Long-term Evaluation of the Effect of Platelet-rich Fibrin on Cartilage Tissue Regeneration: An Animal Model Study

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Introduction: Autologous cartilage graft in rhinoplasty is associated with common challenges, such as resorption, inflammation, and bone metaplasia. This study aimed to assess platelet-rich fibrin's long-term effect, as a superphysiological source of biomolecules, on cartilage regeneration in an animal model. **Material and Methods:** Cartilage and fascia were excised autologously from the ear and leg of thirty-three Wistar rats. They then transplanted with cartilage alone (control group), cartilage wrapped with fascia (fascia group), and cartilage wrapped with PRF (fibrin group) autologously and subcutaneously. **Result:** The histological results demonstrated no significant difference in chondrocyte viability, fibrosis, and resorption between three groups after six months. However, other parameters, including calcification, bone metaplasia, infection, and basophilia, were lower in the fibrin group. **Conclusion:** This study concluded that PRF could be an appropriate autologous cell source for cartilage regeneration and nasal augmentation in the long term.

Keywords: Cartilage; Regeneration; PRF ; Rhinoplasty

Introduction

In rhinoplasty surgery, cartilage tissue is used for augmentation, smoothening, or camouflaging of the nasal dorsum, obtained from various tissue sources (1). Cartilage tissue contains few cells with minimal mitotic activity and without vascular structures; therefore, cartilage regeneration poses challenges that secondary resorption is common after transplantation (1, 2). Despite these challenges, the current gold standard graft used in rhinoplasty is autologous cartilage grafts, preferably taken from the individual auricular, septal or costal cartilage because of its high acceptance rate, high durability, low immunogenic response, extrusion rates, and low infection rate compared to allograft or alloplastic options. Significant limitations of the use of this graft are low tissue volume, donor site pain, ossification in the elderly, and visible scarring at donor sites (3, 4).

Given the disadvantages above, researchers evaluated new approaches in which cartilage grafts are used and supportive tissue/cells, such as oxidized regenerated cellulose, Poly Lactic-co-Glycolic Acid (PLGA) membrane, fascia, and perichondrium (5-8). Several advantages have been reported for the simultaneous use of cartilage in the fascia, including lack of rejection, availability, and minimal resorption. In contrast, they have several disadvantages, including undesired cartilage growth, shrinkage of the fascia, lack of functional correction or structural support, and inability to be used in large amounts (9). Besides, the additional surgical incision is required that leads to increased operating time, cost, and also the risk of infection (10).

Platelet-rich fibrin (PRF), first described by Choukroun *et al.,* in 2000 and it is a second-generation platelet concentrate containing a dense fibrin bioscaffold and a natural source from integrated growth factor and cytokines. This concentrate can promote regeneration and repairing tissues, such as bone, skin, eardrum perforation, and an anti-inflammatory response (11-14).

Previous studies evaluated the safety and efficiency of cartilage with wrapped PRF. These studies showed that PRF increases chondrocytes viability compared with cartilage alone or with oxidized regenerated cellulose and Alloderm graft (5,

15). There is controversy in these studies results about the role of PRF in inflammatory responses and fibrosis. Guler *et al.,* reported that PRF transplantation with unwrapped cartilage to subcutaneous rabbit reduces inflammation and fibrosis after ten weeks (16). They showed no significant differences in the rate of inflammation, fibrosis, and vascularization when PRF was used with cartilage compared with other groups after two months from the transplantation in the subcutaneous rabbit (4).

Due to the disagreements mentioned about PRF effect on cartilage regeneration in the long term, we decided to investigate the effect of PRF on viability and biological behaviour of cartilage tissue in rat animal models at six months. Three groups were considered to achieve this aim, including cartilage graft alone (control group), cartilage wrapped with fascia (fascia group), and cartilage wrapped with PRF (fibrin group).

