Original Article

The Effect of Cell-Deprived Platelet Lysate Growth Factors (PLGF) on the Perichondrium Preserved Cartilage Graft Viability

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Received: 27 Sep 2020
Accepted: 30 Nov 2021

ABSTRACT

BACKGROUND

Finding a suitable pharmacological substance and a surgical method for improving cartilage graft preparation are necessary. This present study was planned to evaluate the effects of PLGF and graft preparation methods on cartilage graft survival.

METHODS

This controlled, experimental study was performed in Kerman University of Medical Science, Kerman, Iran during 2016- 2017 on two groups of rabbits. Group 1 received PLGF (PLGF +) while Group 2 did not receive any PLGF (PLGF -). In each group, three carilage graft preparation methods including Block Cartilage Graft (BCG), Diced Cartilage Graft (DCG), and Crashed Cartilage Graft (CCG) were used. Three months after the intervention, the grafts were re-assessed and weighed. A specimen from each graft was taken for inflammation, fibrosis, necrosis, and viable chondrocyte.

RESULTS

The CCG method had the maximum ossification percentage (OS%) and no change occurred by PLGF. The BCG method had the greatest viable chondrocyte number, attenuated by PLGF. The BCG method had the highest amount of fibrosis, without any change by PLGF. Additionally, the inflammation percentage and necrosis in the PLGF + group were greater than the PLGF - group.

CONCLUSION

The most important effecting factor on the properties of cartilage graft is the method of graft preparation and PLGF only attenuates the methods properties without changing them.

KEYWORDS
Diced; Block; Crushed; PLGF; Cartilage; Graft; GF

Please cite this paper as:
Iranpour M, Khodarahmi A, Farsinejad AR, Jafari E. The Effect of Cell-Deprived Platelet Lysate Growth Factors (PLGF) on the Perichondrium Preserved Cartilage Graft Viability. World J Plast Surg. 2022;11(1):97-110. doi: 10.52547/wjps.11.1.97

INTRODUCTION

The cartilage grafts have been employed in various forms by plastic surgeons for reinstating the volume, structural unity and strength in many parts of the body. The first usage of these grafts dates back to Konig and Goodale at the end of the 19th century. Nowadays, they are

used in many aesthetic and reconstructive surgeries such as rhinoplasty, periorbital reconstruction, and nipple reconstruction.

Similar to other grafts, the critical success point is the preservation of the form and mass of the graft[1]. The cartilage is an avascular tissue composed of few cells with low mitotic activity. Thus, its repair is impaired, and there is no approved drug for improving its injuries[2]. This graft can be prepared as blocked, diced, crushed, and liquid form. Factors such as strength, resorption, pliability, palpability, visibility, infection, and ease of usage determine the benefits and limitations of each form. The overall result of these factors determines which form of cartilage graft be applied.

Bioactive growth factors of Platelet-Riched Plasma (PRP) intensify graft synthesis. This ration supports the 'use of PRP accompanied with grafts' hypothesis[3]. Tissue Engineering and Regenerative Medicine focuses on the recognition of instructive scaffolds to address the adequate healing of injuries. Hemocomponents serve as conveyance structures for growth factors, cytokines, and immune/stem-like cells for immunomodulation, received much attention[4].

Perichondrium is the most important trophotopic component of cartilage and plays an essential role in the nutrition and its repair[5]. The perichondrium-attached diced cartilage shows better vascularization, weight gaining, and viability than perichondrium-deprived diced cartilage[6].

In spite of demonstrated long-term prognosis of the irradiated allograft cartilage and adipose-derived stem cells in nasal reconstruction by some studies, there is no practically accepted solution for improving prognosis of grafts in reconstructions by plastic surgeon community. In fact, the application of these materials for depleting autogenous cartilage has not been generally accepted. This incompatibility of data provoked us to center on the application of stem cells to restore and improve the graft outcome as a new concept and focus of the present study.

PRP encompasses a mixture of growth factors and cytokines including Vascular Endothelial Growth Factor (VEGF), fibroblast growth factor, platelet-derived growth factor, insulin-like growth factor-1, interleukin-1B, interleukin-10, and tumor necrosis factor-B[7]. PRP includes a milieu of bioactive growth factors, which intensify the graft synthesis. This ration supports the 'use of PRP accompanied with grafts' hypothesis[3]. PRP has two conflicting effects leading to the same point, the use of which reduces MMP-3 and MMP-13 activity shortly after injury, which leads to matrix formation and healing process. Delayed PRP administration after injury decreases the secondary matrix damage mediated by a pro-inflammatory process. Each of these effects improves the healing process[8].

Platelet-Riched Fibrinogen (PRF) improves the chemotaxis, proliferation, and viability of the cultured chondrocytes. The gene expression of the type II collagen and aggrecan, as chondrogenic markers, discloses that PRF induces the chondrogenic differentiation of the cultured chondrocytes. PRF increases the formation and deposition of the cartilaginous matrix produced by cultured chondrocytes[9]. Cells in the PRP and PRF require the autologous usage of these products, but acellular growth factors are only some proteins, which should not necessarily be autologous. Preparation of PRP, Platelet-Riched Growth Factor (PRGF), or PRF is a time-consuming process, the commercial production of which will be cheaper than individual preparation for each patient. Acellular growth factor-enriched product with a limited rejection risk and efficient on graft viability is a great opportunity for producing a commercially available and cost-effective allogenic product.

