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The Effect of PRP & Fat Grafting on Viability of Cartilage Grafts: An Experimental Study

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Abstract

Background: Cartilage tissue has limited capacity for regeneration mainly due to its avascular nature. As a result, cartilage deficiencies treatment has always been a difficult problem for surgeons.

Objectives: To evaluate the safety and effectiveness of PRP and fat in cartilage grafting.

Methods: This work was performed as a prospective manner on 20 young male New Zealand rabbits, each was an experimental unit for three subcutaneous pockets for cartilage grafts (plus PRP in first pocket and Microfat in second one while the third one kept without any additives). Weight, volume, and histopathology of inserted cartilage were evaluated after three months of insertion.

Results: Revealed decrease in weight and volume of all cartilage grafts. The findings of histopathology revealed that the tissues of cartilages showing maximum degeneration and losing of chondrocytes in intact cartilage without any additives followed by cartilage injected with PRP while the tissues of cartilages with fat showing minimum degeneration. The thickness of the cartilage grafts injected with fat was the largest followed by cartilage with PRP and cartilage without any additives respectively.

Conclusion: PRP and Microfat are suitable biological wrapping materials for grafting of the cartilage that increases the viability of the grafts. The use of fat on cartilage graft gives the best findings on the volume and weight measurement as well as histopathological score followed by cartilage graft with PRP.

Key Words: *Structural cartilage – PRP and Microfat.*

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Ethical Consideration: The surgical process and the care of rabbits are subject to the rules of the Institutional Animal Care and Use Committee (IACUC), and the ethical committee of the faculty of medicine.

Introduction

Regeneration of cartilage is essential in order to successfully heal damaged cartilage in the nose, the auricle, joints, or during head and neck surgery. The avascular nature of cartilage tissue contributes significantly to the limited regenerative potential of cartilage. As a direct consequence of this, treating cartilage deficits has historically been a challenging issue for medical professionals **[1]**.

It is possible to improve the healing process of wounds in both hard and soft tissues by making usage of a technique known as platelet-rich plasma (PRP) therapy that is performed using the patient's own blood. Platelet-derived growth factor (PDGF), platelet derived angiogenesis factor (PDAF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), platelet-factor (PF-4), insulin-like growth factor (IGF)-I and epidermal growth factor (EGF) are some of the growth factors that are released in substantial quantities as a consequence of the activation of PRP **[2]**.

PRP was previously utilized in the past to increase bone production at the site of a fracture or grafting and to expedite the healing process of chronic wounds that have not healed properly **[3]**.

Throughout the First World War, the fat was applied to wounded soldiers in an attempt to speed up the healing process. However, the application of the fat varied greatly until Sidney Coleman developed clear standards for its usage in the year 1990. The development of new surgical techniques has allowed for the association of the volumizing impact of lipofilling with the regeneration capabilities of natural fat tissue. This has led to a preference for

the reduction, processing, and purification of the tissue to increase the survival rate of the grafts and exaggerate the regenerative capacity of mesenchymal stem cells, also known as MSC [4].

Due to the fact that these cells are capable of both self-renewal and multipotent in vitro differentiation into several mesodermal cell subtypes, it was clear that MSC had the potential to usher in a new era in medical practice. To tell you the truth, research has indicated that trophic, paracrine, and immunomodulatory actions are responsible for many of the remarkable therapeutic results in vivo [5].

In the current research, our goal was to assess the usefulness of platelet-rich plasma (PRP) injections as well as fat injections in cartilage grafting using a rabbit as an animal model.

Methodology

Clinical subjects: A total of twenty young male New Zealand rabbits, each of which is five months old and weighs between two and two and a half kilograms.

Pre-operative care: All the rabbits will be brought to the laboratory five days prior to the surgery so as to minimize the negative effects of transportation and stress.

Surgical technique:

The use of anesthesia:

All the animals are going to be put under general anesthesia utilizing a mixture of ketamine (intramuscular injection at 15mg/kg) and xylazine hydrochloride (intramuscular injection at 5mg/kg). Ceftriaxone will be administered intramuscularly at a dose of 60mg/kg for each of the rabbits.

