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Different Protocols of Combined Application of Photobiomodulation In Vitro and In Vivo Plus Adipose-Derived Stem Cells Improve the Healing of Bones in Critical Size Defects in Rat Models

Armin Khosravipour[1], Atarodalsadat Mostafavinia[2], Abdollah Amini[1*], Rouhallah Gazor[3], Fatemeh Zare[1], Somaye Fallahnezhad[4], Fatemehalsadat Rezaei[5], Mehrdad Asgari[3], Fatemeh Mohammadian[6], Zhaleh Mohsenifar[7], Sufan Chien[8], Mohammad Bayat[1,8*]

[1]Department of Biology and Anatomical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Anatomy, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[3]Department of Anatomy, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[4]Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[5]University of Kentucky, College of Pharmacy, 789 South Limestone, Lexington, Kentucky 40536, USA
[6]Department of Medical Physics and Biomedical Engineering, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[7]Department of Pathology, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[8]Price Institute of Surgical Research, University of Louisville, and Noveratech LLC, Louisville, Kentucky, USA

***Correspondence to**
Mohammad Bayat,
Email: bayat_m@yahoo.com;
Abdollah Amini,
Email: d.amini2008@yahoo.com

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Abstract

Introduction: Long bone segmental deficiencies are challenging complications to treat. Hereby, the effects of the scaffold derived from the human demineralized bone matrix (hDBMS) plus human adipose stem cells (hADSs) plus photobiomodulation (PBM) (in vitro and or in vivo) on the catabolic step of femoral bone repair in rats with critical size femoral defects (CDFDs) were evaluated with stereology and high stress load (HSL) assessment methods.

Methods: hADSs were exposed to PBM in vitro; then, the mixed influences of hDBMS + hADS + PBM on CSFDs were evaluated. CSFDs were made on both femurs; then hDBMSs were engrafted into both CSFDs of all rats. There were 6 groups (G)s: G1 was the control; in G2 (hADS), hADSs only were engrafted into hDBMS of CSFD; in G3 (PBM) only PBM therapy for CSFD was provided; in G4 (hADS + PBM in vivo), seeded hADSs on hDBMS of CSFDs were radiated with a laser in vivo; in G5 (hADSs + PBM under in vitro condition), hADSs in a culture system were radiated with a laser, then transferred on hDBMS of CSFDs; and in G6 (hADS + PBM in conditions of in vivo and in vitro), laser-exposed hADSs were transplanted on hDBMS of CSFDs, and then CSFDs were exposed to a laser in vivo.

Results: Groups 4, 5, and 6 meaningfully improved HSLs of CSFD in comparison with groups 3, 1, and 2 (all, $P=0.001$). HSL of G5 was significantly more than G4 and G6 (both, $P=0.000$). Gs 6 and 4 significantly increased new bone volumes of CSFD compared to Gs 2 (all, $P=0.000$) and 1 ($P=0.001$ & $P=0.003$ respectively). HSL of G 1 was significantly lower than G5 ($P=0.026$).

Conclusion: HSLs of CSFD in rats that received treatments of hDBMS plus hADS plus PBM were significantly higher than treatments with hADS and PBM alone and control groups.

Keywords: Critical size bone defect; Fracture healing; Demineralized bone scaffold; Human adipose-derived stem cell; Photobiomodulation.

Introduction

Long bone segmental deficiencies brought on by big trauma with extensive soft tissue damage are challenging complications to treat. Amputation has often been used as the backbone of management.[1] The latest research in the United States demonstrates dramatic escalations in the occurrence of fractures.[2] Nonunion bone fractures are the main problems in the healing process of fractures and remain challenging for orthopedists.[3] Research-based progress in mechanical and biological courses conducting bone repair has eventuated in the recognition of crucial intermediaries that could possibly be aimed to encourage fracture healing. The development of presently existing bone substitute materials and the innovation of new

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biomaterials have added remarkably to obtainable remedy choices. Additional investigation and identification of unique beneficial tactics with satisfactory results will achieve vital medical essentials; that is, they will hasten fracture healing and correct skeletal deficiencies in people suffering from nonunion.[4] Because nonunion continues to be a vital medical difficulty, scientists are interested in the use of biomaterial for bone tissue engineering (BTE) to enhance the course of fracture healing. Accordingly, effective fracture healing requires the interaction of the following four crucial issues: (1) stem cells, (2) biostimulators (to encourage stem cell proliferation and differentiation), (3) a dynamic and biodigestable scaffold (to support cell attachment, propagation, and development), and (4) a capable blood vessel support. Therefore, an integrative tactic would be considered in the remedy of long bone nonunion.[5]

