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Comparison of the effectiveness of the new generation dual wavelength laser with the conventional laser type in treating rabbit model temporomandibular joint osteoarthritis

Gizemnur Karadayı¹ , Nihat Akbulut² , Faik Alev Deresoy³ and Ahmet Altan^{4*}

Abstract

Background This study compared the effects of low-level laser applications with different wavelengths on rabbits' temporomandibular joint regeneration capacity with experimentally induced osteoarthritis.

Methods This study used 23 male New Zealand white rabbits. The animals were randomly divided into three groups. Osteoarthritis was induced in all groups by injecting sodium monoiodoacetate into the rabbits' joints. The joints of the experimental animals in Group 1 (GRR laser-dual wavelength, $n=8$) and Group 2 (Epic10 laser-conventional laser, $n=8$) were treated with laser for 14 sessions. In the control group ($n=7$), no treatment was performed after osteoarthritis was induced. After the end of laser therapy, the rabbits were sacrificed simultaneously, and the temporomandibular joints were removed. Defect filling percentage, osteochondral junction reconstruction, matrix staining, and cellular morphology were examined histopathologically.

Results There was a connection between the groups with defect filling, and a statistically significant difference was found ($p=0.005$). When we examined whether there was a connection between the osteochondral junction and the groups, it was determined that there was no statistically significant connection ($p=1.000$). It was determined that there was a significant connection between the groups with matrix staining ($p=0.001$). There was a connection between cellular morphology and the groups. It was determined that there was a statistically significant connection ($p=0.018$). The defect filling, matrix staining, and cellular morphology values in both laser groups were better than in the control group.

Conclusions Low-level laser therapy can increase remodeling and improve tissue repair in temporomandibular joint osteoarthritis treatment. There was no significant difference between lasers at different wavelengths in treating temporomandibular joint osteoarthritis.

Keywords Low-level laser therapy, Osteoarthritis, Temporomandibular joint disorders

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Background

Temporomandibular joint disorders (TMD) is a term that includes many closely related conditions, including functional changes and pathological conditions of the temporomandibular joint (TMJ), maxillofacial region, and associated muscles. Clinical studies suggest that 93% of the global population experience at least one TMD symptom at some point during their lifetime, and up to 20% of these individuals subsequently seek treatment for these difficulties [1].

Temporomandibular joint osteoarthritis (TMJ-OA) is an important subtype of temporomandibular disorders (TMDs). The etiology of most TMJ-OA is complex and multifactorial. It may develop secondary to disc displacement, trauma, functional overload, and developmental anomalies [2]. Among TMDs, TMJ-OA accounts for 18-85% of all cases [3].

Osteoarthritis treatment aims to prevent the progression of the disease, eliminate pain and limitation of movement, and restore function. Non-invasive and invasive treatment options are available. Among non-invasive treatments, patient education, occlusal splinting, thermal treatments, laser applications, and physiotherapy are non-pharmacologic treatment methods [4]. Pain relief constitutes an essential part of the treatment. Patients with joint pain may be prescribed non-steroidal anti-inflammatory drugs and muscle relaxants [5]. Invasive treatments such as open surgery (arthroplasty) and temporomandibular joint (TMJ) replacement are only used when all other possible treatment options have been exhausted [6].

Laser applications are important among conservative dentistry and maxillofacial surgery procedures. In the literature, laser applications for bio stimulation are referred to as low-level laser therapy (LLLT). The purposes of LLLT include accelerating the wound healing process, enhancing bone repair and remodeling, restoring normal nerve function after nerve injury, reducing pain, stimulating endorphin release, and modulating the immune system [7]. Recent studies suggest that new-generation dual-wavelength lasers are more effective than conventional lasers. New generation dual wavelength lasers penetrate tissue more than conventional lasers (≤ 5 mm). They can treat tissue or organs deeper (≤ 50 mm) [8, 9].

This study compared the effects of single-wavelength conventional (Epic10 laser, Biolase Inc. California, USA) and dual-wavelength new-generation low-energy laser (GRR LASER[®], 2000, Ankara, Turkey) applications on the regeneration capacity of the rabbit temporomandibular joint.

