AAO Foundation Final Report

1. **Type of Award**: Biomedical Research Award

2. **Name(s) of Principal Investigator(s)**: Phimon Atsawasuwan

3. **Institutional**: University of Illinois at Chicago

4. **Title of Project**: A Novel Nanoparticle Delivery System of miRNAs for Orthodontic Purposes

5. **Period of AAOF support**: 07-01-19 to 11-15-22

6. **Amount of AAOF Funding**: $30,000

7. Summary

Specific Aim #1. To investigate the release rate of miRNA-29 from the miRNA-29 encapsulated PLGA nanoparticles. The release rate of miRNA-29 will be investigated using in vitro under conditions considered to have in vitro-in vivo correlation (IVIVC) to verify the time course during the miRNA delivery.

The first specific aim was successfully performed, and the result was obtained below. We have performed the in vitro-in vivo correlation (IVIVC) to verify the time course during the miRNA delivery. The preparation of miR-29 nanoparticles was successfully performed, and the in vitro release assay was performed over the time course of 105 days (15 weeks). The release study was verified using the nanodrop spectrophotometer and miRNA qPCR using Taqman® microRNA RT-PCR assays specific to hsa-miR-29. We discovered that the release sustainability of microRNA extended through 105 days of the study. The finding verified the potential of PLGA to be used as a slow-release system for miRNA delivery for orthodontic purposes.

As described above, we have performed the proposed specific aim 1 and discovered that the PLGA nanoparticles sustained the release of microRNA for up to 7 weeks. In the original proposal, we proposed to study this aim for 4 weeks (5 time-points; days 1, 7, 14, 21, and 28); however, we would like to evaluate the in vitro dynamic miRNA release ability profile of PLGA nanoparticles. We extended the duration of the study to 7 weeks (105 days) and increased the sample collection time-points (18 time-points) during the experiment. The brief methodology was described below. A hundred microliters of each particle preps were pipetted into the 1 mL of 1X Dulbecco's Phosphate Buffer Salt solution (1X DPBS, Corning) in 1.5 mL Eppendorf tubes. The nanoparticles were incubated at 37ºC on a shaker platform for 105 days. The tubes were centrifuged to pellet the particles at a specific time.
The entire volume of supernatant PBS was removed and stored at -80°C until analysis. The pellets in the Eppendorf tubes were suspended with the fresh DPBS buffer, and incubation was continued until the end of the study. The analyses of miRNA in collected supernatant were performed using a nanodrop spectrophotometer (ThermoFisher) and 7900HT real-time PCR system (Applied Biosystems). First, we determined the concentration of nucleic acid in the supernatant from the in vitro release study using the nanodrop spectrophotometer at 260 and 280 nm. We found that the concentration of nucleic acid in the supernatant is very low or below the detectable level of the spectrophotometer. We solved the low concentration in the supernatant samples by taking 500 µL of the supernatant samples from each batch and aliquoting

them into Eppendorf tubes using Amicon Ultra-0.5 Centrifugal Filter Unit (3,000 MWCO) to concentrate the supernatant to 80 µL volume. We subjected the supernatant to the nanodrop spectrophotometer at 260 and 280 nm to determine the nucleic acid concentration in each supernatant sample. The profile of nucleic acid in the collected supernatant was demonstrated in Figure 1A. The spectrophotometer showed a high amount of nucleic acid in the supernatant of early time-point samples, decreasing at the end of the experiment. The value from the spectrophotometer cannot demonstrate the presence of miR-29 in the supernatant, yet it demonstrated the presence of nucleic acid in the supernatant at each time point. After determining nucleic acid in the supernatant, 1 µL of concentrated samples from each batch was used as a template for RT-realtime PCR assays specific to hsa-miR-29. The concentrated supernatants from each batch of miRNAs were analyzed in triplicate. The profile of miR is shown in Figure 1B. The CT values represent the inverse of the amount of miR-29. The low CT value of real-time PCR represents the high abundance of miRNA, and the high CT value represents the low abundance of miRNA. The result showed that the presence of miR-29 in the early time-point supernatant was high on the first 2 days and then sustained at the same level until the end of the study. The slight difference in the profiles between the one from the spectrophotometer and the one from the real-time PCR may be due to the light absorbance of PLGA polymer or the minute amount of PLGA particles in the solution because the empty particle demonstrated the value of absorbance during the early time-point as well.