Autologous bone grafting (ABG) is the first choice for nonunion bone fractures. ABG requires three elements to encourage fracture healing: an osteoconductive scaffold, endogenous dynamic particles, and cells that are capable of responding to the signs. Regrettably, while ABG is regarded as the first graft choice, noteworthy problems associated with the collecting area (iliac crest) have been described. Additionally, the necessary quantity of the prerequisite graft may occasionally be inadequate.[6] Human demineralized bone matrix scaffolds(hDBMS)s are bone tissue grafts that are extracted from deceased human's bones and include collagen I as well as numerous growth factors. The above-mentioned constituents create both osteoconductive and osteoinductive DBMSs.[7] Nevertheless, additional studies have revealed that DBMS holds insufficient osteoinductive ability.[8,9]

Consequently, scientists could use stem cell therapy for bone healing and/or bone renewal. Mesenchymal-derived stem cells (MSCs) have the differential capability to osteoblasts and are obtainable from an extensive range of origins. Stem cell therapy would propose a novel opportunity to rebuild long bone deficiency, particularly considering their great repeatability and the brilliant mechanical strength of renovated bones.[10] Adipose-derived stem cells (ADSs) have established a dominant role in BTE, supplying several novel answers and great adaptability of usage, as was apparent in both culture systems and animal models. The usage of ADSs to achieve bone renewal and repair goals has demonstrated numerous benefits compared to other MSCs.[11] Dozza et al investigated the efficiency of the impact of DBM plus MSC in a new nonunion simulation. They determined that the demineralized bone matrix (DBM) is a persuasive option for nonunion management, but MSCs do not advance the curing course once cultured on DBM particles before transplantation.[12] Accordingly, Mott et al identified 94 eligible studies in their systematic review and reported that nearly 30% of the involved investigations did not present a precise description of remedy. Moreover, the remedies described and used in most investigations were subjective. The number of measurable evaluations was low. Remedy results should have been better expressed, and additional investigations are necessary to describe and confirm these results. Mott et al concluded that excellent proof to confirm the efficiency of MSCs for bone repair is nonexistent.[13]

One strategy to increase MSC capability and function after transplantation in bone defects could be the use of photobiomodulation (PBM). Similarly acknowledged as low-level laser therapy, PBM is the usage of a laser to enhance curing, mitigate soreness, and decrease inflammation. Ebrahimi et al determined that PBM could quicken fracture repair.[14] PBM is a noninvasive method for inducing cell proliferation and enhancing stem cell differentiation.[11] In addition, the Bayat group reported that PBM significantly encouraged cell survival and reduced population doubling time and apoptosis of human bone marrow (hBM) - MSCs and hADSs in a culture system. Moreover, PBM considerably augmented hADS survival in comparison with the non-treated and laser-radiated hBM-MSC groups.[15]

Therefore, in the current study, it was hypothesized that preconditioning with PBM increases the function of ADSs in the fracture bed of an experimental model of critical size femoral defect (CSFD) which was filled with hDBMS in rats and promotes bone repair after ADS transplantation. Furthermore, it was hypothesized that the combination of human demineralized bone matrix (hDBM) plus hADS plus PBM could considerably promote bone repair in CSFD simulation in a murine model. Recently, the Bayat group assessed the impacts of hDBMS plus hADS plus PBM on bone repair in a CSFD murine model. The outcomes were evaluated with bone strength parameters including maximum force as well as one imaging technique, and it was concluded that the pretreatment of hADS with PBM in a culture system remarkably augmented bone repair.[16]

In the current study, ADSs were first treated with PBM in vitro. Next, the combined impacts of hDBMS plus hADS plus PBM on repairing of CSFDs during anabolic and catabolic phases of bone healing were evaluated with stereology and high stress load (HSL). HSL is a more valid and reliable bone strength evaluating parameter than maximum force.[16,17]

Materials and Methods

Animals and Study Design

CSFDs were made on both femurs in 72 rats, after which hDBMS(s) were engrafted into both CSFDs of all rats. Experiments were conducted on 6 groups (12 rats per group): G1 was the control; in G2 (hADS), hADSs only were engrafted into the hDBMS of the CSFDs; in G3 (PBM), PBM therapy for CSFD only was administered; in

G4 (hADS + PBM in vivo) (hADS + PBM in vivo), hADSs were transplanted on hDBMS of CSFDs, and then they were radiated with a laser in vivo; in G5 (hADS + PBM in vitro), hADSs in a culture system were radiated with a laser, then seeded into hDBMS (in CSFDs); and in G6 (hADS + PBM in vitro + in vivo), the hADSs were exposed to a laser and transplanted into hDBMS, and the CSFDs were then exposed to a laser under in vivo condition. On days 14 (anabolic phase) and 42 (catabolic phase of bone repair) after CSFD induction, the animals were killed and the CSFD areas were dissected. The CSFDs from left limbs were considered for mechanical compression tests, and CSFDs from right limbs were applied for stereological (histologic) analyses.

