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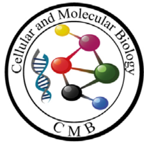

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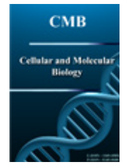

Original Article

# Effect of bone-marrow-derived mesenchymal stem cells on the rate of orthodontic tooth movements in rabbits

**Rawand Jasim Othman[1*], Omar Fawzi Chawshli[2]**

*[1] Department of Orthodontics, Khanzad Teaching Center, Ministry of Health, Erbil, Kurdistan Region- Iraq*
*[2] Department of Pedodontics, Orthodontics and Preventive Dentistry, Collage of Dentistry, Hawler Medical University, Erbil, Kurdistan Region- Iraq*

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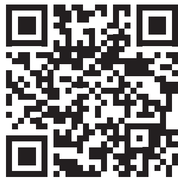

## Abstract

Mesenchymal stem cells from bone marrow, such as bone marrow aspirate concentrate (BMAC) and cultured and isolated bone marrow mesenchymal stem cells (BM-MSCs), have been used as therapeutic alternatives to enhance remodeling in the bone. Objective: This study aimed to evaluate the effects of BMAC and BM-MSCs on orthodontic tooth movements in rabbits. Methods: A100- gram nickel–titanium closed-coil springs were used to initiate orthodontic tooth movement of the lower first premolars in 35 male New Zealand rabbits for 21 days. Using a split-mouth design, autologous BMAC or BM-MSCs were submucosally injected into the right sides of the lower jaw, while the left sides served as the control. On days 7, 14, and 21, a three-dimensional digital model scan was used to measure the amount of tooth movement. The microfocus computed tomography (Micro-CT) and histological findings were examined on day 0 as the baseline measurement and on days 7, 14, and 21. Results: Compared to the control group, the quadrant receiving BMAC and BM-MSCs had a considerably greater amount of tooth movement. Histomorphometric analysis revealed that both BMAC and BM-MSCs had significantly higher numbers of osteoclasts and active bone-resorptive lacunae. The resorptive changes were greater in the BMAC and BM-MSCs groups than in the control group. Conclusion: The submucosal injection of BMAC and BM-MSCs accelerates orthodontic tooth movement (OTM) by decreasing bone density and supplying more osteoclast progenitor cells.

**Keywords:** Bone marrow; Stem cells; Tooth movement; Orthodontics; Rabbit

## 1. Introduction

Complex molecular signaling is recognized as the process by which mechanical stresses are transformed into biochemical events that subsequently lead to bone apposition and/or resorption [1]. As a result of age-related physiological changes in the periodontal ligament (PDL) (which becomes more fibrous and less vascular), alveolar bone (which becomes denser and more calcified), and cellular activity and turnover (which all diminish with age), OTM occurs more slowly in adults than in children [2]. An early inflammatory response characterized by enhanced vascular permeability and leukocyte migration is brought on by orthodontic force [3]. Increased bone turnover also contributes to bone remodeling via the recruitment of osteoclast precursors from blood vessels and through the stimulation of osteoclast formation and activation [4]. It is necessary to alter the ratio of resorption to apposition to accelerate tooth movement and reduce the duration of orthodontic therapy. Evidence from other research suggests that orthodontic tooth movement can be accelerated if there are changes in the density of the bone surrounding the tooth [5, 6]. Some of the methods that have been tried to increase the rate of OTM fall into the physical, surgical, biological, and biomechanical categories [7–10].

Tissue engineering and cell-based treatments have been considered to be novel approaches and therapeutic alternatives to support regeneration in the bone and periodontium [11]. MSCs are self-renewing cells that can differentiate between various cells under suitable conditions; they also show specific surface antigen expression, including CD90 and CD45 [12]. MSCs can be harvested from a variety of tissues, including muscle, dermis, bone marrow, adipose tissue, periosteum, blood, umbilical cord, synovial membrane, and teeth [13]. Bone marrow aspirate (BMA) is a common source for harvesting MSCs, other progenitor cells, and associated cytokines and growth factors to be used in the biological treatment of various pathologies [14, 15]. They have several remarkable properties, such as immunomodulatory potential, proliferation, migration, and multilineage differentiation [16]. Bone marrow MSCs can enhance osteoclastogenesis and increase the effectiveness of OTM, in addition to being recruited to the pressurized side of the periodontal ligament [17].

