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Effect of Adeno-Associated Virus-Transfected Mesenchymal Stem Cells Containing Agents on Bone Formation in Extended Inter-Premaxillary Suture in Rats

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ABSTRACT

Introduction: The aim of this study was to investigate the histomorphometric effects of bone marrow-derived mesenchymal stem cells (MSCs) transfected with Adeno Associated Virus (AAV) and containing bone morphogenic protein 7 (Bmp7) or osteoprotegerin (OPG) on bone formation after injection into inter-premaxillary suture in rats with Bmp7 or OPG alone.

Materials and Methods: In 4 groups (each group n:9), different chemical solutions, namely AAV-Bmp7, AAV-OPG and AAV-Bmp7-OPG and AAV-EGF (control group) were injected into the interpremaxillary suture of rats. Bone volumes (BV), soft tissue volume (STV) and total bone volumes (TBV) of 10µm serial selection with hemotoxylin and eosin staining were calculated according to the Cavalieri principle. Each point in the region of interest was 1000µm^3.

Results: The BV for AAV-Bmp7, AAV-OPG, AAV-Bmp7-OPG and AAV-EGF were 46.98±1.50 mm^3, 49.40±4.72mm^3, 42.58±2.89mm^3 and 38.82±0.76mm^3, respectively. The STV was 11.53±0.99, 13.31±1.88, 8.00±4.43 and 9.57±1.90mm^3 for Bmp7, OPG, AAV-Bmp7-OPG and AAV-EGF, respectively. TBV was 58.34±2.28mm^3, 63.83±5.17mm^3, 53.74±3.34mm^3 and 48.13±1.54mm^3 for AAV-Bmp7, AAV-OPG, AAV-Bmp7-OPG and AAV-EGF, respectively. The comparison between BV, STV, TBV for AAV-OPG showed a statistically significant difference (p=0.001) compared to AAV-Bmp7 or AAV-Bmp7-OPG and AAV-EGF.

Conclusion: During tooth movement and bone remodeling, the ratio of soft and bone tissues is maintained by OPG. Although Bmp7 is not as effective as OPG in bone remodeling, both can reduce the retention time and the risk of recurrence.

Keywords: Histomorphometry; Bmp 7; OPG; Maxillary expansion; Orthodontics; Rats.

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Introduction

The transverse constriction of the maxillary arch and the presence of crossbite are often corrected with the orthopedic expansion of the maxillary arch which is named as rapid maxillary expansion [1]. This procedure results in the correction of the transverse relationship of the dental arches and an increase is seen in the arch length for the alignment of the teeth [2]. It includes the separation of the maxillary halves and activation of bone remodeling in the middle palatal suture [3,4]. The durability of the achieved correction depends on factors such as bone metabolism and the direction of generated stress. The expanded maxilla tends to collapse back to its original width. The recurrence rate can be up to 90% [5]. Long-term retention of the expanded maxilla is mandatory to avoid recurrence [4,6,7]. The retention protocol includes appliances supported by bone and teeth and is increasingly used in current mechanics [8]. Since the maxillary halves tend to relapse, residual forces acting on the supporting teeth may cause unwanted buccal tooth movement, leading to fenestrations and dehiscence [9-11]. The protocol for tooth-borne and tooth-bone-borne retention is almost identical; the appliance should be retained for at least 3 months to ensure minimal relapse of the maxillary halves [8].

