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Evaluation of Low Level Laser-Activated Stromal Vascular Fraction as a Single Procedure for Treatment of Experimental Chondral Defects

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ABSTRACT

Regenerative therapies offer attractive alternatives for the treatment of chondral defects. Adipose-Derived Mesenchymal Stem Cells (AD-MSCs) found in the Stromal Vascular Fraction (SVF) allow the development of one-step surgical procedure by their abundant availability and a minimal invasive technique. Low Level Laser Irradiation (LLLI) of stem cells showed improved results on stem cell growth and proliferation. The aim of this study was to evaluate the treatment of chondral defects using single injection of laser activated SVF in a Platelet Rich Plasma (PRP) vehicle. Chondral defects were created surgically in the femoral condyle of 9 dogs, autologous adipose tissue was harvested from the abdomen and SVF was isolated, added to the PRP, laser irradiated and injected intraarticularly in the right joint. The left joint was injected normal saline as control negative. Evaluation of the treatment was done by physical examination, radiology and histopathology. Treated joints showed marked degree of cartilage regeneration and restoration of chondral histomorphological picture on the contrary of the control joints that showed deterioration over time and defect filling with only fibrous tissue forming a fibrocartilage at the end of 6 months period. The obtained results proved that the use of low level laser activated adipose derived stem cells is a safe, feasible technique as a single step surgical procedure and a very promising option for treatment of chondral defects.

Key words: Adipose, stromal vascular fraction, stem cells, laser, platelet rich plasma, chondral defects

INTRODUCTION

Hyaline articular cartilage plays a crucial role in mammalian joint function (Hunter, 1742). Joint injury is the most common cause of chronic pain in orthopedic diseases and characterized by synovitis and degeneration of the articular cartilage with loss of matrix and can result in complete loss of the cartilage surface (Buckwalter *et al.*, 2005; Hunter, 1742; Luyten, 2004).

Hyaline articular cartilage endogenous healing potentials are limited and true restoration with repair tissue mimicking hyaline cartilage was never observed in humans or in other mammalian species. This is due to the avascular and aneural nature of this tissue and consequent lack of access to a pool of potential reparative cells and growth factors (Hunter, 1742; Luyten, 2004). Several methods that aimed to reform a new chondral surface were attempted such as periosteal

arthroplasty, perichondral arthroplasty, autologous osteochondral transplantation, autologous chondrocyte transplantation, autogenetic cancellous grafts and tendon autografts (Breinan *et al.*, 1997; Buckwalter and Brown, 2004).

In contrast to the commonly attempted drug and surgical procedures, cellular therapies such as stem cell therapy do not rely on a single target receptor or pathway for their action but are thought to mediate repair via five primary mechanisms: (1) Providing an anti-inflammatory effect, (2) Homing to damaged tissues and recruiting other cells, that are necessary for tissue growth, (3) Supporting tissue remodeling over scar formation, (4) Inhibiting apoptosis and (5) Differentiating into bone and cartilage (Parker and Katz, 2006; Schaffler and Buchler, 2007).

Autologous adult stem cells can be harvested from a variety of sources, including bone marrow and adipose tissue (Luyten, 2004) with no ethical issues related to their use. Both Bone Marrow derived Mesenchymal Stem Cells (BM-MSCs) and Adipose tissue Mesenchymal Stem Cells (AD-MSCs) derived from Stromal Vascular Fraction (SVF) have demonstrated broad multipotency with differentiation into a number of cell lineages, including adipo, osteo and chondro-cytic lineages (Parker and Katz, 2006; Schaffler and Buchler, 2007).

Clinically, SVF have the advantage over their bone marrow derived counterparts, because of their abundance in numbers eliminating the need for culturing over days to obtain a therapeutically viable number and the ease of the harvest procedure itself; being less painful than the harvest from bone marrow (Bongso and Lee, 2005; Casteilla *et al.*, 2011).

It means that an autologous transplant of AD-MSCs will not only work in much the same way as the successes shown using BM-MSC transplant, but also be of minimal risk to the patient (Parker and Katz, 2006; Schaffler and Buchler, 2007). Autologous transplant of SVF also poses extremely low risk to the patient when done as a single procedure in a sterile surgical operating room setting (Fraser *et al.*, 2006).