hDBMS

Sanitized hDBMS(s) were obtained from the bones of human dead bodies procured from the Hamanand Saz Baft Kish Company, Kish, Iran. According to information which was released by the company, the DBMS(s) were produced from long bones of human cadavers (from cancellous parts) under standard conditions. DBMS(s) were donated by Hamanand Saz Baft Kish Company (TRC Corporation, Kish, Iran).

The harvested bone tissues were dissected into smaller pieces, and then they were physically and chemically processed for removing the blood, fat and cells from the pieces.

Cultivation and Immunophenotyping of hADSs

The hADSs were cultivated according to a standard method, and flow cytometry exploration to characterize passage-3 hADS (Royan Institute, Tehran, Iran) for MSC markers was done according to an earlier study.[18]

The Treatment of hADSs With PBM Under In Vitro Condition

1×10^5 hADSCs at passage 3 were harvested and moved into 24-well plates. Then, the red laser with 630 nm, 0.05 W, 46 seconds and 1.2 J/cm^2 (Table 1) along with the near-infrared laser (NIR, 810 nm, 0.05 W, 46 seconds, 1.2 J/cm^2, NILTVIR202 Noura Instruments, Tehran, Iran) were applied on alternate days for a total of 3 times. Each time, first the red and then the NIR lasers were applied next to each other.

Seeding hADS on hDBMS

One hDBMS (3 × 3 × 5mm) was put into the 96-well tissue culture plate. Twenty-four hours after laser radiation, 1×10^5 untreated (control) or pretreated hADSCs were trypsinized, transplanted and seeded on each hDBMS in the tissue wells and kept at 37°C for 24 hours. At last, one cube with hDBMS that held hADSs was embedded in each CSFD of the rats from Gs 2, 4, 5 and 6, and one cube of hDBMS (without hADSs) was embedded in CSFD of G3.

The Making of CSFD and Engraftment of hDBMS

CSFDs were produced in the lateral side and distal part of the right and left femoral bones of each rat. Using systemic anesthesia and aseptic settings, a typical 3mm in width and round osteotomy was pierced in the lateral side of the distal part of the bone. Then, a cube of hDBMS (no hADSs) was seeded on the CSFDs of the rats in G1; hDBMSs + hADS were inserted in the CSFDs of G2; and hDBMS + treated-hADSs were seeded on the CSFDs of the rats in Gs 5 and 6 forty-eight hours after laser radiation.[16,19]

In Vivo PBM Protocol

The CSFDs of G3, G4 and G6 were exposed to PBM in vivo (890 nm; peak power = 75 W; frequency = 80 Hz; spot size = 1 cm^2; pulse duration = 180 ns; duration of exposure for each shooting = 900 seconds; energy density = 0.97; 3 shootings in each session, MUSTANG 2000, Technica Co., Russia) (Table 1). PBM was begun directly after surgery and continued 3 sessions each week for 2 and/or 6 weeks.[16]

Biomechanical Test (Compression Test)

The harvested bones were immobilized in a stationary immobilizer of the device (Santam Engineering Design Co., Ltd., Iran). A mobile nail was used on the defect at a continuous speed of 5 mm/min until a break happened. At first, the parameter of maximum force (Newton) was

Table 1. Specifications of In Vitro and In Vivo Photobiomodulation Protocols

Specifications of In Vitro Photobiomodulation							
Laser type	**Wavelength (nm)**	**Power (W)**	**Time of Each Session (s)**	**Energy Density (J/cm^2)**	**Laser Beam Diameter (cm)**	**Laser Beam Area (cm^2)**	**Power Density (W/cm^2)**
Red	630	0.05	46	1.2	1.56	1.91	0.0261
Near infrared	810	0.05	46	1.2	1.56	1.91	0.0261
Specifications of in vivo photobiomodulation							
Peak power output, average power					75 W, 0.001		
Wavelength, pulse frequency, spot size					890 nm, 80 Hz,1 cm^2		
Pulsed duration, time of each exposure					180 ns, 900 s		
Energy density (J/cm^2) one exposure, one session, one week, two weeks, six weeks					0.972, 2.96. 9.74, 19.74, 58.44 (J/cm^2)		

determined using the load deformation curve. Next, the value of it (maximum force) was divided into the surface area of the mobile nail (cm^2) in order to obtain the HSL (Figure 1).