PRF has greater induction effect in cellularity and collagen production on cartilage grafts than PRP. PRF releases growth factors more gradually compared with PRP, attributed to the gelatinous nature of PRF[10].

PRP comprises abundant benefit growth factors for wound repairing. Nowadays, much attention has focused on its usage to repair articular cartilage injuries. Several factors such as unstable biological fixation and burst release of growth factors reduce its therapeutic effects[11].

In the present study, PRGF was selected as a source of growth factors lacking leukocyte or platelet contents, minimizes its rejection risk when used as allograft or heterograft. PRGF is conjoined with a fibrin scaffold to give it a gelatinous form and make it a sustained release source of growth factors.

The acellular effect of PRGF is evaluated on three forms of perichondrial preserved cartilage grafts. The present study aimed to find out whether human allogeneic PRP could serve as a biological scaffold and source of growth factors in cartilage repair.

MATERIAL AND METHODS

Ethics statement

The study protocol was accepted by the Animal Ethics Committee of Kerman University of Medical Sciences. The procedures were adjusted according to the protocols approved by the institutional animal care and Declaration of Helsinki protocol.

Animals

This controlled, experimental study was performed in Kerman University of Medical Science, Kerman, Iran during 2016- 2017. The study was performed on 36 New Zealand white rabbits aged 12-16 wk and weighing 2-2.5 kg. Prior to the study, all animals were screened for common diseases, indicating their full health. None of the rabbits has been used in any previous study. The animals were kept in controlled shelters in an environment with room temperature (22–24 °C) and at a relative humidity of 40%-60% under a 12-h light/12-h dark cycle. They had free access to water and pelleted diet. The caring, feeding, and breeding conditions were the same for all the animals.

Platelet lysate growth factors (PLGF) preparation

Twenty ml whole blood of a volunteer person screened for common diseases was collected by 20 G needles and glass blood tubes previously treated with 3 ml sodium citrate (3.2%) as an anticoagulant. The blood was placed in a centrifuge (Zisco) at 400×g at 22 °C room temperature for 10 min. Then, the uppermost layer was transferred with a sterile pipette to Falcon 15 mL conical centrifuge tube[3]. This product was frozen in a -70 °C freezer (Jal tajhiz Co.Iran). After 30 min, it was transferred to a water bath for thawing at 37 °C. This freezing-thawing cycle was repeated three times (4). Finally, the product was retransferred to a centrifuge (Zisco) at 2700×g at 22 °C room temperature for 20 min. The supernatant layer containing platelet growth factors was separated and held in 2mL bottles in -18 °C (Samsung freezer) for later use, called Platelet lysate Growth Factors (PLGF).

Fibrin scaffold preparation

Twenty ml of human whole blood sample was collected in citrate blood collection tube. After High speed centifugation, Fibrinogen separated from upper plasma layer (Platelet poor plasma) by ethanol precipitation method near pH 7 at low temperature. In connection with thrombin preparation method, 10 ml of citrated blood was combined with calcium chloride and incubated at room temperature for 60 min. The sample was centrifuged, filtered for omitting cellular components, and frozen at –80 °C until used[4]. Fibrin construct was fabricated with pre-gel solutions of fibrinogen, Thrombin and calcium choloride (CaCl2). Briefly, a solution of 4 ml fibrinogen (40 mg/ml), was mixed in equal volume with a human thrombin solution in 50 mM CaCl2, after 1 h at 37 °C in laboratory beshers the gel formation proccess was done. Fibrin scaffold was then obtained in the form of the membrane by squeezing gels in between two sterilized gauze piece (Figure 1).

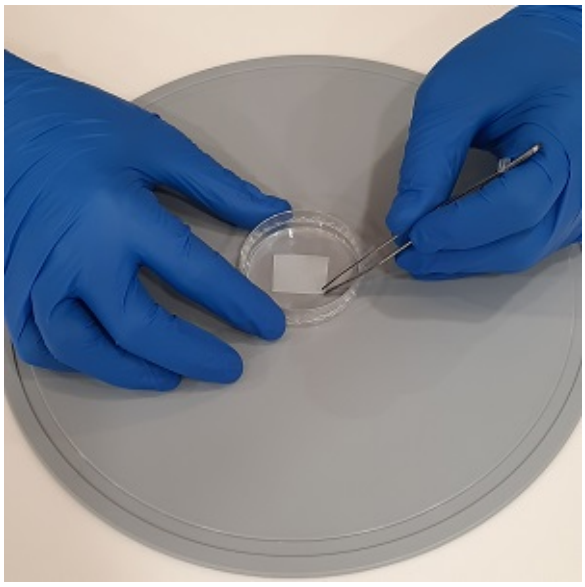

Figure 1: Fibrin scaffold: Fibrin construct obtained by the polymerization of human fibrinogen with the help of thrombin and calcium

Surgery

General anesthesia, applied by Intra Muscular (IM) injection of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg), was done for all surgical procedures. Before the surgery, a single IM dose of cefazoline (50 MG/KG) was injected and repeated as single daily dose for 7-day after operation. Meloxicam (0.2 mg/kg) was used as single daily intramuscular injection until 3 d after surgery for pain relief. After the cefazolin administration, both ears as donor sites and back of the rabbit as the recipient site were shaved by an electrical shaver (Mozer).