Platelet Rich Plasma (PRP) has been used as a vehicle for MSCs as it contains many growth factors and has been successfully used clinically to improve hard and soft tissue healing (Andia *et al.*, 2012; Arora *et al.*, 2009; Mishra *et al.*, 2009). Therefore, adding PRP to MSC- based therapies could increase the regeneration ability and accelerate the healing process. Furthermore, PRP promoted AD-MSCs proliferation and differentiation into chondrogenic cells that strongly expressed collagen II, Sox9 and aggrecan (Van Pham *et al.*, 2013).

Laser irradiation at different intensities has been shown to inhibit and stimulate cellular processes. Recent findings suggest that at the cellular level, laser energy of a particular wavelength can initiate signaling cascades, such as those that promote cellular proliferation (Mvula *et al.*, 2010).

Studies on Low Level Laser Therapy (LLLT) and stem cells have shown that LLLI increased migration of stem cells and suggested that LLL could affect positively the metabolism of stem cells, which in turn, could also be indicative of increased cell proliferation rate and viability and differentiation potential (Mvula *et al.*, 2010).

This study aimed to evaluate the treatment of chondral defects using SVF activated by LLLI in a PRP vehicle as a single step surgical procedure.

MATERIALS AND METHODS

Work was divided into four steps; induction of experimental cartilage defect, acquisition and preparation of SVF and PRP, injection of the low level laser activated SVF/PRP and then the evaluation process.

Induction of chondral defect was one week before treatment and all the steps from adipose tissue collection, preparation, laser activation and injection were done on the same day.

Study design: All animal's experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Faculty of Veterinary Medicine, Cairo University (Approval ID: CUFVM/F/S/2014/38).

Experimental animals: Nine mongrel dogs (2-5 years of age of both sexes) were used in this study. Dogs were housed individually in kennels at the Faculty of Veterinary Medicine, Cairo University and handled by trained personnel.

All dogs went through a pre-study evaluation that included routine physical examination, lameness inspection, joint mobility and notation of pain on manipulation, range of motion and functional disability. Dogs having any signs of joint affections were excluded from the study.

Groups: Animals were equally and randomly allocated into 3 groups. All animals underwent bilateral induction of chondral defect. Treatment using the preconditioned SVF was applied only to the right joint. The left joint was injected an equal amount of normal saline as a self-control negative. Animals were humanely euthanized after $1\frac{1}{2}$, 3 and 6 months, respectively post treatment.

Induction of chondral defect: Under general anesthesia, lateral para-patellar stifle arthrotomy was applied and using a rounded trephine with a 3 mm diameter and 1 mm depth, a partial thickness of the weight bearing articular surface was removed from the lateral femoral condyle without damaging the subchondral bone (Fig. 1) (Mokbel *et al.*, 2011). The induction process was done one week before treatment.



Fig. 1: Femoral condyle after induction of the defect

Stem and regenerative cell preparation

Adipose tissue collection: Under general anesthesia, a small skin incision (2-3 cm) was done and adipose tissue (15-20 g) was collected from sub-cutaneous fat in the abdomen into a 50 mL sterile cup.

Tissue processing and isolation of SVF: The collected fat pad was minced and washed extensively with phosphate-buffered saline and then an equal volume of 0.1% collagenase type 1 (Sigma, Aldrich) was added. The tissue was placed in a rotary incubator at 37°C, with continuous agitation for 1 h. After digestion, the lipoaspirates were centrifuged at 1200 rpm for 10 min to separate the lipoaspirate and the collagenase. The lipoaspirates were then rewashed 3 times to remove any remaining collagenase. After the last round of centrifugation, cells in the aspirates were counted using a hemocytometer and the viability of the cells was assessed using the trypan blue dye exclusion test.

Platelet rich plasma: A blood sample was drawn on acid citrate dextrose as anticoagulant. Gradient density centrifugation was made to obtain the PRP layer and added to SVF preparation (Fig. 2).

Low level laser activation: The prepared cells seeded in the PRP were exposed to low level laser for 20 min using LED technology (Adilight 2[®] Australia) at 3 laser diodes for 3 frequencies one in the red, one in the green and one in the yellow.

Stem cell injection: One week after the first surgery each animal received 6×10^6 nucleated cell of SVF in 0.6 mL of PRP after being activated by LLLI in the right joint and the left joint was injected same volume of normal saline.



