

## **AAO Foundation Award Final Report**

Type of Award: Orthodontic Faculty Development Fellowship Award (2017 Albert P. Westfall Memorial Teaching Fellowship Award).

Name(s) of Principal Investigator(s): Ghada Nimeri

Title of Project: Characterization of the molecular level effects of low level laser therapy irradiation on periodontal ligament cells.

Period of AAOF Support: 07-01-17 to 06-30-18

Amount of Funding: \$20,000

**Summary/Abstract:** The purpose of this study was to characterize the optimized effects of light-emitting Diode (LED) on human periodontal ligament cells, to be accomplished by comparing the molecular effect of different wavelengths, intensities and duration of low-level laser irradiation on human periodontal ligament cells. My specific aims were as follows: 1) obtain specialized training in course development, electronic teaching tools, time management, and visual literacy through Marquette University's Center for Teaching and Learning to become an effective educator for my students; 2) obtain more training in wire bending, by attending the Tweed Foundation Course and enhance my clinical skills; 3) begin a research program to characterize the optimized effects of a low level laser on human periodontal ligament cells, which will be the foundation for future human and animal research projects; and 4) obtain certification from the American Board of Orthodontics in order to launch my clinical practice within Marquette's School of Dentistry. These aims were proposed to meet the PI's career and research goals in teaching, research and clinical practice.

Response to the following questions:

1. Were the original, specific aims of the proposal realized?

To date, I have successfully achieved part of my goals and objectives during the project period, which included working towards aim 1 (courses in educator development are offered sporadically throughout the academic year, and I will continue to attend sessions as they become available). This will develop my understanding and knowledge of pedagogy, learning styles, and practices for teaching and learning in a variety of settings. Regarding aim 2 (I have successfully completed the Tweed Course, which is an intensive 10-day course in advanced edgewise mechanics, differential diagnosis, and the Tweed/Merrifield force system. I have gained valuable information regarding the historical aspect, plus hands-on training on wire bending. This has enhanced my orthodontic knowledge and clinical skills and was a huge step in my educational and clinical development.

In aim 4 (my goal was to complete certification through the American Board of Orthodontics. I have successfully completed the requirements needed for being a board-certified orthodontist). This was a very rewarding experience and helped in enhancing my level of confidence and development as a new graduate orthodontist.

Progress was also made towards completing aim 3, which included calibration of protocol and concentration of PDL cells needed to actively detect some of these biological responses related to this project.

We were delighted to present this abstract at the 2018 AADR meeting. Setting up the protocol took longer than expected, delaying the start of the experiments. Details of the research completed to date are as follows:

**Introduction:** Light-Emitting Diode (LED) irradiation has been proposed to enhance bone metabolism and recently introduced to help move teeth faster during orthodontic treatment. However, previous study results are contradictory, and there is a lack of understanding of the mechanism involved at cellular and molecular levels.

**Objective:** To study the early release of Adenosine Triphosphate (ATP) and the late downstream productions of osteogenic related proteins i.e. Osteoprotegerin (OPG), Receptor activator of nuclear factor kappa-B ligand (RANKL) and Asporin (ASPN) from human periodontal ligament (PDL) cells in response to LED irradiation, in attempt to gain a molecular insight of the LED-induced change of bone metabolism involved in orthodontic tooth movement.

**Materials and Methods:** PDL cells were cultured in alpha-MEM with 10% serum. The cells were seeded at a density of  $2 \times 10^5$  in 6-well culture dishes for 24 hours, starved with serum-free alpha-MEM overnight, then exposed to LED light (660 nm wavelength and  $50 \text{mW/cm}^2$  light intensity) for 88 seconds. Medium samples were collected immediately after LED irradiation to measure ATP release using a Luminescent ATP detection kit. Protein samples were collected after 5 hours post LED irradiation, OPG/RANKL and ASPN were detected using western blot analysis. The results of these variables were compared between the irradiation group and the non-irradiation group using one-way ANOVA ( $P < 0.05$ ). The experiments were repeated three times.

**Results:** ATP release was drastically elevated after LED irradiation 3.3 times higher in the irradiation than in the non-irradiation group ( $P = 0.000$ ). The ratio of OPG/RANKL was decreased by 60% in irradiation group than in non-irradiation group ( $P = 0.002$ ). ASPN production was slightly changed but insignificantly.

**Conclusion:** LED regulates both early signaling molecule - ATP and the downstream osteogenic-related proteins i.e. OPG/RANKL and ASPN in human PDL cells, which sheds light on the possible molecular mechanisms involved in the LED-induced acceleration of orthodontic tooth movement.

#### **Next steps:**

The established protocol and the data obtained to date are very important for our ongoing experiments. Our main goal is to characterize the optimized effects of a low-level laser/ or light-emitting Diode on human periodontal ligament cells. This will be accomplished by comparing the molecular effect of different wavelengths, intensities and duration of low-level laser irradiation on human periodontal ligament cells. We have begun the investigation with one wavelength of LED light (660 nm wavelength and  $50 \text{mW/cm}^2$  light intensity) for 88 seconds, optimizing our protocol and cellular concentration.

A new light-emitting diode device has been built with the help of an optical engineer with specific qualifications needed for the main investigation. With this device we will be able to compare 3 different wavelengths at the same time with the same power and exposure time, allowing me to be able to continue with my research goals in a more timely fashion, expanding to investigation of several additional wavelengths. Additional funding will be sought to complete this crucial next step.

2. Were the results published?

- a. A manuscript is under preparation for the experiments described above. After final checking of the parameters it will be submitted to Lasers Med Sci for publication.
- b. AAOF will be acknowledged in the coming publication.

3. Have the results of this proposal been presented?

Yes.

- a. If so, list titles, author or co-authors of these presentation/s, year and locations.

Oral presentation at the AADR meeting, 2018 Fort Lauderdale, FL.

Abstract number: 0183

LED Irradiation Regulates ATP Release and OPG/RANKL in PDL cells.

Presenter: Ghada Nimeri Co-investigator; Dawei Liu

- b. Was AAOF support acknowledged?

Yes, the acknowledgement was mentioned in the oral presentation and will also be acknowledged in the future publication.

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

AAOF funding has been important for my research project and the establishment of my academic career. The funding obtained has allowed me to develop in my research, and in my training and development as a researcher and educator.

Research: I was able to create a reproducible protocol for the cellular molecular experiments needed to fulfil the main purpose of the investigation. Please see above for more details.

This funding has provided me with a baseline for my research career, enabling me to take the first steps towards an NIH-level application.

Educational and Clinical: It has also enabled me to complete some of my goals that are important in my educational and clinical development (as mentioned above).

I am very grateful to the AAOF, which has been important to the establishment of my career.