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Experimental Histopathological Comparison of Fresh Frozen Allograft and Autograft Cartilage

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Abstract

Background Cartilage grafts are essential in rhinoplasty, particularly in revision cases that require structural support. Fresh frozen cartilage allografts (FFCA) have been proposed as an alternative to autografts to avoid donor site morbidity; however, their biological properties remain insufficiently studied. This study aimed to evaluate the histopathological and immunohistochemical characteristics of FFCA compared with autograft cartilage in a rabbit model, focusing on antigenicity, cellular viability, inflammation, and fibrovascular integration.

Methods Eight adult New Zealand white rabbits underwent bilateral auricular cartilage harvesting. The right ear cartilage from each rabbit was immediately implanted subcutaneously into its own paraspinal region as an autograft. The left ear cartilage was cryopreserved at -80 °C for two weeks and subsequently implanted as an FFCA into the paraspinal region of the next consecutively numbered rabbit. All grafts were harvested and analyzed histologically after three months.

Results Both groups exhibited preserved cartilage architecture and fibrovascular integration. No significant differences were observed in graft resorption, fibrosis, or calcification ($p > 0.05$). However, FFCA showed a significant reduction in viable chondrocytes ($p < 0.05$) and lower glial fibrillary acidic protein expression, suggesting diminished cellular activity. Despite maintaining structural stability, FFCA demonstrated reduced biological viability compared with autografts.

Conclusions FFCA preserves structural integrity and integrates with surrounding tissue but exhibits lower cellular viability than autografts. While it may represent a useful alternative in rhinoplasty, further studies are required to optimize preservation techniques and assess long-term outcomes.

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Keywords Autograft cartilage · Fresh frozen cartilage allograft · Histopathology · Rhinoplasty

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Introduction

Rhinoplasty is a fundamental procedure in facial plastic surgery. In 2020, more than 325,000 rhinoplasty surgeries were performed in the United States [1]. The need for revision rhinoplasty arises in approximately 3.3% to 15.2% of cases following primary procedures [2]. Revision surgeries often require substantial cartilage grafts to achieve both aesthetic and functional success. Although septal

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cartilage is considered the ideal graft material, it is often unavailable or insufficient during revision surgery. Depending on the patient's specific requirements, auricular or costal cartilage may be employed as alternative sources [3].

In revision cases where a large volume of cartilage is required, autologous costal cartilage grafts are generally preferred. Their advantages include abundant availability, biocompatibility, malleability, and robust structural support. However, disadvantages include prolonged operative time, the need for an additional surgical site, higher procedural costs, postoperative donor-site pain, and the risks of pleural injury, pneumothorax, and damage to intercostal vessels and nerves [4]. These potential complications, along with the surgeon's diminished focus on the target site, underscore the need for innovative solutions to overcome these challenges. Irradiated costal cartilage allografts, typically sourced from cadavers, have become increasingly common in clinical practice [5]. However, they are associated with higher rates of resorption and infection compared with autologous costal cartilage [6].

While long-term outcome data remain limited, recent studies have reported promising results with fresh frozen cartilage allografts (FFCA). This technique reduces the risks of resorption and warping associated with irradiated grafts. A considerable number of clinical studies suggest that FFCA functions as a non-immunogenic biological graft source, eliminating the need for intervention at a secondary surgical site [7–12]. However, these clinical studies lack the scientific foundation that should be provided by preliminary experimental research. To address this gap, the present experimental study evaluated the antigenicity, viability, inflammation, and fibrovascular organization of FFCA using histopathological methods.

Methods

Eight healthy adult New Zealand white rabbits, each weighing approximately 4 kg and aged 12 months, were used in the study. The animals were housed individually in cages at room temperature. The study protocol was approved by the Ethics Committee for Experimental Animals at our University Hospital.

Surgical Procedures

Anesthesia was induced with an intramuscular injection of 50 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride. Approximately 10 minutes after administration, the depth of anesthesia was assessed by checking the extremity withdrawal response. The left pinna of each rabbit was disinfected with povidone-iodine. For pain and bleeding control, 1–2 ml of 2% lidocaine with 1:100,000 epinephrine was injected subcutaneously before making a 3 cm incision under sterile conditions. After traversing the skin, subcutaneous tissue, and perichondrium, a 2 × 2 cm cartilage graft was harvested. Hemostasis was achieved at the donor site, and the skin was closed with 4-0 Vicryl sutures. The harvested cartilage grafts were disinfected in a clindamycin-containing antibiotic solution and stored in sterile containers at –80 °C for two weeks (Fig. 1a–d).

