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Article

Effects of Intraoperative Laser Photobiomodulation on Osteogenic Differentiation of Human Jaw Bone Explants Harvested During Cyst Surgery

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Abstract

Background: Surgical removal of jaw cysts frequently results in bone defects that may compromise healing and delay functional rehabilitation. Laser photobiomodulation (PBM) has been proposed as a minimally invasive adjuvant capable of enhancing osteogenic activity; however, translational evidence based on freshly harvested human jaw bone tissue remains limited. **Objective:** To investigate the effects of intraoperative laser photobiomodulation on osteogenic differentiation and cellular viability in human bone explants obtained during cystectomy, using a paired experimental design. **Materials and Methods:** This translational experimental study included 20 patients undergoing surgical treatment for medium to large maxillary or mandibular cysts. From each patient, paired bone explants were harvested intraoperatively, with one explant exposed to diode laser photobiomodulation and the contralateral explant serving as an untreated control. Explants were cultured under standardized conditions and assessed for osteogenic differentiation using morphometric analysis, immunofluorescence staining for alkaline phosphatase (ALPL) and osteocalcin (OCN), and confocal microscopy. Cell viability was evaluated using a live/dead fluorescence assay. **Results:** Biologically viable osteogenic cultures were obtained from 8 patients and included in the final paired analysis. In the majority of responsive cases, laser-treated explants demonstrated an increased number of osteoblast-like cellular structures, greater osteoblastic surface area occupancy, and enhanced expression of ALPL and OCN compared with paired controls. No evidence of laser-induced cytotoxicity was observed. **Conclusions:** Intraoperative laser photobiomodulation enhanced osteogenic activity in human jaw bone explants in a subset of cases, supporting its potential role as a biologically active adjuvant in maxillofacial cyst surgery. Inter-individual variability highlights the need for further optimization of irradiation protocols and larger controlled studies to identify predictive factors for clinical responsiveness.

Keywords: jaw cysts; oral and maxillofacial surgery; bone explant; photobiomodulation; diode laser; osteogenic differentiation; alkaline phosphatase; osteocalcin; confocal microscopy; regenerative medicine

1. Introduction

To date, most evidence regarding photobiomodulation-induced osteogenesis has been derived from animal models or isolated cell cultures, and data based on freshly harvested human jaw bone tissue exposed intraoperatively remain scarce [1,2]. Bone regeneration represents a critical aspect of contemporary oral and maxillofacial surgery, particularly following the surgical management of cystic jaw lesions [1–3]. Although these lesions are generally benign, their surgical removal results in bone defects, which may compromise local structural integrity and delay functional rehabilitation. Consequently, there is interest in therapeutic strategies to enhance bone healing while minimizing surgical morbidity [4,5].

Autologous bone remains the gold standard for skeletal regeneration due to its osteogenic, osteoinductive, and osteoconductive properties [6]. However, the biological behavior of bone tissue following surgical trauma is influenced by multiple factors, including local vascularization, cellular viability, and the microenvironment of the defect [7,8]. In this context, the use of bone explants harvested intraoperatively offers a unique opportunity to investigate osteogenic potential under clinically relevant conditions, closely reflecting the biological reality encountered in maxillofacial surgery [9,10].

Mesenchymal stem cells derived from bone tissue play a central role in bone regeneration through their capacity to proliferate and differentiate into osteoblasts [11–13]. The modulation of osteoblastic activity represents a key target in regenerative strategies, aiming to accelerate matrix synthesis and mineralization [14,15]. Experimental models based on human bone explants allow direct assessment of cellular viability, differentiation markers, and functional activity, providing valuable translational insights [13,14].

Laser photobiomodulation has emerged as a promising adjuvant therapy in regenerative medicine [16,17]. Low-level laser therapy has been shown to influence mitochondrial activity, cellular metabolism, and gene expression, thereby promoting cell proliferation and differentiation [18,19]. In bone tissue, photobiomodulation has been associated with increased osteoblastic activity, enhanced expression of osteogenic markers, and improved mineralization [20,21]. Despite encouraging results, the biological effects of laser therapy remain dependent on irradiation parameters and the biological context in which it is applied [22].

Within the field of oral and maxillofacial surgery, the potential role of laser photobiomodulation as an adjunct to the surgical treatment of jaw cysts remains insufficiently explored, particularly in experimental models using human bone tissue [9,10]. Investigating the response of bone explants obtained during cyst surgery may contribute to the development of minimally invasive, biologically optimized therapeutic protocols [23].

