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Comparison of Mechanical Properties of Nasal Cartilage Preserved in Ethanol at 4 °C versus Normal Saline at -18 °C for Rhinoplasty Reuse

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Abstract

Introduction:

This study aimed to compare the mechanical properties of nasal cartilage preserved in ethanol at 4 °C versus normal saline at -18 °C, with the goal of assessing their suitability for reuse in subsequent nasal surgeries.

Materials and Methods:

Twenty nasal septal cartilage samples from patients who had undergone rhinoplasty were preserved for two months using two different methods. Ten cartilages were stored in alcohol at 4 °C, while the remaining ten were stored in normal saline at -18 °C. Additionally, a control group consisting of ten fresh cartilage samples was included for comparison. The mechanical properties (namely Young's modulus, fracture stress, yield stress, ductility, and tensile strength) were measured using a universal tensile testing machine STM-20 and analyzed to evaluate the effects of the preservation methods.

Results:

Significant differences were observed between the groups in mean tensile strength, ductility, Young's modulus, and yield stress ($P<0.05$). However, no significant difference was found in mean fracture stress ($P=0.318$). The alcohol-preserved group exhibited significantly lower mechanical properties compared to the saline and fresh cartilage groups ($P<0.05$). No significant differences were detected between the frozen (saline) and fresh cartilage groups ($P>0.05$).

Conclusions:

Cartilage stored in normal saline at -18 °C retained its mechanical properties better than cartilage preserved in alcohol at 4 °C. Freezing did not adversely affect the mechanical properties, and the slightly higher values observed in the frozen group suggest that lower temperature may enhance cartilage elasticity.

Keywords: Nasal cartilage, Rhinoplasty, Secondary rhinoplasty, Mechanical properties

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Introduction

Rhinoplasty may require revision for various reasons, including secondary surgeries, septal perforation, or nasal tip deformities. Revision rhinoplasty is a technically complex procedure, and several studies have reported that an incidence of postoperative nasal abnormalities requiring correction ranging from 3.3% to 15.2% after primary rhinoplasty (1,2). A significant challenge in reconstructive surgery is the limited availability of septal cartilage for grafting (3). An ideal cartilage graft should be cost-effective, easily available at the donor site, and free from complications (4). Preserving cartilage in an optimal solution at appropriate temperatures offers a practical alternative to harvesting new grafts during revision surgeries (3). Clinical experience suggests that preserving cartilage harvested during primary septorhinoplasty is a simple and effective practical alternative to reharvesting new cartilage during subsequent procedures. Generally, cartilage harvested during primary rhinoplasty is returned to the nasal septum when sufficient tissue remains to serve as a structural graft or primary shield. However, when the remaining cartilage (such as from the nasal septum, alae, or dorsum) are small and insufficient for primary grafting, preservation becomes necessary (5).

Temperature and the preservation solution are crucial parameters that must be carefully controlled (6). The mechanical properties of cartilage are typically evaluated under various loading conditions, including compression, tension, and shear (7,8). Several studies have investigated optimal preservation environments, proposing different methods. One study advocates alcohol preservation as the most effective approach, whereas another recommends low-temperatures storage as a suitable alternative (5,6). It has been concluded that freezing does not negatively affect cartilage's mechanical properties and can serve as an alternative to fresh allografts, especially given its capacity to extend storage time (9). Conversely, another study reported that necrosis rates in alcohol-preserved cartilage (46.7%) were significantly lower than in other solutions, suggesting that the reduced necrosis and minimal metachromasia in alcohol could make it a more suitable preservation medium (3). Martino et al also reported that frozen cartilage exhibited better performance in tensile and compression tests compared to cartilage preserved in glycerol (10). Hanna et al concluded that frozen cartilage was a safe (11), convenient, and patient-centered option for transplantation in nasal surgery, further supporting the utility of frozen cartilage in clinical practice. According to previous studies, Hicks et al reported that the preservation of nasal septal cartilage at reduced temperatures in a normal saline solution enhanced the survival of chondrocytes (6). Graham et al examined the mechanical properties of nasal cartilage preserved in phosphate-buffered saline and decellularized at 23°C, comparing them with fresh samples (12).

Their mechanical testing revealed that the structural integrity of the preserved samples was maintained, with no significant differences observed between the preserved and fresh cartilage in terms of mechanical performance. Galicia et al discovered that cartilage preserved in alcohol exhibited varying degrees of nuclear and cytoplasmic changes after 30 days (13).

