Am J Stem Cells 2024;13(2):75-86
www.AJSC.us /ISSN:2160-4150/AJSC0153360

Original Article

The impact of acemannan, an extracted product from *Aloe vera*, on proliferation of dental pulp stem cells and healing of mandibular defects in rabbits

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Received September 10, 2023; Accepted March 25, 2024; Epub April 25, 2024; Published April 30, 2024

Abstract: Objectives: Dental pulp stem cells (DPSCs) were shown to play an important role in regenerative medicine including reconstruction of various bone lesions. This study determined the impact of acemannan, an extracted product from *Aloe vera*, on *in vitro* proliferation of DPSCs and *in vivo* healing of mandibular defects in rabbits. Methods: DPSCs were isolated and characterized. The growth kinetics of cells exposed to acemannan (8 mg/mL) and Hank's balanced salt solution (HBSS) were compared *in vitro*. Fifteen male rabbits were divided into 3 groups. Five animals were left as control group without any therapeutic intervention. Five rabbits were considered as experimental group 1 and received 20 µL of a cell suspension containing 10^6 DPSCs in the bone defect. Another 5 rabbits were regarded as experimental group 2 and were injected in the bone defect with 20 µL of a cell suspension containing 10^6 DPSCs treated with acemannan for 24 h. After 60 days, the animals were assessed by radiography and histologically. Results: The mesenchymal properties of DPSCs were confirmed. Population doubling time (PDT) of DPSCs treated with acemannan (29.8 h) was significantly shorter than cells were just exposed to HBSS (45.9 h). DPSCs together with acemannan could significantly accelerate the healing process and osteogenesis in mandibular defects. Conclusions: As DPSCS showed an increased proliferation when treated with acemannan and accelerated the healing process in mandibular defects, these findings can open a new avenue in dentistry regenerative medicine when remedies of bone defects are targeted.

Keywords: Acemannan, dental pulp stem cells, mandibular defect, healing, rabbit

Introduction

It has always been a goal for oral, maxillofacial and orthopedic surgeons to reconstruct maxillofacial bone defects due to trauma, congenital malformations, cancer or infection [1, 2]. Various bone graft materials and also autologous bone grafts have been introduced for osteogenic, osteoinductive, and osteoconductive properties to facilitate the new bone formation [3], but restricted available amounts of bone grafts, donor site morbidity and deformities have increased the infection risk and expenses as drawbacks to use these methods [4].

In bone tissue engineering, natural polymers like fibrin, collagen, chitosan, and glycosaminoglycans have been employed with various outcomes [5]. As another alternative treatment in reconstruction of bone defects, mesenchymal stem cells (MSCs) alone or together with scaffolds have been successfully used [1-3], because these cells were shown to have multi-

https://doi.org/10.62347/UAFC3719

potential, antiinflammatory and immunomodulating properties [6]. MSCs have been isolated from various tissues such as bone marrow [7], adipose tissue [8], menstrual blood [9], endometrium [10], and dental pulp [6]. Dental pulp stem cells (DPSCs) were demonstrated to have mesenchymal characteristics, to be easily obtained from dental pulp and have high plasticity and multipotential properties making them a candidate in regenerative therapies [11].

Several solutions such as Hank's balanced salt solution (HBSS) and herbals like *Aloe vera* have been used as transport media for tooth storage and to support the viability of DPSCs [12] for cell transplantation purposes [13]. An immediate transfer is necessary for an avulsed tooth to promote the cell viability and if any extra-oral time is considered for transplantation of DPSCs, HBSS can be a storage medium of choice [14], as HBSS maintains the pH and osmotic balance and provides water and essential inorganic ions for the stem cells [15].

Among herbals as transport media for tooth storage and to support the viability of DPSCs, Acemannan β(1→4)-acetylated polymannose is a polysaccharide found in the inner leaf gel of the *Aloe vera* (*Aloe barbadensis* Miller) that can accelerate healing in oral wounds, promote dentin formation and stimulate proliferation and differentiation of gingival fibroblasts [16], dental pulp fibroblasts [17], cementoblasts [18], bone marrow stem cells [19], and periodontal ligament cells [20]. As acemannan has various polysaccharides, anti-inflammatory properties, can stimulate bone and periodontal ligament regeneration, accelerate proliferation and differentiation of osteoblasts, and adjust expressions of vascular endothelial growth factor, alkaline phosphatase, bone sialophosphoprotein, bone morphogenetic protein 2 and type I collagen; it can be a suitable transport media for tooth storage and to support the viability of DPSCs and a candidate to participate in bone regeneration especially in the oral and maxillofacial regions [19]. So the current study was undertaken to determine the impact of acemannan, an extracted product from *Aloe vera*, on proliferation of DPSCs and healing of mandibular defects in rabbits.