The present study evaluates the effects of laser photobiomodulation on human bone explants harvested during surgical treatment of jaw cysts, with a focus on osteogenic differentiation, cellular viability, and functional activity. By integrating surgical practice with experimental laboratory analysis, this research aims to provide a translational framework for optimizing bone regeneration in maxillofacial surgery [24,25].

2. Materials and Methods

2.1. Study Design

This study was designed as a translational experimental investigation evaluating the effects of intraoperative laser photobiomodulation (PBM) on the osteogenic behavior of human jaw bone

explants harvested during surgical treatment of cystic lesions. A paired-sample experimental design was employed, whereby laser-treated and non-treated explants were obtained from the same patient, allowing direct intra-individual comparison and minimizing biological variability inherent to human tissue-based models.

The primary outcomes were osteogenic differentiation, osteoblast-like cellular organization, and cellular viability, assessed under standardized in vitro conditions.

2.2. Study Population and Ethical Approval

Bone explants were harvested from 20 consecutive patients undergoing surgical treatment for medium to large cystic lesions of the maxilla or mandible at the Department of Oral and Maxillofacial Surgery, Emergency County Clinical Hospital of Târgu Mureș, Romania, between January and December 2023.

Eligibility criteria included patients presenting cystic lesions of sufficient size to permit safe harvesting of adjacent bone tissue through the standard surgical approach [26]. Exclusion criteria included a history of radiotherapy or chemotherapy in the head and neck region, osteonecrosis or osteoradionecrosis, active cystic infection or suppuration, and systemic diseases or treatments known to impair bone metabolism significantly [27].

The study protocol was approved by the Institutional Ethics Committee (approval no. 13555/21.06.2022) and conducted in accordance with the Declaration of Helsinki. Bone harvesting did not require additional surgical maneuvers beyond routine clinical procedures. All specimens were anonymized before laboratory processing.

2.3. Bone Explant Harvesting Procedure

Bone explants were collected intraoperatively during cystectomy procedures. Following surgical exposure of the cystic cavity via a standard vestibular approach, small cortical bone fragments were obtained from the surgical access area, without removing perilesional bone tissue or modifying the standard surgical protocol.

Only cystic lesions of medium to large dimensions were included, as smaller lesions do not allow reliable or reproducible explant harvesting. Immediately after collection, bone fragments were placed under sterile conditions in transport medium and prepared for experimental processing (Figure 1).



Figure 1. Handling of a tissue-like specimen in a liquid medium under controlled laboratory conditions. The sample was transferred using sterile forceps into a test tube and prepared for subsequent analyses. All procedures were performed under standard aseptic conditions.

2.4. Experimental Groups and Laser Photobiomodulation Protocol

For each patient, two morphologically comparable bone explants were harvested and assigned as follows:

- Control explant: no laser exposure

- Laser-treated explant: subjected to intraoperative photobiomodulation
- Laser irradiation was performed using a diode dental laser (Litemedics Prime, Litemedics, Romania) (Figure 2) with the following parameters:
- Wavelength: 980 nm
 - Output power: 1 W
 - Emission mode: pulsed
 - Operating mode: biostimulation
 - Fiber diameter: 320 μm
 - Irradiation distance: 50 mm (non-contact)
 - Exposure time: 40 seconds



Figure 2. Experimental setup for dental laser application under controlled laboratory conditions. A Lite Medics laser unit was operated in therapy mode at an output power of 1.0 W, delivering focused laser energy to a tissue-like sample positioned in a sterile Petri dish and stabilized with precision forceps, illustrating laser–tissue interaction in a simulated clinical environment.

The laser fiber was positioned perpendicular to the explant surface to ensure uniform irradiation while avoiding direct contact and thermal tissue effects. Based on the output power (1 W), exposure time (40 s), and estimated irradiation area at a distance of 50 mm, the calculated energy delivered per explant was approximately 40 J, corresponding to an estimated energy density within the biostimulatory range reported for bone tissue (J/cm^2). These parameters were selected to promote photobiomodulation while minimizing the risk of thermal effects.

The selected parameters were chosen to promote cellular photobiomodulation while minimizing the risk of thermal alteration.

2.5. Transport and Laboratory Processing

Bone explants were transported to the laboratory within a maximum interval of three hours following harvesting. All subsequent experimental procedures were carried out at the Department of Immunology, Center for Advanced Medical and Pharmaceutical Research (CCAMF), George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș.

