Histological and Molecular Assessment of Bone Regenerative Potential of Human Dental Pulp Stem Cells' Secretome on Rabbits' Tibial Bone Defects

Original Article

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ABSTRACT

Introduction: Bone fractures are a global public health burden as there are more than 150 million bone fracture incidence per year. The therapeutic effect of mesenchymal stem cells (MSCs) is mediated by the secretion and release of trophic molecules, which are called 'secretome'. There are only a handful of studies that evaluated the effect of dental MSCs-derived secretome in bone healing and regeneration.

Aim of Study: This study was designed to investigate the regenerative potential of human dental pulp stem cells' (hDPSCs') secretome on rabbits' tibial bone defects at three- and six-weeks follow-ups.

Materials and Methods: 42 rabbits received 84 six-mm-diameter tibial bone defects; 21 for each outcome; 7 defects for each group per each time interval. Bone defects were either left to heal spontaneously (Group A) or received either collagen scaffold (Group B) or hDPSCs' secretome loaded on collagen scaffold (Group C). Animals were euthanized at three and six weeks. Histomorphometric evaluation of newly formed bone area percent and degree of bone maturation using Hematoxylin and Eosin (H&E) and Masson Trichrome (MT) stains respectively was constructed in addition to quantitative real time polymerase chain reaction (qRT-PCR) to evaluate osteocalcin levels.

Results: Histomorphometric analysis of H&E- & MT- stained sections showed significant increase in bone area percent as well as bone maturation in the group C in comparison to the other two groups ($P < 0.05$) at both time intervals. qRT-PCR results supported our abovementioned results as they showed that osteocalcin levels were higher in group C as compared to the other two groups at both time intervals ($P < 0.05$).

Conclusions: hDPSCs' secretome showed superior results and significant improvement of bone healing.

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Key Words: Bone regeneration, dental pulp stem cells, secretome, tibial bone defects.

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INTRODUCTION AND REVIEW

Bone defects usually arise from infections, trauma, congenital malformations, tumor resection and reconstructive surgery[1]. The prevalence of fractures was found to be 21% in a cross-sectional study done to investigate fracture risk among older persons living in geriatric facilities in Egypt[2]. Recurrent falls were the most common risk factor for fractures (49%) overall.

Delayed or non-union of bone defects may be affected by multiple risk factors including old age, smoking, genetic disorders, metabolic disorders and nutritional deficiencies. Non-unions are challenging to treat; therefore, all strategies aim to reduce healing time to improve the prognosis and allow patients to resume their normal life activities[3].

Regenerative medicine has been introduced to enhance bone defects' healing. Mesenchymal stem cells (MSCs) are the osteoblastic lineage cells' progenitors and are characterized by their great ability to regenerate multiple mesenchymal tissues[4,5].

Dental MSCs are regarded as one of the most promising safe non-invasive sources of human stem cells[6]. Still multiple reasons to be concerned as these cells are not yet fit for experimental purpose. As in *vitro* expansion culture conditions might affect their regenerative ability as oxygen, glucose concentration and pH levels[7,8]. Safe and effective engraftment of transplanted cells in the host is questionable, due to the immune response triggering leading to the rejection of transplanted cells or worsening of the disease state[9,10]. Furthermore, anticipated oncogenic

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potential of cultured stem cells due to in *vitro* genetic manipulation should be considered[11].

Thus, there is an increased interest in the MSCs' secretome (which includes exosomes, large extracellular vesicles and microvesicles) as an alternative treatment modality[12]. The secretome has less immunogenicity due to lower cell surface protein expression[13] and could be produced at higher rates[14].

Interestingly, human dental pulp stem cells' (hDPSCs') secretome has stronger neurotrophic, angiogenic, antiapoptotic, neurite outgrowth, migratory activity and immunomodulatory effects than the secretome of bone marrow mesenchymal stem cells[15,16].

hDPSCs' secretome promotes nerve regeneration, by increased colony formation and neurite extension, which leads to appropriate neuronal differentiation and maturation[16,17]. It enhances proliferation, migration and mineralization potential of hDPSCs in *vitro*, and stimulates anti-aging action[18]. Yet, to the best of our knowledge, there are no studies assessing the efficiency of hDPSCs' secretome in bone healing. Therefore, this study aimed to assess the bone regenerative potential of hDPSCs' secretome in rabbits' tibial bone defects.