Materials and Methods

Study design

The present study was approved by the Ethics Committee (Approval code: IR.SBMU.MSP.REC.1396.123) of the Shahid Beheshti University of Medical Sciences. In this study, 33 normal male Wistar rats (weighing 240-280 grams) at eight weeks of age were purchased from the Experimental Animal Center of Shahid Beheshti University, Thran, Iran. They also kept in cages in 12 hours light/12 hours darkness at 18oC and 50-70% humidity, according to the Principles of Laboratory Animal Care recommended by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals.

Three groups of graft were designed in each ectopic rat model, including cartilage graft wrapped with PRF in a 1:1 ratio (n=33, Fibrin group), cartilage graft wrapped in the fascia in a 1:1 ratio (n=33, Fascia group), or cartilage alone (n=33, control group). After six months, chondrocyte viability, resorption, bone metaplasia, and immunological response were evaluated through histological analysis. This study design showed in Figure 1.

Preparation of PRF

PRF was prepared according to the protocol described by Choukroun *et al.,* (17). Briefly, 2ml of the whole blood sample was taken from the rat tail and collected in a sterile tube without any anticoagulant reagent. After 10 min of centrifugation at 3000 rpm, the middle PRF layer was separated by tweezers. The fibrin was pressed between two sterile microscope slides to obtain a fibrin membrane to remove serum and red blood cells.

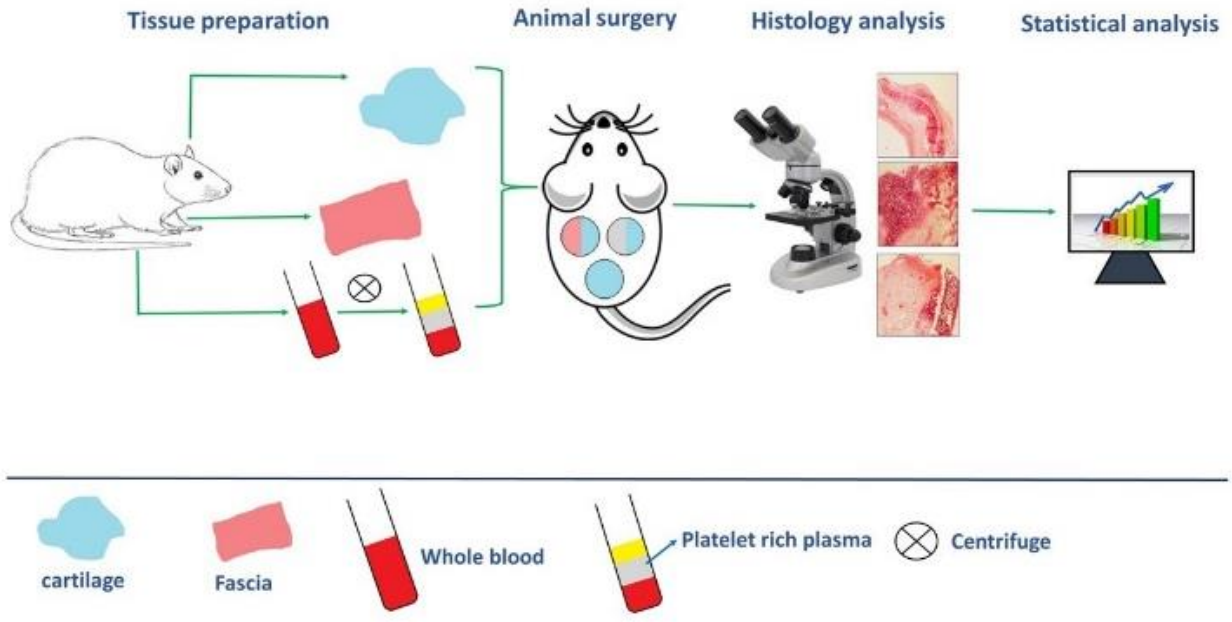

Figure 1. Graphic abstract from all study design

Preparation of cartilage and Fascia graft

The rats were anaesthetized through an intramuscular injection from 35 mg/kg ketamine hydrochloride (Ketavett, Bayer, and Leverkusen, Germany) and 5 mg/kg xylazine hydrochloride (Bayer, Leverkusen, Germany). The rats' right ear and the posterior leg were shaved and cleaned with povidone-iodine for isolation of autologous cartilage and fascia, respectively. An incision (approximately 1- 2 cm) was made on the posterior ear side to harvest a composite cartilage-perichondrium. Finally, these incisions were closed with 4-0 silk sutures.

