

Proceedings of Congress IBOSCON 2018

On 30th and 31st March

Venue: Hotel Taj, Sector 17, Chandigarh

Organized by: Department of Orthopedics, PGIMER and Professor DS Grewal Memorial Orthopedics Society

Theme of conference: "Consensus in Biologics"

Multiple PRP Injection vs Single PRP Injection in Early OA Knee: An Experimental Study in a Guinea Pig Early Knee OA Model

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INTRODUCTION

Platelet-rich plasma (PRP) has emerged as the forerunner among disease-modifying treatment options of early osteoarthritis (OA) of knee.¹ However, there is currently no consensus on the number and frequency of PRP injections, and their dosing schedules are widely debated. A few clinical studies^{2,3} have shown that multiple injections of PRP are better than single injection; however, the biological basis of this effect is not yet clear.

PURPOSE OF STUDY

To determine whether multiple injections of PRP (3) are better than single injection of PRP in a spontaneous guinea pig knee OA model in the short and long term.

STUDY DESIGN

Controlled laboratory study.

MATERIALS AND METHODS

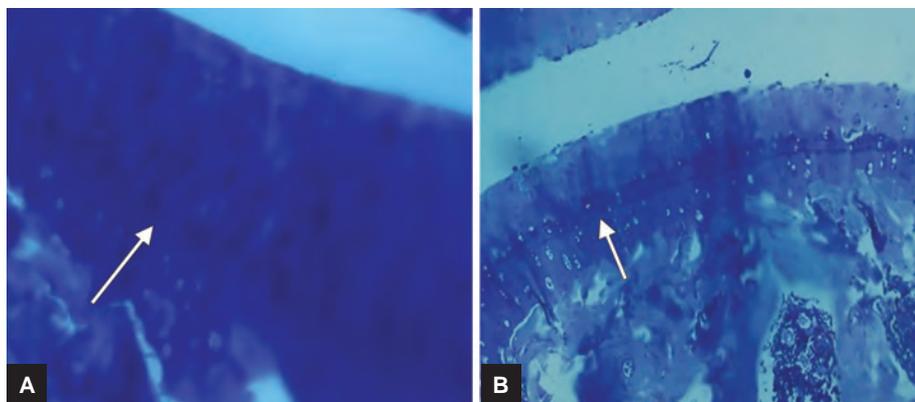
A total of 32 Dunkin–Hartley guinea pigs (weighing ~ 700 gm) were chosen for this study. The animals were divided into group I (disease control group), group II (single PRP group), and group III (multiple PRP group) containing 10, 10, and 12 animals respectively. Groups II and III received one and three injections of PRP (at weekly intervals) in the intervention knee, while the contralateral knee acted as control and was treated with normal saline. Group I acted as the disease control group and no intervention was done for either of the knees in this group. Half of the animals from each group (subgroup IA, IIA, and IIIA) were euthanized at 3 months and the remaining half (subgroup IB, IIB, and IIIB) at 6 months postintervention. Upon euthanasia, both knee joints from each animal were harvested for histologic assessment of articular cartilage and synovium. The articular cartilage and synovium were stained with the help of toluidine blue and hematoxylin and eosin stains respectively. The scoring was using OARSI outlined scoring systems for articular cartilage and synovium for guinea pigs.⁴

RESULTS

The mean initial weight, weight at sacrifice, and the mean weight gains were comparable among various groups at 3 and 6 months. The mean synovial scores of single and multiple PRP group were significantly better than disease control group at 3 months. There was no difference between single and multiple PRP group at 3 months. At 6 months, the multiple PRP group was significantly better than single PRP and disease control group in terms of mean synovial scores. However, the mean synovial scores between single PRP group and disease control group were comparable at 6 months. The mean articular cartilage scores in multiple PRP group were significantly better than single PRP and disease control group at 3 months. There was no significant difference between the mean articular cartilage scores of single PRP group and disease control group at 3 months. However, at 6 months there was no significant difference among any of the groups in terms of mean total articular scores.

CONCLUSION

Both single and multiple injection of PRP exert similar anti-inflammatory effect on the synovium in the short term. However, this effect is sustained in the long term only for multiple injections. Multiple injections of PRP exert a chondroprotective effect but only in the short term. This effect is not seen with single injection of PRP.



Figs 1A and B: Toluidine blue stain for cartilage. (A) Knee treated with multiple injections of PRP at 3 months showing no loss of proteoglycan, no fissures, and no duplication of tidemark (white arrow) with normal cellularity. (B) Knee treated with single injection of PRP showing patchy loss of proteoglycan, superficial fissures, and duplication of tidemark (white arrow) with regions of hypercellularity

CLINICAL RELEVANCE

Our study gives new insight into the histological basis of superiority of multiple injections of PRP and may help in deciding the number and frequency of injection of PRP in OA knee.

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The Effect of Intra-articular Allogeneic Platelet-rich Plasma in Dunkin–Hartley Guinea Pig Model of Knee Osteoarthritis

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INTRODUCTION

Osteoarthritis (OA) is a chronic arthropathy characterized by debilitating pain and consequent hampering of day-to-day activity, most commonly affecting the knee and the hip joints. Treatment modalities directed to modify the course of disease are lacking adequate evidence for widespread usage and the final outcome is often resorting to invasive procedures like joint replacement surgery for alleviation of pain.

Platelet-rich plasma (PRP) has been introduced in an attempt to modify the disease process and aid in healing of ailing joint and symptomatic relief. Many preclinical and clinical studies have been undertaken to investigate its role as a therapeutic agent for OA and most of them have produced promising results.¹ Studies giving a concrete proof of disease-modifying effect of PRP in *in vivo* settings are scarce.^{2,3} Clinical studies only focus on symptomatic improvement.