Fig. 2: Stromal vascular fraction appearing in the bottom the falcon tube after addition of the platelet rich pasma

Evaluation: The evaluation process comprised physical and a histopathological testing.

Physical examination: Lameness examination was done including joint mobility and notation of pain on manipulation, range of motion and functional disability according to the following score (Black *et al.*, 2007, 2008).

Radiological examination: Each animal was submitted to x-ray evaluation of the stifle joint at 1½, 3, 6 months according to Crawford radiographic scoring system (Mokbel *et al.*, 2011).

Histopathological evaluation

Macroscopically: The collected samples were evaluated according to the macroscopic evaluation scoring system of Rudert (Rudert *et al.*, 2005).

Microscopically: All samples were fixed in 10% neutral buffer formalin. De-calcification specimens was done by using 8% formic acid decalcifying solution in distilled water. The decalcifying solution was renewed every 48 h until softening of the specimens. The decalcified specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 μ m thickness and stained with Haematoxylene and Eosin (HE) as well as Masson's Trichrome (MT) stain for detection of collagen fibers and degree of matrix staining (Bancroft, 2008). Then evaluated according to score system of Mainil-Varlet *et al.* (2010).

RESULTS

All animals tolerated the surgery well and remained healthy throughout the whole study period, lameness on both limbs occurred for 3 days after the induction of the defects (dogs were unwilling to walk).

Physical examination: All animals suffered from lameness during trotting, mild pain on manipulation with normal range of motion and normal activity at 1½ months at the left limb. The later groups showed no lameness on both limbs and no detectable pain on manipulation.

Radiological evaluation: Radiological evaluation revealed minor changes in the stifle joints with evidence of cartilage loss at the site of the defect at 1½, 3 and 6 months in the left joint (Fig. 3a) whilst showing restoration of the articular surface in all the treated groups (Fig. 3b). No signs of joint distension or swelling were observed in all animals.

Histopathological evaluation

Macroscopically: All animals were healthy at the time of euthanasia, furthermore no joints showed any signs of infection or osteoarthritis. The cartilage samples were collected and evaluated according to the corresponding scoring system and grossly the results were in Table 1.

At $1\frac{1}{2}$ months the left joints showed no signs of regeneration, the defect area was covered by a thin transparent layer under which the subchondral bone was easily seen as a brownish area underneath this layer (Fig. 4a). In a single case, the defect area tended to expand to reach the subchondral bone and bleeding occurred. On the contrary the right joint at the same period was filled by a tissue resembling the surrounding normal cartilage and began to regain its glistening appearance but the borders of the defects were still noticeable (Fig. 4b).



Fig. 3(a-b): (a) Radiographic appearance of the left joint. Notice the site of the defect is not continuous and evidence of cartilage loss and (b) Radiographic appearance of the right joint showing continuous articular surface without any signs of cartilage or bone damage

Average	Group 1 (1 ¹ / ₂ months)		Group 2 (3 months)		Group 3 (6 months)	
	Right	Left	Right	Left	Right	Left
Filling of the defect	2	1	3	2	3	3
Color of the defect filling	2	1	2	1	3	2
Surface texture	1	1	2	1	2	1

Table 1: Average evaluation of macroscopic scores of cartilage regeneration

Filling score: 1: Underneath the surrounding cartilage, 2: Up to surrounding cartilage with central depression, 3: Flush with surrounding cartilage, Color score: 1: Brown to yellow, 2: White, 3: Same as surrounding cartilage, Surface score, 1: Rough, 2: Smooth

At 3 months the left joints showed widening of the defects and cracks began to appear in the surrounding cartilage while the defect area was filled with a dark-whitish tissue with a central depression (Fig. 4c). The right joints were filled by a smooth glistening tissue resembling the host normal cartilage and the articular surface continuity was maintained (Fig. 4d).

At 6 months the left joints showed complete filling of the defect area with a whitish/opaque tissue with widening of the defect size and cracking in the adjacent articular cartilage (Fig. 4e). While in the right joint the defects tended to disappear and the articular surface was smooth and glistening (Fig. 4f).

