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Original Article

The Condylar Effects of Mesenchymal Stem Cells, Low-Level Laser Therapy and Grape Seed Extract on Functional Mandibular Advancement of the Rat Mandible

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Main Points
- In rats, condylar remodeling can be achieved using experimental functional appliances.
- The injected adipose tissue -derived mesenchymal stem cells successfully remained in the condylar area and were found to be effective.
- The use of low -level laser therapy and grape seed extract increases the effects of adipose tissue -derived mesenchymal stem cells.
- The combination of adipose tissue -derived stem cells, low -level laser therapy, and grape seed extract with mandibular advancement is the most effective.
- Adipose tissue -derived mesenchymal stem cells are a promising cell source in bone tissue production and regeneration.

ABSTRACT

Objective: Functional treatment of Class II malocclusion is expected to lead to adaptation in the condyle. This study aimed to evaluate the effects of adipose tissue-derived mesenchymal stem cells (ADMSCs), low-level laser therapy (LLLT), and grape-seed extract (GSE) on condylar growth after functional mandibular advancement.

Methods: Forty-five rats were randomly divided into 8 groups. Functional appliances were applied to all groups (n=6) except the control group (n=3). One group was treated with appliances only; the other six groups received various combinations of ADMSCs, LLLT, and GSE. Analyses for new osteoblasts and new bone formation, vascular endothelial growth factor, and Type II collagen were performed on condylar tissues, after an experimental period of four weeks. The quantitative data obtained from the results of the experiments were evaluated by H-score and analyzed using One-Way ANOVA by Tukey-Kramer multiple comparisons test ($p \leq 0.05$).

Results: Levels of all investigated parameters increased in all groups ($p \leq 0.05$). The highest increases were achieved by a combined application of functional appliance, ADMSCs, LLLT and GSE ($p \leq 0.05$). Single LLLT administrations or single GSE applications did not create a statistical difference from appliance alone ($p > 0.05$). A positive effect of ADMSCs or LLLT on osteoblast formation, neovascularization, and Type II collagen level was apparent ($p \leq 0.05$), however, neither affected new bone formation ($p > 0.05$).

Conclusion: This study shows that ADMSCs with LLLT and GSE applications provide differing levels of new osteoblast and bone formation, new vascular formation, and Type II collagen formation in rat condyles after functional mandibular advancement.

Keywords: Functional mandibular advancement, grape-seed extract, laser therapy, mandibular condyle, stem cell therapy

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INTRODUCTION

Class II malocclusion with mandibular retrognathism is one of the most common clinical orthodontic problems that can be addressed using functional appliances. These appliances reposition the mandible in a forward-and-downward direction, which may accelerate and increase mandibular growth.[1] Previous studies have shown that forward positioning of the mandible leads to increased new bone formation in the condyle and the posterior region of the glenoid fossa.[2] This new bone formation can be reached its maximum level within four weeks in the rats.[3,4] The mandibular condylar cartilage can respond to environmental stimuli, such as ultrasound application, laser application,[4,5] systemic administration of hormones[6] and steroids,[7] as well as mechanical stimulation with functional appliances.[8]

The development of genetic research has facilitated investigations into the effects of adipose-derived mesenchymal stem cells (ADMSCs) on growth and development. ADMSCs have the ability to differentiate into various types of cells with mesenchymal origins, including osteoblasts, chondroblasts and myoblasts and are easily obtained from adult bone marrow, cartilage and adipose tissue. They are considered vital components for new bone formation.[9] Bone-marrow stem cells have been used to increase osteogenic differentiation in orthodontically expanded maxilla in rats. Local administration of ADMSC led to new bone formation, osteoblast formation and vascularization in the maxilla.[10]

LLLT has been used to increase the tissue regeneration.[11] Previous research on rabbit condyles indicated that LLLT had beneficial effects in accelerating condylar remodeling and enhancing new bone formation during mandibular advancement.[12] In rats, increased osteoblast and chondroblast activities resulting in condylar growth and mandibular length increase were observed after functional mandibular advancement and LLLT.[5]

Various physical and chemical stimuli can induce the differentiation of ADMSCs and LLLT is one such stimulus that allows ADMSCs to remain in the implanted area for longer periods, aiding regenerative events by increasing the release of various growth factors by ADMSCs.[13]