Under sterile conditions, auricles were amputated, the skin of excised, perichondrium-preserved cartilage was harvested under *3 loop magnification, and the donor sites were repaired (Figures 2,3). In each rabbit, the harvested cartilages were incidentally and equally divided into 6 segments. Two segments were used intact as Block Cartilage Graft (BCG), mildly crushed by a manual crusher as Crushed Cartilage Graft (CCG) and the remaining was separately minced to 1-2 mm pieces by scalpel no11 as two Diced Cartilage Grafts (DCG). In each rabbit, grafts were incidentally divided into two equal groups. Each group contained BCG, DCG, and CCG as discrete grafts. One group received PLGF and fibrin scaffold (PLGF + group) while the other group received nothing.

Three distinct subcutaneous packets were created on each side of the spinal columns. Each packet was created by a 1 cm transverse skin incision (Figure 5). The grafts of PLGF + group were inserted on the pockets on the left side of the backbone of each rabbit while the grafts of the other group were inserted on the right side. The BCG, CCG, and DCG were placed in the cephalic, middle, and caudal pocket in each group, respectively.

PLGF (0.5 ml), thrombin (0.5 ml), and fibrinogen (0.5ml) were added to each graft of the PLGF + group, which were then incubated in water tub at 37 °C for 10 min. The resulting clot was inserted into the created subcutaneous packet. All grafts were weighed before insertion in the subcutaneous packets and before the addition of PLGF (Figure 4).

After the surgery, the rabbits were confined for 3 months in the previously described condition. After

