Enhancement role of Adipose Derived Stem Cells Vs Platelet Rich Fibrin in an induced bony defect

Original Article

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ABSTRACT

Autologous Adipose derived stem cells (ADSCs) are seen to be safe and abundant source of mesenchymal stem cells. Platelet rich fibrin (PRF) is platelet distillate that used usually in dental surgery setting, it contains many growth factors that help in alveolar bone healing. The experiment aimed to compare the bone formation in bone defects on either side of the maxillae of 2 dogs, after application of ADSCs (on the left side) and PRF (on the right side). Each defect was labelled as a group, as follows: group 1 and group 2 designated for the right sides, treated with PRF on Gelfoam®, of the brown dog and the black dog respectively, groups 3 and 4 were for the brown and black dogs respectively, and they were treated with the ADCSCs on a Gelfoam. After six months, the bone formation in the four groups was assessed using Computed Tomography (CT), and level of gene expression of Aggrecan, and collagen type two using PCR. CT indicated an increase in bone formation in the left sides treated with ADSCs in comparison with the right sides supplemented with PRF. The same goes for gene expression of Collagen type 2 and Aggrecan genes. We concluded that ADSCs generated more mineralized tissue than PRF over six months' period.

Key Words: Adipose derived Stem Cells (ADSCs), Platelet Rich Fibrin (PRF), Computed Tomography (CT), Aggrecan, Collagen type 2.

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INTRODUCTION

Basically, bone has an inherent capability of regeneration and healing itself after an injury or an infection; significant bone loss - from trauma, surgical procedures, and pathologies such as, infections as well as tumors - requires tissue engineering interventions, whether it is cell based therapies, bone grafts or guided bone regeneration (GBR) [1,2] . Autologous bone grafting is still a gold standard for large bone defects treatment because of its safety, high postoperative success, and clinicians can obtain the graft from the same subject [3]. Bone marrow stem cells (BMSCs) are the most common type of mesenchymal stem cells (MSCs) deployed in cell based therapies for bone regeneration [4].

Adipose tissue is a type of connective tissue that is present in bone marrow (yellow bone marrow), around internal organs (visceral fat), under the skin (subcutaneous fat), and in breast tissues. It constitutes a large portion of the adult human body mass (at least 4%) and it plays a key role in energy balance regulation. White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT) are the forms in which adipose tissue is found. WAT is where triglyceride is stored primarily storage, while BAT is specified for energy spending and has the ability to offset obesity [5,6]. There are two types isolated from subcutaneous fat: cultured/relatively homogeneous adipose-derived stromal/stem cells (ASCs), and cultured/heterogeneous stromal vascular fraction (SVF) cells [7].

Choukroun et al [8] was the first to report that Platelet Rich Fibrin (PRF) can be used in maxillofacial surgery. Platelet-rich fibrin (PRF) is formed when platelet cytokines, platelets and growth factors entrapped in fibrin meshwork [9].The entrapped growth factors contribute in wound healing with many factors like collagen production, blood vessels growth, cell mitosis, recruitment of other cells migrating to the injury site, and cell differentiation induction [10,11]. When Mazor et al. [12] used PRF only as a filling in sinus lifting procedure, considerable bone has been detected in the sinus cavity.

Despite being the gold standard for treatment of large bone defects, the autologous bone grafting may bear complications such as, donor site compromise, patient discomfort, and risk of graft resorption as well as dislodgement [3,13–15].

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Bone marrow stem cells (BMSCs) on the other hand are difficult to obtain, their acquirement procedure may cause pain and discomfort, and their overall quantities are finite [4,16]. Notwithstanding its abundance in the human body, ADSCs transition toward the clinical application is challenging. Large scale ADSCs transplantation studies on animal and human subjects are still much needed in order to expedite ADSCs approval for clinical application and subsequently make this, feasible stem cell source, universally accessible [17].

Aim of the study was to assess bone formation after creation of the bone defect in the maxilla of two dogs in both sides: platelets rich fibrin in the right side and in left side Adipose derived stem cells. This was achieved through Radiographic and gene expression profile of bone formation.

How ADSCs treated site will fare in safety as well as in the induction of bone formation in comparison with the platelet rich fibrin treated sites?