Materials and methods

Acemannan preparation

After washing the external surface of the leaves of *Aloe vera Linn* (*A. barbadensis* Miller), they were disinfected by 70% ethanol and under sterile conditions by applying a knife, the inner gel was separated from the outer leaf. The inner gel was later homogenized and diluted in DMEM (Dulbecco's Modified Eagle Medium, Sigma-Aldrich, St. Louis, MO, USA) culture medium. It was then filtered by a 0.45 μm filter mesh; while the pH and osmolality were balanced by a vapor pressure (Vapro 5520; Wescor, Inc., Logan, UT, USA) and an Orion pH meter (model 720 A; Orion Research, Inc., Boston, MA, USA), while calibrating the osmometer from 100 to 500 mosm kg^{-1} [12].

Isolation and culture of DPSCs

Pulp tissues were obtained from male New Zealand white rabbits (*Oryctologus cuniculus*, age =1 year, weight =2.5 kg) provided from the Animal Facility of Shiraz University of Medical Sciences, Shiraz, Iran. All animals were caged under 12 h light and 12 h dark cycle and at temperature of 20-22°C and had free access to food and water. The pulp tissues were removed from extracted incisor teeth (n=12) under aseptic conditions using a sterile blade and under a class I laminar flow hood. They were washed in sterile phosphate-buffered saline (PBS, Sigma-Aldrich, USA), and transferred to 15-mL falcon containing 5 mL of DMEM until further studies. Under a class I laminar flow hood, the pulp was mechanically cut into small pieces and was transferred into a 15-mL falcon containing 5 mL of DMEM. The chopped tissue was centrifuged at 200 g for 10 min, the supernatant was removed, and the precipitate was exposed to a solution containing 1.5 mg/mL of collagenase type I (Sigma-Aldrich, USA) for 30 minutes at 37°C.

The collagenase type I enzyme was further inactivated utilizing 5 mL of DMEM, and was later centrifuged at 200 g for 7 minutes. The supernatant was removed, and the cell pellet was re-suspended in 1 mL of DMEM and was transferred to a culture flask containing 4 mL of DMEM enriched with 10% fetal bovine serum (FBS, Sigma-Aldrich, USA) and 1% peni-

cillin/streptomycin (Sigma-Aldrich, USA). The culture flask was finally placed in 5% CO_2 incubator at 37°C and saturated humidity, while media was changed every three days until the cells were 80% confluent observed under an invert microscope. Cells at 80% confluence were subcultured until passage 3 by treating the cells with 0.25% (w/v) trypsin-EDTA (Gibco BRL, Life Technologies, USA). Images were provided from culture flask employing a digital camera (ELWD 0.3/OD75, Nikon, Tokyo, Japan).

Characterization of DPSCs

Characterization of DPSCs was carried out morphologically, by adipogenic and osteogenic differentiation properties and finally by use of reverse transcription-polymerase chain reaction (RT-PCR) technique for mesenchymal (CD73) and hematopoietic markers (CD34 and CD45). To study adipogenic differentiation of DPSCs, they were cultured in 6-well plates containing complete DMEM culture medium and 15% FBS and were later supplemented with adipogenic medium consisted of 100 nM dexamethasone (Sigma-Aldrich, USA), 100 µM ascorbic acid (Sigma-Aldrich, USA), and 200 µM of indomethacin (Sigma-Aldrich, USA) for 3 weeks; while the medium was replaced every 3 days. After 21 days, the cells were fixed in 10% formalin for 20 min, washed three times with deionized water and finally were treated with fresh 0.5% Oil Red-O (Sigma-Aldrich, USA) solved in 2-propanol solution (Merck, Germany) for 2 h. Differentiated cells must appear in red color as red oil droplets [14].

For osteogenic induction, DPSCs were cultured in 6-well plates containing complete DMEM culture medium and 15% FBS that were later supplemented with 50 µM ascorbic acid (Merck, Germany), 100 nM dexamethasone (Sigma-Aldrich, USA), and 10 mM glycerol 3-phosohate (Merck, Germany) for 3 weeks, while the medium was changed 2 times per week. Then, by addition of 10% formalin for 20 min, the cells were fixed, washed three times with deionized water, and finally stained with 1.4% Alizarin Red solution solved in deionized water at pH of 4.1 (Sigma-Aldrich, St. Louis, USA). Differentiated DPSCs appeared in red color based on presence of calcium deposits in the cells [14].