GSE is a flavonoid derivative with important antioxidant properties.[14] It has been shown to stimulate angiogenesis and possess anti-inflammatory, anti-oxidative, anti-cancer, anti-diabetic, anti-allergic, cardioprotective, vasodilator, cholesterol-lowering, and dermal wound healing mechanisms.[15] Proanthocyanidins, which are the active components of GSE, have not been found to have toxic and mutagenic effects at high-doses (1400-1500 mg/kg/day).[14] Alternatively, use of GSE combined with calcium has been shown to increase bone formation by enhancing osteoblast differentiation.[16]

The aim of the present study is to investigate the effects and synergy of LLLT, and GSE on the action of ADMSCs, in functionally induced condylar growth. The use of these stimulants in various combinations was evaluated in terms of their effects on condylar growth, with respect to the amount of new osteoblast and bone formation, condylar vascularization, and Type II collagen. The hypothesis of the study was that the applications of ADMSCs with LLLT and GSE would not significantly change the investigated parameters in rat condyles after functional mandibular advancement.

METHODS

Animals

All animal experiments were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and ethical permission was obtained from the Manisa Celal Bayar University Ethics Committee of Experimental Animal Use and Research Scientific Committee (approval no: 71, date: 22.11.2016).

Forty-five Wistar-albino rats (6-week old, weighing 250±50 gr) were randomly divided into 8 groups. Functional appliances were administered to all study groups (n=6) except the control group (group C; n=3). Groups were treated with appliances only; the other six study groups received single administration or various combinations of ADMSCs, LLLT, and GSE, as shown in Table 1.

All the rats were housed in the same well-controlled environment, maintained under 12-hour light-dark cycles (with lights on 7:00 AM to 7:00 PM), at room temperature of 18-22 °C and a relative humidity of 40-60%. The study groups were housed in separate cages and provided with a soft diet to prevent weight loss due to the appliances. Water was available ad libitum to all rats throughout the experimental period.

Appliance Fabrication

Plaster models were prepared from impressions of the lower anterior teeth. 1 mm thickness vacuum-formed clear acrylic plates were the fabricated (Clear Advantage, OrthoTechnology, USA) on the models to create an inclined plane, which would move the mandible forward. The acrylic appliances were positioned in a way that caused mandibular forward-downward positioning during both resting rest and functional bite in the rats. When the appliance was bonded, the lower incisors of the subjects were positioned in front of the upper incisors, creating an anterior crossbite. In this state, the subjects were unable to retract their lower jaw.[3,8] The subjects were anesthetized intraperitoneally using a mixture of 20% lidocaine and 80% ketamine-hydrochloride. The lower incisors were washed, dried, and coated evenly with Transbond™ Plus self-Etching Primer (3M Unitek, Monrovia, California, USA) and the acrylic appliances were bonded to lower incisors using light-cured composite (3M™ESPE™Z100™, USA) (Figure 1).

Preparation and Administration of ADMSCs

ADMSCs were collected from adipose tissue of two rats not included in the study groups, following the procedures described

by Aydemir et al.[17] The cells were cultured until passage P3, and ADMSC characterization was performed immunocytochemically using Stro-1, c-kit, CD45, and CD105 markers (Figure 2).

After the characterization of ADMSC, each condylar region received a dose of $1x10^6$ ADMSCs/mL through intra-articular injections under general anesthesia. To locate the temporomandibular joint (TMJ), the mandible was positioned forward to allow palpation of the TMJs, which are located approximately 5-10 mm posterior to the lateral canthus of each eye. A 30-gauge needle was then inserted posterior to the zygomatic process of the temporal bone and moved medio-anteriorly into the TMJ spaces[18] where ADMSCs were slowly injected (Figure 3A). In addition to local administration, ADMSCs were also given intraperitoneally[19] on the same day and at the same hour to prevent the decline of ADMSCs and to enhance the involvement of the condyle and the efficacy of ADMSCs. The intraperitoneal injection was intended to improve the treatment's effectiveness.