MATERIALS AND METHODS :

The study was done in accordance with the U.K. Animals (Scientific Procedures) Act, 1986. The experiment was approved ethically by Banha University. The approval number is BUFVTM 06- 11- 21.

2.1 Collection of adipose tissue: -

Two male Mongrel dogs (The first dog is brown in color and weighted 12.8 kg and the second dog color is black and weighted13 kg) were kept in the dogs' kennel at the department of surgery, Anesthesiology and Radiology faculty of veterinary medicine Benha university. We premedicated the dogs with atropine sulfate in a dose rate of 0.04 mg/kg body weight followed by xylazine hydrochloride (Xyla-Ject, Adwia, Egypt) at a rate of 1.5mg/kg body weight, both were given intramuscularly at 10 minutes' interval. General anesthesia was induced by Ketamine Hydrochloride (ketamax-50, Troikaa pharmaceuticals ltd, India) at a rate of 5mg/kg body weight via intramuscular injection. The anesthesia was maintained by intravenous propofol (Diprivan 10mg, Astra Zeneca, UK) injection in a dose rate of 5mg/kg [18–21].

Lapatotomy incision was performed in the skin and subcutaneous tissue till reaching to the level of subcutaneous fat. The fat was autologous because they are collected from the same animals. The site of collection subcutaneous fat in the abdominal area, shown in (Figure 1). The sample was washed with warm Phosphate buffer saline (PBS) and NaCl very well to remove blood cells and any tissue remains. The samples were collected in tubes containing PBS [22]. The weight of collected fat was 15 mg.

2.2 Adipose derived stem cell preparation: -

Sample was prepared at general histology department laboratory, Banha University. We washed the fat sample with phosphate buffer saline (PBS) and used scalpel to mince the fat into smaller pieces.

0.075% Collagenase type II (Serva Electrophoresis GmbH, Mannheim), in Hank's Balanced Salt Solution was used to digest extracellular matrix for one hour at 37°C in a 5% CO2 incubator with at 10 min intervals shake. Digested tissue was filtered and centrifuged, and erythrocytes were removed by treatment with erythrocyte lysis buffer. We obtained cellular pellets after using a cell strainer to filter out the debris and centrifugation at 3000 r/m for 10 minutes. Resuspension in culture medium [23].

The cells were transferred to tissue culture flasks with Dulbecco Modified Eagle Medium (DMEM, Gibco/ BRL, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (Gibco/BRL) and, after an attachment period of 24 hours, non-adherent cells were removed by a PBS wash.

Attached cells were cultured in DMEM media supplemented with 10% fetal bovine serum FBS, 1% penicillin-streptomycin (Gibco/ BRL), and 1.25 mg/L amphotericin B (Gibco/BRL), and expanded in vitro. At 80-90% confluence, cultures were washed twice with PBS and the cells were trypsinized with 0.25% trypsin in 1 mM EDTA (Gibco/BRL) for 5 min at 37oC. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50 cm2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures and expanded in vitro until passage three. ADSCs in culture were characterized by their adhesiveness and fusiform shape, by flow cytometry for ADSC surface markers CD45− and CD29+ [24].

2.3 Collection of PRF: -

A phlebotomy was performed from in the forelimb vein to obtain 10 mL venous blood via 18-gauge needle, as shown in (Figure 2). The blood divided equally on two coagulant-free, and sterile vacutainer tubes in order to achieve the balance around rotor axis of the centrifuge. We centrifuged the blood at 3000 rpm (400 g), for 12 min.

After centrifugation, the blood settled in three different layers: the upper straw-colored layer was the acellular plasma, the middle portion contained the fibrin clot, and the red-colored lower portion, which was the red blood cells. We discarded the upper straw colored portion, then the middle portion containing the fibrin clot was obtained, just 2 mm below to the dividing line between lower and middle portions [25,26].

2.4 Creation of the bone defect: -

First, local anesthesia with adrenaline (Carpulemepecaine-L mapecaine HLC levonoardefrine 1/20000, Alexandria Company for Pharmaceuticals & Chemical Industries) for the of hemostasis and pre-analgesia. It was done by the infiltration technique; 1.2 ml of the anesthesia carpule was injected above canine root - at the junction of labial mucosa and the attached mucosa - on the labial surface of the dogs 'canines. The rest of the carpule, 0.6ml, went into the rugae area at a point between the palatal gingiva of the upper canines and median palatine raphe on the hard palate.