Histological and Stereological Tests

The bone defects were harvested and fixed in the formalin saline solutions, and then they were decalcified by ethylenediaminetetraacetic acid (EDTA). The paraffin blocks were serially sectioned (5 micro-millimeters thickness) and stained by hematoxylin and eosin. Here 10 sections were randomly selected for histological and stereological tests.

Stereological Measurement

The total volume (V, mm^3) of new bone formation was calculated using Cavalieri's method:

$V = \Sigma P$ (total count of the volume profiles for each rat) $\times$ a/p (the examined area) $\times$ t (the interval between the 2 sections).

Statistical Analysis

The obtained data were reported as mean ± SD. The independent-samples *t* test and one-way analysis of variance (ANOVA), and post hoc test of LSD (least significant difference) were used. $P < 0.05$ was considered statistically significant.

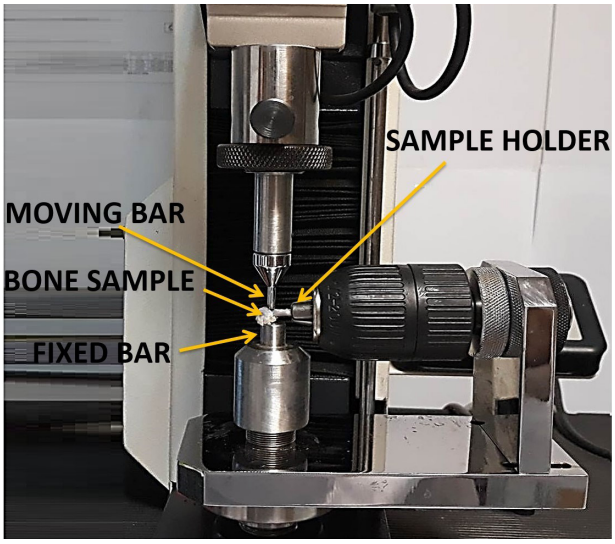

Figure 1. Compression Testing Machine and Specimen. During testing, the specimen is fixed to ensure no movement of the specimen.

Results

Gross Observations

The first and last body weights of the rats of the study groups after 2 and 6 weeks are shown in Table 2. Some groups showed significant changes in body weights.

hADS Surface Marker Characterization

The hADS expressed MSC markers (CD 105 and CD73) almost completely (84.8% and 99.8% respectively), and hADS expressed few hematopoietic markers (CD 45 and CD 31) (0.43% and 0.36% respectively).

Results of Biomechanical Tests at Week Two

Briefly, at week six, all PBM plus hADS groups had significantly increased HSLs of CSFD in comparison with groups 3, 1, and 2 (all, $P = 0.000$). At the same time, G5 was remarkably superior to all other groups ($P = 0.000$).

In this test, all *P* values corresponded to the post hoc test of LSD. In Figure 2, panel A displays that the HSLs of CSFD were remarkably higher in all experimental groups than the control group (all, $P = 0.000$, with the exception of G3, whose *P* value = 0.002). At the same time, the HSLs of groups 5, 6, and 4 were significantly higher than those of groups 2 and 3 (all, $P = 0.000$). Concurrently, the HSL of G2 was significantly higher than that of G3 ($P = 0.005$).

Results of Biomechanical Tests at Week Six

In Figure 2, as shown in panel B, groups 5, 4, and 6 had significantly increased HSLs of CSFD in comparison with groups 3, 1, and 2 (all, $P = 0.000$). Increased HSLs in G5 were significantly more than G4 and G6 (both, $P = 0.000$). Treatments of PBM and ADS alone did not have significant effects on the compression test results in comparison with G1 (control group).

Outcomes of the New Bone Volume at Week Two

Briefly, at week six, groups 3, 4, and 6 had meaningfully more new bone volumes of CSFD in comparison with groups 1 and 2 (Figure 3).

In Figure 4, panel A indicates that groups 2 and 6

Table 2. Comparison of Body Weights in the Studied Groups at 2nd and 6th Weeks After Inducing Critical Size Femoral Defect by Student *T* Test

Factors	Groups					
	Control	ADS	PBM (In Vivo)	PBM + ADS (In Vivo)	PBM-In Vitro + ADS	PBM + ADS (In Vitro + In Vivo)
Initial body weight (g) at week 2	236.8 ± 14.1	256.1 ± 21.1	258.44 ± 14.4	278.5 ± 38.87	248.5 ± 21.63	277.25 ± 39.16
Final body weight (g) at week 2	281.6 ± 11.25***	277.66 ± 26.68*	230.3 ± 16**	245.5 ± 26.16	255.5 ± 26	275 ± 20.18
Initial body weight (g) at week 6	229.8 ± 11.97	225.6 ± 31.89	256.6 ± 10.57	269.1 ± 19.58	243.8 ± 16.32	277 ± 33.26
Final body weight (g) at week 6	283 ± 15.68***	291.1 ± 27.24**	250.7 ± 16.11	269.2 ± 29.47	280.8 ± 30.18**	255.7 ± 17.43