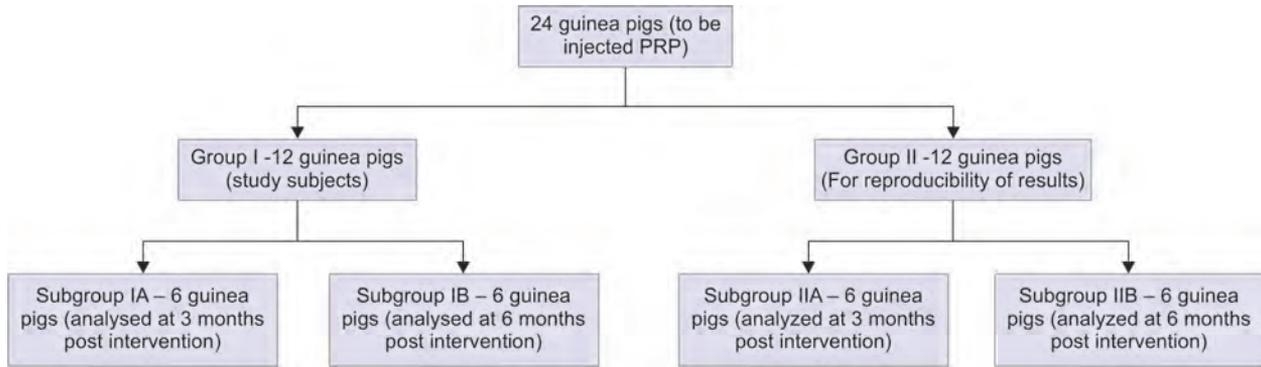
This study aims to investigate the chondroprotective and anti-inflammatory properties of PRP in Dunkin–Hartley guinea pig model of spontaneously occurring knee OA in objective terms and to demonstrate its disease-modifying potential.

MATERIALS AND METHODS

This study was a blinded controlled experimental study performed on male Dunkin–Hartley guinea pigs, proven to be a model in which knee OA occurs spontaneously and is bilaterally symmetrical, induced by increasing weight and age.

Two experimental models (group I and II) of 12 Dunkin–Hartley guinea pigs each were enrolled as part of a prospective controlled experimental study (Flow Chart 1). One knee was enrolled for intervention and the other knee of the same animal was used as control, the intervention being three intra-articular allogeneic PRP injections given at a weekly interval. Equal volume of isotonic saline injection were given simultaneously in the control knees. Six animals from each model (subgroup IA, IIA) were

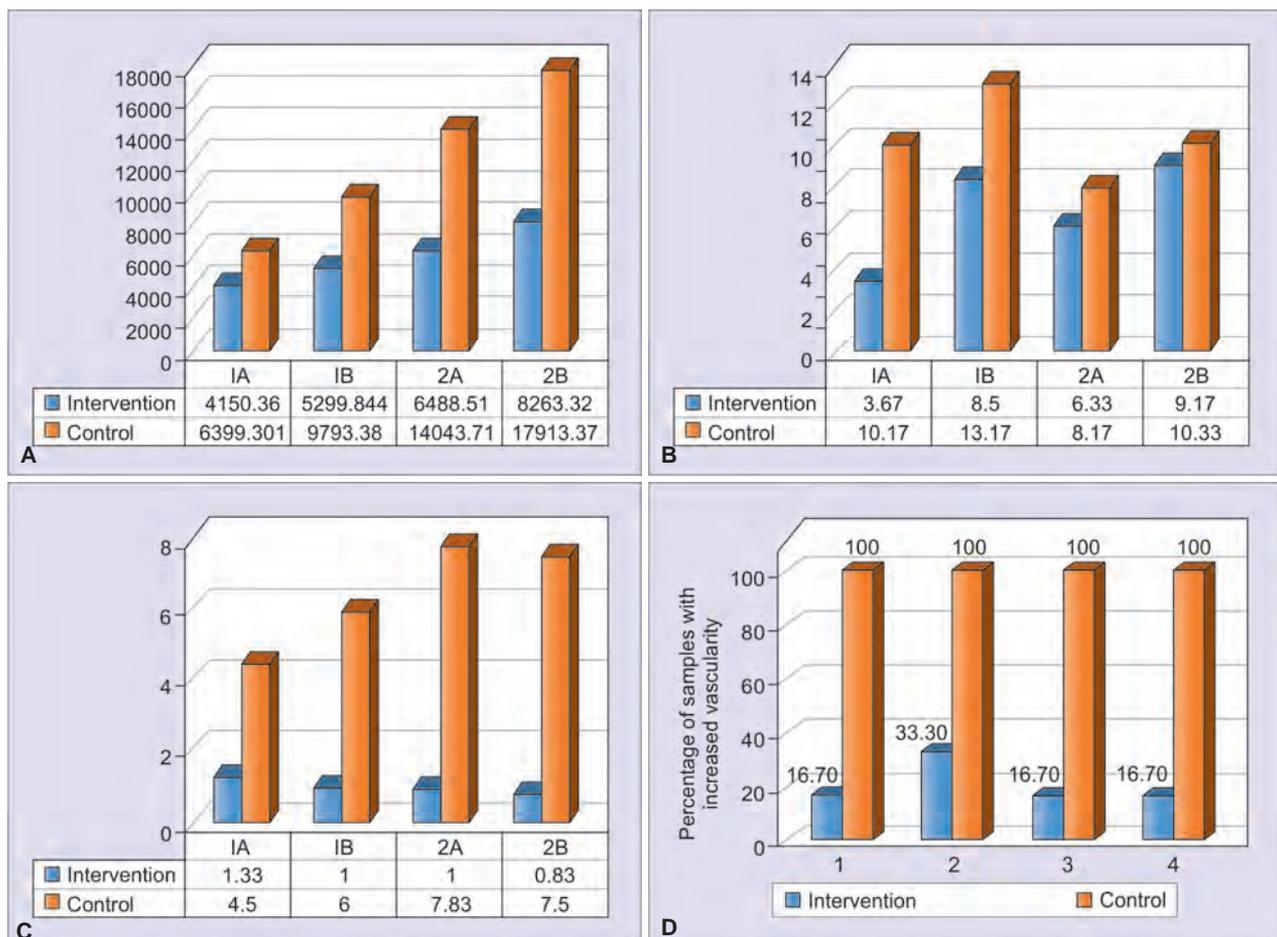
Flow Chart 1: Distribution of study subjects in various groups and subgroups



ethanized at 3 months and the remaining six (subgroup IB, IIB) at 6 months postintervention. Samples of synovial fluid were collected from each knee joint for Cartilage Oligomeric Matrix Protein (COMP) level analysis by enzyme-linked immunosorbent assay and bilateral knee joints were harvested for histopathological assessment of articular cartilage and synovium at the time of euthanasia. The articular cartilage was assessed for degeneration using modified Mankin score and the synovium was assessed for inflammation using a score devised by Pelletier et al.⁴