MATERIALS AND METHODS

Experimental animals

Forty-two male New Zealand white rabbits that weigh around two and half to three and half kilograms were utilized in this study. The entire experimental procedures were carried out after ethical approval from the Institutional Animal Care and Use Committee (IACUC) – Cairo University. Approval number (CU/III/F/58/19)

Sample size

To evaluate the bone regenerative potential of hDPSCs' secretome in rabbits' tibial bone defects, area percentage of newly formed bone was measured. It was found that 21 bone defects for each duration (seven rabbits per each group) was an adequate sample size for histomorphometric analysis of area percentage of newly formed bone with total sample size 42 defects (three weeks and six weeks). For qRT-PCR analysis, the same number of rabbits was use, with total of 42 bone defects for each outcome. This sample size was sufficient to detect an effect size of 0.74 with an 80% power, and α error probability =0.05. G power software was used to determine the proper sample size.

The magnitude of the detected effect was calculated from the scientific literature using the mean and standard deviation (SD) of concerning variable[19].

Materials

Scaffold

Collagen scaffolds (collacone, Bottiss dental, Germany) were purchased and cut during the surgical procedure into equal pieces about 5-6 mm using sterile scissors to fit in the surgically-induced defects.

Preparation of hDPSCs' secretome

hDPSCs' secretome was prepared at the Stem Cell Lab, National Research Centre. hDPSCs at the fifth passages were grown till reaching confluency of 60%; then the culture medium was shifted to serum deprived DMEM resulting in starvation of cells were for a whole day. Amicon Ultra-15 centrifugal filter unit with an ultracel-3 membrane (Millipore, Billerica, MA) was used to collect and concentrate the medium about 40-fold. Then, 10 µL/mL halt protease inhibitor cocktail (Thermo Scientific, USA) was applied to the secretome that has been collected[20].

Coomassie (Bradford) protein assay kit (Thermo Scientific, USA) was used to determine the concentration of protein. The final protein concentration used was 3 µg/ml[21].

Tibial bone defects induction and animal grouping

All the surgical procedures were carried out while rabbits being under general anaesthesia using ketamine chlorhydrate (0.08 mL/100 gm body weight) combined with xylazine 2% (0.04 mL/100 gm body weight)[22].

Each rabbit received two tibial bone defects. Iodate alcohol was used to disinfect the tibiae before being shaved under sterile conditions. In the medial direction, a two-three cm cutaneous incision was created until the proximal ends of the tibiae were exposed, then the periosteum was raised. A bone defect with a diameter of six mm was created using a round surgical carbide bur (Meisinger, Germany) attached to a low-speed hand piece connected to a 2000-rpm micro-motor while being irrigated constantly using a solution of saline to avoid excessive heating till reaching the medullary canal[23].

The defects were classified into three groups. For group A, the bone defects were left for spontaneous healing (negative control group). The collagen scaffold was either immediately placed in the bone defects (group B; collagen scaffold group) or immersed in 500 µl of previously prepared hDPSCs' secretome for at least five minutes before being applied into bone defects (group C; (hDPSCs' secretome group) (Table 1). The collagen scaffold was adapted to the defect using a sterile tweezer and then covered with the periosteum and muscles. Then resorbable #4.0 catgut was used for suturing. Finally, to avoid leakage of materials, interrupted #4.0 silk sutures were used to suture the skin[24,25].

Group Number of defects Intervention Euthanization date

Group A (negative control group) 28 (14 for each outcome) Defects were left to heal spontaneously 14 defects were dissected at three weeks and the other 14 at six weeks

Group B (collagen scaffold group) 28 (14 for each outcome) Defects received collagen scaffold only 14 defects were dissected at three weeks and the other 14 at six weeks

Group C (hDPSCs' secretome group) 28 (14 for each outcome) Defects received hDPSCs' secretome loaded on collagen scaffold 14 defects were dissected at three weeks and the other 14 at six weeks

Table 1: Showing animal grouping

Group A (negative control group)	28 (14 for each outcome)	Defects were left to heal spontaneously	14 defects were dissected at three weeks and the other 14 at six weeks
Group B (collagen scaffold group)	28 (14 for each outcome)	Defects received collagen scaffold only	14 defects were dissected at three weeks and the other 14 at six weeks
Group C (hDPSCs' secretome group)	28 (14 for each outcome)	Defects received hDPSCs' secretome loaded on collagen scaffold	14 defects were dissected at three weeks and the other 14 at six weeks

Postoperative care

Immediately after the surgical procedures, the rabbits received systemic antibiotic Amikacin at a dose of 15 mg/kg every 12 hours intramuscularly for one week[26]. Additionally, the rabbits received Ketofan® (1 mg/kg) per day as an analgesic[27]. The animals were free to move in their cages with no external support during the postoperative stage. Throughout the study, the animals were checked for limb fractures, illness, and any adverse effects. If any were detected, the animals were discarded.