Blood sampling: Following the induction of anesthesia, a total of 5 milliliters of blood will be extracted from the rabbit's ear central vein and then combined with 3.2% sodium citrate in the proportion of 1 milliliter of sodium citrate to every 5 milliliters of whole blood. The platelet-rich plasma (PRP) is prepared using this blood.

Preparation of PRP: The process of preparing PRP is carried out concurrently with the surgical operations. PRP is made in the manner that was explained. Each animal's platelet-rich plasma (PRP) is prepared using 5 milliliters (ml) of blood. As was discussed before, a solution containing sodium citrate at a concentration of 3.2% is utilized to inhibit coagulation. As a consequence of this process, the bottom portion of the blood becomes red and opaque. This portion, which is composed of red and white blood cells as well as platelets, is referred to as the blood cell component (BCC). The upper portion of the blood becomes yellow and turbid, and it contains plasma as well as platelets. This portion is known as the serum component (SEC). The whole of the SEC as well as the top 6-8mm of the BCC is pipetted into a sterile vacurette that does not contain citrate. Once again, this material is centrifuged for five minutes at a speed of 2000 revolutions per minute for 5 minutes. It is necessary to eliminate the top yellow SEC, also known as platelet poor plasma (PPP). Platelet-rich plasma, sometimes known simply as PRP, is the last material that is left behind after everything else has been removed.

Microfat preparation: The liposuction process is performed utilizing specially made liposuction cannulas made of surgical-grade stainless steel, 2mm diameter and about 20cm length with a round tip and 3 openings on each side (Fig. 1). A 10-cc syringe was used for suction. Each rabbit is put on the operating table in a supine position in a way that is analogous to that which is used in clinical practice. The regions of the belly and flanks are then shaved. Injections of 50-60mL of normal saline, or about 10% of the animal's total weight, are made into the fatty deposits located in the flank and abdominal regions of the rabbit. After roughly half an hour has passed since the injection of the tumescent solution, liposuction may begin (Video). In order to allow fat aspiration in the subcutaneous layer and to prevent harm to the surrounding tissues, just a small incision and dissection are made on both sides of the groin. This is done throughout the procedure. The liposuction procedure is subsequently carried out in the standard professional fashion for a total of one hour, consisting of thirty minutes spent on each side. To avoid injuring the surrounding tissues, particularly the muscle, it is imperative that the cannula be kept inside the subcutaneous fat deposit layer at all times. In order to do this, the cannula has to be positioned such that it is just below the surface of the skin. After the process is complete, the aspirate that was collected in the syringe is then transferred into a 50-mL syringe. After collecting the fat, it is centrifuged for four minutes at 2000 rpm to separate the fat layer, and the fat layer is then filtered through a fine filter to produce a "microfat" aliquot that is ready to be injected into 1-mL lock syringes.

The preparation of the cartilage: Involves cutting the ear that will be utilized for blood sample and then suturing the stump using nylon suture 4-0. Following this step, the skin and perichondrium that were attached to the auricular cartilage are cut away. The prepared cartilage is cut into three pieces that are each two centimeters by two centimeters. After that, each cartilage is given a weight using a scale that has a sensitivity of 0.01 grams. After inserting the cartilage without any damage into an insulin syringe by slight wrapping, a second insulin syringe is used to inject 1 milliliter of normal saline to the cartilage in order to bring the total volume up to 1 milliliter. It was determined that the volume of the cartilage was equal to the amount of the saline that was left over in the second solution.

Implantation of the grafts combined with PRP or microfat: Auricular cartilage will be extracted and implanted on the rabbit's back. Then, at the rear of the rabbit, we will have three packs (Fig. 2), which will be distributed as follows:

- Intact cartilage without any additive (a control group).
- Intact cartilage with PRP.
- Intact cartilage with fat graft.

PRP or fat was added into the pocket after insertion of cartilage.