Methods

This study was conducted at Tokat Gaziosmanpaşa University Experimental Medicine Research Unit with the approval of Tokat Gaziosmanpaşa University Animal Experiments Local Ethics Committee dated 01.12.2020, file number 51879863/141. The project was financially supported by Tokat Gaziosmanpaşa University Scientific Research Unit dated 16.03.2021 and coded 2021/31. The rabbits used in the study were purchased from the Saki Yenilli Experimental Animals Production Laboratory (Ankara, Turkey).

Animals

Our study was conducted on 23 adult male white New Zealand rabbits weighing 2.5-3 kg. The rabbits were housed in compartmentalized cages at 21°C in an environment with continuous warm and fresh air, 12 h day and 12 h night with artificial lighting and sunlight. Animals were randomly divided into three groups. In all groups, osteoarthritis was induced by injecting sodium monoiodoacetate (SMIA) into the joints of rabbits. The joints of the experimental animals in Group 1 (GRR laser, $n = 8$) and Group 2 (Epic10 laser, $n = 8$) were treated with laser for 14 sessions. In the control group ($n = 7$), no treatment was performed after osteoarthritis was induced. After the end of the laser therapies, the rabbits were sacrificed simultaneously, and the temporomandibular joints were removed and examined histopathologically.

Experimental osteoarthritis

For anesthesia, 50 mg/kg ketamine hydrochloride (Ketalar[®] fl., Pfizer, 50 mg/ml solution) and 5 mg/kg xylazine hydrochloride (Rompun[®] inject. 2% solution Bayer, Germany) were administered intramuscularly. After anesthesia, the hair in the left TMJ region was shaved. The joint area was wiped with povidone-iodine (Betadine[®] solution Kansuk, Turkey) for antisepsis. To induce osteoarthritis, SMIA (Sigma I 2 512-25G, St. Louis, MO, United States of America) was prepared by mixing with saline at 3 mg per ml following the study by Güler et al. [10]. To inject directly into the joint capsule, an incision was made 1 cm lateral to the orbital corner with a number 15 scalpel. Soft tissues were dissected, and the capsule was reached. A 29-gauge insulin injector was used for intra-articular injections. Control Group, Group 1 (GRR laser), and Group 2 (Epic10 laser) rabbits were injected intra-articularly with 1 ml of 3 mg/ml MIA solution in the left TMJ (Fig. 1). After waiting four weeks for osteoarthritis to develop, laser applications were started.



Fig. 1 Injection of MIA



Fig. 2 Application of GRR Laser

Treatment protocol

Group 1 (GRR laser)

In this group, the GRR LASER device, which is a combination of a 22 mW IR (infrared) LED with a wavelength of 904-940 nm and a 10 mW GaAlAs (Gallium Aluminum Arsenide) laser with a wavelength of 632-650 nm, was used (GRR LASER®, 2000, Ankara, Turkey). This probe contains one red laser and four infrared LEDs. The energy transfer time for a one-minute application is 16 J. Following the manufacturer's instructions, the laser was applied for five consecutive days (Monday to Friday), and for the other three weeks, the laser was applied on Mondays, Wednesdays, and Fridays for a total of 14 (5 + 9) sessions of 10 min laser application for four weeks. (Fig. 2).

Group 2 (Epic10 laser)

The Epic10 laser (Biolase Inc. California, USA) is a GaAlAs laser operating at a wavelength of 940 nm and an energy density of 5 J. The area of the external probe used is 2.8 cm² (35 mm × 8 mm). For the left joint region, each rabbit received 14 sessions of 10-min laser treatment. (Fig. 3).

Histopathological evaluation

An overdose (100 mg/kg) of thiopental sodium (Ekipental, Tüm-Ekip İlaç A.Ş., Istanbul) was administered intracardiac to the rabbits for euthanasia. The left joint was resected en bloc under saline irrigation with a size of 2*1 cm. (Fig. 4).

