A pilot study of fat allograft transplantation in immunocompetent rabbits for potential neurosurgical applications

Laboratory investigation

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Object. The authors investigated the feasibility of using fat allografts (chemically treated to reduce the host immune response) for neurosurgical applications.

Methods. Subcutaneous fat specimens collected from New Zealand White rabbits were treated with DNase I and sodium deoxycholate to reduce immunogenicity before subcutaneous, midscapular implantation in immunocompetent recipient rabbits. Allograft incorporation and the host-allograft response were examined at 1, 6, and 11 weeks by histopathological analysis. Control specimens of autograft and untreated fat allograft implants were examined for comparison.

Results. The host immune response was markedly reduced in the region around the chemically treated fat allografts when compared with untreated allografts, and was similar to the tolerant host response to autografts.

Conclusions. Based on their results, the authors suggest that fat allografts processed for reduced immunogenicity may be a convenient, viable alternative for neurosurgical applications. (DOI: 10.3171/2009.10.JNS08259)

Key Words • cerebrospinal fluid leak • fat allograft • rabbit • transsphenoidal surgery • wound repair

This work is an initial feasibility study to explore the potential use of fat allografts for surgical applications such as CSF leak repair. Human fat allografts are readily available because undesired adipose tissue is frequently removed and discarded via liposuction and other cosmetic procedures. Successful tissue allograft transplantation requires suppression of the host immune response, either with an immunosuppressive drug regimen or by reducing or eliminating the immunogenic properties of tissue allografts. We demonstrate that after chemical processing to minimize immunogenicity by digestion of antigenic proteins and nucleic acids, fat allografts are successfully incorporated in recipient immunocompetent rabbits in a fashion comparable to that of autografts, and without significant immunological rejection.

Methods

Experimental Animals

These experiments were performed under an approved

Abbreviation used in this paper: HSC = Hospital for Sick Children.
animal utilization protocol of the Animal Care Committee at the HSC, University of Toronto. Eighteen adult New Zealand White rabbits weighing between 2.5 and 3.5 kg (Riemans Co.) were housed and monitored under standard conditions at the HSC Animal Care Facility. Four rabbits were implanted with fat allograft and killed within 1 week for acute-phase analysis; 4 were implanted with fat allograft and killed at 6 weeks for intermediate analysis; and 6 were implanted with fat allograft and killed at 11 weeks for chronic reaction analysis. As controls, 2 rabbits received either fat autograft directly transplanted from their midthoracic region to their midscapular “dewlap” region, or fat autograft implanted after 1 week of processing as described below. Only 2 additional rabbits received untreated fat allograft due to obvious discomfort, inflammation, and clearly obtained histological results.

**Surgical Procedures**

Following overnight fasting, rabbits were anesthetized with Akmezine premedication (ketamine, atravet, and atropine), induced with 3.5% halothane in a 2:1 O2/NO mixture, and then intraoperative anesthesia was maintained with 1.5% halothane. The animals also received prophylactic antibiotics (150,000 U penicillin G and 100 mg ceftazolin), pulse oximetry monitoring, and warming blankets until after awakening from surgeries. Fat grafts (1–1.5 g) were marked at 1 end with a 2-0 Prolene suture for orientation, and implanted in a subcutaneous midscapular pocket that was created to minimize wound manipulation and facilitate wound monitoring in recipient rabbits. Meticulous aseptic techniques were used for harvesting and implanting fat grafts. Wounds were copiously irrigated before closure in multiple layers, dressed with antibiotic ointment, and checked twice daily until well healed. On postoperative Day 2, rabbits were allowed to resume normal diets. No rabbits died due to surgical complications during this study. The rabbits were humanely killed for examination via ear vein injection of Euthanyl (Biomed-MTC Animal Health, Inc.).

