DISSERTATION ABSTRACT

STUDY ON ELECTROMYOSTIMULATION INFLUENCING MECHANICAL AND MICROSTRUCTURAL PROPERTIES OF BONES BEYOND THE STIMULATED SITE

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Abstract

Electromyostimulation is a nonpharmacological prevention method for osteoporosis that is safe and feasible for the elderly and people with physical disabilities. In the previous study, the random pulse train (RdPT) electromyostimulation of rat quadriceps induces the mechanical properties not only at the stimulated femoral neck but also the unstimulated contralateral femoral neck. This brought a new hypothesis about the possibility of electromyostimulation in inducing the mechanical properties beyond the stimulated site. The aim of this study is finding the possibility if the electromyostimulation could induce the mechanical properties of bones beyond the stimulated site. In the first study, the RdPT electromyostimulation hadn't shown its effectivity in inducing the mechanical properties of the long bones' diaphyseal in a whole-body scale. In the second study, the RdPT shows its capability to influence the mechanical properties of vertebra but it worked specifically. Only the stiffness of L2 was increased. Additional comparator testing with μCT scan also shows the influence of RdPT electromyostimulation on the mineral content or the bone volume of the L2, but not the bone mineral density. This influencing on distant bones suggests nerve involvement in this process. On the other hand, PrPT electromyostimulation did not any effect on these bones. In conclusion, RdPT electromyostimulation is effective not only in the stimulated femur but also in the lumbar vertebrae depending on the vertebra's location.

1. Introduction

Osteoporosis is the bone disorder that is characterized by low bone mass density or deterioration of bone's microarchitecture. It is caused by age, lack of physical activity, or long-duration spaceflight[1], that resulting in bone fragile and easy to be broken, finally reducing the quality of life and life expectancy. Although exercising, such as walking or running, is often suggested in preventing osteoporosis than drugs because of their side effect[2], the exercising has limitation for people who have poor movement ability such as old people or bedridden patients. It proposes the mechanical stimulation, which has a capability in suppressing bone loss, as an alternative treatment. Unfortunately, a high value of the minimum bone strain which induces osteogenesis-induced osteogenesis, is afraid will cause bone fracture. Finally, it suggests electromyostimulation as an alternative treatment by mimicking exercising, when the electric-induced muscle contraction induces mechanical force in the bone via tendons, thus resulting in increasing the bone formation[3]or suppressing the bone loss suppressing of the osteoporosis model[4].

2. Electromyostimulation

At the previous studies of electromyostimulation (the electric stimulation on muscle), this stimulation not only can suppress bone loss[4–6] and muscle loss[7] but also induce the osteogenesis[3]. Interestingly, although *in vivo* studying with rats showed the highest number of muscle contraction was at the 40 Hz and the highest number of average peak-to-peak force was at 2 Hz, but the highest gene expression of osteocalcin was shown by 20 Hz[3]. It suggests a specific frequency of muscle contraction is more appropriate to induce osteogenesis. Moreover, the generated bone strain because of electric-induced muscle contraction was below 1050 μstrain[8], indicates the electromyostimulation is more effective and safe than mechanical loading stimulation.

As well as the Periodic Pulse Train (PrPT) electromyostimulation, the Random Pulse Train (RdPT) electromyostimulation has shown its capability in influencing bone remodeling. Comparing the PrPT, the RdPT has the same pulse train physical condition but different in the time period. The duration of each RdPT pulse train appearance is determined from the probability of geometric distribution[9]. The studying of the PrPT and the RdPT electromyostimulation at left quadriceps has shown the capability the PrPT and the RdPT electromyostimulation in inducing osteogenesis at the mid-diaphyseal and the femoral neck of stimulated femur. Moreover, it is interesting because the RdPT could increase the mechanical properties of both the stimulated femoral neck and the unstimulated contralateral femoral neck[9]. Base on the RdPT phenomena, the next study will bring this study to a new hypothesis that the bones could be stimulated by stimulating in one location only.