Two weeks later, the same surgical steps were repeated on the right pinna of each rabbit. Cartilage grafts (2 × 2 cm) were harvested without perichondrium for use as autografts. The grafts obtained from the left ears two weeks earlier were thawed at room temperature and numbered for use as FFCAs.

After appropriate positioning, a 40 mm^2 area of the paraspinal region was shaved and cleansed with povidone-iodine. Two 1.5 cm incisions were made bilaterally to create subcutaneous pockets. The graft from the right ear was implanted in the right paraspinal region of the same rabbit (Autograft group). In the FFCA group, the thawed graft from another rabbit was placed in the pocket in the left paraspinal region. All incisions were closed with 4-0 Vicryl (Fig. 2a–c).

No complications occurred during follow-up. After three months, the grafts were excised from the paraspinal region for comparison of FFCAs and autografts (Fig. 3a–d). Each specimen was numbered and placed in 10% formaldehyde for fixation, then sent for histopathological evaluation.

Histopathological Evaluation

Cartilage specimens were fixed in 10% formaldehyde for 72 hours, followed by processing through a graded alcohol series (70%, 90%, 96%, and 100%) and xylene. The samples were embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E) and Masson's trichrome. Evaluation was performed using a light microscope (Carl Zeiss, Axio Observer) at 200× magnification. Samples were assessed for loss of chondrocyte nuclei, peripheral proliferation, fibrosis, inflammation, graft resorption, calcification, and extracellular matrix (ECM) formation. Parameters were scored as a percentage of the analyzed material: 0% (none, 0), 1–25% (minimal, 1+), 26–50% (moderate, 2+), 51–75% (moderate-high, 3+), and 76–100% (high, 4+).

Immunohistochemical Staining

Anti-glial fibrillary acidic protein (GFAP) immunohistochemistry was used to assess chondrocyte matrix synthesis, indicated by GFAP quantity and distribution. Sections were incubated overnight at 37 °C, then deparaffinized in xylene

