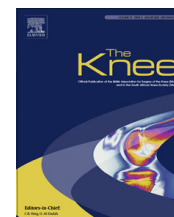




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Articular chondroprogenitors in platelet rich plasma for treatment of osteoarthritis and osteochondral defects in a rabbit knee model



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ABSTRACT

Background: Articular chondroprogenitors are a promising contender for cartilage repair due to their inherent nature which stands primed for chondrogenesis and minimal hypertrophic preponderance. Platelet rich plasma (PRP) has been extensively used for treating cartilage defects and osteoarthritis (OA), due to its chondro-inductive properties and abundant pool of growth factors. The aim of this study was to assess the efficacy of chondroprogenitors injected with PRP versus PRP alone in the healing of experimentally created early OA and osteochondral defects (OCD) in a rabbit model.

Methods: Adult New Zealand White male rabbits were used for cell and PRP isolation. Chondroprogenitors were isolated by fibronectin adhesion assay, labelled with iron oxide, characterized for surface markers, differential potential and expanded. PRP was isolated by double spin centrifugation using a TriCell kit. Study groups included (a) Monosodium iodoacetate induced early OA and (b) critical OCD. Following intervention (test arm: PRP+ chondroprogenitors and control arm: PRP), assessment was performed at 6- and 12-weeks which included histopathological examination and scoring (OARSI and Modified Wakitani score), immunohistochemistry analysis (Collagen type II and X) and synovial fluid S100A12 levels.

Results and conclusion: Comparable, evident healing was noticed in both test and control arms when the OA group samples were assessed at both time points. In the OCD group, PRP alone exhibited significantly better results than the test arm, although repair was notable in both interventions. Further evaluation of chondroprogenitors is required to assess their role as a standalone therapy and in combination with PRP to further cartilage regeneration.

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1. Introduction

Treatment of articular cartilage pathologies remains a significant challenge due to limited self-repair seen in this tissue. Osteoarthritis (OA), a common degenerative condition of joints characterized by cartilage deterioration, progressive chondral bone erosion and synovial inflammation [1]. It has also been noted that chondral defects occurring secondary to trauma/disease may lead to osteoarthritic changes when left untreated. In scenarios where cartilage injury remains superficial, demonstrating loss of non-collagenous matrix without collagen damage, complete repair of the injured matrix has been reported [2]. However, when fibrillar network damage is accompanied with chondrocyte death, healing is hampered. With reference to full-thickness defects involving the cartilage and subchondral bone, repair tissue generated contains higher levels of collagen type I and other non-specific proteoglycans resulting in a fibrocartilaginous structure leading to sub-optimal biomechanical properties [3]. Currently, trends in cell-based therapy focus on mesenchymal stem cells and chondrocytes for the treatment of cartilage afflictions such as focal osteochondral defects and OA [4]. However, numerous limitations such as donor site morbidity, upregulation in hypertrophic marker expression and fibrotic tissue formation still warrant optimization of current protocols in use or identification of cell types with improved chondrogenic phenotype and minimal terminal differentiation.

Multiple studies investigating the potential of articular cartilage derived chondroprogenitors have proposed its applicability as a promising alternative for cartilage regeneration given its inherent *in-vitro* properties. Characteristic features of these progenitors include differential adhesion to fibronectin, high replicative potential, enhanced chondrogenesis and low inclination to hypertrophy in addition to being classified as mesenchymal stem cells (MSC) [5–8]. Several *in vitro* studies involving mechanical stimulation of chondroprogenitors under physioxia, have demonstrated higher expression of chondrogenic genes and elevated migration to the injury site in response to SDF-1 [9–11]. Reports comparing chondroprogenitors to chondrocytes and MSCs have exhibited superior potential for cartilage repair in chondroprogenitors, in terms of high PRG4 expression with low levels of RUNX2 and COL10 [12,13].

In an attempt to ease cellular delivery to the cartilage defect or disease site, various scaffolds, which are either natural derivatives, biomimetics or synthetic have been in use [14]. Platelet-rich plasma (PRP), a byproduct of blood is extensively used as a stand-alone therapeutic tool for symptomatic relief in cartilage pathologies. It has also gained recognition as a bio-scaffold for applications in cell delivery [15]. The rationale for its use depends on its constituent elements, which comprise of multiple growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF β), which are known to stimulate cellular anabolism and activate endogenous repair. The super-added anti-inflammatory properties and cross interaction with fibrinogen contribute to its scaffolding effect [16]. Since, the end goal in cartilage repair is symptomatic relief and healing with hyaline-like tissue formation, our study aimed to assess the potential of articular cartilage derived chondroprogenitors suspended in platelet-rich plasma in experimentally created early grade osteoarthritis and osteochondral defects for neo-cartilage formation.

2. Methods

2.1. Study design

Study approval by the Institutional Review Board and Animal Ethics Committee was obtained. All experimental procedures were performed following the Institutional animal care and use guidelines (Fig. 1, Graphical abstract). Rabbits were chosen for the study as they are commonly used for assessing cartilage repair. Ease of manipulation of joint, synovial fluid volume, cost and space effectiveness were further considerations when choosing the animal model. Following veterinarian clearance, 23 mature New Zealand White rabbits (males, mean age = 1-year, average weight = 2 kg) were recruited, tagged with a unique identification number, and maintained at our Institutional animal house facility. Twenty rabbits were randomized into two experimentally created study groups namely (a) OA (n = 10) and (b) osteochondral defect (OCD, n = 10). All experiments involving animals were initiated in the morning. Chondroprogenitors were isolated from the articular cartilage of one rabbit for this study. Cells were isolated using fibronectin adhesion assay, labelled with micron-sized superparamagnetic iron-oxide, and expanded to confluence. Before the transplant, the chondroprogenitors were evaluated for MSC markers and trilineage differentiation potential. For PRP acquisition, whole blood from two rabbits was subjected to double spin centrifugation.

To create OA, a single dose of monosodium iodoacetate (MIA, I2512, Sigma Aldrich, US) was injected bilaterally into the knee joints of the hindlimbs, and 35 days was fixed for the development of disease. OCD was created on the day of the intervention, for which joint was exposed, and interventions delivered directly into the created defects. Simultaneously, no exposure was required for the OA arm, and an intra-articular injection of the treatment sufficed. The experimental groups included (a) a control arm where PRP alone was injected/delivered into the left knee joint, and (b) a test arm which composed of chondroprogenitors suspended in PRP, injected/delivered into the right knee joint. At the end of 6 and 12 weeks, five rabbits from each group were euthanized at each time point, the knee joints were harvested and subjected to histopathological analyses and grading by three individual blinded observers using Osteoarthritis Research Society International (OARSI) grading and modified Wakitani scoring system for OA and OCD respectively. For the OA study group, synovial fluid was also harvested and assessed for an OA biomarker, S100A12 using an ELISA kit.