Unprocessed BMA has rarely been used, as only about

* Corresponding author.
E-mail address: Rawandothman052@gmail.com (R. J. Othman).
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0.001% of nucleated cells from BMA are MSCs [18], and it also contains platelets, red and white blood cells, and hematopoietic and nonhematopoietic precursors [19]. To obtain sufficient numbers of cells for clinical applications, MSCs have to be expanded ex vivo [20]. Centrifugation of the aspirate is believed to produce differential density gradients, which will raise the concentration of MSCs in an injectable volume that is suitable for immediate administration [14]. This process has been termed “bone marrow aspirate concentrate” (BMAC). Alternatively, bone marrow aspirate can be isolated, cultured, and expanded to form bone marrow mesenchymal stem cells (BM-MSCs); this yields a more homogenous population with a larger quantity of cells for injection [21].

Because stem cells can develop into several cell types, they may enhance bone remodeling during orthodontic processes, so this study aimed to determine the effects of bone-marrow-derived MSC submucosal injection on the OTM rate of the mandibular first premolar of rabbits by utilizing digital model analysis, histological analysis, and Micro-CT analysis.

## 2. Materials and Methods

### 2.1. Study Design

Included in the study were thirty-five 16-week-old healthy male white New Zealand rabbits with body weights of 2.5–3.5 kg and with normal dental development. Five rabbits were randomly selected for the negative group at day 0; this group did not undergo orthodontic treatment or receive any injections and served as a baseline group for histological and micro-CT analysis. The other thirty rabbits were randomly divided via simple random allocation into two groups: the BMAC injection group (n =15) and the cultured BMAC injection group (n =15). Three time points were studied: days 7, 14, and 21. Both the BMAC and BM-MSCs injection groups consisted of 5 animals per time point. A split-mouth design was used, and the right side of each animal's mandible served as the experimental group (BMAC and BM-MSCs), whereas the left side served as a positive control group after receiving an injection of normal saline solution. All procedures were completed under general anesthesia through an intramuscular injection, with the rabbits being weighed to calculate an accurate dose of ketamine hydrochloride (25 mg/kg) in combination with xylazine hydrochloride (5 mg/kg). Every week, the rabbits were weighed, and if one of them lost more than 20% of its starting body weight, it was excluded from the research and replaced with the other.

### 2.2. Preparation of autologous BMAC

The skin of the right femur was trichotomized and, after appropriate asepsis and antisepsis, the puncture from the head of the femurs was performed, using an 11G * 100 mm needle with a trocar (TSK BMB Surelock, Japan). After this, the trocar was removed, connecting to the needle a 10 ml disposable plastic syringe that was previously soaked in a heparin solution at 1:1.000. Immediately thereafter, the tube was centrifuged at 2000 RPM for 30 minutes at room temperature. Three fractions were formed in the tube: the top layer contained plate-let-poor plasma, and, in the middle, there was the so-called buffy coat containing nuclear cells (BMAC) and sedimented cells with high density at the bottom. The supernatant was removed and the 300 µl of BMAC (above the erythrocyte fraction) was aspirated using a sterile Pasteur pipette and made ready for direct application [22].