Next, two mucoperiosteal semilunar flaps – one on the right side and one on the left side - was performed on each dog. They were cut using scalpel number 3 with parker blade number 15. The flaps were raised using mucoperiosteal elevator. The flaps are located on the attached gingiva above roots of canines (teeth number 204, 203, and 202) [27].

The bone defects were created in the safety area mesial to the canines far away from maxillary sinus. Surgical round bur mounted on straight hand piece, connected to micro motor, was used to create four stop cuts to delineate the defect as well as to avoid unwanted overcutting. After that, a fissure surgical bur was used to connect between the stop cuts and create the final form of the defect. All was done under copious irrigation of saline.

The shape of the bone defects was circular; their depth was 0.5 cm and width was 0.5 cm. Their volume was 125 ml as shown in (Figure 3). The defects were named and divided into four groups: First group (first created bone defect) would be the right side of brown dog treated with PRF, second group (second defect) would be the right side of the black dog treated with PRF, the third group (third defect) was the left side of the brown dog treated with ADSCs, and finally the forth group (forth bone defect) was the black dog's left side treated with ADSCs.

2.5 Insertion of ADSCs and PRF

After the defects were completed, in the same session, PRF was obtained after gentle pressing with sterile dry gauze and put into the bone defect of the right side. Subsequently, repositioning of the mucoperiosteal flap was done and resorabable vicryl material interrupted suture was done.

After one week, we received ADSCs from the laboratory in a falcon tube. We put the two dogs again through the same anesthesia protocol we applied during the first session. We opened the mucoperiosteal flap on the left side and exposed the bone defect Adipose derived stem cells were carried on the transmitting media (Gelfoam®) and put into the bone defect on the left side of both dogs as shown in (Figure 4). Then, the corresponding mucoperiosteal flap was repositioned and closed by vinyl interrupted suture [28,29]. (Figure 5).

2.6 Post-operative care: -

Postoperative care was done by putting animals in separate boxes, with administration of antibiotics for seven days, Synulox (Amoxicillin and clavenolic acid)1cm/ 20kg subcutaneously, and analgesic (Carprofen 5%, 50mg, Adwia, Egypt) for three days [30]. Diet Restriction for three days to the half the amount was achieved. The dogs were kept under observation for any local or systemic reactions and complications [31].

2.7 Radiographic Imaging and biopsy: -

After six months, the dogs were prepared for the biopsies as well as Computed tomography (CT) radiographs. They were generally anesthetized using Ketamine Hydrochloride (ketamax-50, Troikaa pharmaceuticals ltd, India) at a rate of 5mg/kg body weight via intramuscular injection. The anesthesia was maintained by intravenous propofol (Diprivan 10mg, Astra Zeneca, UK) injection in a dose rate of 5mg/kg.

Mucoperiosteal Flaps were performed at the same location and biopsies were taken in order to test the genes expression profile, and the placed immediately in cryo tubes and stored in RNA Later solution (by 10 µL per 1 mg of tissue) (Qiagen-GmbH, Germany) at-80°C. Mucoperiosteal Flaps were repositioned and closed by resorablevicryl material interrupted suture.

Then, the dogs transported for CT radiographs. They were taken by CBCT machine is planmeca model proface Finland. field of view 17*20 with voxel size 200 microns the arrows showed us the density tool measurements in the romexis software in Hounsfield units.

Animals were put in separate boxes to achieve postoperative care. Antibiotics were administered for seven days, Synulox (Amoxicillin and clavenolic acid)1cm/ 20kg subcutaneously, with analgesic (Carprofen 5%, 50mg, Adwia, Egypt) for three days. Half amount of the diet administered for three days. The animals were observed for any local or systemic reactions.

2.8 Gene Expression profile: -

2.8.1 Total RNA Extraction

Total RNA extraction was done by using total RNeasy Mini (Qiagen-GmbH, Germany). Rotor Tissue Ruptor was used to homogenize the sample and was used according to the manufacture instructions (Qiagen-GmbH, Germany). We eluted total RNA in nuclease- free water, and used Nanodrop spectrophotometer (Thermoscientific, 2000 USA) to quantify total RNA spectrophotometrically. Pure RNA has an A260/A280 ratio of 1.8-2.0, and kept at -80 °C until use.