Acceleration of bone regeneration in the expanded suture may be effective in reducing the retention time and preventing recurrence [12,13]. There are several studies testing agents and methods in promoting osteogenic activity [12-23]. There are several studies on the effect of bisphosphonates, known as inhibitors of bone resorption, on the relapse and durability of rapid palatal expansion. The authors concluded that bisphosphonates have positive effects on bone formation and remodeling of the rat sagittal suture after examining rapid expansion [13,14]. Uysal et al. used vitamins C, D, and E on inter-premaxillary expanded suture region.15-18 Vit C stimulates bone when administered systemically and local injection has negative effects [15]. They found that Vit D has positive effects on early phase of bone regeneration in the mid-palatal suture [16]. They also concluded that Vit E stimulates bone formation in the early stages of orthopedically expanded premaxillary suture and shortens the retention time [17]. Local injection of resveratrol to orthopedically expanded inter-premaxillary suture area may shorten the retention time by stimulating bone formation [18]. Systemic thymoquinone use has a positive effect on new bone formation due to expansion [19]. Periosteal stimulation [20] and light-emitting diode photobiomodulation [21], applications after rapid inter-maxillary suture expansion were also determined as different methods. Sawada and Shimizu [3] have suggested an increase in active bone deposition with a single dose of TGF-β1. In a study conducted by Altan et al. In 2015, low-level Ga-Al-As diode laser application to the sutural region also resulted in increased bone activity in rats [22]. The early use of antioxidants in healing bone fractures had also induced active bone remodeling.

It will be important to accelerate osteogenic activity and shorten the retention time. Osteoclastogenesis, also known as bone resorption, is regulated by OPG (osteoprotegerin), RANK (receptor of the same origin) and RANKL (receptor activator nuclear kappa B ligand) [23-26]. The biological effect of RANKL occurs by binding to RANK on the osteoclast surface, OPG binds to RANKL, inhibits its binding to RANK and protects bone from excessive resorption [27,28]. Some studies have evaluated the bone regeneration effect of OPG [26,27]. In the study of Wlodarczyk et al.'s [29] osteoblastic activity is increased when OPG diffuses into bone tissue in rheumatoid arthritis patients or when serum OPG concentration increases in arthritis patients. Jayash et al [30]. used OPG on the critical-sized calvarial bone defects of rabbits and found out that it is a kind of osteoclastic bone resorption suppressor. The effect of orthognathic surgery on the OPG level was measured and OPG has been found to have an important role in bone development, induction and repair [30]. Bone morphogenetic protein 7 (Bmp7) is a protein of the transforming growth factor beta (TGFβ) family that transforms mesenchymal cells into bone and cartilage tissue. It can convert mesenchymal cells into osteoblasts and increase osteoblastic activity, which is why it is used in the treatment of spinal fusion, osteochondral defects, bone fractures and diaphyseal fractures [31]. There are several studies in which Bmp7 was used to increase osteoblastic activity in scaphoid bone defects [32] or femur fracture healing [33] and also with iliac graft as a scaffold [34]; histological examination results showed that it increased mineralisation in bone tissue regeneration. A stem cell is defined as a self-renewing and differentiating cell derived from haematopoietic stem cells, which have been used in treatments for many years [35]. Over the past decade, there has been considerable growth in the understanding of stem cell classifications, fundamental biological characteristics, and plasticity, pertaining to their capacity to differentiate in various cell types. Mesenchymal stem cells (MSCs), which can be harvested from a range of sources such as bone marrow,

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periosteum, trabecular bone, adipose tissue, cartilage, skeletal muscle and lung, have gained particular attention during this time [36,37]. Human tooth follicle cells are also considered one of the newly recognized sources of stem cells [38-40]. These cells possess the capability to differentiate into bone, adipose tissue, cartilage, and muscle. Evidence suggests that the osteogenic potential escalates in human MSC cultures when supplemented with growth factor components such as fibroblast growth factor FGF-2. Bone Morphogenetic Proteins (BMPs) can stimulate osteoblastic activity in pluripotent and mesenchymal stem cells. Research has shown that, in vivo, bone and cartilage formation can be observed when human MSCs are implanted subcutaneously with fibronectin-coated hydroxyapatite in mice [41]. Currently, retroviral vectors are frequently utilised to guarantee sufficient and long-term gene transfer into cells. Nevertheless, random genomic access to these vectors can result in the formation of cancer [42]. Recent studies have shown that viruses identified as Adeno-Associated Virus (AAV) can be used much more safely in gene-cell therapy. These viruses belong to the Parvovirus family and are viruses with small DNA [43]. Wild-type AAVs do not randomly integrate into the genome; instead, they integrate into a specific region on chromosome [19]. However, recombinant AAVs used in gene therapy lack viral replication genes and never integrate into the cell genome [44]. Additionally, research has indicated that AAV does not elicit significant immune responses within the organism, and to date, approximately 60 clinical trials have demonstrated no notable side effects associated with the use of viruses [45].