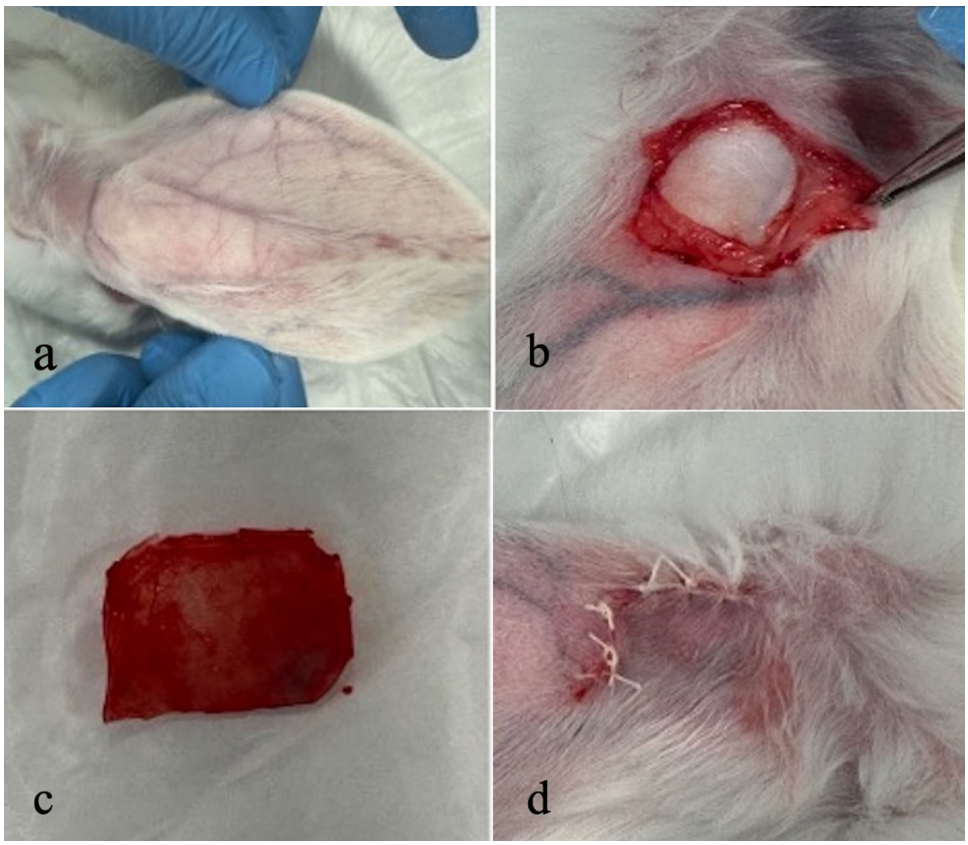

Fig. 1 Stages of harvesting allograft cartilage. **a** The ear of the rabbit. **b** Exposure of the auricular cartilage. **c** Excised cartilage measuring 2×2 cm. **d** Closure of the incision in the ear

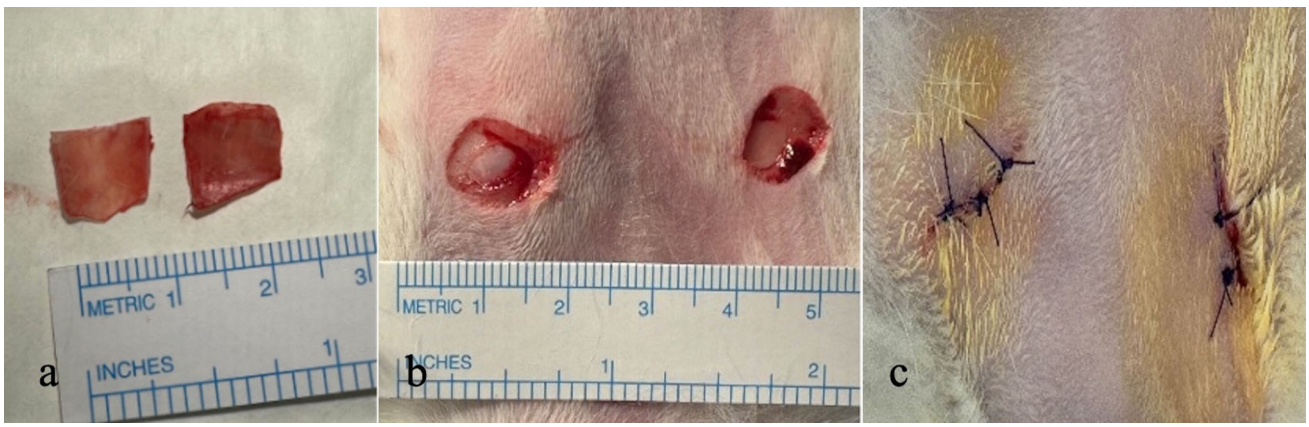

Fig. 2 Placement of autograft and allograft cartilages in the paraspinal region. **a** Autograft and allograft cartilages. **b** Pockets are created in the paraspinal region (The right pocket contains the rabbit's own auricular cartilage, while the left pocket contains cartilage obtained from another rabbit). **c** Closure of the paraspinal incisions

(3 × 5 min), followed by immersion in absolute and 96% alcohol for 10 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 20 minutes. Antigen retrieval was performed by heating sections in citrate buffer at 200 W in a microwave for 10 minutes. After cooling, sections were washed three times with phosphate-buffered saline (PBS) for 5 minutes each and incubated for 10 minutes in blocking solution. The primary antibody (Anti-GFAP, 1:500, Sigma-Aldrich, G3893) was applied, and sections were incubated overnight at +4 °C. After primary antibody treatment, sections were incubated with anti-mouse Alexa Fluor 555 secondary antibody for 1 hour at room temperature. Finally, samples were mounted with DAPI-containing medium (Sigma-