Post-operative care: Following the rabbits have returned to a state of relative consciousness, they are placed back in their cages and have a suspension of co-amoxiclav added to the water they drink for the next five days. The animals are kept in the laboratory research housing facilities until the conclusion of the study. While they are there, they continue to get food, water, and healthcare as required.

Evaluation: After three months based on 3 items:

1- Graft volume

2- Graft weight

3- Histological examination of graft.

Statistical analysis: The SPSS program (Version 19, developed by SPSS Inc. and located in Chicago, Illinois, USA) was used to do the analysis on the collected data. The data were shown using mean and standard deviation. After being checked for normalcy, it was found that all of the data were normally distributed. One-way ANOVA was utilized for the purpose of contrasting the quantitative parameters. Tukey Post Hoc was utilized for the purpose of analyzing the variations among the groups. It was determined to have statistical significance if the *p*-value was lower than 0.05.

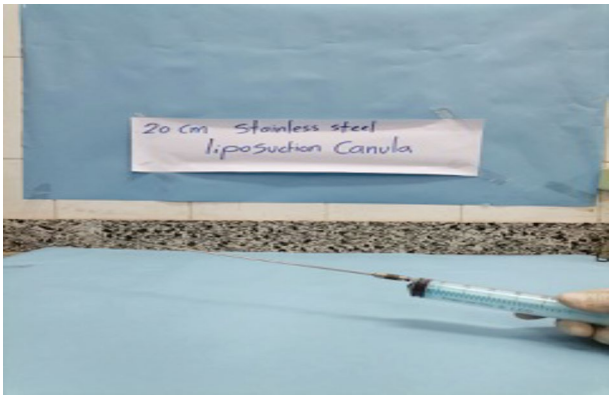

Fig. (1): Liposuction canula.

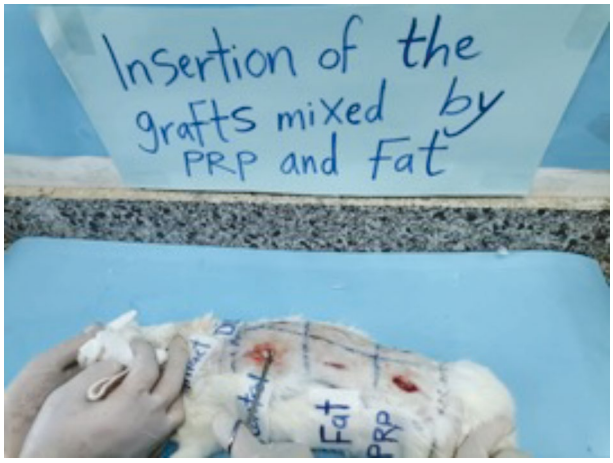

Fig. (2): Insertion of the grafts.

liposuctio
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Results

Macroscopic evaluation:

Upon excision of skin covering the grafts, we observed that all grafts were in place, none of cartilage pieces in any group migrated and there was slight vascularization in tissue in close proximity to grafts in all groups.

When compared to the adhesion that cartilages have to the structures that surround them, cartilages have lower adherence, which makes extracting cartilages much simpler. In this aspect, there was no discernible variation noticed among cartilages treated with PRP and those not treated with it.

As regard to volume measurements:

We observed a decrease in weight and volume in all groups (Table 1).

Group (Cartilage without any additives) show more significant decrease in volume mean 1.71± 0.25ml (–57.2% decrease).

Average of initial and final volume in group (Cartilage with PRP) 4ml and 3.54±0.05ml (-11.3% decrease), respectively (p=0.00).

Average of initial and final volume in group (Cartilage With fat) 4ml and 3.61±0.24ml (–9.7% decrease), respectively (p=0.00) 6 (Fig. 3).