ADS, adipose derived stem cells; PBM, photobiomodulation.
Data are presented as mean ± standard deviation.
*$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

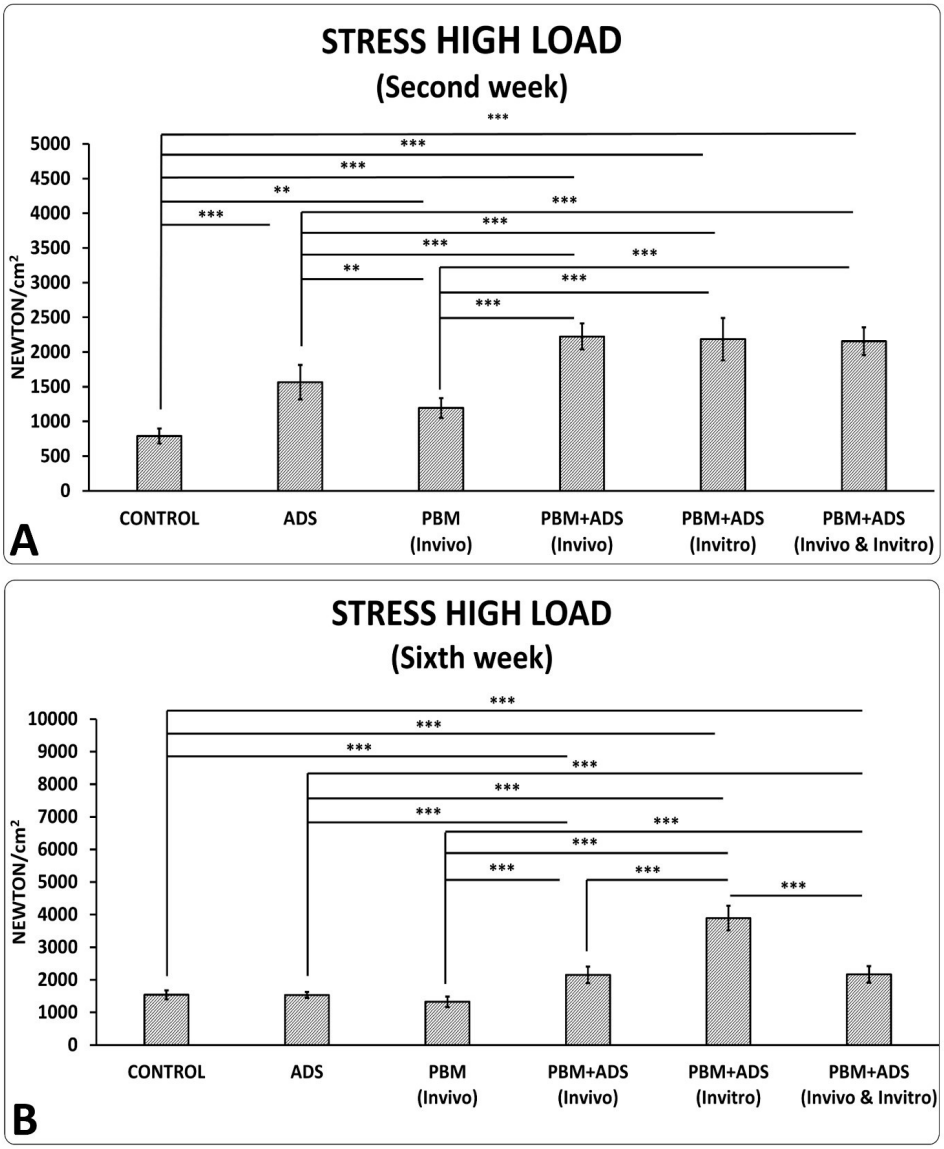

Figure 2. Mean ± SD of High Stress Load (N/cm²) of Critical Size Femoral Defect 2 and 6 Weeks After Surgery in the Studied Groups Compared by the LSD test. ***$P<0.001$.