Microscopically: Table 2 is showing the average values of microscopic chang as of the caitilage. The control joints showed no tendency to regenerate throughout the whole study period, after 1½ months the defect was partially filled with unorganized fibrous connective tissue and the subchondral bone showed hypercellularity and thickening (Fig. 5a and b). At 3 months, the articular surface was still uneven and the fibrous tissue became more mature, more organized and



Fig. 4(a-f): (a) Macroscopic appearance of the articular cartilage in the control group after 1.5 months. Notice that the defects became deeper and reached subchondral bone without any signs of regeneration, (b) Articular cartilage in the treated group after 1.5 months. Notice the defects were filled with a whitish tissue resembling the surrounding cartilage and began to be glistening, the defect outline can still be seen, (c) Macroscopic appearance of the articular cartilage in the control group after 3 months. Notice the defect site became wider with central depression and covered by opaque fibrous rough tissue. Cracks in the adjacent cartilage began to appear, (d) Treated group after 3 months. Notice the defects were filled with smooth glistening tissue resembling the adjacent cartilage and the defect outlines began to disappear to maintain the continuity of the articular surface, (e) Macroscopic appearance of the articular cartilage in the control group after 6 months. Notice the defect filling is fibrous opaque tissue covering the whole defect site with cracking of the adjacent articular cartilage and (f) Articular cartilage in the treated group after 6 months. Notice that the defect filling is completely resembling the adjacent normal tissue and the articular surface is smooth

Table 2: Average values of the microscopic ch	anges of the ca	artilage				
	(Group 1) 1 ¹ / ₂ months		(Group 2) 3 months		(Group 3) 6 months	
Average	Right	Left	Right	Left	Right	Left
Percentage of defect filling	3	2	4	3	4	3
Articular surface continuity	1	0	2	1	2	2
Restoration of osteochondral architecture	2	2	3	2	4	2
Cellular morphology of regeneration tissue	3	0	3	1	4	2
Matrix staining	2	2	2	2	3	2

Percentage of defect filling score: 1:25, 2:50, 3:75, 4:100%. Articular surface continuity score, 0: Discontinuous, 1: Continuous but rough, 2: Continuous and smooth. Restoration of architecture score,1: Poor, 2: Unclear (heterologous), 3: Clearly differentiable, Cellular morphology score: 0: Fibrous tissue, 1: Fibrocartilage, 2: Hyaline fibrous hybrid, 3: Hyaline without zonal architecture, 4: Hyaline with zonal architecture, Matrix staining score, 1: Markedly reduced, 2: Slightly reduced, 3: Normal as adjacent cartilage

blood vessels began to appear while the subchondral bone was still showing more thickening and hypercellularity (Fig. 5c and d). After 6 months, the joints showed an advanced degree of repair as the defect site began to form a fibrocartilage with rounded chondrocytes dispersed in between 2 layers of fibrous tissue with even articular surface (Fig. 5e and f).

On the other hand, the treated joints showed a significant degree of regeneration over time. After 1½ months the defect site was filled with a hyaline like matrix (Fig. 6a) with differentiated chondrocytes but the collagen fibers were unorganized with fibrillated surface containing a large number of chondroblasts (Fig. 6b). At 3 months, the collagen fibers were organized with a homogenous hyaline like matrix resembling the adjacent normal cartilage tissue (Fig. 6c) with less number of chondroblasts and increased number of differentiated mature chondrocytes that are singly located inside the lacunae (Fig. 6d) with well-developed territorial collagen fibers and there was no clear demarcation between the defect site and the host's normal cartilage (Fig. 6e). While after 6 months there was no major differences between the 3 months samples, the chondrocytes tended to divide more to form clusters with more development of territorial and interterritorial collagen fibers (Fig. 6f).

DISCUSSION

Articular cartilage repair is still a challenge in orthopedic surgery despite of many surgical treatment options that have been developed in the last decade with limited success. While further studies are necessary to fully characterize the use of cell-based therapeutics for treatment of chondral defects, stem cells functions trophically by secreting cytokines and growth factors (Black *et al.*, 2008; Murphy *et al.*, 2003) promoting cellular differentiation into the resident lineages (Black *et al.*, 2007). In addition, stem cells suppress immunoreactions and inhibit apoptosis (Black *et al.*, 2007, 2008), which is needed to stop the progression of cartilage degeneration.

Autologous MSCs can be harvested from a variety of sources, including bone marrow and adipose tissue (Jurgens *et al.*, 2013) with no ethical issues related to their use and have demonstrated broad multipotency with differentiation into a number of cell lineages, including adipo-, osteo- and chondro-cytic lineages. However, the easy and repeatable access to subcutaneous adipose tissue, the simple isolation procedure and the approximately 500-fold greater numbers of fresh MSCs derived from equivalent amounts of fat versus bone marrow provide a clear advantage in using AD-MSCs over BM-MSCs (Parker and Katz, 2006; Schaffler and Buchler, 2007; Tholpady *et al.*, 2006).