Half of the rabbits was euthanized after three weeks and the other half was euthanized after six weeks from the start of the experiment[28]. Euthanization was carried out by an overdose of Ketamine/Xylazine mixture injected intraperitoneally[29]. The tibiae were dissected and soft tissues were removed. Under continuous irrigation, bone specimens from each group, including the defect, were sliced using a disc with caution to include the whole defect site.

Histological and Histomorphometric assessment

For 48 hours, bone specimens of the primary assigned to obtain the area percentage of newly formed bone were fixed in 10% calcium formol solution followed by immersion in a solution of 10% EDTA for around four-five weeks to achieve proper demineralization. Then the samples were embedded into paraffin blocks after being dehydrated in increasing concentrations of alcohol and cleaned by xylol. Hematoxylin and eosin (H&E) and Masson's trichrome (MT) staining were employed to stain successive 5-6 μm paraffin cross sections.

The area percentage of the bone trabeculae that were newly formed was measured in the H&E stained sections, while MT stained sections were used to measure the mature bone area percentage. The histomorphometric data were obtained using image analysis software Image J using an objective lens of magnification x20 non-overlapping fields from H&E-stained and MT sections respectively from each specimen.

qRT-PCR analysis for osteocalcin

The mRNA levels of osteocalcin were analyzed as follows; qRT-PCR was used to determine total RNA containing genes. RNAs were isolated by Qiagen tissue extraction kit (Qiagen, USA). High capacity cDNA reverse transcription kit from Fermentas, USA) was used to generate cDNA, and qRT-PCR was performed utilising an ABI 7600 Fast Thermocycler with SYBR Green (BioWhittaker Molecular Application, Rockland, ME, USA). The expression of the osteocalcin gene was normalized in relation to the mean critical threshold (CT) values of the mRNA for β -actin as an internal control by the ΔΔCt technique. Primers for osteocalcin and β-actin are listed in Table (2).

Table 2: Primers' sequence specific for each gene

	Primer sequence
β-actin	Forward primer :5'- AGA CCT GTA CGC CAA CAC AG -3'
	Reverse primer: 5'- CGA TCC ACA CGG AGT ACT TG -3'
Osteocalcin	Forward primer: 5'- GGGCAATAAGGTAGTGAACAG -3'
	Reverse primer: 5'- GCAGCACAGGTCCTAAATAGT -3'

Statistical analysis

Statistics was used to statistically express the results of the histomorphometric investigation as mean and SD values. To evaluate different follow up times within the same group, a two-way ANOVA test was employed. Tukey's post hoc test was then performed to assess several comparisons between each two groups. The level of significance was set at $p < 0.05$. The previous statistical tests were conducted using IBM SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA).

RESULTS

There weren't any discarded animals till the end of the study as there wasn't any limb fractures, illness or other adverse effects. So, the whole sample size was used to obtain the results.

Histological (H & E and MT stains) results

H & E stain

Three weeks following the surgery, the spontaneously healed bone defects and the defects that received collagen scaffold only were almost filled with granulation tissue with the parent bone edges hanging on both sides (Figure 1A,B).

New bone formation (woven bone) was evident only at the bottom and the sides of the defects as dispersed bony spicules in groups A and B. However, the woven bone was more organized in group B. Chronic inflammatory cell infiltration and blood vessels were observed within the granulation tissue in addition to many adipocytes in the

medullary cavity of groups A and B. Moreover, collagen scaffold remnants were observed in group B. On the flip side, the defects in group C were almost filled with interconnected network of thin newly formed woven bone trabeculae. Partial regeneration of the periosteum was noticed in this group as well (Figure 1C).

Six weeks postoperatively, defects in group A were still entirely filled with granulation tissue with the parent bone edges extending from both sides, nevertheless, there was a minimal increase in the quantity of the newly formed bone. Multiple reversal lines as well as resting lines were observed. Group B revealed considerable amounts of thickened newly formed bone trabeculae that grew on the defect margins and center trying to bridge the surgical defect forming a trabecular network with interconnected marrow cavities lined by osteoblasts in many areas. Periosteum was reformed and connected to the margins of the parent bone. Group C showed more organized thick trabecular bone with osteoblast lining the marrow cavities filling the entire defect. Haversian system (osteonal pattern) and lamellar bone were seen denoting advancement in the healing process. Limited areas of woven bone were noticed. Reforming periosteum was noticed connected to that covering the old bone (Figure 1 D,E,F).