The joint specimens taken from the subjects and sent to the laboratory were first fixed in 10% formalin solution and allowed to be fixed for at least seven days. Subsequently, they were decalcified in 10% formic acid and 10% formalin solution (prepared one to one). The specimens were again placed in 10% formalin solution for macroscopic examination and sampling, embedded in hot paraffin, and blocked. Sections were taken randomly from some of these blocks and stained with Hematoxylin and Eosin stain to check for inflammation. Subsequently, the blocks were cooled, and



Fig. 3 Application of Epic10 Laser

sections of 4-5µ thickness were taken on three different slides (Shandon Finesse ME +). One of the sections from each sample was stained with routine Hematoxylin and Eosin, and the other was stained with Gomori's

Trichrome Green Collagen (Histomed) for histochemical staining. Hematoxylin and Eosin stained specimens were analyzed for defect filling, osteochondral junction, and cellularity. Pineda's semiquantitative scoring system evaluated healing in the osteochondral defect areas [11].

The literature includes various scoring systems for evaluating healing in osteochondral defect areas. The Pineda Scoring System is among the most frequently used. Our study preferred the Pineda Scoring System because it is a semiquantitative scoring system consisting of four categories and is generally used in the follow-up of osteochondral defect healing.

Statistical analysis

In the study where the relationship between qualitative variables in three groups would be sought, it was decided to use at least 24 samples in total, 8 in each group, with a power of 80%, a margin of error of 5%, and an effect size of 0.65. The power analysis was performed with the G*Power 3.1.9.6 program. Data were analyzed with IBM SPSS V23. The effect of independent variables on the absence of results was examined with Binary Logistic Regression analysis. Fisher's Exact Test with Monte Carlo Correction examined the relationship between categorical data. Median (minimum-maximum) was used to display quantitative data. Frequency and percentage were used to display categorical data. The significance level was taken as $p < 0.05$.



Fig. 4 Joint specimens taken

Results

Defect filling, osteochondral junction, matrix staining, and cellular morphology findings were evaluated in joint sections obtained from rabbits. (Fig. 5).

In the defect filling, the rate of those who said it was acceptable in the control group was 42.9%, while the rate of those who said it was unacceptable was 57.1%. In the defect filling, the rate of those who said it was acceptable in both Group 1 (GRR laser) and Group 2 (Epic10 laser) was 100%, while the rate of those who said it was unacceptable was 0%.

In the case of osteochondral junction, the rate of those who said it was acceptable in the control group was 42.9%, while the rate of those who said it was unacceptable was 57.1%. In the case of osteochondral junction, the rate of those who said it was acceptable in Group 1 (GRR laser) was 50%, while the rate of those who said it was unacceptable was 50%. In the case of osteochondral junction, the rate of those who said it was acceptable in Group 2 (Epic10 laser) was 37.5%, while the rate of those who said it was unacceptable was 62.5%. The effect of independent variables on the risk of no result was examined using binary logistic regression with a Univariate model. The univariate model result did not find a statistically significant effect of the independent variables on the risk of no result ($p > 0.050$).

In the case of matrix staining, the rate of those who said it was acceptable in the control group was 28.6%, while the rate of those who said it was unacceptable was 71.4%. In the case of matrix staining, the rate of those who said it was acceptable in both Group 1 (GRR laser) and Group

2 (Epic10 laser) was 100%, while the rate of those who said it was unacceptable was 0%.

In the case of cellular morphology, the rate of those who said it was acceptable in the control group was 57.1%, while the rate of those who said it was unacceptable was 42.9%. In the case of cellular morphology, the rate of those who said it was acceptable in both Group 1 (GRR laser) and Group 2 (Epic10 laser) was 100%, while the rate of those who said it was unacceptable was 0%. (Table 1).

When the defect-filling distributions in the control group were examined, the rate of 100% was 42.9%, while the rate of 75% was 57.1%. When the defect filling distributions in both Group 1 (GRR laser) and Group 2 (Epic10 laser) were examined, the rate of 100% was 100%, while the rate of 75% was 0%. When examining whether there was a connection between the groups with defect filling, it was determined that there was a statistically significant connection ($p = 0.005$). (Fig. 6).

