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Upturn Strategies for Arachidonic Acid-Induced MC3T3-E1—625 nm Irradiation in Combination with NSAIDs: Dissipating Inflammation and Promoting Healing

Danyang Liu [1,†], Byunggook Kim [2,†], Wenqi Fu [1], Siyu Zhu [1], Jaeseok Kang [1], Oksu Kim [3,4] and Okjoon Kim [1,*]

[1] Department of Oral Pathology, School of Dentistry, Chonnam National University, Gwangju 61186, Republic of Korea
[2] Department of Oral Medicine, School of Dentistry, Chonnam National University, Gwangju 61186, Republic of Korea
[3] Department of Periodontology, School of Dentistry, Chonnam National University, Gwangju 61186, Republic of Korea
[4] Hard-Tissue Biointerface Research Center, School of Dentistry, Chonnam National University, Gwangju 61186, Republic of Korea
* Correspondence: js3894@jnu.ac.kr; Tel.: +82-62-530-4830; Fax: +82-62-530-4839
† These authors contributed equally to this work.

Abstract: Oral surgery, such as tooth extractions and dental implantations, can cause inflammation in the surrounding tissue, especially in bones. Anti-inflammatory drugs are crucial for pain relief and wound healing. Nonsteroidal anti-inflammatory drugs (NSAIDs) and light-emitting diode irradiation (LEDI) at 625 nm have been used as therapies to reduce inflammation, which ultimately promotes wound healing. The mechanism of these two methods, however, is different, which possibly makes the combined use of the two approaches effective. Therefore, the efficacy of 625 nm LEDI, NSAIDs, or a combination of both on anti-inflammatory and wound healing effects were analyzed in MC3T3-E1. In this study, piroxicam, ibuprofen, indomethacin, and celecoxib were selected as the NSAIDs. The effect of LEDI at 625 nm was investigated by cell viability, prostaglandin E_2 (PGE_2) release, and the expression of inflammation-related proteins and cell migration-related proteins were evaluated. Additionally, alkaline phosphatase staining with activity, cell migration assay and BrdU cell proliferation assays were performed. Both LEDI and NSAIDs reduced cyclooxygenase-2 (COX-2) and PGE_2. Additionally, LEDI promoted cell migration, proliferation, and bone formation as well, but not by NSAIDs. Thus, a combination of LEDI and NSAIDs can benefits the cells in inflammation, which provides upturn strategies for bone healing after tooth extraction.

Keywords: bone healing; inflammation; NASIDs; 625 nm irradiation

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1. Introduction

Oral surgery includes tooth removal, dental implantations, and orthognathic surgery, during which postoperative complications can occur in 30% of cases, such as swelling, pain, infections, etc. [1,2]. Clinically, a number of strategies can be chosen to help relieve the patient's pain [3]. Local anesthetics are administered during the procedure [4], ice packs are offered to the patient after the procedure [5], and analgesic agents are recommended [6].

After surgery, the tissue enters a recovery period. The alveolar bone healing process can be divided into three phases: inflammation, proliferation, and modeling/remodeling, which often occur sequentially [7]. However, the body's natural healing processes may interfere with the inflammation caused by the pain [8]. Uncontrolled acute inflammation may become chronic and lead to a variety of chronic inflammatory diseases [9], which also increases the risk of impaired bone healing [10]. Therefore, effective pain management can help reduce inflammation and promote healing, resulting in a faster and more successful recovery [11].

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to be effective in postoperative pain treatment [12] and are the first-line drug of choice for pain relief after tooth extractions and implants [13]. However, previous research has shown that NSAIDs can delay fracture healing, bone formation, and increase the proportion of nonunion, especially in acute treatments [14–16]. In terms of mechanisms, studies have shown that NSAIDs reduce bone healing capabilities due to Prostaglandin E_2 (PGE_2) reduction [17,18], which has a regulatory effect on bone reorganization [19]. However, the goal of controlling bone inflammation is to reduce pain and shorten the time needed to restore the bone. An adjunctive method, such as ice pack therapy, only relieves the swelling [20]. Therefore, NSAIDs need to be assisted by alternative methods to compensate for their negative effects on bone healing.

Photobiomodulation (PBM), which is also known as low-level laser therapy (LLLT), is a method of stimulating cells and tissues using a low-intensity laser or light source [21]. Light-emitting diode irradiation (LEDI) has been applied to periodontitis [22], temporomandibular disorder (TMD) [23], herpes labialis [24], and mucositis [25]. Irradiation with a low power energy density ($\geq$5 J/cm^2) may be more suitable for postoperative analgesia [26]. Low-level diode lasers promote fibroblast proliferation and osteogenic differentiation as well as regulate inflammation, with positive effects [27].

Regarding anti-inflammatory mechanisms, NSAIDs act by inhibiting cyclooxygenase (COX). Red LEDI has an anti-inflammatory effect through ROS scavenging [28,29]. Thus, NSAIDs and irradiation go through different mechanisms. Therefore, the effect of the combined use of the two methods has attracted the interest of many researchers. Laser phototherapy and piroxicam treatment reduced the arthralgia caused by the temporomandibular joint [30]. A randomized clinical study showed that the combination of low-intensity laser therapy and ibuprofen produced the best results in reducing postendodontic pain [31]. In a rat model, a combination of LLLT and diclofenac achieved more remarkable results in the treatment of acute-phase muscle strains [32]. However, the effects of phototherapy and NSAIDs combined use on anti-inflammation and bone recovery are still unclear.

In this study, we established an in vitro model of inflammation in MC3T3-E1, then verified the effects of 625 nm LEDI with NSAIDs on bone cells. We analyzed the effects of cell migration, proliferation, and bone formation using the two methods combined. The combined use of 625 nm LEDI with NSAIDs promoted cell proliferation and restored the adverse effects of NSAIDs on wound healing and bone formation.

2. Materials and Methods

2.1. Chemical Reagents and Instruments

Arachidonic acid (AA), ibuprofen, and indomethacin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Celecoxib and piroxicam were obtained from Tocris Bioscience (Tocris Bioscience, Bristol, UK). Monoclonal antibodies against E-cadherin and Vimentin were purchased from Santa Cruz (Santa Cruz, CA, USA). Antibodies for COX-1, N-cadherin, COX-2, and β-actin were obtained from Cell Signaling Technology (Danvers, MA, USA).