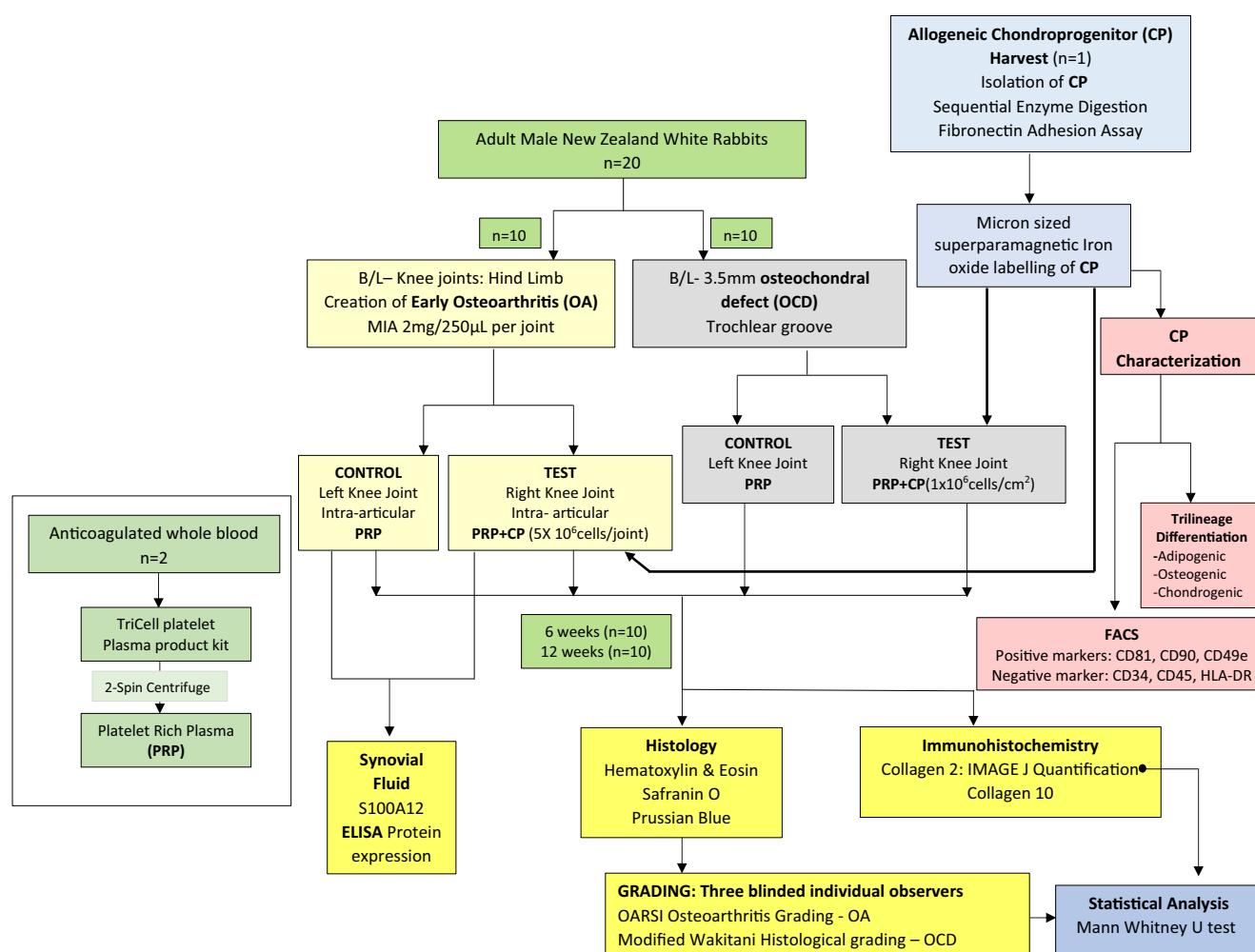


Fig. 1. Study algorithm depicting isolation of chondroprogenitors, isolation of platelet rich plasma, creation of osteoarthritis and osteochondral defects, intervention, and subsequent evaluation parameters.

2.2. Animal care

Before any procedure, the rabbits were anesthetized using an intramuscular injection of 50 mg/kg of ketamine (K03593, Troikaa Pharmaceuticals Ltd, Ahmedabad, India) with 4 mg/kg of 2% Xylazine (Medvet Biolinks Ltd.) and a top-up dose was administered based on the requirement. Postoperatively, 0.2 mg/kg of Meloxicam (S03298, Intas Pharmaceuticals, Ahmedabad, India) was administered subcutaneously for analgesia and 10 mg/kg of intramuscular Enrofloxacin (Virbac Pharmaceuticals, France) once daily for 3 days. All the rabbits were carefully observed and maintained in a controlled environment (1 animal/cage) ensuring adequate ventilation, room temperature, free mobility (provided in turns) with unlimited access to food and water.

2.2.1. Creation of OA using MIA

After confirming adequate anesthetic depth, knee joints of rabbits ($n = 10$) were prepared for the procedure. Under aseptic precautions, a single dose of 2 mg MIA reconstituted in 250 μ l of 0.9% normal saline was injected intra-articularly on both sides. To ensure uniform distribution, the knee joints were ranged multiple times. These animals were then allowed 35 days for the development of OA.

2.3. Isolation and culture of rabbit articular cartilage derived chondroprogenitor cells

Cartilage shavings obtained from rabbit knee joints were minced to a size of 1 mm^2 and washed. The shavings were subjected to sequential overnight digestion with 12 IU of pronase (Mat no: 10165913103, Roche, Switzerland) and 100 IU of collagenase type II (Cat no: LS004176, Worthington, USA). The released chondrocytes were added to precoated fibronectin (10 μ l/ml, F1141, Sigma Aldrich, Missouri, USA; containing CaCl_2 and MgCl_2) wells at a concentration of 4000 cells per well and incubated at 37 $^\circ\text{C}$ for 20 min as described by Nelson et al. [7]. Non-adherent cells and media were removed and replaced with standard growth media. The medium used for expansion comprised of DMEM-F12 Glutamax (AL155G, Himedia,

Mumbai, India) with 10% fetal calf serum (10270106, Gibco, ThermoFischer Scientific, California, USA), 0.1 mM ascorbic acid (Sigma Aldrich, Missouri, USA), 100 IU/ml penicillin–streptomycin (Gibco, ThermoFischer Scientific, California, USA) and 2 µg/ml amphotericin-B (Gibco, ThermoFischer Scientific, California, USA). After 11 days in culture, colonies containing a cluster of more than 32 cells were trypsinized and transferred to a T-25 flask and cultured to 50% confluence (Fig. 1).