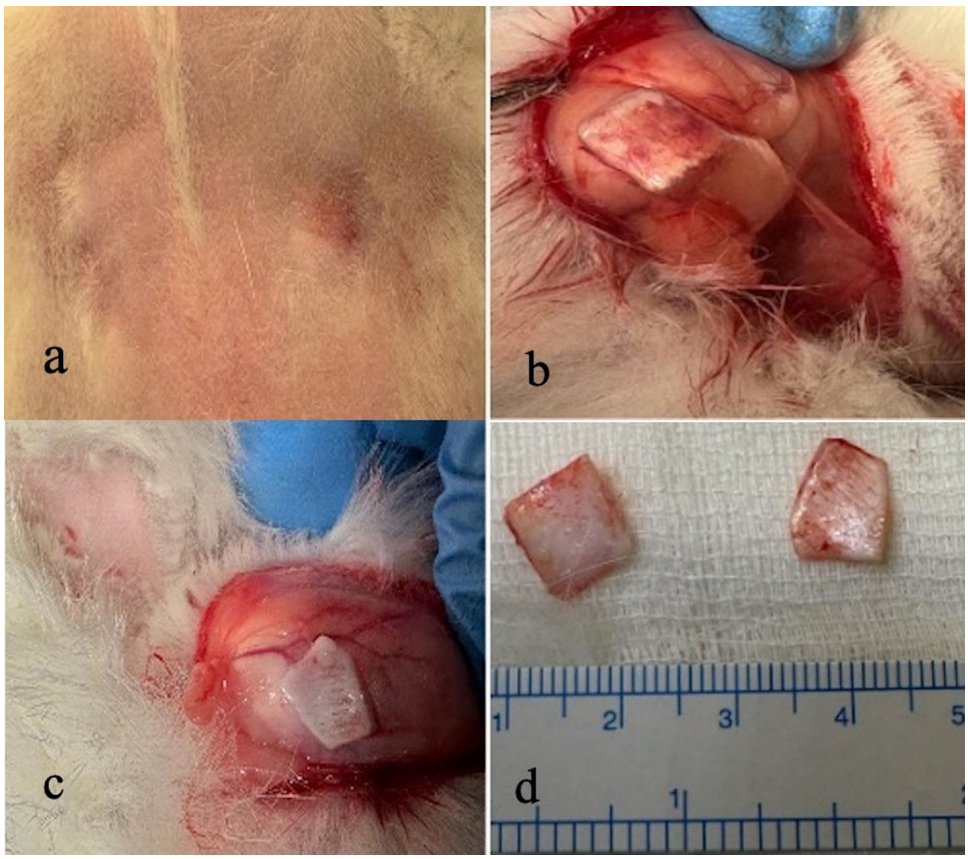

Fig. 3 Extraction and visualization of cartilages from the paraspinal region during the third month. **a** External appearance of the skin. **b** FFCA after skin elevation. **c** Autograft after skin elevation. **d** Cartilages extracted from the paraspinal region

Aldrich, F6057) and examined under a confocal microscope (Zeiss LSM 880) using 405 and 543 nm excitation lasers.

Statistical Analysis

Normality of the data was assessed using the Shapiro–Wilk test. The Mann–Whitney U test was applied to compare histopathological scores and GFAP cell counts between groups. P values were not adjusted for multiple comparisons, as the number of comparisons was limited. All statistical analyses were performed using GraphPad Prism software, version 9.1.

Results

Eight FFCA specimens and eight autograft cartilage specimens were examined macroscopically. No graft resorption was observed in either group. Fibrovascular structures and macroscopic viability were evident around the cartilage tissue (Fig. 3b–d).

On hematoxylin and eosin (H&E) staining, chondrocytes and chondroblasts in the lacunae of the autograft group appeared regular, with intact interterritorial matrix and normally organized collagen fibers. Some large lacunae showed vacuolization and loss of chondrocytes. In the FFCA group, the lacunae, pericellular and territorial matrix, as well as the pale basophilic interterritorial matrix, were also preserved, and collagen fibers followed their normal structure. However, unlike the autograft group, the FFCA group exhibited a marked loss of chondrocytes and chondroblasts (Fig. 4a–d).

Anti-GFAP immunohistochemical staining was performed to evaluate the matrix-synthesizing capacity of chondrocytes. In the autograft group, chondrocytes showed strong GFAP expression, indicating healthy cellular activity. In contrast, the FFCA group displayed cytoplasmic remnants of fragmented chondrocytes within the lacunae (Fig. 5a–b). Quantitative evaluation revealed that the number of viable chondrocytes was significantly lower in the FFCA group than in the autograft group (Fig. 6).

Table 1 summarizes the histopathological parameters and group comparisons. Loss of chondrocyte nuclei was significantly greater in the FFCA group, whereas peripheral proliferation was significantly higher in the autograft group.