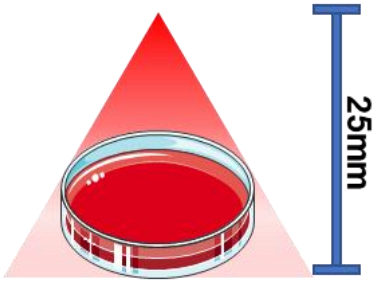
2.2. LEDI Treatment

The density power of the manufactured irradiation system (K&C wellbeing, Gwangju, Republic of Korea) was 5 mW/cm^2 and wavelength was 625 nm. The machine was installed in the cell incubator and filled with a 5% CO_2 humidified atmosphere at 37 °C. The lid of the cell culture dish was removed during the irradiation. A schematic of the irradiation system used in this experiment is shown in Figure 1.

(A)



(B)



(C)



(D)

The irradiation device	
Wavelength	625 nm
Light source	LED
Power Density	5 mW/cm^2
Current	125 mA
Voltage	4V
Frequency	Continuous wave
Exposure duration	3600(second)

Figure 1. Schematic of the irradiation system. (**A**) LEDI machine schematic; (**B**) schematic of irradiation; (**C**) irradiation physical diagram; (**D**) parameters of irradiation instrument.

2.3. Cell Culture and Design

MC3T3-E1 were cultured in the Minimum Essential Medium α (αMEM, Thermo Scientific, CA, USA) supplemented with 1% penicillin/streptomycin (Welgene, Gyeongsan, Republic of Korea) and 10% fetal bovine serum (FBS) (Atlas Biologicals, Fort Collins, CO, USA) in a 5% CO_2 humidified atmosphere at 37 °C. Cells were irradiated at a wavelength of 625 nm for 1 h, and then, exposed to AA for 6 h. Cell viability was detected at indicated timepoints. Furthermore, to detect the anti-inflammatory effects of LEDI and NSAIDs, with or without LEDI, MC3T3-E1 were treated with AA for 6 h and 10 nM–1 μM of piroxicam, 0.1–10 μM of ibuprofen, 50 nM–0.5 μM of indomethacin, and 10 nM–1 μM of celecoxib for 1 h, after 8 h, the cell supernatants were harvested.

The groups in the present study are as follows: Cells without any treatment: control group; only AA-treatment: AA group; only 625 nm LEDI: IR group; 625 nm LEDI pretreatment with AA treatment: PreIR+AA group; 625 nm LEDI and AA treatment simultaneously: SimulIR+AA group; AA treatment and post-treatment with 625 nm: PostIR+AA group; AA treatment and NSAIDs treatment: AA+NSAIDs group; 625 nm LEDI pretreatment, AA treatment followed by NSAIDs treatment: PreIR+AA+NSAIDs group.

2.4. Cell Viability Assay

MC3T3-E1(2×10^6 cells/mL) were seeded into 96-well plates (Corning, NY, USA) in αMEM with 10% FBS and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 atmosphere. MC3T3-E1 were treated with 0–400 μM AA for 6 h. At the end of the experiment, each well was rinsed using 1× PBS, which had been prewarmed to 37 °C, to remove any dead cells. At the appointed times, 10 μL of the Cell Viability Assay Kit reagent (EZ-Cytox, DoGen,

Republic of Korea) was added to each well, and incubated at 37 °C in 5% CO_2 for 30 min, the absorbance was measured at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). Cell viability was analyzed using the following formula [33]:

$$\text{Cell viabiliy} = \frac{\text{Optical density of treated}}{\text{Optical density of control}} \times 100\% \quad (1)$$

2.5. PGE_2 Release Assay

MC3T3-E1 cells were pretreated with or without LEDI, treated with 0, 10, 20, 50, 100, and 200 μM AA for 6 h. Next, the cell culture medium was harvested and centrifuged at 1000 rpm for 5 min and the supernatant was collected for further analysis. The PGE_2 kit (R&D Systems, Minneapolis, MN, USA) was used to measure PGE_2 levels, according to the manufacturer's instructions. The absorbance was tested at 450 nm using a microplate reader for data analysis.

2.6. Western Blotting

The cells were rinsed with 1× PBS, prechilled at 4 °C, and lysed in radioimmunoprecipitation (RIPA) buffer (Biosesang, Seongnam, Republic of Korea), supplemented with a protease inhibitor cocktail (PIC) (Takara, Shiga, Japan), and phenylmethylsulfonyl fluoride (PMSF) (Sigma Aldrich Corp., St. Louis, MO, USA) at a ratio of 1000:1:1. Protein extracts were normalized using the BCA assay (Thermo Scientific, Santa Clara, CA, USA), and 30 μg extracted proteins were electrophoresed on either a 7.5% or 10% SDS-PAGE gel, then, transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, Piscataway, NJ, USA). The PVDF membranes were blocked in a 5% non-fat skim milk–0.1%-Tween (TBST) buffer at room temperature for 1 h to reduce the non-specific binding of the incubated antibodies to the PVDF membrane.

After blocking, the membranes were probed with monoclonal primary antibodies overnight at 4 °C, at the following concentrations: anti-COX-1 (1:1000), anti-COX-2 (1:1000), anti-β-actin (1:2000), anti-PCNA (1:2000), anti-Vimentin (1:500), anti-E-cadherin (1:500), and anti-N-cadherin (1:1000). Afterwards, the membranes were washed four times with 0.05% TBST. Then, the PVDF membranes were incubated in the specific secondary antibodies, either in anti-rabbit IgG (1:10,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-mouse IgG (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at RT for 2 h. Then, the membranes were visualized using ECL Western Blotting Substrate (Thermo Scientific, Santa Clara, CA, USA). All antibodies were reconstituted according to the manufacturer's instructions. The 1× TBS was configured from a 20× TBS stock solution, which was dissolved in DDW. Likewise, a 10× PBS stock solution was dissolved to 1× PBS using autoclaved DDW.