Upon arrival, explants were processed under sterile conditions according to a standardized protocol applied uniformly to all samples.

2.6. Explant Culture and Cell Isolation

Bone fragments were rinsed with Dulbecco's phosphate-buffered saline (DPBS) to remove blood residues and transferred to culture flasks containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum and antibiotics.

Explants were cultured using the explant technique, allowing spontaneous migration of cells from the bone fragments. Cultures were maintained at 37°C, in a 5% CO₂ humidified atmosphere, with periodic medium replacement. Cellular outgrowth from the explants was monitored microscopically.

Once sufficient cell confluence was achieved, bone fragments were removed, and adherent cells were detached using enzymatic dissociation and prepared for further expansion or differentiation assays.

2.7. Osteogenic Differentiation Protocol

Cells derived from explant cultures were induced toward osteogenic differentiation using a standard osteogenic induction medium containing β -glycerophosphate, L-ascorbic acid, and dexamethasone.

Cultures were maintained under controlled conditions with regular medium changes until the appropriate stages of osteogenic differentiation were reached, allowing assessment of early and late osteoblastic markers.

2.8. Immunofluorescence Staining and Confocal Microscopy

Osteogenic differentiation was evaluated by immunofluorescence staining for alkaline phosphatase (ALPL) and osteocalcin (OCN), established markers of osteoblastic differentiation and matrix maturation.

Cells were fixed, permeabilized, and incubated with primary antibodies against ALPL and OCN, followed by fluorochrome-conjugated secondary antibodies. Nuclear counterstaining was performed using 4',6-diamidino-2-phenylindole (DAPI).

Image acquisition was performed using a Leica TCS SP8 confocal microscope (Leica Microsystems, Germany). Identical acquisition parameters, including magnification, laser power, detector gain, and field of view, were applied to all samples to allow reliable qualitative and morphometric comparisons between laser-treated and control cultures.

Morphometric analysis was performed using ImageJ/Fiji software (National Institutes of Health, USA). Osteoblast-like structures were identified through manual and threshold-assisted segmentation based on fluorescence intensity and morphological criteria, including cell size, shape, and marker positivity. Identical segmentation thresholds and analysis parameters were applied uniformly across paired control and laser-treated samples to ensure consistency.

2.9. Cell Viability Assessment

Cell viability was assessed using a live/dead fluorescence assay based on Calcein AM and Ethidium Homodimer III. Viable cells were identified by green cytoplasmic fluorescence, whereas non-viable cells exhibited red nuclear staining.

Stained cultures were imaged immediately using confocal microscopy, and viability was assessed qualitatively by comparison between paired laser-treated and control samples.

2.10. Experimental Yield and Data Analysis

Due to the inherent biological variability associated with primary human bone explant cultures, measurable osteogenic differentiation and viable cell populations were obtained in 8 out of 20 patients. These cases constituted the final experimental cohort included in the comparative analysis.

Data analysis followed a paired descriptive approach, with each laser-treated explant directly compared to its corresponding control. Results were expressed as cell counts, morphometric parameters, and relative differences between groups.

Because of the natural biological variability of primary human bone explant cultures, detectable osteogenic differentiation and viable cell populations were achieved in samples from 8 of the 20 patients. This yield is consistent with previously reported success rates for primary cultures derived

from surgically harvested human bone tissue, where variability in cellular viability, vascularization, and local bone quality can significantly affect culture establishment.

The remaining explants failed to generate sufficient cellular outgrowth or osteogenic differentiation for reliable morphometric and immunofluorescence analysis and were therefore excluded before comparative evaluation.

Given the limited number of biologically responsive paired samples and the exploratory nature of this translational study, formal inferential statistical testing was not performed. Instead, results were analyzed descriptively on a paired basis, focusing on consistent biological trends across multiple outcome measures. This approach was selected to avoid overinterpretation of statistically underpowered data while preserving biological relevance.

3. Results

3.1. Study Cohort and Experimental Yield

Bone explants were harvested from 20 patients undergoing surgical treatment for cystic lesions of the maxilla or mandible and processed according to the standardized experimental protocol.

Analysis of excluded samples indicated no systematic clustering with respect to anatomical localization (maxilla versus mandible), patient age, or cyst type. Both maxillary and mandibular explants were represented among excluded cases, suggesting that explant viability was influenced predominantly by local biological factors rather than demographic or anatomical variables.