Reverse transcription-polymerase chain reaction (RT-PCR) was employed to examine the presence of mesenchymal stem cell marker of CD73 and hematopoietic stem cell markers of CD34 and CD45 (12). In brief, the 3rd passage of DPSCs was lysed to extract the total RNA applying the column RNA isolation kit-III according to the protocol (DENAzist column RNA isolation kit-III, Tehran, Iran). RNA was later quantified through addition of 2 µL of sample to 2 µL of DR4 buffer from the employed kit. The absorbance was further estimated applying a spectrophotometer (Thermo Scientific Nanodrop 1000 spectrophotometer; Cambridge Scientific, Watertown, MA, USA) at 260 nm. The primers used for the RT-PCR were CD34 and CD45 as hematopoietic markers, and CD73 mRNAs as mesenchymal marker utilizing the ALLELE-ID software version 7.0 (Premier Biosoft International, Palo Alto, CA, USA). Following RNA isolation, cDNA synthesis was done employing a cDNA synthesis kit (AccuPower CycleScript RT PreMix dN6; BIONEER, Seoul, South Korea). Finally, the RT reaction was carried out by addition of one microgram of total RNA and 20 µL of distilled water to AccuPower CycleScript RT PreMix microcentrifuge tube containing all components of thermostable CycleScript Reverse Transcriptase including dNTPs, reaction buffer, primer (random hexamer: dN6), and stabilizers. The mixture was heated for 30 s at 25°C (primer annealing), 4 min at 45°C (cDNA synthesis), and 30 s at 55°C (melting secondary structure and cDNA synthesis). The reaction was subsequently inactivated by heating the mixture for 5 min at 95°C and the obtained cDNA was used as a template in the PCR. The 20 µL prepared mixture of PCR consisted PCR buffer, 10 mM of each dNTP, 5.0 mM magnesium chloride, 1 unit of Taq DNA polymerase (CinnaGen, Tehran, Iran), 1 µM of the first-strand cDNA reaction, and 10 µM of each rabbit-specific primer sets including CD73, CD34 and CD45 (**Table 1**). DNA was amplified in a PCR thermal cycler (Veriti Thermal Cycler, USA) for 5 min at 95°C as one cycle and then for 30 s at 95°C, for 40 s at 64°C, and for 40 s at 72°C during 35 cycles, and finally for 10 min at 72°C. After amplification, 5 µL of each PCR product with 2 µL of loading buffer were assessed utilizing 1.5% agarose gel electro-

Table 1. The sequences of mesenchymal and hematopoietic markers

Gene	Primer sequence	Size (base pair)
CD73	Forward: 5'-TACACCGGCAATCCACCTTC-3'	212
	Reverse: 5'-CTTGGGTCTTCGGGAATGCT-3'	
CD34	Forward: 5'-ACCATCTCAGAGACTAGACTG-3'	512
	Reverse: 5'-GAAAGTTCTGTTCTGTTGGC-3'	
CD45	Forward: 5'-CAGTACTCTGCCTCCCGTTG-3'	269
	Reverse: 5'-TACTGCTGAGTGTCTGCGTG-3'	

phoresis. Visualization was undertaken by a DNA safe staining (CinnaGen, Tehran, Iran).

Growth kinetics

The growth kinetic of DPSCs was determined utilizing trypan blue exclusion method as previously explained [14]. The cell viability was investigated after culturing in 24-well plates (22,000 cells/well) for 6 days. Briefly, the 0.4% trypan blue solution (Sigma-Aldrich, USA) was transferred to the cell suspension and homogenized. The cells were examined and counted under a phase contrast microscope (Olympus, Tokyo, Japan) applying a Neubauer hemocytometer slide. The population doubling time (PDT) was estimated and the growth curve was displayed by use of a defined formula, where T is the incubation time in h, Xb is the cell number at the beginning of the incubation time, and Xe is the cell number at the end of the incubation time, Ln = $\log_e$ and e = the Euler's number. PDT = T × ln2/lnXe/lnXb; N = n1 + n2 + n3 + n4/4 × 2 × v × 100 [14]. PDT of DPSCs was compared in Hank's balanced salt solution (HBSS) and in acemannan (8 mg/mL).

Cell viability assay

DPSCs in the third passage were cultured in 24 well plates (30,000 cells/mL) and after 48 h, the culture media was replaced as follows with Hanks' Balanced Salt Solution (HBSS) as positive control, distilled water as negative control, and 2 and 8 mg/mL of acemannan as experimental groups 1 and 2, respectively. The storage time was considered 45 min, 90 min, 3 hours, 6 hours, 12 hours and 24 hours in incubator with 5% CO_2 at 37°C and saturated humidity and then the media was removed and replaced with 500 µg/mL of (dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole (MTT) (Sigma-Aldrich, USA) and again incubated at 37°C for 4 h. In order to dissolve the formazan crystals, a mixture of 0.01 M glycine and DMSO (Sigma-Aldrich, USA) was added. Finally, the cell viability was estimated by measuring the optical density at 540 nm on a microplate reader.

Animals and grouping

Fifteen healthy one-year-old male New Zealand white rabbits with average weight of 2.4±0.3 Kg were purchased from the *Animal Facility of Shiraz University of Medical Sciences, Shiraz,* Iran. Under deep anesthesia using 50 mg/kg of 10% ketamine and 8 mg/kg of 2% xylazine, both sides of each rabbit's maxilla were shaved, disinfected and finally, a 1 cm longitudinal incision was made to reach the bone. It was drilled to the marrow cavity to induce a 10 × 2 × 1 mm defective site. Then, all covering tissues were sutured. Five animals were left as control group without any therapeutic intervention. Five rabbits were considered as experimental group 1 and received 20 µL of a cell suspension containing 10^6 DPSCs in the bone defect. Another 5 rabbits were considered as experimental group 2 and were injected in the bone defect with 20 µL of a cell suspension containing 10^6 DPSCs treated with acemannan for 24 h. Oxytetracycline and piperacillin spray (22000 IU/kg) were administered on the bone defect area to prevent any probable infection. Flunixin (1.1 mg/kg) was injected to control any probable pain. After 60 days, radiography was undertaken for the defective area and also, the defective region was examined histologically.

