HISTOMORPHOMETRIC ANALYSIS OF THE EFFECT OF THERAPEUTIC ULTRASOUND ON THE DENTOALVEOLAR STRUCTURES DURING ORTHODONTIC FORCE APPLICATION IN-VITRO

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1. INTRODUCTION

Contact between odontoblasts and the dentin matrix is required to maintain the phenotypic morphology and secretory activity of odontoblasts. [1] To accurately assess the phenotypic morphology and secretory activity of odontoblasts, a longer term model of the dentin-pulp complex is required. Magloire et al. were able to culture human tooth slices such that the cell viability was maintained for a significant period; however the model allowed limited experimental manipulation [2]. In 1998 Sloan *et al* studied the process of dentinogenesis in rat incisor for up to 14 days *in vitro*. They demonstrated that the dentin-pulp complex from mature rodent tissues can be cultured successfully for substantial periods of time and would provide a useful model for the study of dentinogenesis and tissue repairDhopatkar introduced a variation of the tooth slice model which allowed investigation of the dentin pulp complex undergoing orthodontic force in vitro [4].

Low intensity pulsed ultrasound (LIPUS) has been found to stimulate angiogenesis during wound healing and to enhance bone growth into titanium porous-coated implants [5] to enhance bone healing after fractures [6, 7] and bone formation after mandibular osteodistraction [8, 9]. LIPUS can enhance mandibular growth in growing patients with hemifacial microsomia. The expression of bone proteins: osteonectin, osteopontin, and bone sialoprotein has been found to respond to therapeutic ultrasound in a dose dependent manner.

El-Bialy et al. reported that LIPUS minimized orthodontically-induced root resorption and accelerated healing by reparative cementum in 12 patients over a 4 week period of simultaneous tooth movement and LIPUS application. It was reported that LIPUS exposure affected cementoblasts by regulating mRNA expression of alkaline phosphatase, which plays a role in the mineralization process but had no effect on cell proliferation [10]. In addition, it has been reported that LIPUS stimulates odontoblast matrix production and gene production in vitro [11]. This suggests that LIPUS may have distinct effects of cell viability, cell adhesion and gene expression of odontoblasts in culture.

We hypothesize LIPUS will enhance dentine and cementum matrix modelling formation as well enhance alveolar bone and periodontal ligament remodelling.

2. METHODS

2.1 Sample Collection and Preparation

The mandibles were dissected from 28-day-old male SD rats euthanized by cervical dislocation. Transverse sections of approximately 1.5 mm thickness were cut with a 0.006" diamond wafer saw (ISOMET® Wafering Blade, Series 15HC, 3" x 0.006" x 1/2").

The sections were washed several times in washing medium at 37°C immediately after cutting. Slices were placed in culture medium (100 ml), containing DMEM, vitamin C (0.15 mg/ml), 10% heat inactivated fetal calf serum, L-glutamine (200mM) and 1% penicillin/ streptomycin solution. The mandible slices were cultured at 37°C in an atmosphere of 5% CO_2 in air, in a humidified incubator for 24 hours. After changing the media, springs consisting of 0.016 × 0.025 were applied to each slice. This is what is referred to as the mandible slice organ culture (MSOC). The springs were calibrated to deliver 50 grams of force to each slice across the slice passing through the tooth and periodontal ligament inside the mandible slice. Upon application of the compression force by the spring, the mandible slice organ culture (MSOC) were deformed and resulted in two areas of periodontal ligament compression as well as two areas of tension.

2.2 Application of Ultrasound

After the application of the springs one hour LIPUS was applied using a 2.5 transducer producing incident intensity of 30 mW/cm^2 of the transducer's surface area to the slices in the treatment group. The sample was divided into three groups Control (n=7), 5 minutes US (n=10), 10 minutes US (n=10) for one week.

2.3 Assessment

Once the treatment period was completed, histomorphometric analysis was performed. The images were captured using a digital camera [(CCD), Leica, Wetzlar, Germany)] with 40X magnification lenses. Field of view for each image was standardized using a calibration ruler of 2mm in length. Histomorphometric analysis was performed using Meta-Morph software (Molecular Devices Corporation, Sunnyvale, CA , U.S.A.). Predentine and cementum thicknesses and cell counts for the odontoblastic and the preodontoblastic layers as well as for the periodontal ligament spaces were calculated for the compression sides and the resultant tension sides. All data collected was analyzed by Multi-Variate Analysis Of Variance (MANOVA) test with Bonferroni test as the sample size was relatively small. Data analysis was performed using SPSS statistical package (Version 15, Chicago, IL, USA).

3. RESULTS

It was observed that both cementum and predentin thickness were increased in the ultrasound group. [Figure 2] In addition, the number of odontoblast, preodontoblast, and periodontal ligament (PDL) cells were increased in the ultrasound groups. The observed cell counts and thickness measurements may be dependent on the amount of ultrasound exposure.

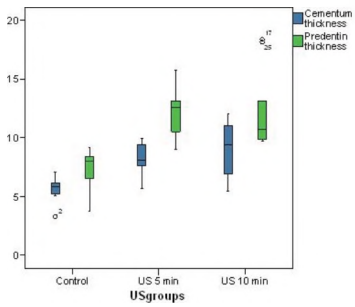

Figure 1

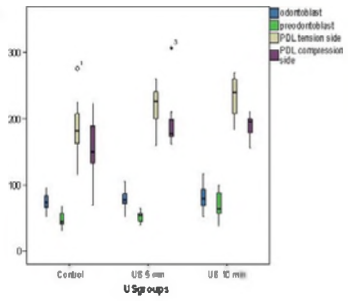

Figure 2

Figure 1. Graphical presentation of cementum and predentine thicknesses
Figure 2. Graphical presentation of cell count in the odontoblast, preodontoblast, PDL in both tension and compression sides.

4. DISCUSSION

Mandible slice organ culture is an effective means of investigating the dentin-pulp complex while allowing experimental manipulation. The application of the springs allowed compression and tension of the complex to replicate effects of orthodontic movements. LIPUS can be applied to this model in such that the entire complex received even distribution of the treatment.

The increase in cementum thickness may be a result of altered mRNA expression of alkaline phosphatase or increase in matrix production by cementoblasts [10]. As stated in the introduction, LIPUS may have stimulated odontoblast matrix production resulting in an increase in preodontoblast and odontoblast cells. Previous studies suggest that LIPUS stimulates PDL cell proliferation [12] which was observed in our results.

From a molecular and cellular level, it can be observed that LIPUS may be beneficial to the restructuring of the mineralized tissue along the dentin-pulp complex when undergoing orthodontic force.

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