2.3.1. Superparamagnetic iron-oxide labelling (M-SPIO)

At 50% confluence, M-SPIO (BM547, 3–12 µm; Bangs laboratories, Indiana, USA) at a concentration of 12.75 µg/ml of culture medium, was added to the cells for a period of 18 h as described by Vinod et al. [17]. Following this, the cells were washed thoroughly to remove non-incorporated M-SPIO particles and further expanded to passage 2.

2.4. Characterization studies

2.4.1. Flow cytometric studies

To determine surface marker expression, chondroprogenitors were harvested with 0.25% trypsin-EDTA (25200072, GIBCO, ThermoFischer Scientific, California, USA) and washed with phosphate-buffered saline (PBS, M1866, Himedia, Mumbai, India). Flow cytometry was used for assessment against the following conjugated rabbit (BD Pharmingen, New Jersey, USA) surface antigens (a) MSC and chondroprogenitor markers: CD81-APC, CD 90-FITC, CD49e-PE, (b) negative markers associated with MSCs: HLA-DR-FITC, CD45-FITC and CD34 PE [6,18]. The cells were subjected to flow cytometric analysis using BD-Calibur and BD-Celesta flow cytometers. Appropriate unstained and IgG controls were run for each conjugate and gating applied using BD FACS Diva v 5.0.2 FACS.

2.4.2. Trilineage differentiation and staining

Chondroprogenitors were subjected to multilineage differentiation using Stem Pro (A1007001, A1007101, A1007201, ThermoFischer Scientific, California, USA) kits. To establish adipogenic and osteogenic differentiation, the chondroprogenitors were expanded in two-dimensional monolayer cultures up to a confluence of 50% using a standard growth medium. This was replaced with either adipogenic Stem Pro or osteogenic Stem Pro differentiation medium and further culture for a period of 21 days was performed, with medium change once every 3 days. To confirm the acquisition of oil droplets and differentiation to adipocytes, the cells were fixed with 10% buffered formalin for 1 hour and stained with Oil red O (O0625, Sigma Aldrich, Missouri, USA) for 20 min. For osteogenic differentiation, cells were fixed with 70% ethanol and stained with 2% Alizarin red solution (A5533, Sigma Aldrich, Missouri, USA) for 20 min. For chondrogenic differentiation, 0.5 million cells were cultured in an Eppendorf for 48 hours to form a stable pellet and differentiated using a chondrogenic Stem Pro medium for 21 days. The pellets were fixed for a short duration and sectioned. The slices were further processed for Alcian blue staining (pH = 4.2, J60122, Alfa Aesar, ThermoFischer Scientific, California, USA) and immunohistochemical analysis for collagen type II.

2.5. Creation of OCD

Under aseptic precautions, a parapatellar skin incision was made, followed by lateral dislocation of the patella. A critical OCD, 3.5 mm in diameter and depth, was created in the central area of the femoral trochlear groove using a drill bit. Following a thorough saline wash to remove debris, the site was prepared for transplant.

2.6. Platelet rich plasma (PRP) isolation

PRP isolation was performed based on the unique dial-in hourglass technology using the TriCell Plasma Kit (PA34-190322, TriCell Biologics Private Limited, Chennai, India). After confirmation of deep anaesthesia, 27 ml of whole blood was drawn in a prefilled syringe containing 3 ml of the anticoagulant. The blood was injected into the TriCell unit, adjusted using the RBC chamber cap and subjected to a 5 min centrifugation at 3200 rpm. Following separation into three layers i.e: plasma, buffy coat and RBC, the admixture containing plasma and buffy coat was further centrifuged at 3300 rpm for 5 min to obtain the final PRP yield (PRP obtained following TriCell kit processing yielded plasma containing approximately 1×10^6 platelets/ml). It was ensured that the utilization of PRP was completed within 2 hours of its harvest.

2.7. Intervention

2.7.1. OA study arm

Following confirmed anaesthesia, the knee joints of the hindlimbs were prepared and positioned in full flexion. Fresh chondroprogenitors (5 million cells suspended in 250 µl of PRP) were introduced as a single intra-articular injection into the right knee joint, which served as the test arm (Video 1). Simultaneously, the same volume of PRP alone was injected into the left joint which served as the control. Knee joints were ranged to ensure uniform distribution.

2.7.2. OCD study arm

Fresh chondroprogenitors (1 million cells suspended in 25 µl of PRP) were delivered using a pipette into the critical osteochondral defect of the right knee (test arm). In contrast, the same PRP volume was delivered into a defect of the left knee joint (control arm). In order to activate PRP, CaCl₂ solution (final concentration 15 mM) was also added to the defect which was further secured with fibrin glue ([Video 2](#)).

All rabbits were allowed to move freely following the procedure. Animals from both intervention arms were euthanized with a lethal intracardiac dose of ketamine at 6 weeks (n = 5 for each arm) and 12 weeks (n = 5 for each arm).

2.8. Synovial fluid S100A12 analysis

Synovial fluid analysis was performed on samples collected from animals in the OA study arm (both control and test). Under aseptic precautions, 250 µl of 0.9% normal saline was injected into the knee joints, which were flexed and extended multiple times for optimal admixture. A midline incision and a medial para-patellar arthrotomy were performed to expose the joint. After incising the synovium, fluid was collected into cryovials. The level of the biomarker S100A12 was assessed as per the manufacturer's instructions using a commercial ELISA kit (EL020626RB, Cusabio, Texas, USA). 0.9% normal saline, which was used as the sample diluent was designated as the negative control. Absorbance was detected using Bio-Rad microplate reader at 650 nm, with wavelength correction. The readings were interpreted using a standard curve fit, and the S100A12 concentration (dilution factor included) was calculated for each sample. Each sample was performed in duplicates.

2.9. Histopathological assessment

Joints were fixed with 10% buffered formalin for a week and were washed with tap water for 24 h. The specimen was decalcified by complete immersion in 10% EDTA solution (2964750918, Qualigens, ThermoFischer Scientific, California, USA) pH 7.4, continually stirred at 25°C for a total of 35 days and paraffin-embedded. 4 µm transverse sections were prepared and stained with Hematoxylin & Eosin, Safranin O and Prussian blue. Prussian blue staining was performed to localize the presence of M-SPIO labelled chondroprogenitors.

