Comparison of AlloDerm, Fat, Fascia, Cartilage, and Dermal Grafts in Rabbits

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Objective: To compare various graft materials in the rabbit model, including autologous cartilage, dermal tissue, fat, and AlloDerm (a cadaver-derived material).

Methods: Twenty-five New Zealand white rabbits were used. Equally sized autogenous (fat, fascia, cartilage, and dermal) grafts and AlloDerm were implanted into subcutaneous dorsal pockets on the rabbits. Animals were killed 1, 2, 3, and 4 months after surgery. The grafts were examined microscopically for thickness, resorption, fibrosis, neovascularization, inflammation, eosinophilia, and the presence of multinucleated giant cells or microcysts.

Results: The cartilage grafts revealed excellent viability with no resorption. The fascial grafts showed negligible volume loss. The dermal grafts developed epidermoid cysts. The AlloDerm grafts demonstrated graft thickening at 1 month and total resorption at 3 and 4 months. The fat grafts demonstrated 30% to 60% partial resorption.

Conclusions: The major disadvantage of using an autogenous fat graft was partial resorption, whereas cyst formation was observed with dermal grafts. AlloDerm caused tissue reaction and resorption. The best graft material was cartilage, with a low absorption rate, good biocompatibility, and minimal tissue reaction or fibrosis, followed by fascia, with a minimal shrinkage capacity and tissue reaction.


DORSAL CONTOUR IRREGULARITIES after various procedures, such as hump resection, are a major concern in patients who have undergone rhinoplasty. Dorsal onlay grafts may be used to mask these contour irregularities and to achieve a better aesthetic result. Several types of autografts, homografts, and allografts have been used for this purpose. The advantages of using autogenous grafts include excellent biocompatibility with minimal stimulation of the immune response and low infection and rejection risks compared with alloplastic and homologous materials. Autogenous grafts, including cartilage and various soft-tissue grafts, such as temporalis fascia and dermal grafts, have been commonly used to cover underlying dorsal irregularities. AlloDerm (LifeCell Corp, Branchburg, New Jersey), which is derived from cadaveric skin, is another material often used to achieve a smooth nasal dorsum. However, each material has disadvantages, including donor site morbidity and variable resorption risk, depending on its source. Autogenous cartilage is widely considered to be an ideal material for all kinds of grafting in rhinoplasty operations. This material survives as living tissue, does not stimulate an immune response, and seldom undergoes resorption. However, these characteristics are not shared by the other dorsal onlay graft materials, which include fat, fascia, dermis, and AlloDerm. The literature contains contradictory data about the resorption rates and the predictability of these graft choices. Temporalis fascia grafts have been reported to be excellent dorsal contour grafts and also excellent viable bulk, whereas shrinkage of fascia and the need for overcorrection have been reported by some researchers. Resorption rates of 20% to 90% have been reported by different authors for autografts of fat. Some authors advocate using dermal grafts owing to their limited postoperative absorption rates and stability, whereas others report resorption rates varying from 11% to total volume loss. Similarly, although AlloDerm has been suggested to be an ideal graft material for secondary rhinoplasty surgery to create a smooth dorsum with no resorption, partial graft resorption has been reported by some authors. As a result of this conflicting information and the differing resorption rates of these graft materials, most surgeons are hesitant to use these materials for contour restoration in rhinoplasty.

The purpose of this study is to compare the outcomes and absorption rates of
autologous cartilage, dermis, fat, fascia, and AlloDerm in a rabbit study. Clarifying graft resorption rates would be helpful in choosing better graft material and would help reduce controversy regarding its selection.

**METHODS**

Twenty-six New Zealand white rabbits, aged 15 to 18 months and weighing 2300 to 4100 kg, were used. One rabbit died before the end of the study and was thus excluded from all analyses. The ethics committee of Baskent University approved the study protocol, and all the procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.  