RESULTS

Statistical analysis showed that mean synovial fluid COMP concentration was significantly lower in PRP-treated knees ($p < 0.05$) at 3 months. On histological examination, mean synovitis scores and synovial vascularity were significantly lower in PRP-treated knees as compared with controls at both 3 and 6 months ($p < 0.05$). Additionally, mean articular cartilage degeneration was significantly lower in PRP-treated knees in group I only ($p < 0.05$) (Graph 1).



Graphs 1A to 1D: (A) Mean synovial fluid COMP concentration [standard deviation: intervention group (IA = 1733.45, IB = 5299.84, IIA = 3606.04, IIB = 8047.74), control group (IA = 2377.96, IB = 5985.20, IIA = 8401.64, IIB = 1.40)]. (B) Mean articular cartilage scores [standard deviation: intervention group (IA = 1.366, IB = 1.64, IIA = 2.160, IIB = 2.927), control group (IA = 3.488, IB = 1.472, IIA = 2.401, IIB = 4.546)]. (C) Mean synovitis scores [Standard deviation: intervention group (IA = 1.03, IB = 0.894, IIA = 0.632, IIB = 0.753), control group (IA = 3.2, IB = 2.683, IIA = 0.753, IIB = 2.258)]. (D) Percentage of samples with increased vascularity in each group

CONCLUSION

Our study showed that intra-articular PRP injection results in reduction of synovial inflammation and vascularity as compared with controls, which may be the biological basis of improvement in pain after PRP injection, in addition to short-term chondroprotective effect.

The anti-inflammatory effect was present at both 3 and 6 months postintervention, which can correspond to short-term and long-term effect in humans respectively, keeping in view the short lifespan of the guinea pigs and the fact that knee OA in guinea pigs is temporally proportionate to that found in human beings. However, the effect on articular cartilage remains somewhat equivocal, however, since the significant results of histological analysis of the cartilage were not reproducible in all subjects in our limited study. Nevertheless the evaluation of COMP levels, which is a marker of cartilage metabolism, demonstrated that the chondroprotective effect of PRP is present at short-term analysis, but it may not be present over a prolonged duration.

This opens up avenues for future research regarding the timing of PRP injections to allow its effect over prolonged durations.

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Efficacy of Platelet-rich Plasma (PRP) with Chitosan Gel vs PRP in an Experimental Guinea Pig Knee Osteoarthritis Model

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INTRODUCTION

Multiple studies have proven the efficacy of platelet-rich plasma (PRP) in early osteoarthritis of knee. However, the effects of PRP are not longlasting, leading to use of multiple injections of PRP for clinically relevant effects.¹ Another approach to enhance the duration of effect and efficacy of PRP has been to combine it with biomaterials. Chitosan is a biomaterial which has been used with PRP in a variety of applications like wound healing, bone defects, etc.^{2,3} However, their intra-articular use and effect on cartilage has not been studied.

PURPOSE OF STUDY

To investigate whether Chitosan gel with PRP is better than PRP in an *in vivo* experimental study on spontaneous guinea pig knee osteoarthritis model.

STUDY DESIGN

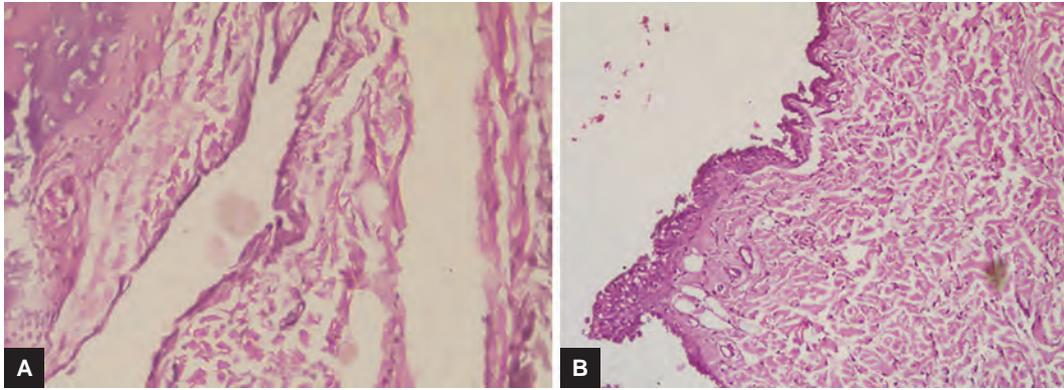
A controlled laboratory study.

MATERIALS AND METHODS

Forty Dunkin–Hartley guinea pigs were taken to conduct this study (weighing ~ 700 gm). Ten animals (group I) were randomized into the disease control where no intervention was done in either knee. Ten animals (group II) were given a single intra-articular allogeneic PRP injection in one knee with simultaneous isotonic saline injection to the contralateral knee to act as control. Groups III and IV, which had 10 animals each, were given Chitosan gel alone and Chitosan gel with PRP in one knee respectively. An isotonic saline injection was given in the other knee to act as control. Five animals from each group (subgroup IA, IIA, IIIA and IVA) were euthanized at 3 months and the remaining five (subgroup IB, IIB, IIIB and IVB) at 6 months postintervention. Upon euthanasia, knee joint synovial fluid samples were collected from each joint for Cartilage Oligomeric Matrix Protein (COMP) estimation by enzyme-linked immunosorbent assay and bilateral knee joints were harvested for histologic assessment of articular cartilage and synovium.