Figure 2: Auricle amputation

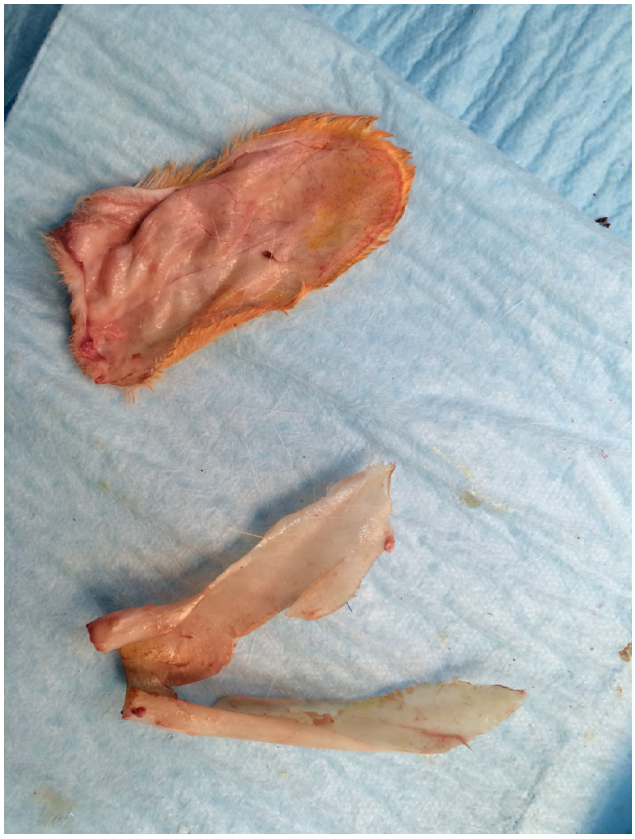

Figure 3: Process of the cartilage harvest

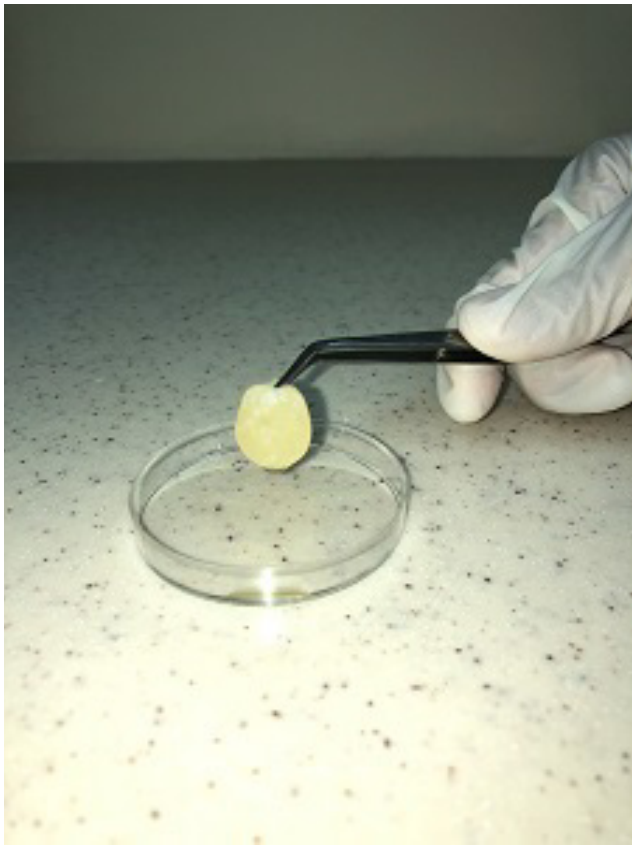

Figure 4: Diced cartilage added PLGF rolled in the fibrin scafold

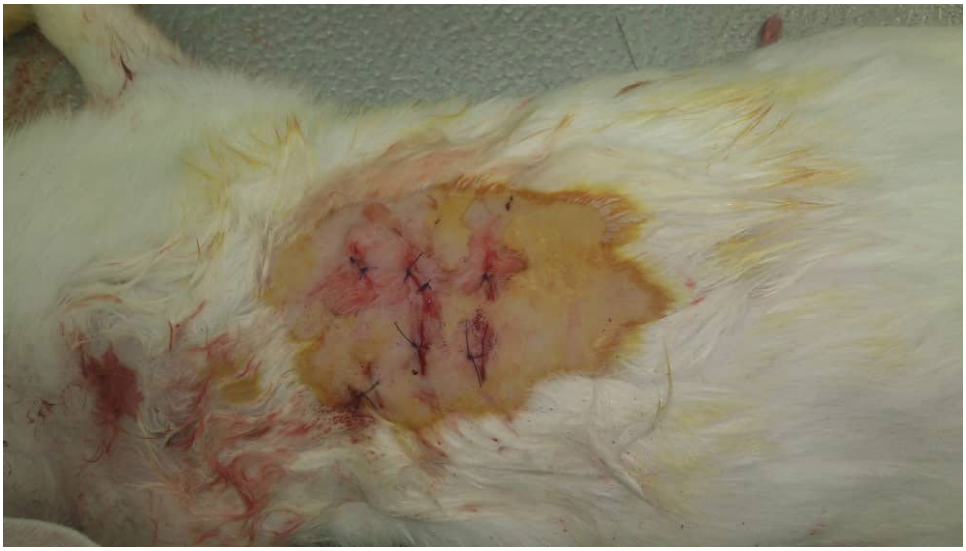

Figure 5: graft implantation on the back of rabbit

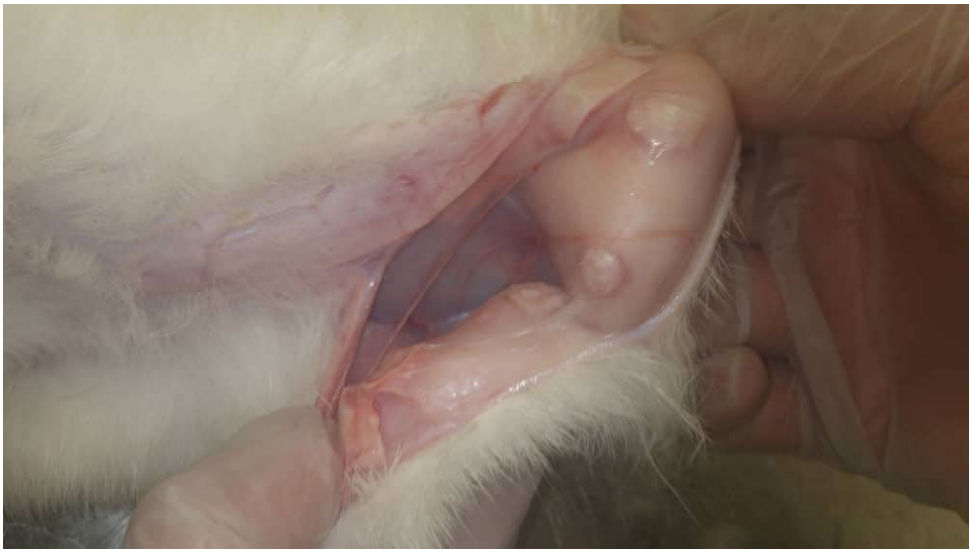

Figure 6: Graft appearance on the back of rabbits after 3m

3 months, general anesthesia was applied for all rabbits, as previously done and grafts were harvested from their pocket under loop magnification without their surrounding fibrosis (Figure 6). Each graft was weighed and fixed in formalin 10% solution for histopathological evaluations. Finally, all rabbits were killed by intraperitoneal injection of high-dose sodium thiopental.

Histological Staining

The specimens were fixed with 10% neutral formalin solution and placed in paraffin. Six-Km-thick tissues were prepared and stained with hematoxylin-eosin. A pathologist who was unknown of the treatment group checked necrosis, ossification, viable chondrocyte, and fibosis. Immunohistochemical staining (trichrome staining) was performed for a more precise evaluation of the fibrosis (Figure 7).

Statistical Analysis

SPSS-26 was used to analyze the collected data (Chicago, IL, USA). The *P* level was used to indicate the statistical significance, where $P<0.05$ was accepted as significant.

T test and ANOVA were used to examine the variables with normal distribution while Mann-Whitney U test was utilized to assess variables with a non-normal distribution.