Each rabbit was given general anesthesia via an intramuscular injection of ketamine hydrochloride, 50 mg/kg, and xylazine hydrochloride, 5 mg/kg. The skin on the rabbit’s back and interscapular donor site was shaved, cleansed with povidone-iodine (Betadine; Purdue Pharma LP, Stamford, Connecticut) solution, and draped in a sterile manner. The same procedure was applied to the left ear to harvest the cartilage graft, and the perichondral layers were removed from all the cartilage grafts. The interscapular area was infiltrated with lidocaine hydrochloride, 1%, with 1:100 000 epinephrine solution. A 5-cm interscapular midline dorsal skin incision overlying the fat pad was made to take the fat, dermis, and thoracodorsal fascial grafts. Dermal grafts were harvested from the interscapular dorsal skin. The epidermal elements were removed from the surface of the graft using a No. 10 scalpel blade to achieve in situ de-epithelialization of the skin paddle. Care was taken to derm-abrade to the deep dermis to devitalize all the epithelial elements. All fat tissue was removed from the underlying dermis. The fat pad was dissected to harvest the fat graft. The fascial grafts were harvested from the thoracodorsal fascia of the rabbits and were prepared in multiple layers to have an approximate graft thickness of 1.5 mm. Grafts of acellular dermis (AlloDerm) (3 × 7 cm; 0.79–1.02 mm thick) were used. Each graft was rehydrated in isotonic sodium chloride 20 minutes before implantation. The graft materials (autogenous fat, fascia, cartilage, dermis, and AlloDerm) were prepared as 1 × 1-cm pieces (Figure 1).

Five skin incisions, approximately 1.5 cm long, were made in the recipient’s paraspinal region, and small subcutaneous pockets were created. The 5 grafts were implanted into the 5 subcutaneous pockets and were sutured using 5-0 polypropylene sutures (Prolene; Ethicon Inc, Somerville, New Jersey) on the dorsum, and the skin incisions were sutured using 4-0 polyglactin 910 sutures (Vicryl; Ethicon Inc). Each rabbit was given penicillin G, 40 000 IU/kg/d intramuscularly, for 1 week after the operation. There were no infections, seromas, or hematomas at the recipient sites during the postoperative period. Animals were killed by using lethal doses (150 mg/kg) of thiopenicil sodium 1 month (n=5), 2 months (n=4), 3 months (n=4), and 4 months (n=12) after surgery. The recipient sites were excised immediately after each animal was killed, and the specimens were fixed in 10% formaldehyde and processed routinely (Figure 2).

**HISTOPATHOLOGIC EXAMINATION**

Serial sections of each graft were stained with hematoxylin-cosin and Mason trichrome. All representative sections were examined histologically using a light microscope by a researcher (B.H.O.) blinded to the study variables for the degree of fibroblast proliferation, neovascularization, and inflammation. Also, the thickness and resorption rate of the grafts were determined using a microscopic grid. In addition, the presence of eosinophil infiltration, granulation tissue, and multinucleated giant cells was evaluated. Histopathologic findings for each group were recorded, and the findings in each group 1, 2, 3, and 4 months after implantation were compared (Figure 3).

**GRADES OF FIBROBLAST PROLIFERATION, INFLAMMATION, AND NEOVASCULARIZATION**

Fibroblast proliferation is graded as follows: F0, no fibroblast proliferation, normal collagen morphologic features; F1, mild fibroblast proliferation, mild irregularity of collagen bundles; and F2, moderate to severe fibroblast proliferation.

Inflammation is graded as follows: i0, no inflammation; i1, biopsy specimen with fewer than 25 inflammatory cells; and i2, biopsy specimen with 25 or more inflammatory cells.