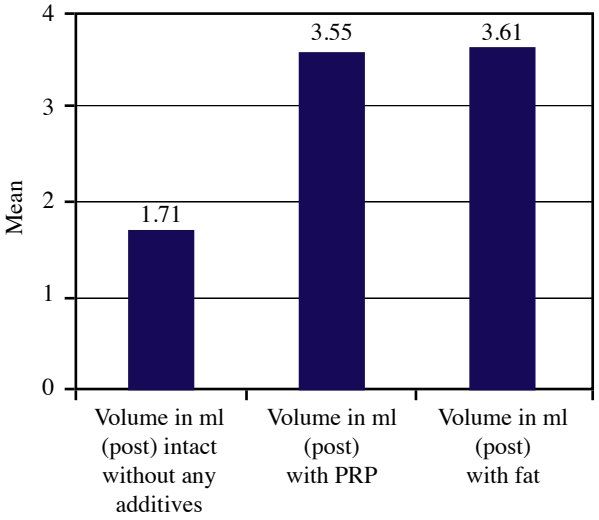

Fig. (3): Show means of volume between groups post-operative.

Statistical analysis of volume measurements revealed that there was statistical significance among groups post-operative (p=0.00), except relation between group (Cartilage With PRP) and group (Cartilage With fat) show no statistical significance (p=0.48).

Table (1): Volume measurement in (ml) of cartilage graft before and at the end of study.

Group	Before	After	Difference %
Cartilage without any additives	4	1.71	–57.2
Cartilage With PRP	4	3.54	–11.3
Cartilage With fat	4	3.61	–9.7

As regard to weight measurements: (Fig. 4)

Group (Cartilage without any additives) show more significant decrease in weight mean 0.1194± 0.00135gm (-60.19% decrease).

Average of initial and final weight in group (Cartilage With PRP) 0.3gm and 0.1782±0.0012 gm (-40.58% decrease), respectively (p=0.00).

Average of initial and final weight in group (Cartilage With fat) 0.3gm and 0.2000±0.00gm (–33.33% decrease), respectively (p=0.00) (Table 2).

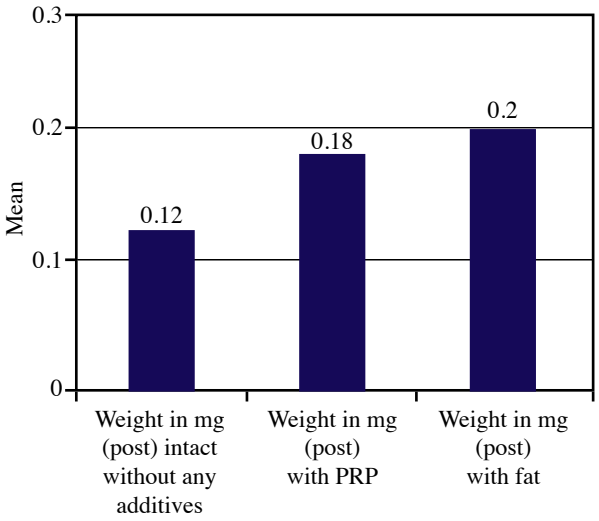

Fig. (4): Shows means of weight between groups post-operative.

Statistical analysis of weight measurements revealed that there was statistical significance among groups post-operative (p=0.00).

Table (2): Weight measurement in (gm) of cartilage graft before and at the end of study.

Group	Before	After	Difference %
Cartilage without any additives	0.3	0.1194	–60.19
Cartilage With PRP	0.3	0.1782	–40.58
Cartilage With fat	0.3	0.2000	–33.33

Histopathological examination:

Photomicrograph of the control cartilage graft (intact cartilage without any additive) H&E X 200, showing normal elastic cartilage with numerous aggregated lacunae containing chondrocytes and a moderate amount of matrix in between. Most chondrocytes are viable (C) while others are degenerated with empty lacunae (Astrix). The perichondrium appears on both sides with its two layers fibrous (black arrows) and cellular (red arrows) (Fig. 5).