When the osteochondral junction distributions in the control group were examined, the complete rate was 42.9%, while the approximate rate was 57.1%. When the osteochondral junction distributions were examined in Group 1 (GRR laser), the rate of completely present was 50%, while the rate of approximately present was 50%. When the osteochondral junction distributions in Group 2 (Epic10 laser) were examined, the completion rate was 37.5%, while the approximate rate was 62.5%. When the connection between the osteochondral junction and the groups was examined, it was determined that there was no statistically significant connection ($p = 1.000$). (Fig. 7).

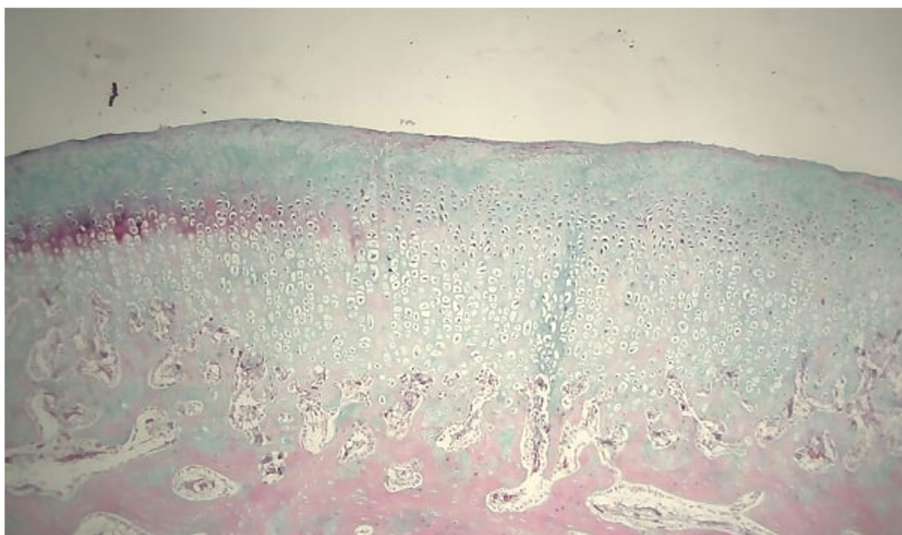


Fig. 5 Normal joint section-Gomori trichrome Collagen Green Stain. Cartilage areas were stained green, bone areas were stained red, and nuclei were stained black. These sections evaluated the intensity of green staining in cartilage repair areas

Table 1 Binary Logistics results for no result case

Parameters	Groups	Results		Univariate	
		Acceptable	Unacceptable	OR (%95 CI)	p
Defect Filling	Control	3 (42,9)	4 (57,1)	-	-
	Group 1 (GRR laser)	8 (100)	0 (0)	-	-
	Group 2 (Epic10 laser)	8 (100)	0 (0)	-	-
Osteochondral Junction	Control	3 (42,9)	4 (57,1)	Reference	
	Group 1 (GRR laser)	4 (50)	4 (50)	0,75 (0,1 - 5,77)	0,782
	Group 2 (Epic10 laser)	3 (37,5)	5 (62,5)	1,25 (0,16 - 9,92)	0,833
Matrix Staining	Control	2 (28,6)	5 (71,4)	-	-
	Group 1 (GRR laser)	8 (100)	0 (0)	-	-
	Group 2 (Epic10 laser)	8 (100)	0 (0)	-	-
Cellular Morphology	Control	4 (57,1)	3 (42,9)	-	-
	Group 1 (GRR laser)	8 (100)	0 (0)	-	-
	Group 2 (Epic10 laser)	8 (100)	0 (0)	-	-