**Fat Graft Processing**

Subcutaneous fat was harvested after planned death in 3 New Zealand White rabbits (from shoulder, flank, back, buttocks, and limb regions), divided into 20-g aliquots, and then rinsed in phosphate-buffered saline containing ampicillin, gentamicin, and amphotericin B before processing. The following treatment was designed to remove immunogenic cellular components.14 In brief, fat allografts were rinsed in distilled H2O for 72 hours at 4°C, followed by 3 applications of the following protocol: 4% sodium deoxycholate (Sigma) for 4 hours, then 2000 Kunitz units of DNase I (Roche) for 3 hours. Processed fat grafts were stored in distilled H2O plus antibiotics at 4°C and regularly monitored for infection or degradation. Untreated fat autografts were similarly rinsed and stored without undergoing chemical processing.

**Histopathological Analysis**

Rabbits were killed within 1 week, at 6 weeks, and at 11 weeks (6 animals at each interval, including controls) to examine the acute, chronic, and late effects on the implanted fat grafts and the host-graft interface by using histopathological analysis. The entire fat graft, along with a 0.5–1 cm specimen of surrounding host connective tissue, was excised in each rabbit for analysis.

**Results**

Surgical wounds in the recipient rabbits’ midscapular regions created by implanting chemically treated fat allografts were unaffected by the allograft’s enzymatic processing, and the wounds healed well over time, showing Grade 0–1 inflammation (Figs. 1 and 2). The allografts were observed as discrete implants at the end of postoperative Week 1, with no surrounding gross inflammation or necrosis—at most a Grade 1 inflammation (Fig. 1). By the end of Week 6, treated allografts were surrounded by fibrotic capsules, and at Week 11, they were incorporated by still distinguishable from the surrounding tissues by their yellow color (Fig. 2), showing Grade 0 inflammation at both extended time points. The degree of tissue inflammation was graded as follows: Grade 0 (no inflammation, mature fibrous tissue, presence of fibroblasts); Grade 1 (mild inflammation, macrophages or plasma cells present, immature fibrous tissue); Grade 2 (moderate inflammation, 30–60 macrophages or plasma cells or neutrophils, occasional leukocyte foci); or Grade 3 (severe inflammation, focal necrosis, dense leukocyte infiltrate, > 60 macrophages or plasma cells or neutrophils).5,17

In contrast, the wounds of control rabbits receiving unprocessed fat allografts were erythematous and inflamed by postoperative Day 3, and those rabbits were killed according to the approved animal use protocol and examined. The untreated fat allografts were surrounded by inflammatory and necrotic tissue that extended into and involved the adjacent fascia and muscle, exhibiting areas of moderate Grade 2 to severe Grade 3 inflammation (Fig. 3D–F). Control rabbits had well-healed wounds exhibiting only Grades 0 or 1 inflammation, and showed that untreated and processed autografts were incorporated in a fashion similar to processed allografts (data not shown).

The protocol used to process allografts reduces immunogenicity by creating an anuclear lipid matrix through digestion of nucleic acids and antigenic proteins (Fig. 3A). Implanted processed allografts only cause a slight acute inflammatory reaction and infiltration in small regions with a small number of macrophages and neutrophils at Week 1, and no reaction is observed in adjacent muscle (Fig. 3A–C). Treated allografts elicited a similarly mild acute host immune response as in control autografts. In contrast, an intense acute immune response was noted in the untreated allografts by Week 1, with the elicited acute host inflammatory response resulting in significant macrophage infiltration and damage to the adjacent recipient muscle (Fig. 3D–F).

When examined at 6 and 11 weeks postimplantation, processed allografts were grossly incorporated into the recipient sites, but were distinguishable from the surrounding recipient pale adipose tissue and muscle by the grafts’ yellow color. Untreated autografts showed fibroblast and vascular infiltration (Fig. 4A), whereas processed autografts were even more densely infiltrated, with dense replace-
ment of portions of the anuclear lipid matrix with fibroblasts and neovascular tissue (Fig. 4B–D). In comparison, the processed allografts had regions with incorporation, infiltration, and neovascularity similar to the processed autografts, with other peripheral regions bordering dense fibroblastic neovascular scar that did not infiltrate into the graft (Fig. 4E–G).

