miRNA networks regulating gene expression in response to tension or compression forces in the cells of the periodontal ligament

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***Abstract*—In response to force application, biological reactions occur in the periodontal ligament that causes the orthodontic tooth movement. Differential expression of genes such as ALPL, FGF7, PDRM1, and NR4A2 are involved in the exposure to tension or compression forces by contributing to bone remodeling. In this study, a systems biology approach is used in order to identify a modulator of gene expression when mechanical loadings are applied in periodontal ligament cells. We developed a mathematical model of the interaction network of ALPL and FGF7 with several miRNAs and the transcription factor modulators in BioNetUCR, Cytoscape and COPASI software. We found that miR129-2 has a negative control in ALPL, FGF7, NR4A2 and PRDM1 genes, while miRNA192 has a negative control in ALPL and FGF7 genes in compression and tension side. Both miRNAs are proposed for future experimental validation.**

***Index Terms*—Orthodontics, periodontal ligament cells PDL, Systems Biology**

I. Introduction

Orthodontic tooth movements (OTM) are based on stimulation in the periodontal ligament (PDL) cells and alveolar bone by using applied forces [1]. It is a synergistic sequence of physical phenomena and biological tissue remodeling, which includes several signaling pathways [2]. A theoretical model after the application of an external force in the tooth includes first the matrix of the PDL and alveolar bone strained due to the fluid flow alteration and cellular stress, which results in the release of proinflammatory, angiogenic, and osteogenic agents. Finally, a combination of remodeling of the PDL and alveolar bone enables dental movements [3].

The capacity of tooth movement through the dentoalveolar complex relies on the PDL, which attaches the tooth to the adjacent bone [2], as this structure is an aligned fibrous network that connects the tooth root cementum and alveolar bone with a diameter of 100-400 m [4]. PDL consists of an extracellular matrix of fibers (principally collagen) and a ground substance that contains cells, blood vessels, and nerves [5]. These include bone cells, cementum cells, Epithelial Rest Cells of Malassez, endothelial cells, neural cells, and the precursor cells for bone, PDL proper, and cementum [4], [6], however, the predominant cell type is the PDL fibroblast, which mainly secretes type I collagen [5].

PDL cells produce numerous growth factors, prostaglandins, cytokines and osteogenic factors, such as Insulin-Like Growth Factor 1 (IGFI), Bone morphogenetic proteins (BMPs), platelet-derived growth factor subunit B (PDGF), Interleukin-1 Beta (IL-1), transforming growth factor beta 1 (TGFs). TGFs, Receptor activator of nuclear factor kappa-ligand (RANKL), Osteoprotegerin (OPG), and alkaline phosphatase, as a result, stimulate the synthesis of collagen, and influence bone cell activity [5], [7]. Regarding tooth movement, within a few seconds upon force being applied, the tooth moves its position within the PDL space, as a result, in some areas PDL is compressed and PDL is tensioned in others; therefore, blood flow is decreased on the compression side and maintained or increased on the tension side. If this force is maintained, the chemical environment changes by releasing biological agents, which affect cellular activities in the compression vs tension areas [2]. This means that site-specific bone metabolism takes place simultaneously: osteoclastic bone resorption in the compressed zone and osteoblastic bone formation in the tension zone of the periodontal ligament [7], [8].

Several numbers of studies were performed with the aim of understanding the process of OTM based on biological mechanisms at the cellular, tissue, and molecular levels over 100 years ago [9], [10]. Recently with the new sequencing technologies and bio-informatics, studies can perform differential gene expression in order to identify genes responding to load application, providing more information regarding genes and pathways regulating these biological mechanisms. In one of the studies, the genes Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2), Nuclear Receptor Subfamily 4 Group A Member (NR4A3), Nicotinamide Phosphoribosyltransferase (NAMPT), Phosphoglycerate kinase (PGK1), and DNA Damage Inducible Transcript 4 (REDD1) are suggested as novel biomarkers for orthodontic tooth movement [10], [11].