Currently, very few clinical studies on MSC transplantation for cartilage repair have been reported, though animal experiments on MSC use in the prevention and treatment of experimental OA have showed encouraging results (Mokbel *et al.*, 2011; Murphy *et al.*, 2003). Where Mokbel *et al.* (2011) showed the formation of hyaline cartilage with differentiated chondrocytes surrounded by lacunae with homogenous hyaline matrix forming hyaline cartilage to prove that use of mesenchymal stem cells is a viable option for treating partial thickness chondral defects in dogs.

The SVF cells allow the development of one-step surgical procedures by their abundant availability and high frequency. Jurgens *et al.* (2013) evaluated the *in vivo* safety, feasibility and efficacy of this concept using scaffolds seeded with freshly isolated (SVF) or cultured AD-MSCs and compared these to their acellular counterparts. Osteochondral regeneration was evaluated by macroscopy, immunohistochemistry, biomechanical analysis, microCT analysis and biochemistry. The SVF cells tended to perform best on all parameters for cartilage regeneration.



Fig. 5(a-f): Histomorphological appearance of control joints during the different periods (a) Junction between the defect site and the normal adjacent cartilage. The defect filling is mainly fibrous tissue with more densely stained subchondral bone, Haematoxylene and Eosin 100x, (b) Defect site is filled with unorganized fibrous tissue with increased condrification in the lower part, Masson's trichrome 200x, (c) Filling of the chondral defect with organized mature fibrous tissue with deeply stained subchondral bone, Haematoxylene and Eosin 200x, (d) Organized fibrous tissue with newly formed blood vessels with differentiated chondrocytes to begin the formation of fibrocartilage, Masson's trichrome 400x, (e) Arrangement of chondrocytes between fibrous tissue forming a fibrocartilage, 200x and (f) 2 layers of fibrous tissue containing rounded chondrocytes forming a fibrocartilage, Masson's Trichrome 200x



Fig. 6(a-f): Histomorphological appearance of treated joints during the different periods (a)
Fibrillated surface with high number of chondroblasts and unorganized collagen fibers
Haematoxylene and Eosin 200x, (b) Fibrillated surface containing large number of
chondroblasts, Masson's Trichrome 400x, (c) Defect site filled with hyaline matrix
resembling the adjacent normal cartilage with even continuous articular surface,
Haematoxylene and Eosin 100x, (d) Hypercellular regenerated cartilage. Notice the
chondrocytes are mature and surrounded by lacunae, Haematoxylene and Eosin 200x,
(e) Regenerated cartilage resembling the adjacent normal tissue with homogenous
hyaline matrix, Masson's trichrome 200x and (f) Clusters of chondrocytes surrounded
by with well-developed territorial collagen taking a deeper stain around the lacunae,
H and E 400x

The PRP contains (and releases through degranulation) several different growth factors and other cytokines that stimulate healing of bone and soft tissue (Andia *et al.*, 2012; Arora *et al.*, 2009;

Mishra *et al.*, 2009; Sutter, 2007) and can be used to promote ADSC proliferation and differentiation into chondrogenic cells that strongly expressed collagen II, Sox9 and aggrecan.

PRP-pretreated AD-MSCs improved healing of injured articular cartilage in murine models (Van Pham *et al.*, 2013). When AD-MSCs were subjected to low level laser irradiation *in vitro*, cell viability, cell proliferation and the expression of β 1-integrin increased (Mvula *et al.*, 2008, 2010). For those reasons and to further promote tissue regeneration, low level laser activated SVF in PRP were used in this study.

The rounded trephine with a predetermined diameter and depth provided two main advantages; the first was that the trephine-shape made it easier to locate the area of interest for histo-sampling at the end of the experiment period. The second was that the selected depth (1 mm) was very successful in preventing the penetration of the subchondral bone in all operated joints to avoid liberation of progenitor cells from the bone marrow. So avoiding any influence or contribution in the reparative process to ensure that the any chondral repair is entirely due to the injected cells.