No significant differences were found between groups in terms of graft resorption ($p = 0.2821$), calcification ($p = 0.5692$), fibrosis ($p = 0.4452$), or ECM formation ($p >$

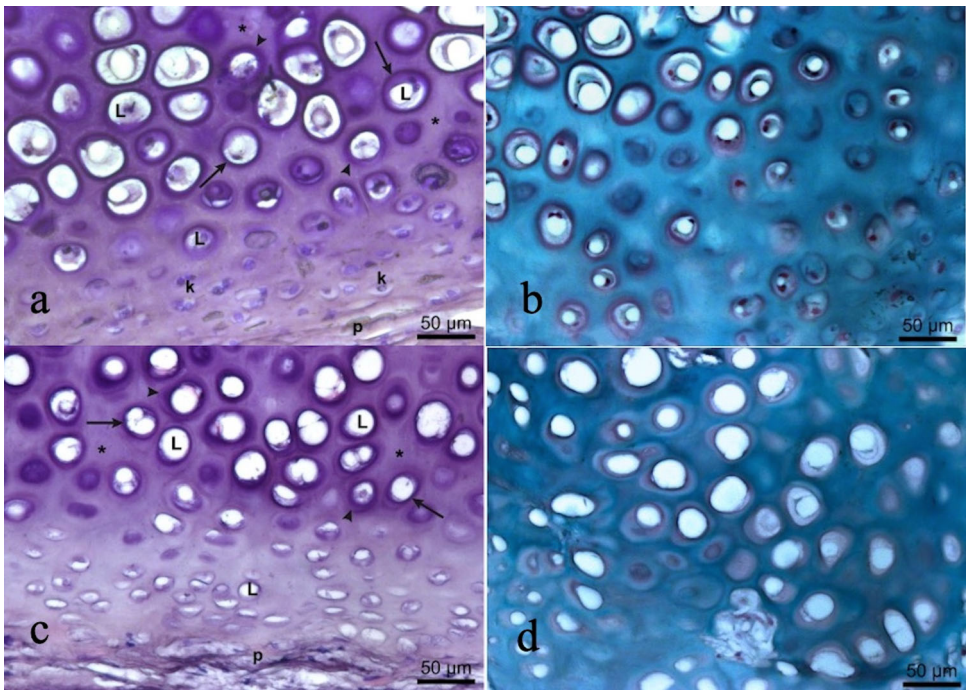

Fig. 4 **a** Hematoxylin & eosin staining, x200 magnification, light microscope: in the autograft cartilage, chondrocytes and chondroblasts are observed, surrounded by densely basophilic stained pericellular matrix (arrow), with territorial matrix between the lacunae (arrowhead), and pale basophilic stained interterritorial matrix (*) where regular collagen fibers are seen. **b** Masson trichrome staining, x200 magnification, light microscope: in the autograft cartilage, the extracellular matrix areas and the chondrocytes within the lacunae are observed in their regular structures. **c** Hematoxylin & eosin staining, x200 magnification, light microscope: FFCA- Different sizes of lacunae (L) are seen within the cartilage, surrounded by densely basophilic stained pericellular matrix (arrow), with territorial matrix between the lacunae (arrowhead), and pale basophilic stained interterritorial matrix (*) appearing regularly. Collagen fibers surrounding the cartilage are observed in their normal structure. The loss of chondrocytes and chondroblasts within the cartilage is notable. Fragmented remnants of chondrocytes are visible within the lacunae. **d** Masson trichrome staining, x200 magnification, light microscope: In the FFCA, while the extracellular matrix areas and lacunae are regularly observed, the loss of chondrocytes within the lacunae is notable