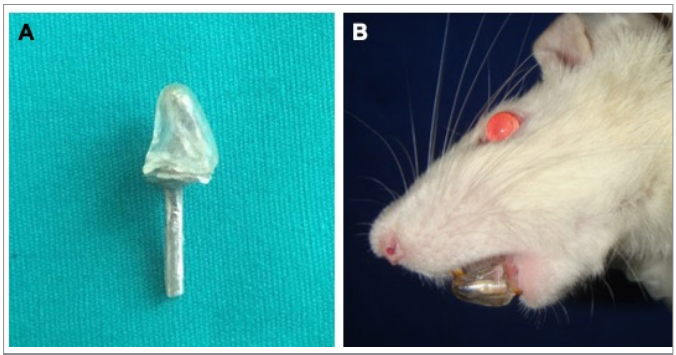

Figure 1. Acrylic appliance (A) was cemented on the lower incisors of experimental rats to move the mandible into a forward position during rest and function (B)

LLLT Irradiation

A low-level diode laser (SiroExtend Laser, 8 J/cm^2, 970 nm, 0.5 watt, 16 seconds) was used to irradiate each rat condyle once in every two days for four weeks (Figure 3B) (Table 1).[8]

Administration of GSE

GSE was obtained from grapes (Vitis-Vinifera L.) of the Denizli province in Turkey, known for their large and isomorphic seeds and prepared according to the method described by Erdemli et al.[20]. The extracts were stored at +4 °C and administered systemically by way of orogastric gavage, diluted with distilled water. The amount of extract given to each group and the number of applications are shown in Table 1 (Figure 3C).[20]

Histopathological Analyses

All rats were euthanized after four weeks through a high-dose of anesthesia. Mandibles were dissected and divided into right

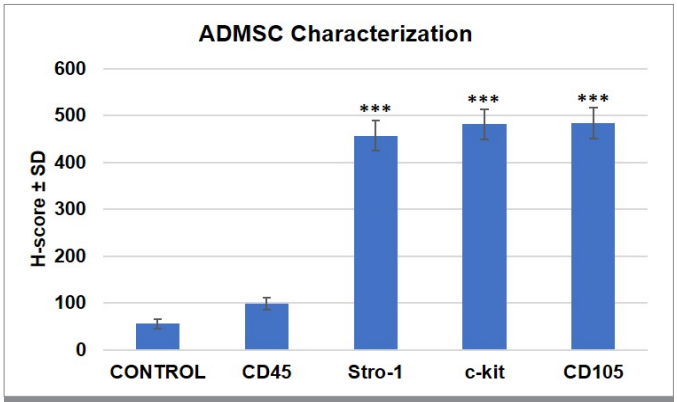

Figure 2. The characterization of ADMSCs immunocytochemically via CD45, Stro-1, CD44, and CD90 markers. Because of the H-score evaluation, Stro-1, c-kit, and CD105 positivity and CD45 negativity were detected ADMSCs, Adipose tissue-derived mesenchymal stem cells

Table 1. Applications and procedures for the experimental groups

Group (n)	Procedure	Application type	Dose	Frequency	Total application number
Control group (3)	-	-	-	-	-
Appliance group (6)	-	-	-	-	-
ADMSCs-LLLT group (6)	**MSCs**	Local	$1x10^6$ UI/mL	1/4 weeks (begining)	1
		Systemic	$1x10^6$ UI/mL	1/week	4
	LLLT	Local	8 J/cm^2	1/2 day	15
ADMSCs-GSE group (6)	**MSCs**	Local	$1x10^6$ UI/mL	1/4 weeks (begining)	1
		Systemic	$1x10^6$ UI/mL	1/week	4
	GSE	Systemic	300 mg/kg/day	1/day	30
ADMSCs-LLLT-GSE group (6)	**MSCs**	Local	$1x10^6$ UI/mL	1/4 weeks (begining)	1
		Systemic	$1x10^6$ UI/mL	1/week	4
	LLLT	Local	8 J/cm^2	1/2 day	15
	GSE	Systemic	300 mg/kg/day	1/day	30
LLLT group (6)	**LLLT**	Local	8 J/cm^2	1/2 day	15
LLLT-GSE group (6)	**LLLT**	Local	8 J/cm^2	1/2 day	15
	GSE	Systemic	300 mg/kg/day	1/day	30
GSE group (6)	**GSE**	Systemic	300 mg/kg/day	1/day	30