Surgical procedure

Three subcutaneous pockets were created on the backbone of each rat using blunt dissection. The samples, i.e., fibrin, fascia, and control, were implanted autologously in each pocket. Then the incisions were closed with 4-0 silk suture. After six months, the rats were sacrificed using CO2 inhalation, and death was confirmed by cervical dislocation. All samples were then excised carefully to avoid obtaining subcutaneous skin tissue with the grafts for histologic analysis.

Histological analysis

The samples were fixed in 4% paraformaldehyde and dehydrated in a graded series of ethanol (50-100%) and xylol (Sigma-Aldrich, St. Louis, Missouri, USA). These samples were immersed in paraffin (Sigma-Aldrich, St. Louis, Missouri, USA), and the 5µM sections were obtained with a microtome (DID SABA CO, Tehran, Iran). These sections were then fixed, deparaffinized, and rehydrated using xylene/ethanol and a graded ethanol series, respectively. Finally, staining was performed by Hematoxylin (H130, BD Biosciences, and San Jose, CA, United States) & Eosin (H&E) (E12169, e Biosciences, San Diego, CA, USA) stain.

Then, the specimens were evaluated by an experienced pathologist, who was not aware of the group allocations. The number of lacunae with nuclear chondrocyte to the total number of lacunae was calculated and expressed as a percentage of the chondrocyte viability. Bone metaplasia and classification were measured to assess undesirable differentiation into bone tissue. The extracellular matrix change was evaluated in terms of fibrosis, infection, basophilia, and resorption. These parameters were then scored based on the previous studies for each sample (4, 16, 18) (Table 1).

Table 1. Percentage of observation of histological parameters and their score in each microscopic field

Percentage of observation	score
0	0 or none
1 to 25	1^+ or minimal
26 to 50	2^+or moderate
51 to 75	3^+ or moderate to severe
76 to 100	4^+ or severe

Statistical analysis

Results of the quantitative variables were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by Tukey test, was used to compare multiple sample means. All statistical analyses were performed by the statistical software IBM SPSS Statistics for Windows version 21.0 (IBM Corp. 2012. Armonk, NY: IBM Corp.). *P*-values <0.05 were considered statistically significant.