2.7. BrdU Cell Proliferation Assay

Cells were incubated in 96-well plates, with or without irradiation, and treated with AA for 6 h. Then, the cells were harvested, at the indicated timepoints. The BrdU Cell Proliferation assay kit (Roche, Indianapolis, IN, USA) was used, according to the manufacturer's instructions. A 0.01% H_2SO_4 solution was used as the stop solution. The absorbance of each well was measured at 450 nm using a microplate reader.

2.8. Migration Assay

MC3T3-E1 were seeded at 2 × 10^6 cells/mL in 6-well plates and incubated for 24 h to enable the cells to become 100% confluent. Then, a 10 μL pipette tip was used to scratch the bottom of the well to create a wound. Afterwards, each well was rinsed with 1× PBS and images of each well were taken, at each timepoint, using a 4× PL FL phase microscope (Lionheart FX, Winooski, VT, USA) with the phase contrast channel. The area of migration

in the control group was set to 100%. The initial migration area was set to A(t0), and the endpoint migration area was A(t). Migration area was calculated as follows [34]:

$$\text{Migration area} = \frac{A(t0) - A(t)}{A(t0)} \times 100\% \quad (2)$$

2.9. ALP Activity and ALP Staining

Cells were inoculated at 2×10^6 cells/mL in a 35 mm dish until the cell confluency reached 70%. Then, MC3T3-E1 with or without irradiation were treated with 100 μM AA for 6 h, followed by NSAIDs for 1 h. After 8 h, the media were replaced with the bone differentiation medium. The medium was changed every 3 days. After 7 days of using the differentiation medium, ALP activity was evaluated using the ALP kit (Anaspec, Inc., Fremont, CA, USA). The fixed cells were stained using the ALP staining reagent (Thermo Scientific, Santa Clara, CA, USA). The liquid was aspirated from the cells, and images of the staining in the cell dishes were scanned. The crystals in the culture dish were dissolved using 10% cetylpyridinium chloride (Sigma Aldrich Corp., St. Louis, MO, USA) reagent, and the absorbance was tested at 570 nm using a microplate reader. Differentiation medium content αMEM was supplemented with 10% FBS, 1% penicillin/streptomycin, 100 nM dexamethasone, 50 μg/mL l-ascorbic acid, and 10 mM β-glycerophosphate (Sigma Aldrich Corp., St. Louis, MO, USA).

2.10. Statistical Analysis

The experimental results were expressed as mean ± standard deviation from at least two independent experiments. Statistical analysis was performed using a one-way ANOVA test with SPSS software (version 22.0 SPSS Inc., Chicago, IL, USA). *p*-values were set as follows * $p < 0.1$, ** $p < 0.01$, and *** $p < 0.001$.

3. Results

3.1. Effects of AA on MC3T3-E1

To understand the effects of AA on the cell viability and inflammatory status of the MC3T3-E1, they were treated with 0–400 μM AA for 6 h. Figure 2A shows that AA concentrations of 0–200 μM do not affect cell viability. However, treatment of 300 μM AA for 6 h decreased the cell viability to approximately 60%.

COX-1/COX-2 was analyzed in AA-treated MC3T3-E1 by Western blotting (Figure 2B,C). COX-2 increased in a dose-dependent manner of AA. However, the level of COX-1 was constant. Figure 2D shows increased PGE_2 levels in dose-dependent manner, which agreed with COX-2 expression. In total, 100 μM AA was chosen for the further experiment.

