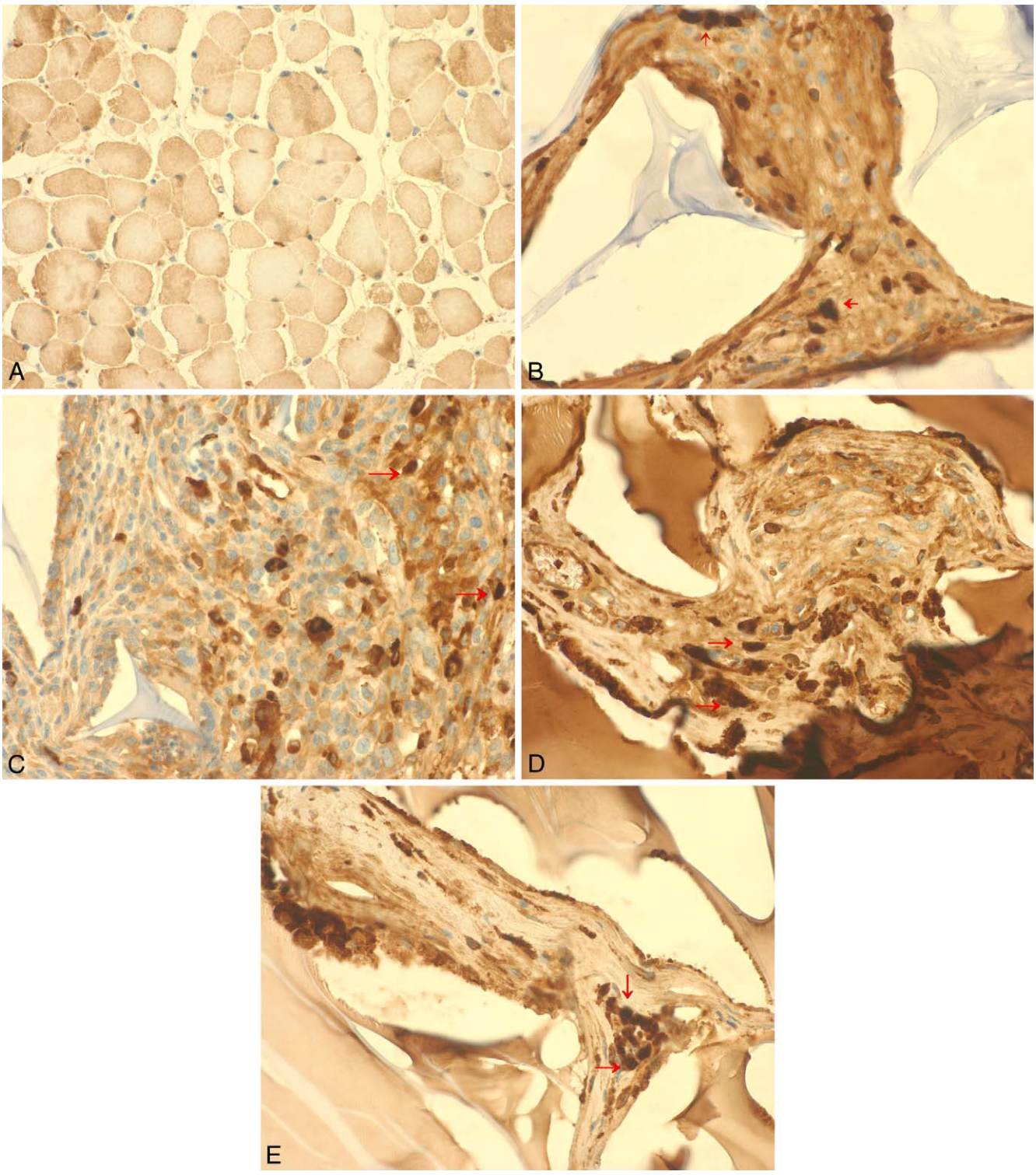

FIGURE 8. Immunohistochemical findings in adipose tissue after injection of HAG + hoSVF, and HAG + hoADSCs on 5 μm sections cut from formalin-fixed, paraffin-embedded tissue using anti-human nuclei and chromosomes, histone H1 protein antibody (mouse monoclonal, clone 1415-1, 1:200 dilutions; Milipore). The visualization system used was the BenchMark XT (Ventana) with heat-induced epitope retrieval (CC1 solution; Ventana) and the ultraView Universal DAB detection kit (Ventana). Negative control of rabbit gluteus muscle (**A**). Immunohistologically, the presence of the survived human adipose stem cells was identified as strong stained *dark brown nuclei* (*red arrows*) in contrast to the rabbit fibroblasts with negative stained *blue nuclei* in the orbital tissue four weeks after injection of HAG + hoADSCs (**B**) and HAG + hoSVF (**C**). There was persistence of these cells (*red arrows*) at 12 weeks after injection in the HAG + hoADSCs group (**D**) and HAG + hoSVF group (**E**) regarding the tracking of human origin adipose-derived stem cells. All images are ×400 magnification.

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included longevity, biocompatibility, minimal adverse reactions, and low risk for migration.[22,23]

Another injectable alternative is autologous fat transfer as an intraconal, extraconal, or periorbital injection.[21] This technique has the advantages of no risks for rejection, sensitivity reactions, or nodules.[20] However, the duration of its effect was limited when it was injected in the face.[20,24]

In our study, HAG + hoADSCs were injected safely and produced satisfactory volume augmentation. During the first 4 weeks after injection, the increase in exophthalmos was similar among all three groups. However, owing to the absorption of hoSVF and HAG, the changes in exophthalmometric values differed significantly at 12 weeks: values increased by 2.51 ± 0.76 mm in the HAG + hoADSCs group versus 0.24 and 0.05 mm in the HAG + hoSVF and HAG only groups, respectively.

The hyaluronic acid gel used in our study was characterized as a cross linked type HAG, which was approximately 400 to 700 μm, 20 mg/mL. HAG was absorbed at a regular rate in all rabbits. So, the difference of measured exophthalmometric values compared to the HAG only group suggested the effect of hoADSCs. Regarding the role of scaffolds including HAG, to our knowledge it currently is completely unknown