RESULTS

Single injection of PRP and Chitosan PRP resulted in better mean total synovial scores than Chitosan gel and disease control groups at 3 months (p-value <0.05). The anti-inflammatory effect was present only in the short term and there were no significant



Figs 1A and B: Hematoxylin and eosin stain showing synovial morphology. (A) Chitosan PRP treated knee showing no synovial hyperplasia and minimal inflammation. (B) Disease control knee showing 3 to 5 layered synovial hyperplasia and marked inflammation

differences in the mean synovial scores at 6 months among any of the groups. However, there was no difference in mean articular scores among intervention groups of various groups at 3 and 6 months. The synovial COMP levels did not show any significant difference on intra- or intergroup comparison at 3 and 6 months. Chitosan PRP was similar to PRP in terms of its anti-inflammatory effect. There was no significant difference between the mean synovial scores of Chitosan PRP and PRP groups at 3 or 6 months.

CONCLUSION

Our study shows that a single injection of PRP and Chitosan PRP exerts an anti-inflammatory effect in the short term but not in the long term. The anti-inflammatory effect of PRP may be the primary mechanism of its efficacy for a single injection and does not offer significant chondroprotective effect. Addition of Chitosan gel does not seem to offer any additional advantage.

CLINICAL RELEVANCE

Our study is one of the earliest studies to investigate the combination of chitosan with PRP for intra-articular application in OA knee. It opens up new avenues for future research into combination of other preparation of various biomaterials with PRP to enhance the effects of PRP on the joint.

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Bone Marrow Aspirate Concentrate in Nonunion of Bones: Methodology and Our Experience

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INTRODUCTION

This study is based on the research and the results of “Stem cell and cell based therapy” undertaken for the last 5 years in the Nitte University Centre for Stem Cell Research and Regenerative Medicine and the associated hospitals, the Justice K.S. Hegde Charitable Hospital and Tejasvini Hospital, Mangaluru, Karnataka, India, from 2012 to 2017.

PURPOSE OF THE STUDY

Orthopedic application of stem cells in critical bone defects and nonunion is known. We worked in our research lab and our hospitals to arrive at the potential applications of bone marrow aspirate concentrate (BMAC)/stem cells in nonunion of long bones.

METHODOLOGY

For the last 10 years, many publications have come to light where BMAC was directly injected into delayed/nonunion and chondral defects and platelet-rich plasma (PRP) to osteoarthritic knee joints and the site of lateral epicondylitis, plantar fasciitis, etc.

The aspirated bone marrow (BM) will have cellular components of blood including red blood cells (RBCs), white blood cells (WBCs), platelets, plasma, and even stem cells with limited number present per area. It is observed that approximately 0.001% of nucleated cells from BM aspirate are multipotent stem cells. Moreover, the presence of RBCs, WBCs, and plasma will retard the activity of stem cells.

Hence, the aspirate is centrifuged to increase the proportion of stem cells. The method of aspiration and the needle used, and the density gradient centrifugation thereby separating the stem cell population from RBCs and plasma is fairly standardized now, after it was initially reported by Kim and Shetty et al.¹

It is also important that the stem cells injected into the site require scaffolding for the cells to remain in the place and potentially differentiate into cells with osteoplastic activity.

METHODOLOGY OF BMAC PREPARATION

SPRP Kit, separation of BM from RBCs and plasma by centrifugation. For aspirating BM, ensure a 1:5 ratio of acid citrate dextrose-A (ACD-A) to BM, usually collect 20 mL of BM with ACD-A solution at 1:5 volume ratio.

Separation and collection of PRP/buffy coat—Step 1: Mix BMAC with Cartifill/Surgifill/Fibrin using 2- or 3-way connector. Step 2: Inject mixture of BMAC and Cartifill/Surgifill/Fibrin to damaged site of bone or cartilage.

Advantages of Surgifill: Triple helix structure of atelocollagen—optimal cellular scaffold, ideal biocompatibilities, no antigenicity, atelocollagen, pepsin-solubilized type I collagen solution is a water-soluble form of collagen, it can be engineered into a wide array of physical shapes.

MATERIALS AND METHODS

Study was undertaken between August 2013 and July 2016.

Role of BMAC in nonunion of long bones between July 2013 and July 2016:

Table 1: Pre-intervention patient demography

Age from 19 to 60	26 men	2 women	38.5 mean age
Nonunion of tibia	16	Shaft of femur	4
Fracture humerus	4	Fracture radius	2
Mean time since injury	7 months	Range	3–18 months

All these cases were treated as nonunion, when there was no likelihood of fracture uniting irrespective of the duration of fracture.

Patient selection criteria: All patients with nonunion of long bones with a stable implant *in situ* with a gap less than 1 cm and with atrophic and oligotrophic horse hoof type of nonunion.

Exclusion criteria: Patients with infected nonunion, bone gap of more than 1 cm, metaphyseal and intra-articular fracture, pre-existing deformity and shortening of bone, associated diseases like tuberculosis and psychiatry patients, and patients not willing to enter the study.

POSTOPERATIVE FOLLOW-UP

Follow-up every 6 weeks with X-ray for 9 months to assess the callus and for clinical and radiological union.

CALLUS SCORE

The method described by Kim et al³ was followed. Callus score of 5 and above was considered as fracture united.

Points	Anterior cortex	Posterior cortex	Medial cortex	Lateral cortex
0	No callus	No callus	No callus	No callus
1	Slight callus	Slight callus	Slight callus	Slight callus
2	Bridging callus	Bridging callus	Bridging callus	Bridging callus

RESULTS

We compared the outcome with the age and duration of the fracture though it was statistically not significant because of the small number of different types of cases. It is noteworthy to mention that the age and the duration of the fracture and the type of fracture had a significant role to play in the union and the callus score of the fracture.

Out of 28 cases,

- Below age of 35 united
 - Fracture united with excellent callus
- All
1 (above 6)

- United fractures 20
- Nonunited fractures 8 (3A type)
- Duration of fracture more than 9 months 4
- Smokers 7

As far as the duration is concerned, all fractures except 2, below 6 months duration, fractures were united with good callus score.