2.9.1. Immunohistochemistry analysis: Collagen type II and type X

The sections were rehydrated, collagen type II was retrieved using pronase (Mat no: 10165913103, Roche, Switzerland; 1 mg/ml: 30 min) and hyaluronidase (5 mg/ml: 30 min, H3506, Sigma Aldrich, Missouri, USA). Collagen type X was retrieved using chondroitinase ABC (0.1 units/ml: 1 h, C3667, Sigma Aldrich, Missouri, USA) and pepsin (pH 2.2, 1 mg/ml: 15 min, R2283, Sigma Aldrich, Missouri, USA). Thereafter sections were subjected to 3% hydrogen peroxide incubation for blocking endogenous peroxidase activity. Following protein block (1% bovine serum albumin and 6% FCS), the slides were incubated with primary antibodies against collagen type II (5 µg/ml, mouse monoclonal antibody at 4°C, overnight, DSHB II-II6B3, Iowa, USA) and collagen type X (1 in 200 dilution, 37°C for 4 h, ab49945, Abcam, Cambridge, UK). The secondary antibody incubation was performed using HRP labelled goat anti-mouse immunoglobulin (31430, Pierce, Wisconsin, USA; 1:100 dilution). Following this, staining was performed with a 3,3'-diaminobenzidine solution and counterstained with hematoxylin.

All histological images were taken using an Olympus microscope and were scored by three blinded individual observers. The severity of OA was evaluated using the Osteoarthritis Research Society International (OARSI) assessment system where the product of grade (varying from 1 to 6) and stage (percentage involvement) provided the arthritis score [19]. A higher number indicated severe disease and vice versa. A modified Wakitani histological scoring system (Table S1) was used for assessing cartilage repair in the OCD group following the intervention [20]. The variables considered included cell morphology, metachromacy, surface regularity, cartilage thickness and integration with adjacent cartilage leading to a maximum score of 14.

2.9.2. Quantification of collagen type II

To quantify the staining intensity of Collagen type II, the stained section's total area in the OA study group and area limited to defect in the OCD study group, were selected and compared between the corresponding joints at 6 and 12 weeks. Quantitative analysis of positive intensity staining (in arbitrary units) was measured by Image J software version (DAB-H vector).

2.10. Statistical analysis

Analysis and statistical comparison of synovial fluid S100A12 expression, OARSI grading, modified Wakitani scoring and Collagen type II levels at 6 and 12 weeks between control and test arms were performed with non-parametric Mann-Whitney U test. Statistical Analysis was done using SPSS 15.0 and a P value < 0.05 was considered significant. All data are expressed as mean ± standard deviation.

3. Results

3.1. Characterization of chondroprogenitors

Chondroprogenitors isolated via fibronectin adhesion assay grew clonally, achieving 5 population doublings by day 11 and were successfully labelled with M-SPIO at passage 0, and continued to multiply up to passage 2 (Fig. 2).

3.1.1. FACS

Flow cytometric analysis of clonally expanded passage 2 chondroprogenitors showed moderate to high expression of CD81 (98.25%), CD90 (61.4%), CD49e (64.7%) and minimal expression of HLA-DR (0.9%), CD45 (0.2%) and CD34 (0.1%) (Fig. 3).

3.1.2. Trilineage differentiation

Representative microphotographs show that rabbit chondroprogenitors were able to differentiate into adipogenic lineage verified by positive intracellular Oil Red O accumulation (Fig. 4A), osteogenic lineage verified by positive Alizarin red uptake (Fig. 4B) as well as chondrogenic lineage by Safranin O uptake indicative of glycosaminoglycan accumulation (Fig. 4C) and positive collagen type II protein uptake (Fig. 4D).

3.2. Gross appearance of the joint following intervention

The appearance of joints in the OA group following the intervention demonstrated similar findings in both test and control arms at 6 weeks, with minimal cartilage abrasion, low osteophyte formation, focal subchondral bone exposure (at the trochlear groove) and synovial thickening. The condition of the joints was comparatively better in the PRP + chondroprogenitor arm on subjective visual analysis. At 12 weeks, joints from both the study arms showed comparable findings and significant healing compared to 6 weeks. Regarding the OCD group, both 6- and 12-week assessments revealed better healing in the PRP arm. Filling of the defects was comparatively better in the PRP arm with improved integration, whereas the defect in PRP + chondroprogenitor arm was filled with repair tissue displaying increased redness. (Fig. 5).

3.3. S100A12 protein expression

Both study arms demonstrated of the OA group comparable levels of S100A12 protein expression with no significant difference between them in synovial fluid samples obtained at 6 and 12 weeks (Fig. 6).

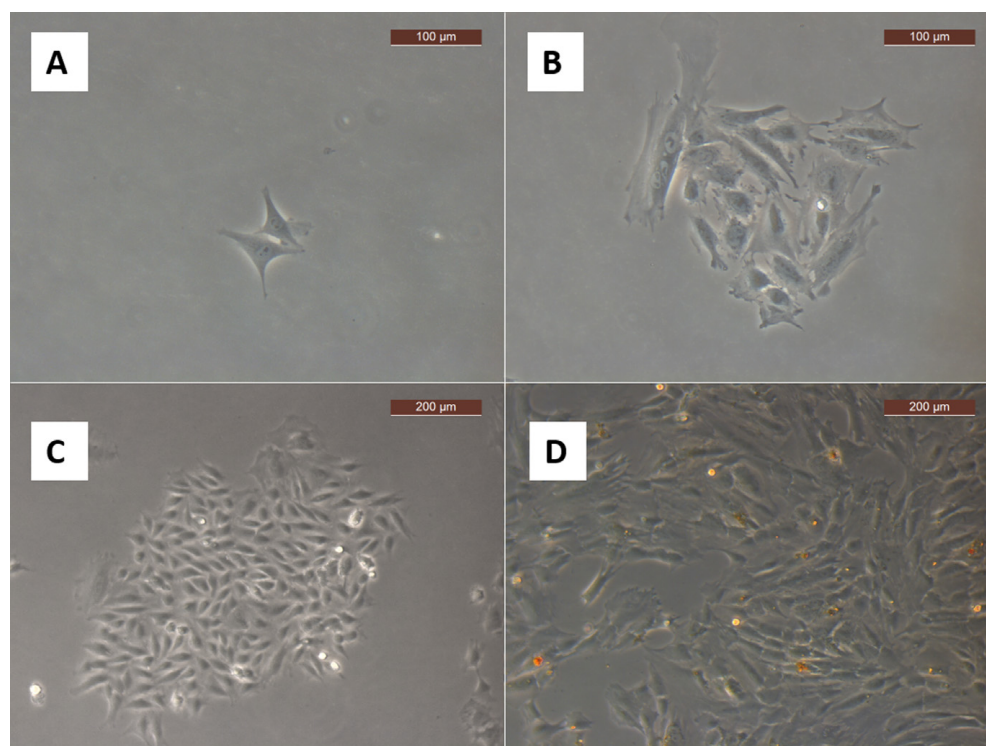


Fig. 2. Culture of clonally derived rabbit articular chondroprogenitors. A clone at day 1 (A), day 4 forming a loose cluster of few cells (B) and day 8 forming a tighter cluster with more than 5 population doublings (C). Iron labelled passage 0 chondroprogenitors at sub confluence. Mag: scale bar.