**Discussion**

In this study, we showed that a method of chemical processing to degrade nucleic acids and antigenic proteins creates a fat allograft consisting of an acellular lipid matrix with highly reduced immunogenicity. Processed fat allografts were well tolerated and incorporated into immunocompetent recipient animals without the use of drug immunosuppression, similarly to control fat autografts. Furthermore, the chemical processing of grafts may also result in more dense fibroblastic infiltration and neovascularization of implants. Recent reports of experimental models of fat harvest have shown that intact grafts, rather than adipose cells or liquefied adipose tissue, are better for wound healing.\(^9,18,25\) More work needs to be performed to investigate whether use of adipocytes and/or mesenchymal stem cells will optimize fat graft incorporation rather than resorption over time.\(^18,21\)

Wound repair using fat grafts is a time-proven method to help seal CSF leaks, and also for improving cosmesis in many surgical procedures.\(^11,12,24\) Harvesting autografts from another body site is usually well tolerated in most patients, but requires more surgery time, is associated with additional risks of complications, and may be limited by a patient’s adipose tissue reserves. In contrast, the use of artificial dural sealants has been significantly
associated with higher costs and multiple reported risks of wound infection and other complications.\textsuperscript{2,13,16}

Abundant adipose tissues are harvested and discarded via liposuction and other procedures. If a practical processing method can be developed for these allografts to remove infectious risks and eliminate their immunogenicity, “fat banks” would be a convenient and potentially inexpensive resource for use in repairing CSF leaks in neurosurgical procedures such as transsphenoidal craniotomies or other skull base approaches that require dural reconstruction and have a high risk of CSF fistula formation. Repair of large spinal dural defects would also be facilitated by “off-the-shelf” fat grafts, although autologous fat grafts are readily available in lumbar spine surgeries. Large volumes of processed fat allografts may also be used in sculpting and repairing cosmetic tissue defects, and may be useful to provide temporary coverage and serve as a lipophilic barrier in burn injuries. Future studies will also need to address the question of what amount of inflammation is beneficial for wound healing and CSF leak repair, versus whether excessive inflammation resulting from untreated allograft implants may be deleterious to wound healing.

Future investigations are needed to validate the use of this chemical processing method with human fat tissue, and to improve uniformity in the treatment and production of these low-immunogenic allografts. Different methods may need to be developed to create multiple formulations of the allografts (semisolid, gel, or malleable forms) to optimize their various surgical and cosmetic applications. There are ongoing studies on the optimal method for long-

![Fig. 3. A–C: Processed fat allografts cause only a slight inflammatory response in grafts and adjacent muscle during the 1st week after implantation. D–F: Control (untreated) fat allografts cause significant inflammatory response in grafts and adjacent muscle tissue during the 1st week after implantation. H & E, original magnification × 40 (A and B), × 100 (C and F), × 200 (D and E).](image)

![Fig. 4. A: Processed fat allografts incorporate into host tissue in a fashion similar to autografts after 6 and 11 weeks. Untreated autografts show fibroblast and vascular infiltration. B–D: Processed autografts show denser infiltration with fibroblasts and neovascular tissue. E–G: Processed allografts showed similar regional incorporation, infiltration, and neovascularity, with other peripheral regions bordering dense fibroblastic neovascular scar that did not infiltrate into the graft. H & E.](image)
Fat allograft pilot study

term fat graft storage. Clinical trials would be needed to examine the long-term persistence and efficacy of these processed fat allografts in specific clinical applications, such as CSF leak ablation.

Conclusions

Chemical processing to degrade nucleic acids and antigenic proteins creates a fat allograft consisting of an acellular lipid matrix with highly reduced immunogenicity. Processed fat allografts are tolerated and incorporated into immunocompetent recipient animals without the use of drug immunosuppression, and behave comparably to control fat autografts. Treated allografts may have many viable and convenient uses in neurosurgical applications, such as CSF leak repair.

Disclosure

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Author contributions to the study and manuscript preparation include the following: Conception and design: JS Kuo, MH Weiss. Acquisition of data: JS Kuo. Analysis and interpretation of data: JS Kuo, C Hawkins. Drafting the article: JS Kuo. Critically revising the article: JS Kuo, C Hawkins, JT Rutka, MH Weiss. Final approval of the article: JS Kuo, C Hawkins, JT Rutka, MH Weiss. Administrative/technical/material support: JT Rutka. Study supervision: JT Rutka, MH Weiss.

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References


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