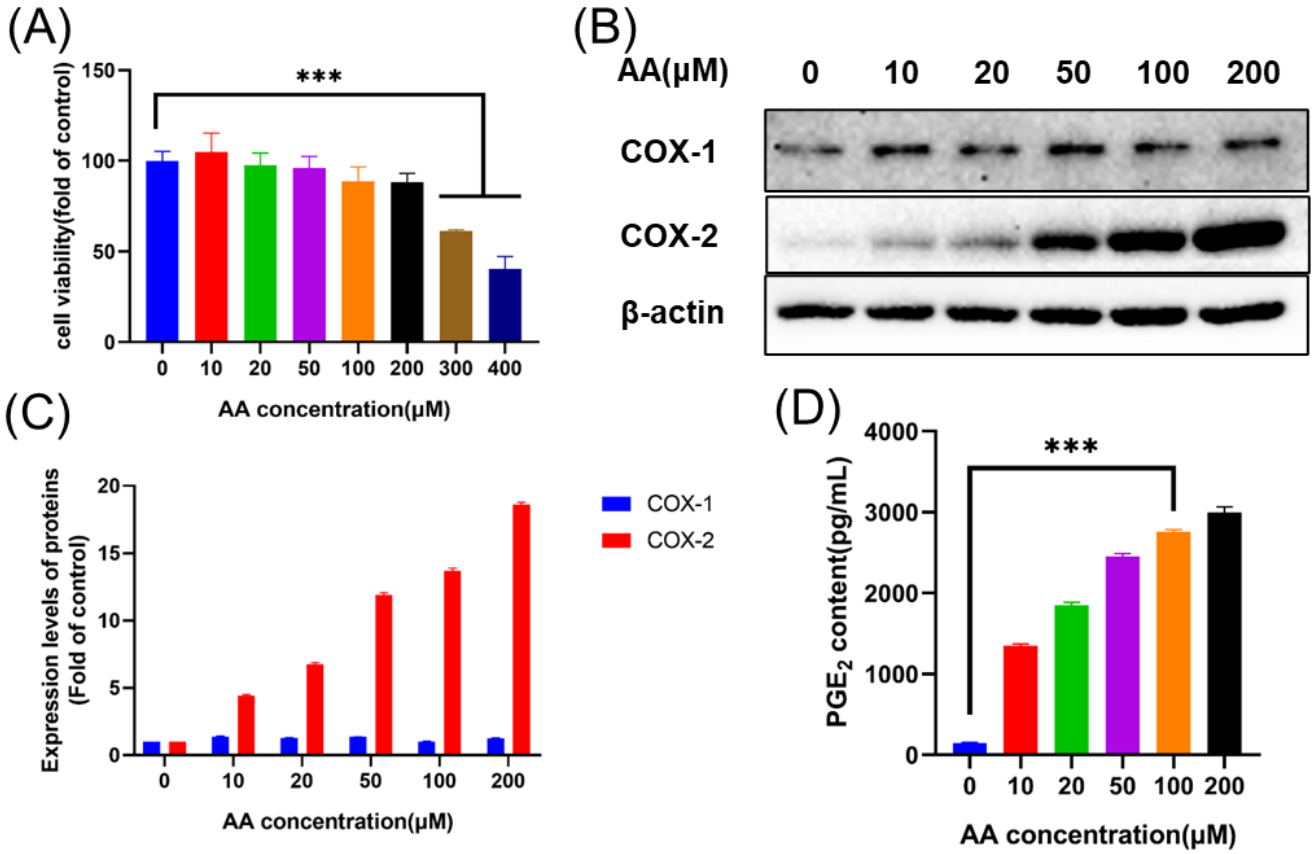


Figure 2. Effects of AA on MC3T3-E1. (**A**) MC3T3-E1 were treated with 0–400 μM AA for 6 h for cell viability. (**B**) The expressions of COX-1 and COX-2 were analyzed by Western blotting in a dose-dependent manner of AA. (**C**) The relative expression levels of the proteins (protein/β-actin) were shown using Image J software. (**D**) After AA treatment, the release of PGE_2 was detected using an ELISA kit. The PGE_2 release increased after AA induction, and the levels of PGE_2 were positively correlated with the AA concentrations. *** $p < 0.001$.

3.2. Effects of 625 nm LEDI on AA-Induced Inflammation in MC3T3-E1

To investigate the anti-inflammatory effect of LEDI in present study, the cells were irradiated before, simultaneously, or after 100 μM AA treating and cell viability and PGE_2 release were evaluated.

Regardless of the irradiation or indicated AA treatment, cell viability was not affected (Figure 3A). Both the PreIR+AA and SimulIR+AA group had a decreased PGE_2 release. However, PGE_2 release was not affected in the PostIR+AA group. The PreIR+AA group had the most decreased PGE_2 in the present study (Figure 3B), and was used for further experiments.

COX-2 expression was increased in AA group. The PreIR+AA group, however, had a decreased COX-2 expression (Figure 3C,D), which is consistent with PGE_2 release (Figure 3B).

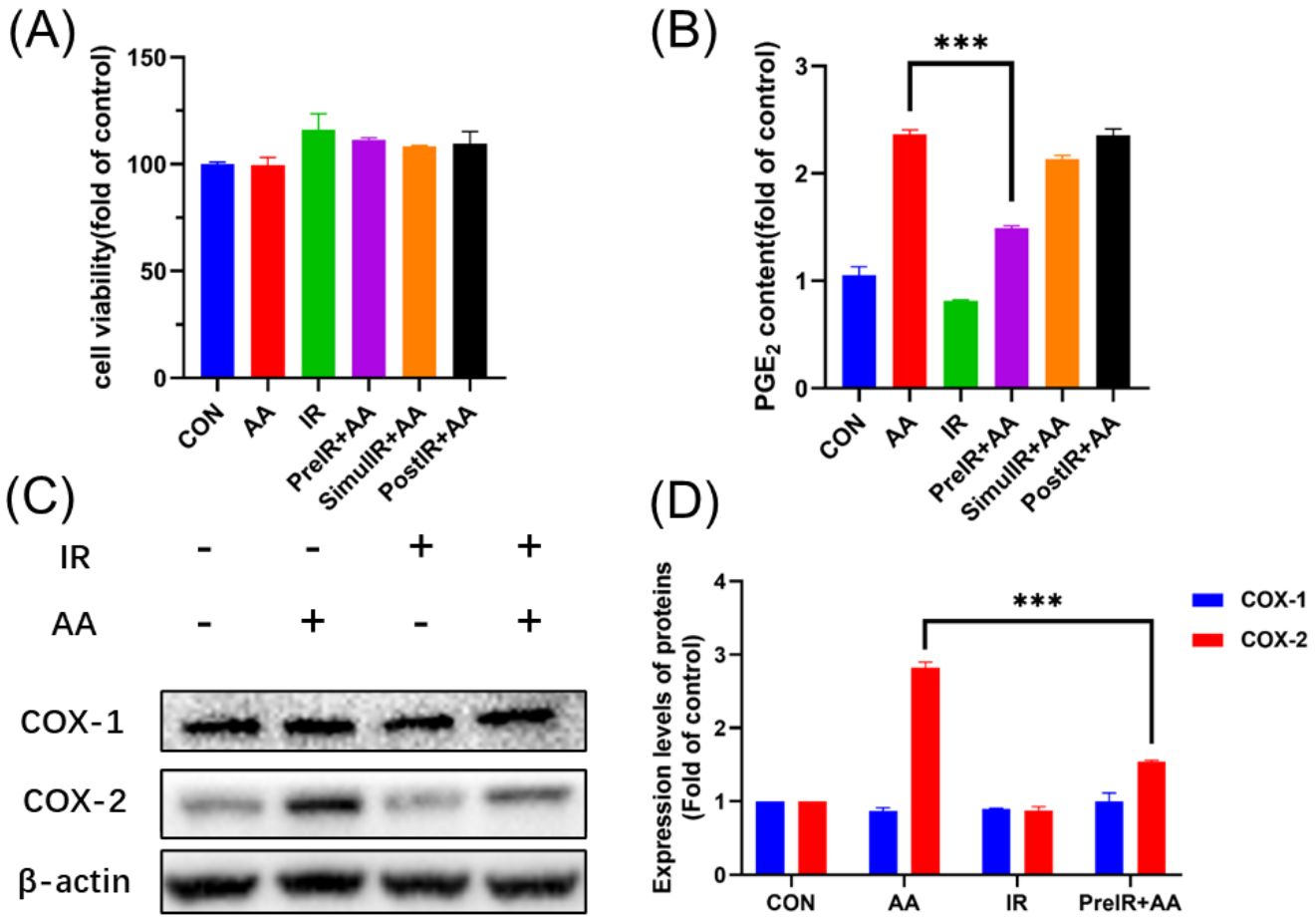


Figure 3. Effects of 625 nm LEDI on cell viability and PGE_2 in AA-induced MC3T3-E1. (**A**) MC3T3-E1 were treated with irradiation before AA treating (PreIR+AA), AA treating and irradiation simultaneously (SimulIR+AA), and irradiation after AA treating (PostIR+AA) for cell viability and (**B**) PGE_2 assay. PreIR+AA group had the most decreased PGE_2 release. (**C**) Effects of irradiation on COX-1 and COX-2 expression in AA-induced MC3T3-E1 analyzed by Western blotting. (**D**) The relative expression levels of proteins (protein/β-actin) were shown using Image J software. Compared to the AA group, PreIR+AA group significantly decreased COX-2, which was consistent with PGE_2 release. *** $p < 0.001$.