### 2.3. Isolation, cell culture, and seeding of BM-MSCs

Similar to the BMAC group, the rabbit marrow in the BM-MSCs group was aspirated three weeks in advance. It was then centrifuged for ten minutes at 500 g and resuspended in Dul-becco's modified Eagle's medium (DMEM), a low-glucose medium supplemented with 1% penicillin–streptomycin and 10% fetal bovine serum (Lonza, Walkersville, MD). The red blood cells were lysed via the addition of autoclaved water. After adding an extra maintenance medium and gently mixing the marrow for 30 seconds, the suspension was centrifuged at 500g for 10 minutes. The cells were counted, plated on 100-mm plates at a density of 107 cells per plate, and incubated at 37°C with 5% CO2 after the pellet was resuspended. A phosphate-buffered saline (1x PBS) wash step was followed by four days of culture before the first medium change, which was carried out every three days thereafter. At 70% to 80% confluency, the cells were subcultured after 2.5 weeks. At around 90% confluence, the adherent cells were enzymatically detached, incubated with monoclonal anti-rat antibodies against CD45 (orb312177 Mouse Anti-Rat PE, Biorbyt, UK) and CD90 (ab225 Abcam, USA), and sorted using flow cytometry (FACS ARIA III, BD Bioscience, San Jose, CA) to produce a subpopulation of CD45-CD90+ BM-MSCs. The cells were trypsinized and centrifuged, and then the cell pellet was suspended in one milliliter of the culture media to count the cells. To achieve this, a 1.5 ml microtube was filled with 80 µl of growth media, 10 µl of the homogenized cell suspension was added, and then 10 µl of trypan blue dye was added.

### 2.4. Orthodontic procedures

For the orthodontic intervention, a custom-made fixation apparatus was used for orthodontic interventions in rabbits with a specially designed check retractor made of hot-cured acrylic (Figure 1a,b). The appliance used to encourage OTM resembled that described by Pithon and Ruellas [23].

Stainless steel ligature wire was used to stretch the nickel-titanium closed-coil springs, which were 14 mm long and had a diameter of 1.5 mm (International Orthodontic Services, Houston, Tex, United States) between the mandibular first premolars and mandibular incisors bilaterally (Figure 2a). To prevent the slippage of the appliance, a notch was made 0.5 mm away from the gingival margin level on the distal surfaces of the right and left lower incisors with a diamond-round disc (Ortho Technology, Inc.; Tampa, FL, United States) (Figure 1c). The ligature wires were

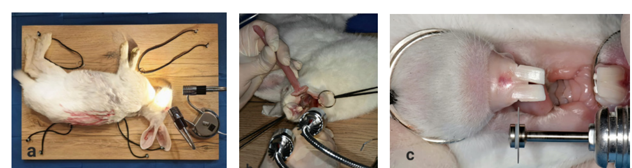


**Fig. 1.** Rabbits' preparation (a) Custom-made retraction and lighting apparatus for orthodontic interventions; (b) Appropriate mouth opening with key rings and check retractors. (c) notch creation with a diamond-round disc

placed in the notch and fixed to the incisors with a flowable composite (Filtek Bulk 3M ESPE Dental Products, Saint Paul, Minn. United States). The force was measured using a force gauge (Federwaage, 25–250 g, stress, and tension gauge; Dentaurum, Ispringen, Germany) to generate 100 g of activation (Figure 2b). A 0.5 mL insulin syringe with a 31-gauge ultrafine needle (Insumed 31G Insulin Syringe 31G 3 8 mm; Pic-solution, Artsana, Grandate, Italy) was used to give the submucosal injection close to the mesial root of the mandibular first premolars. The solution was delivered 300 µl, 150 µl on the buccal side, and 150 µl on the lingual side. All injections were administered only once, on day 0, and an equivalent amount of saline was injected on the control side. In the BM- MSCs group, each rabbit received 106 cells/kg in a 300 µl volume of normal saline (Figure 2c).

### 2.5. Measurement of the distance of tooth movement

In 7, 14, and 21-day observational period, mandibular dental impressions of the rabbits were taken with custom-made trays that had previously been constructed and color-coded; they were filled with injection-type silicone vinyl polysiloxane impression material (3M ESPE Dental Products, Saint Paul, Minn, United States). The impressions were then poured with the use of an improved die stone (Elite Rock Dental Stone, Zhermack, Badia Polesine, Rovigo, Italy). Then, the casts were scanned directly and used to construct three-dimensional models with a 3D scanner a Zirkonzahn S600 ARTI (Zirkohnzahn GmbH, Gais, Italy). A stereolithography (STL) file was produced by the scanning process and was used to create 3-dimensional (3D) models in the STL file format with the use of Orthoanalyzer software, version 1.7.1.0 (3Shape, Copenhagen, Denmark). Briefly, the shortest distance between the first premolar's distal surface and the second premolar's mesial surface of the crown was measured using the 3Shape Ortho Analyzer digital caliper feature (Figure 3).