LLLT, Low-level laser therapy; ADMSCs, Adipose tissue-derived mesenchymal stem cells; GSE, Grape-seed extract

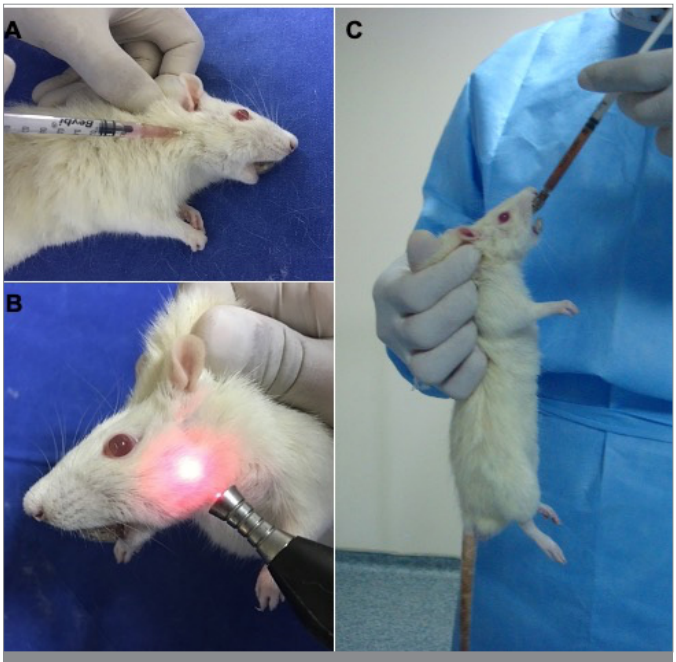

Figure 3. The applications of ADMSCs in the temporomandibular joint spaces (A), LLLT (B), and GSE via orogastric gavage (C)

LLLT, Low-level laser therapy; ADMSCs, Adipose tissue-derived mesenchymal stem cells; GSE, Grape-seed extract

and left parts at symphysis, and kept in a 10% formaline solution (Merck, Germany) until histochemical analyses were performed. The condyle was chosen as the side for cell counting because the most prominent cellular responses to mandibular repositioning occur in the condyle.

The left side condyle of each mandible was used for examination. Samples were embedded in parafine and cut into 5 µm thickness, and sections were taken from the posterior region of the condyles. Tissue sections were stained with hematoxylin-eosin and Masson's Trichrome dyes.[17] Sections were evaluated with a light microscope (BX43, Olympus, Japan) and photographed (SC50, Olympus, Germany) to investigate two histological parameters (Figure 4). The histomorphometrics evaluation was conducted by two histologists. The number of new osteoblasts was classified as mild (+, 0–15 cells), moderate (++, 15–30 cells), and strong (+++, >30 cells). The bone formation was scored between +1 to +5.[17]

Immunohistochemistry

To detect vascularization and collagen formation, primary antibodies anti-VEGF (Sigma V1253, St Louis, Mo , USA) and anti-Type-II collagen (Sc7763, Santa-Cruz Biotechnology, Calif, USA) were used. After dewaxing in xylene, the sections were dehydrated with ethanol. They were then incubated with 0.5% trypsin at 37 °C for 15 minutes and endogenous peroxidase activity was inhibited using hydrogen peroxide (Merck). Blocking serum was applied for 1 hour, followed by incubation with primary antibodies anti-VEGF and anti-Type-II collagen at 4 °C overnight. The sections were then treated with the anti-mouse biotin-streptavidin hydrogen peroxidase secondary antibody (85–9043 Zymed Histostain kit). Immunoreactivity was made