The size of the induced defect was similar to that induced by Mokbel *et al.* (2011) and the number of the nucleated cells to be injected was similar to that used by Black *et al.* (2007, 2008) to detect the pathological progression of the healing process.

Physical and radiological evaluation revealed minor changes where physical examination was insignificant and radiological pictures confirmed that the defect was still present in all the control groups and that the treated joints showed continuous articular surface resembling the normal joints as early as $1\frac{1}{2}$ months which confirms (Mokbel *et al.*, 2011).

Macroscopic evaluation of the regenerated cartilage revealed a significant filling of the lost articular cartilage after treatment using the preconditioned SVF when compared to the control ones. Where the defect site was filled with a tissue resembling the surrounding articular cartilage after $1\frac{1}{2}$ months and its borders began to disappear after 3 months and the defect filling was smooth and glistening to nearly disappear after 6 months of treatment leaving an even, smooth and continuous articular surface. These findings agree with previous publications (Buckwalter and Brown, 2004; Dell'Accio and Vincent, 2010). On the contrary of the untreated joints where the gross appearance after $1\frac{1}{2}$ month showed a marked increase in depth of the defect with cracking of the surrounding normal cartilage and the defect after 3 months was filled by a whitish opaque tissue that was distinctly underneath surrounding cartilage. At 6 months the defect filling was rough but flushed to the surrounded cartilage and with increased cracking in the adjacent normal cartilage. It is likely that the cumulative effect of the abnormal load imposed as a result of the severed cartilage resulted in progressive cartilage damage that was not completely prevented by the repair process (Gotterbarm *et al.*, 2008).

Microscopic evaluation confirmed what appeared macroscopically and revealed that after $1\frac{1}{2}$ month the treated joints showed a hyaline like matrix filling the defect site with fibrillated surface and increased number of chondroblasts as a marked sign of regeneration, while the control ones revealed deepening of the defect that reached the subchondral bone with more damage to the surrounding cartilage and the defect began to be filled with unorganized fibrous tissue with a thickened hypercellular subchondral bone in a trial to compensate the lost articular surface. These findings confirmed what previous publications revealed (Jurgens *et al.*, 2013) that after injection of SVF early healing is achieved as early as 1 month and also confirmed that the untreated cartilage showed no tendency to regenerate and was unable to compensate the loss of the articular cartilage leading to extensive loss of adjacent and underlying cartilage (Gotterbarm *et al.*, 2008).

At 3 months from treatment the articular surface was even with mature chondrocytes in a hyaline like homogenous matrix and the territorial collagen fibers began to appear around the lacunae of chondrocytes while the untreated ones showed only organization of the newly formed fibrous connective tissue that was confirmed by Masson's trichrome stain, these findings are similar to the reports of Gotterbarm *et al.* (2008).

At 6 months of treatment there was no marked difference in the histological structure of the regenerated bone but the chondrocytes tended to be more mature forming clusters in a homogenous hyaline matrix with well-developed collagen on the contrary that control joints showed more organization of fibrous tissue and appearance of rounded chondrocytes forming a fibrocartilage that is incapable of withstanding the biomechanical environment of a loaded joint over time (Buckwalter *et al.*, 2005; Murphy *et al.*, 2003). Even if we did not see intraoperative bleeding, histomorpholgical analysis revealed repair tissue merging from the subchondral space, indicating a disruption of the subchondral bone plate. It was reported by Breinan and co-workers in the dog model that there is in fact a significant correlation between the depth of a chondral defect and reparative effect (Breinan *et al.*, 1997).

It was noticed that the mean histopathological scores of the treated group was better than the control group mean score in the 3 studied groups. It reflected the presence of less conspicuous degenerative injuries, assuming that Laser-activated SVF cells in the PRP vehicle stimulated the reparative process.

CONCLUSION

Taken together from all the previous findings and interpretation, this study demonstrated that the efficacy, safety and feasibility of a one-step surgical procedure for chondral cartilage regeneration using laser-activated adipose-derived stromal cells in PRP vehicle. Moreover, the freshly isolated adipose stromal cells are at least equal to cultured AD-MSCs to regenerate chondral and osteochondral defects. The isolation process of SVF following the enzymatic process and PRP preparation and photoactivation using low level laser was an easy procedure, not time consuming and with minimal contamination level. Low level laser irradiation could enhance the viability and proliferation of the stem cell properties of stem cells and hence can maximize the effect of AD-MSCs therapies.

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