MT stain

MT-stained bone specimens were analyzed to assess bone maturation, MT stains collagen fibers and newly formed osteoid tissue in blue; however, the mature well-mineralized bone was stained red (Figure 2).

Three weeks postoperatively, group C showed adequate stain reaction versus the other two groups. When it comes to the specimens at six weeks postoperatively, group C showed bone tissue with marked elevated maturation expressed in the red color and superior bone trabeculae arrangement while group A showed intense stain reaction with increased expression of the blue stain and group B revealed a moderate stain reaction. Overall, these observations were consistent with those from H&E-stained sections (Figure 2).

Histomorphometry analysis of H&E and MT stained sections

Histomorphometry analysis of H&E stained sections

Regarding bone area percent, two-way ANOVA revealed a statistically significantly greater mean value in group C in relation to groups A and B within both timelines (three and six weeks postoperatively) (P<0.05). Within the three weeks' time, pair wise comparison demonstrated significantly higher mean area percent in group C in relation to both groups B and A, additionally higher mean area percent was detected in group B as compared to group A. Regarding the six weeks' time, a significantly higher mean area percent was recorded in group C in relation to both groups B and A, while the difference between groups B and A was insignificant (Figure 3). Within each group, a statistically significant improvement in the area percent of bone was observed in groups A and C within the six weeks' time interval as compared to the three weeks' time interval, while the difference between three weeks and six weeks for group B was insignificant (Figure 3).

Histomorphometry analysis of MT stained sections

According to two-way ANOVA test, group C presented statistically significantly higher mean area percent of mature bone in relation to group A and group B within both timelines (three and six weeks) (P<0.05). Within the three weeks' time, pair wise comparison demonstrated a significantly higher mean area percent of mature bone in group C in relation to both group B and group A, also higher mean area percent of mature bone was detected in group B in comparison to group A. Regarding the six weeks' time, the mature bone area percent was significantly higher in group C in relation to both group B and group A, as well as in group B in relation to group A (Figure 4).

A statistically significant improvement in mature bone area percent was detected in the three groups within the six weeks' time in comparison to the three weeks' time (Figure 4).

Evaluation of genetic expression of osteocalcin using qRT-PCR

qRT-PCR analysis showed that there was an increase in osteocalcin levels from three to six weeks in all groups. It was evident that group C had the highest osteocalcin levels followed by groups B and A, either at three weeks or six weeks.

Two-way ANOVA testing revealed that the gene expression was significantly greater in group C as compared to groups B and A within both time periods (three and six weeks) (P<0.05). Within both time intervals, gene expression was significantly higher in group C as compared to both groups B and A as well as in group B as compared to group A.

A statistically significant increase in osteocalcin gene expression was detected within six weeks' as compared to three weeks' in groups B and C, while the difference between the three weeks and six weeks for group A was insignificant (Figure 5).