2.8.2 Synthesis of cDNA

We used 10 µL 2X Reverse Transcriptase Master Mix (Applied Bio system, USA) with 2 µg of total RNA in 10 µL for each sample for the purpose of synthesizing First-strand cDNA by the process of reverse transcription [32].

2.8.3 Assessment of Genes Expression

Quantitative Real Time PCR amplifications were performed with RT² SYBR Green qPCRMastermix (Qiagen-GmbH, Germany), in the thermocycler Real time PCR (Applied Biosystem 6900 Real time PCR, USA), under the following cycle conditions: 10 minutes at 95 ˚C for initial denaturation, followed by 40 cycles of 95 ˚C, 15 seconds, then 60 ˚C for one minute. For cDNA amplification primers of Aggrecan, and Collagen II were used and normalized by housekeeping genes GAPDH. All primers were designed using NCBI Primer- BLAST software listed in table 1 [33,34].

2.8.4 Data Analysis and Statistics

Calculate the Threshold Cycle (ΔCT) for each well using the real-time cycler software. Dissociation (melting) curve analysis was performed to verify PCR specificity. A melting curve program was run and generated a first derivative dissociation curve for each well using the real-time cycler software. The fold change for each gene calculated using the formula: 2(-ΔΔCT) (Livak and Schmittgen, 2001). The p values are calculated based on a Student's ttest of the replicate 2^ (-Delta Ct) values for each gene in the control group and treatment groups and p values less than 0.05.

RNA purification kit (Gene JET, Kit, #K0731, Thermo Fisher Scientific Inc. Germany) was used to isolate total RNA. Then, reverse transcriptase transcribed RNA into complementary DNA (cDNA), After that the cDNA was used as the template for the qPCR reaction. Real-time reverse transcription polymerase chain reaction (RT-qPCR) was used for RNA quantification and it was achieved by SensiFast, SYBR Hi-ROX One-step Kit, catalog number. PI- 50217 V, Bioline, UK.

2.8.5 Statistical Analysis

We entered and coded our data by utilizing the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data was interpreted by Scheffé's statistical method.

RESULTS

3.1 Stem cells preparation results

Flow cytometric analysis for identification of adipose derived stem cells showed the cells were CD 29 positive as well as CD 45 negative.

Stem cells in the third passage appeared to have fibroblast cells appearance (spindle shaped with long anastomosing cells' processes.

Stem cell differentiation into osteal tissue in the created bone defect site was indicative that the isolated cells are indeed stem cells.

3.2 Radiographic results

Computed tomography (CT) of the two dogs revealed that bone density on the stem cell treated sites is higher than that of the PRF treated sites. The CT image for the black dog shows the fourth group (ADSCs treated bone defect of the left site) with higher bone density of 357 HU. The bone density of the second group (the black dog's right side treated with PRF) is 164 HU, which is significantly lower than the left stem cell treated site. (Figure 7, A). The CT image for the brown dog shows the bone density of the left site treated with adipose derived stem cells (group 3) is 275 Hounsfield unit (HU), while the right site which treated with PRF (group 1) demonstrated much lower bone density, 189 HU, showed in (Figure 7, B).

3.3 Gene expression results

Gene expressions of aggrecan& collagen II genes are presented in the (Figure 8). PRF caused an increase in expression of aggrecan and collagen II genes in the first and second group. In the other hand, Adipose derived stem cells (ADSCs) caused significant upregulation of both aggrecan and collagen II gene expression in the third and fourth groups, that is according to quantitative reverse transcriptase-PCR analysis. Overall, there was a significant upregulation of aggrecan, collagen II expression in the third and fourth groups, treated with ADSCs, in comparison with the first and second groups, treated with PRF.

Table 1: Primer sequences used for the Real-Time PCR.