3.3. Effects of NSAIDs on Cell Viability and PGE_2 in MC3T3-E1

To compare the anti-inflammatory effects of several NSAIDs in AA-induced MC3T3-E1, cell viability and PGE_2 assay were performed. Cell viability was not affected within 10 μM NSAIDs (Figure 4A–D). 10 nM–1 μM of piroxicam, 0.1–10 μM of ibuprofen, 50 nM–0.5 μM of indomethacin, and 10 nM–1 μM of celecoxib were determined by PGE_2 assay. NSAIDs significantly reduced PGE_2 release in a dose-dependent manner (Figure 4E–H). Additionally, 1 μM of piroxicam, 10 μM of ibuprofen, 0.5 μM of indomethacin, and 1 μM of celecoxib decreased PGE_2 to the control level, which was used in further experiments.

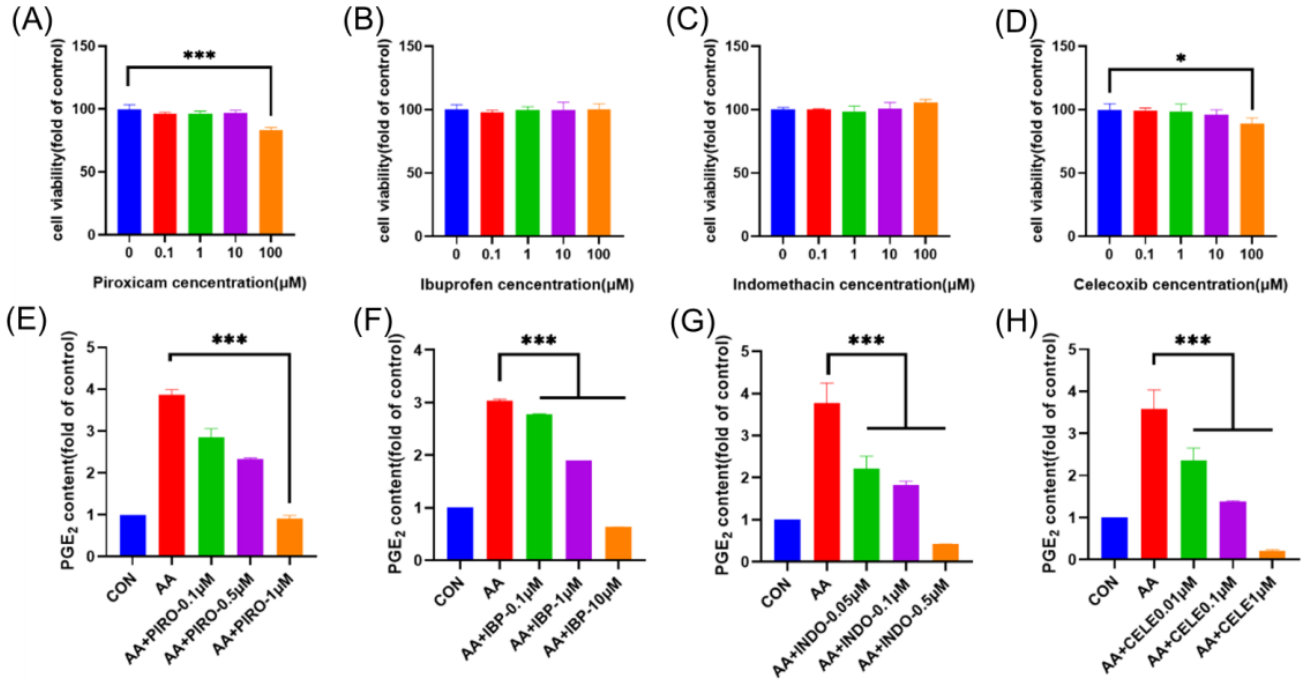


Figure 4. Effects of NSAIDs on MC3T3-E1 cell viability and PGE_2 release. (**A**–**D**) MC3T3-E1 were treated with four kinds of NSAIDs: Piroxicam, ibuprofen, indomethacin, and celecoxib at different concentrations for 1 h, and the cell viability was evaluated. (**E**–**H**) Among them, after treating with 10 nM–1 μM of piroxicam, 0.1–10 μM of ibuprofen, 50 nM–0.5 μM of indomethacin, and 10 nM–1 μM of celecoxib, PGE_2 was detected using an ELISA kit. PIRO: piroxicam; IBP: ibuprofen; INDO: indomethacin; CELE: celecoxib; * $p < 0.1$, *** $p < 0.001$.

3.4. Effects of 625 nm LEDI and NSAIDs on Cell Migration in MC3T3-E1

The IR group mostly increased to the area covered, whereas the scratch remained in both AA and AA+NSAIDs groups (Figure 5A,B). In the presence of AA, four kinds of NSAIDs decreased the area covered, but irradiation increased the migration area. Meanwhile, to further verify the effects of 625 nm LEDI and NSAIDs on cell migration, the expression levels of proteins related to cell migration were analyzed by Western blotting (Figure 5C,D). Both the AA and AA+NSAIDs group had an increased E-cadherin but decreased Vimentin and N-cadherin expression, compared to the control group. Compared to the AA group, the N-cadherin expression was decreased in the AA+NSAIDs group. However, the PreIR+AA group had an increased expression of Vimentin but a decreased E-cadherin.