Due to the inherent biological variability associated with primary human bone explant cultures, measurable osteogenic differentiation and viable cell populations were obtained in 8 out of 20 patients. These 8 cases constituted the final experimental cohort included in the paired comparative analysis between laser-treated and non-treated explants. The remaining samples did not yield sufficient cellular viability or osteogenic differentiation for reliable morphometric and immunofluorescence evaluation and were therefore excluded from further analysis.

A flow diagram summarizing patient inclusion, explant processing, and final sample selection is presented in Figure 3.

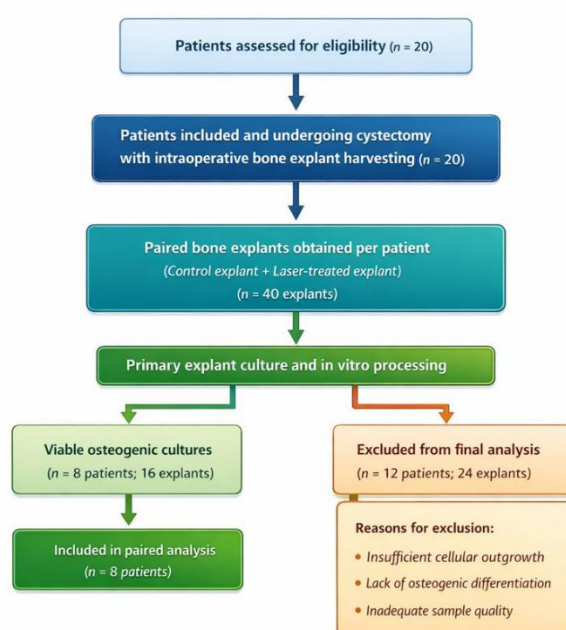


Figure 3. Flow diagram of patient inclusion and explant processing (included vs. excluded samples).

The demographic characteristics and anatomical harvesting sites of the patients included in the final experimental group are summarized in Table 1.

Table 1. Demographic and clinical characteristics of patients included in the final experimental analysis.

Patient Number	Patient Age	Patient Gender	Anatomical Localization
Patient 1	26	female	maxilla
Patient 2	29	male	maxilla
Patient 3	19	female	maxilla
Patient 4	39	female	maxilla
Patient 5	40	male	mandible
Patient 6	46	female	mandible
Patient 7	50	male	mandible
Patient 8	55	female	mandible

3.2. Osteoblast-Like Cellular Structures: Quantitative Paired Analysis

Quantitative morphometric analysis revealed consistent differences between laser-treated and control explants in terms of the number of osteoblast-like cellular structures.

In 7 out of 8 patients, explants exposed to photobiomodulation demonstrated a higher number of osteoblast-like cellular structures compared with their paired controls. This increase suggests a stimulatory effect of laser photobiomodulation on osteogenic cellular activity. The magnitude of this effect varied among patients, reflecting individual biological variability.

Across the analyzed samples, laser-treated explants generally exhibited a greater density of smaller, more uniformly distributed osteoblast-like cellular structures, whereas control explants displayed fewer but larger cellular aggregates.

Detailed quantitative values for each paired comparison are provided in Table 2, and a graphical comparison of cellular structure counts between experimental groups is illustrated in Figure 4.

Cumulative area values represent pixel-based measurements obtained from digital image analysis and are expressed in relative units, reflecting the number of pixels occupied by segmented osteoblast-like structures within a defined field of view; these values were used exclusively for paired intra-sample comparisons and not as absolute dimensional measurements.

Table 2. Paired quantitative and relative morphometric analysis of osteoblast-like cellular structures in control and laser-treated human bone explants.

Slice	Count	Cumulative Area (Relative Units)
Patient 1 LASER +	2201	554123.852
Patient 1 LASER -	1215	1441762.407
Patient 2 LASER +	3901	644989.447
Patient 2 LASER -	2408	634447.68
Patient 3 LASER +	4917	533615.553
Patient 3 LASER -	2760	482853.707
Patient 4 LASER +	1837	239164.02
Patient 4 LASER -	682	133335.969
Patient 5 LASER +	4583	1079814.891
Patient 5 LASER -	2878	793648.215
Patient 6 LASER +	1922	621348.193
Patient 6 LASER -	1256	435137.329
Patient 7 LASER +	929	178271.067
Patient 7 LASER -	320	48918.086
Patient 8 LASER +	441	73910.588
Patient 8 LASER -	94	2791900.064