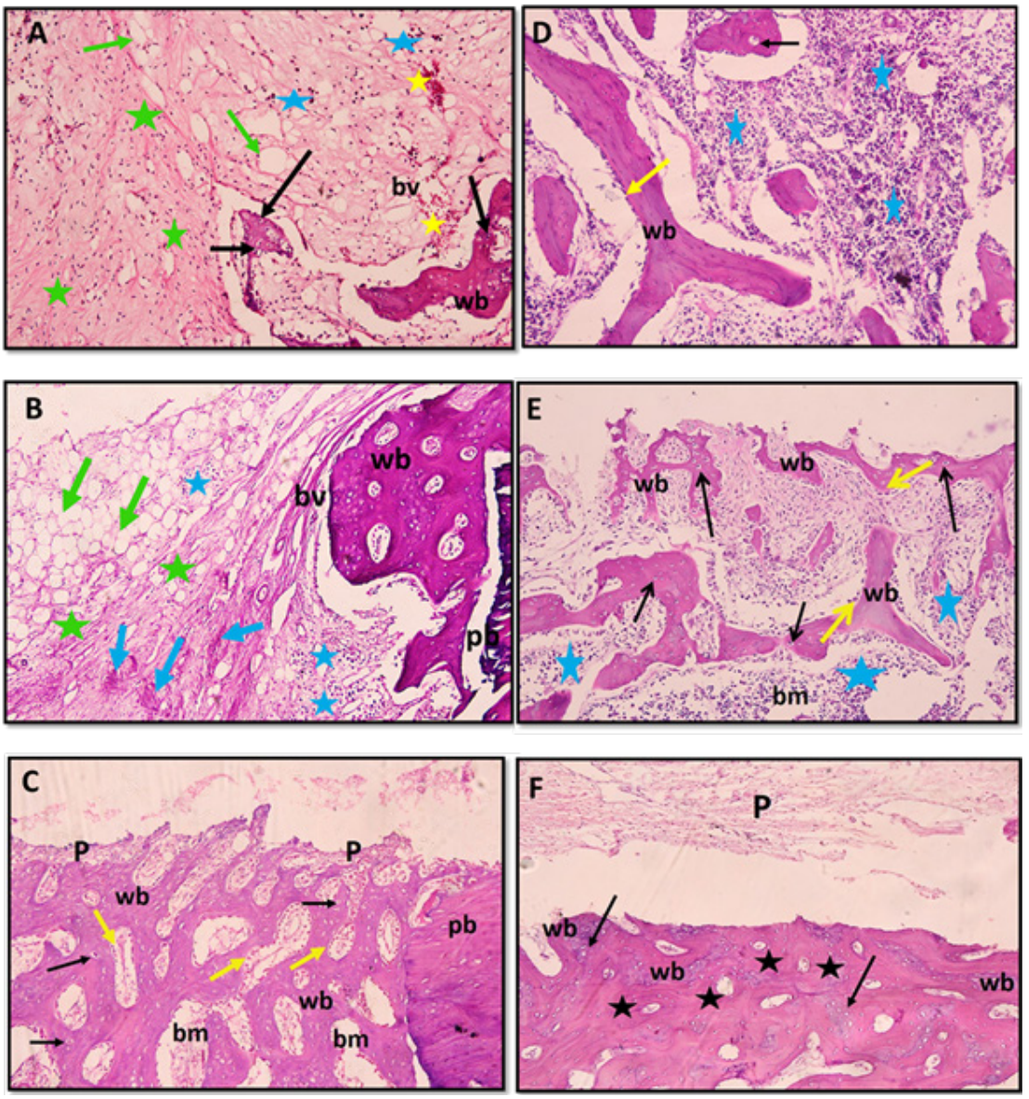

Fig. 1: photomicrographs showing sections of all groups at both time intervals;

A (group A at 3 weeks): defects filled with granulation tissue with few areas of woven bone, B (group B at 3 weeks): granulation tissue filling the defects and some woven bone at the defects' edges, C (group C at 3 weeks): woven bone trabeculae filling the defect entirely, D (group A at 6 weeks): thin woven bone trabeculae lined by osteoblasts, E (group B at 6 weeks): thin woven bone trabeculae outlining indefinite bone marrow cavities lined by osteoblasts, and F (group C at 6 weeks): well organized newly formed bone filled the defect with islands of woven bone and some areas of osteon formation. wb: woven bone, pb: parent bone, black arrow: osteocytes, yellow arrow: osteoblasts, blue star: inflammatory cells, green arrow: adipocytes, black star: osteon, P: periosteum, yellow star: extravasated RBCs, green star: collagen fiber bundles, bm: bone marrow cavities, blue arrow: collagen scaffold remnants, bv: blood vessel. (H&E, orig. Mag.X20)