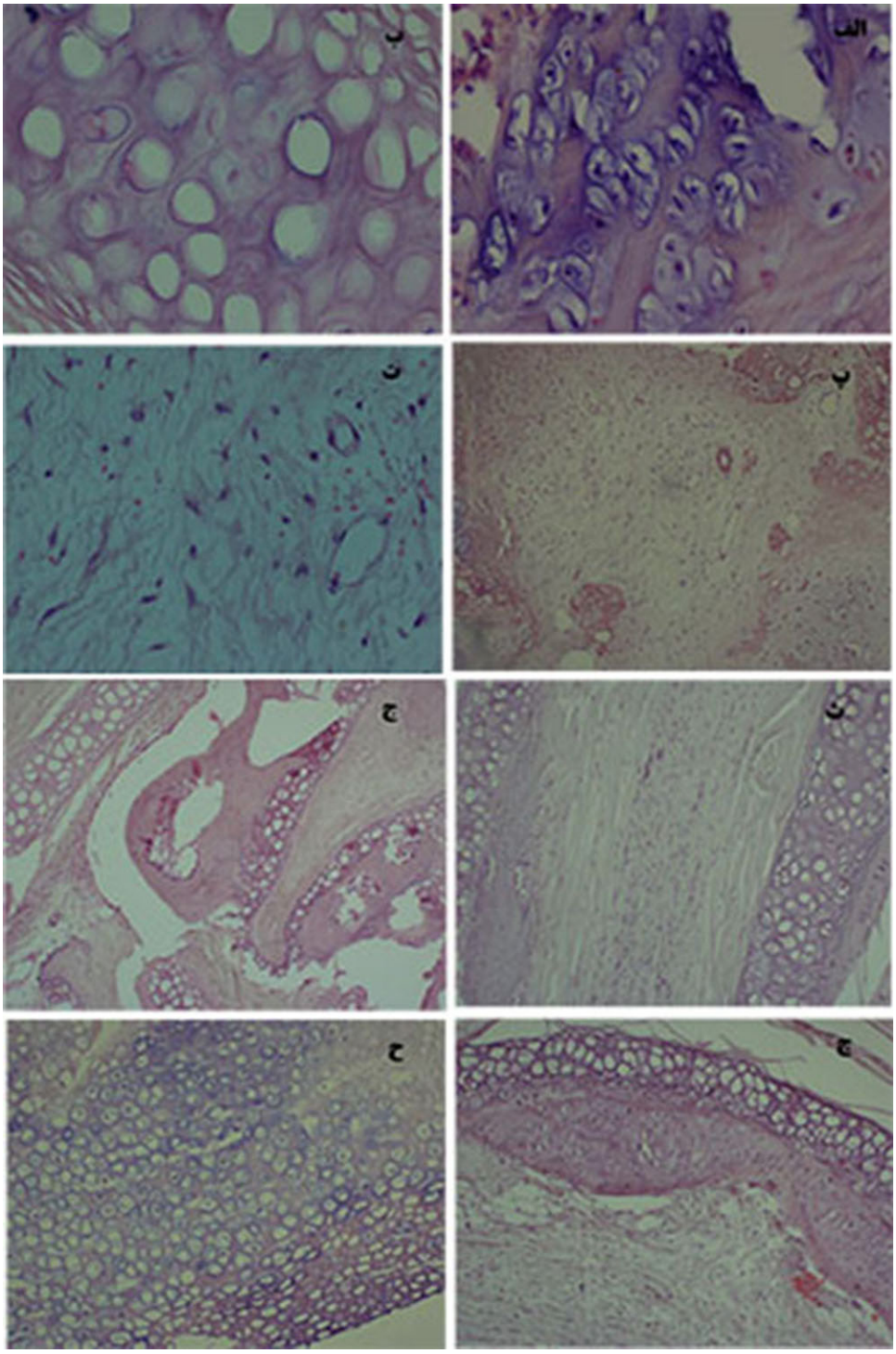

Figure 7: Histopathological evaluation of the specimens

RESULTS

The variables were analyzed once based on the graft preparation method by removing the confounding effect of PLGF and another time based on the effect of PLGF by removing the cofounding effect of the graft preparation method. The results of the Kolmogorov-Smirnov test indicated no statistically difference in the initial weight of the grafts, showing the incidental and equal distribution of grafts between the groups and the methods (Tables 1, 2). There was no necrosis in the PLGF - group (Tables 3, 4). However, there was a statistically significant difference between PLGF - and PLGF + groups,

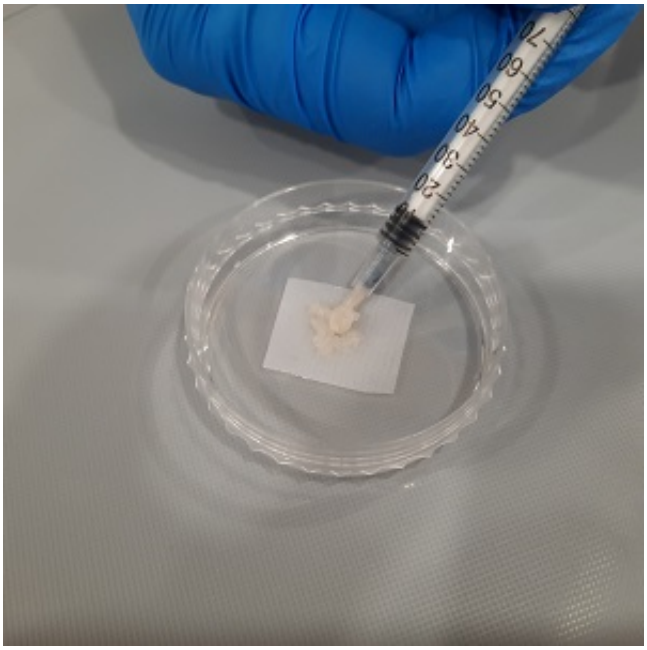

Figure 8: Rolling diced cartilage by fibrin scafold

Table 1: Comparison of primary graft weight between the three cartilaginous methods of block, crushed and diced after removing the disruptive effect of PLGF

Variable		Blocked	Crushed	Diced	*P* value
Primary weight	PLGF+	0.6419±0.08	0.6288±0.06	0.6155±0.07	0.313
	PLGF-	0.6194±0.08	0.6404±0.08	0.6825±0.07	0.332

Table 2: Results of primary weight in groups with and without PLGF after eliminating the confounding effect of methods

Variable		PLGF+	PLGF-	*P*- value
Primary weight	blocked	0.6419±0.08	0.6194±0.08	0.26
	diced	0.6155±0.07	0.6125±0.07	0.865
	crushed	0.6288±0.06	0.6404±0.08	0.521

which obviated the cofounding effect of the method indicating its significant difference in DCG (Tables 5, 6).