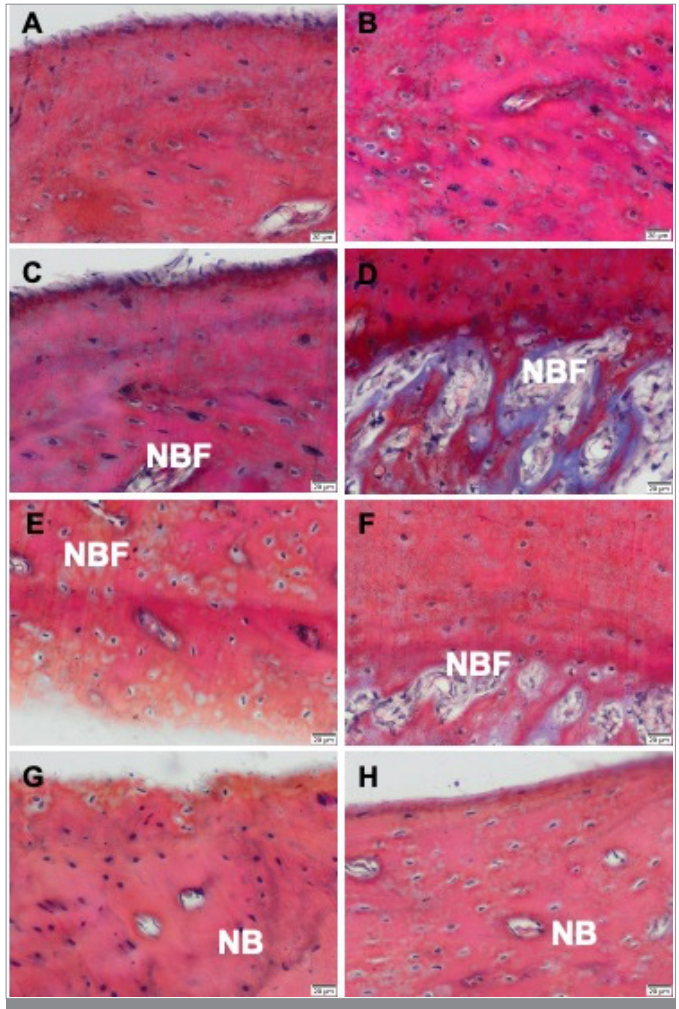

Figure 4. Histochemical staining of experimental groups with Masson's Trichrome; A) Control group, B) Appliance group, C) GSE group, D) LLLT group, E) LLLT-GSE group, F) ADMSCs-GSE group, G) ADMSCs-LLLT group, H) ADMSCs-LLLT-GSE group

LLLT, Low-level laser therapy; ADMSCs, Adipose tissue-derived mesenchymal stem cells; GSE, Grape-seed extract; NBF, New bone formation; NB, New bone; Scale bars, 20 µm

visible using diaminobenzidine (DAB, 00–2014, Invitrogen), and counterstaining was performed using Mayer's hematoxylin (800-729-8350, ScyTek). The sections were coated with entellan, and evaluated using a light microscopy (BX43, Olympus, Japan) by three independent researchers.[17] The procedure was performed three times. The immunoreactivity was evaluated as no (0), weak (+), moderate (++), and strong (+++), and stained cells were counted for each staining degree. The H-score value was calculated using the formula: Pi (intensity of staining + 1), where Pi is the percentage of stained cells for each intensity.[17]

Statistical Analysis

The data were presented as mean±standard deviation and analyzed using GraphPad software (San Diego, USA) after performing a normality test. Statistical significance was considered at $p \leq 0.05$, and the One-Way ANOVA followed by Tukey-Kramer multiple comparisons test was performed for data analysis.[17]

RESULTS

Clinical Observations

Daily observations were performed for appliance irritation on tissues and feeding behaviors. During the experiment, an appliance was broken and replaced. The rats were weighed on the first day of the experiment and immediately before they were euthanized. None of the animals showed any weight loss at the end of the experiment.

Histochemical and Immunohistochemical Results

Descriptive statistics of the groups for all four parameters (New osteoblast formation, new bone formation, VEGF and Type II collagen) and test results of paired group comparisons can be found in Tables 2 and 3.

Compared to the control group, all study groups showed an increase in all parameters, with the most significant increase observed in ADMSCs-LLLT-GSE group ($p<0.001$). Although all combinations of stimulants resulted in significant differences compared to the appliance only group (Appliance vs ADMSCs-LLLT; Appliance vs ADMSCS-GSE; Appliance vs ADMSCs-LLLT-GSE; Appliance vs LLLT-GSE groups), single LLLT administrations or single GSE applications did not create a statistical difference (Appliance group vs LLLT group; Appliance group vs GSE group) ($p>0.05$).