Gabori et al also noted that despite significant progress in cartilage tissue engineering (14), it is still not possible to replicate septal cartilage with the same molecular composition, mechanical properties, and volume retention after transplantation. As a result, autologous cartilage transplantation remains the preferred choice. Despite these findings, a comprehensive and practical comparison of different preservation methods remains lacking. In this study, we aimed to the compare the mechanical properties of nasal cartilage preserved in ethanol at 4 °C versus normal saline at -18 °C for potential reuse in nasal re-surgery.

Materials and Methods

After approval by the local ethics committee and obtaining written informed consent, in this experimental (laboratory-based) study, patients aged 18–35 years who presented to Godarz Hospital for rhinoplasty and had no history of prior nasal surgery, medication use affecting coagulation, or bleeding disorders were involved (during 2024-2025.). A total of 30 human nasal cartilage specimens were randomly allocated to one of three groups (n = 10 per group): (1) fresh controls (tested immediately after harvest), (2) stored in 70% ethanol at 4°C, or (3) frozen at –18°C. Both preservation groups were maintained under these conditions for 2 months. Randomization was performed using computer-

generated sequences (Random Allocation Software v2.0).

Sample size was determined a priori using G*Power (version 3.1), based on a two-sided α = 0.05, power = 90%, a standard deviation of 0.3 for tensile strength (derived from a prior study (15)), and a target mean difference of 0.5 between groups (effect size = 0.79), yielding a required sample size of 10 specimens per group. Exclusion criteria encompassed autoimmune diseases such as Wegener's granulomatosis, severe systemic illnesses like diabetes, and use of medications like aspirin or non-steroidal anti-inflammatory drugs (NSAIDs). This study evaluated and compared the mechanical properties of nasal cartilage preserved using two storage methods: (1) in alcohol-containing environment at 4 °C, and (2) in normal saline at -18 °C. Cartilage samples, excised during primary nasal surgeries and potentially reusable for revision procedures, were collected from eligible patients. After excision, cartilage deemed insufficient for primary grafting was rinsed in saline to blood. The perichondrium was carefully peeled off to prevent interference with testing. To standardized collection, all samples were obtained simultaneously, with collection dates recorded on each container.

Samples were divided into three groups:

- **Alcohol Storage Group:** Ten samples placed in 50 ml of 70% isopropyl alcohol, sealed, labeled, and stored at 4 °C for two months.
- **Saline Storage Group:** Ten samples placed in 50 ml of normal saline, sealed, labeled, and stored at -18 °C for two months.
- **Control Group:** Ten fresh samples stored temporarily at 5.37 °C in saline, tested immediately.

Throughout preservation, refrigerator and freezer temperatures were monitored daily, and solution volumes checked biweekly. Additional isopropyl alcohol was added under sterile conditions to maintain volume when needed. Cartilage pieces measured approximately 10–12 mm, cut into uniform 2 mm thick slices before testing to ensure consistency. Post-preservation, samples were transported to the Polymer and Petrochemical Laboratory in a cooled environment. Before mechanical testing, thawed samples stored in saline at -18 °C were brought to room temperature, with the evaluator blinded to preservation method. Each sample was positioned between tensile testing jaws with a 2 mm gap, and loaded until failure (Fig.1).

Fig. 1. Universal Tensile Testing Machine STM-20. When the load cell (attached to the upper clamp) registers tension the lower clamp moves downward.

The protocol for freezing and thawing was based on previous studies showing no impact on cartilage's dynamic mechanical properties (16-18). Force and displacement data were continuously recorded during tensile testing to generate engineering and true stress-strain curves and derive key mechanical parameters. Engineering stress was calculated by dividing the applied force by the initial cross-sectional area, while engineering strain was obtained by dividing displacement by the initial length. The stress-strain curve was plotted with strain on the horizontal axis and stress on the vertical axis, followed by the computation of the true stress-strain curve using established methodologies. These analyses provided values for ductility, tensile strength, Young's modulus, and fracture stress (19, 20):

- **Young's modulus:** The slope of the elastic region of the true stress-strain curve, expressed in Pascal's (Pa).
- **Failure stress:** The maximum stress sustained before rupture.
- **Ductility:** The area under the true stress-strain curve.
- **Tensile strength:** The maximum stress during the test.