In accordance with prior research, this study involved the transfer of OPG and Bmp7 genes into stem cells utilizing AAVs. OPG and Bmp7 transgenic stem cells, as well as AAVs containing OPG and Bmp7 genes, were introduced to the enlarged region in the upper jaw of rats. Subsequently, the effect of active bone formation in the intermaxillary suture following rapid maxillary expansion was examined through gene and gene-cell applications to facilitate new bone formation.

Materials and Methods

Animals and groups

Our study protocol was approved by Yeditepe University, Regional Animal Research Ethics Committee (protocol approval number 13.01.2012-233) and was carried out in the Yeditepe Experimental and Clinical Research Centre. In the current study, 36 Wistar rats weighing about 172.6±6.3 grams each (Table 1) were fed ad libitum in polycarbonate cages at a temperature of 23±2°C in a 12-hour light, 12-hour dark cycle. The sample consisted of 4 groups.

Appliance placement

Before each intervention, the rats were anesthetised intramuscularly using xylazine (10mg/kg) and ketamine (80mg/kg). To exert a 0.5 N force, a helical appliance comprised of 0.014 inch stainless steel wire was attached to the maxillae, as depicted in Figure 1. The appliance was fixed to the teeth using 0.25mm ligature wire, after drilling small holes in the anterior teeth and closing the cervical region. Rapid maxillary expansion was carried out for five consecutive days among each group of rats, as indicated in Figure 1. The helical expansion appliance was not reactivated during this period. Once the expansion was done, the distance between the anterior teeth was measured using a digital caliper. Subsequently, the helical expansion appliance was removed and the rectangular wire was used to maintain the distance between the teeth as depicted in Figure 1. The timeline of the study is presented in Figure 2.

Preparation of solutions

The study utilised stem cells derived from the bone marrow tissues of 6-8 week old Wistar rats. Following in vitro evaluation of their proliferation and differentiation capabilities and characterisation, the stem cells were transfected with AAVs containing OPG, Bmp7 and EGF genes (Figure 3). Technical abbreviations, such as AAVs, will be explained upon first usage throughout. RT-PCR (Reverse Transcription Polymerase Chain Reaction) technique was used to confirm the presence of mRNA of OPG/Bmp/ and EGF genes in transfected cells. The RNA's were isolated from the transfected cells, reverse transcribed into cDNA, and then PCR was performed using gene-specific primers.

Administration of solutions

Each group is comprised of 9 rats. The first group received 1x1012 iu (infectious unit) of AAV-OPG to the expanded mid-palatal suture. The 2nd group received 1x1012 iu AAV-Bmp7, 3rd group received 1x1012 iu AAV-Bmp7+OPG, 4th group and which was the control group of the AAV, received 1x1012 iu AAV-EGF. Micro-syringes (Hamilton Injection syringe, Hamilton Company, Nevada, USA) were used for injections.

Tissue Processing And Bone Histology

After the 10th day of injection, Wistar rats were euthanized under deep sedation before their mandibles

were dissected and sent for histological assessment. The collected tissue specimens were fixed by immersion in 10% neutral formaldehyde in 0.1 M phosphate buffer (pH 7.4) for fixation at 40°C. After fixation, decalcification was carried out by Morse solution (10% sodium citrate and 22.5% formic acid) for 28 days. Further, the decalcified samples were rinsed in tap water for 12 hours, and then dehydrated using an alcohol series before being embedded in paraffin. Sagittally cut paraffin jaw blocks measuring 10µm thickness were subjected to sectioning using a rotary microtome (Leica RM 2245 model; Leica Instruments, Germany). The jaw sections were then mounted on poly-L-lysine-coated slides and stained using the haematoxylin and eosin staining technique [46]. (Figure 4).

Histomorphometric Analysis

The alveolar bone volume was estimated by the Cavalieri principle [46-48]. Same blinded researcher performed all countings and 10 of the samples of each groups were recounted after 10 days for intraclass correlation (ICC) tests.