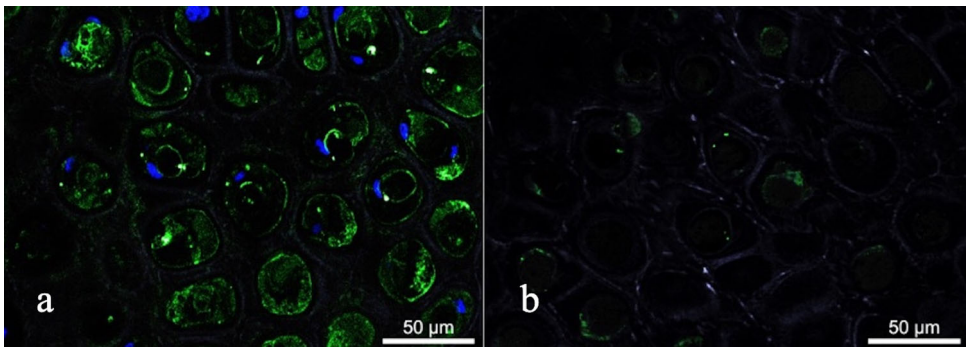

Fig. 5 **a** Autograft cartilage group **b** fresh frozen cartilage allograft group glial fibrillary acidic protein (GFAP) (green), the nuclei of the chondrocytes stained with 4′,6-Diamidino-2-Phenylindole (DAPI) (blue), and the extracellular matrix structure between the chondrocytes (autofluorescence, gray) is visible. Chondrocytes exhibiting high levels of GFAP expression are seen to be healthy cells capable of matrix production

0.9999). Inflammation was not observed in either group (Table 1).

Discussion

Cartilage grafts are widely used in nasal surgery, craniofacial reconstruction, and orthopedic procedures [13]. To repair cartilage defects, autografts, allografts, and alloplastic implants are the main options [3]. In this study, FFCA was compared with autograft cartilage using a rabbit auricular cartilage model evaluated macroscopically, histopathologically, and immunohistochemically. Auricular (elastic) cartilage was chosen in rabbits because of its lower morbidity; however, in clinical rhinoplasty, the standard graft source is costal (hyaline) cartilage. Elastic cartilage contains elastic fibers in addition to type II collagen, which makes it more flexible and less matrix-dense, whereas hyaline cartilage is structurally denser and mechanically more resistant. Despite the use of elastic cartilage in our study, no mechanical degradation or graft degeneration was observed. Moreover, as the primary aim of this study was to evaluate antigenicity, elastic cartilage was selected owing to its higher cellular density. Comparative studies of human auricular cartilage with auricular cartilage from various species have shown that rabbit auricular cartilage provides the greatest structural and biochemical similarity. For instance, Chi et al. reported that porcine and bovine auricular cartilage contain higher levels of glycosaminoglycans and collagen compared with human cartilage, whereas rabbit auricular cartilage demonstrated the closest biochemical resemblance [14]. Therefore, rabbit auricular cartilage was considered an appropriate model in terms of both histocompatibility and biochemical similarity. The three-month follow-up period was selected in accordance with similar experimental studies reported in the literature [15].

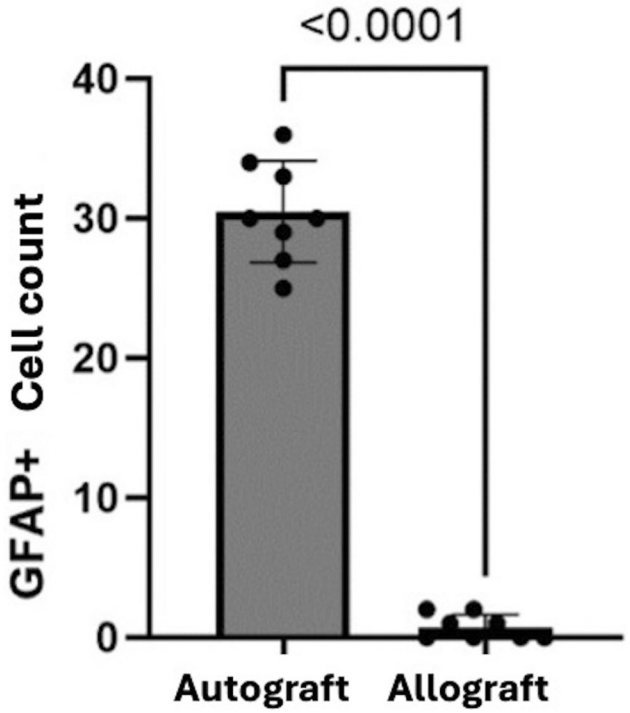

Fig. 6 Comparison of the number of glial fibrillary acidic protein (GFAP)-positive cells per area (78,400 μm^2) visualized with a confocal microscope in groups after GFAP immunohistochemical staining

In rhinoplasty, cartilage grafts are frequently used to reshape and support the nasal structure [13]. Septal autografts are generally preferred because of their availability and biocompatibility. However, in primary or revision rhinoplasty cases with insufficient septal cartilage, additional sources are required [2]. In such situations, costal cartilage is often chosen, as it provides strong structural support and maintains nasal stability. Particularly when septal and auricular cartilage are inadequate, costal cartilage becomes a critical option [16].