The animals were sacrificed by inhalant overdose of isoflurane anesthetic for histological examination, while a high concentration of isoflurane could lead to loss of consciousness and later the respiratory and cardiac arrest. Animals were sacrificed based on instructions of Animal Care Committee of Islamic Azad University, and upon regulations and laws of Iran Veterinary Organization for laboratory animals. This study was ethically approved and financially supported from a grant provided by National Institute for Medical Research Development of Ministry of Health, Treatment, and Medical Education to reach DPSCs (Grant No: NIMAD943645).

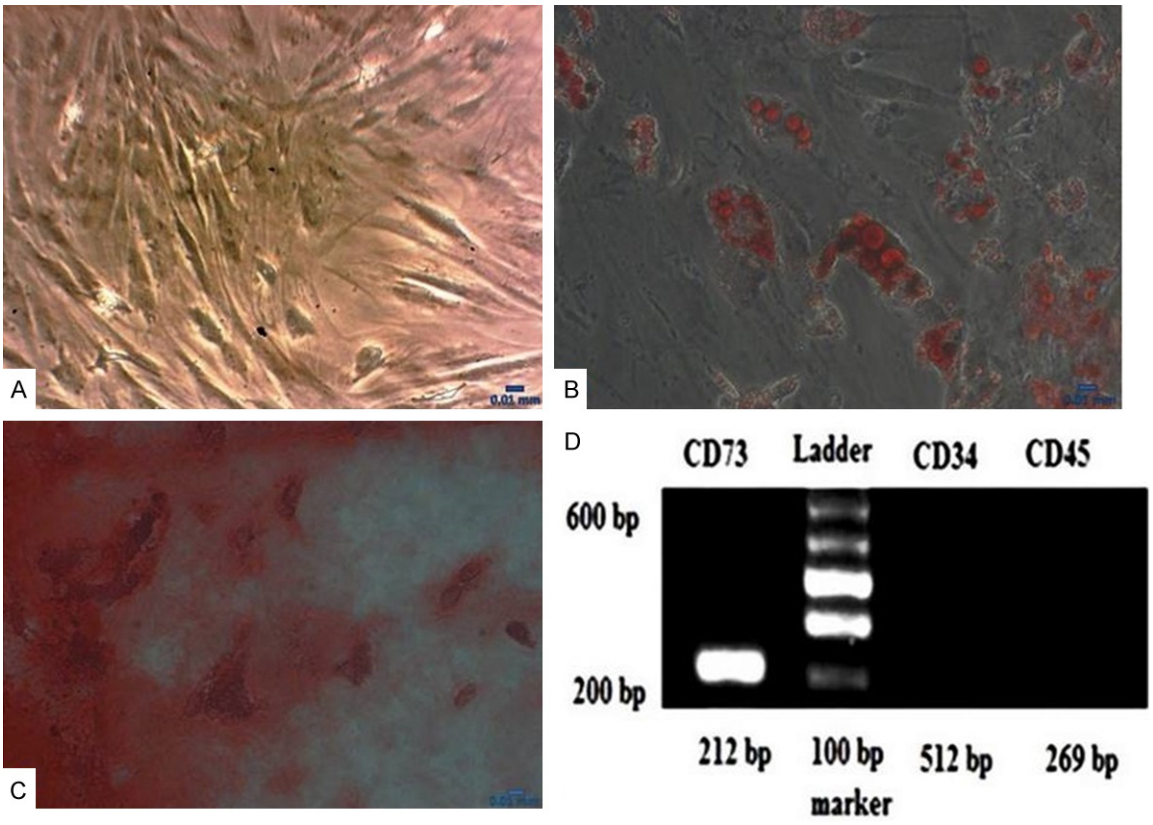

Figure 1. A. Rabbit dental pulp stem cells (DPSCs) with fibroblast-like morphology at 3rd passage (20 ×). B. Adipogenic differentiation of DPSCs revealing presence of intracellular lipid droplets in red color (20 ×). C. Osteogenic differentiation of DPSCs demonstrating intracellular calcium deposits in red color (40 ×). D. Reverse transcription-polymerase chain reaction (RT-PCR): Positive expression of CD73 as mesenchymal marker in absence of hematopoietic markers of CD34 and CD45.

Radiographic evaluation

A plain radiography (Kodak CR X-ray system) was carried out for the 3 groups after 60 days of interventions.

Histological assessment

The bones were fixed in 10% buffered formalin solution (Sigma-Aldrich, USA) for one week. The bones underwent a demineralization process using 12% nitric acid solution for 2 days. After demineralization, the bones were dehydrated and later soaked in paraffin. Blocks were decalcified for 72 hours in Shandon TBD-2 decalcifier, while cut into 5 µm sections, and stained with hematoxylin and eosin (H&E).

Statistical analysis

SPSS software (Version 20, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. To compare the groups, two-way analysis of variance (ANOVA) and Tukey post hoc test were employed. A P value ≤0.05 was considered statistically significant.