Equipment

Stereo Investigator version 7.5 (Microbrightfield, Colchester, UK) was used to calculate volumes and generate images using a computer system linked to a light microscope (Leica DM 4000B, Wetzlar, Germany), CCD digital camera (Optronics Microfire 1600x1200P, Goleta, CA) and image capture card (ATI FireGL Advance Micro Device, Camberly, UK). A motorised specimen table controlled by a computer (Bioprecision, Howtrone, NY) and an electronic microcontroller (Heidenhein, Traunreut, Germany) were utilised to manipulate movement along the x, y, and z planes utilizing an attached joystick. The Leica C Plan X10 objective (NA=0.22) was adopted to delineate the region of interest and estimate volume.

Sampling

Every 25th sample section was chosen from the jaw to measure the amount of alveolar bone through the systematic random sampling approach. The initial section of jaw tissue was selected systematically and at random. For all study groups, the same consecutive sections were taken [48].

Estimate of Bone Volume

To obtain maximum efficiency of volume estimation was performed with a point counting grid (PCG) (d=100µm). The sampling areas per point (a/p) were 1000 μm^2. PCG was applied to the sampled sections in a systematic-random fashion, and after that the numbers of points hitting alveolar bone and soft tissues in the sample were calculated. Results were used for the estimation of bone volumes using the following formula:

Bone volume= t x ssf x a/p x Σp.

Where "t" represents the mean thickness of the section.; "ssf" is a systematic random sampling fraction (1/8); "a/p" represents the area of each point on the point counting grid; "ΣP" is the total number of points hitting the sectioned area. Section sampling fraction to estimate bone volume investigated from each jaw for calculation of the total volume of sampled sections. The volume estimates obtained represent an unbiased and accurate measurement of the structure under investigation. The method's proficiency and dependability were gauged by evaluating the Coefficient of Error (CE) and Coefficient of Variation (CV) values for each individual study. In this investigation, all CE and CV values were within an acceptable range (<0.05 and <0.10, respectively). Therefore, the volumetric estimations implemented in this study can be deemed as dependable and accurate..

Statistical Analysis

Data were processed using NCSS software (version 2007; NCSS, Kaysville, Utah, United States). Mean and standard deviation were used to express results. To compare multiple groups of recalculated measurements, the Friedman test was employed, while group differences were analysed with Kruskal-Wallis test, one-way analysis of variance, and Tukey multiple comparison test. The significance level was set at $p<0.05$.

Results

During the study, four out of 40 rats were excluded. Three of them had teeth fractured during weaning and one died due to anaesthesia-related respiratory problems. There were no significant differences in body weight between groups at baseline, day 5 or day 15 ($p>0.05$), as shown in Table 1. Additionally, the amount of expansion did not differ significantly between the groups, as shown in Table 2. The results of the histomorphometric evaluation reveal a significant difference ($p=0.01$) in mean bone volume among the groups. The mean bone volumes of the AAV-EGF, AAV-Bmp7, AAV-OPG, and AAV-Bmp7-OPG groups were 38.82±0.76mm3, 46.98±1.50mm3, 49.40±4.72mm^3, and 42.58±2.89mm3, respectively (re-

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fer to Table 3). The AAV-OPG and AAV-Bmp7 groups had a greater bone volume compared to the AAV-EGF group. There was no statistically significant difference between the bone volumes of AAV-Bmp7-OPG and AAV-EGF groups (p=0.122), as indicated in Table 4. There was no significant difference in mean alveolar bone volume between the AAV-Bmp7/AAV-OPG and AAV-Bmp7-OPG/AAV-Bmp7 groups. In comparison, the mean bone volume of AAV-OPG was higher than that of AAV-Bmp7-OPG (p=0.003), as shown in Table 4.