All data were entered into SPSS version 25 for statistical analysis, which included descriptive statistics, ANOVA, and Tukey's multiple comparisons. Normality of data distribution was verified using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Significance was set at $P < 0.05$.

Key formulas used (19-22)

- Strain: $\varepsilon=\Delta L/L_0$ (where ΔL and L_0 represent the change in length and initial length, respectively).
- Stress: $\sigma=F/A_0$
- Ductility: Area under the true stress-strain curve
- Failure stress: Maximum stress recorded during testing

Results

The results showed a significant difference in mean tensile strength among the three groups (P=0.004). Post hoc analysis revealed that frozen group had a significantly higher tensile strength compared to the other groups, while there was no significant difference between the fresh and alcohol-preserved groups. The fracture stress showed no significant difference between the groups (P=0.318), with Frozen group exhibiting the highest value (4.79 MPa), followed by the alcohol group (4.32 MPa), and then the fresh group (3.51 MPa). Fig.2 represents the stress-strain curves for the respective groups. Additionally, there are significant difference in the mean ductility between the groups (P=0.003).

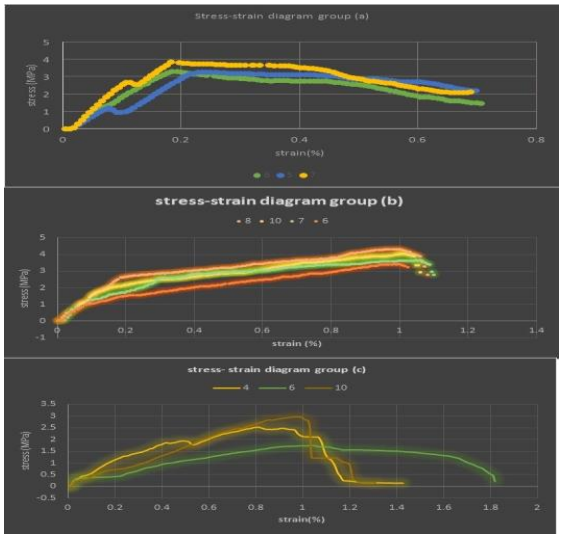

Fig. 2. Stress/strain curves related to the (a) frozen, (b) fresh, and (c) alcohol groups.Stress–strain curves for Group (a) frozen specimens. Strain (x-axis) versus stress (y-axis). Blue: specimen #5; green: specimen #6; yellow: specimen #7. Stress–strain curves for Group (b) specimens. Strain (x-axis) versus stress (y-axis). Brown: specimen #6; yellow: specimen #10; orange: specimen #8; green: specimen #7. Stress–strain curves for Group (c) specimens. Strain (x-axis) versus stress (y-axis). Green: specimen #6; brown: specimen #10; orange: specimen #4.

The frozen group had the highest mean ductility (2.51 %), followed by the alcohol and fresh groups, with no significant difference between the latter two. The compared the results also indicate a significant difference in Young's modulus among the groups (P=0.001) fresh group the highest mean value (20.67 MPa), followed by the frozen group (20،37 MPa), and then the alcohol group (12.58 MPa). The did not have significant the Post hoc testing showed that the alcohol group was significantly different from both the frozen and fresh groups, while there was no significant difference between the frozen and the fresh groups. Finally, the study found a significant difference in yield stress among the groups (P = 0.001).

Discussion

This study aimed to compare the mechanical properties of nasal cartilage preserved in ethanol at 4°C versus normal saline at -18°C for potential reuse in nasal re-surgery. The results indicated that the tensile strength, Young's modulus, yield stress, and ductility of cartilage stored in normal saline at -18°C were significantly different from those stored in 70% alcohol at 4°C. These findings suggest that cartilage is better preserved with decreasing temperature than in 70% alcohol, which aligns with the results presented by Maha Ead et al. (9). In their study of preserved and fresh cartilage samples, they concluded that freezing did not negatively affect the mechanical properties of the samples and that fresh samples exhibited lower mechanical properties than the control samples. As demonstrated in the present study, the tensile properties of cartilage stored in normal saline at -18°C were not significantly different from those of fresh cartilage, but they were slightly higher and better preserved. The results of Santin et al also support these findings (23), as they reported that long-term frozen cartilage, in addition to maintaining its mechanical properties, showed enhanced properties compared to fresh cartilage. On the other hand, Wan et al concluded in a study on frozen cartilage used in rhinoplasty that frozen cartilage is a promising option for nasal surgery (24). It is biocompatible compared to synthetic implants and helps prevent donor site complications, which are often associated with autologous cartilage. The results of the present study also demonstrated that frozen cartilage retained superior mechanical properties. Raja