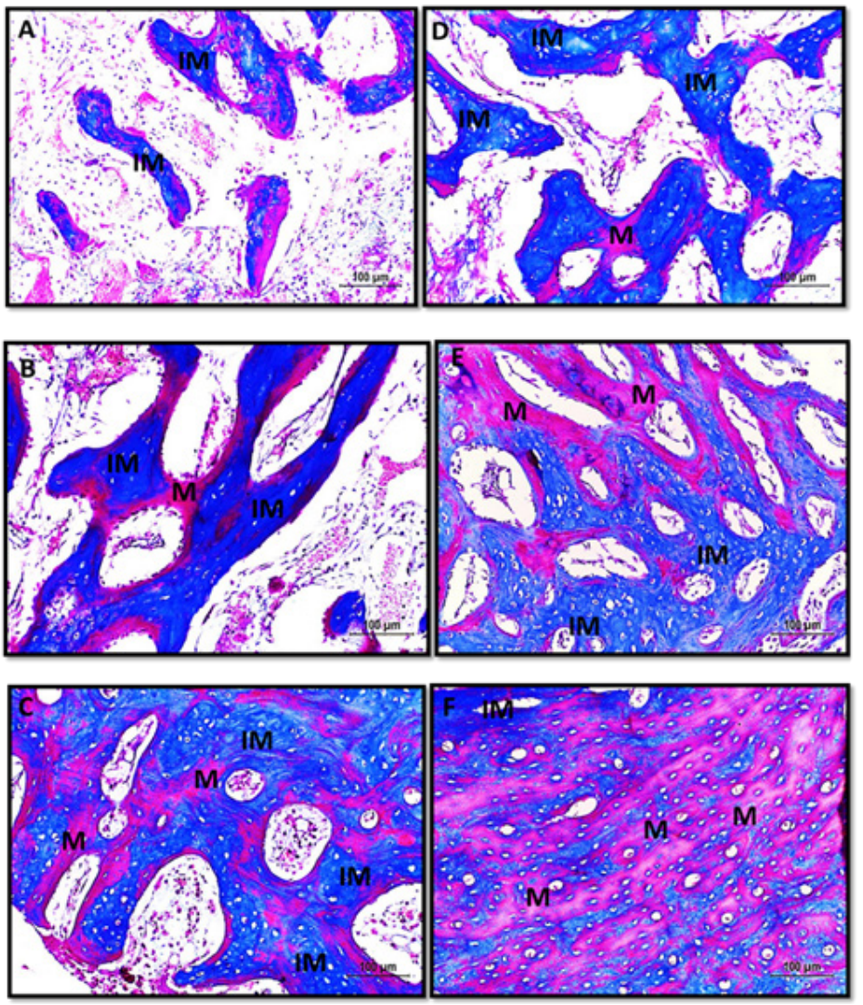

Fig. 2: photomicrographs showing sections of all groups at both time intervals;

Immature newly formed bone was stained in blue (IM) while mature newly formed bone was stained in red (M). A & D: group A at 3&6 weeks respectively, B & E: group B at 3&6 weeks respectively, C& F: group C at 3&6 weeks respectively. (M): mature newly formed bone; (IM): immature newly formed bone. (MT, orig. Mag.X20)

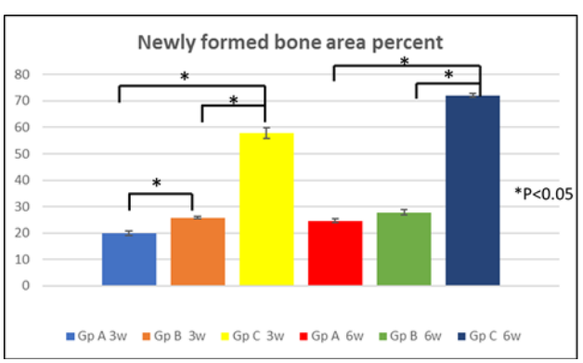

Fig. 3: Bar chart showing mean value of newly formed bone area percent with SD error bars. Significance level *P*<0.05, *significant.

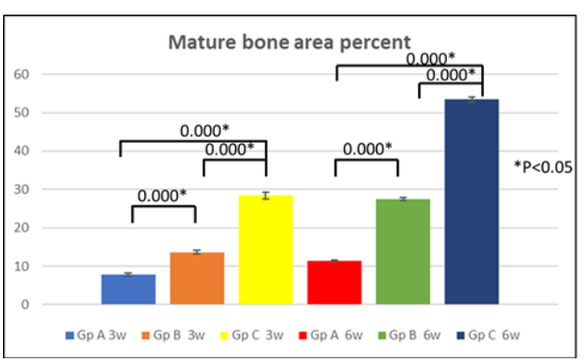

Fig. 4: Bar chart showing mean value of mature bone area percentage with SD error bars. Significance level *P*<0.05, *significant.

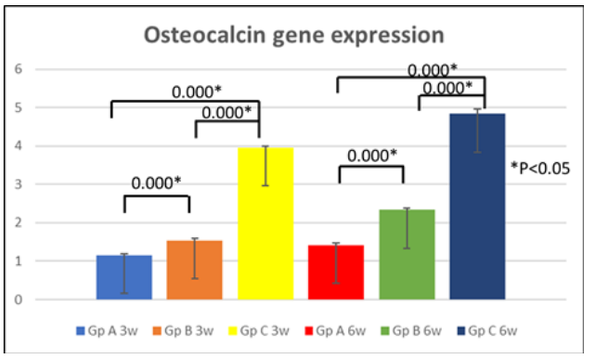

Fig. 5: Bar chart showing mean value of qRT-PCR analysis of osteocalcin with SD error bars. Significance level $P<0.05$, *significant.