### 2.6. Micro-CT Analysis

Five rabbits from each group were sacrificed at 7, 14, and 21 days using a lethal dose of thiopental sodium. After that, in under 48 hours, the mandible was harvested and preserved in 10% neutral buffered formalin to perform

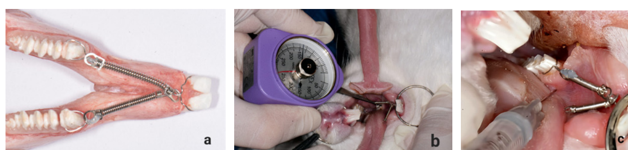


**Fig. 2.** Orthodontics procedure and injection of experimental solutions (a) Occlusal views of the experimental orthodontic appliance in situ. (b) A force gauge to measure 100g of a force. (c) Submucosal injection.

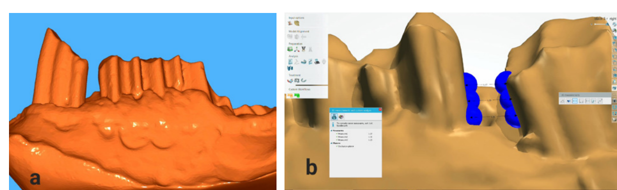


**Fig. 3.** Three-dimensional models and tooth movement measurement (a) STL file format (b) 3Shape Ortho Analyzer digital caliper feature.

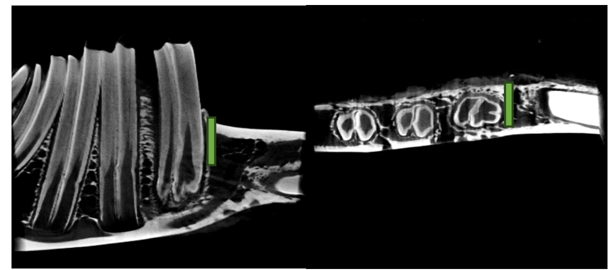

**Fig. 4.** The 3D micro-CT images of the sample and the region of interest (a) lateral view; (b) occlusal view (ROI-green box).

the micro-CT analysis and histologic investigations. An in vivo X-ray micro-CT scanner was utilized in the present investigation. The LOTUS in vivo apparatus was equipped with a flat panel detector and a cone beam micro-focus X-ray source. The X-ray tube's voltage and current were set to 60 kV and 130 µA, respectively, and the frame exposure time was set to 1 second with 1.4 magnification to provide the highest possible image quality. The slice thicknesses of the reconstructed images were set to 50 micrometers and the scan took 28 minutes to complete.

The alveolar bone's region of interest (ROI) was selected using the previously described method, presenting the integrated microstructure of the bone [9]. The trabecular bone, which is mesial to the cervical third of the root of mandibular first premolars, was selected for examination (Figure 4). The LOTUS in vivo- ACQ software controlled all the protocol setups and a typical Feldkamp, Davis, and Kress (FDK) technique was used to reconstruct the collected 3D data using LOTUS in vivo-REC [24]. Then, the following parameters were calculated: bone mineral density (mg/cm3), trabecular number (mm-1), and bone volume fraction (%).

### 2.7. Histomorphometric analysis

Upon scanning, the mandible was decalcified and fixed, and its soft tissues were stripped. It was also dissected and divided into two pieces. Then, 10% formaldehyde was used to fix the specimens, and 10% formic acid was used to decalcify them for 48 hours (Sigma-Aldrich, St. Louis, MO, USA). Before total decalcification occurred, the decalcifying solution was agitated ten times a day and replaced twice a week. After that, they were fixed in paraffin blocks and dehydrated using ethanol then a microtome (Leica, Wetzlar, Germany) was used to slice the specimens sagittally, parallel to the long axis of the mandibular first premolar, at a thickness of 5 micrometers [25]. The tissue specimens were stained with hematoxylin and eosin, and the slides were viewed at 100x magnification using a light microscope (Eclipse E400, Nikon, Japan). The approach previously reported by Igarashi et al. was used to evaluate the histomorphometric characteristics, such as the number of osteoclasts and the area of active bone-resorptive lacunae inside a specified square area [26].