Comparing ossification percentage (OS%) among various methods, irrespective of the PLGF as well as after removing the confounding effect of PLGF, indicated a statistically significant difference (Tables 3, 7, 8, 9). In addition, CCG represented the maximum ossification percentage, and DCG had its minimum amount. Furthermore, no statistically significant difference was observed although the evaluation of this variable between PLGF + and PLGF - groups indicated such a pattern.

In addition, the comparison of the viable chondrocyte percentages among DCG, CCG, and BCG indicated a statistically significant difference. BCG had the highest percentage of viable chondrocytes while CCG had minimum amount (Tables 3,4,7,8,9). Regarding PLGF + and PLGF - groups, a statistically significant difference was observed for BCG (Tables 5, 6). The evaluation of the inflammation percentage among PLGF + and PLGF - groups demonstrated a statistically significant increase in PLGF + group of DCG (Tables 5, 6). Additionally, weight gain between PLGF + and PLGF - groups indicated a statistically significant difference for CCG. The comparison of the fibrosis percentage demonstrated that BCG had the highest amount while the DCG had the lowest

Table 3: Comparison of quantitative variables between three methods of crushed, blocked and diced cartilage regardless of receiving or not receiving PLGF

Variable	Crushed	Diced	Blocked	*P*- value
	M ± SD	M ± SD	M ± SD	
Fibrosis percentage	22.81±8.25	**21.41±7.79**	**25.62±8.14**	**0.002***
Necrosis percentage	0	**1.25±4.88**	**0.31±1.75**	**0.123**
Ossification percentage	12.66±13.85	**4.84±9.1**	**8.28±9.80**	**<0.001***
Vable chondrocyte percentage	32.97±14.95	39.06±14.11	43.31±16.80	0.002*
Primary graft weight	0.6346±0.072	0.6140±0.068	0.6306±0.79	0.244
Final graft weight	1.1561±0.11	1.1527±0.13	1.1267±0.13	0.320
Graft weight gain	0.5213±0.13	0.5390±0.15	0.4960±0.13	0.201
Graft weight gain percentage	84.62±27.80	90.24±31.39	80.88±26.09	0.177

Table 4: Comparison of quantitative variables between the three cartilaginous methods of block, crushed and diced after removing the distorting effect of PLGF

Variable		Blocked	Crushed	Diced	*P*- value
Primary weight	PRGF+	0.6419±0.08	0.6288±0.06	0.6155±0.07	0.313
	PRGF-	0.6194±0.08	0.6404±0.08	0.6825±0.07	0.332
Final weight	PRGF+	1.1660±1.41	1.1875±0.1	1.1780±0.12	0.780
	PRGF-	1.0874±0.09	1.1247±0.11	1.1274±0.12	0.276
Weight difference	PRGF+	0.5240±0.15	0.5587±0.1	0.5632±0.13	0.414
	PRGF-	0.4679±0.09	0.4838±0.15	0.5149±0.16	0.391
Weight gain percentage	PRGF+	84.28±30.69	90.09±20.68	93.26±26.79	0.389
	PRGF-	77.49±20.44	79.15±32.87	87.22±35.57	0.394
Necrosis percentage	PRGF+	0.62±2.45	0	2.50±6.72	0.111
	PRGF-	0	0	0	1
Fibrosis percentage	PRGF+	26.56±7.97	22.50±10.21	22.81±6.21	0.076
	PRGF-	24.69±8.32	23.12±5.6	20±8.9	0.018
Ossification percentage	PRGF+	9.06±10.65	11.88±12.42	5±9.33	0.008
	PRGF-	7.5±8.98	13.44±15.31	4.69±9.06	0.024
Viable chondrocyte percentage	PRGF+	48.19±17.68	33.44±16.23	40±11.21	0.004
	PRGF-	38.44±14.56	32.5±13.79	38.12±16.64	0.258

rate irrespective of the PLGF usage, which indicated a statistically significant difference.

DISCUSSION

The present study aimed to evaluate the effect of PLGF on three cartilage graft preparation methods by considering variables such as fibrosis, ossification, viable chondrocyte, necrosis, inflammation, weight gain amount, and percentage of weight gain. The variables were analyzed in four ways to help remove the bias effects.