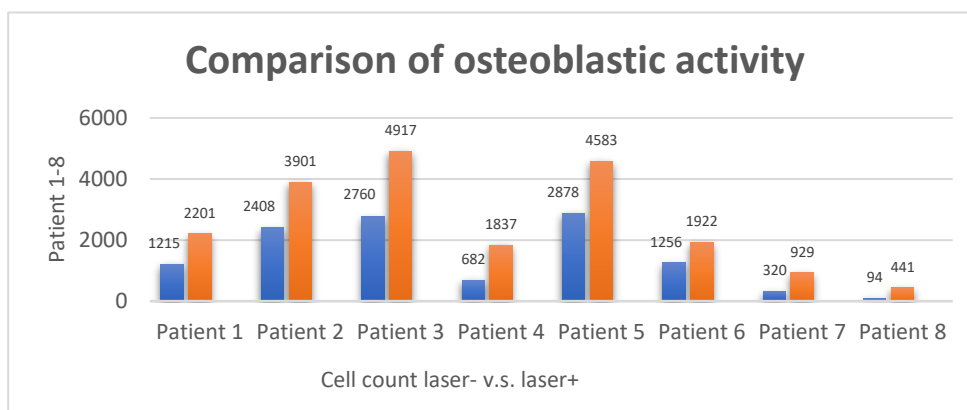


Figure 4. Comparison of osteoblast-like cellular structure counts between control and laser-treated bone explants.

Paired quantitative analysis demonstrates a higher number of osteoblast-like cellular structures in laser-treated explants compared with corresponding non-treated controls in the majority of analyzed patients.

3.3. Morphometric Analysis of Osteoblastic Area Occupancy

To further characterize osteogenic behavior beyond simple cell counts, a morphometric analysis was performed to assess the percentage of surface area occupied by osteoblast-like cellular structures in control and laser-treated cultures.

In the majority of cases, laser-treated explants demonstrated an increased percentage of osteoblastic area occupancy compared with paired controls. This finding suggests that photobiomodulation affects not only the number of osteogenic cells but also their spatial organization and surface coverage, which are crucial features of effective osteogenic differentiation.

Notably, in several patients, laser-treated explants exhibited both increased cellular density and improved distribution of osteoblast-like cellular structures across the culture surface. Individual morphometric values are presented in Table 3, and a comparative graphical representation of osteoblastic area occupancy is shown in Figure 5.

The percentage of osteoblastic area (%Area) was calculated as the proportion of the image surface occupied by osteoblast-like structures relative to the total analyzed field, based on pixel-based morphometric analysis.

Table 3. Paired morphometric evaluation of osteoblastic area occupancy in control and laser-treated human bone explants.

Slice	Cumulative Osteoblastic Area (Relative Units)	Osteoblastic Area Occupancy (%)
Patient 1 LASER +	554123.852	11.556
Patient 1 LASER -	1441762.407	28.777
Patient 2 LASER +	644989.447	13.292
Patient 2 LASER -	634447.68	12.136
Patient 3 LASER +	533615.553	42.039
Patient 3 LASER -	482853.707	35.527
Patient 4 LASER +	239164.02	19.231
Patient 4 LASER -	133335.969	11.216
Patient 5 LASER +	1079814.891	22.55
Patient 5 LASER -	793648.215	16.206

Pacient 6 LASER +	621348.193	12.85
Pacient 6 LASER -	435137.329	7.259
Pacient 7 LASER +	1782710067	18.906
Pacient 7 LASER -	48918.086	4.722
Pacient 8 LASER +	73910.588	7.541
Pacient 8 LASER -	279190006.4	0.685

Figure 5. Comparison of the percentage of osteoblastic area occupancy between control and laser-treated explants.

3.4. Immunofluorescence Assessment of Osteogenic Marker Expression

Immunofluorescence staining demonstrated the presence of alkaline phosphatase (ALPL) and osteocalcin (OCN) in both control and laser-treated explants, confirming osteogenic differentiation in cultured cells derived from human bone tissue.

In laser-treated explants, ALPL and OCN signals were generally more intense and more widely distributed compared with paired controls. This pattern is consistent with enhanced osteoblastic differentiation and matrix maturation following photobiomodulation.

Confocal microscopy revealed that laser-exposed cultures exhibited a higher number of ALPL-positive cells at earlier differentiation stages, as well as increased OCN expression in more mature osteoblast-like cells.