whether and under which conditions scaffolds containing human adipose stem cells, such as preadipocytes, develop or not into a stable and fully integrated soft tissue when autotransplanted in humans. It only is known from older experiments using several techniques that transplantation of fat tissue is not a suitable approach to reach the goal of augmentation and even is associated with severe complications.[25]

The aim of our project was to develop soft tissue filler that is able to serve several clinical needs, for example, the orbital volume augmentation after orbital fracture or orbital fat atrophy after tumor removal or enucleation. Our present results represent only the first step towards this ultimate target.

Rabbits tolerated the transplantation of hoADSCs well, without any signs of postoperative graft-versus-host inflammation, rejection, or tumorigenicity at the time of this report. Histopathologic examination confirmed that no teratoma formation, hyperproliferation of cells, or ectopic tissue formation had occurred.

Our findings suggest that the injection of hoADSCs is a safe and effective treatment modality for orbital volume augmentation. However, the limitations of our study should be considered. This experiment was performed in rabbits with an orbital contour within normal limits. To prove a corrective effect, rabbit models with enophthalmos are required. Moreover, there was the limitation of the measurement technique, such as differences in head positioning or ruler placement. However, we tried to make bias less by using pictures and the Image J program, which is a widely used method for analysis of measurable parameters in the picture.

In our study, the injected hoADSCs were identified using anti-human nuclei and chromosomes, histone H1 protein antibody in the orbital tissue at least 12 weeks after injection. However, it was unclear whether proptosis was induced solely by transplanted cell growth or through inflammatory volume expansion. Nevertheless, it was clear that the volume expansion was sustained after the inflammation subsided. To confirm the role of stem cells regarding the volume expansion, a long-term follow-up would better clarify the longevity of hoADSCs in the fibrotic period. An expanded study is needed with regard to the various types of HAG scaffolds, which can differ in particle size, density, and morphology, to investigate the relationship between scaffold type and stem cell differentiation.