### 2.8. Statistical Analysis

The data were analyzed using SPSS version 21 (IBM, Armonk, NY, USA) with one-way ANOVA and Tukey's post-hoc test to investigate the differences between each group's means. Statistical significance was set at $p<0.05$.

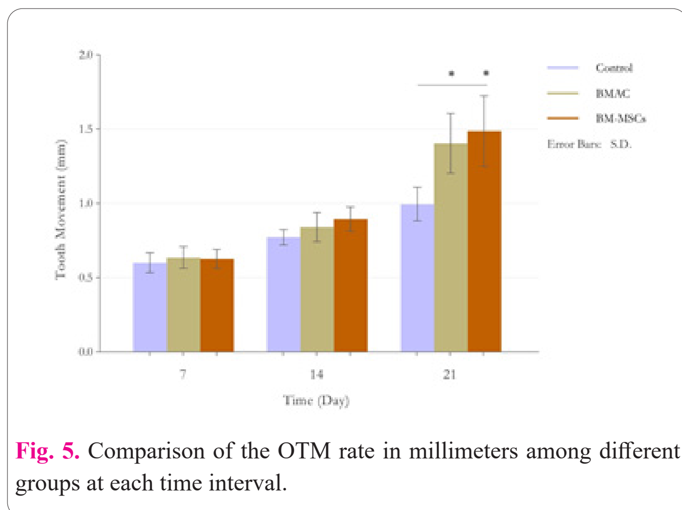


**Fig. 5.** Comparison of the OTM rate in millimeters among different groups at each time interval.

## 3. Results

### 3.1. Characterization of BM-MSCs

The BM-MSCs isolated from the rabbit bone marrow cavity exhibited multipotential differentiation characteristics. Flow cytometry revealed that the BM-MSCs expressed high levels of the MSCs surface marker CD90 but did not express the hematopoietic stem cell surface marker CD45. High percentages of unmarked CD45 (95.6%) and marked CD90 (5.4%) cells were seen in the BM-MSCs (Figure 5).

### 3.2. Effects of the Autologous Injection bone-marrow-derived MSCs on OTM.

Data that describe the amount of OTM across all groups are shown in Figure 5. Significant differences were seen between the groups in OTM (P < 0.05) following 21 days of orthodontic force application. The mean distance of tooth movement for the first premolar in the BMAC injection group was (1.40 mm); for the BM-MSCs group, it was (1.48mm). Meanwhile, the saline injection group showed a significantly shorter distance (0.99mm). The amount of OTM was at its minimum in the positive control group (0.601mm) on day 7 and at its maximum in the BM-MSCs group on day 21 (1.487mm). At day 21, a significant difference existed between the positive control group and BMAC in terms of OTM. Moreover, significant differences existed between the positive control and BM-MSCs (P < 0.05). However, the difference between the BMAC and BM-MSCs groups on days 7, 14, and 21 was not significant (P > 0.05).

### 3.3. Effect of bone-marrow-derived MSCs on microstructural parameters

#### 3.3.1. Bone mineral density (BMD)

On day 21, BMD was highest in the negative control group (1434.66±38.37) and lowest in the BM-MSCs group (515.33±26.54). During each of the three time periods, there were significant differences in the BMD between the other groups and the negative control (P < 0.05). Additionally, a significant difference existed between the positive control and the BMAC and BM-MSCs groups for BMD on days 14 and 21 (P< 0.05). The difference between the BMAC group and the BM-MSCs group was only significant on day 21; see Table 1.

#### 3.3.2 Trabecular number (Tb. N)

As shown in Table 1, the microstructural parameters regarding Tb. N was lowest in the BMAC group on day 21 (1.21 ± 0.08) and highest in the negative control group (6.18 ± 0.77). Significant differences existed between the negative control and other groups in the Tb. N at all three time points (P < 0.05).