3.5. Cell Viability Analysis

Live/dead fluorescence assays demonstrated preserved cellular viability in both experimental groups. In laser-treated explants, viable cells predominated, with a high proportion of Calcein AM-positive cells and minimal Ethidium Homodimer III nuclear staining.

No evidence of laser-induced cytotoxicity or increased cell death was observed under the applied photobiomodulation parameters. In contrast, several control explants exhibited areas of reduced cell density and sporadic non-viable cells, particularly in cultures with limited cellular outgrowth.

These observations indicate that the applied photobiomodulation protocol was biologically safe and did not adversely affect cell viability.

3.6. Summary of Experimental Findings

Collectively, the results demonstrate a consistent trend toward enhanced osteogenic activity in human bone explants exposed to intraoperative laser photobiomodulation. Compared with paired non-treated controls, laser-treated explants exhibited:

- Increased numbers of osteoblast-like cellular structures
- Greater osteoblastic surface area occupancy
- Enhanced expression of osteogenic markers (ALPL and OCN)
- Preserved cellular viability

Despite inter-individual variability and a limited number of biologically responsive samples, the concordance of findings across quantitative, morphometric, and qualitative endpoints supports a biologically relevant osteostimulatory effect of photobiomodulation in human jaw bone tissue.

4. Discussion

Bone regeneration following surgical management of cystic lesions of the maxilla and mandible remains a fundamental concern in oral and maxillofacial surgery, particularly in cases involving medium to large defects where spontaneous bone healing may be delayed or incomplete [28,29]. Although cystic lesions are generally benign, the resulting bone defects can compromise structural stability, delay functional rehabilitation, and complicate subsequent implant or prosthetic treatment

[31,32]. Consequently, considerable interest has been directed toward adjuvant strategies to enhance osteogenesis while maintaining a minimally invasive surgical approach [31–33].

In this translational experimental study, we evaluated the effects of intraoperative laser photobiomodulation (PBM) on osteogenic behavior in human jaw bone explants harvested during cystectomy procedures [34]. By employing a paired-sample design, laser-treated explants were directly compared with non-treated controls obtained from the same patient, thereby minimizing inter-individual biological variability and strengthening the internal validity of the findings [35].

4.1. Photobiomodulation-Induced Osteogenic Activity in Human Bone Explants

The results of the present study demonstrate a consistent trend toward enhanced osteogenic activity in bone explants exposed to photobiomodulation [18,19,21,22,25,34]. Laser-treated samples exhibited increased numbers of osteoblast-like cellular structures, greater osteoblastic surface area occupancy, and enhanced expression of osteogenic markers when compared with paired controls. These findings support the hypothesis that PBM can positively modulate osteogenic differentiation in human bone tissue [18,19,21,22,25,34].

Previous experimental studies have shown that low-level laser irradiation stimulates osteoblastic proliferation and differentiation through mitochondrial activation, increased adenosine triphosphate synthesis, and modulation of intracellular signaling pathways involved in bone metabolism [25,34,35]. The present findings are consistent with these reports and extend existing knowledge by demonstrating similar biological effects in freshly harvested human jaw bone explants, a model that preserves native cell–matrix interactions and more closely reflects the clinical environment [34,35].

Importantly, the use of human explant-derived cells distinguishes this study from many investigations based on animal models or immortalized cell lines, which, although valuable, do not fully reproduce the complexity of human bone tissue [36,37]. The observed osteogenic response, therefore, carries particular translational relevance for oral and maxillofacial surgery.

The applied irradiation parameters fall within ranges previously reported to enhance osteogenic activity without inducing cytotoxic or thermal effects, as described in established photobiomodulation guidelines and experimental studies.

4.2. Morphometric and Organizational Effects of Laser Photobiomodulation

In addition to increasing the number of osteoblast-like cellular structures, photobiomodulation was associated with changes in cellular organization and surface distribution, as reflected by the morphometric analysis of osteoblastic area occupancy [38,39]. In most cases, laser-treated explants exhibited a greater percentage of surface area covered by osteoblast-like cellular structures, suggesting a more organized and functionally active osteogenic environment [38–40].

Effective bone regeneration depends not only on the presence of osteogenic cells but also on their spatial distribution and coordinated matrix deposition. The tendency toward smaller, more uniformly distributed osteoblast-like cellular structures in laser-treated cultures may indicate a more proliferative and metabolically active osteogenic phenotype, potentially favoring subsequent mineralization and tissue maturation [40,41].