Conclusion

We are encouraged by our favorable early results for volume augmentation with hoADSCs. Continued follow-up and further study are required. The ultimate therapeutic goal is to use hoADSCs injection successfully to treat patients with enophthalmos arising for various reasons.

Acknowledgments

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References

1. Ahn JM, Mao JJ. Adipose tissue engineering from adult human stem cells: a new concept. *Facial Plast Surg*. 2010;26:413–420.
2. Bunnell BA, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: isolation, expansion and differentiation. *Methods*. 2008;45:115–120.
3. Gimble JM. Adipose tissue-derived therapeutics. *Expert Opin Biol Ther*. 2003;3:705–713.
4. Beltrami AP, Urbanek K, Kajstura J, et al. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med*. 2001;344:1750–1757.
5. Gussoni E, Soneoka Y, Strickland CD, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature*. 1999;401:390–394.
6. Kotton DN, Fine A. Derivation of lung epithelium from bone marrow cells. *Cytotherapy*. 2003;5:169–173.
7. Petersen BE, Bowen WC, Patrene KD, et al. Bone marrow as a potential source of hepatic oval cells. *Science*. 1999;284:1168–1170.
8. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284:143–147.
9. Korn BS, Kikkawa DO, Hicok KC. Identification and characterization of adult stem cells from human orbital adipose tissue. *Ophthal Plast Reconstr Surg*. 2009;25:27–32.
10. Yoshimura K, Shigeura T, Matsumoto D, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol*. 2006;208:64–76.
11. Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. *Regen Med*. 2009;4:265–273.
12. Estes BT, Diekman BO, Gimble JM, Guilak F. Isolation of adipose derived stem cells and their induction to a chondrogenic phenotype. *Nat Prot*. 2010;5:1294–1311.
13. Guilak F, Estes BT, Diekman BO, Moutos FT, Gimble JM. Nicolas Andry Award: multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. *Clin Orthop Relat Res*. 2010;468:2530–2540.
14. Aust L, Devlin B, Foster SJ, et al. Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy*. 2004;6:7–14.
15. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. *J Cell Physiol*. 2001;189:54–63.
16. Wickham MQ, Erickson GR, Gimble JM, Vail TP, Guilak F. Multipotent stromal cells derived from the infrapatellar fat pad of the knee. *Clin Orthop Relat Res*. 2003;412:196–212.

17. McIntosh K, Zvonic S, Garrett S, et al. The immunogenicity of human adipose-derived cells: temporal changes in vitro. *Stem Cells*. 2006;24:1246–1253.
18. Mitchell JB, McIntosh K, Zvonic S, et al. Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells*. 2006; 24:376–385.
19. Kishi K, Imanishi N, Ohara H, et al. Distribution of adipose-derived stem cells in adipose tissues from human cadavers. *J Plast Reconstr Aesthet Surg*. 2010;63:1717–1722.
20. Hardy TG, Joshi N, Kelly MH. Orbital volume augmentation with autologous micro-fat grafts. *Ophthal Plast Reconstr Surg*. 2007;23:445–449.
21. Zamani M, Thyagarajan S, Olver JM. Adjunctive use of hyaluronic acid gel (restylane sub-q) in anophthalmic volume deficient sockets and phthisical eyes. *Ophthal Plast Reconstr Surg*. 2010;26:250–253.
22. Malhotra R. Deep orbital Sub-Q restylane (nonanimal stabilized hyaluronic acid) for orbital volume enhancement in sighted and anophthalmic orbits. *Arch Ophthalmol*. 2007;125:1623–1629.
23. Goldberg RA, Lee S, Jayasundera T, et al. Treatment of lower eyelid retraction by expansion of the lower eyelid with hyaluronic acid gel. *Ophthal Plast Reconstr Surg*. 2007;23: 343–348.
24. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg*. 1995;19:421–425.
25. Halbleib M, Skurk T, de Luca C, et al. Tissue engineering of white adipose tissue using hyaluronic acid-based scaffolds. I: in vitro differentiation of human adipocyte precursor cells on scaffolds. *Biomaterials*. 2003;24:3125–3132.

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