#### 3.3.3. Bone volume fraction (BV %)

The micro-CT data showed that there was a significant (P<0.05) difference in the amount of BV between the groups. On day 21, the BV fraction was lowest in the BM-MSCs group and highest in the negative control group. At various time intervals, there were significant differences (P < 0.05) between the negative control group and the other groups in this regard. The BMAC and BM-MSCs groups showed a significantly lower bone volume than the positive control group on days 7 and 14 (P < 0.05). However, no significant difference existed between the positive control and BMAC group on day 21 (P>0.05). No significant difference was found between the BMAC and BM-MSCs groups on days 14 and 21 (P > 0.05); see Table 1.

**Table 1.** Mean and standard deviations of the microstructural parameters of trabecular bone at different time points.

| Groups | N. Control | P. Control | BMAC | BM-MSCs | P value |
|---|---|---|---|---|---|
| **BMD (mg/cm³)** | | | | | |
| Day 7 | 1434.66±38.37[bcd] | 1012.33±66.51[a] | 1004.33±39.11[a] | 990.00±19.00[a] | <0.001 |
| Day 14 | 1434.66±38.37[bcd] | 948.00±20.88[acd] | 848.66±41.40[ab] | 750.66±35.23[ab] | <0.001 |
| Day 21 | 1434.66±38.37[bcd] | 915.00±46.70[acd] | 637.00±29.61[abd] | 515.33±26.54[abc] | <0.001 |
| **Tb. N (1/mm)** | | | | | |
| Day 7 | 6.18± 0.77[bcd] | 2.14± 0.56[a] | 1.60± 0.17[a] | 1.48± 0.20[a] | <0.001 |
| Day 14 | 6.18± 0.77[bcd] | 1.79± 0.24[a] | 1.36± 0.23[a] | 1.23± 0.07[a] | <0.001 |
| Day 21 | 6.18± 0.77[bcd] | 1.73± 0.15[a] | 1.21 ± 0.08[a] | 1.32 ± 0.03[a] | <0.001 |
| **BV %** | | | | | |
| Day 7 | 62.64 ±4.13[bcd] | 37.54± 2.05[acd] | 27.75 ±1.78[abd] | 20.46±1.51[abc] | <0.001 |
| Day 14 | 62.64 ±4.13[bcd] | 34.08 ±2.47[acd] | 22.88 ±2.10[ab] | 16.85 ±2.84[ab] | <0.001 |
| Day 21 | 62.64 ±4.13[bcd] | 24.89 ±3.51[ad] | 17.95±1.18[a] | 11.078±1.38[ab] | <0.001 |

One-way ANOVA test followed by Tukey's test was used. N=Negative, P= Positive. [a] significant difference compared with N. Control (P < .05). [b] Significant difference compared with P. Control (P < .05). [c] Significant difference compared with BMAC (P < .05). [d] Significant difference compared with BM-MSCs (P < .05).

### 3.4. Effect of bone-marrow-derived MSCs on histomorphometric analysis.

#### 3.4.1 Active bone resorptive lacunae

The number of Howship's lacunae was highest in the BM-MSCs group (0.84 ±0.07) and lowest in the negative control group (0.017 ±0.005). Significant differences existed between the negative control and other groups in terms of the number of areas of active bone resorptive lacunae at all three-time points (P < 0.05). Additionally, a significant difference existed between the positive control and other groups in terms of the number of areas of active bone resorptive lacunae for all time points, days 7, 14, and 21 (P< 0.05). The difference between the BMAC group and the BM-MSCs group was not significant for all time points (days 7, 14, and 21) (P> 0.05) (Table 2).

#### 3.4.2. Osteoclasts

Table 2 shows that the osteoclast numbers among all the groups, except in the negative control group, increased from day 7 to day 21. On day 14, the BM-MSCs group had the highest number of osteoclasts (8.20 ± 0.84), while the negative control group had the lowest number (1.40 ± 0.54). For all three time periods, there was a statistically significant difference (P<0.05) in the number of osteoclasts between the negative control group and all other groups. The BMAC and BM-MSCs groups showed significantly higher osteoclast numbers than the positive control group on day 14 and day 21 (P < 0.05). No significant difference was found between BMAC and BM-MSCs on all time points.