mohan et al (25), in their study on the role of frozen cartilage in revision rhinoplasty, found that frozen cartilage allografts were an emerging and reliable source of cartilage for revision procedures, offering a lower complication rate. Similarly, kadhum et al reviewed 544 patients undergoing revision rhinoplasty and concluded that frozen cartilage could be a safe and reliable alternative to autologous rib grafts. In another review (26), Wan Wiseman et al conducted a long-term study comparing the outcomes of autologous rib grafts and frozen cartilage grafts in revision rhinoplasty among 50 patients (27). They concluded that frozen cartilage was a useful, safe, and reliable source of cartilage for reconstructive and cosmetic rhinoplasty when compared with autologous rib grafts. Milkovich et al also emphasized that the use of frozen rib cartilage can prevent donor site complications, reduce operative time (28), and maintain a low complication rate, making it a suitable alternative to autologous rib cartilage in complex primary or secondary rhinoplasty cases with insufficient autologous nasal cartilage. The present study supports the findings of the aforementioned studies, demonstrating that frozen cartilage retains its mechanical properties better and does not significantly differ from fresh cartilage. In addition to the benefits mentioned in previous studies, such as lower complication rates, reduced surgical time, and lower patient costs, our study also highlights that the mechanical properties of cartilage are better preserved when frozen. Ruderman et al in their study on rabbit cartilage (29), concluded that cartilage removed from the septum during the initial rhinoplasty surgery, if well-preserved, can be as useful for transplantation as freshly harvested cartilage during a second surgery, with no significant differences. This conclusion aligns with the present study, as we also found no significant difference between frozen and fresh cartilage. However, the present study findings suggested that the preservation method is crucial in maintaining mechanical properties. The results showed that cartilage preserved in an alcohol-containing environment exhibited significantly lower mechanical properties compared to the other groups. In another study by Salzano et al (30), it was concluded that frozen cartilage in nasal revision surgery was a suitable alternative to autologous rib grafts and irradiated homologous rib grafts. The present study similarly found that although frozen cartilage did not differ significantly in mechanical properties compared to fresh samples, it showed higher tensile strength and ductility. As Martino's and Hannah's studies(26), The findings of the present study showed that frozen cartilage outperformed samples preserved in alcohol and demonstrated slightly better properties than fresh samples in factors such as Young's modulus and ductility (27). Our study is also at the same side of Hicks study and indicated that mechanical factors increased with decreasing temperature (6), and while the elastic properties of frozen cartilage were not significantly different from fresh samples, they were nonetheless at a higher level. In this study, cartilage preserved in alcohol showed the greatest mechanical changes, with lower values for tensile strength, ductility, fracture stress, and Young's modulus compared to both frozen and fresh groups. The alcohol-preserved samples underwent more negative changes in their mechanical properties within the two-month period that it is at the same side whit Galicia et al discovered. In this study, no significant difference was observed in the mechanical properties between fresh and frozen cartilages (10), which suggest that frozen cartilages, like decellularized samples, undergo minimal mechanical changes as Graham et al Sayed in their study(12,31). Khorasani et al noted that allograft cartilage had the advantage of preventing scarring at the donor site, while offering the same benefits as autologous costal cartilage with a comparable complication rate. Therefore, it can serve as a reliable alternative material for nasal surgeries. The present study further supports this idea, showing that frozen cartilage is slightly superior in a few variables compared to fresh cartilage, although no significant differences were found. This indicates that frozen cartilages can be a suitable alternative as homo grafts in nasal surgeries in terms of their mechanical properties. Additionally, a study by Toussaint et al yielded that the use of allografts did not differ in outcome from autografts (32), and in patients with more extensive cartilage defects, surgeons may prefer allogeneic cartilage grafting to reduce operative time and avoid additional complications and scarring. Our findings align with this, showing that frozen and fresh samples exhibited no significant differences in mechanical properties,