DISCUSSION

Natural body processes usually fail to heal severe bone injury which dictates clinical intervention. Every year, four million people throughout the world require bone transplantation or bone replacement surgery[30]. Several strategies have been used to restore bone defects they are deemed a major problem affecting the patient's life quality[31,32].

In the present study, collagen sponge was the scaffold of choice for the hDPSCs' secretome owing to its biocompatibility, safety, low immunogenicity, hemostatic effect as well as promoting bone regeneration by preserving space for bone growth and acting as a delivery vehicle for the secretome[33,34]. Collagen sponge was also chosen as it is one of the natural main components of bone[35]. Previously, collagen has been used successfully as a scaffold for bone morphogenic protein-2 to achieve bone regeneration where the effect of collagen itself was obvious in terms of better bone response when compared to spontaneously healed defects. However, its osteogenic ability was poor when used on its own compared to being combined with bone morphogenic protein -2[36,37].

Thus, the present work attempted to investigate the bone regenerative potential of hDPSCs' secretome in rabbits' tibial bone defects by histological examination in addition to histomorphometric analysis to the area percentage of newly formed bone and bone maturation using H&E and MT stained sections respectively.

At both intervals, hDPSCs' secretome on collagen scaffolds loaded bone defects (group C) showed better bone regenerative capacity than the scaffold alone (group B) or the spontaneously healing (group A) defects. Histologically, groups C presented closure of the defect with densely packed bone tissue having osteonal and non-osteonal pattern of bone lamellae six weeks postoperatively. Our results came in line with Buss *et al*, 2023[38] who reported that lyophilized secretome from hDPSCs resulted in better bone regeneration in clavarial bone defects as compared to xenografts 14 and 42 days after the surgery. It was reported that bone remodeling starts once newly formed bone tissue completely bridges the bone defect area, which was noticed in group C in this study[39].

Interestingly, histomorphometric results revealed significantly higher bone area percentage as well as a higher degree of bone maturation in H&E and MT-stained sections of group C when compared to the other two groups. Those superior results of hDPSCs' secretome were in accordance with a study that showed that mandibular cartilage lesions of mice were improved by using intravenous injection of hDPSCs' secretome. They reported a decrease in interleukin 1-beta (IL-1β) and matrix metalloproteinase-13 levels in the secretome-treated group when in relation to the control group. Moreover, a decline in inflammatory cytokines and matrix-degrading factors levels was noticed which indicated inhibition of degradation of cartilage matrix. Moreover, there was an increase in the levels of proliferating cell nuclear antigen-positive cells in the group treated by secretome compared to the control group, which demonstrates signaling and cell division stimulation[40].

Periosteum was reformed partially in group C at three weeks and almost completely at six week postoperatively. This finding might explain the superior results in this group as it was proved that within two weeks of treatment with periosteum substitute through delivering periosteal factors, the periosteum pluripotent cells, which have excellent regenerative abilities, can fill in bone defects in the bovine femur[41]. While no signs of periosteum regeneration at three weeks in groups A and B. At six weeks postoperatively, partially reformed periosteum in group B only was observed. Furthermore, in accordance with our results that showed increase in the degree of bone maturation in MT stained sections of group C, a study reported that secretome derived from human exfoliated deciduous teeth increase the amount of newly formed bone as well as the degree of bone maturation as revealed by H&E and MT stain[42].

Regarding qRT-PCR of this study, our findings were consistent with those of an earlier study that evaluated osteocalcin levels after guided tissue regeneration in tibia of rats at six, eight and ten days. They demonstrated that while osteocalcin mRNA levels in the control group steadily increased over time, they dramatically increased in the experimental group. They suggested that these results could be due to accelerated osteogenic cell differentiation in the experimental group. Elevated levels of osteocalcin mRNA could be explained by higher number of cells differentiating from pre-osteoblasts to osteoblasts[43].

The therapeutic effect of hDPSCs' secretome could be achieved through the elimination of inflammation and matrix-degradation factors thus preventing the pro-inflammatory phase from proceeding to the inflammatory phase. hDPSCs' secretome promotes the expression of the macrophage M2 gene which is characterized by its paracrine activity in promoting cell survival, enhancing repair of axons, producing angiogenesis factors and preventing fibrosis[44].

The regenerative effect of hDPSCs' secretome is suggested to be attained through regulating several processes

as neuroprotective, anti-inflammatory, antiapoptotic and angiogenic processes which make them good candidates for bone repair[45,46]. It inhibits the expression of pro-inflammatory cytokines which include tumour necrosis factor alpha, IL-1β, IL-4, IL-6, IL-13, IL-17, IL-18 and interferon gamma as reviewed in[15,17]. In addition to increasing expression of anti-apoptotic B cell lymphoma-2 in parenchymal cells, the secretome of hDPSCs inhibits the production of pro-apoptotic proteins such as p53 and B cell lymphoma-2 Associated X-protein[20,47,48].