However, we observed that there was an increase in callus score in 24 patients out of 28.

CONCLUSION

The BMAC (stem cell therapy) is a safe and simple option for nonunion of long bones with gap less than 1 cm. Based on the findings, the percutaneous BM injection did not promote rapid healing when compared with standard operative autogenous grafting. However, it showed many distinctive benefits over the other techniques.

The donor site issue is avoided and could be done repetitively, as it is performed under local anesthesia, the method evades the risk of general anesthesia, infection, and open surgery. Collectively, this is a simple and effective method of providing cellular reactivation of osteogenesis and can positively be employed as an early intervention.

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Scaffold: The Story behind Every Successful Repair—Experience of using Ingenious *Burger Technique* for Cartilage Repair

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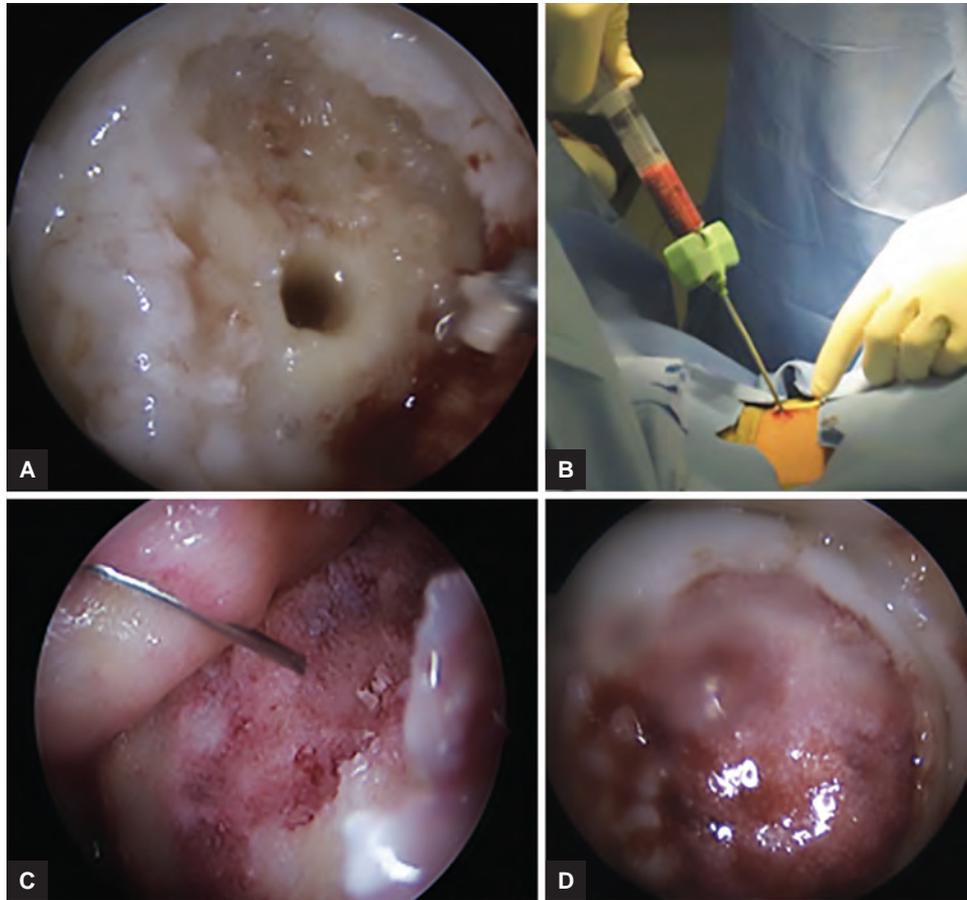
INTRODUCTION

Tissue engineering has three primary components: stem cells, growth factor, and scaffolds. In cartilage, the reparative process involves the following steps:

1. Basilar integration of the repair construct with the underlying bone and cartilage
2. Marginal integration and filling of the defect to the level of surrounding cartilage, and finally
3. Natural stratification. Scaffolds, apart from providing the structural framework for repair, are also responsible for two key functions: they promote extracellular matrix formation and facilitate cellular interactions. All in all, they are temporary structures that are expected to behave as the physiological powerhouse.

For a product to be known as bio-scaffold, it is expected to be nonimmunogenic, nontoxic, biocompatible, and biodegradable. In addition, for universal acceptability and accessibility, it is expected that the product be reasonably priced, be freely available, have a simple surgical technique for application, and have less postoperative restrictions. With availability of lots of commercial products, how to distinguish among various products is always something that is in the minds of clinicians. The properties on which these products can be scored are their porosity, interconnectivity, surface properties, and mechanical strength. All these supposedly are important as they influence cell adhesion, proliferation, reorganization, and neovascularization.

While various natural and synthetic products are available, we devised our own *Burger* technique that is performed using universally available, approved, inexpensive products. In our technique, as the name suggests, three products are used: fibrin glue, absorbable gelatin sponge, and bone marrow concentrate. After the bed is prepared by debriding the area of cartilage defect, the field is dried using CO₂ insufflation and the fibrin glue is injected. After injecting, an absorbable gelatin sponge (Abgel™) is stuck on the cartilage bed over the fibrin glue. After 1 min, the bone marrow concentrate is injected onto this scaffold combination of fibrin gel and gelatin sponge. A setting time of 7 min is observed followed by a gentle range of movement to confirm the construct stability. In our small series of cases, we found this to be a simple, inexpensive method of combining two easily available, approved scaffold materials and harnessing the individual properties of both to ensure a stable biological construct for cartilage repair.