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Figure 1. One set of 5 grafts (from left to right): autogenous cartilage, dermis, fat, fascia, and AlloDerm (LifeCell Corp, Branchburg, New Jersey). The upper rule is in inches; lower rule, centimeters.
The number of microvessels was evaluated by using microscopic grade, and the results of microvessel density analyses are given as the number of microvessels per unit of area. According to these results, 3 degrees of neovascularization are defined: V1, fewer than 25 microvessels per unit of area; V2, 25 to 50 microvessels per unit of area; and V3, more than 50 microvessels per unit of area.

STATISTICAL ANALYSES

Statistical analyses were performed using a software program (SPSS version 11.0; SPSS Inc, Chicago, Illinois). For quantitative variables, values are given as mean (SD). Mean values were calculated for each group and compared using the 1-sample t-test for normal distribution. The Kruskal-Wallis test was used to compare variables with normal distribution. Results were considered to be significant at $P < .05$.

RESULTS

MACROSCOPIC EXAMINATION

Cartilage Grafts

Cartilage grafts preserved their macroscopic appearance and volume and showed no significant changes at the different evaluation times during the 4 months.

AlloDerm Grafts

At 1 month, all the AlloDerm grafts were larger than their original size on macroscopic examination. However, at the end of the second postoperative month, AlloDerm grafts were smaller and were only barely palpable. At postoperative months 3 and 4, no visible or palpable grafts were found in the recipient sites.

Fascial Grafts

Fascial grafts did not show noticeable volume loss during the study in macroscopic evaluation after implantation.

Dermal Grafts

At each assessment time, the dermal grafts appeared as cystic masses, which were larger than their preoperative appearances. There were no significant differences in their appearance at 1, 2, 3, or 4 months.

Fat Grafts

At each assessment time, there was significant volume loss in all the fat grafts. Also, the fat grafts were inconsistent in shape, with surface irregularities noted.

HISTOPATHOLOGIC EXAMINATION

Cartilage Grafts

No significant differences were noted regarding cartilage graft thickness (Figure 3). The mean thickness of the cartilage graft was 1.8 mm at the first month and 2.0, 1.7, and 1.7 mm at the second, third, and fourth months, respectively. No significant resorption was seen in the cartilage grafts during these months ($P = .65$). The viability of all the cartilage grafts, as assessed histologically, appeared very good, with normal cartilage architecture and normal chondrocytes (Figure 4).

Only mild neovascularization and inflammation were evident in the cartilage grafts at the first month, and as time passed, the degree of neovascularization (Figure 5) and inflammation (Figure 6) significantly decreased ($P < .01$). Cartilage grafts did not show eosinophil infiltration, fibroblast proliferation (Figure 7), granulation tissue formation, or the presence of multinucleated giant cells at any time.

AlloDerm Grafts

At the first month, we noticed that all the grafts showed significant thickening (Figure 3). The mean (SD) thickness of the grafts was 2.0 (0.1) mm at the first month, although their original thickness was 0.79 to 1.02 mm. With time, this process reversed: 2 of the 4 grafts totally resorbed, 1 graft resorbed at a rate of 30%, and the fourth graft remained unresorbed at the second month postoperatively. At the third postoperative month, all the AlloDerm grafts ($n = 4$) were totally resorbed. In the last group, microscopic examination at the end of the fourth postoperative month again showed total resorption of all grafts ($n = 12$).

In the first month, there was intense eosinophil infiltration, neovascularization, inflammation, and fibroblast proliferation in the sections with the AlloDerm grafts (Figures 4-7). At the second, third, and fourth months, the degree of eosinophil infiltration, neovascularization, inflammation, and fibroblast proliferation decreased significantly compared with the first month (Figures 5-7) ($P < .01$ for all). In addition, multinucleated giant cells were observed in 3 of the 5 AlloDerm grafts during the first month, but no grafts showed granulation tissue or multinucleated giant cells during the second, third, and fourth months.
Fascial Grafts

None of the grafts showed histologic evidence of significant resorption (Figure 4). Although the fascial grafts were prepared with an approximate graft thickness of 1.5 mm, the mean thickness was 1.0 mm in the first month and 0.9 mm in the second, third, and fourth months (Figure 3). There was a moderate degree of neovascularization and inflammation in the fascial grafts at the first, second, and third months (Figures 5 and 6). At the fourth month, the degree of neovascularization and inflammation was less than in the previous months, but no significant difference was found ($P = .09$ for neovascularization; $P = .12$ for inflammation).