Gene symbol	Primer sequence from 5′- 3′	Genbank accession number
Collagen Type 2	Forward primer: 5'-TGAATGGAAGAGCGGAGACT-3' Reverse primer: 5'-CCACCATTGAT-GGTTTCTCC-3'	NG_008072.1
Aggrecan	Forward primer: 5'-ACCCCT-GAGGAACAGGAGTT-3' ReversePrimer: 5'-GTGCCAGATCATCACCACAC-3'	NG_012794.1
GAPDH	Forward primer: 5'-GATTGTCAGCAATGCCTCCT-3' Reverse primer: 5'-GTGGAAGCAGGGATGATGTT-3'	NC_000012.12

Figure1: Adipose tissue collection. A) Hair removal of the abdominal area before the incision was made. B) Adipose tissue being cut from subcutaneous fat .C) Fat tissue after being collected. D) Incision was closed after the adipose collecting procedure.

Figure 2: - Phlebotomy was done in the forelimb vein to prepare PRF.

Figure 3: - A) Bone defect on the left side of the black dog, before ADSCs insertion. B) Bone defect on the right side of the brown dog, before insertion of ADSCs.

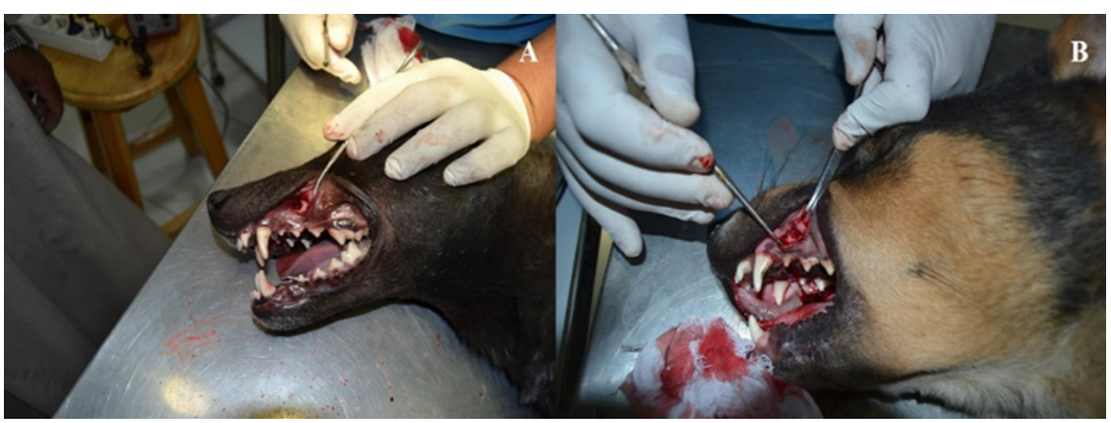


Figure 4: - A) Insertion of Gelfoam loaded with ADSCs on the left side of the black dog. B) Insertion of Gelfoam loaded with ADSCs on the left side of thee brown dog.

Figure 5: - A) Left side of the brown dog after gel foam loaded with ADSCs. B) Black dog left side bone defect after insertion of Gelfoam loaded with ADSCs. C) Brown dog left side after closure. D) Black dog left side after suturing and closure.