Results

Characterization of DPSCs

DPSCs were adherent to the culture plates and visible as fibroblast like spindle-shape morphology verifying their mesenchymal characteristics (**Figure 1A**). When DPSCs were subjected to adipogenic differentiation media, presence of intracellular lipid droplets in red color was demonstrated confirming their mesenchymal properties (**Figure 1B**). In osteogenic differentiation media, intracellular calcium deposits in red color appeared that confirmed the mesenchymal characteristics of DPSCs (**Figure 1C**). RT-PCR displayed positive expression of CD73 as mesenchymal marker, in absence of any

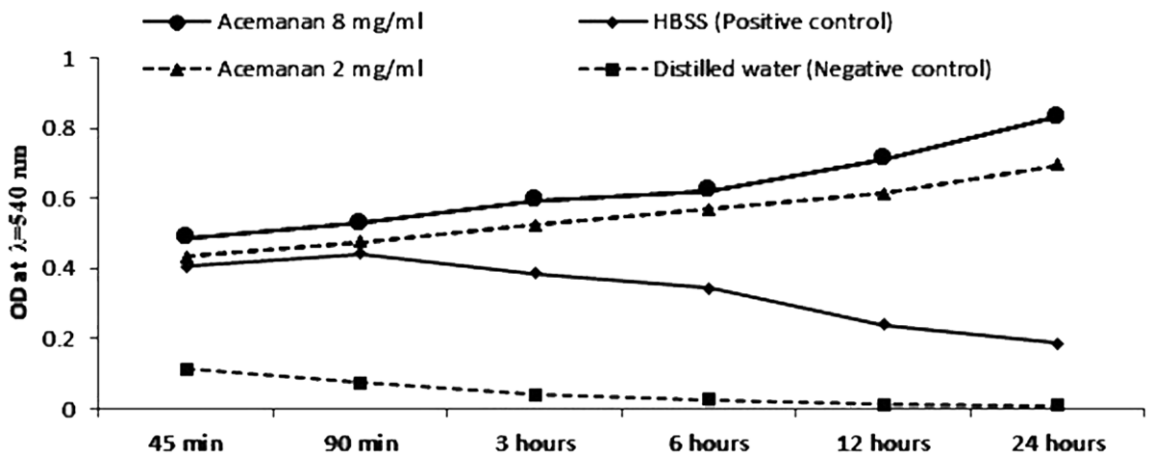

Figure 2. Optical density (cell viability) of rabbit-DPSCs after treatment with acemannan in comparison to the control groups in different time intervals. At λ=540 nm, when OD was 0.4, viability was 100%.

expression for CD34 and CD45 as hematopoietic markers (**Figure 1D**).

Growth kinetics

At logarithmic phase, PDT of DPSCs when subjected to HBSS was 45.9 hours, while after treatment of DPSCs with acemannan (8 mg/mL), it was 29.8 hours (**Figure 2**).

Viability of DPSCs

After 3 hours of incubation, the MTT assay demonstrated that acemannan at concentrations of 2 and 8 mg/mL could significantly increase the proliferation of DPSCs when compared to HBSS ($P<0.001$). After 12 hours, acemannan at concentration of 8 mg/mL was able to significantly increase proliferation of DPSCs ($P<0.01$, **Table 2**). The optical density (OD) value for each media and each time period, which represented the viability of DPSCs, was time-dependent (**Figure 3**). Distilled water as negative control had the lowest potential to maintain cell viability in all time periods ($P<0.001$). After 3 hours of incubation, 2 mg/mL of acemannan presented higher indices of absorbance when compared to the HBSS as positive control group ($P=0.001$, **Table 2**). At time periods of 12 and 24 hours, 8 mg/mL of acemannan was found to act with absorbance rates higher than the 2 mg/mL of acemannan ($P=0.018$ and $P=0.020$, respectively) and the control group ($P<0.001$) and was considered as the best storage solution. At all time periods, the cells treated with 2 and 8 mg/mL of acemannan illustrated a higher viability in comparison to the control group ($P<0.001$). After 45 min ($P=0.178$), 90 min ($P=0.158$), 3 h ($P=0.059$) and 6 h ($P=0.369$), the viability of DPSCs treated with 2 and 8 mg/mL of acemannan was not statistically different.

Radiography

Sixty days after surgery, radiography in control group disclosed a linear lucence in the medullary bone indicative of persistent bone defect with no obvious changes. In the second group receiving only DPSCs (20 µL of a cell suspension containing 10^6 cells), partial repair with areas of bone formation was visible in the same region. Bone defect treated with combination of DPSCs (20 µL of a cell suspension containing 10^6 cells) and acemannan (8 mg/mL) in the third group revealed a complete improvement with almost no appreciable bone defect on imaging assessment (**Figure 4**).

Histologic assessment

Histologic examination in control group showed a small area of fibroblastic proliferation with new bone formation (**Figure 5A**). In experimental group treated with just DPSCs, an area of fibroblastic proliferation with new bone formation in absence of complete osteogenesis were noticed (**Figure 5B**). I experimental group treated with DPSCs together with acemannan, a complete osteogenesis in absence of any defective symptoms were observed (**Figure 5C**).