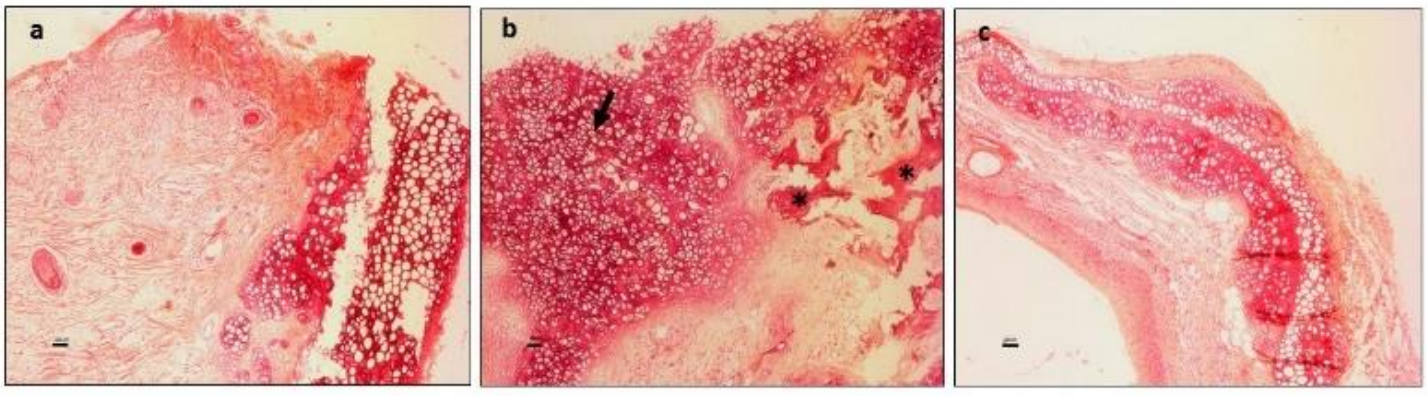

Figure 2. Hematoxylin and eosin stain (original magnification: ×400) show chondrocyte cell viability and cartilage regeneration. a) Cartilage graft alone, as control groups, b) Fascia-cartilage grafts, as fascia group and c) PRF-cartilage graft, as fibrin group.

Results

After six months, the grafts were assessed using H&E staining (Figure 2). Results of chondrocyte viability showed that there were no significant differences between control (37.31%±22.50), fascia (38.62%±21.502), and Fibrin groups (26.20%±13.38) (Figure 3).

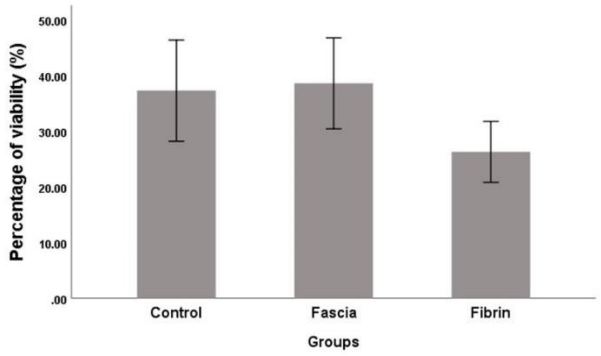

Figure 3. Graph showing the percentage of cartilage viability in the groups. Error bars are standard division (N=33), $p \leq 0.05$ comparison between groups. Error bars are standard division (N=33).

Moreover, this study demonstrated no evidence of ectopic bone formation, such as calcification and bone metaplasia in the fibrin group. The calcification rate was similar in the fascia and control groups (0.32±0.65 and 0.62±1.87, respectively). It can be noted that bone metaplasia was observed only in the fascia group (0.79±1.29) (Figure 4). The mean score of infection and basophilia in the fibrin group (0.76±0.43 and 1.36±0.70, respectively) were significantly lower compared to other groups (control: 1.23±0.42 and 2.15±0.73, fascia: 1.56±0.81 and 2.31±0.66, respectively). However, no significant difference was observed between control and fascia groups (Figure 5). Also, the score of fibrosis and resorption was lower in the fibrin group (2.00±0.40, 0.31±0.47, respectively) than control (2.23±0.65 and 0.72±0.22, respectively) and fascia (2.00±0.40 and 0.31±0.47, respectively) groups. This difference was not statistically significant between all groups (Figure 5). The P-value of histologic parameters was reported in Table 2.

Discussion

Tissue reconstruction is a complex process that needs cell participation and additional growth factors (19-21). However, these processes take a long time in cartilage tissue because it lacks blood vessels to obtain growth factors and nutrition from the surrounding environment (21). Therefore, the present study aimed to evaluate the effect of PRF, as an excellent source out of many kinds of growth factors and cytokines, in cartilage regeneration in rhinoplasty surgery for long-term by an animal model. In this regard, autologous cartilage with PRF was implanted in the rat animal models and then histological results compared with conventional treatment, including autologous cartilage with and without fascia.

Autologous cartilage graft is a standard gold technique for rhinoplasty surgeries to form a hyaline-like tissue with mechanical and histological properties similar to the native tissue (22).