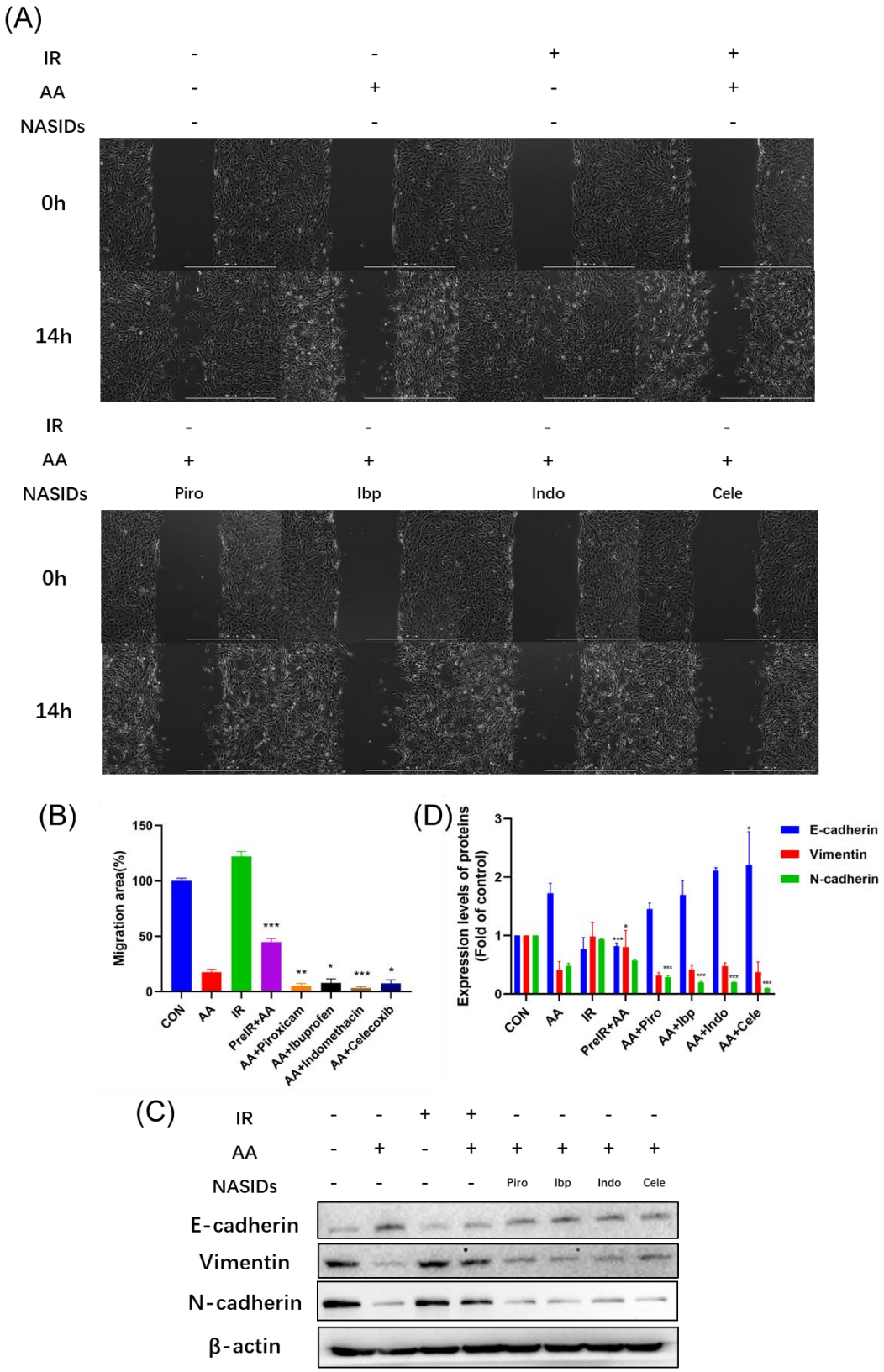


Figure 5. Effects of 625 nm LEDI and NSAIDs on cell migration in AA-induced MC3T3-E1. (**A**) Pictures of the cell migration process. (**B**) Migration area was measured by Image J software. (**C**) The expressions of E-cadherin, Vimentin and N-cadherin by Western blotting. (**D**) The relative expression levels of proteins (protein/β-actin) were shown using Image J software. * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$. Compared with the presence or absence of AA.

3.5. Effects of Combined 625 nm LEDI and NSAIDs on Cell Migration and Proliferation in AA-Induced MC3T3-E1

To investigate the effect of the PreIR+AA group, AA+NSAIDs group, and PreIR+AA+NSAIDs group on cell migration, the expression of N-cadherin, E-cadherin, and Vimentin were analyzed (Figure 6A,B). The AA and AA+Cele group had an increased expression of E-cadherin but a decreased Vimentin and N-cadherin expression. Compared to the AA+group, the PreIR+AA group had an increased PCNA expression, which is consistent with the BrdU result. Compared to the AA+Cele group, the PreIR+AA+Cele group had a significantly increased expression of PCNA (Figure 6C,D). The PreIR+AA group had a significantly reduced PGE_2 in AA-induced MC3T3-E1. The AA+Cele group had a decreased PGE_2, compared to the PreIR+AA group. PGE_2 was decreased to the control level in the PreIR+AA+Cele group (Figure 6E).