with the properties of the frozen cartilage trending slightly higher due to the decreased ambient temperature. Vargas et al also highlighted the proven usefulness of cartilage allografts in improving the aesthetic outcomes of patients (33). The present study, in conjunction with this research, demonstrated the potential for cartilage banking, which could reduce the need for additional incisions and scars in patients. Furthermore, Armen et al emphasized that the use of allogeneic nasal septal cartilage in rhinoplasty could offers stable (34), positive functional and aesthetic results while avoiding complications and additional surgeries, making it a safe and viable alternative to autologous cartilage. Therefore, maintaining the mechanical properties of cartilage in an optimal condition is essential for successful transplantation, whether as autografts or allografts, leading to favorable surgical outcomes. The results of the present study suggested that freezing preserves the mechanical properties of cartilage more effectively than other methods. This preservation method enables cartilage to be stored in a cartilage bank, making it available for autografts in surgeries requiring immediate reconstruction or as allografts for use in primary or secondary surgeries. However, the findings of this study contrast with those of Wong et al (5), who stored nasal cartilages in alcoholic solutions for two years and concluded that the best storage method was a 70% alcohol environment. This discrepancy could be due to the longer storage duration in their study compared to the present study. Sari et al also found that cartilage preserved in alcohol exhibited less necrosis (3), likely because ethanol, as an organic solvent, can help preserve the three-dimensional structure of cartilage and prevents degradation. On the other hand, Hex et al proposed freezing as an alternative to storing cartilage in saline or other environments, which aligns with the findings of the present study (6). Our results uncovered a significant difference between the frozen preservation method and the alcohol-based method. In a case report, Roerich et al used fresh frozen cartilage grafts for correcting nasal deformities (35), such as retracted columella in revision rhinoplasty. They highlighted that using fresh frozen cadaver cartilage grafts could reduce donor site complications associated with autologous cartilage harvesting and deemed them a promising source of graft material. The present study also showed no significant difference between frozen and fresh cartilage, further supporting the notion that frozen cartilage can reduce the need for autologous harvesting and prevent associated donor site complications. In contrast, Agir Hakan et al examined the simultaneous implantation of alcohol-preserved cartilage grafts and fresh autologous grafts in a patient undergoing rhinoplasty surgery (36). They concluded that alcohol-preserved autologous cartilage may fail as a graft material after prolonged storage, suggesting that isopropyl alcohol is not an optimal solution for cartilage preservation. According to our findings, the mechanical properties of cartilages preserved in alcohol were more significantly altered compared to the other two groups, exhibiting lower levels of mechanical performance. Russell et al (37), in a study on rib cartilage homo grafts preserved in normal saline and irradiated, found that irradiated homo grafts were quite stable and generally retained their structural and support properties. They suggested irradiated homo grafts as an alternative to rib cartilage autografts or even as primary graft material. Similarly, Drake et al concluded in a retrospective study that irradiated rib cartilage did not differ from autografts in terms of efficacy and safety (38).

Hilary et al also found that irradiated homologous costal cartilage grafts were as safe and effective as autologous cartilage in rhinoplasty (39), offering the advantage of availability and eliminating donor site complications. In line with these studies, the present study showed that frozen cartilages did not differ significantly in mechanical properties from fresh cartilage, suggesting that cartilages removed from individuals can be stored as autografts for the same individual or as homo grafts for other individuals. However, Marcia et al conducted a study on three groups of rabbit cartilages and concluded that fresh autograft cartilages were better preserved in terms of chondrocyte viability compared to two groups of homo grafts stored in alcohol (40), both crushed and uncrushed. As in the present study, fresh cartilages retained their mechanical properties significantly better than those preserved in alcohol. A case report by Sancho et al on the use of auto grafts and homo grafts demonstrated the long-term survival of homologous cartilage grafts in the nose (41). They found that there was

