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# Platelet rich concentrate enhances mesenchymal stem cells capacity to repair focal cartilage injury in rabbits

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ABSTRACT

*Background:* It has been previously suggested that the use of regenerative promoters, which include bone marrow-derived mesenchymal stem cells (MSCs) or natural growth factors supplement such as platelet-rich concentrate (PRC) could promote cartilage regeneration. However, the notion that the concurrent use of both promoters may provide a synergistic effect that improves the repair outcome of focal cartilage injury has not been previously demonstrated. This study was thus conducted to determine whether the concomitant use of PRC could further enhance the reparative potential of MSCs encapsulated in alginate transplanted into focal cartilage injury in rabbits.

*Methods:* Artifically created full thickness cartilage defects were made on the weight-bearing region of medial femoral condyles in bilateral knees of New Zealand White rabbits (N = 30). After one month, the right knee was treated with either i) PRC (n = 10), ii) MSCs (n = 10), or, iii) a combination of PRC and MSCs (PRC + MSC) (n = 10), all encapsulated in alginate. The left knee remained untreated (control). Rabbits were sacrificed at 3 and 6 months after treatment. Cartilage tissue regeneration was accessed using ICRS morphologic scoring, histologic grading by O'Driscoll scoring, immunohistochemical staining and quantitative analysis of glycosaminoglycans (GAG) per total protein content.

*Results:* At 3 months, transplantation using PRC alone was equally effective as MSCs in inducing the repair of cartilage defects. However, PRC + MSC resulted in significantly higher ICRS and O'Driscoll scores ($p < 0.05$) as compared to other groups. The regenerated tissues from the PRC + MSC group also had stronger staining for Safranin-O and collagen type II. By 6 months, in addition to superior ICRS and O'Driscoll scores as well as stronger staining, glycosaminoglycan per total protein content was also significantly higher ($p < 0.05$) in the PRC + MSC group ($3.4 \pm 0.3$ μg/mg) as compared to the MSC ($2.6 \pm 0.2$ μg/mg) or PRC ($2.1 \pm 0.2$ μg/mg) groups.

*Conclusion:* PRC enhances the reparative effects of MSC in treating focal articular cartilage injuries.



## Introduction

Focal injury or defect of articular cartilage is a prevalent orthopaedic problem, particularly amongst individuals participating in high impact sports activities [1]. If left untreated, the initial chondral or osteochondral lesion may progress further, predisposing the individual to early onset of osteoarthritis. Successful treatment of articular cartilage defect in the knee has continued to be a formidable challenge for clinicians, owing to the well-known poor intrinsic healing capacity of normal cartilage tissue. Nevertheless, as the knowledge of cartilage biology expands, many novel potential treatment strategies for cartilage repair have been increasingly explored in the recent years. This includes the use of regenerative promoters such as mesenchymal stem cells (MSCs) and growth factors. MSCs have been shown to promote cartilage regeneration through the production of cartilage matrix, whilst several growth factors have been shown to stimulate cartilage resident cells i.e. chondrocytes to produce repaired tissues [2]. The ability of MSCs to undergo self-renewal and multi-lineage differentiation, as well as having immunomodulatory and anti-inflammatory properties, renders them as an attractive potential therapeutic agent for tissue repair [3]. Accordingly,

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many approaches have been developed to augment the reparative potential of MSCs. One such effort is through the use of naturally occurring source of growth factors such as platelet-rich concentrate (PRC). PRC in essence, is a concentrated suspension of platelets above baseline levels. In many instances, PRC may be prepared from the re-suspension of platelets in liquid suspensions such as normal saline. PRC contains various mitogenic and angiogenic growth factors such as platelet-derived growth factor (PDGF) – AA, –BB, –AB, transforming growth factor β1 (TGFβ –1) and –2, insulin growth factor-1 (IGF-1), and fibroblast growth factor (FGF), which have been shown to stimulate cell proliferation, cellular protein expression, and tissue regeneration [4]. Supplementing platelet-rich plasma (PRP) to culture medium has been shown to enhance chondrocyte proliferation and matrix synthesis [5] as well as improve chondrogenic differentiation of MSCs *in vitro* [6]. In animal studies, PRP has been shown to enhance articular cartilage repair when used in conjunction with microfracture for the repair of chondral defects [7–10], or when used for treatment of osteochondral defects [11–15]. Direct PRP injections administered after subchondral bone drilling for the repair of chondral defects have also been shown to produce excellent functional outcome in clinical studies [16–18]. In all instances, the therapeutic potential of platelet-rich preparation *in vivo* has been demonstrated only when it was used in conjunction with some form of surgical intervention, such as following bone marrow-stimulation procedure. Considering the fact that microfracture or subchondral bone drilling would itself stimulate cells from the bone marrow to migrate and initiate *in situ* cartilage repair, it remains unclear to what extent platelet-rich preparation on its own is sufficiently efficacious in promoting healing of cartilage injury *in vivo* without the influence of other potential confounding factors. More importantly, it is also unclear whether the use of platelet-rich concentrate could further enhance the capacity of MSCs to repair focal cartilage injury *in vivo* since the synergistic effect of both factors have not been previously demonstrated in an animal model of this injury. Therefore, the aim of this study was to determine the effectiveness of platelet-rich concentrate (PRC) encapsulated in alginate beads in enhancing articular cartilage repair in an *in vivo* rabbit model of focal cartilage injury, and to investigate whether implantation of PRC together with MSCs encapsulated in alginate into the defect site could further enhance repair of the injury, as reflected by gross morphology, histology, and glycosaminoglycan levels of the regenerated tissue. We hypothesize that treatment with a combination of PRC and MSCs encapsulated in alginate will enhance the quality of repair of the full thickness chondral defects as compared to PRC and MSC in alginate alone.