Table 2. Comparison of new osteoblast and bone formations between groups

		Mean±SD	Differences between appliance group	Differences between ADMSCs-LLLT group	Differences between ADMSCs-GSE group	Differences between ADMSCs-LLLT-GSE group	Differences between LLLT group	Differences between LLLT-GSE group	Differences between GSE group
Control group	**New Osteoblast Formation**	15.0±2.0	-4.8*	-13.0***	-12.0***	-14.5***	-8.3***	-11.0***	-5.7**
	New Bone Formation	1.4±0.6	-1.0*	-3.2 ***	-2.3***	-3.3***	-1.8**	-2.2***	-1.3*
Appliance group	**New Osteoblast Formation**	19.9±2.1		-8.2***	-7.1***	-9.7***	-3.5 NS	-6.2***	-0.8 NS
	New Bone Formation	2.4±0.4		-2.2***	-1.3**	-2.3***	-0.8 NS	-1.2*	-0.3 NS
ADMSCs-LLLT group	**New Osteoblast Formation**	28.2±1.7			-1.0 NS	-1.5 NS	-4.7**	-2.0 NS	-7.3***
	New Bone Formation	4.6±0.5			-0.8 NS	-0.2 NS	-1.3**	-1.0 NS	-1.8***
ADMSCs-GSE group	**New Osteoblast Formation**	27.4±1.9				-2.5 NS	-3.7*	-1.0 NS	-6.3***
	New Bone Formation	3.7±0.6				-1.0 NS	-0.50 NS	-0.2 NS	-1.0 NS
ADMSCs-LLLT-GSE group	**New Osteoblast Formation**	29.4±2.3					- 6.2**	-3.5 NS	-8.8***
	New Bone Formation	4.7±0.5					-1.5**	-1.2*	-2.0***
LLLT group	**New Osteoblast Formation**	23.3±1.9						-2.7 NS	-2.7 NS
	New Bone Formation	3.2±0.9						-0.3 NS	-0.5 NS
LLLT-GSE group	**New Osteoblast Formation**	26.0±1.4							-5.3***
	New Bone Formation	3.6±0.6							-0.8 NS
GSE group	**New Osteoblast Formation**	20.7±1.9							
	New Bone Formation	2.7±0.6							

Tukey-Kramer multiple comparisons test. *p<0.05; **p<0.01; ***p<0.001; NS: p>0.05 and SD: Standard deviation. The units for New Osteoblast Formation: Cell count; for New Bone Formation: Trabeculae count

Table 3. Comparison of VEGF and Type-II Collagen immunoreactivities between groups

		Mean±SD	Differences between appliance group	Differences between ADMSCs-LLLT group	Differences between ADMSCs-GSE group	Differences between ADMSCs-LLLT-GSE group	Differences between LLLT group	Differences between LLLT-GSE group	Differences between GSE group
Control group	**VEGF**	56.7±3.2	-6.8*	-24.2***	-21.7***	-28.5***	-10.5***	-12.5***	-8.5**
	Type-II Collagen	45.3±3.5	-13.1***	-39.7***	-33.8***	-44.3***	-19.7***	-22.5***	-17.3***
Appliance group	**VEGF**	63.5±1.8		-17.3***	-14.8***	-21.7***	-3.7 NS	-5.7*	-1.7 NS
	Type-II Collagen	58.5±4.3		-26.5***	-20.7***	-31.2***	-6.5 NS	-9.3**	-4.2 NS
ADMSCs-LLLT group	**VEGF**	80.5±2.9			-2.5 NS	-4.3 NS	-13.7***	-11.7***	-15.7***
	Type-II Collagen	85.0±2.9			-5.8 NS	-4.7 NS	-20.0***	-17.2***	-22.3***
ADMSCs-GSE group	**VEGF**	78.3±3.3				-6.8**	-11.2***	-9.2***	-13.2***
	Type-II Collagen	79.1±4.2				-10.5***	-14.2***	-11.3***	-16.5***
ADMSCs-LLLT-GSE group	**VEGF**	85.3±2.6					-18.0***	-16.0***	-20.0***
	Type-II Collagen	90.8±2.1					-24.7***	-21.9***	-27.0***
LLLT group	**VEGF**	67.1±2.6						-2.0 NS	-2.0 NS
	Type-II Collagen	65.0±3.2						-2.9 NS	-2.3 NS
LLLT-GSE group	**VEGF**	69.1±2.4							-4.0 NS
	Type-II Collagen	67.8±3.8							-5.2 NS
GSE group	**VEGF**	65.2±2.1							
	Type-II Collagen	62.7±3.9							