The soft tissue volume of the different groups exhibited statistically significant differences (p=0.011) as evidenced by Table 3. AAV-OPG group's soft tissue volume was greater compared to AAV-EGF (p=0.043) or AAV-Bmp7-OPG (p=0.01). Nonetheless, there was no statistically significant difference between AAV-EGF and AAV-Bmp7 or between AAV-EGF and AAV-Bmp7-OPG as well as AAV-Bmp7 and AAV-OPG or AAV-Bmp7 and AAV-Bmp7-OPG groups, as shown in Table 4. Statistically significant differences were found in the total bone volume of the groups. The following total bone volumes were observed for the respective groups: AAV-EGF (48.13±1.54 mm^3), AAV-Bmp7 (58.34±2.28 mm^3), AAV-OPG (63.83±5.17 mm^3), and AAV-Bmp7-OPG (53.74±3.34 mm^3) (refer to Table 3). The AAV-OPG, AAV-Bmp7-OPG and AAV-Bmp7 groups all demonstrated higher total bone volume in comparison to the AAV-EGF group (refer to Table 3). The difference between AAV-Bmp7 and AAV-OPG groups was not statistically significant (p=0.066). There was no statistically significant difference in overall bone volume observed between the AAV-Bmp7 and AAV-Bmp7-OPG groups (p=0.154). However, the mean volume of alveolar bone for AAV-OPG was greater when compared to that of AAV-Bmp7-OPG (p=0.0001).There was a statistically significant difference (p=0.047) in the ratio of bone volume to total volume between the study groups. Table 3 shows that the mean alveolar bone volume ratios for the AAV-EGF, AAV-Bmp7, AAV-OPG and AAV-Bmp7-OPG groups were 80.49±3.41%, 79.70±0.41%, 78.11±3.22% and 84.73±8.90%, respectively. It is noteworthy that no statistically significant difference between the AAV-EGF group and AAV-OPG, AAV-Bmp7, or AAV-Bmp7-OPG groups was observed. The AAV-Bmp7 group showed no statistically significant difference between the AAV-OPG and AAV-Bmp7-OPG groups. However, the AAV-Bmp7-OPG group exhibited a higher volume fraction relative to the AAV-OPG group (refer to Table 4). The intraclass correlation coefficient (ICC) value was above 0.98 for all groups, confirming the reliability of the measurements.

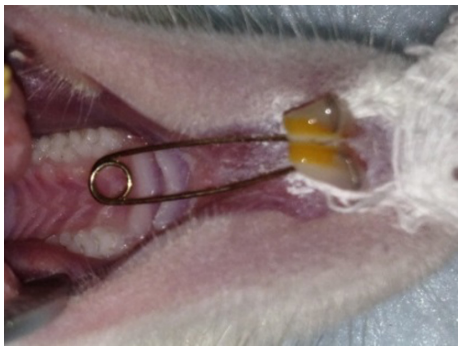

Figure 1. Appliance in-situ.

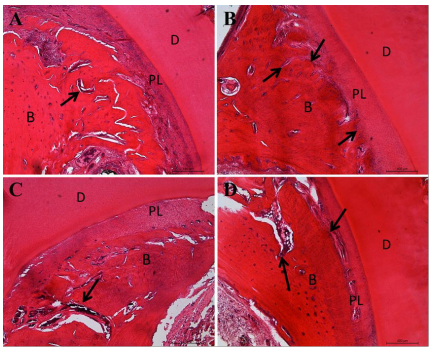

Figure 2. Experimental design.

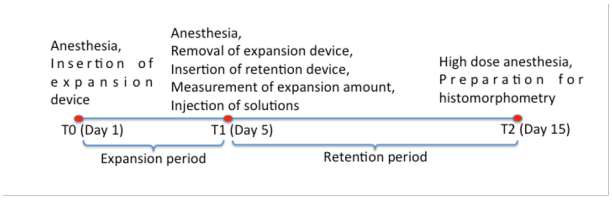

Figure 3. Wistar rats transfected with AAVs carrying the OPG, Bmp7 and EGF genes. The figure shows the experimental setup illustrating the process of gene transfer using adeno-associated vectors (AAVs) in Wistar rats.