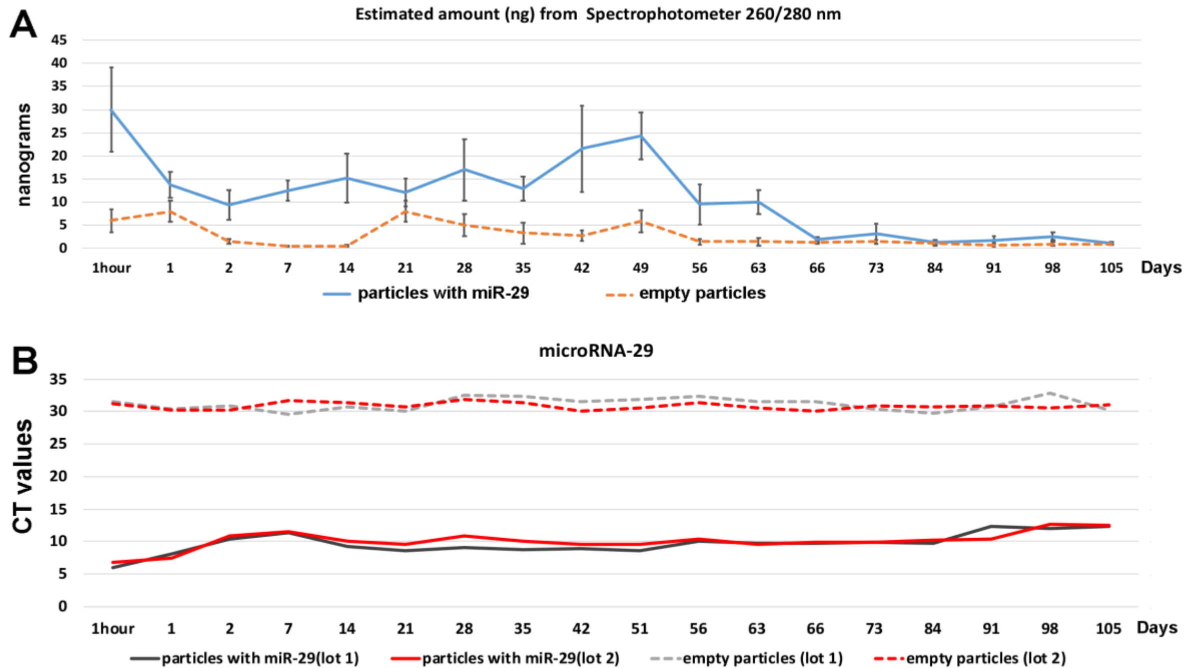

Figure 1. Profiles of prolonged miRNA-29 release from the PLGA particles over the time course of the study. (A, the amount of nucleic acids and B, the presence of miR-29).

Specific Aim #2. To investigate the effect of miRNA-29 mimic or inhibitor encapsulated PLGA nanoparticles on osteoclastic activities in vitro and in vivo. The nanoparticles will be tested in human osteoclasts in a dose-dependent manner. TRAP, H&E, and phalloidin staining techniques, MTS cell proliferation assay, and osteoclast marker expression will be evaluated. In addition, the distance of tooth movement after in vivo local delivery of miR-29 mimic or inhibitor encapsulated PLGA nanoparticles in a mouse model will be evaluated.