Although some of the fascial grafts showed a minimal amount of eosinophil infiltration and fibroblast activity at the first month, neither of the fascial grafts showed eosinophil infiltration or fibroblast activity at the second, third, or fourth months (Figure 7). There was no significant difference between the groups regarding eosinophil infiltration or fibroblast activity. None of the fascial grafts showed granulation tissue formation or the presence of multinucleated giant cells at any time.
Dermal Grafts

The most prominent finding was the formation of epidermoid cysts in all of the dermal grafts (Figure 4). These epidermoid cysts were surrounded by intense neovascularization and inflammation, accompanied by increased fibroblast cells in all of the grafts (Figures 4-7). In the first and second months, we did not observe granulation tissue or multinucleated giant cells in any of the sections, but in the third and fourth months, formation of significant granulation tissue with multinucleated giant cells was observed in all of the grafts. The grafts showed a moderate amount of eosinophil infiltration only at the third month.

Fat Grafts

The histologic evidence of resorption of each fat graft was determined using a microscopic ocular meter. The areas with inflammatory cell infiltration and fibrosis were accepted as the loss of fat cells in the graft area. Thus, the resorption value of each animal was given as the percentage of lost fat cells to the total graft area. The mean (SD) resorption values for fat grafts were 60 (7) for the first month, 48 (13.2) for the second month, 30 (4) for the third month, and 39 (7.9) for the fourth month ($P = .38$).

Increased neovascularization, moderate cosinophil infiltration, severe inflammation, and moderate fibroblast proliferation were noted in the first-month grafts. The degree of neovascularization and inflammation decreased across time (Figures 5 and 6). A significant difference was found between the groups regarding neovascularization and inflammation ($P < .01$ for all). However, the amount of fibroblast proliferation was similar in all the groups, and no significant differences were found.

The most common finding in all the groups was the development of fat cysts in the grafts (Figure 4). Fat cysts were reported in all the fat grafts in the first, second, and third months and in 8 of 12 grafts in the fourth month. Usually, multinucleated giant cells and many histiocytes accompanied these fat cysts. In addition, almost all the grafts in all the groups showed multinucleated giant cells.

Comparison of Graft Groups

During the first month, the degree of neovascularization and inflammation was very mild in the sections of the cartilage grafts, and neither showed fibroblast proliferation. However, the degree of neovascularization and inflammation was significantly higher in the other graft groups (AlloDerm, dermis, fascia, and fat) compared with the cartilage grafts. In addition, these grafts showed accompanying fibroblast proliferation.

Regarding fibroblast proliferation, neovascularization, and inflammation during the first, second, third, and fourth months, the cartilage grafts showed significant differences from the AlloDerm, dermal, and fat grafts ($P < .001$ for all). Although there were significant differences between the fascial grafts and the cartilage grafts regarding inflammation, neovascularization, and fibroblast proliferation at the first month ($P < .05$), there was no significant difference between the cartilage grafts and the fascial grafts regarding fibroblast proliferation, neovascularization, and inflammation during the second, third, and fourth months.