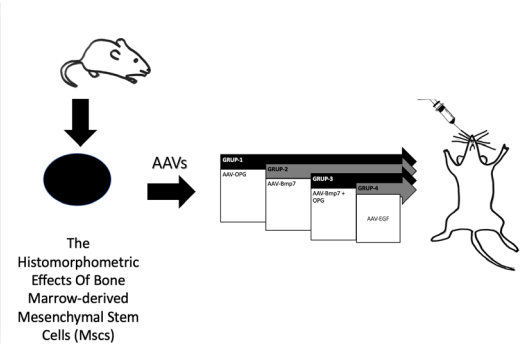

Figure 4. Photomicrographs show all groups (A=MSC, B= MSC-Bmp7, C= MSC-OPG, D= MSC-Bmp7-OPG). Bone (B), dentin (D) and areas of vascularization (arrows) are shown. Scale bar represents 400µm.

Table 1. Body weight changes (gr) comparison between groups and time points.

Groups	T0 (mean±standard deviation)	T1 (mean±standard deviation)	T2 (mean±standard deviation)	p*
AAV-EGF	170.6±5.51	172±2.24	170.8±1.3	0.549
AAV-Bmp7	179.2±7.79	180±8.06	179.2±7.79	0.368
AAV-OPG	172.2±4.97	172.6±5.98	172.2±5.85	0.662
AAV-Bmp7-OPG	170.6±3.78	171±3.08	170±3.08	0.678
p‡	0.31	0.257	0.293	

T0, initial weight; T1, weight at the end of expansion (5-day); T2, weight at the end of retention (10-day); $p<0.05$ significance level, ‡Kruskal Wallis Test, * Friedman test.

Table 2. Comparison of expansion amount between groups.

Groups	Amount of expansion (mm)
AAV-EGF	1.12±0.08
AAV-Bmp7	1.15±0.04
AAV-OPG	1.17±0.02
AAV-Bmp7-OPG	1.15±0.06
p*	0.275

Kruskal Wallis Test, *significance level $p<0.05$.

Table 3. Comparison of mean alveolar bone volume (mm^3), total volume (mm^3), bone to total volume ratio (%) of the groups.

Groups	Bone volume (mm^3)	Soft tissue volume (mm^3)	Total volume (mm^3)	Bone/Total volume ratio (%)
AAV-EGF	38.82±0.76	9.57±1.90	48.13±1.54	80.49±3.41
AAV-Bmp7	46.98±1.50	11.53±0.99	58.34±2.28	79.70±0.41
AAV-OPG	49.40±4.72	13.31±1.88	63.83±5.17	78.11±3.22
AAV-Bmp7-OPG	42.58±2.89	8.00±4.43	53.74±3.34	84.73±8.90
p	0.001*	0.011*	0.001*	0.047*

One way variance analysis, *significance level $p<0.05$.

Table 4. Group comparisons of mean bone volume (mm^3), total volume (mm^3), and bone to total volume ratio (%) of the groups.

Group's comparisons	Bone volume (mm^3)	Soft tissue volume (mm^3)	Total volume (mm^3)	Bone/Total volume ratio (%)
AAV-EGF/AAV-Bmp7	0.001*	0.544	0.001*	0.991
AAV-EGF/AAV-OPG	0.0001*	0.043*	0.0001*	0.823
AAV-EGF/AAV-Bmp7-OPG	0.122	0.699	0.045	0.440
AAV-BmpV/AAV-OPG	0.446	0.615	0.066	0.937
AAV-Bmp7/AAV-Bmp7-OPG	0.089	0.108	0.154	0.069
AAV-OPG/AAV-Bmp7-OPG	0.003*	0.01*	0.0001*	0.047*

Tukey multicomparison test, *significance level $p<0.05$.

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Discussion

Orthodontic expansion of the maxilla is utilised in patients with transverse deficiency of the maxillary arch. This procedure necessitates an additional retention period of 3 to 6 months to prevent the loss of expansion gained by the collapse of the maxillary halves [49]. The expanded maxilla tends to collapse back to its original width [5]; therefore prolonged retention of the expanded maxilla is mandatory to avoid a relapse [6,7]. The appliance must be retained in the mouth for at least 3 months to ensure minimal relapse [8]. There are numerous studies in the literature that aim to accelerate osteogenic activity in order to reduce retention time [12-22].