Figs 1A to D: Steps of cartilage repair using Burger technique

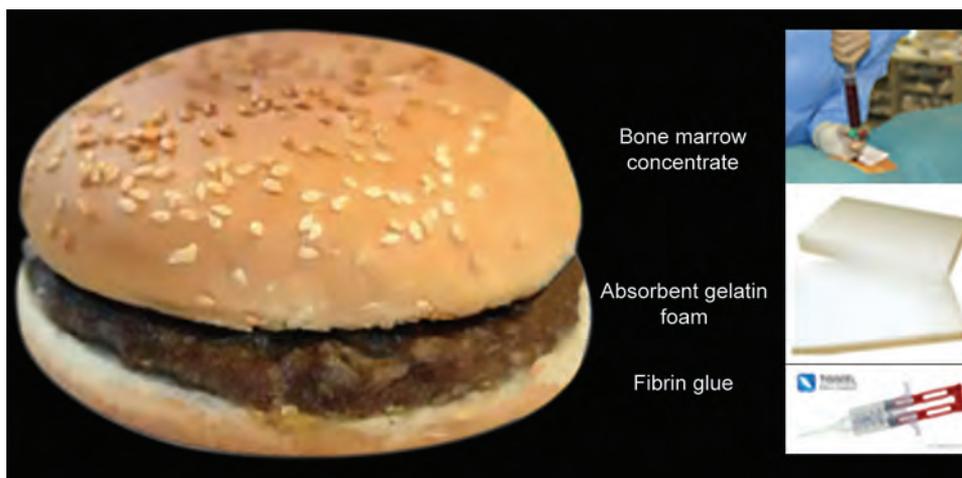


Fig. 2: Illustration of Burger technique

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Efficacy of Platelet-rich Plasma in Recalcitrant Lateral Epicondylar Tendinopathy (Tennis Elbow): A Randomized Controlled Trial

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INTRODUCTION

Lateral epicondylar tendinopathy (tennis elbow) is a nagging problem. While most of the patients benefit from analgesics, counterpressure bracing, physical therapy, rest, and activity modification, there are few who do not improve with any of these modalities. Local corticosteroid injection in such patients was once popular; however, the possibility of permanent damage to the wrist extensor tendons by such injections has brought them into disrepute.

Mishra et al¹ were the first to demonstrate the efficacy of platelet-rich plasma (PRP) in patients of tennis elbow. Since then, several investigators have attempted to evaluate the same. We conducted a randomized controlled trial² in consenting patients with recalcitrant tennis elbow, who were satisfying our inclusion and exclusion criteria. Randomization was done by envelope method and patients in the cases group received 2 to 3 mL of autologous type 4B PRP,³ while the control group received 2 to 3 mL of bupivacaine by peppering technique under ultrasound guidance into the substance of extensor carpi radialis brevis tendon. Physical therapy was initiated in every patient under the supervision of a physiotherapist. Follow-up visits were done at 6 weeks, 3 months, 6 months, and 1 year. Patients were allowed strenuous activities in a staged manner. Visual analog scale (VAS) for pain, modified Mayo clinic performance index for elbow (MMCPPIE), and Nirschl's scores were recorded before injection and at each follow-up visit by a separate observer.

There were 15 patients in the PRP group and 10 in the bupivacaine group. One patient of the bupivacaine group was lost to follow-up. Patients in both the groups had comparable baseline characteristics. An improvement in all the three parameters was noted in patients of both the groups. While the improvement in scores of the PRP injection group was gradual, sustained, and longlasting, that in the bupivacaine group was ill sustained. From a statistical viewpoint the improvement in VAS, MMCPPIE, and Nirschl scores was statistically significant at 6 months and 1 year after the injections. None of the patients complained of increased injection site pain and no one had any significant adverse reaction.

Orthobiologics especially PRP are now being investigated for their probable healing potential in many orthopedic conditions, and the results have been predominantly promising. A summary² of the studies evaluating the role of PRP has been shown in Table 1. These previously published studies along with our present study have all reported a favorable response of PRP in tennis elbow without any significant adverse reaction. In our study, we noted that PRP injection resulted in a better short-term improvement in pain and functional abilities than a bupivacaine injection. Also, the injections were safe and in fact were devoid of any injection site pain which was reported in few earlier studies. Separation of leukocytes might have contributed to the same, as we used a leukocyte-poor PRP.⁴

Table 1: Comparison of studies of PRP injection for recalcitrant lateral epicondylar tendinopathy

Study	Design	No. of patients	Control	PRP type	% improvement in cases versus controls
Mishra and Pavelko ⁷	Cohort study	15 cases vs 5 controls	Bupivacaine	1A	Visual analogue scale (VAS) for pain at 6 months (81% vs unknown)
Peerbooms et al ¹⁰	Randomised controlled trial	51 cases vs 49 controls	Cortisone	1A	VAS for pain at 6 months (53% vs 24%) and 12 months (63% vs 24%)
Creaney et al ⁹	Prospective randomised trial	80 cases vs 70 controls	Autologous blood	1B	Patient-rated Tennis Elbow Evaluation score at 6 months (35.8 vs 46.8); higher conversion to surgery in autologous blood group
Thanasas et al ¹¹	Randomised controlled trial	14 cases vs 14 controls	Autologous blood	1A	VAS for pain at 6 months (70.8% vs 57.8%)
Mishra et al ¹⁴	Randomised control trial	116 cases vs 114 controls	Bupivacaine	1A	VAS for pain (71.5% vs 56.1%)
Present study	Randomised controlled trial	15 cases vs 10 controls	Bupivacaine	4B	At one year, VAS for pain (83.1% vs 45.6%), modified Mayo clinic performance index for elbow (46.97% vs 21.17%), and Nirschl scores (76.6% vs 56.3%)

While several commercial kits are now available for producing PRP, they are costly. The PRP which we used was prepared with the help of the Department of Transfusion Medicine and was highly cost-effective.

The major limitations of the study are the low number of patients who could be included and the short duration of follow-up. Strict following of inclusion and exclusion criteria led to only few patients eligible for being included. The improvement in the pain and the functional capabilities of the patients receiving PRP with no adverse reaction make PRP in recalcitrant tennis elbow a suitable treatment modality.