The fascial grafts showed significant differences from the dermal ($P = .042$, .02, and .02 in the neovascularization, inflammation, and fibroblast proliferation groups, respectively), AlloDerm ($P = .02$, .048, and .043 in the neovascularization, inflammation, and fibroblast proliferation groups, respectively), and fat ($P = .048$, .028, and .03 in the neovascularization, inflammation, and fibroblast proliferation groups, respectively) grafts regarding fibroblast proliferation, neovascularization, and inflammation during the first, second, third, and fourth months. In the fascia group, we noticed that the degree of fibroblast proliferation, inflammation, and neovascularization was moderate in the first month. During the second and third months, there was no significant inflammation, vascularization, or fibroblast proliferation in the fascia group. However, fibroblast proliferation, neovascularization, and inflammation were moderate to severe in the fat and dermis groups during the first, second, third, and fourth months.

Comment

Many grafts have been used for nasal contour augmentation and to achieve a smoother dorsal surface in rhinoplasty. None of the graft materials universally satisfies all the requirements of an ideal grafting material. Autogenous grafts have the advantages of excellent biocompatibility, of not stimulating the immune response, and of carrying the lowest infection and rejection risks. Owing to these advantages, autogenous cartilage and various soft-tissue grafts, such as temporalsis fascia, dermal, and fat, have been used to cover underlying dorsal irregularities. However, these materials have the disadvantages of donor site morbidity and a variable resorption risk depending on their source.
Autogenous cartilage is the most preferred material of all the alternatives. Donor site morbidity is often not a concern for this material because nasal septal cartilage is usually within the surgical field in rhinoplasty. In addition, autogenous cartilage survives as living tissue and seldom undergoes resorption, as reported in clinical and experimental studies. Similarly, we found excellent viability for the cartilage groups throughout the study, with no evidence of resorption, as demonstrated by macroscopic and microscopic examinations, which showed normal cartilage architecture and normal chondrocytes. We found no significant differences in cartilage grafts regarding graft thickness in any of the groups (Figure 3). Examinations of all the groups revealed a lack of eosinophil infiltration, fibroblast proliferation (Figure 7), granulation tissue, and multinucleated giant cells. We did not detect significant neovascularization or inflammation at any time in any of the cartilage groups (Figures 5 and 6). However, when using a dorsal onlay graft, the edges of the cartilage graft may become apparent years after the surgery, especially in thin-skinned patients. To prevent this, autogenous cartilage may be crushed before implantation. Previous studies in rabbits and human cell cultures show that slight crushing of the cartilage creates excellent graft material for filling defects and creates a smoother surface without causing visible or dorsal irregularities.

Adipose tissue is an alternative autogenous material that may be obtained in large amounts with minimal donor site morbidity. However, this material has very high resorption rates that vary from 20% to 90%. Because of these high resorption rates, overcorrection has been suggested as being 35% in clinical observations. However, this may vary considerably among patients. In a rabbit study, surgically excised fat maintained its volume (42.2%) better than suction-assisted fat grafts (31.6%) 9 months after implantation. In another study using a rabbit model, the viability of the fascia and, therefore, has recommended a slight overcorrection. In the present study, fascial grafts showed negligible volume loss. Because we did not observe histologic evidence of significant resorption, this result could be attributed to the shrinkage capacity of the fascial grafts. We also did not detect any eosinophil infiltration, fibroblast activity, granulation tissue, or multinucleated giant cells at any time in either of the fascial grafts.

Some researchers have advocated using dermal grafts because of the availability, the ease of manipulation, and the limited postoperative absorption and stability. However, Thompson reported finding partial resorption of the dermal graft in 11.6% and total resorption in 9.3% of their 33 patients in a clinical and histologic study. He also stated that an absorption rate of 20% might be expected in the first postoperative year. In a clinical study, loss of dermal graft volume in 9 of 182 patients (5%) was detected, with recurrence of the preoperative deformity. In another review, 23% of all the patients needed further augmentation within a year of dermal grafting. It has been reported that epidermoid cyst formation is commonly observed on histologic examination and that these cysts derive from sebaceous glands; however, most cysts have been shown to arise from hair follicles. Reich reported that no cyst formation was noted in clinical observations. In contrast to this study, all of the present dermal grafts developed epidermoid cysts and significant inflammation, fibrosis, and neovascularization around these cysts. In the present study, intense neovascularization and inflammation, with an increased number of fibroblast cells surrounding epidermoid cysts, was detected in all the grafts (Figures 5-7). This finding may be due to harvesting of the dermal grafts from the rabbit’s hairy dorsal skin, although meticulous care was used when preparing the dermal grafts. Significant granulation tissue and multinucleated giant cells were observed in the third and fourth months in all grafts, which are indicative of a tissue reaction. Significant cystic formation prevents us from commenting on the resorption rates of the dermal grafts.