Few studies involve systems biology and dentistry, generally only imply oral cancer research, inflammatory pathways

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correlated to periodontal disease, bacterial behavior, and pathology area. For example, the study of Stephen J. Hagen and Minjun Son uses stochastic simulations in order to understand the single-sensitive cell response of *Streptococcus mutans*, bacteria predominant in cavities [12]. Another study describes potential biomarkers and signaling pathways associated with Primary Ameloblastoma [13]. However, studies by using systems biology related to OTM have not been performed. Furthermore, the relationship between mechanic forces and microRNA (miRNA) expression has recently become clearer in periodontal cells. A study found that miR-3198 is upregulated by compression and is downregulated by tension, suggesting that miR-3198 downregulates osteoprotegerin expression in response to mechanical stress in osteoblastic cells [7]. However, the role of miRNA in PDL cells under mechanical forces remains unclear, and new methodologies based on systems biology are necessary to understand the level of miRNA involvement in the molecular mechanisms in OTM. Besides, understanding the gene expression biology of periodontal ligament cells in tension and compression conditions has profound clinical implications, especially in the area of accelerating orthodontic tooth movement [15]. A study of differential gene expression profile of human in vitro cultured periodontal ligament cells (PDLCs) submitted to mechanical forces [10] showed the genes Alkaline Phosphatase (ALPL), Collagen Type VI Alpha 1 chain (COL6A1) and Fibroblast Growth Factor (FGF7) showed a switch up- grade/downgrade behavior in 3D culture PDLCs [10]. ALPL participates in the osteogenesis process and bone deposition [16], along with COL6A1, both related to bone development and maturation of osteoblast by establishing communication between cells [17]. Regarding FGF7 released by PDL cells, it regulates the proliferation and osteogenic differentiation of mES (mouse embryonic stem) cells via the secretion of FGF through a mechanism that involves mitogen-activated protein kinase-mediated signaling [18].

A complex network of biological processes in two different scenarios, which are tension/compression, allows orthodontic tooth movement. However, these processes are still unclear, especially the relationship between miRNA and loading. For this reason, this systems biology-based study is fundamental to comprehend the regulation of gene expression associated with OTM in order to obtain a better understanding of the regulation of molecular and cellular signaling and the processes occurring in compressing and tension scenarios. The aim of this study is to identify a possible miRNA- target as a differential modulator of gene expression in response to tension or compression forces in the cells of the periodontal ligament through a system biology approach.

II. Methods

A. Criteria of gene selection

A systematic review regarding the evidence of differential gene expression profile of human in vitro cultured periodontal ligament cells (PDLCs) submitted to static mechanical loading compared to a control group [10] was used as a reference to obtain the most common gene expression in compressive and tension conditions. ALPL, COL6A1, and FGF7 showed switch upgrade/downgrade behavior in 3D culture PDLCs after 2 h of tension force and downregulated after 1 and 12 h of compressive force.

In order to include diversity and the relation of miR, miR1246 and miR654-5p on 2D culture were included in the network. Finally, NR4A2 and PRDM1 were included because they are the most common transcription factors involved in compression and tension forces in PDL [10] according to BioNetUCR data.

B. Network construction and visualization

BionetUCR software [19] was used in order to construct the network with ADD BACKWARD and ADD FORWARD as command lines. By using only these command lines, 222 genes and 375 nodes were obtained. However, DELETE SOURCES IN NODES and DELETE NODES IN FILE were used in order to remove nodes not regulated by other nodes and remove nodes in the specified file interest genes respectively. Then the obtained network was visualized by using Cytoscape version 3.9 software, in which interested genes were selected with layout commands to review better biological interactions.

C. Simplification and analysis of Model

BioNetUCR network was exported into COPASI version 4.29 software. In the section of Parameter Estimation, NL2SOL method with 2000 iterations was used in order to decrease the best value and adjust boundaries alerts. Then, Hooke and Jeeves, and Particle Swarm method were used to obtain three genes, six Transcription Factors, six miRNA, fifteen nodes, and twenty-one edges, and the difference between the experimental and the simulated data is not significant (T-student, p¿0.05) by using the next link: https://www.larmcr.com/tools/visualizer. In order to reduce model complexity, Sensitivity task on COPASIversion4.29 with parameters such as steady-state and all variables of the model was used and then with larmcr, the sensitivity analysis was plotted against the p-values obtained with the t-test.

However, after performing Sensitivity task, no species had sensitivity over COL6A1 gene, for this reason, this gene was eliminated. After adjustment of the model, Task Parameter Scan, Steady State, and Time Course were programmed to obtain different results. By using Parameter Scan we observed the behavior of increasing the copy number of miR129-2 and miR 192 over species ALPL and FGF7. miR129-2 and miR192 were decreased and kd was altered in order to simulate the effect of their perturbation across time.

