The Anabolic Effects of Electrical Stimulation on Endochondral Bone

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Introduction: Electrical stimulation (ES) is used to treat slow and non-healing long bone fractures and to enhance rates of spinal fusion. Direct current ES (DC) increases osteoblast function; capacitively coupled ES (CC) stimulates voltage-gated calcium channels [1-3]. The effect of ES on endochondral bone formation has also been studied both in vivo and in cell culture [4,5]. Depending on the type of current and voltage used, ES can have either an inhibitory or stimulating effect [6, 7]. However, the use of ES for the treatment of human growth plate disorders is not currently the standard of care. Surgery remains the only viable option for treating disorders that have failed conservative management. The purpose of this investigation is to further examine the anabolic effects of ES on endochondral bone formation. The authors hypothesize that through the use of DC and CC, endochondral ossification of murine growth plates in long bone organ culture will be accelerated through distinct mechanisms.

Methods: Femurs for culture from 3-week old C57BLK6 mice were obtained from another IACUC approved investigation (11-034). They were suspended on stainless steel mesh and maintained in a BGJb medium for one week. One femur from each animal received ES treatment; the contralateral femur served as a non-treatment control (NT). After 2 days, the ES femurs were transferred daily to a silicone chamber filled with phosphate buffered saline (PBS) and subjected to 10 minutes of ES using either DC or CC at 16Hz. The NT femurs were placed in PBS for 10 minutes a day without ES. After 7 days, all specimens were terminated and either placed in ethyl alcohol (EtOH), followed by 4% paraformaldehyde (PFA), for micro-computed tomography (μCT) analysis and histomorphometry, or frozen immediately in liquid N\(_2\) for RNA isolation and gene expression analysis using quantitative polymerase chain reaction (qPCR). In each group, 6-8 bones were used for μCT (4-6 of which were used for histology and/or histomorphometry) and 2 for gene analyses. All data was analyzed using ANOVA with p < 0.05 considered significant.

Results: Compared to the NT control specimens, there was a significant increase in bone volume/tissue volume (BV/TV) and bone area (B.Ar) in all ES groups. There was also a significant increase in trabecular thickness (Tb.Th) in the DC treatment groups. Significant increase was also found in trabecular number (Tb.N) and decrease in the trabecular spacing (Tb.Sp) in the CC treatment group, indicating increased bone formation. Histomorphometric analysis of the growth plate using von Kossa-stained specimens demonstrated comparable growth plate height in all specimens. However, there was a significant decrease in the percent of the growth plate taken up by the hypertrophic zone and increase in resting and proliferative zones for both DC and CC specimens. The zone of provisional calcification in the DC group also was found to be significantly larger. Masson’s trichrome staining showed a significant increase in the osteoid area/bone area (O.Ar/B.Ar) on both the DC and CC stimulated sections. We
found a deeper staining and increased area of red staining indicative of a higher proteoglycan content within matrix collagen on the Safranin O-stained sections in both ES groups compared to the control. Finally, on qPCR analysis, there was a statistically significant increase in Col1 expression in the DC group and an increase in Sox9 and Col10 genes in the CC group, when compared to controls (Figure 1).

**Discussion:** Endochondral ossification is a complex process involving the evolution of chondrocytes within a growth plate into calcified matrix. This study demonstrates and corroborates other studies that ES applied under controlled conditions stimulates endochondral bone formation and the type of current (DC, CC) acts through distinct mechanisms [1, 2]. Compared to NT control specimens, all ES specimens demonstrated a significant increase in overall bone formation. However, the different currents used (DC, CC) stimulated different types of bone formation. DC and CC current showed increased expression of Col1 and Sox9, Col10 respectively. Additionally, while DC stimulation enhanced Tb.Th, CC increased Tb.N. This corroborates previous studies and suggests that DC and CC stimulation work through different mechanisms in stimulating endochondral bone formation, specifically that DC stimulation enhances osteoblast function while CC stimulation increased chondrocyte function [1-3].

**Significance:** ES may in the future prove to be an alternative method of growth plate modulation; however, further investigation is warranted.