4.3. Osteogenic Marker Expression and Cellular Viability

Immunofluorescence analysis confirmed the expression of alkaline phosphatase (ALPL) and osteocalcin (OCN) in explant-derived cultures, validating successful osteogenic differentiation under the applied experimental conditions. Laser-treated explants generally exhibited more intense and widespread expression of these markers, consistent with enhanced osteoblastic differentiation and matrix maturation [42,43].

Equally important, the live/dead viability assays demonstrated that the applied photobiomodulation protocol did not induce cytotoxic effects [43,44]. On the contrary, laser-treated

explants frequently displayed preserved or improved cellular viability compared with controls. This finding supports the biological safety of intraoperative PBM and is particularly relevant in the surgical context, where maintaining cell viability is essential for successful regeneration [18,19,21,22,25,34,43,44].

4.4. Clinical and Translational Significance

From a clinical perspective, the observed enhancement of osteogenic parameters following PBM supports its potential role as an adjuvant therapy in the surgical management of jaw cysts [44,45]. Improved osteogenic activity at the cellular level may translate into faster bone healing, improved defect filling, and more predictable regenerative outcomes, particularly in extensive defects where spontaneous regeneration may be unreliable [22,25,44,45].

The intraoperative application of PBM, as used in this study, offers practical advantages. It can be easily integrated into standard surgical workflows, does not require additional surgical sites, and adds minimal operative time or morbidity. As such, PBM represents a promising strategy for biologically optimizing bone healing without increasing the invasiveness of treatment [40,45,46].

4.5. Biological Variability and Response Heterogeneity

A notable observation in this study was the variability in biological response among harvested explants. Although bone samples were obtained from 20 patients, biologically valid osteogenic responses were observed in a subset of cases. This variability reflects the inherent heterogeneity of human bone tissue and the challenges associated with primary explant culture models [47].

Factors such as patient age, anatomical location, local bone quality, vascularization, and systemic conditions may influence cellular viability and differentiation potential [48]. Rather than representing a limitation alone, this heterogeneity underscores the importance of human tissue-based translational research and suggests that individualized or optimized photobiomodulation protocols may be necessary to achieve consistent regenerative outcomes [47,48].

4.6. Study Limitations and Future Perspectives

Several limitations should be considered when interpreting the results of this study. The limited number of biologically responsive samples restricts statistical generalization and precludes definitive conclusions regarding clinical efficacy. Additionally, the study focused on short-term cellular and morphometric outcomes, without longitudinal assessment of mineralization dynamics, biomechanical properties, or clinical healing parameters.

Future investigations should aim to include larger patient cohorts, standardized laser dosimetry protocols, and correlations between cellular responses and clinical or radiological outcomes. The integration of molecular analyses may further clarify the mechanisms by which photobiomodulation influences osteogenic pathways in human bone tissue [17,46].

Within the limitations of this translational experimental model, the present study provides evidence that intraoperative laser photobiomodulation can enhance osteogenic differentiation, cellular organization, and viability in human jaw bone explants. The consistency of findings across multiple outcome measures supports the biological relevance of PBM as a modulator of bone regenerative processes.

These results contribute to the growing body of evidence supporting photobiomodulation as a minimally invasive adjunct in oral and maxillofacial surgery and provide a foundation for future clinical studies aimed at optimizing bone regeneration following cystectomy [34,49].

5. Conclusions

This translational experimental study demonstrates that intraoperative laser photobiomodulation can enhance osteogenic differentiation, cellular organization, and osteogenic marker expression in human jaw bone explants harvested during cystectomy. Using a paired design,

laser-treated explants exhibited increased osteoblast-like cellular structures and improved osteogenic surface occupancy compared with untreated controls, without evidence of cytotoxic effects.

Although biologically responsive cultures were obtained in a limited subset of patients, the consistency of findings across morphometric, immunofluorescence, and viability assessments supports the biological relevance of photobiomodulation in human bone tissue. These results suggest that laser photobiomodulation may serve as a minimally invasive adjunct for optimizing bone healing following cystic lesion removal.

Further studies with larger cohorts, refined laser dosimetry, and correlation with clinical and radiological outcomes are required to establish the clinical efficacy and reproducibility of photobiomodulation-assisted bone regeneration in oral and maxillofacial surgery.

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