The primary advantage of costal cartilage is its ability to provide robust and durable support, especially along the

Table 1 Histopathological comparison of autograft and allograft cartilage groups

Histopathological parameters	Autograft group (n= 8)					Allograft group (n=8)					*P*-values for the intergroup comparison
Scoring	0	+1	+2	+3	+4	0	+1	+2	+3	+4	
Loss of chondrocyte nucleus	–	6	2	–	–	–	–	–	2	6	**0.0002**
Graft resorption	7	1	–	–	–	4	4	–	–	–	0.2821
Calcification	7	1	–	–	–	5	3	–	–	–	0.5692
Inflammation	8	–	–	–	–	8	-	–	–	–	1.000
Fibrosis	6	2	–	–	–	4	3	1	–	–	0.4452
Peripheral proliferation	–	–	–	6	2	5	3	–	–	–	**0.0002**
ECM formation	–	–	–	1	7	–	–	–	2	6	>0.999

Bold values indicate statistically significant differences ($p < 0.05$)

nasal dorsum. It is frequently used in Afro-American and Asian patient populations, where greater structural reinforcement is needed [17]. However, harvesting costal cartilage carries specific risks, including postoperative donor-site pain, scar formation, injury to the intercostal neurovascular bundle, pleural injury, and, most importantly, pneumothorax, which represents the most serious complication [4].

Another major drawback is the tendency of costal cartilage to warp in the postoperative period, potentially compromising rhinoplasty outcomes. Reported warping rates vary from 3% to 26.1%, depending on follow-up duration [18]. Severe warping usually appears within 6 to 12 months after surgery, whereas minor deformities may develop years later and sometimes necessitate revision [19].

Given these disadvantages, alternative graft options such as FFCA have gained attention. Allograft cartilage eliminates the need for donor-site harvesting, thereby shortening surgery and reducing donor-site morbidity. Nevertheless, allografts are still associated with a potential risk of immunological reactions, and ongoing research continues to investigate this issue.

Chondrocyte Antigenicity and Irradiation Techniques

Chondrocyte antigenicity is an important factor that increases the risk of immunological responses in allografts. Although cartilage tissue has traditionally been considered relatively immune-privileged, studies have shown that when isolated chondrocytes are transplanted as allografts, they can trigger a cellular immune response and interact with host immune cells [20]. Langer and colleagues demonstrated in a rat model that transplantation of isolated chondrocytes induced humoral antibody production and immune reactivity against the chondrocytes [21]. By contrast, when the cartilage matrix remains intact, the extracellular matrix acts as a protective barrier, limiting the interaction of chondrocytes with NK and T cells [20].

Irradiation techniques have been employed to reduce infection risk and decrease antigenicity in allografts. Irradiated costal cartilage exhibits lower antigenicity and has been reported to support fibrovascular ingrowth from host connective tissue [22]. However, orthopedic studies indicate that the durability of irradiated grafts depends on the gamma radiation dose [23]. Wee and colleagues reported that irradiated costal cartilage contained fewer chondrocytes than autografts, with reduced collagen content and disrupted cellular organization [6]. Moreover, irradiation is believed to generate free radicals that damage collagen structure, potentially increasing the risk of graft resorption over time [24].

Reduction of Antigenicity in FFCAs through Freezing Process

After appropriate health screenings and tests, cartilage tissues are harvested from donors under sterile conditions. Before transplantation, the allograft is stripped of its perichondrium and sterilized to eliminate pathogens. It is then rapidly frozen in a controlled environment, typically at –80 °C, to preserve its biological structure. The tissue is continuously stored within a cold chain and thawed immediately prior to use.

Freezing is a widely used method to reduce the antigenicity of fresh allograft cartilage. Friedlander demonstrated that not only chondrocytes but also collagen, a key matrix component, may contribute to the antigenicity of allografts [25]. The freezing process can render collagen more resistant to immune-mediated degradation. Overall, freezing provides several advantages for cartilage grafts, including prolonged preservation and reduced immunogenicity, making them a safer and more practical option for rhinoplasty. However, fresh cartilage allografts still present certain limitations, such as transportation logistics, risk of communicable diseases, and the need for bacterial testing. Despite these drawbacks, freezing helps minimize these concerns, supporting safer and more reliable clinical use [21].