Tukey-Kramer multiple comparisons test. *p<0.05; **p<0.01; *** p<0.001; NS: p>0.05 and SD: Standard deviation. The units for VEGF and Type-II Collagen: H-scores

The positive effects of ADMSCs or LLLT (ADMSCs-GSE vs ADMSCs-LLLT-GSE; LLLT-GSE vs ADMSCs-LLLT; LLLT-GSE vs ADMSCs-GSE; GSE vs ADMSCs-GSE groups) were evident on neovascularization (VEGF), and Type II collagen levels compared to other groups (ADMSCs-GSE vs ADMSCs-LLLT-GSE; LLLT-GSE vs ADMSCs-LLLT; LLLT-GSE vs ADMSCs-GSE; GSE vs ADMSCs-GSE) (p≤0.05). However, neither ADMSCs nor LLLT affected new bone formation [LLLT group vs ADMSCs-GSE group, (p>0.05)]. Comparisons of the triple combination group (ADMSCs-LLLT-GSE group) with LLLT-GSE group indicated that the addition of ADMSCs increased new bone formation (p≤0.05), but had no visible effect on the number of osteoblasts (p>0.05). Osteoblast formation was high in all stimulant combinations, and the increase in the triple combination group was not statistically different.

GSE did not increase the effect of LLLT or ADMSCs for any of the four parameters being examined (ADMSCs-LLLT vs ADMSCs-LLLT-GSE; ADMSCs-GSE vs ADMSCs-LLLT; LLLT vs LLLT-GSE; GSE vs LLLT groups) (p>0.05). When applied together, ADMSCs and LLLT proved more effective than a single GSE administration (GSE vs ADMSCs-LLLT; GSE vs ADMSCs-LLLT-GSE groups) (p<0.001). ADMSCs and GSE together were very effective; they even increased the effectiveness of LLLT on all parameters, although new bone formation was slightly lower than the increase in the other three parameters. (Appliance vs ADMSCs-GSE; LLLT vs ADMSCs-LLLT-GSE groups). When applied together, LLLT and GSE were more effective compared to an appliance only (Appliance group vs LLLT-GSE group) (p≤0.05); but not compared to single LLLT or single GSE administration (LLLT vs LLLT-GSE; GSE vs LLLT-GSE groups) (p>0.05).

DISCUSSION

Many studies have contributed to our knowledge of condylar growth stimulation in rats, including an increase in the number of osteoblasts and new bone formation in the condyle,

increased growth potential of the mandible,[8,9,21] an increase in neovascularization,[22] and Type-II collagen expression.[2] These findings are consistent with with the results of this study and support the deduction that osteoblast formation, new bone formation, VEGF, and Type-II collagen might be enhanced with the active advancement of the lower jaw in rats. Different combinations of ADMSCs, LLLT and GSE in addition to appliance use, increased these findings to varying degrees. While the increases due to the use of LLLT and GSE alone or together, did not reach statistically significant levels, the highest statistically significant increases were seen in the groups administered with ADMSCs. These results lead us to assume that appliance use, LLLT or GSE, can activate ADMSCs, which in turn, increases chondroblastic and osteoblastic activity.

Transformation of ADMSCs into osteoblasts and chondroblasts can increase bone and cartilage production.[10] Additionally, ADMSCs can increase vascularization.[9] Similarly, in this study, the number of osteoblasts increased significantly in all ADMSC groups. In order for to the ADMSCs to be activated and able to differentiate, they must be stimulated. They remain in a non-proliferative silent phase until they are activated by stimulatory signals initiated by remodeling forces or tissue damage, which prompts them to differentiate into the desired cell type for repair and remodeling.[23] The effects of intra-articular injection of ADMSCs combined with low-intensity pulsed ultrasound on developing rats increased the growth of the mandibular condyle according to CBCT and histological analyses. This change was not significant when ADMSCs were applied alone.[24] Therefore, in this study, ADMSCs were not administered alone, but rather in combination with a functional appliance, LLLT and GSE to achieve activation of ADMSCs.