OR Odds ratio

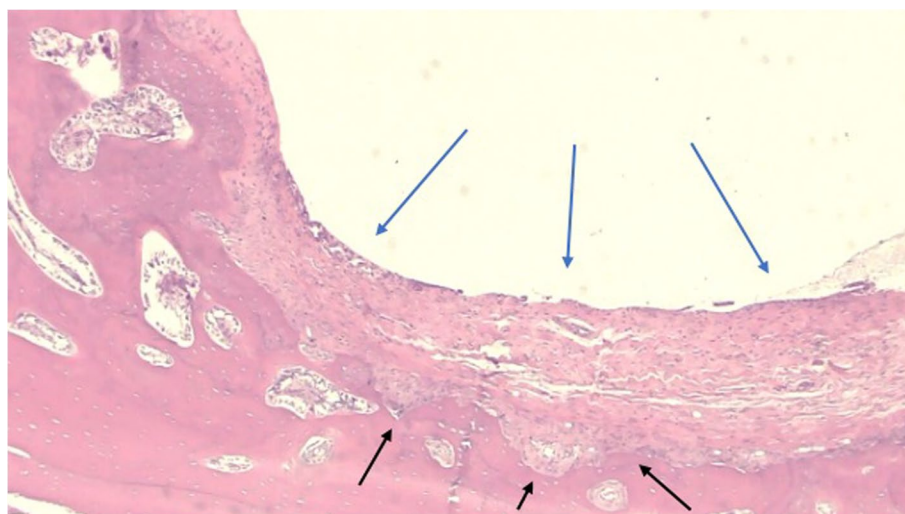


Fig. 6 Preparation from Group 1. Relatively irregular osteochondral junction (black arrows) and defect area dominated by fibroblastic cells (blue arrows)

When the matrix staining distributions were examined in the control group, the normal rate was 28.6%, while the slightly decreased rate was 71.4%. When the matrix staining distributions were examined in both Group 1 (GRR laser) and Group 2 (Epic10 laser), the normal rate was 100%, while the slightly decreased rate was 0%. It was determined that there was a significant connection between the groups with matrix staining ($p = 0.001$). (Fig. 8).

When the cellular morphology distributions were examined in the control group, the normal rate was 57.1%, while the rate of frequently hyaline and fibrous cartilage cells was 42.9%. When the morphology

distributions in both Group 1 (GRR laser) and Group 2 (Epic10 laser) were examined, the rate of normal cells was 100%, while frequent hyaline and fibrous cartilage cells were 0%. When it was examined whether there was a connection between the groups with cellular morphology, it was determined that there was a statistically significant connection ($p = 0.018$). (Table 2) (Fig. 9).

Discussion

TMJ-OA, one of the temporomandibular joint disorders, is a locally occurring chronic degenerative disease. It usually results in degeneration and loss of articular cartilage accompanied by pathologic changes in the subchondral

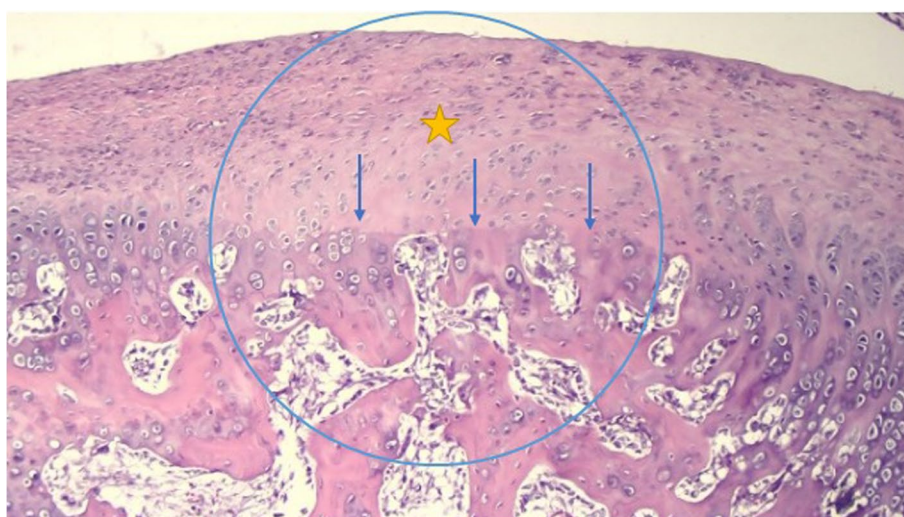


Fig. 7 Preparation from Group 2. The defect area shows full filling (blue ring inside), and the osteochondral line is regular (blue arrows), but cellularity is predominant in favor of fibrous cartilage (yellow star)