Table 2. The mean absorbance, standard deviation (SD) and percentage of cell viability in various media at different time intervals

Groups	Mean	SD	Viability (%)	*P* value[1]	*P* value[2]	*P* value[3]	Time
HBSS	0.407	0.040	100.0				45 min
DW	0.113	0.015	27.9	<0.001			
Acemannan (2 mg/mL)	0.433	0.021	106.6	0.672	<0.001		
Acemannan (8 mg/mL)	0.487	0.031	119.7	0.036	<0.001	0.178	
HBSS	0.443	0.025	109.0				90 min
DW	0.073	0.012	18.0	<0.001			
Acemannan (2 mg/mL)	0.477	0.025	117.2	0.485	<0.001		
Acemannan (8 mg/mL)	0.530	0.040	130.3	0.020	<0.001	0.158	
HBSS	0.387	0.032	95.1				
DW	0.040	0.010	9.8	<0.001			3 h
Acemannan (2 mg/mL)	0.523	0.042	128.7	0.001	<0.001		
Acemannan (8 mg/mL)	0.593	0.015	145.9	<0.001	<0.001	0.059	
HBSS	0.343	0.049	84.4				
DW	0.027	0.015	6.6	<0.001			6 h
Acemannan (2 mg/mL)	0.570	0.036	140.2	<0.001	<0.001		
Acemannan (8 mg/mL)	0.623	0.042	153.3	<0.001	<0.001	0.369	
HBSS	0.240	0.030	59.0				
DW	0.013	0.012	3.3	<0.001			12 h
Acemannan (2 mg/mL)	0.613	0.049	150.8	<0.001	<0.001		
Acemannan (8 mg/mL)	0.713	0.021	175.4	<0.001	<0.001	0.018	
HBSS	0.187	0.045	45.9				
DW	0.010	0.000	2.5	0.005			24 h
Acemannan (2 mg/mL)	0.697	0.035	171.3	<0.001	<0.001		
Acemannan (8 mg/mL)	0.833	0.065	204.9	<0.001	<0.001	0.020	

P value[1]: Comparison between groups. *P* value[2]: Comparison between negative control group and acemannan groups. *P* value[3]: Comparison between two acemannan groups. DW: Distilled water, HBSS: Hank's balanced salt solution. At λ=540 nm, when OD was 0.4, viability was 100%.

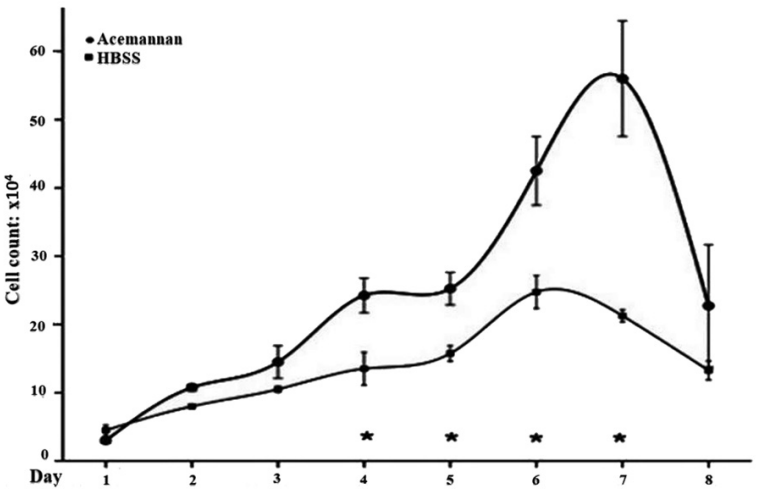

Figure 3. Comparison of the growth kinetic and population doubling time (PDT) of rabbit dental pulp stem cells (DPSCs) subjected to 8 mg/mL of acemannan or Hank's balanced salt solution (HBSS) (P=0.01).

Discussion

In the present study, DPSCs were spindle shape in morphology, were positive for adipogenic and osteogenic inductions and in RT-PCR were positive for CD73 as mesenchymal marker and negative in CD34 and CD45 hematopoietic markers confirming that isolated cells to have mesenchymal characteristics [6, 11, 12, 14]. Sholehvar *et al.* found that DPSCs can be easily obtained from dentistry clinics, preserved for a long period, have less ethical/legal issues to be provided, and are