The data were first analyzed based on cartilage graft preparation methods, irrespective of PLGF usage and then based on cartilage graft preparation methods, by considering the confounding effect of PLGF administration. Finally, the data were compared according to PLGF by taking into account the confounding effect of the cartilage graft

Table 5: Comparison of quantitative variables between PLGF + and PLGF- groups regardless of graft preparation method

Variable	PRGF+	PRGF-	*P*- value
	M ± SD	M ± SD	
Fibrosis percentage	23.96± 8.45	22.6± 7.94	0.254
Necrosis percentage	1.04± 4.22	0	0.017*
Ossification percentage	8.65± 11.13	8.54± 11.96	0.95
Vable chondrocyte percentage	40.54± 16.30	36.35± 15.1	0.067
Primary graft weight	0.6287± 0.69	0.6241± 0.78	0.666*
Final graft weight	1.1772± 0.12	1.1131± 0.11	<0.001**
Graft weight gain	0.5486± 0.13	0.4889± 0.14	0.002**
Graft weight gain percentage	89.21± 26.36	81.29± 30.34	0.055

Table 6: Results of quantitative variables in groups with and without PLGF after eliminating the confounding effect of methods

Variable		PRGF+	PRGF-	*P*- value
Primary weight	Blocked	0.6419±0.08	0.6194±0.08	0.26
	Diced	0.6155±0.07	0.6125±0.07	0.865
	Crushed	0.6288±0.06	0.6404±0.08	0.521
Final weight	Blocked	1.1660±0.14	1.0874±0.09	0.012
	Diced	1.1780±0.13	1.1274±0.12	0.109
	Crushed	1.1875±0.09	1.1247±0.11	0.018
Weight difference	Blocked	0.5240±0.15	0.4679±0.09	0.078
	Diced	0.5632±0.13	0.5149±0.16	0.195
	Crushed	0.5587±0.10	0.4838±0.15	0.022
Weight gain percentage	Blocked	84.28±30.69	77.49±20.44	0.301
	Diced	93.26±26.79	87.22±35.57	0.446
	Crushed	90.09±20.68	79.15±32.87	0.116
Necrosis percentage	Blocked	0.62±2.45	0	0.154
	Diced	2.5±6.72	0	0.040
	Crushed	0	0	1
Fibrosis percentage	Blocked	26.56±7.97	24.69±8.32	0.888
	Diced	22.81±6.21	20±8.98	0.148
	Crushed	22.50±10.31	23.12±5.64	0.322
Ossification percentage	Blocked	9.06±10.66	7.50±8.98	0.546
	Diced	5±9.33	4.69±9.06	0.294
	Crushed	11.88±12.42	13.44±15.31	0.740
Viable chondrocyte percentage	Blocked	48.19±17.68	38.44±14.56	0044
	Diced	40±11.21	38.12±16.64	0.427
	Crushed	33.44±16.23	32.50±13.79	0.934

preparation method.

The comparison of the data among the cartilage graft preparation methods with and without the removal of the confounding effect of PLGF administration indicated that some variables had statistically significant differences in different methods in terms

Table 7: Comparison of quantitative variables between three methods of crushed, blocked and diced cartilage regardless of receiving or not receiving PLGF

variable / Analysez groups		Compression between block & diced graft preparation method	Compression between block & crushed graft preparation method	Compression between crushed & diced graft preparation method
Fibrosis percentage	***P*- value**	0.001	0.013	0.187
Ossification percentage	***P*- value**	0.014	0.137	<0.001
Viable chondrocyte percentage	***P*- value**	0.261	0.001	0.022

Table 8: Quantitative comparison of quantitative variables between three methods of crushed, blocked and diced cartilage in PLGF+ group

variable / Analysez groups		Compression between block & diced graft preparation method	Compression between block & crushed graft preparation method	Compression between crushed & diced graft preparation method
Fibrosis percentage	***P*- value**	0.055	0.047	0.908
Ossification percentage	***P*- value**	0.065	0.435	0.001
Viable chondrocyte percentage	***P*- value**	0.143	0.001	0.041

Table 9: Quantitative comparison of quantitative variables between three methods of crushed, blocked and diced cartilage in PLGF+ group

variable / Analysez groups		Compression between block & diced graft preparation method	Compression between block & crushed graft preparation method	Compression between crushed & diced graft preparation method
Fibrosis percentage	***P*- value**	0.010	0.131	0.073
Ossification percentage	***P*- value**	0.094	0.199	0.008
Viable chondrocyte percentage	***P*- value**	0.192	0.099	0.252

of fibrosis percentage, ossification percentage, and viable chondrocyte percentage.

Comparing the data among the PLGF usage groups with and without removing the bias effect of method reveals some statistically significant differences for viable chondrocyte percentage, cell necrosis percentage, inflammation percentage, and weight gain amount.

In the present study, four forms of cartilage grafts including block, diced, crushed, and morselized were compared. The cartilage graft of both crushed and morselized groups were crushed by different devices, where the crusher of morselized group was similar to the crusher device used in the present study. The diced cartilage and morselized cartilage groups had higher concentration of viable cells than the block cartilage group. The diced and crushed cartilage groups had the maximum and minimum viable cells, respectively. The dead cell amount was statistically similar to the diced and block groups; although block grafts had the lowest dead cells[12].

In addition, a significant difference was reported in viable chondrocyte percentage among block, diced and crushed methods. The block method and crushed cartilage had the highest and lowest viability

percentages, respectively. Further, the differences between block and crushed methods as well as the crushed and diced methods were statistically significant.

In spite of wide acceptance of crushed cartilage for masking irregularities and eliminating slight deficits in rhinoplasty, there is no agreement about the optimal degree of crushing and rate of graft resorption over time.