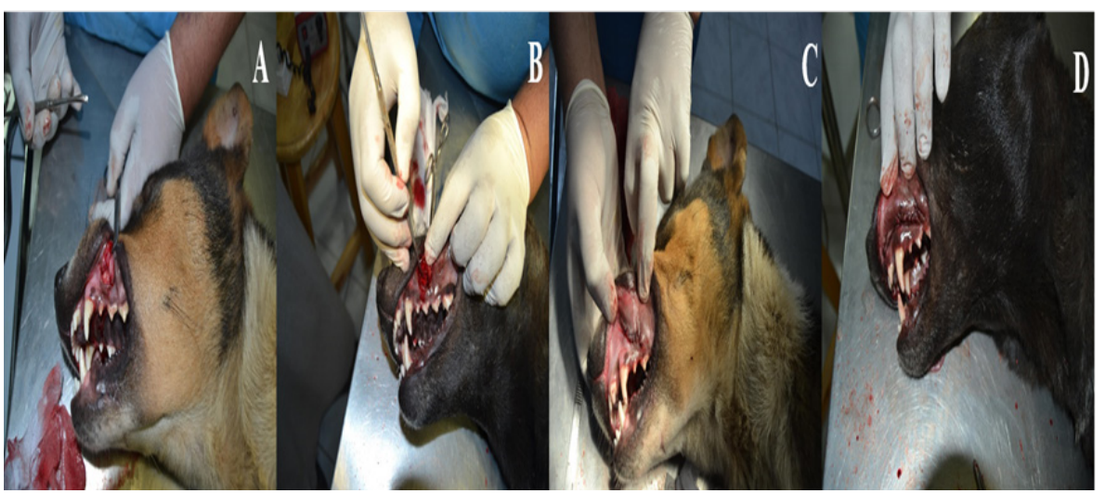


Figure 6: - A) Right side of the brown dog after one week of healing. B) Right side of the black dog after one week of healing.

Figure 7: - A) CT image for the black dog. It shows higher bone density on the left side than the right one. B) CT image for the brown dog. It demonstrates higher bone density on the left side than the right one.

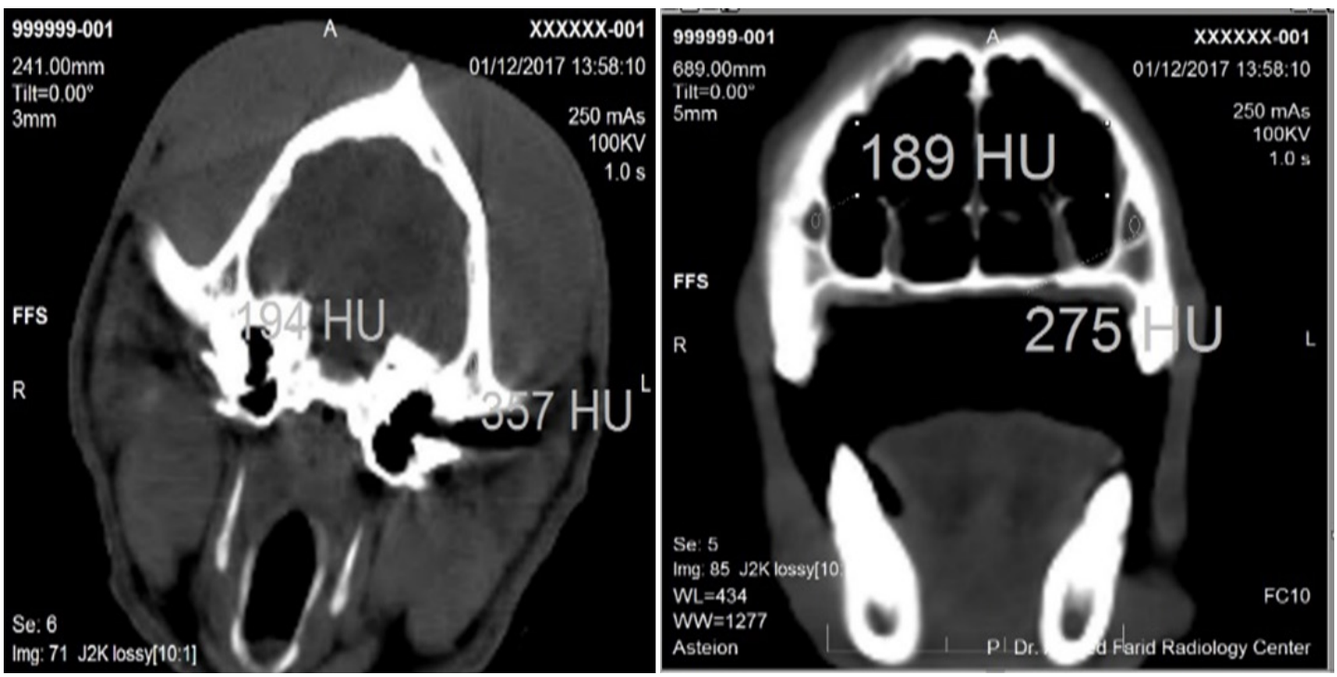
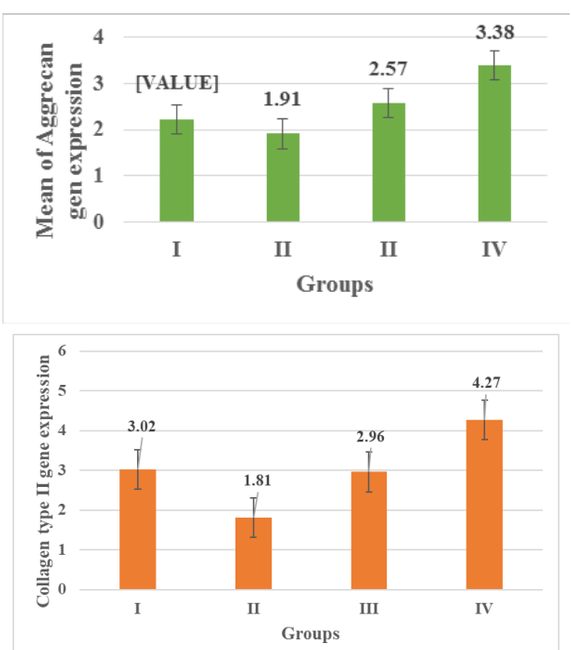