The maxillae of cats and monkeys resemble those of humans, making them useful animal models for studying maxillary enlargement. Rabbits and rats are often used for histological studies of sutures and bone under stress [49]. In animal studies, it is customary to employ the smallest possible animal to examine the effect. These animals are easy to produce and maintain, affordable, and enable efficient histological assessments. Consequently, the experimental protocol utilised rats in this study. Only adult male rats aged 12 weeks were selected to limit the influence of metabolic variations and the estrous cycle. The expansion process lasted for approximately five days, with a retention time of 10 days. This technique is utilised in literature involving animal models [16]. There are various methods for expansion. However, the intermaxillary suture is commonly separated under pressure to create an orthopaedic effect. It was also confirmed during sectional examinations that the suture was orthopaedically opened. The expansion observed was osseous rather than dental. In our study maxilla was fitted with a helical appliance of 0.014-inch stainless steel wire giving 0.5 N of force. In the study of Guerrero et al. [50] loops were bonded to the first and second maxillary molars on both sides and 0.28 N, 0.42 N and 0.56 N distracting forces were used at the midpalatal suture for 7 and 14 days. Micro-computed tomography, histomorphometry and quantitative polymerase chain reaction (qPCR) analysis were used to assess expansion efficiency, midpalatal suture remodelling, osteoblast, osteoclast and chondrocyte cell counts and expression of bone remodelling markers. A standardised protocol for RME in mice was identified, with force magnitudes of 0.28 N, 0.42 N and 0.56 N occurring on days 7 and 14. Time and force were found to be dependent on bone microstructure and expression of target genes. Martins et al. [51] observed that RME causes some functional limitations in children at the start of treatment but is nonetheless well tolerated by them and does not cause notable oral symptoms or significant social and emotional well-being changes. Our study's results demonstrated that the treatment did not adversely affect the body weight of rats, which provides support for the quality of life at the beginning of treatment. The study's samples underwent histomorphometric analysis for the assessment of new bone formation, instead of micro-CT evaluations, due to the unsuitability of the micro-CT tissue sampling preparation procedure for histomorphometry.

Rungcharassaeng et al. [52] carried out a study using cone beam computed tomography scans of 30 participants who underwent the rapid maxillary expansion procedure. The study reveals that the participants experienced buccal crown tipping, a reduction in buccal bone thickness, and bone loss at the margins 3 months after the RME protocol. The buccal tipping of the crowns is attributed to both the forces exerted during active expansion and the residual forces that act on the teeth during the retention phase. A study using CBCTs on patients who underwent RPE demonstrated a reduction in both the buccal alveolar bone density and height during the retention period [53].

To prevent potential side effects of prolonged retention, the current study aimed to investigate the effects of EFG, OPG, and Bmp7 on bone hardness. To achieve this, AAVs carrying stem cells, OPG, and Bmp7 genes were locally transferred to the inter-maxillary suture of rats that had undergone expansion in the present study. It is believed that OPG and Bmp7 can reduce osteoclastogenesis [41-43]. Furthermore, it is established that stem cells derived from adipose tissue or bone marrow contribute to new bone formation. This investigation monitored the sustained osteoblastic and decreased osteoclastic activity by locally injecting stem cells carrying OPG and Bmp7 genes and AAVs carrying these genes into the inter-maxillary suture of rats.42 Thus, the acceleration of new bone production was expected. Numerous studies have been conducted to assess agents and methods for osteogenic activity. Some of these studies have demonstrated positive effects locally or systemically. The method employed in our study is genetically based and elicits osteoblastic activity [12-23]. When the studies carried out in recent years are examined, Sun et al. [54] conducted a study to evaluate if Pyk2-deficiency, a tyrosine kinase marker for osteoclastic activity, increases mid-palatal suture bone mass and preserves sutural integrity after maxillary expansion. This study has the advantage of increasing bone density [54]. Also, OPG and Bmp-