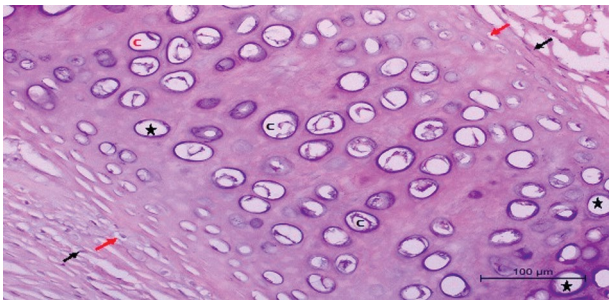

Fig. (5): Histopathological examination of (intact cartilage without any additive).

Photomicrograph of a cartilage graft (intact cartilage with PRP) H&E X 200 showing viable chondrocytes (c) all through the cartilage plate. New proliferating small fusiform chondroblasts (black arrows) are seen in their lacunae adjacent to the perichondrium at one side. Some lacunae containing dividing cells (red arrows) can be noticed (Fig. 6).

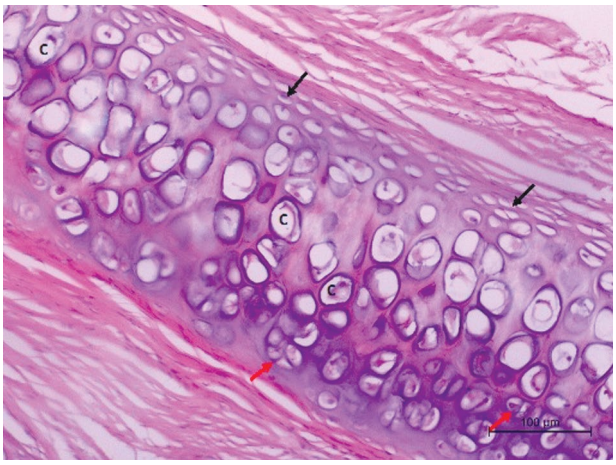

Fig. (6): Histopathological examination of (cartilage with PRP).

Photomicrograph of a cartilage graft (cartilage with fat graft) H&E X 200 showing viable chondrocytes (c) all through the cartilage plate. New proliferating small fusiform chondroblasts (black arrows) are seen in their lacunae adjacent to the perichondrium on both sides. Many lacunae containing dividing cells (red arrows) can be noticed (Fig. 7).

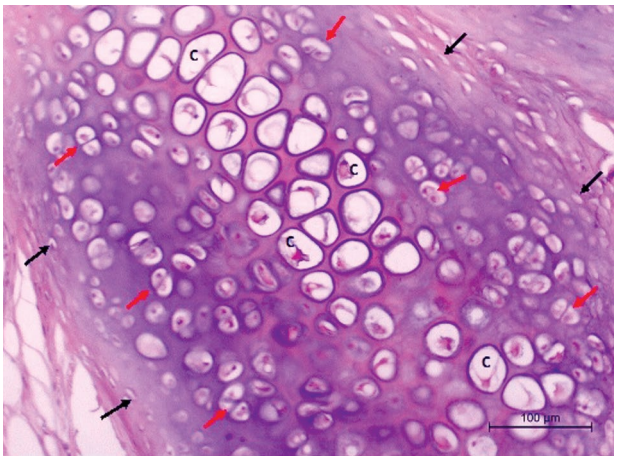

Fig. (7): Histopathological examination of (cartilage with fat graft).

Histopathological lesion score of cartilage in different groups (Table 3):

Histopathological scoring of cartilage tissue injury was scaled in degrees as follows: 0=no change; 1 ≤25% tissue damage; 2=26-50% tissue damage; 3=51-75% tissue damage; 4=76-100% tissue damage.

The findings of histopathology revealed that: The tissues of cartilages showing maximum degeneration and losing of chondrocytes in the group (cartilage without any additives) by average 51-75% followed by cartilage injected with PRP while the tissues of cartilages at the group (cartilage with fat) showing minimum degeneration. The thickness of the cartilage grafts injected with fat was the largest by average 789.319±8.14 followed by cartilage with PRP and cartilage without any additives respectively.

Table (3): Histopathological lesion score of cartilage in different groups.