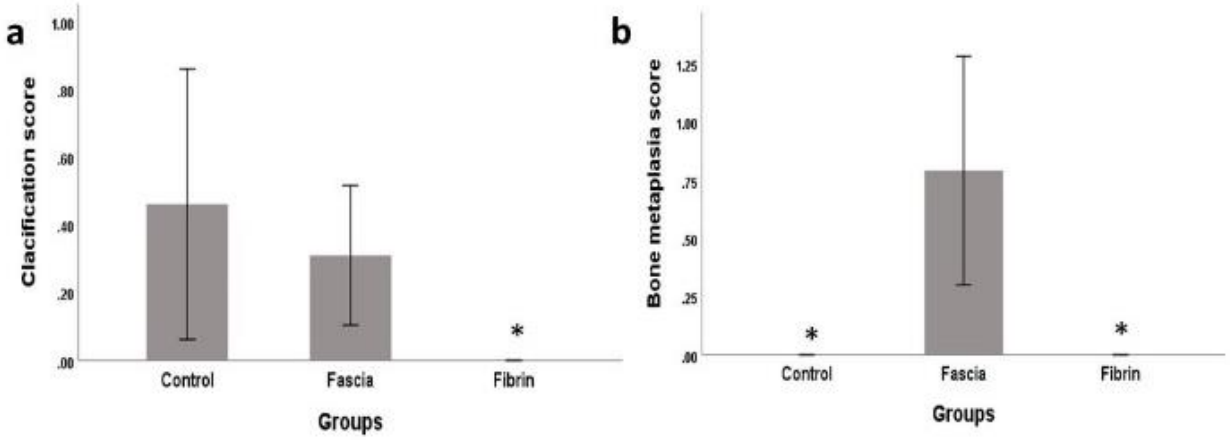

Figure 4. Graph showing the score of calcification score (a) and Bone metaplasia sore (b) in the groups. * $P \leq 0.05$ comparison between groups. Error bars are standard division (N=33).