In recent years, interest in the use of FFCAs for rhinoplasty has grown. Rohrich and colleagues reported successful outcomes with rib-derived FFCA, highlighting the influence of donor age and storage conditions. In their series of 50 cases, no graft resorption was observed [7]. Similarly, Mohan and colleagues followed 50 patients for four months and reported only one case of infection, with no evidence of resorption or warping. These results suggest that FFCAs may represent a reliable option in rhinoplasty [8]. In Asian populations, Wan and colleagues noted a significant increase in patient satisfaction scores over a one-year follow-up period after functional rhinoplasty using fresh frozen costal allografts [9].

Despite these encouraging clinical results, the studies lack a scientific foundation that could be established through preliminary experimental research. To date, no histopathological assessments of the antigenicity, viability, inflammation, and fibrovascular organization of FFCA in rhinoplasty have been published. This study sought to address this gap through experimental histopathological evaluation.

Macroscopically, no cartilage resorption was observed in either the autograft or FFCA groups. In both groups, the cartilage surface appeared healthy and well-integrated with surrounding tissues, forming fibrovascular structures. Histopathological evaluation of the autograft group revealed regular cartilage architecture, intact lacunae, and

normal collagen fibers. In contrast, the FFCA group exhibited significant chondrocyte loss despite preservation of the basophilic matrix between lacunae (Figs. 4a–d, 5a–b).

GFAP is predominantly expressed in tissues containing elastic cartilage. Since elastic cartilage has a higher cellular density compared to hyaline cartilage, GFAP expression was also assessed in this study as part of the antigenicity evaluation. In this context, GFAP served as a supplementary indicator of matrix production capacity and cellular viability [26, 27]. Anti-GFAP immunohistochemistry was then performed to evaluate chondrocyte matrix production. In the autograft group, viable chondrocytes exhibited strong GFAP expression, whereas the FFCA group showed fragmented cellular remnants within lacunae (Fig. 6). These findings suggest that autografts preserve greater cellular viability than FFCAs.

This study has several limitations that should be considered when interpreting the findings. First, mechanical properties such as tensile strength, flexibility, and rigidity were not evaluated. Future research incorporating biomechanical assessments before and after implantation, as well as longer in vivo durations, would provide valuable insights into the structural stability and longevity of FFCA grafts. Second, the rabbit model used elastic cartilage, whereas clinical FFCAs are typically derived from hyaline cartilage. The difference in cartilage type may influence antigenicity, cartilage viability, resorption patterns, and fibrovascular integration; therefore, further studies are needed to clarify its clinical applicability. Third, the three-month follow-up period may not capture long-term changes such as delayed resorption or loss of structural integrity, underscoring the need for extended follow-up in future studies to fully assess graft viability and long-term integration. Fourth, the sample size (n = 8) is consistent with previous similar experimental animal studies [28] and was kept to a minimum for ethical and practical reasons. While appropriate for histopathological evaluation, this sample size may limit statistical power and generalizability. Finally, both experimental groups were tested within the same animal. Although this design was chosen for ethical and practical considerations, it may introduce potential implications—such as differences in immunologic response—compared with assigning each experimental group to separate animals.

In conclusion, this experimental animal study is among the first to compare autografts and FFCAs in the context of rhinoplasty. The results suggest that FFCAs maintain structural integrity and could be considered a potential alternative, although their cellular viability is lower than that of autografts.

Conclusions

In this study, no resorption was observed in either the autograft or FFCA groups, and both demonstrated fibrovascular organization with the surrounding tissues. Histopathological examination revealed a marked loss of chondrocyte nuclei in the FFCA group. No significant differences were found between groups regarding graft resorption, calcification, or fibrosis, and inflammation was absent in both. Although this study has limitations, including its experimental nature and the use of a different cartilage type, these findings provide valuable preliminary data. While FFCA may represent a useful alternative in rhinoplasty, further clinical and experimental studies are required to establish its safe application.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

Ethical Approval Ethical approval for this study was obtained from the Local Animal Experiments Ethics Committee of our university (Approval No: 2023-51).

Informed Consent For this type of study, informed consent is not required.

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