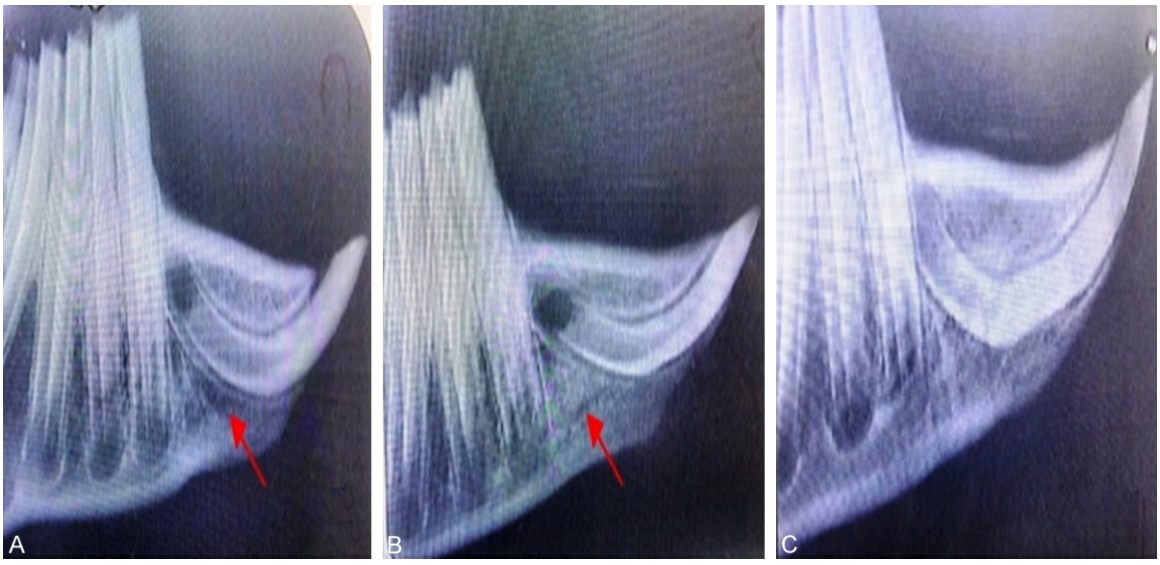

Figure 4. Plain X-ray of rabbit mandible undertaken 60 days after surgery. A. Linear lucence with almost no sign of osteoid formation and repair at the site of medullary defect. B. Partial healing with decreased size of bone defect on the second group treated with dental pulp stem cells (20 µL of a cell suspension containing 10^6 cells). C. Marked healing with almost no residue in bone defect on the group with combination therapy of stem cells (20 µL of a cell suspension containing 10^6 cells) and acemannan (8 mg/mL).

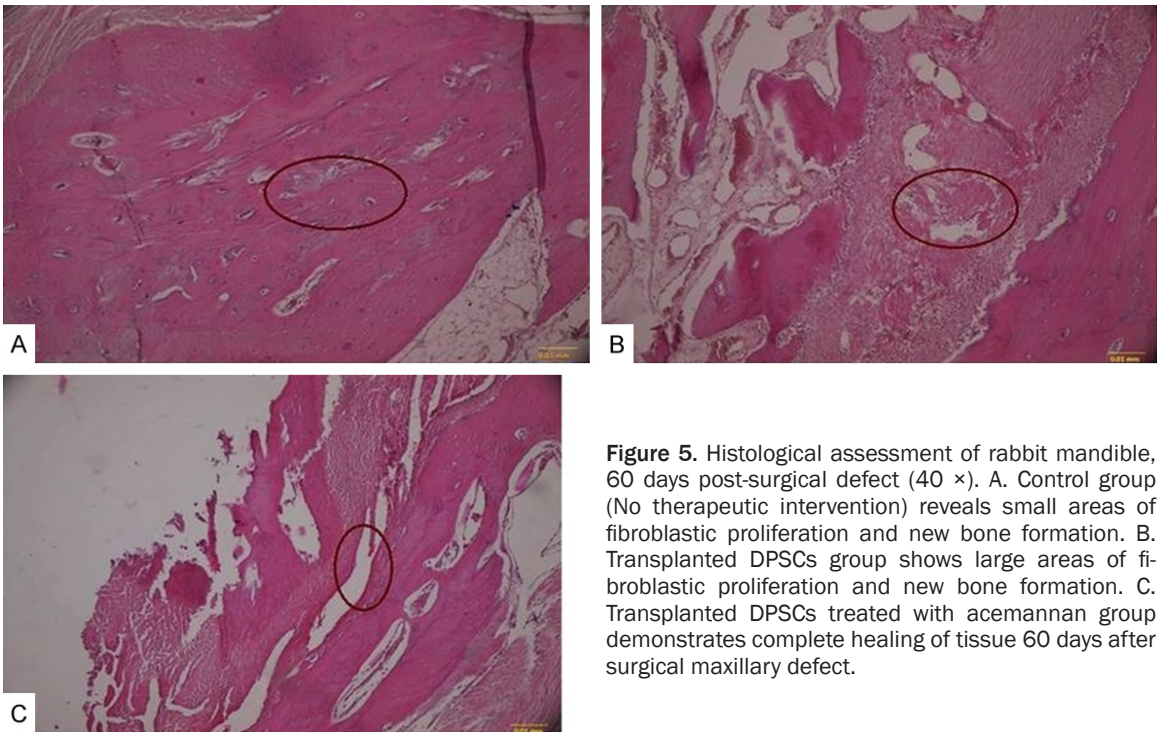

Figure 5. Histological assessment of rabbit mandible, 60 days post-surgical defect (40 ×). A. Control group (No therapeutic intervention) reveals small areas of fibroblastic proliferation and new bone formation. B. Transplanted DPSCs group shows large areas of fibroblastic proliferation and new bone formation. C. Transplanted DPSCs treated with acemannan group demonstrates complete healing of tissue 60 days after surgical maxillary defect.

multipotent [12]; confirming the important role of DPSCs in regenerative medicine [6, 11, 12, 14], especially in repair of bone defects [1-3, 21]. It is necessary to mention that the physiological osmolality and pH of used biomaterials can affect the viability of DPSCs, while the optimum condition to increase the cell viability was reported for osmolalities from 230 to 400 mosmol/kg and for the pH from 6.6 to 7.8 [22].