Groups	Degeneration of chondrocytes	Loss of Chondrocyte	Proliferation chondrocytes	Cellularity (group of cells in one lacuna)	Condensation of matrix	Thickness of cartilage (μm)
Group I (Cartilage without any additives)	3	3	0	0	1	478.6±3.21
Group II (Cartilage with PRP)	2	2	1	1	2	729.74±6.28
Group III (Cartilage with fat graft)	1	1	2	2	3	789.319±8.14

Discussion

The regular aging process and acute injury are both major causes of cartilage damage.

In order to encourage cartilage repair and stop deterioration, there is increased interest in nonoperative therapies and biological supplements to surgical therapies **[6]**.

PRP has attracted a great deal of attention as a complement to surgery and as a non-operative therapy due to the relative simplicity of acquiring a blood sample and the security of its autologous

origin [7]. PRP is an interesting possibility for the therapy of cartilage lesions because of its possible impacts on immune response regulation, angiogenesis, and cell differential. However, a deeper comprehension of PRP's mechanism of action as an adjuvant to cartilage repair and as a non-operative therapy option for osteoarthritis is necessary for the best therapeutic usage of PRP for cartilage disease. Smyth et al.'s earlier thorough evaluation of the fundamental science literature on PRP for cartilage disease [8] showed the need for uniformity in research design so that accurate comparisons and analyses could be conducted.

Throughout the First World War, fat was utilized to treat army wounds, but up until Sidney Coleman established strict rules in 1990, usage was quite varied. The development of surgical methods linked the volumizing effects of lipofilling to the regenerative capabilities of natural adipose tissue, favoring the processing, reduction, and purification of the tissues to improve the survival of the grafts and increase the regenerative potential of mesenchymal stem cells (MSC) [9]. In a non-vascularized region, MSCs may develop into chondrocytes [10]. As a result, there has lately been discussion on the differentiation of MSCs to chondrocytes for application in cartilage tissue engineering [11].

This study's major objective was to compare the impact of adding PRP to cartilage grafts compared to fat grafting on both their resorption and regeneration in a rabbit model.

This prospective animal research used 20 young, male New Zealand rabbits that were 5 months old and weighed between 2 and 2.5kg. It was carried out at the Beni-Suef University Hospital's Plastic Surgery Department with assistance from the school's college of science and veterinary medicine.

In line with our findings, Manafi et al. [12] looked into the impacts of PRP on the ability of the cartilage grafts to regenerate as well as their survival in a rabbit model. 15 white New Zealand rabbits were utilized in the investigation, and 4 pieces of cartilage (each measuring around 2 x 2 cm) were produced using auricular cartilage. According to the research, intact cartilages with PRP saw considerably less volume loss than intact cartilages without PRP ($p<0.05$).

Oh et al. [13] also sought to determine if adipose-derived stem cells (ASCs) might aid in the regeneration of the rabbit's auricular cartilage. ASCs were administered into the middle of a surgically made auricular cartilage lesion in the rabbit to evaluate their capacity for differentiation. Saline solution was administered into the control group. The removed auricles were histopathologically and immunohistochemically analyzed after one month. According to the study's findings, ASCs may help the rabbit with an auricular cartilage defect heal. The regeneration of injured cartilage tissue in vivo is predicted to be considerably aided by these effects.

Plastic surgeons did many attempts to solve the problem of cartilage grafts resorption as diced cartilage alone or wrapping diced cartilage with connective tissue that was an elegant and efficient solution over the long run to safeguard their volume, [14] adding microfat or PRP to cartilage grafts may be a safe and effective option that increases the viability of the grafts.

Conclusion:

The current study showed that both PRP and fat injection were safe and effective in cartilage grafts. PRP and fat injection are suitable biological wrapping materials for cartilage transplantation that increases the viability of the grafts.

The use of fat graft on cartilage graft gives the best findings on the volume and weight measurement as well as histopathological score followed by diced with PRP. Additional clinical research is required to support the hypothesized advantages of this non-invasive, biological method.

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