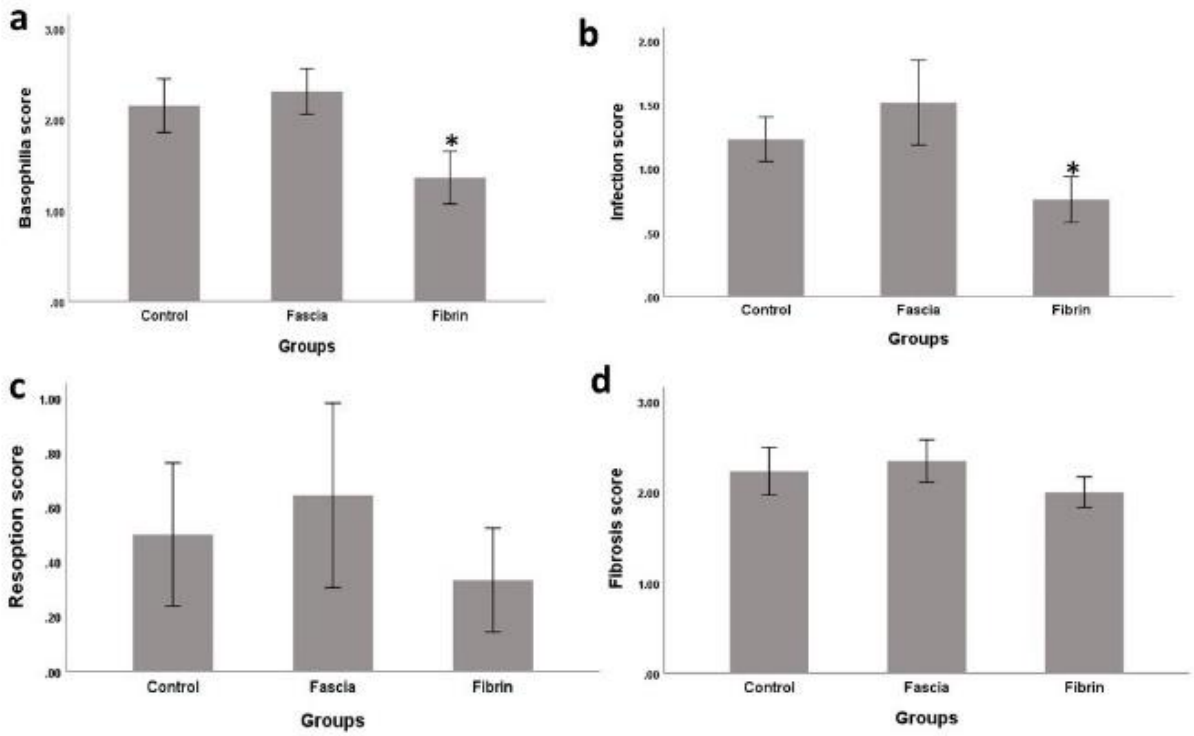

Figure 5. Graph showing the score of basophilia (a), infection (b), resorption (c), and fibrosis (d) in the groups. * $P \leq 0.05$ comparison between groups. Error bars are standard division (N=33).

Several studies have demonstrated several critical challenges: lack of required cartilage volume, long-term survival, and increased graft resorption after the surgery. Hence, using cartilage grafts and supporting materials, for example, autogenous soft tissue grafts (e.g., fascia and dermis), various stem cells and growth factors (7, 16, 23-25) were introduced to solve these challenges. As a cellular supporter and cartilage grafts, the fascia was widely applied to increase cartilage viability and prevent resorption in several studies (2, 16, 26). However, this approach was associated with critical disadvantages, such as a decrease in graft volume over time, expensive, time-consuming, and an increased risk of infection, donor site morbidity (16).

On the other hand, researchers' attention has been drawn to "minimally manipulated" approaches considered by the United States Food and Drug Administration (FDA). According to the FDA regulation in 21 CFR part 1271, all cellular/tissue products used without altering their biological structure and autologous are considered this approach. They do not require FDA approval for clinical application (27).

Table 2. The results of statistical assessment for histological parameters

Histologic examination	Control versus RRF	Fascia versus RRF
Cartilage viability (%)	0.132	0.071
Calcification	0.038	0.203
Bone metaplasia	1.000	0.002*
Infection	0.033*	0.000*
Basophilia	0.001*	0.000*
Resorption	0.679	0.255
Fibrosis	0.359	0.094

Recently, PFR is introduced as a biological tool that releases a superphysiological source of cytokines and growth factors in various medical fields, such as implantology, orthopaedics, cosmetic and oral-maxillofacial surgery. Several studies demonstrated that PRF-derived growth factors, such as bFGF, TGF-β1, and PDGF-BB, can increase the proliferation and differentiation potential of chondrocytes *in vitro* and *in vivo* (14, 28).

However, the present study showed that chondrocyte viability was not significantly different in the fibrin group than the fascia and control groups after six months. Gular *et al.,* revealed that the PFR matrix could improve the cartilage viability of diced cartilage grafts in the subcutaneous rabbit model after ten weeks (16). Another study showed that PRF increased cartilage viability in autologous cartilage grafts compared to oxidized regenerated cellulose grafts after eight weeks (5). This result corresponds with an *in vitro* study to evaluate the chemotactic effects of PRF on rabbit chondrocytes. The study provided evidence that PRF improved viability, proliferation, and chemotaxis of the cultured chondrocytes and increased gene expression of the chondrogenic markers, including type II collagen and aggrecan. Combining PRF and autologous cartilage graft repaired articular chondral defects led to the fast release growth factors in the rabbit animal model after three months (22). Given that the amount of absorption between the groups did not make a significant difference in our study. It can be deduced that PRF keeps cells viable and also prevents future resorption in the long term.