REPORT OF RESULTS

We conducted the experiments on the murine preosteoclasts RAW264.7 and checked the biocompatibility of nanoparticles to the cells. We performed some preliminary experiments on these cells. We also performed the delivery of miRNA- 29 mimic nanoparticles into the preosteoclast cells and observed the differentiation. We established and investigated the phenotypes during the differentiation of murine-preosteoclast (RAW264.7) cell lines (Figure 2). The RAW 264.7 cell preosteoclast was cultured in DMEM supplemented with 33ng/ml recombinant human RANKL and cultured for 7 days to observe the multinucleated giant cell phenotype of osteoclasts. The cells exhibited an osteoclast-like appearance on day 7. The cells were further subjected to the PLGA nanoparticles containing rhodamine and control mimic labeled DY457 to demonstrate the biocompatibility of the nanoparticles. The cells were stained with TRAP and Phallodine staining to elucidate the acid phosphatase activity and cytoskeletal staining. The cells subjected to the PLGA particles on day 7, exhibited positive staining of TRAP and Phalloidin, as shown in Figure 3.

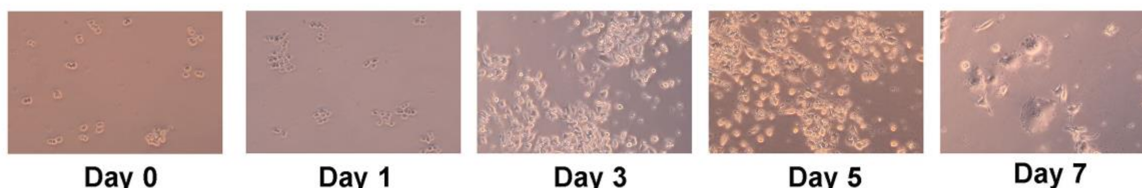

Figure 2. The differentiation pattern of RAW 264.7 preosteoclastic cells in the differentiation medium with RANKL protein (50ng/ul) for 7 days.

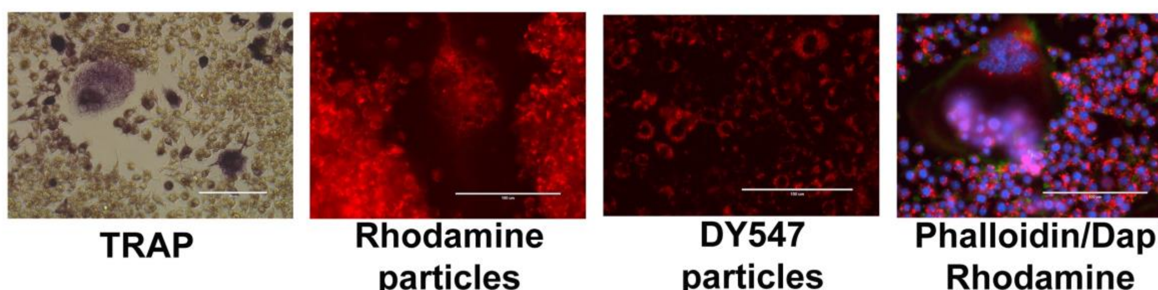

Figure 3 Staining of RAW 264.7 cells after differentiation into osteoclastics cells. Tartrate resistant acid phosphatase showed purple color with multinucleated giant cells (far left). Rhodamine PLGA particles was delivered into osteoclasts (red) (middle left). DY547 labelled particles (red) (middle right). Phalloidin staining (green) to demonstrate cytoskeleton of cells and DAPI (blue) to demonstrate the nuclei and rhodamine nanoparticles in the cell (red).