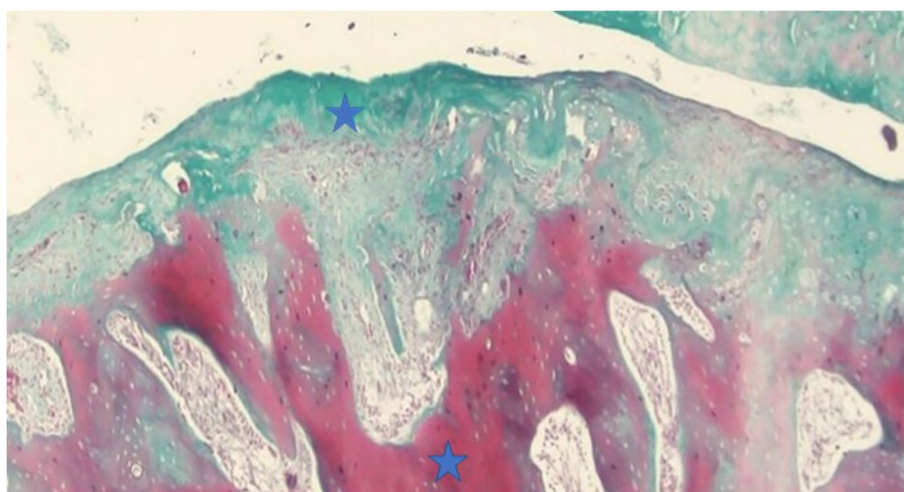


Fig. 8 Preparation from Group 1. Staining intensity evaluation. Intense collagen deposition and complete staining in starred areas

bone and other soft and hard tissues [12]. More than 60% demineralization is required for erosions in TMJ-OA to become radiographically evident. It is not possible to establish a relationship between radiographic and clinical findings. The joint and fossa surfaces are covered with fibrous tissue that cannot be seen on a standard radiograph, and early changes may not be seen [13, 14]. Therefore, histopathologic examination was performed to evaluate the study's results, and defect filling, osteochondral junction, matrix staining, and cellular morphology were assessed at the follow-up of healing. Sodium mono iodoacetate (SMIA), which acts as a glycolysis inhibitor and has been shown to induce chondrocyte death, is

frequently used in experimental studies [10, 15–18]. The animal model of MIA-induced OA almost precisely mimics the pain and structural changes that accompany the disease in human patients [19]. The histopathology of OA in humans and the histopathology of joint degeneration induced by MIA injection are very similar [20].

SMIA was used in our study to create experimental osteoarthritis. Various studies in the literature show that SMIA causes changes in osteoarthritis in joints [15–18]. Güler et al. [10] injected MIA (50 ml doses at concentrations of 1.5, 2, 2.5, and 3 mg/ml) into New Zealand white male rabbits' temporomandibular joints by arthrocentesis. They obtained computerized tomography (CT)

Table 2 Comparison of categorical features by groups

Parameters		Groups			Total	Test statistics	p*
		Control	Group 1 (GRR laser)	Group 2 (Epic10 laser)			
Defect Filling	100%	3 (42,9) ^a	8 (100) ^b	8 (100) ^b	19 (82,6)	8,302	0,005
	75%	4 (57,1) ^a	0 (0) ^b	0 (0) ^b	4 (17,4)		
Osteochondral Junction	Completely	3 (42,9)	4 (50)	3 (37,5)	10 (43,5)	0,404	1,000
	Approximately	4 (57,1)	4 (50)	5 (62,5)	13 (56,5)		
Matrix Staining	Normal	2 (28,6) ^a	8 (100) ^b	8(100) ^b	18 (78,3)	11,656	0,001
	Slightly Reduced	5(71,4) ^a	0 (0) ^b	0(0) ^b	5 (21,7)		
Cellular Morphology	Normal	4 (57,1)	8 (100)	8(100)	20 (87)	5,556	0,018
	Often hyaline and fibrous cartilage cells	3(42,9)	0 (0)	0 (0)	3 (13)		

*Fisher’s Exact Test with Monte Carlo

n (%)