On the other side, our results demonstrated no significant difference between groups in terms of fibrosis scores. Also, the score infection and inflammation were significantly lower in the fibrin group than the cartilage and fascia groups. According to other studies, these results showed that PRF could decrease inflammation and fibrosis and prevent infection when implanted with autologous cartilage graft compared to Alloderm, Surgical, fascia tissues (16, 25). Nevertheless, Goral *et al.,* reported no difference in inflammation, fibrosis, or vascularization between Fibrin, Fascia, and control groups in a histopathologic examination after two months (4).

Considering these studies, we suggested that one of these discrepancies' fundamental causes could be follow-up time. Thus, we evaluated cartilage tissue's biological behavior for six months versus one to three months in other studies. Moreover, other causes include sample size, the PRF preparation methods' differences, and the studied population characteristics (rats vs rabbits).

Therefore, the present results indicated that RPF is a suitable cell product for cartilage regeneration due to having a low level of side effects, including inflammation, bone metaplasia, and infection risk, in the long term. However, this graft should be investigated in future studies in terms of other biological and mechanical properties, such as collagen distribution with Masson's trichrome staining and Young's modulus. Also, these results were evaluated in the clinical trial study.

Conclusion

The present study showed that PRF could be an appropriate autologous cell source for cartilage regeneration and nasal augmentation in the long term. It is also necessary to evaluate PRF effect in clinical studies with a larger sample size to reconstruct cartilage.

Acknowledgement

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Conflict of Interest: 'None declared'.

References

1. Kreutzer C, Hoehne J, Gubisch W, Rezaeian F, Haack S. Free diced cartilage: a new application of diced cartilage grafts in primary and secondary rhinoplasty. Plast Reconstr Surg. 2017;140(3):461-70.
2. Brenner KA, McConnell MP, Evans GR, Calvert JW. Survival of diced cartilage grafts: an experimental study. Plast Reconstr Surg. 2006;117(1):105-15.
3. Gordon CR, Alghoul M, Goldberg JS, Habal MB, Papay F. Diced cartilage grafts wrapped in AlloDerm for dorsal nasal augmentation. J Craniofac Surg. 2011;22(4):1196-9.
4. Göral A, Aslan C, Bolat Küçükzeybek B, Işık D, Hoşnuter M, Durgun M. Platelet-rich fibrin improves the viability of diced cartilage grafts in a rabbit model. Aesthetic Surg J. 2016;36(4):NP153-NP62.
5. Kazikdas KC, Ergur B, Tugyan K, Guneli E, Kaya D, Sahan M. Viability of crushed and diced cartilage grafts wrapped in oxidized regenerated cellulose and esterified hyaluronic acid: an experimental study. Laryngoscope. 2007;117(10):1728-34.
6. Firat C, Gurlek A, Aydn NE. Viability of cartilage grafts in various forms. J Craniofac Surg. 2011;22(5):1666-70.
7. Kemaloğlu CA, Tekin Y. A comparison of diced cartilage grafts wrapped in perichondrium versus fascia. Aesthetic Plast Surg. 2014;38(6):1164-8.
8. Liao J-L, Chen J, He B, Chen Y, Xu J-Q, Xie H-J, *et al.,* Viability and biomechanics of diced cartilage blended with platelet-rich