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Is Platelet-rich Plasma Better than Platelet-poor Plasma in stimulating Human Anterior Cruciate Ligament Cell Growth? Preliminary Data in an Experimental Setting

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INTRODUCTION

Despite our understanding of the effects of platelet-rich plasma (PRP), the effectiveness of individual growth factors on stimulating cell growth are still not clear; the effectiveness of platelet-poor plasma (PPP), consisting of plasma proteins without platelets, and containing growth factors other than those that are platelet derived, is also being documented as equally effective. Anterior cruciate ligament (ACL) injuries are a common occurrence, and the future may consist of ligament tissue engineering with orthobiologics, which is being explored as an alternative approach in place of ACL reconstructions. Investigators have used PRP injected into graft material or as a gel scaffold along with the graft with variable results. While some of the investigators have reported good ligamentization of grafts and good clinical results, others have not had much improvement in outcomes.

In an attempt to evaluate the effectiveness of PRP and PPP individually, a prospective study was designed to evaluate which one of the two was more effective on human ACL cell growth; the characteristics of both were evaluated in cultured cells under experimentally controlled conditions, to see if one was better than the other in modulating or stimulating cell growth.

MATERIALS AND METHODS

The ACL remnants were collected from 11 patients during ACL reconstruction surgery; PPP and PRP were prepared from blood of these patients. Cells were isolated, identified, and cultured and were then divided into six groups.

Groups I to IV had fetal bovine serum (FBS) added to them along with different concentrations of PRP and PPP. Groups V and VI had 5 and 10% PRP respectively, added, but lacked FBS. Cell viability was assayed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye assay, along with Annexin V assay for cell apoptosis. Cell deoxyribonucleic acid (DNA) content was evaluated by propidium iodide staining and flow cytometry.

RESULTS

Patients undergoing ACL reconstruction surgery were between 18 and 40 years (mean 22 years) of age and the duration between injury and surgery was between 4 and 7 months. A successful primary culture was obtained in 11 out of 28 samples of ACL remnant tissue. The analysis of cultured cells showed that addition of PRP (5 or 10%) increased the viability of ACL cells in 4 out of 11 and promoted cell proliferation in 8 of 11 donor samples; 10% PRP was more effective than 5% PRP. However, the difference in effectiveness of 10% PRP was not significantly better than that of 5% PRP. The 5% PPP had no significant effect on cell viability, but it led to an increase in DNA content in 5 of 11 samples. However, there was no statistically significant effect of either PRP or PPP in preventing cell death (depicted by apoptosis rate).

CONCLUSION

Platelet-rich plasma may have an enhancing effect on ACL cell viability and promotion of cell proliferation, but the ideal concentration of PRP for these positive effects needs to be determined before it could be used in clinical settings for enhancing primary repair of torn ACLs. Even though the study design and execution was not completely flawless, our study can be taken as a work in continuation of the work done by Fallouh et al. Both studies suggest that PRP in correct amounts could have a proliferative effect on ACL cells *in vitro* and this could be potentially harnessed for preparing a scaffold with cells and PRP as ACL ligaments, thus changing reconstruction techniques from allograft/autograft tendon reconstructions to real-time ACL reconstruction. However, larger, more controlled, and better applied studies are needed to confirm the clinical utility of this concept.

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Medicolegal Issues Concerning Orthobiologics

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BACKGROUND

There has been an intense global debate on the ethical, moral, and legal aspects of the use of cell-based regenerative therapies as a potential cure, and it is hard to ignore their potential benefits.

Doctors have professional responsibilities—a commitment to medical competence, an evidence-based practice, and avoiding financial conflicts of interest. The clinical applications of novel cell-based therapy, stem cells, and their direct derivatives in orthopedics (orthobiologics), including the use of platelet-rich plasma, hematopoietic stem cells, and other somatic stem cells, raise concern about the physical, psychological, and financial harm to patients who received these cell-based therapies and the general lack of scientific evidences, professional accountability, and regulatory norms of these.

INTRODUCTION

Not all patients are satisfied with their treatment. Some patients are seeking remedies under civil law and regulatory enforcement actions against doctors and providers who have violated laws and best practice standards due to the lack of resources, legal ambiguity, and fraudulent practices, demanding compensation for injuries caused by unproven treatments and financial losses due to false advertising and fraud.

Recently, patients in Japan have initiated legal action following unsatisfactory results from autologous adipose-derived cell treatments, while others in the US have sued for failure to deliver on claims, and there is an ongoing fraud investigation into Stamina Foundation's activities in Italy. The governments in Germany and Italy had to shut down their centers due to death of patients in certain cases.¹

Recently, in the US, nine individual and class action lawsuits were filed against stem cell interventions; these cases have a stronger basis for liability, relying on physical injury rather than financial injury and claiming that the advertised therapies are actively harmful, not merely ineffective.²

Orthopedicians have to practice within the ambit of many law and regulations. They have to be aware of the legal system of the nation and Statutory Law, Indian Laws and Regulations related to these therapies.

In India, no litigation/complaints against cell therapy have been yet reported. In the future, actions can be taken against the practicing clinicians/entities as per the various existing rules and regulations.

Misleading advertising and publicity is prohibited by various acts, such as the Indian Medical Council (Professional Conduct, Etiquettes and Ethics) Regulation³; the Drugs and Magical Remedies (The Objectionable Advertisements) Act, 1954; Drugs and Cosmetics Act, 1940⁴; and code by the Advertising Standards Council of India (ASCI).⁵

The Indian Medical Council (Professional Conduct, Etiquettes and Ethics) Regulations³: Evasion of Legal Restrictions of State Acts, special qualification in specific branch, existing ICMR guidelines of research.

Civil Tort law and Consumer Protection Act: charges for negligence because of lack of care, injury to patient, invading right to information regarding the safety and efficacy record of the cell treatment, right to informed consent: patients have a right to a true informed consent process that includes all the elements and lack of science-based evidence.

CONCLUSION

The efforts of doctors in framing autologous stem cell therapies under the protection of medical practice do not safeguard these treatments from further legal investigations. Doctors have significant amount of freedom in choosing how to best treat their patients, yet certain professional norms restrain the scope of these voluntary benefits.

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Bone Marrow Aspirate Concentrate in Avascular Necrosis Femur Head: How and When?