Indeed, hDPSCs' secretome displays a high expression of proangiogenic factors such as fibroblast growth factor, granulocyte macrophage colony stimulating factor, insulin derived growth factor-1, vascular endothelial growth factor, platelet derived growth factor, transforming growth factor β1 and IL-8 that bind to their corresponding receptors on endothelial cells, leading to their proliferation which improve angiogenesis during process of tissue repair[15,17,20,49]. Particularly, the high expression of vascular endothelial growth factor shows a direct impact of hDPSCs' secretome on angiogenesis[49,50].

Furthermore, hDPSCs' secretome contains bone morphogenic protein-7 and dentin sialophosphoprotein that play a crucial role in new bone formation and mineralization[51]. It has been suggested that hDPSCs' secretome enhances osteogenesis by stimulating the differentiation and proliferation of MSCs in addition to their migration and mineralization potential through transforming growth factor-β1 as well as upregulation of osteogenic genes expression such as osterix, osteopontin and osteocalcin[52,53].

CONCLUSION

hDPSCs' secretome loaded on collagen scaffold showed a superior pattern of bone regeneration, in comparison to the spontaneously healed (negative control) defects and defects that received collagen scaffold only, confirming their regenerative osteogenic potential. Therefore, hDPSCS' secretome is able to repair tibial bone defects, making it a promising regenerative tool in clinical applications.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تجدد العظام و تمعدنها باستخدام سيكريتوم الخلايا الجذعيه من لب السنه في العيوب العظميه في قصبة عظم الساق

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المقدمة: تشكل كسور العظام عبئًا عالميًا على الصحة العامة حيث يوجد أكثر من ١٥٠ مليون إصابة بكسور العظام سنويًا. التأثير العلاجي للخلايا الجذعية الوسيطة يتم عن طريق إفراز وإطلاق جزيئات تسمى "الإفراز". لا يوجد سوى عدد قليل من الدراسات التي قيمت تأثير الإفراز المشتق من الخلايا الجذعية الوسيطة في شفاء العظام وتجديدها.

الهدف من العمل: تم تصميم هذه الدراسة للتحقيق في الإمكانات التجديدية للافراز المشتق من الخلايا الجذعية للب الأسنان البشرية على عيوب عظام الظنبوب للأرانب عند المتابعة لمدة ثلاثة وستة أسابيع.

المواد و طرق البحث: تلقي ٤٢ من الأرانب ٨٤ عيوبا في عظام الظنبوب يبلغ قطرها ستة ملم ؛ ٢١ لكل نتيجة ؛ ٧ عيوب لكل مجموعة في كل فترة زمنية. تُركت عيوب العظام إما للشفاء تلقائيًا (المجموعة أ) أو تلقت إما سقالة كولاجين فقط (المجموعة ب) أو سقالة الكولاجين محمله بالافراز المشتق من الخلايا الجذعية للب الأسنان البشرية (المجموعة ج). تم قتل الحيوانات في ثلاثة وستة أسابيع. تم إنشاء التقييم النسيجي لقياس مساحة العظام المشكلة حديثًا بنسبة ودرجة نضج العظام باستخدام صبغات Hematoxylin and Eosin و Masson Trichrome على التوالي (النتيجة الأولية) بالإضافة إلى تفاعل البوليميراز الكمي في الوقت الفعلي (qRT-PCR) لتقييم مستويات النتيجة الثانوية.

النتائج: أظهر التحليل النسيجي للأقسام المصبوغه ب Hematoxylin and Eosin و Masson Trichrome زيادة كبيرة في مساحة العظام بنسبة مئوية بالإضافة إلى نضج العظام في المجموعة ج مقارنة بالمجموعتين الأخريين ($P < 0.05$) في كلا الفترتين الزمنيتين. دعمت نتائج qRT-PCR نتائجنا المذكورة أعلاه حيث أظهرت أن مستويات osteocalcin كانت أعلى في المجموعة ج مقارنة بالمجموعتين الأخريين في كلتا الفترتين الزمنيتين ($P < 0.05$).

الخلاصه: أظهر الافراز المشتق من الخلايا الجذعية للب الأسنان البشرية نتائج متفوقة وتحسنًا كبيرًا في التئام العظام.