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III. Results

After several model reductions, the final network finishes with two genes (ALPL and FGF7), five transcription factors, two miRNAs, for a total of nine nodes and twelve edges. The

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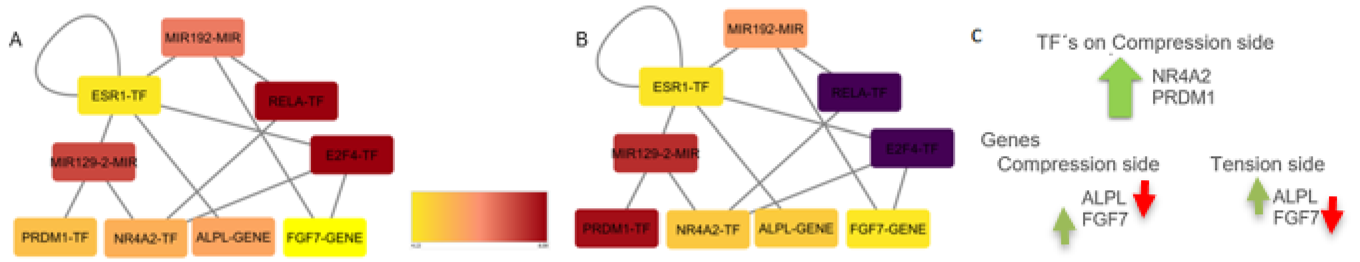

Fig. 1. Fig. 1. Final Network including interested genes.A)Cell line CNS SNB 75 as compressing representation. B)Cell line LE SR as tension representation.C)Results according to experimental data obtained.