Current studies indicate that LLLT increased osteoblast differentiation and cellular proliferation, as well as collagen deposition,[25] leading to new bone formation.[5,12] Based on these findings, LLLT is expected to increase bone and cartilage formation, promoting faster and more permanent remodeling of bone during orthodontic treatment.[26] Therefore, different protocols for LLLT application have been reported in the literature. The safe dose range for LLLT applications is known as 6-10 J/cm^2 .[27] In this study, an 8 J/cm^2 protocol was selected as it fell within the middle of the safe dose range reported in the literature.[4,8] However, in this study, the increase in four parameters using LLLT and appliance, was not statistically significant. This may be attributed to differences in doses and methods used between studies, as well as the short duration, terminating the experiment at an early stage of bone formation. Nevertheless, the addition of LLLT to the ADMSCs-GSE combination managed to increase VEGF and Type-II collagen levels.

Some studies that investigated the effects of LLLT on ADMSCs, with some reporting a bio-stimulatory effect of LLLT on ADMSC proliferation under favorable conditions,[28] while others failed to confirm this synergic effect.[29] In the present study, the use of LLLT combined with ADMSCs resulted in increased levels of all parameters. These findings suggest that the application protocols for LLLT and ADMSCs may have an impact on the outcomes obtained.

The administration of GSE has been shown to induce bone formation, accelerate osteoblast differentiation, increase the amount of cortical bone and mineral content of trabecular bone,[30] particularly in the condyle,[15] when combined with dietary calcium.[15,30] It is believed that proanthocyanidin is responsible for inhibiting bone resorption by inhibiting proteolytic enzyme activity.[31] In this study, GSE was systemically administered, and while its use in combination with the appliance increased the effect on all parameters in the condyle, the increase was not statistically significant. It is possible that differences in methodology may have a greater impact on the results than anticipated.

Study Limitations

A limitation of this study was the absence of a single ADMSC group. This decision was based on previous studies that demonstrated that ADMSCs are not effective without an external stimulant and to avoid increasing the number of animals used in the study.

Another potential limitation of this study is the relatively short duration of the experiment, which may have been insufficient to fully evaluate new bone formation. In future studies, longer durations could be considered to more thoroughly evaluate the effects of the interventions on bone formation. Additionally, more accurate methods such as micro-CT could be used to evaluate new bone formation in a more precise manner.

The results of the present study suggest that the combination of all three stimuli (appliance, LLLT and GSE) resulted in the most effective activation of ADMSCs. The triple group demonstrated the highest statistically significant increase in new bone formation, VEGF and Type-II collagen. ADMSCs appear to be a promising cell source for osteogenic, chondrogenic and vascular tissue generation. However, further animal studies are necessary before clinical application can be considered.

Based on the results of the present study, it is believed that vascularization could be enhanced with various combinations interventions, resulting in increased bone formation in a shorter time in the condyle. These clinical interventions may compensate for factors that prolong the treatment period, leading to more successful outcomes in a shorter time, reducing the need for patient cooperation and increasing the comfort of both the patient and physician.

CONCLUSION

The data obtained from this study suggest that the effects of LLLT, and GSE on the action of ADMSCs in functionally induced condylar growth were significant. The combined use of these stimulants showed the greatest synergy in terms of their effects on condylar growth, including the amount of new osteoblast and bone formation, condylar vascularization, and Type-II collagen.

Ethics

Ethics Committee Approval: Ethical permission was obtained from the Manisa Celal Bayar University Ethics Committee of Experimental Animal Use and Research Scientific Committee (approval no: 71, date: 22.11.2016).

Informed Consent: Animal experiment study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.N.E.; Design - M.N.E.; Data Collection and/or Processing - M.N.E., I.A., M.İ.T.; Analysis and/or Interpretation - A.G., I.A.; Literature Review - A.G.; Writing - M.N.E.; Critical Review - A.G.

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