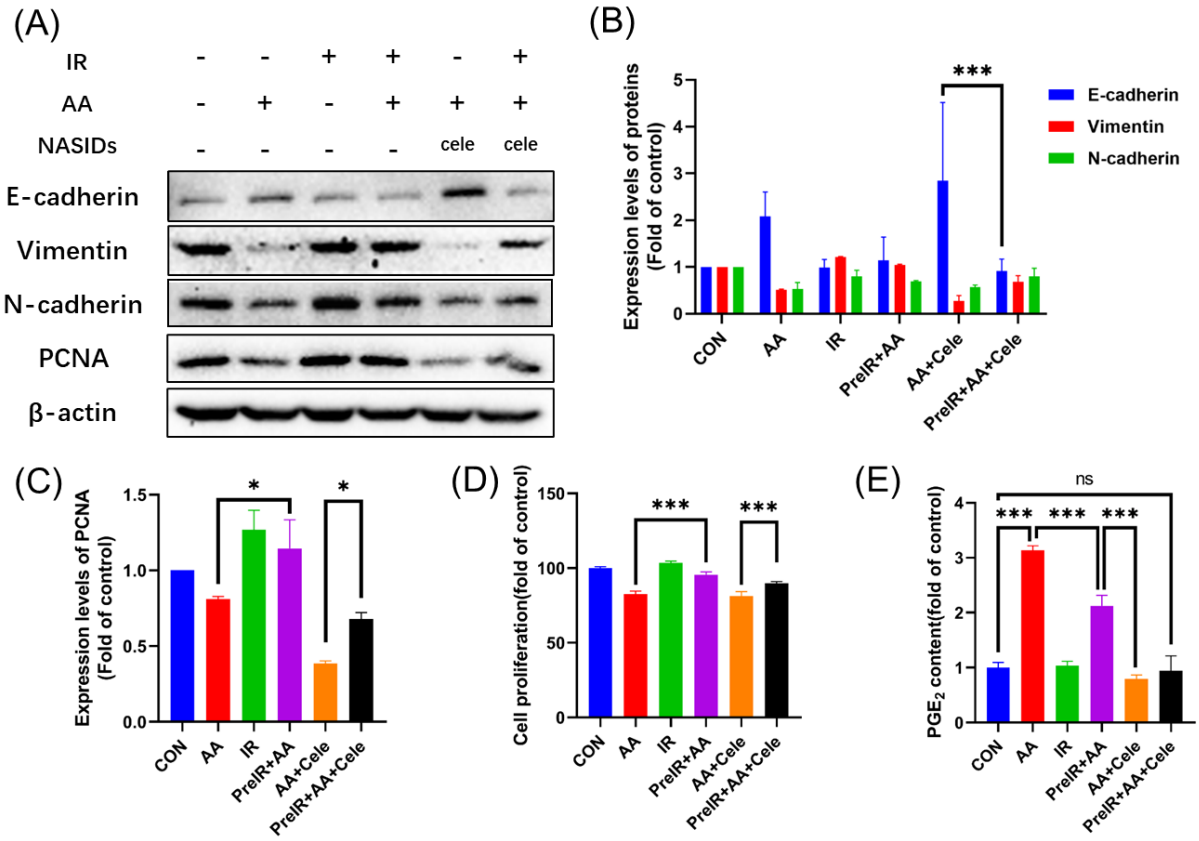


Figure 6. Effects of combined 625 nm LEDI and NSAIDs on cell migration and proliferation in AA-Induced MC3T3-E1. (**A**) The expressions of E-cadherin, N-cadherin, Vimentin and PCNA were analyzed by Western blotting. (**B**,**C**) The relative expression levels of proteins (protein/β-actin) were shown using Image J software. (**D**) Cell proliferation was evaluated with the BrdU Cell Proliferation Assay Kit. (**E**) After treatment, the release of PGE_2 from MC3T3-E1 was detected by an ELISA kit. * $p < 0.1$, *** $p < 0.001$.

3.6. Effects of the Combined Use of 625 nm Irradiation and NSAIDs on the Bone Formationin AA-Induced MC3T3-E1

To investigate the effects of LEDI, NSAIDs, and the combination of both on bone formation in the presence of AA, ALP staining (Figure 7A,B) and ALP activity (Figure 7C) were performed. ALP staining and ALP activity levels were decreased in the AA and AA+Cele group. However, the PreIR+AA+Cele group had an increased ALP staining and activity, compared to the AA+Cele group. The PreIR+AA+Cele and PreIR+AA group results were similar. Both the PreIR+AA+Celecoxib and PreIR+AA group had an increased ALP staining and activity, compared to the AA group.