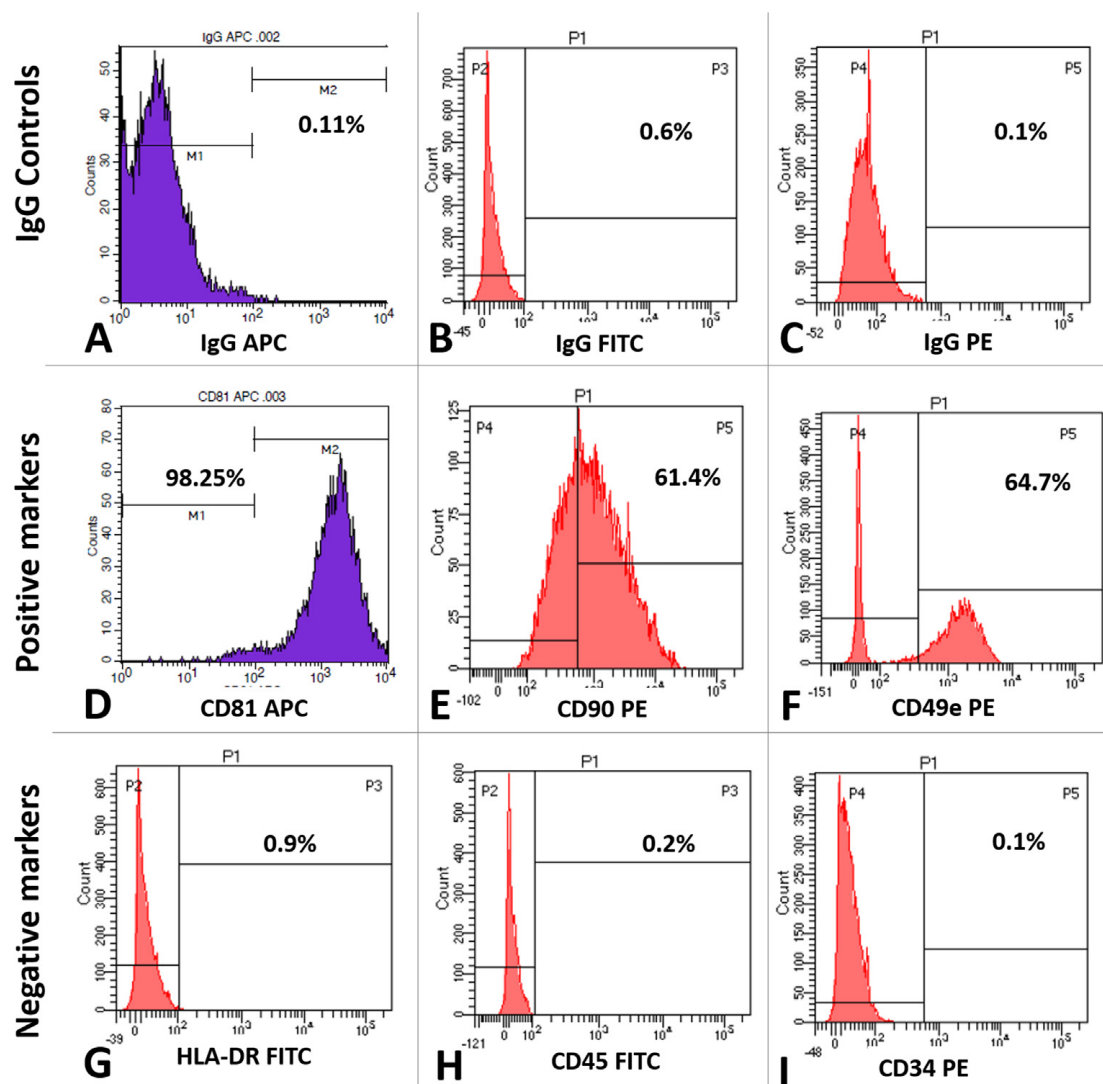


Fig. 3. Flow cytometric analysis of articular cartilage derived chondroprogenitors for mesenchymal stem cell positive markers: CD 81(D), CD90(E), CD49e(F), negative markers: HLA-DR(G), CD45(H), CD34(I) and IgG controls (A-C).

3.4. Histopathological analysis and scores

3.4.1. OA study group

Joints harvested at both 6 and 12 weeks showed changes typically suggestive of early OA, including minimal cell death and clustering, depletion of cationic stain (Safranin O) limited to the upper 2/3rd of the cartilage and intact superficial zone. There was no significant difference observed between test and control arms in terms of morphology (OARS scoring, Fig. 7A & 8), collagen type X uptake, and collagen type II stain intensity (Fig. 9A).

3.4.2. OCD study group

Samples analyzed at both 6 and 12 weeks demonstrated significantly better scores in the control arm when compared to the test (6 weeks: PRP = 4.2 ± 0.9 , PRP + chondroprogenitor = 8.3 ± 1.6 , $P = 0.02$; 12 weeks: PRP = 4.9 ± 2.0 , PRP + chondroprogenitor = 8.9 ± 2.0 , $P = 0.04$, Fig. 7B). Healing with PRP alone exhibited regeneration with tissue primarily composed of hyaline cartilage spanning > 2/3rd of the adjacent healthy tissue with a slight reduction in matrix staining, good surface regularity and integration on both ends (Fig. 10). However, PRP + chondroprogenitors caused comparatively reduced regeneration, as evident with decreased cartilage thickness, marker reduction in matrix staining and moderate surface irregularity. When Collagen type II was quantified, the control arm exhibited significantly higher stain intensity than the test arm (6 weeks and 12 weeks: $P = 0.01$, Fig. 9B).

All test arms in both study groups demonstrated Prussian blue staining which was indicative of M-SPIO labelled chondroprogenitors. Staining was also seen to be present in the deeper zone of regenerated cartilage, indicative of cell migration (Fig. 8&10).