A moderate degree of crushing provides better results in terms of flexibility and stability over time[13]. No statistically significant was found difference between the crushed and block cartilage grafts in the final chronic inflammation, fibrosis, cartilage mass viability, and vascularization. The mildly crushed cartilage maintained the structural integrity and was more predictable for usage in rhinoplasty[14].

A statistically significant difference was noticed in the percentage of fibrosis among the three cartilage preparation methods in the present study. The maximum and minimum fibrosis production were in the block and diced methods, respectively. In addition, the differences in the percentage of fibrosis between block and crush methods as well as block and diced methods were statistically significant.

Liao et al. reconstructed auricle using diced cartilage wrapped in porous, hollow Materialise Magics v20.03. After 4 months, they found high chondrocyte viability and production of collagen II, glycosaminoglycans, and other cartilaginous matrix components. The appearance, stiffness, and flexibility of the newly formed auricles were similar to a normal auricle, which indicates the acceptable viability and fusion of diced cartilage graft wrapped in porous, hollow Materialise Magics v20.03 without significant resorption[15].

In the present study, the highest and lowest ossification percentages were in the crushed and diced methods, respectively. The differences were statistically significant.

A two-months study was conducted to compare bare diced and block cartilage grafts. The results showed no obvious resorption in any of the cartilage graft groups and no significant difference in weight changes, chondrocyte viability, and matrix formation. Furthermore, modulus values of both elasticity and stress for the costal cartilage grafts were higher than those of the diced cartilage grafts with a statistically significant difference[16], which is in line with the results of the present study. Further, more viable chondrocyte was found in the block method in comparison to the diced method, which was not statistically significant.

Erdogmuş et al. compared block, diced cartilage viability, and concluded that peripheral chondrocyte proliferation was more prominent in the diced cartilage grafts than the block cartilage [17]. The degree of resorption of the diced cartilage wrapped in fascia was considerably greater than the resorption of the one-piece block grafts[18].

PRP contains a cocktail of growth factors activating TGFβ, which lead to chemotaxis, proliferation, and differentiation of mesenchymal stem cells. PRP stimulates cellular anabolism, anti-inflammatory properties, chondrocyte proliferation, and extracellular matrix production. In addition to the aforementioned properties of PRP, interaction of PRP with fibrinogen produces a scaffolding effect on PRP, justifying the PRP application for the treatment of cartilage pathologies[19]. PRF can enhance the healing process in a variety of injuries including cartilage repair, rotator cuff surgery, and anterior cruciate ligament surgery. However, the results are inconclusive. A Previous study generally supports "the benefits of PRF as a useful adjuvant for a range of chronic muscle, tendon, bone, or other soft tissue injuries"[20].

After administering PRP and PRF in two groups of animal cartilage injury, the histopathological studies revealed better and earlier cartilage regeneration in the PRF group compared with the PRP group. The finding was observed in the 4th postoperative week and continued until the 12th postoperative week[21], which is variable with the findings of the present study indicating the viable chondrocyte percentage is statistically higher after the administration of PLGF in block cartilages. Adding PLGF to the diced cartilage causes a statistically important increase in the cell necrosis percentage.

PRFM significantly enhanced the chondrocyte viability of diced cartilage grafts. Additionally, there were lower inflammation and fibrosis, attributed to PRFM biocompatibility[22].

A compression study between bared diced cartilage, diced cartilage wrapped with poly (lactic-co-glycolic) acid (PLGA), and blended PRP-diced cartilage wrapped with PLGA revealed no significant differences in chondrocyte nucleus loss, inflammation, fibrosis, biomechanical analysis, and gross graft resorption between the bared diced cartilage and the PRP blended-diced

cartilage wrapped with PLGA. However, all of these parameters had the worst condition in the diced cartilage wrapped with PLGA membrane group[23].
Based on the results of the present study, increasing PLGF to cartilage graft, especially crushed grafts, caused a statistically significant improvement in increasing the weight of the cartilage graft.
The study of Ali G. focused on four forms of diced cartilage grafts, namely, unwrapped, wrapped with fascia, wrapped with oxidized cellulose, and wrapped with PRF. The group wrapped with oxidized cellulose had the lowest significant viability. There were no statistically significant differences among other groups in terms of the mean percentages of inflammation, fibrosis, and vascularization[24].
Furthermore, the fibrosis and ossification percentage increased after adding PLGF to the cartilage grafts, which was not statistically significant.
Topkara et al. compared nucleus loss, calcification, inflammation, giant cell formation, and chondrocyte proliferation among five diced cartilage groups including bare diced cartilage, diced cartilage wrapped with fascia, diced cartilage wrapped with fenestrated fascia, diced cartilage wrapped with concentrated growth factor (CGF), and diced cartilage wrapped with blood glue. The lowest loss of chondrocyte nuclei was in the Concentrated Growth Factors (CGF) group, but no significant difference was reported between the CGF group and the bare diced cartilage group. The highest chondrocyte proliferation was observed in the CGF group. However, bone metaplasia had the lowest value in the bare diced cartilage group[25].
In the present study, a statistically significant difference was reported in the inflammation percentage when the PLGF was added to the crushed cartilage. In addition, this variable increased when PLGF was added to the diced and block cartilage. However, there was no statistically significant difference in these cases.

CONCLUSION

The PLGF only attenuates the properties of each cartilage preparation method and cannot change them.

ACKNOWLEGMENTS

Kerman University of Medical Sciences suported all financial demands of this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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