## 4. Discussion

Many studies have evaluated the impact of various medications and surgical techniques on OTM. In some studies, certain drugs or surgical techniques have been found to accelerate OTM, whereas other investigations have shown that OTM is slowed down by certain stimuli [5, 6, 9, 10]. In orthodontics, tissue engineering using stem cells is a fast-emerging field that has seen widespread applications [27]. Bone marrow stem cells are thought to constitute a promising combination for healing alveolar bone defects following a tooth extraction, reducing the risk of periodontal disease, bone dehiscence, and fenestration during tooth movement [28].

The application of MSC in dentistry has recently emerged due to the ease of access to sources such as the extracted wisdom teeth, the periodontal and pulp tissues of primary teeth, and oral cavity adipose tissue [29]. Laboratory rabbits are a good choice for studying OTM and bone remodeling in response to mechanical forces because of the similarities between their cellular and tissue physiology and those of human MSCs [14].

The present study assessed the effect of bone-marrow-derived MSCs on OTM in rabbits, using a split-mouth technique to reduce the variation in the response to metabolic activity between animals. Throughout the trial, the local administration of BMAC and BM-MSCs increased the average weekly incremental rate of tooth movement; nevertheless, only on day 21 was there a significant difference between the experimental groups and the control group in terms of tooth movement These results are in agreement with earlier research, which found that the slow tooth movement in the rabbit model was due to the jaw bones' 2% annual bone turnover rate, whereas the canine alveolar bone is thought to undergo 20–36% annual bone remodeling [30]. Moreover, the theoretical acceleration of OTM predicted by stem cell implantation into the pressure site depended on the hypothesis of the prevention of the delay period brought on by the elimination of hyalinized necrotic tissue [29]. Consistent with these hypotheses are the remarks made by Huang et al. [17], who states that "because of stem cells' capacity for proliferation and differentiation, PDL stem cells may be utilized to expedite the orthodontic treatment process"; this confirmed our findings regarding OTM acceleration through MSC transfer. However, the assessment of the amount of OTM is complicated by a number of variables, including cranial development throughout the research period, the mesial move of the second molar, and the deformation of the device during food consumption [31].

MSCs can proliferate and differentiate, making them a promising tool for the remodeling of alveolar bone, which, in turn, accelerates the orthodontic treatment process [32]. Bone density and tooth movement rate are generally inversely correlated. The fact that children's teeth move more quickly than adults' does provide support for this idea [33]. Micro-CT results of the present study showed that an injection of bone-marrow-derived MSCs had a resorptive effect by decreasing the bone density on the pressure site. The resorptive change in the alveolar bone around the mesial root of the mandibular first premolar demonstrated that the BV% in the BMAC and BM-MSCs groups was significantly lower than that in the control group on days 7, 14, and 21 after the application of force, which

**Table 2.** Mean and standard deviations of the number of active bones resorptive lacunae and osteoclast at each time interval (mm2).

| Groups | N. Control | P. Control | BMAC | BM-MSCs | P value |
|---|---|---|---|---|---|
| **Active bone resorptive lacunae** | | | | | |
| Day 7 | 0.017 ± 0.005[bcd] | 0.07 ± 0.01[acd] | 0.09 ± 0.01[ab] | 0.10 ± 0.01[ab] | <0.001 |
| Day 14 | 0.017 ± 0.005[bcd] | 0.24 ± 0.03[acd] | 0.74 ± 0.07[ab] | 0.83 ± 0.06[ab] | <0.001 |
| Day 21 | 0.017 ± 0.005[bcd] | 0.35 ± 0.05[acd] | 0.77 ± 0.08[ab] | 0.84 ± 0.07[ab] | <0.001 |
| **Osteoclasts** | | | | | |
| Day 7 | 1.40 ± 0.54[bcd] | 3.40 ± 0.55[ad] | 4.00 ± 0.71[a] | 4.40 ± 0.55[ab] | <0.001 |
| Day 14 | 1.40 ± 0.54[bcd] | 4.25 ± 0.50[acd] | 7.20 ± 0.84[ab] | 8.20 ± 0.84[ab] | <0.001 |
| Day 21 | 1.40 ± 0.54[bcd] | 3.45 ± 0.84[acd] | 6.16 ± 0.70[ab] | 7.167 ± 0.71[ab] | <0.001 |