Figure 8: - Different groups on the graph demonstrates Higher expression of Aggrecan and Collagen 2 genes in groups treated with ADSCs (Groups, 3 and 4) in comparison with the groups treated with PRF (First and second groups).



DISCUSSION

We chose to utilize autologous ADSCs as a source for stem cells in our experiment for numerous reasons: they are easy accessible, they are from the same living organism, thus, eliminating the consequences of immune rejection and complications of cross infection, abundance of fat tissue in most organisms, post-operative pain and complications are minimal in comparison with BMSCs, studies demonstrated their capacity of inducing mineralized tissue formation in vivo [35,36]. Our study used PRF as control to determine if ADSCs would stimulate bone formation; PRF is frequently used in wound healing, as it contains cytokines, growth factors, and can act as a matrix to guide stem cells and signals to induce bone generation [37–39].

Gel foam was also used as a scaffold to convoy ADSCs, as well as PRF to the bone defect sites, and to test their effectiveness and biocompatibility with ADSCs. Gelfoam has been used in clinical setting for some time now, because it mainly has a good safety record, biodegradability, biocompatibility in surgical sites, low cost material, and approved by most regulating bodies. All these properties are significant if we want to establish a safe protocol for deployment of stem cells to wound sites without worrying about safety and costly experimental scaffolds [40–42].

We performed the study on a large animal model, since there were a very few studies that experimented on larger organisms. Testing on larger animals would give an insight into the effectiveness of ADSCs in inducing mineralization of a bone defect, and whether ADSCs are safe to be implanted in vivo or not [43–45]. It would also give us an understanding on how the bone defect size of large mammals would affect the administration of ADSCs, as there is no universal protocol that determine whether the bone defect size is critical or mild [46].

Our experiment determined that bone formation in the Adipose derived stem cells (ADSCs) treated site is much more than the bone formed in Platelet rich fibrin site (PRF) treated site. Our findings were drawn from the assessment of computed tomography (CT) radiographs - there were not many studies that used CT radiograph in clinical evaluation of bone formation in case of ADSCs - as well as the genes - involved in the osteogenic differentiation - expression profile. A significant increase in bone density in groups four and three, which treated with Adipose derived stem cells, can be detected in the CT radiographs. It is evidenced by increased Hounsfield unit (HU): 275 HU for group 3 and 357 HU for group 4, while group 1 and 2, which treated with PRF, would be 189 HU and 164 HU respectively. Beside the bone formation in the created defects, the CT radiographs showed no abnormal growths or lesions. That means that the autologous ADSCs used in in that experiment are safe and they have osteogenic potential.

Gene expression profile showed an increased expression of Collagen 2 and aggrecan genes. Collagen 2 gene is associated with osteogenesis, while aggrecan gene is associated with chondrogenesis [47]. Which indicates that the cartilage tissue might be formed either alongside the osteal tissue or preceded it.