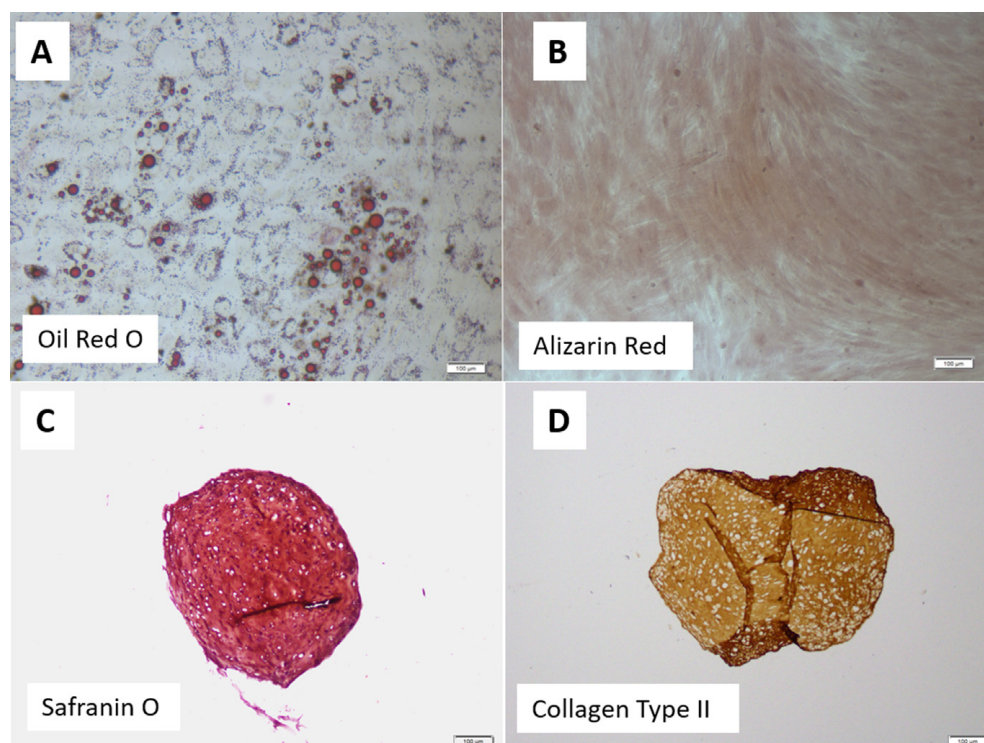


Fig. 4. Trilineage differentiation studies of CP. Oil red O (A) and Alizarin Red (B) staining of monolayer differentiated CPs. Safranin O(C) and Collagen type II (D) immunohistochemical staining of differentiated CP pellets. Mag: scale bar. CP: chondroprogenitor.

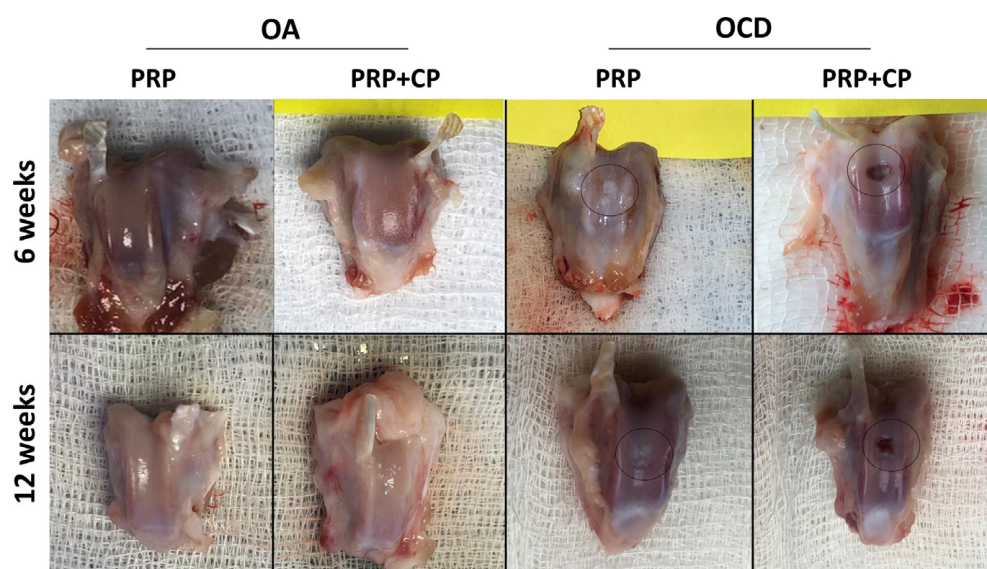


Fig. 5. Macroscopic examination of (A) effect of PRP and PRP + CP on the progression of Monosodium Iodoacetate induced OA and (B) effect of PRP and PRP + CP on OCD of femoral condyles harvested at 6 weeks and 12 weeks. PRP: Platelet rich plasma, CP: chondroprogenitors, OA: osteoarthritis and OCD: osteochondral defect.

4. Discussion

Cell-based repair can be the therapeutic tool to address the finite healing capacity of cartilage, once it is affected by disease or trauma, like that seen in osteoarthritis and osteochondral defects. The ability of articular cartilage derived chondroprogenitors to cause regeneration of hyaline cartilage in early OA and osteochondral defects have not been explored. With this aim in mind, an ideal bio-scaffold was required to facilitate cell delivery in the area of interest. Therefore, this study included PRP as the scaffold and the control arm, while cells impregnated in PRP served as the test arm to assess healing of MIA induced early OA and OCD in a rabbit model.

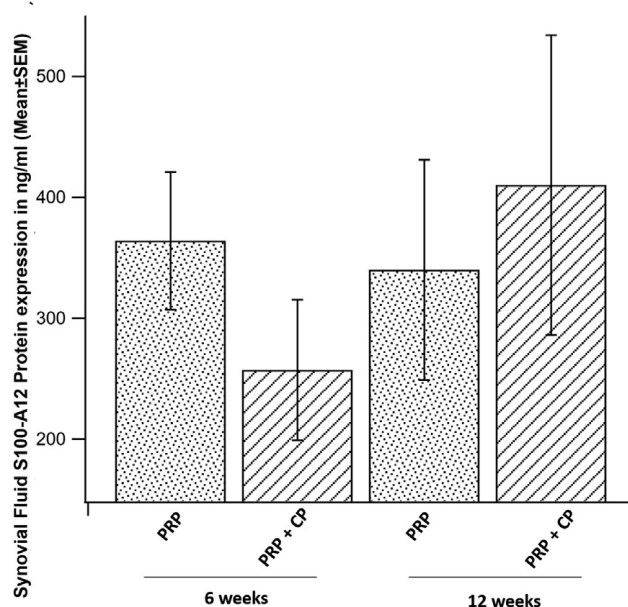


Fig. 6. Comparative synovial fluid S100A12 protein expression levels (ng/μl) between PRP and PRP + CP joints at 6 weeks and 12 weeks. Data expressed are Mean + SD, n = 5.