^{a-b} There is no difference between ratios with the same letter

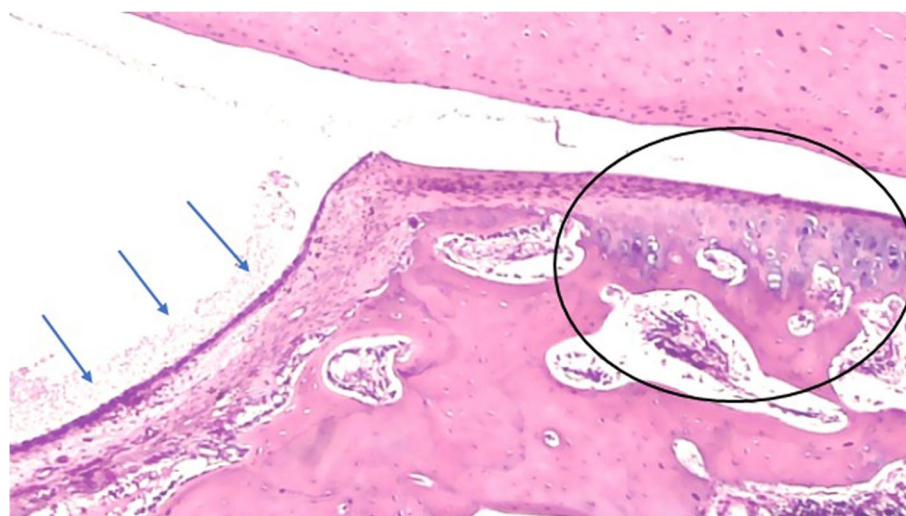


Fig. 9 Preparation from Group 2. The defect area is showing partial filling (blue arrows). Fibroblastic cell proliferations are present in the ground. Adjacent normal areas show hyaline cartilage cells and regular osteochondral junction (black circles)

images at 2, 4, and 6 weeks after injection. Early osteoarthritic changes were found histologically in the rabbit TMJ at 4 weeks and a concentration of 3 mg/ml MIA. Subchondral bone invaginations and chondrocyte clusters (hypertrophic reaction) with disrupted osteochondral connection and increased trabeculation structure in the subchondral bone were observed with 3 mg/ml MIA solution. In our study, 3 mg/ml MIA was applied and waited 4 weeks for OA symptoms to develop, per the literature.

It is mainly acute exacerbations and a decreased range of motion that require urgent and active intervention

in the treatment of osteoarthritis. Conservative, non-invasive, or surgical methods are preferred in treatment [5]. Non-invasive treatments include patient education, occlusal splinting, thermal therapies, laser applications, physiotherapy, and non-pharmacologic treatment [4]. Pain relief is an essential part of treatment, and patients with joint pain may be prescribed non-steroidal anti-inflammatory drugs (NSAIDs) and muscle relaxants [4].

Our study used a GaAlAs laser (EPIC 10) and new generation dual-wavelength (GRR Laser with GaAlAs and LED combination) laser devices. The diode laser used in our study has been frequently used for bio stimulation in

osteoarthritis and other temporomandibular joint disorders [17, 21–24]. Çetiner et al. [25] found a statistically significant reduction in maximal mouth opening amount, pain, and chewing difficulty in patients receiving LLLT as a result of their study in 39 patients with orofacial pain, restricted mandibular movement, chewing difficulties, and tender points. They evaluated LLLT as an alternative treatment method for TMD [25]. In a similar study by Desai et al. [21] applied LLLT to patients who complained of TMJ pain and had previously received self-treatment, physiotherapy, and occlusal splint treatment that failed to relieve pain. The study evaluated pain, mouth opening, and lateral movements; they obtained better treatment results in the study group, where they applied a laser for 20 sessions [21]. Yanık et al. [24] conducted a study in which arthrocentesis and LLLT were combined in 36 patients diagnosed with TMJ-OA. They observed greater improvements in muscle palpation scores and mandibular movements in patients who underwent diode laser and arthrocentesis combined compared to patients who underwent arthrocentesis alone. They observed that the combination with LLLT had an additional benefit for myofascial components. Since our study was an animal study, pain and mandibular movements could not be evaluated. However, according to histopathological results, statistically significant results were obtained in treating TMJ-OA in groups treated with laser at different wavelengths.