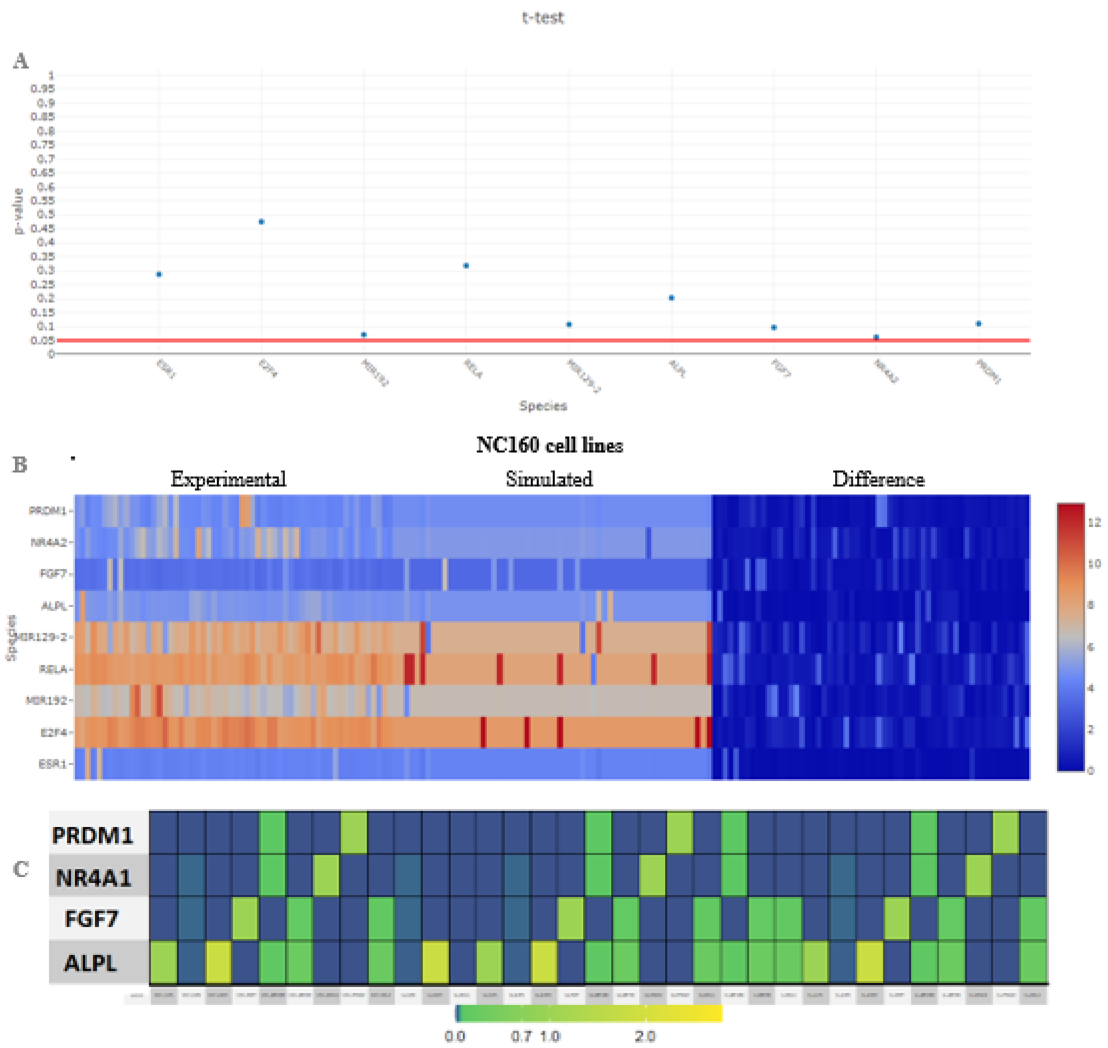

Fig. 2. Fig. 2 A)T-test of all species(p¿0.05). B)Differences between experimental and simulated data by using NC160 panel. C)Sensitivity test on the species of interest

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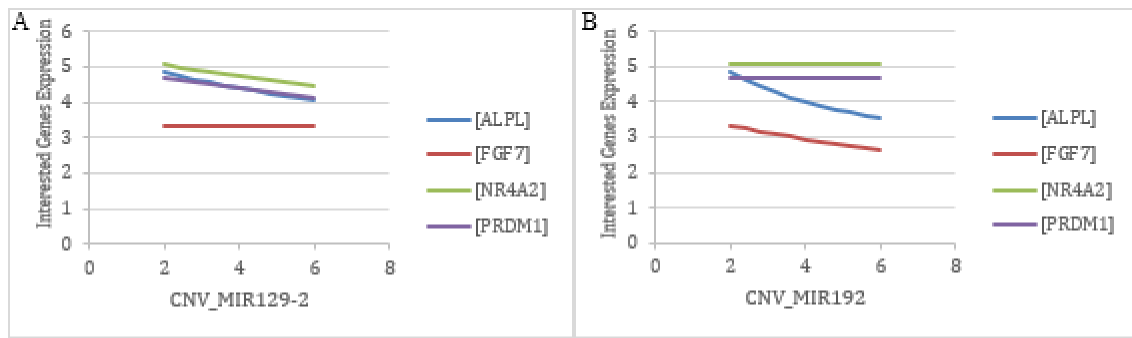

Fig. 3. Fig. 3. A)Effect of increased the copy number of miR129-2 on interested species. B)Effect of increased the copy number of miR192 on interested species

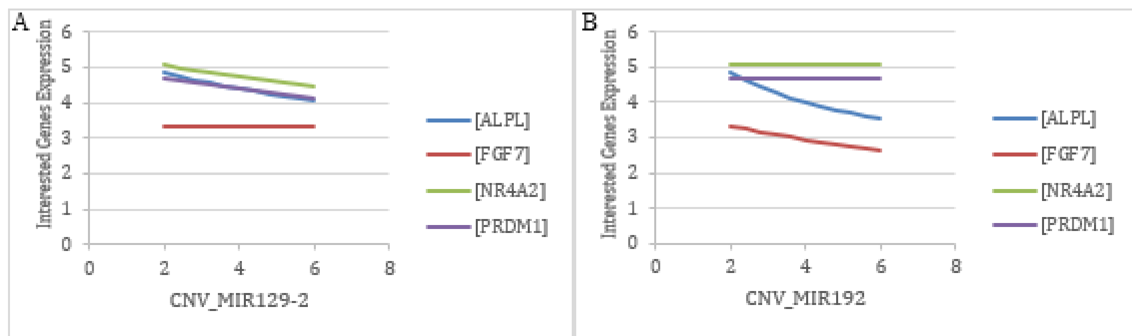

Fig. 4. Fig. 4. A)Effect of time when miR129-2 is inhibited on interested genes over control. B)Effect of time when miR192 is inhibited on interested genes over control. C)Effect of time when miR129-2 and miR192 are inhibited on interested genes over control.

first figure shows the cell line that resembles the simulated tension side by showing ALPL with medium expression and FGF7 has low expression. However, PDRM1 and NR4A2 have medium expression. In contrast on the compression side, PRDM1 has high expression and FGF7 maintains low expression(Figure 1).

According to the t-test, all species are fitted in this model (p¿0.05), and no significant differences between experimental and simulated data are present. By using a sensitivity analysis miR129-2 and miR 192 were determined to have control over PRDM1, NR4A2, FGF7 and ALPL (Figure 2).