One-way ANOVA test followed by Tukey's test was used. N=Negative, P= Positive. [a] Significant difference compared with N. Control (P < .05). [b] Significant difference compared with P. Control (P < .05). [c] Significant difference compared with BMAC (P < .05). [d] Significant difference compared with BM-MSCs (P < .05).

corresponded to greater decreases in Tb. N in the results. Furthermore, the results showed that the BMAC and BM-MSCs groups had a higher decrease in BMD, which may be related to osteoclast-induced bone resorption on the trabecular bone's surface. The osteoclasts absorb the surface of the alveolar bone, creating an acidic environment. The release of various hydrolases contributes to the acidity's effects on the neighboring bone, which results in mineral loss and collagen matrix breakdown [34].

The current study showed that the injection of BMAC and BM-MSCs enhanced the number of osteoclasts and resorptive lacunae. Localized resorption of bone, an integral component of the process of tooth movement, is facilitated by resorptive lacunae. This substantial effect on the number of osteoclasts and areas of bone resorptive lacunae may be due to the diffusion of the submucosal local injections of bone-marrow-derived MSCs, from where it passed subperiosteally at the level of the alveolar crest and proceeded along the vascular canals to reach and act directly on the bone. This could provide a reasonable rationale for the observed rise in the magnitudes of tooth movement seen in the group that received local injections of MSCs produced from bone marrow. According to a prior publication, an injection of MSCs with orthodontic force increases osteoclast counts and stimulates osteoclastogenesis [17, 35].

This finding agrees with the study conducted by Huang et al. [36], which revealed that short-term compression, or applying force for one hour, has the potential to encourage osteogenic differentiation, but long-term compression, or applying force for twelve hours or more, inhibits osteogenesis and increases osteoclastogenesis [36].

Both BMAC and BM-MSCs had almost the same effect because there was no statistically significant difference between them in most areas of the study; this finding concurs with that of Kohno et al. [35], who showed that BMAC has osteogenic abilities equivalent to or even greater than BM-MSCs, suggesting that crosstalk among the various cells in the BMAC leads to more pronounced osteogenesis. Costa et al. stated that bone remodeling was enhanced equally by both BM-MSCs and BMAC cell movement. Nevertheless, using cultivated BM-MSCs resulted in early bone remodeling [37].

Among the study limitations is the need for larger sample sizes to evaluate the impact of bone-marrow-derived MSCs on OTM. Another limitation of this study was the lack of an evaluation of new bone formation and osteoblast activity along the tension side of the root. Finally, other types of bone-marrow-derived MSCs were suggested and other protocols may be used in future studies.

## 5. Conclusions

In summary, the study findings showed that autologous submucosal injection of bone-marrow-derived mesenchymal stem cells (BMAC and BM- MSCs) may accelerate orthodontic movement and decrease the duration of treatment by increased osteoclastic behavior in conjunction with the greater number of resorption lacuna. Additionally, their impact on more resorptive change in microstructural parameters of alveolar bone, including the volumetric bone mineral density, the bone volume fraction, and trabecular number. Both BMAC and BM-MSCs had nearly similar osteoclastogenesis and resorptive effect on the pressure side of the alveolar bone and approximately equivalent OTM rate with slightly greater in BM-MSCs group.

## Conflict of Interests

The author has no conflicts with any step of the article preparation.

## Consent for publications

The author read and approved the final manuscript for publication.

## Ethics approval and consent to participate

The experiment was approved by the Hawler Medical University's College of Dentistry's Ethics and Scientific Committee, and it was conducted following the guidelines set by the Spanish Royal Decree 53/2013, which addresses the protection of animals used in research (Boletín Oficial del Estado, 2013).

## Informed Consent

The authors declare not to use any patients in this research.

## Availability of data and material

Data are available from the corresponding author upon request.

## Authors' contributions

Rawand J. Othman and Omar F. Chawshli have given substantial contributions to the conception or the design of the manuscript, Rawand J. Othman to acquisition, analysis and interpretation of the data. All authors have participated in drafting the manuscript, Rawand J. Othman and Omar F. Chawshli revised it critically. All authors read and approved the final version of the manuscript. All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

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