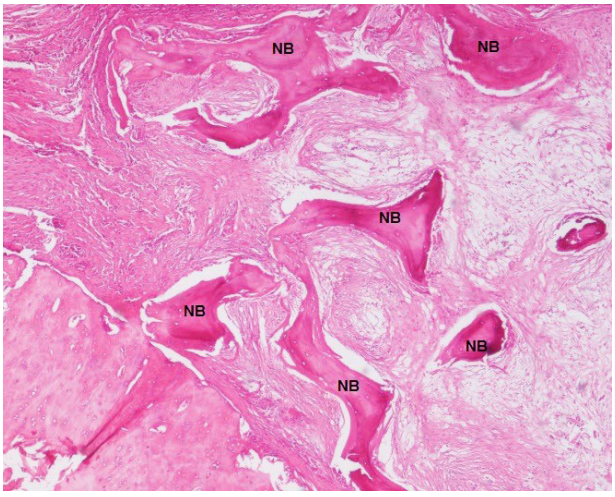

Figure 3. A Histological Slide of New Bone in Repairing Tissue of the Current Study (*Hematoxylin and Eosin,* X40). NB: new bone.

had meaningfully more new bone volumes of CSFD in comparison with G3 (both $P=0.000$), G1 ($P=0.003$, $P=0.000$), and G5 ($P=0.001$, $P=0.000$). At the same time, G4 had significantly increased new bone volume of CSFD compared with groups 3 and 5. Groups 4 and 5 were statistically lower than G1 ($P=0.005$ and $P=0.014$ respectively).

Outcomes of the New Bone Volume at Week Six

In Figure 4, panel B shows that groups 6 and 4 had significantly increased new bone volumes of CSFD compared to G2 (all, $P=0.000$) and G1 ($P=0.001$, $P=0.001$, $P=0.003$). G1 was significantly lower than G5 ($P=0.026$).