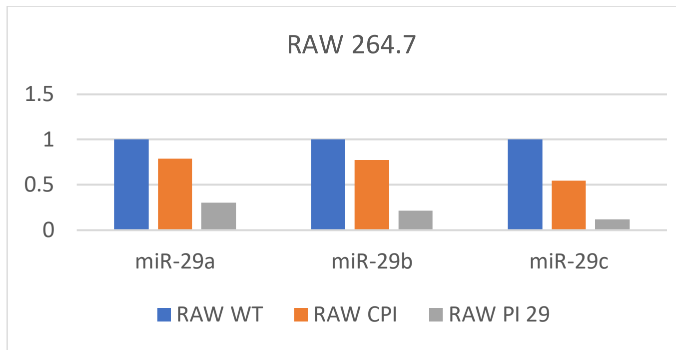

Figure 4. Expression of miR-29a/b/c in RAW264.7 murine preosteoclasts after transfected with miR-29 power inhibitor (RAW PI29) and control (RAW CPI) and no transfection (RAW WT).

The nanoparticles containing mimic and inhibitor of miRNA-29b were administered into the RAW264.7 by adding the particles into the culture media. The results showed that the administration of PLGA particles without any chemical transfection exhibited the miR-29 family (a,b and c) expression in RAW264.7 cells was suppressed to the range of 0.26-0.25 folds of endogenous expression. This experiment demonstrated the biocompatibility of the PLGA particles to the cells and the effect of miR-29 power inhibitor (Qiagen) on the suppression of the expression of all miR-29 family members in these cells.

We have performed the administration of miR-29b mimic and power inhibitor PLGA nanoparticles in the tooth movement C57BL6 mice model and found no differences between the controls (169+88 μm) and PLGA nanoparticles containing miR-29b mimic (178+ 100 μm) or inhibitors (140+ 122 μm) over 21-day of tooth movement. However, the bone resorption rate around the alveolar bone seems different. The miR-29b mimic showed more bone resorption compared to the control, while the miR-29 power inhibitor showed the effect of the resistance of their alveolar bone to bone resorption under loading. We have analyzed the bone parameter data from microCT from all samples and found no signficant differences among different groups (n=3). We plan to present our findings at scientific conferences such as the annual IADR meeting and prepare a manuscript for publishing in a peer-reviewed journal. AAOF support will be acknowledged in the publication. This discovery is essential for the proof-of-concept (POC) for developing a slow-release delivery system for the microRNA to develop an innovative modality for orthodontic care. The fund from AAOF have been completely used and no remaining fund to be returned.

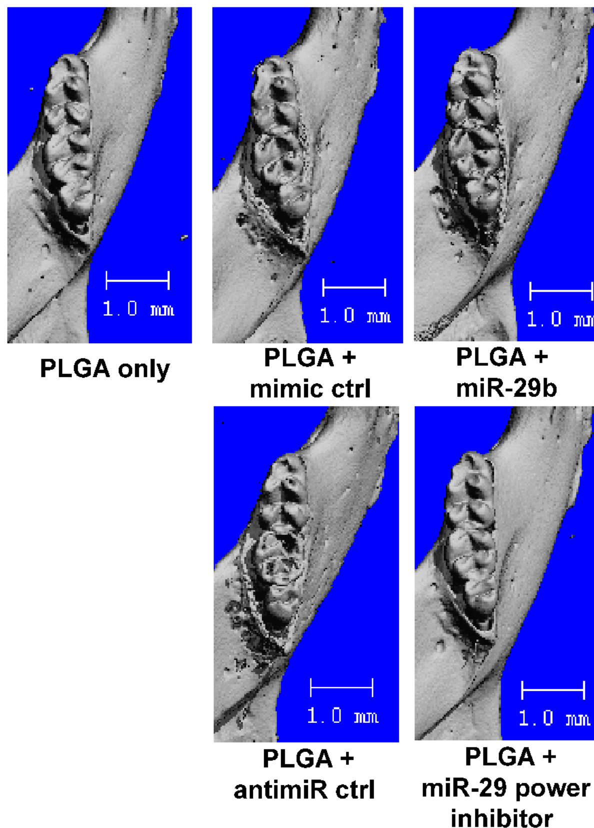

Figure 5. The microCT images of mouse mandible after injection with PLGA particles containing different types of miRNAs