plasma and wrapped with poly (lactic-co-glycolic) acid membrane. J Craniofac Surg. 2017;28(6):1418-24.
9. Singh S, Sood T, Sabarigirish K, Ramakrishnan N, Rajguru R, Kumar KM. A New Era of diced cartilage rhinoplasty: Our experience. INDIAN J OTOLARYNGOL. 2015;67(4):338-40.
10. Tasman A-J. Advances in nasal dorsal augmentation with diced cartilage. Curr Opin Otolaryngol Head Neck Surg. 2013;21(4):365-71.
11. Choukroun J, Diss A, Simonpieri A, Girard M-O, Schoeffler C, Dohan SL, *et al.,* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2006;101(3):e56-e60.
12. Habesoglu M, Oysu C, Sahin S, Sahin-Yilmaz A, Korkmaz D, Tosun A, *et al.,* Platelet-rich fibrin plays a role on healing of acute-traumatic ear drum perforation. J Craniofac Surg. 2014;25(6):2056-8.
13. Bastami F, Khojasteh A. Use of leukocyte-and platelet-rich fibrin for bone regeneration: a systematic review. Regeneration, Reconstruction & Restoration. 2016;1(2):47-68.
14. Abd El Raouf M, Wang X, Miusi S, Chai J, Mohamed AbdEl-Aal AB, Nefissa Helmy MM, *et al.,* Injectable-platelet rich fibrin using the low speed centrifugation concept improves cartilage regeneration when compared to platelet-rich plasma. Platelets. 2019;30(2):213-21.
15. Tarhan E, Cakmak O, Ozdemir BH, Akdogan V, Suren D. Comparison of AlloDerm, fat, fascia, cartilage, and dermal grafts in rabbits. Archives of Facial Plastic Surgery. 2008;10(3):187-93.
16. Güler İ, Billur D, Aydin S, Kocatürk S. Efficacy of platelet-rich fibrin matrix on viability of diced cartilage grafts in a rabbit model. Laryngoscope. 2015;125(3):E104-E11.
17. Saluja H, Dehane V, Mahindra U. Platelet-Rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. Ann Maxillofac Surg. 2011;1(1):53.
18. Manafi A. Effects of platelet-rich plasma on cartilage grafts in rabbits as an animal model. WJPS. 2012;1(2):91.
19. Liu Q, Huang Y, Lan Y, Zuo Q, Li C, Zhang Y, *et al.,* Acceleration of skin regeneration in full-thickness burns by incorporation of bFGF-loaded alginate microspheres into a CMCS–PVA hydrogel. J TISSUE ENG REGEN M. 2017;11(5):1562-73.
20. Mehendale HM. Role of Tissue Repair and Death Proteins in Liver Injury. Drug-Induced Liver Disease: Elsevier; 2013. p. 195-213.
21. Xu F, Yang Y, Yang T, Dai T, Shao X, Xu H, *et al.,* The use of allogenic adipose-derived stem cells in combination with platelet-rich fibrin for the treatment of cartilage defects in rabbit ear. Am J Transl Res. 2018;10(6):1900.
22. Wong C-C, Chen C-H, Chan WP, Chiu L-H, Ho W-P, Hsieh F-J, *et al.,* Single-stage cartilage repair using platelet-rich fibrin scaffolds with autologous cartilaginous grafts. Am J Sports Med. 2017;45(13):3128-42.
23. Bahmanpour S, Ghasemi M, Sadeghi-Naini M, Kashani IR. Effects of platelet-rich plasma & platelet-rich fibrin with and without stromal cell-derived factor-1 on repairing full-thickness cartilage defects in knees of rabbits. IJMS. 2016;41(6):507.
24. Kemaloğlu CA, Tekin Y. The effect of sildenafil on fascia-wrapped diced cartilage grafts. Laryngoscope. 2015;125(5):E168-E72.
25. Topkara A, Özkan A, Özcan RH, Öksüz M, Akbulut M. Effect of concentrated growth factor on survival of diced cartilage graft. Aesthetic Surg J. 2016;36(10):1176-87.
26. Dubay DA, Wang X, Kirk S, Adamson B, Robson MC, Franz MG. Fascial fibroblast kinetic activity is increased during abdominal wall repair compared to dermal fibroblasts. WOUND REPAIR REGEN. 2004;12(5):539-45.
27. Food, Administration D. Regulatory considerations for human cells, tissues, and cellular and tissue-based products: Minimal manipulation and homologous use; guidance for industry and food and drug administration staff; availability. FDA. 2017;82(221):54290-2.
28. Brandl A, Angele P, Roll C, Prantl L, Kujat R, Kinner B. Influence of the growth factors PDGF-BB, TGF-β1 and bFGF on the replicative aging of human articular chondrocytes during *in vitro* expansion. Journal of Orthopaedic Research. 2010;28(3):354-60.

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