Discussion

Briefly, at the catabolic step of bone healing, all PBM

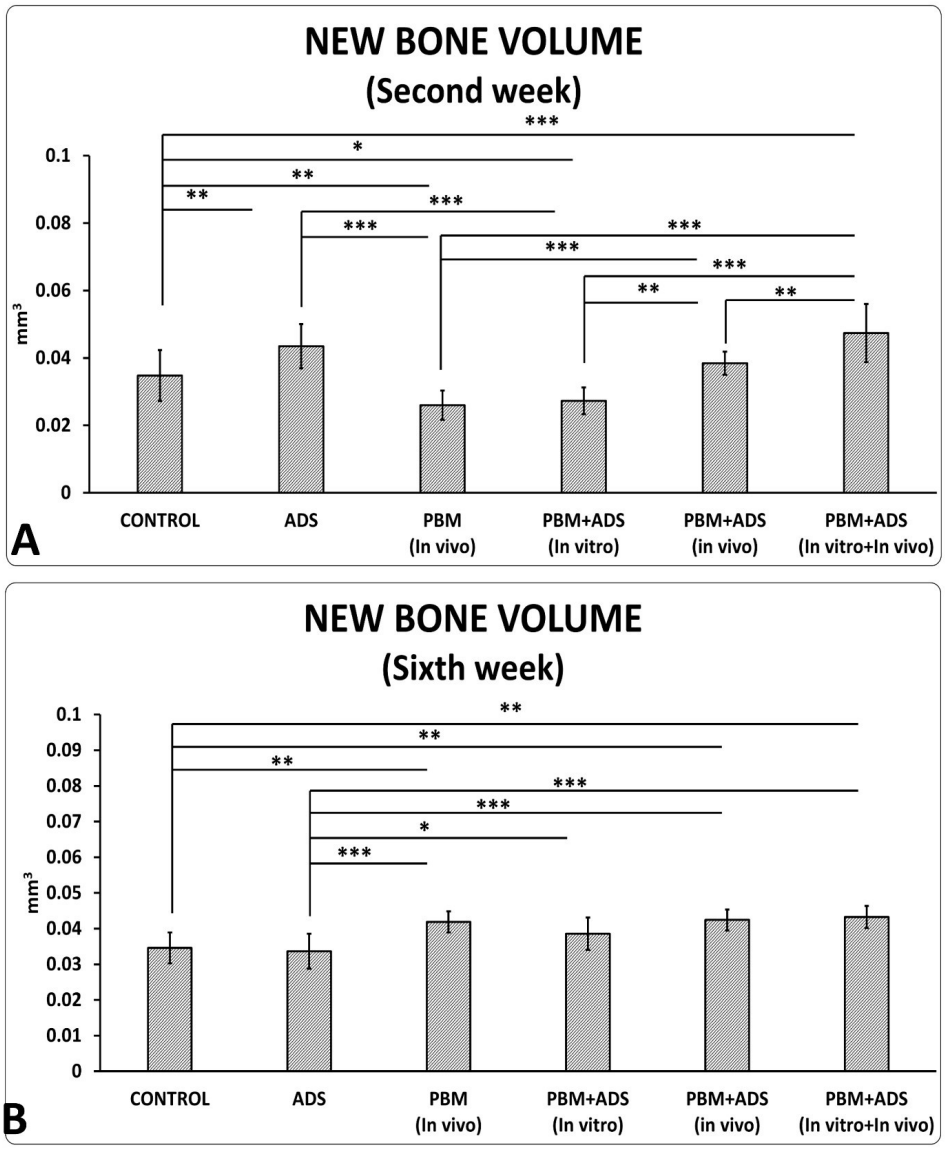

Figure 4. Mean ± SD of New Bone Formation Volumes of Critical Size Femoral Defect 2 and 6 Weeks After Surgery in the Studied Groups Compared by the LSD Test. *$P<0.05$, ** $P<0.05$, *** $P<0.001$.

plus hADS groups had remarkably augmented stress high loads compared to groups 3, 1, and 2. At the same time, G5 was significantly superior to all other groups. Simultaneously, groups 4, 5, and 6 had meaningfully more new bone volumes of CSFD in comparison with groups 1, 2, and 3.

In terms of kinesiology, bone repair characterizes a balanced escalation in the power and firmness of a fractured bone. Only when bone power and firmness parameters are adequately great to support unlimited weight-bearing can it be claimed that a broken bone is repaired. Data on the ratio of escalation of the biomechanical features of a repaired bone defect is thus valued by ascertaining the ratio at which a broken bone is repaired and in assisting to delineate the criteria and quantifiable endpoint of restoration.[20] Thus, in the present study, biomechanical compression tests were applied to evaluate bone power by evaluating the HSL of a repaired bone defect, and the results were compared to those of histological examination.

The restoration of big bone deficiencies continues to be a chief medical difficulty, and BTE is an encouraging method for solving this complication.[21,22] Nevertheless, only a small number of BTE protocols have been transitioned into medical settings, and none of them have been developed into an approved approach for regenerative medicine.[23,24]

In spite of the growing use of MSCs in human studies, the beneficial aids persist in being negligible.[25] MSC-based treatments have the potential to modernize existing remedies for illnesses with the abundant occurrence and associated financial and community threats. Regrettably, human studies using MSCs have resulted in a limited number of developments in typical repairing actions for deteriorating tissues. This restriction can be explained partially by the loss of engrafted MSCs during several hours

after engraftment due to a combination of mechanical, cellular, and host factors.[26] Accordingly, several studies have shown the lost survival and function rates of MSC after transplantation. Torres-Espín et al used two tactics to increase the number of MSCs in damaged spinal cords in rats. They determined that MSC engraftment joined with FK560 extends the viability of transplanted MSCs and restores physiologic and morphological properties after spinal cord injury.[27] Similarly, it was stated in a review article that bone healing is a composite course that needs the propagation and growth of MSCs for effective tissue restoration. Even though a pool of MSCs stays obtainable for bone restoration throughout the life, this important populace of MSCs is exposed to the collected impacts of aging, alterations in the micromilieu, environmental exposure, and illness. Total comprehension of the composite interaction of environmental exposure, aging, illness, and genetics on the propagation and differentiation of MSC populaces is fundamental to creating bone reformative treatments to treat bone illnesses in the human populace.[28] The favorable impacts of MSC treatment for regenerative medicine ultimately rely on the quantity of the transplanted MSCs getting the selected area, their survival, and their advancement of tissue restoration. Thus, approaches targeting the improvement of surviving MSC transplantation are vital for regenerative medicine.[26] To advance the viability of engrafted MSCs, policies to control apoptotic signing and improve MSC adhesion have been advanced, for example, preconditioning with cytokines or hypoxia (or PBM).[29] ADSs are encouraged by their advocates for their far superior availability and the existence of larger amounts of colony forming unit-fibroblast (CFU-f) per unit volume compared to that observed in bone marrow. Medical proof of the efficiency of ADS-based treatment shows that adipose tissue is a brilliant origin for MSC for the creation of bony tissue.[30] From the perspective of fracture healing, irrespective of MSC origin, presently living MSC-based grafts show a tendency to be greater than cell-free and decellularized substitutes at restoring bony tissue.[29]

Overall, it was hypothesized that preconditioning with PBM will increase ADS function in the fracture bed of a CSFD model plus hDBMS in rats and promote bone repair after hADS transplantation.