When the copy number of miR129-2 is increased from two to six, the species ALPL, NR4A2, and PRDM1 decreased their concentrations. However, the FGF7 gene is maintained without perturbation. In the case of miR192, when it is increased to six, the species ALPL and FGF7 decreased their concentrations, however, NR4A2, and PRDM1 remain without perturbation, showing a similar result in the compression side(Figure 3). To confirm these results, a time course simulation was run upon a reduction of miR129-2 copy number to zero. Our simulations show that the species ALPL, NR4A2, and PRDM1 increased their concentrations during 500 seconds. FGF7 gene is maintained without perturbations. When the copy number of miR192 is reduced to zero, the species ALPL and FGF7 increased their concentrations, however, NR4A2, and PRDM1 maintained without perturbation. In the third scenario, the copy number of miR129-2 and miR192 were reduced to zero, and all interested genes increased (Figure 4). These results indicate that when the concentrations of miR192 are reduced, the behavior of the gene expression profile resembles that of present at the compression side.

IV. Discussion

After applying mechanical forces, signaling molecules associated to inflammatory conditions, osteoclastogenic and remodeling processes are produced by PDL fibroblasts. These cells play a crucial mediating role in orthodontic tooth movement (OTM) [20].

Orthodontic tooth movement involves the reconstruction and alteration of bone architecture and collagen network. Previous studies have suggested that PDL cells are able to develop osteoblastic characteristics under certain conditions, for example, mechanical forces [21]. Mature osteoblasts and hPDL fibroblasts secreted ALPL pro- tein during bone formation [20]. ALPL, alkaline phosphatase, is used as an indicator for osteoblastic activity due to its function in tissue calcification and new bone formation [21]. ALPL expression plays a central role in osteogenic mineralization, bone calcification, and mineralization [22]. However, other studies indicate ALP expression reduction appears at 2 and 12 hours after compressive stress after application by using the compressive force continuously in the chondrocytes of the mandibular condyle.

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Secondly, the intermittent compressive force increases the ALP activity in many cell types. As a result, ALP expression has contradictory effects on depends of the nature of mechanical stress [23]. In our study, when miR 129-2 and miR 192 are inhibited, ALPL gene increases, which potentially enhances bone formation on tension side.

FGF7 contributes with regulation of actin cytoskeleton, positive regulation of cell proliferation, extracellular matrix organization and it is correlated with MAPK signaling pathway. Previous studies have shown that loading PDLCs with either cyclic compression or static tension might be related with down-regulation of the cell cycle genes such as FGF7 [23]. In case of FGF7, only miR192 have shown control when this miR is altering their value of copy number as shown in figure 3. Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2) has relevance in the response to hypoxia due to the immediate early stress response, and it is up-regulated after 6, 24, and 72 h of compression.

Furthermore, NR4A receptors are associated as important regulators of bone homeostasis and may be involved in regulating RANKL, and osteoclast activation[24]. PRDM1 (PR/SET Domain 1)is a transcriptional osteoclastogenic factors when it is overexpressed lead to an increase in osteoclast formation, which is induced by RANKL through NFATc1 during osteoclastogenesis[25]. According to systems biology theory, it is essential to assess whether the model's predictions are reasonable during the fitting pf a mathematical model. Therefore, it is necessary to know if the model predicts steady states, if it makes correct predictions for the cases of limit values (very low or very high values) [26]. The kinetic stability analysis based on the eigenvalues of the Jacobian matrix in steady states indicated that this model is asymptotically stable, no imaginary numbers are present, and only negative real parts are present without showing bi stability or bifurcation.

COPASI software allows simulating the dynamics of the defined model through the time course, using a stochastic formalism where the events of the individual reactions are brought from probability distributions using various algorithms [27].

The result of the time course with and without the alteration of miR192-2 and miR192 under interested genes allows an analysis of parameters to examine how the model behaves when some parameters vary in a certain interval [28]. This study suggested miR 129-2 and miR192 are fundamental miRNAs on OTM. In previous studies mechanical stretch regulated miRNA expression in PDLSCs, and these miRNAs were involved in osteogenic differentiation[29].

Computational biological simulations or mathematic models of the biological process of the OTM have not been studied, even when this industry was valued at $2,767.4 million in 2020 and it estimates 10% of growth in 2023 [30]. For this reason, our study proposes both miR129-2 and miR192 as potential modulators of OTM, however, they are not reported as molecules with a role in OTM in previous investigations. This study provides the basis of research for further investigations in orthodontics area.

V. Conclusion

miR129-2 has a negative control on ALPL, NR4A2 and PRDM1 gene expression in compression and tension side. miR192 has a negative control in ALPL and FGF7 genes and simulated compression side by decreasing bone formation related to ALPL and FGF7. Further experimental validation is required in order to understand the involvement of these miRNAs in OTM.

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