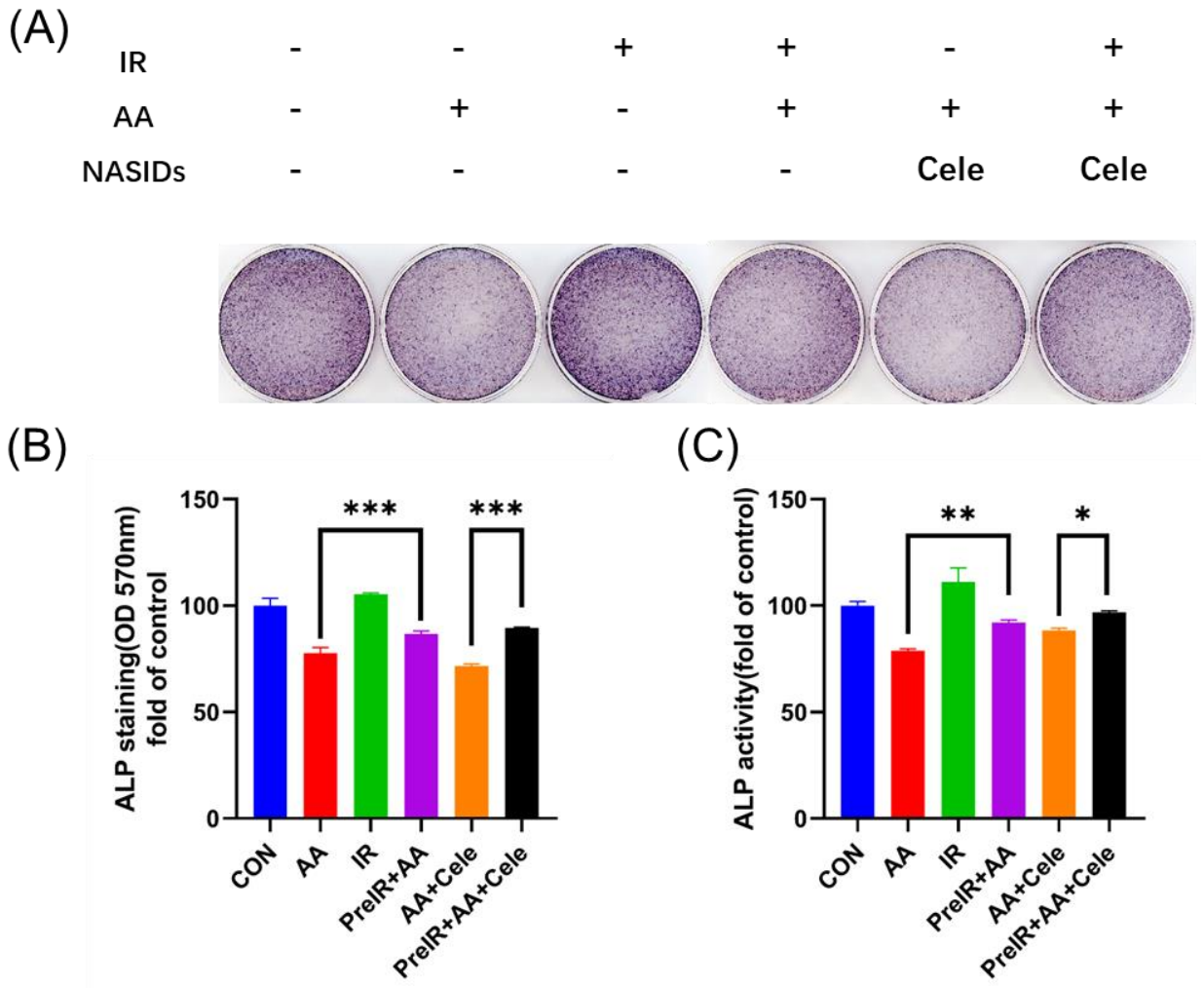


Figure 7. Effects of combined use of 625 nm LEDI and NSAIDs on bone calcification in AA-induced MC3T3-E1 (**A**,**B**) ALP staining and their relative expression was measured by absorbance. (**C**) ALP activity was detected by an ELISA kit. Cele: celecoxib * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

NSAIDs are commonly used in dentistry for pain relief and anti-inflammation by reducing the synthesis of prostaglandins, which are needed in bone formation [35]. Therefore, NSAIDs are known to inhibit bone healing [36,37]. Thus, there is a need for alternative therapies.

PBM can reduce the levels of inflammation by increasing the expression of anti-inflammatory genes, enhancing regeneration potential, and reducing oxidative stress through enhanced ROS scavenging [29,38,39]. The positive effect of PBM on wound healing has previously been demonstrated by shorter defect of skin wound [40,41], increased levels collagen synthesis [42], and tensile strength [43,44].

Owing to the different anti-inflammatory mechanisms and wound-healing properties, their combined application may potentially reduce inflammation and enhance wound healing as well.

For tooth extraction and dental implant surgery, bone formation is required for complete healing. In this study, the effects of NSAIDs and LEDI were compared for both anti-inflammation and wound healing. Moreover, the combined effects of these two treatment options were evaluated by cell migration, cell proliferation, and bone formation.

PGE_2 and COX-2 levels were increased in a dose-dependent manner following AA treatment in MC3T3-E1 (Figure 2). COX-2 is an inducible enzyme that can be induced by

inflammatory inducements [45], while COX-1 is a structural enzyme [46]. Furthermore, both PGE_2 and COX-2 were reduced in the PreIR+AA group, which suggests that irradiation has anti-inflammatory properties (Figure 3). Pretreatment with LEDI decreased PGE_2 and COX-2, which agreed with a previous report [47]. NSAIDs were commonly used as clinical agents, acting as anti-inflammatory and analgesic agents by inhibiting COX to reduce PGE_2 release [48]. PGE_2 was reduced in a dose-dependent manner of NSAIDs (Figure 4).

Several reports showed that inflammation delays cell migration [49,50]. Additionally, the ability of cells to migrate is an essential physiological process involved in wound healing [51]. In the present study, the AA+NSAIDs group failed to the cover area between the scratches, while the PreIR+AA group increased the migration area (Figure 5A,B). In addition, cell migration requires alterations in cell shapes, such as extension, attachment, retraction, and release [52]. E-cadherin, Vimentin and N-cadherin have crucial roles in junctional attachment [53], cell extension formation [54], and cell adhesion [55], respectively. During cell migration, E-cadherin expression is suppressed [56], although Vimentin and N-cadherin expressions are often promoted [57,58]. Based on this present study, celecoxib increased E-cadherin expression, which lead to tighter cell–cell junctions followed by a reduction in cell movement. The expression of E-cadherin was decreased, but Vimentin was increased in the PreIR+AA group compared to the AA group (Figure 5C,D). Due to the different effects of these two methods on cell migration, the combination used in the subsequent experiments obtained better wound healing results.

Indeed, using a combination of NSAIDs and LEDI, the MC3T3-E1 expressed increased N-cadherin and Vimentin expression, although decreased E-cadherin, compared to AA+Cele group (Figure 6A,B). Moreover, the combination treatment reduced PGE_2 levels compared to the PreIR+AA group (Figure 6E). PCNA expression increased during cell proliferation [59,60]. In the present study, the PreIR+AA group was shown to increase the expression of PCNA, consistent with the BrdU result (Figure 6C,D), which agree with a previous report [61]. Cell migration is essential for bone healing [62] and ALP is highly detected during bone formation [63]. In a previous report, NSAIDs inhibited osteogenesis [18], however, irradiation enhanced bone regeneration [64]. ALP activity and staining were reduced in the AA + Cele group but recovered in the PreIR+AA+Cele group (Figure 7). After applied irradiation, a combined use may promoted bone formation in inflammation and NSAIDs condition.

5. Conclusions

In this study, evidence was provided that supports the combined use of LEDI and NSAIDs on an inflammation-induced MC3T3-E1 in vitro system. The combined use of LEDI and NSAIDs achieved an anti-inflammatory effect and promoted wound healing simultaneously. For future applications, a 625 nm LEDI should be conducted prior to tooth extraction or dental implantation, as it may help relieve pain and promote bone healing. In summary, the combined use of LEDI and NSAIDs could potentially represent an upturn strategy for bone healing process.

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