AlloDerm, derived from cadaveric skin, has been designated as a dermal replacement graft material. The epidermis and all of the dermal elements are detached from the cadaveric skin and, via a freeze-dried process, form an acellular graft. Owing to its lack of major histocompatibility antigens, AlloDerm should not have immunologic properties. Jackson and Yavuz reported prolonged mild edema after implantation, which resolved in 3 to 7 days. Achauer and coworkers reported the resolution of this edema in 4 months. They did not report any resorption of their grafts. Similarly, in a series of 15 patients, it has been reported that recurrent irregularity was not a problem, although it was difficult to assess how
much resorption occurred. However, partial graft resorption has been reported in rhinoplasty and lip augmentation in clinical series.22,23 Shrinkage of 15% to 20% has been reported, which became stable after 4 to 6 weeks.23 Similarly, Gryskiewicz and coworkers11 did not observe complete absorption; however, 45% of their patients demonstrated partial absorption within 1 year. In the present study, the first month’s examinations revealed that the dimensions of the AlloDerm grafts were larger than their original size, with significant thickening (Figure 1). We suggest that this increased thickness might have been due to severe edema secondary to tissue reaction during the first month. Subsequently, this process subsided, and the edema was nearly resolved at the second month’s examination. However, this study showed complete resorption of the AlloDerm grafts by the third and fourth postoperative months. Although AlloDerm is considered to be nonimmunogenic, we observed intense eosinophil infiltration, neovascularization, inflammation, and fibroblast proliferation in sections, as demonstrated by a foreign body reaction in the tissue at the first postoperative month (Figures 5–7). One explanation for this foreign body reaction is that human cadaveric dermics (AlloDerm) may create an immunemediated response when implanted into rabbits. The degree of eosinophil infiltration, neovascularization, inflammation, and fibroblast proliferation decreased significantly at later assessments compared with the first month (Figures 5–7).

An important consideration regarding the animal studies is that their results can be only partially applied to humans because of the differences in metabolism between the species. Although this study is based on an animal model, its results provide valuable information regarding the comparison of different graft materials. These results suggest that the cartilage graft is the first choice of graft material, with a low absorption rate, high biocompatibility, negligible tissue reaction, and minor generation of fibrosis. The second choice of graft material is the fascia, with a minimal shrinkage capacity and a limited tissue reaction. Owing to high resorption rates, long-term results when using fat grafts can be unpredictable, and overcorrection may be needed. However, most patients may not be willing to adjust to overcorrection. Dermal grafts may give rise to severe problems with epidermal cyst formation. Although AlloDerm is considered to be a nonimmunogenic material, it caused a severe tissue reaction in rabbits in this study. This discordance may be due to the hypersensitivity of the rabbit immune system against AlloDerm. Further comparative clinical investigations are required to assess the clinical efficacy of AlloDerm in humans.

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Author Contributions: All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Cakmak. Acquisition of data: Tarhan, Ozdemir, Akdogan, and Suren. Analysis and interpretation of data: Tarhan and Ozdemir. Drafting of the manuscript: Tarhan, Ozdemir, Akdogan, and Suren. Critical revision of the manuscript for important intellectual content: Cakmak. Statistical analysis: Ozdemir. Administrative, technical, and material support: Tarhan, Ozdemir, Akdogan, and Suren. Study supervision: Cakmak.

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