Lemos et al. [22] evaluated the efficacy of LLLT in rats with experimentally induced OA by histologic and biochemical analysis. The rats were divided into two groups; the control group was not treated with a laser. Matrix metalloproteinases (MMP) and sulfated glycosaminoglycans (GAG) levels were measured in joint sections. They found that active MMP levels were statistically significantly lower in the laser-treated group [22]. Our study showed that LLLT had better and more significant results regarding defect filling, matrix staining, and cellular morphology parameters in arthritis-induced TMJ compared to the control group. It showed that LLLT, although at different wavelengths, could increase TMJ remodeling and improve tissue repair.

Cepera et al. [26] evaluated the effects of LLLT on bone regeneration in rapid maxillary expansion procedures by applying a diode laser to 10 points around the mid-palatal suture. In the evaluation of bone density, the results showed that the laser improved the opening of the mid-palatal suture and accelerated the bone regeneration process. Shanei et al. [27] evaluated the combined effect of LLLT and leukocyte- and platelet-rich fibrin (PRF) on new bone formation in the rabbit calvaria defect they created. The bone formation percentage was significantly higher in the group in which PRF and LLLT were

combined. Consistent with the findings in our study, LLLT increased the percentage of bone regeneration and defect filling.

Madani et al. [28] investigated the effect of a low-level 810 nm laser on improving TMJ osteoarthritis in their clinical study. The penetration depth of the laser they used in their study was approximately 2–3 cm. They found no difference in reducing pain and improving mouth opening in patients with TMJ osteoarthritis who underwent LLLT compared to the placebo group. In their study, Marini et al. [29] randomly divided 99 patients with temporomandibular joint disorders, nonreducible disc displacement, or osteoarthritis into three groups. They applied superpulsed LLLT, ibuprofen, and placebo as sham lasers to these groups. They reported that mandibular function improved in patients who underwent superpulsed LLLT, and significant improvement was observed in clinical signs and symptoms of nonreducible temporomandibular joint disc displacement and osteoarthritis at the end of the treatment. New generation dual wavelength lasers (≤ 50 mm) penetrate more tissue than traditional lasers (≤ 5 mm) [8, 9]. There may be clinical differences in efficacy if traditional and new-generation laser types are applied to large human joints much deeper than 5 mm. We believe that the lack of difference between dual wavelength and traditional lasers in our study is due to the more superficial location of rabbit TMJ compared to human TMJ. Clinical studies to be conducted with new generation dual wavelength lasers will more clearly demonstrate the efficacy of this laser in clinical parameters such as pain and mouth movements.

Conclusion

This study shows that LLLT can increase remodeling and improve tissue repair in TMJ-OA treatment. There was no significant difference between lasers at different wavelengths in treating temporomandibular joint osteoarthritis. As may be the limitation of this study, the findings between the dual laser and the conventional laser with no existing differences may be that the rabbit Tmj experimental model is under 5mm deep. Effect differences may exist if both conventional and new-generation laser types are applied to major human joints much deeper than 5 mm. Further studies are needed to examine the temporomandibular joint complex, measure clinical parameters, and apply different laser bio stimulation protocols to patients with TMJ or other major human joints-OA symptoms.

Authors' contributions

G.K. Conceptualization, Methodology, Validation, Investigation, Writing-Original Draft, Visualization, Project administration. N.A. Conceptualization, Methodology, Software, Formal analysis, Data Curation, Writing-Review &

Editing, Supervision, Project administration. F.A.D. Methodology, Software, Formal analysis, Investigation, Resources, Data Curation. A.A. Methodology, Investigation, Writing-Review & Editing, Supervision, Writing - Original Draft, Visualization.

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Data availability

Data cannot be shared publicly. Data are available from the corresponding author for researchers who meet the criteria.

Declarations

Ethics approval and consent to participate

This study was conducted at Tokat Gaziosmanpaşa University Experimental Medicine Research Unit with the approval of the Tokat Gaziosmanpaşa University Animal Experiments Local Ethics Committee, dated 01.12.2020, file number 51879863/141. Because it is an animal study, informed consent is not required.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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