There are numerous studies that have shown the osteogenic and chondrogenic potential of ADSCs in both in-vitro and in animal models. The exact pathway for bone formation by ADSCs is not yet understood. It is established that, in a controlled environment - in vitro - the ADSCs either differentiate into bone and cartilage forming cells directly, which secrete mineralized tissue. In order for the Adipose derived stem cells to have osteogenic potential, osteogenic medium, containing 1 nM dexamethasone, 2 mM β-glycerolphosphate and 50 μM ascorbate-2-phosphate for 14 days, should be added to the stem cells after reaching 80–90% confluence, and the medium should be replaced every two to three days [23,48]. The golden standard of identifying osteogenic tissue after differentiation is by 40 mM Alizarin Red (pH 4.1) after fixation in 10% formalin [49].

To get chondrogenic differentiation from ADSCs after reaching confluency they are supplemented with insulin growth factor, ascorbate-2-phosphate, dexamethasone, L-proline, BMP6 and 7, and TGF-β. In the other hand, they can also be seeded into cartilage inducing scaffold such as, polyglycolic acid scaffold to induce the ADSCs to differentiate into chondrocytes [50–52].

In the event that stem cells are implanted in vivo, as in our experiment with Gelfoam scaffold, they migrate and secrete cytokines and growth factors that induce differentiation of the microenvironment's cells into osteoblast and chondrocytes. Tumor necrosis factor α (TNFα), b-FGF (basic Fibroblast Growth Factor), interleukin one β, interleukin six, and Growth differentiation factor 11 (GDF11) are the specific proteins secreted by ADSCs to induce cells to differentiate into osteoblasts and chondrocyte [53].

It was demonstrated in the literature that the majority of transplanted mesenchymal stem cells do not survive in the new environment and most likely do not differentiate, in the contrary of the controlled in-vitro setting, which requires adding proliferation, as well as differentiation stimulating proteins [54–60].

Our experiment's new formed mineralized tissue result is consistent with many animal models experiments - rodents, rabbits and canines – in which ASCs are implanted either in a created bone defect or around dental implant: Evaluation of the resultant mineralized tissue may differ, nevertheless, a significant mineralized tissue was formed after ADSCs are implanted with a scaffold.

An example of a study which its result is consistent with ours, is a small animal model experiment was done by Hakan Orbay et al. in which they created critical size calvarial defects in rats, then tried to fill the defects by hydroxyapatite/poly (lactide-co-glycolide) [HA-PLG] scaffolds seeded with adipose derived stem cells. Although the assessment of the results are different than our experiment - they evaluated the results by using micro computed tomography (micro CT) and histologically by hematoxylin and eosin staining method to analyze the resultant mineralized tissue - a significant mineralized tissue created in ADSC with a scaffold groups [60].

In another study on rabbits a critical tibial defect was created and filled with hydroxyapatite scaffold loaded with ADSCs in one group and the other group involved only hydroxyapatite scaffold, it was shown that the first group has more mineralized tissue than the second group and was evaluated using biomechanical, histomorphometric, immunohistochemical methods [61].

Despite our study confirms the paradigm that ADSCs are capable of inducing mineralized tissue in a bone defect, the experiment came with some limitations: histological assessment of the newly formed mineralized tissue should have been done to figure out its the quality and quantity and to find any abnormalities histologically, and more osteogenic and chondrogenic genes, other than aggrecan and collagen type 2 genes, should have been tested, and periodic radiographic assessment should have been done after six months, but the dogs were not available, after our experiment.

CONCLUSION

ADSCs derived from dogs' adipose tissue are implanted with Gelfoam inside a created bone defect, they generated more mineralized tissue than PRF, which is derived from the dogs' blood, over six months' period. The ADSCs are not approved for clinical use yet, so more experiments on large mammals and periodic histological and radiographic assessment are required to determine ADSCs implantation safety and effectiveness [62,63]. Protocols, that govern autologous and allogenous ADSCs preparation, preservation, and banking, should be standardized. More research is required to determine how exactly ADSCs affect the diseased cells in the microenvironment to differentiate into required cell lineages, by studying exosomes secreted by ADSCs in vivo, and cytokines.

AUTHOR CONTRIBUTIONS:

Samir Halawa, Abdelhaleem Elkasapy, and Ahmed Othman conceptualize the experiment. Samir Halawa, Abdelhaleem Elkasapy constructed, supervised, visualized the study, and analyzed the data. Ahmed Othman is responsible for writing—original draft preparation, writing—review and editing. Olla Khalifa validated both the data and methodology.
All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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