## Methods

### *Animals*

New Zealand male white rabbits, 6–7 months old, weighing 2–3 kg, were used in this study in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) Review Board (Ethics approval reference number: FIS/12/09/2014/SS (R)). Animals were housed separately under controlled temperature and light conditions. Animals were also provided free access to drinking water and pellet food. They were kept in standard cages with resting boards that allowed unrestricted weight-bearing activity. During housing, animals were monitored twice daily for health status. In this study, no adverse events were observed. Three rabbits were used for bone marrow isolation. Power analysis indicated that in order to detect an increase of at least 35% in glycosaminoglycan content in the treatment group compared to controls at 6 months, as previously reported in the literature [20], with 80% power and an $\alpha$ value of 0.05, a minimum of 4 animals per group were deemed sufficient. Assuming a 25% animal drop-out rate, which is consistent with other rabbit surgical studies, five animals would be needed in each group. Therefore, a total of 30 rabbits divided into 6 groups were used in the study. The animals were randomly assigned into three treatment groups: PRC encapsulated in alginate group (PRC; n = 10), MSC encapsulated in alginate group (MSC; n = 10) and a combination of PRC and MSC encapsulated in alginate group (PRC + MSC; n = 10). The animals in each treatment group were further divided into 2 groups such that 5 rabbits were sacrificed at each time point (3 and 6 month). The use of alginate as a carrier was necessary for ease of delivery of both compounds into the defect sites. The choice of alginate as a carrier was also in line with that of previously established studies [20,21].

### *Isolation of allogeneic MSCs*

Three rabbits were sacrificed using an intravenous overdose of pentobarbital sodium (Boehringer, Germany). Femur and tibia of both lower limbs of the rabbits were removed, and any adherent tissues were scraped off. All harvested bones were kept on ice in 1X phosphate buffer saline (PBS, pH 7.2, Invitrogen-Gibco, USA) supplemented with 4% penicillin-streptomycin until they were processed [20]. Within 3 hours, bone marrow was harvested from the femur and tibia. MSCs were isolated according to the protocol described by Pittenger et al. [22]. Briefly, 2 mL of bone marrow was diluted with the same amount of 1X PBS and slowly layered over 3 mL Ficoll-paque (GE Healthcare – Amersham Biosciences, Piscataway, New Jersey) in a 15-mL Falcon tube (Corning, USA). After 25 min of centrifugation at 360 x *g*, mononuclear cells were collected from the interphase. Cells were then washed with pre-warmed Dulbecco's modified eagle medium (DMEM, Gibco) and centrifuged at 200 x *g* for another 10 min. The cell pellet was resuspended in DMEM containing 10% FBS (Invitrogen-Gibco) and 1% penicillin-streptomycin (100 U/mL, Invitrogen-Gibco); seeded in a T75 flask (Nunc, Thermo Fisher Scientific, MA, USA) and maintained in monolayer culture at 37 °C, 95% humidity, and 5% $CO_2$ for 3 weeks. The culture medium was replaced every 2–3 days. The cells were then serially passaged until passage 3 where they were finally used for further experiments. The cells isolated were confirmed to be MSCs based on their potential to differentiate to adipogenic, osteogenic and chondrogenic lineages (Supplementary Figure S-1).

### *Preparation of alginate-MSC beads*

In addition to being a carrier that aids in delivery of the treatment compounds into the site of injury, alginate provides a conducive 3-dimensional environment that helps in maintaining a spherical chondrocytic morphology of the encapsulated MSCs [23]. Rabbit MSC in alginate beads were prepared by re-suspending the cells in 1.2% low-viscous alginate (Sigma-Aldrich, St. Louis, MO) in 0.15 M sodium chloride as previously described by Kamarul et al. [24]. Briefly, hMSCs were first detached from the T75 flask surface using TypLE™ express (Invitrogen), pelleted and re-suspended in sterile alginate at cells densities of $1 \times 10^6$ cells/mL. The cell suspension was dropped into a 102 mM calcium chloride solution using a micropipette tip. The resulting beads were washed with 0.15 M sodium chloride after 10 min of polymerization [20]. Two beads were transferred to a low attachment 24 well plate (Corning® Costar®, USA) under sterile conditions and then transplanted into the defect(s).

### *Isolation and preparation of PRC and PRC + MSC in alginate beads*

To prepare autologous PRC, 17 mL blood was drawn from the central vein of the ear of rabbits from PRC and PRC + MSC groups.

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