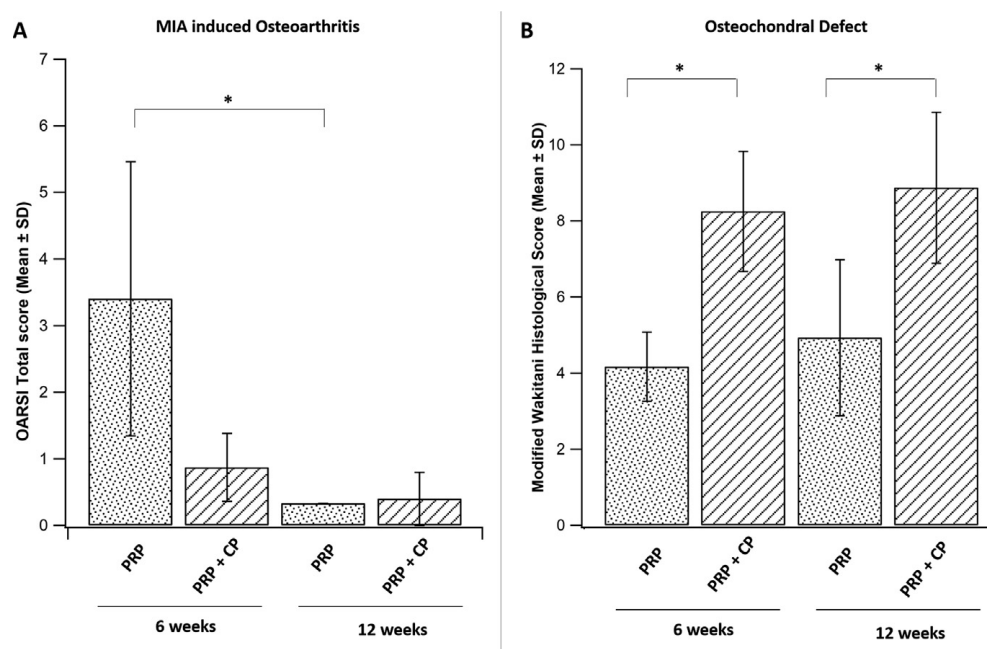


Fig. 7. Histological grading: (A) OARSI mean scores and (B) Modified Pineda and Wakitani scores at 6 weeks and 12 weeks between control (PRP) and test joints (PRP + CP) by three individual blinded observers.

Flow cytometric analysis revealed results comparable to surface marker expression as described by previous reports [6,18]. However, a slight discrepancy regarding CD90 and CD49e can be explained, as the studies mentioned above employed rabbit BM-MSCs or human chondroprogenitors, whereas the current study was performed using rabbit chondroprogenitors. Our results showed evident healing seen in both control and test arms of the OA group at both 6- and 12-weeks, indicated by similar OARSI scores, S100A12 protein levels, and collagen type II staining intensity. Previous literature involving human data has reported improved patient symptoms and radiological scores on the treatment of knee osteoarthritis with various cell types embodied in PRP, albeit lacking a direct comparison with PRP alone [21,22]. In contrast, an in-vivo report studying the rabbit knee OA model demonstrated comparable results when using PRP or PRP laden MSCs [23]. Regarding OCD repair, our study displayed that the PRP arm demonstrated a significantly better quality of repair, integration and staining when compared to the test arm at both 6- and 12-weeks. Previous studies have also reported the superiority of PRP in the regeneration of chondral defects when compared to microfractures and normal saline [24,25]. A recent

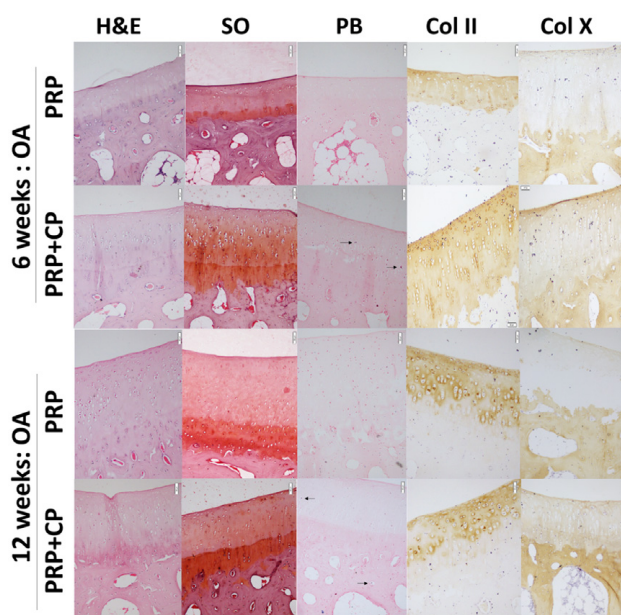


Fig. 8. Histological Analysis of OA joints: Representative images of femoral condyles at 6- and 12- weeks post intervention, were assessed by hematoxylin (H&E), Safranin O(SO), Prussian blue(PB), Collagen type II(Col II) and Collagen type X(Col X). OA: osteoarthritis, PRP: platelet rich plasma and CP: chondroprogenitors. Magnification x200.