no tissue degradation in homo grafts compared to autologous cartilage, and they recommended septal cartilage as the best choice for nasal reconstruction, ahead of ear or rib cartilage. They also emphasized that when cartilage transplantation is necessary and sufficient autologous septal cartilage is not available, homologous septal cartilage is a valuable alternative. Consistent with these findings, the present study revealed no significant difference in mechanical properties between fresh and frozen cartilages. This suggests that frozen cartilages can be used as substitutes for autologous rib or ear cartilages in cases requiring immediate cartilage regeneration. On the other hand, Caffrey et al who compared human cartilage with engineered cartilage, concluded that the mechanical properties of human cartilage were superior (42). Based on the findings of the present study and Gabori et al study(14), since there was no significant difference between frozen and fresh samples, storing individual cartilages in a frozen state can serve as an alternative to rib cartilage or engineered cartilages for nasal re-surgeries that require cartilage transplantation. As previously mentioned, the results of previous studies have indicated that the preservation method plays a crucial role in maintaining the mechanical properties of nasal cartilage. Yang et al demonstrated that storing cartilage in a humid environment (43), such as normal saline at low temperatures, can help preserve both its biological and mechanical properties. The present study also highlighted the positive effect of storage in normal saline on tensile strength and Young's modulus. However, no significant difference was observed in fracture stress between the groups. Lan et al found that the fracture stress of human nasal cartilage was not significantly influenced by different disinfection and storage methods (44), suggesting that this parameter is less affected by storage conditions. Overall, the results of this study suggest that frozen storage of nasal cartilage is more effective in preserving its mechanical properties for reuse in rhinoplasty. However, to establish the optimal storage protocol, further studies are needed to evaluate other important factors, such as storage duration, solution concentration, histological changes in the tissue, and biological characteristics. The increase in ductility and yield stress observed in frozen group may be attributed to better preservation of the collagen and proteoglycan structure of the cartilage. Conversely, the increase in tensile strength and Young's modulus in fresh group may be related to water penetration into the cartilage and enhanced hydration. Cartilage hydration is known to significantly influence its strength and stiffness (45). This study adopts an innovative approach, as no similar research has been conducted domestically or internationally. Based on the findings, it is recommended to prioritize frozen cartilage preservation over traditional serum and alcohol storage methods. This shift can enhance surgical outcomes and reduce patient costs. Furthermore, in cases requiring cartilage transplantation, frozen homo grafts present a viable alternative to harvesting cartilage from the patient's rib, hence minimizing costs, surgery time, and complications associated with rib cartilage transplantation in nasal revision procedures.

Key strength of the study

A key strength of this study is its clear demonstration-under controlled experimental conditions-that frozen nasal cartilage allografts retain tensile properties comparable to fresh (autologous-like) cartilage, with no statistically significant difference observed. Moreover, frozen grafts performed significantly better than those preserved in 70% ethanol, supporting low-temperature storage as a superior preservation method over alcohol-based techniques. These findings provide practical, evidence-based guidance for tissue banking in reconstructive rhinoplasty.

Limitations

Limitations included the inability to perform compressive testing due to equipment constraints, which precluded a full biomechanical profile. Additionally, the study was limited to short-term tensile assessment and did not evaluate histological structure, cellular viability, or long-term in vivo behavior (e.g., resorption or integration). Future work should address these factors to confirm the clinical reliability of frozen allografts beyond mechanical equivalence in tension.

Conclusion

This study indicates that storing cartilage in normal saline at -18 °C better preserve its

mechanical properties, including tensile strength, Young's modulus (elasticity), and ductility, than storage in alcohol at 4 °C. Additionally, cartilage stored frozen tends to retain these properties slightly better than in fresh cartilage, though the differences were not statistically significant. Collectively, these findings suggest that lowering storage temperatures in a normal saline solution enhances chondrocyte survival and tissue integrity. The data support the broader view that frozen cartilage allografts can serve as a safe, effective, and readily available alternative to autologous rib or septal cartilage, reducing donor-site morbidity, shortening operative time, and lowering patient costs. The mechanical equivalence and in some cases the superiority of frozen cartilage to fresh tissue highlights the potential for establishing cartilage banks for clinical use. In contrast to some reports that advocate alcohol preservation, the present results do not support that approach under the conditions tested; differences in storage duration and conditions likely underlie the discrepancy. Overall, these findings favor shifting from traditional alcohol storage toward low-temperatures saline storage as a more effective strategy for preserving nasal cartilage for surgical reuse. Future work should optimize storage protocols by evaluating long-term effects, histological integrity, and post-transplantation biological behavior to further validate this approach in clinical practice.

"Compliance with Ethical Standards"

*Authors have no conflicts of interest. *Study protocol was in accordance with the latest Declaration of Helsinki for medical research involving human subjects and was approved by the local ethics committee. *This article does not contain any studies with animals performed by any of the authors. *Informed consent was obtained from all participants of the study.

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