Sholehvar *et al.* showed that the PDT of DPSCs was 35.1 h when treated with 50% *Aloe vera*,

and 49.5 h after exposure to HBSS denoting to the increase in viability of DPSCs in presence of *Aloe vera* in comparison to HBSS [12]. We demonstrated a PDT of 45.9 hours for DPSCs subjected to HBSS, while after exposure to acemannan (8 mg/mL), it reached 29.8 hours revealing an up-regulated proliferation potential of DPSCs in shorter period of time when stem cells were treated with acemannan. Acemannan was demonstrated to significantly increase the proliferation of bone marrow stem cells (BMSCs), alkaline phosphatase (ALP) activity, mineralization, and expression of main factors for bone formation such as vascular endothelial growth factor (VEGF), and bone morphogenetic protein 2 (BMP-2) [23], while VEGF and BMP-2 have important roles in formation of new capillaries and osteoblast differentiation and regeneration of injured tissues [24].

Identically, Chantarawaratit *et al.* demonstrated that acemannan at concentrations of 0.5-8 mg/mL could significantly enhance the proliferation of periodontal ligament cells [25]. In our study, a high proliferation rate and viability were noticed when DPSCs were treated with acemannan at a concentration of 8 mg/mL. Similarly in another study, acemannan could significantly increase cellular proliferation, VEGF and BMP-2 expressions, and mineralization [26]. When studying the effect of acemannan on dentin formation, it was found that acemannan could significantly increase proliferation of DPSCs, BMP-2, ALP activity, dentin sialoprotein expression, and mineralization [27]. All these studies and our findings reveal when shorter time is targeted for cell proliferation and transplantation, treatment of stem cells with acemannan may be a desirable choice.

Our histological and radiographical assessments showed that DPSCs treated with acemannan could improve tissue regeneration, ossification and healing in mandibular bone defects. As bone reconstruction with a proper quality and quantity for dental implant replacement in the alveolar ridge has been a challenge in dentistry, DPSCs can be a perspective in bone reconstruction and to reduce the necessity of autogenous bone grafts and the donor site morbidity [28]. Various sources of stem cells have been successfully used in repair of bone defects including bone marrow [29], adipose tissue [3], amniotic fluid [30], and dental pulp [28]. It was shown that combining osteogenic, angiogenesis and gene transfection factors, and biocompatible scaffolds as elements of bone tissue engineering can enhance the bone regeneration potential of DPSCs [28]; because DPSCs have self-renewal and multilineage differentiation capacities, a high proliferation potential and clonogenic efficacy. These characteristics for DPSCs reveal that they are one of the most promising MSCs for clinical application, even there may be some challenges to be employed for human [11, 12, 14, 31, 32]. However, when comparing DPSCs to other stem cell sources, no significant difference was visible for bone regeneration in repair of bone defects [28].

Regarding the osteogenic potential, adipose tissue-derived MSCs and amniotic fluid MSCs could significantly increase bone regeneration identical to DPSCs [33]. Seeding of DPSCs on an appropriate scaffold together with growth factors and gene therapy was demonstrated to lead to a maximum bone regeneration [33]; which is of great importance, especially when bone loss happens subsequent to degenerative or traumatic diseases, including periodontal diseases as the first cause of tooth loss in the elderly, mandible necrosis or tumor resections that cannot be amended by conventional treatment methods [34-36].

There were some limitations in our study. One of our limitations was our small sample size of included animals and also the short follow-up of clinical outcome. Future investigations on long-term efficiency and cost-effectiveness of DPSCs together with biomaterials for regenerative purposes seem essential. Also, based on the limited clinical trials carried out for bone regeneration by DPSCs, they may not be still recommended to be used in clinical practices. There is a need to quantitatively evaluate bone regeneration by DPSCs including analysis of the amount of bone volume, bone area, trabecular number, bone mineral density, and mineral content regenerated by DPSCs. Also, in future studies, there is a need to focus on samples closer to humans, such as dogs and sheep, rather than mice, rats and rabbits.

In summary, we showed the mesenchymal properties of isolated DPSCs and the increas-

ing proliferative and viability effects of acemannan on DPSCs. We could also induce a reproducible critical-sized mandibular tissue defect in rabbit model with a successful, fast and efficient outcome in bone regeneration after mandibular tissue defect by using DPSCs treated with acemannan that were confirmed histologically and by X-ray radiography. So in regenerative medicine, DPSCs can be considered as one of the promising ways in bone regeneration. These findings can be added to the literature when DPSCs are targeted in repair of mandibular bone defects.

Acknowledgements

We acknowledge the Stem Cell Technology Research Center of Shiraz University of Medical Science, Shiraz, Iran, for providing necessary research facilities. This research was supported from the grant provided by National Institute for Medical Research Development of Iran Ministry of Health, Treatment, and Medical Education regarding dental pulp stem cells (Grant No: NIMAD943645) that hereby their kind support is appreciated.

Disclosure of conflict of interest

None.

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