The current findings are in parallel with those of Asgari et al and the Bayat group findings. Asgari et al investigated the impact of human hDBMS plus hADS plus PBM on CSFDs in ovariectomy-induced osteoporosis in rat models. They found that ADS and PBM remarkably augmented repairing bone strength (high stress load) in an investigational simulation of DBMS-transplanted CSFDs in the phase of catabolic fracture healing in rats with osteoporosis. However, the effects of PBM + hADS were dominant to the other protocols.[16] Recently, the Bayat group evaluated the impacts of hDBMS plus hADS plus PBM on fracture healing of CSFDs in rats. They used some compression test parameters such as maximum force, elastic modules and energy absorption as well as computed tomography (CT) scans. They concluded that the pretreatment of hADS with PBM in a culture system statistically improved bone healing in the rat CSFDs model under in vivo condition.[16] In the current study, the HSL parameter was used to evaluate bone strength, which is more valid and reliable than maximum force.[16,17]

In their review paper, Kushibiki et al reported that today, scientists are leading concentrated basic and medical investigations in the PBM field with the purpose of discovering new beneficial agents. With the appropriate application of PBM, the propagation ratio of cultured MSCs could be improved, which could be more valuable in regenerative medicine and BTE fields. Kushibiki et al showed that PBM is a suitable method for the pretreatments of MSCs in a culture system before MSCs implantation.[31] The present study found that while the applications of hADS and PBM alone did not meaningfully augment HSLs of CSFD in comparison with the control group, the mixed effects of hADS plus PBM were significantly more persuasive and meaningfully increased the HSL. The results of this study are in line with the results of Wang et al,[32] who evaluated the bony restoration impacts of PBM and hADS regimes throughout bone healing by means of a rat skull bony deficiency simulation. The hADSs were cultivated on the scaffold and transplanted into the CSFDs. They found that the regimes of both PBM and hADSs treatments displayed enhanced skull bony deficiency repair in comparison with the control one. Furthermore, the hADS + PBM treatment displayed meaningfully augmented bone volume in comparison with the ADS and PBM alone treatments. Although Wang et al performed their study on rats, they did not conduct mechanical examinations.

The current study found that treatment with hADS alone did not increase the HSL at the catabolic phase compared to the control rats. These observations are in contrast with other reports. Li et al[33] and Yao et al[34] assessed the impacts of resident endothelial progenitor cells (EPCs) treatment on the microstructure and mechanical features of segmented bone deficiency in a murine model. The remedy group was injected with EPCs planted on a Gelfoam scaffold at the location of the bone deficiency, and control murine received Gelfoam and salt-water. Assessments were done with a CT scan and mechanical testing (torsional strength and stiffness) methods. Li et al recommend that native EPC therapy meaningfully improves bone deficiency repair in a segmented deficiency simulation in a murine femur. The differing results might be attributed to some differences in methodology between the two studies, such as bone defect model, stem cell type, scaffold type, and biomechanical test.[33] Yao et al aimed to define what remedy with LLP2A-Alendronate (LLP2A-Ale) or a

mixture of LLP2A-Ale and MSCs could hasten fracture repair in a murine closed broken bone simulation. They found that the LLP2A-Ale remedy augmented exogenic MSC homing to the broken bone holes and improved the contribution of MSCs to repairing tissue formation. The ADS and LLP2A-Ale mixture remedy was greater on repairing tissue than MSC alone. The differing results might be attributed to some differences in methodology between the two studies, such as special animal model (Reporter mice), bone defect model (drop-weight blunt guillotine device), special treatment of the ADS, and biomechanical test (loaded to failure).[34]

Conclusion

Compression biomechanical examination on the catabolic phase of bone healing revealed that HSLs of CSFD in rats that received treatments of hDBMS plus hADS plus PBM were significantly higher than treatments with hADS and PBM alone and control groups. At the same time, pretreatment with hADS and PBM together in vitro resulted in remarkably augmented HSLs than other combinations of ADS + PBM.

The authors suggest that pretreatment of hADS with PBM be examined in translational and human studies to promote the healing of large bone defects. The fine points of the molecular and cellular functions that worry about the effects of PBM and hADS together on the healing of fractures in CSFD in rats could be clarified by additional studies. Hopefully, these findings will support the use of PBM plus ADS to help BTE procedures achieve their full therapeutic potential in big fractures in healthy injured people and patients who suffer from diabetes and osteoporosis.

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Authors' Contribution

MB researched the data and wrote the manuscript. AK, AM, AA, RG, MA, FZ, SF, and FR performed the experiments. ZM, RG and AA made some suggestions incorporated in article submission. SC scientifically edited the manuscript. AM performed the data analysis and interpretation.

Conflict of Interests

All authors declare that there is no conflict of interest.

Ethical Considerations

The IRB of Shahid Beheshti University of Medical Sciences (SBMU) approved the project (IR.SBMU.MSP.REC.1397.484).

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