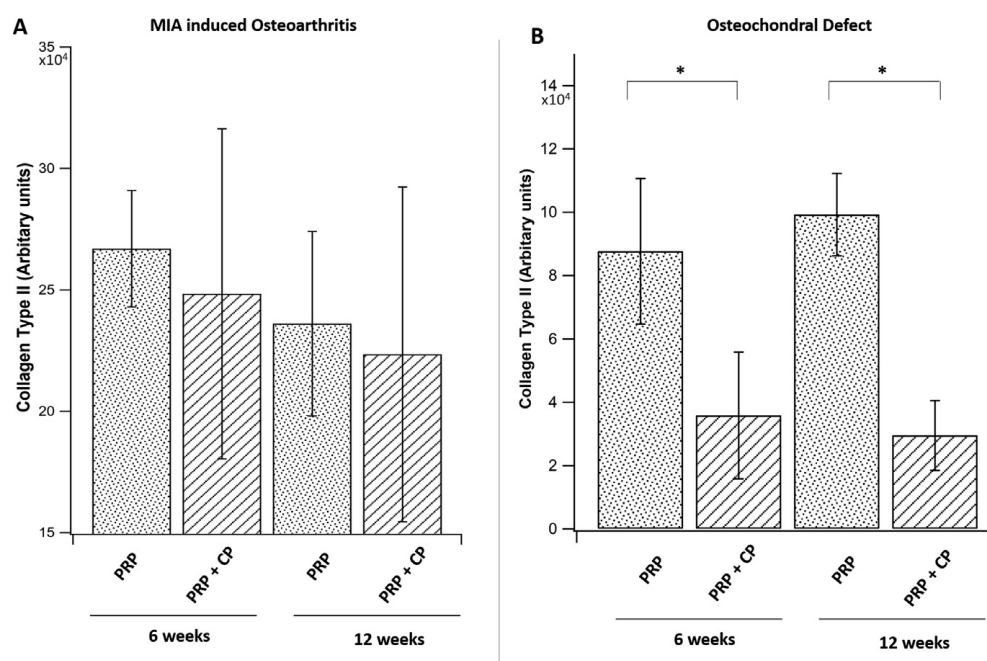


Fig. 9. Comparative values of collagen type II staining of articular cartilage (a and b: Quantified using Image J and expressed in arbitrary units at 6 weeks and 12 weeks between control (PRP) and test joints (PRP + CP). Data expressed are Mean $\pm$ Standard deviation, n = 5. MIA: monosodium iodoacetate, PRP: platelet rich plasma, CP: chondroprogenitors, OA: osteoarthritis and OCD: osteochondral defect. *P < 0.05.

study demonstrated that migratory chondroprogenitors suspended in PRP showed superior healing of chondral defect to PRP alone [26] while Liu et al. reported better meniscal wound healing in a rabbit model when using MSCs with PRP than PRP alone [27]. As there is a lack of information about the use of fibronectin derived chondroprogenitors, scaffolded with PRP versus stand-alone use of the biomaterial itself, coupled with the limitations encountered, it was difficult to form an inference from the contrasting results of this study. The effect of the microenvironment on the cell behavior *in vivo*, and the influence of PRP itself, due to its allogenic nature, could not be accounted for. Furthermore, PRP utilized in the present study was not further processed to eliminate leucocytes, control temporal release of growth factors or obtain cell-free fractions (e.g. exosomes), which may positively influence cartilage repair *in-vivo* [28,29]. To facilitate cellular tracking of allogenic chondroprogenitors, a safe dose of M-SPIO, as reported by Vinod et al., was employed to label the cells [17]. However, the

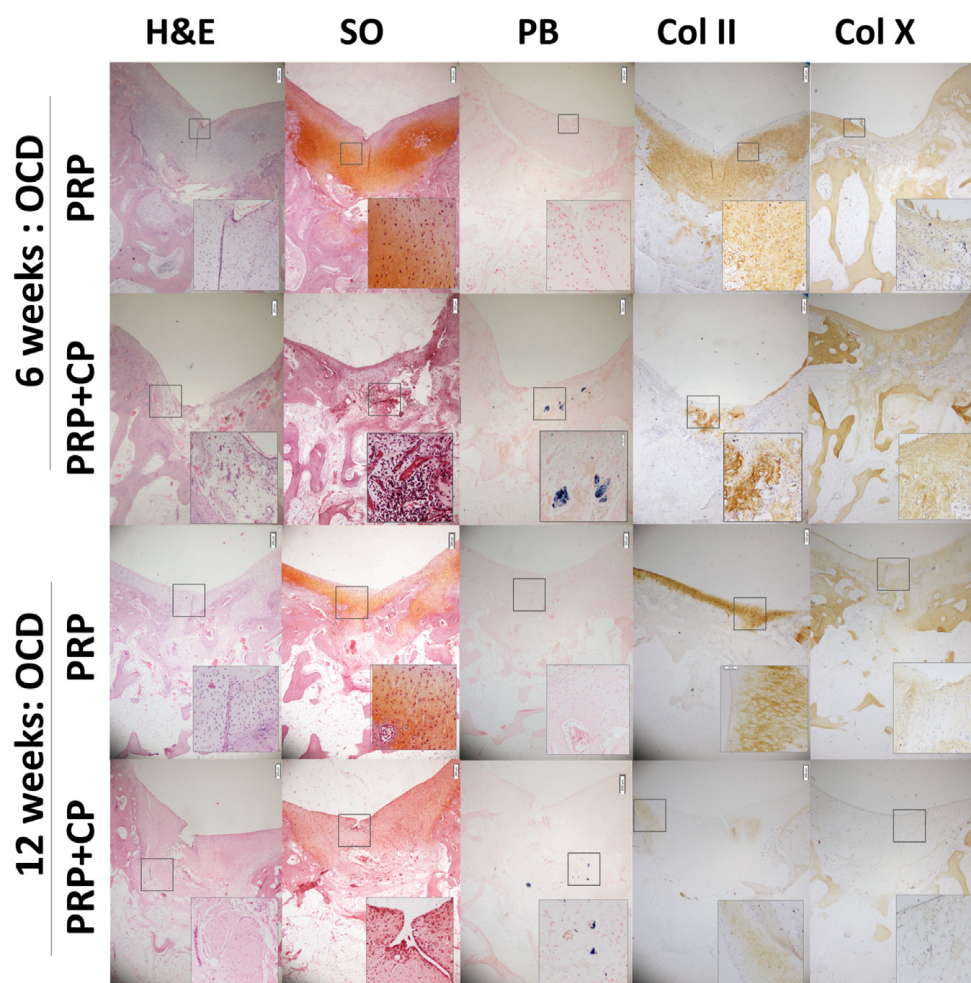


Fig. 10. Histological Analysis of OCD joints: Representative images of femoral condyles at 6- and 12-weeks post intervention, were assessed by hematoxylin (H&E), Safranin O(SO), Prussian blue(PB), Collagen type II(Col II) and Collagen type X(Col X). OCD: osteochondral defect, PRP: platelet rich plasma and CP: chondroprogenitors. Magnification x40, lower box x200.

long-term *in vivo* influence of iron particles on cell health was not assessed. Moreover, due to the *in vivo* nature of the study, control over cell survival was challenging and healing with repeated doses of chondroprogenitor injections could not be assessed as the intervention was performed at a single time point.

This is the first study to report findings related to cartilage repair seen using fibronectin adhesion assay derived chondroprogenitors suspended in PRP for early OA and OCD. It is clear from the data that both interventions demonstrate efficient healing, although PRP was superior to chondroprogenitors when OCD repair was in question. It would be prudent further to optimize chondroprogenitor expansion *in vitro* and targeted cellular delivery, as these progenitors exhibit promise for cartilage repair in the field of cell-based therapy.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.knee.2021.03.010>.

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