OPG combination that we used have a parallel effect with this agent. Baudhuin et al. [55] have concluded that the expression of OPG reduces osteoclastogenesis and promotes bone hardening, resulting in the inhibition of bone softening. We found statistically significant results in the comparison of bone volume and total volume values between the AAV-EGF/AAV-OPG and OPG/AAV-Bmp7-OPG groups. These values indicate a positive effect of OPG on bone hardness, consistent with Baudhuin et al.'s findings [55]. In the study of Chen et al. [56], the dermal fibroblast cells of human origin were transfected with recombinant Bmp7 via adenovirus. The outcomes of soft X-ray, histologic and immunohistochemical analyses indicate significant support of osteogenesis by Bmp7. However, a comparison of the histomorphometric method with previous studies regarding the Bmp7 group reveals conflicting results. Our current research demonstrates Bmp7 to be ineffective in contrast to OPG.

In a retrospective study, Werle et al. investigated the efficacy of using rhBmp7 in conjunction with autologous bone grafts to treat symptomatic lumbar pseudoarthrosis patients. They concluded that this approach could be used in therapeutic interventions for these patients. In our study, bone volume was compared using AAV-EGF/AAV-Bmp7, AAV-Bmp7/AAV-OPG, and AAV-Bmp7/AAV-Bmp7-OPG values. Results showed that only AAV-EGF/AAV-Bmp7 had a statistically significant difference in bone volume. This difference may be attributed to the fact that OPG is potentially as effective as Bmp7, as previously mentioned.

Bilic et al. [32] applied autologous or allogeneic bone grafts with Bmp7 to scaphoid nonunion at the proximal pole. Radiographic, scintigraphic, and clinical evaluations revealed that Bmp7 improved the performance of both bone grafts and decreased healing time. OPG, an osteoprogenitor cell that produces bone matrix upon ossification, demonstrated greater bone volume than the other groups, indicating that Bmp7 is less effective than OPG in bone formation. The significant and unexpected finding is that the EGF, serving as the control group of AAVs, does not demonstrate a distinct effect compared to the Bmp7+OPG group. OPG and Bmp7 present inhibitory effects on each other's bone formation effect instead of synergising. For the total volume, which includes both soft tissue and bone volume, the bone volume gave comparable results. It was as expected that EGF would be less effective than Bmp7+OPG. Figure 3 in the histological sections already shows that EGF does not induce bone formation as much as OPG. There were no differences in soft tissue between control EGF and Bmp7 or Bmp7+Opg. The only group that demonstrated superiority to EGF was OPG. There was no difference between the Bmp7 and OPG or Bmp7+OPG groups. However, OPG was more effective in the Bmp7+OPG group with greater capillary formation. Given these results, a significant increase in soft tissue was predicted. Sadıkoğlu et al. [58] Sadikoglu et al. performed a study examining the histomorphometric impacts of hyaluronic acid of varying molecular weights on bone development in rats after interpremaxillary suture expansion. The authors concluded that applying high molecular weight hyaluronic acid locally to the interpremaxillary suture following rapid maxillary expansion instigates new bone formation. This may lead to a reduction in the retention time and the risk of recurrence. Low molecular weight hyaluronic acid has no impact on bone formation in interpremaxillary sutures. Our study has determined that OPG and Bmp7 demonstrate efficacy in promoting bone formation. To minimize or eliminate systemic side effects, the application was administered locally to assist in bone formation within a specific region and timeframe. However, some studies in the literature have shown different local and systemic effects [15,19]. A comparison can be made by studying the systemic effects of this agent in the future. As studies indicate that corticotomy enhances retention, future research can analyse the combined effect of these injections and corticotomy.

Conclusion

During bone remodelling, OPG can be employed to stimulate bone formation in the mid palatal suture. While not as efficacious as OPG, Bmp7 also participates in bone formation. The simultaneous use of OPG/BMP7 did not affect bone formation. Thus, gene and gene-cell applications were examined for effect of active bone formation in the intermaxillary suture after rapid maxillary expansion or surgical-assisted rapid maxillary expansion. The expansion protocol shows a distraction phase and this agent helps for retention protocol.

Conflict of Interest

There is no conflict of interest to declare.

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