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BACKGROUND

Avascular necrosis (AVN) of the femoral head is a disease with gradual progression that ends in collapse of the articular surface. Several conditions are known to result in spontaneous cell death, leading to a reduction of trabecular bone and the development of AVN. The disease passes through two main stages: precollapse (ARCO 0-II) and postcollapse stage (ARCO III-IV, crescent sign). While in the postcollapse phase, total hip arthroplasty remains the treatment of choice in most patients, in the precollapse phase, core decompression (CD), with or without the addition of bone marrow (e.g., bone marrow aspirate concentrate, BMAC) or bone graft, is being considered as a standard treatment alternative. Sen et al¹ found that patients with poor preoperative Harris hip score (HHS), X-ray changes, edema, and effusion on magnetic resonance imaging had better results in the CD with bone marrow instillation. Hernigou et al² have well established that with BMAC instillation, there occurs intraoperative enrichment of mesenchymal and progenitor stem cells as a single-stage procedure.

PURPOSE OF STUDY

Retrospective comparative evaluation of hip function score and quality of life index in patients of AVN femur head getting CD with or without instillation of bone marrow stem cells.

MATERIALS AND METHODS

In the last 14 years we have been using standard CD alone or with addition of BMAC as management protocol in early stages (ARCO stage I and stage II) of AVN femur head. In a retrospective analysis we have accumulated the experience of managing more than 250 patients at precollapse stage of AVN with a follow-up of 6 months to 11 years. The outcomes have been analyzed using standard HHS for functional status and World Health Organization quality of life (WHOQoL) score for overall outcome of the disease process.

RESULTS

Out of these, a group of 98 patients with mean age of 34.5 years had AVN at ARCO stage 1 and after treatment with CD alone ended with HHS 84 and WHOQoL as 85.5. A total of 90 patients having ARCO stage II with a mean age of 36.7 years had instillation of BMAC along with CD and resulted in HHS as 83.3 and WHOQoL as 83.4 at last follow-up. This means that in spite of advanced stage of AVN ARCO II, the eventual outcome with addition of BMAC was not worse than ARCO stage I patients who had the procedure of CD alone.

CONCLUSION

While patients with AVN femur head ARCO stage I can expect good clinical outcomes with CD alone, the AVN ARCO stage II patients also can expect similar outcome if additional instillation of bone marrow stem cells is made along with CD.

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Short-term Outcome of Autologous Bone Marrow Mesenchymal Cell-induced Chondrogenesis in Young Athletes with Traumatic Chondral Defect

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BACKGROUND

Bone marrow is a good source of progenitor cells and growth factors. The progenitor cells only account for about 0.01 to 0.001% of the bone marrow, but there is a high concentration of growth factors like platelet-derived growth factor, transforming growth factor- β , and bone morphogenetic protein (BMP)-2 and BMP-7. It also contains a good amount of interleukin-1 receptor antagonist, which helps in providing pain relief whereas the growth factors have an anabolic and anti-inflammatory effect.

Traumatic articular cartilage defect is one of the most common problems encountered by young athletes. It is a progressive lesion that is difficult to treat. Mesenchymal cell-induced chondrogenesis is a single-staged arthroscopic procedure that combines bone marrow aspirate concentrate (BMAC) injection with microfracture chondroplasty, aimed at treating the chondral defect.

AIM

To study short-term outcome of mesenchymal cell-induced chondrogenesis along with microfracture chondroplasty in athletes having posttraumatic grade IV chondral defect of knee.

MATERIALS AND METHODS

This is a prospective study of six athletes with a follow-up of 2 years (mean age 26.5 years; mean body mass index 25.16). All the patients had isolated chondral lesions without ligament or meniscal involvement. Postoperative magnetic resonance imaging (MRI) showed exposure of the subchondral bone that was considered to be grade IV chondral lesion according to Outerbridge classification. Under general anesthesia, a diagnostic arthroscopy was done to confirm the MRI findings. Once confirmed, 40 to 60 mL of bone marrow was aspirated from the ipsilateral anterior superior iliac spine using two syringes with preloaded anticoagulant citrate dextrose solution. Using a dual centrifugation device, the aspirate was centrifuged twice and BMAC was obtained. Microfracture chondroplasty was done. Normal saline was drained out of the joint and CO₂ gas was pumped for joint visualization. The BMAC was injected along with hyaluronic acid and fibrin glue, which act as scaffold and prevent the scatter of the mesenchymal cells. Postoperatively, the patients were advised nonweight bearing walking for 6 weeks along with a structured rehabilitation protocol for 4 months and sports-specific rehabilitation thereafter. Postoperative MRIs were taken for comparison with the preoperative MRIs. The outcome measurements were done using visual analog scale (VAS), Tegner's activity scale, and Lysholm score compared with preoperative values.

Table 1: Preoperative and postoperative outcome scores in the six athletes

	Age	Sport	Visual analog scale		Tegner activity		Lysholm score	
			Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative
Athlete 1	24/M	Football	6	0	Level 9	Level 9	70	95
Athlete 2	23/M	Kabaddi	4	0	Level 8	Level 8	84	90
Athlete 3	21/M	Volleyball	4	1	Level 8	Level 8	80	95
Athlete 4	26/M	Football	5	1	Level 9	Level 5	55	85
Athlete 5	28/M	Tennis	3	0	Level 7	Level 7	85	90
Athlete 6	29/M	Cricket	3	0	Level 6	Level 6	89	95

RESULTS

Six male athletes from different sports disciplines, all having isolated grade IV chondral lesions, were followed up at regular intervals. They all showed significant improvement at 2 years follow-up. Five of the athletes returned to recreational play at 9 months and match-level play at the end of 2 years. One athlete reached recreational play at 1 year and thereafter quit sport for academic reasons.

CONCLUSION

Mesenchymal cell-induced chondrogenesis with microfracture chondroplasty showed satisfactory short-term outcomes in terms of patient becoming asymptomatic and return to play. Preliminary outcomes have shown it to be a useful technique in the treatment of isolated grade IV chondral lesions.

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