Effect of Electromyostimulation on Mechanical Properties of Diaphyseal Long Bones apart from The Stimulated Sites

The purpose of this study was to investigate the influence of the electromyostimulation on the mechanical properties of bones beyond the stimulated site which was in a whole-body scale by using the diaphyseal long bones as the samples. The experiment procedure was performed as the same as previous procedure[9] for considering experimental efficiency. The 7-weeks-old female Sprague-Dawley rats were purchased a week before treatment. They were divided into three groups of treatment, which were the Control group, the PrPT group, and the RdPT group. The stimulation groups rats received the stimulation, which was generated from windows PC to needle electrodes via I/O board, 30 minutes/day for 3 days continuously, at the left quadriceps, in anesthetized condition. Differently than the previous study[9], fifteen days after stimulations days, femora, tibiae, humerus, and ulna-radius were harvested and tested immediately in mechanically with the 4-P bending test (figure 1a). The maximum load, the strain energy, and the stiffness were obtained from load-deformation curves (figure 1b).

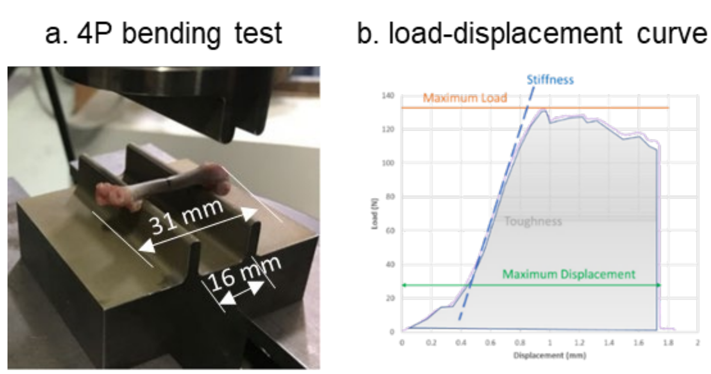

Figure 1. Bending test calculation to obtain the maximum load, the maximum displacement, the toughness (the strain energy) and the stiffness.

Furthermore, the four-point bending testing demonstrated a significant increase of the mechanical property which was the strain energy of simulated left femurs, because of the RdPT, by 46.32% ($p < 0.05$) compared than unstimulated contralateral femur (figure 2). Other mechanical properties such as the maximum load and the stiffness of the femur did not show significant differences between the left and right sides. Moreover, stimulations at the left quadriceps did not significantly influence the mechanical properties of the diaphysis of the other long bones, namely, the tibiae, humeri, and ulnas–radii.

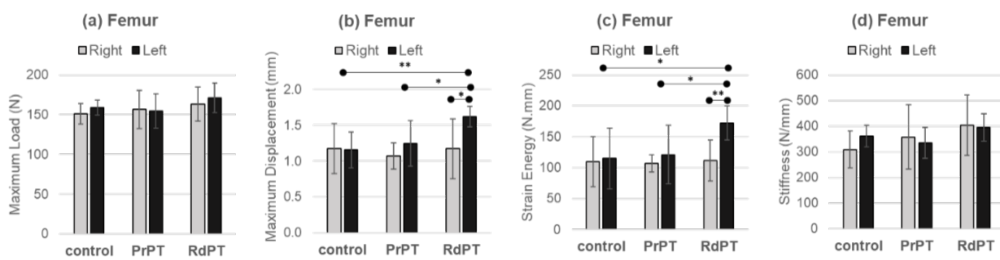

Figure 2. Only maximum displacemement and strain energy at stimulated femoral neck were influenced by the RdPT stimulation.

In this study, interestingly, even though it's difficult to induce cortical bone than trabecular bone[10,11]. But, in this study, the RdPT influenced the strain energy at the femoral diaphysis. Unlikely the PrPT, the RdPT is a no routine signal and the bone cell is difficult to custom this signal[12]. Unfortunately, unlikely previous study at femoral neck which was the investigation of stimulation effect on influencing mechanical properties at the femoral neck[9], both stimulation didn't influence the mechanical properties at the diaphyseal of the unstimulated contralateral femur. This could explain the site-depending osteogenic effect of the RdPT electromyostimulation. Additionally, the innervation density of nerve system is higher in the epiphysis area than diaphysis area[13], suggesting a possibility of signal transduction through the nerve system to stimulate the bone formation in the contralateral bone better in the epiphysis. Base on the clinical experience that the osteoporosis fractures occurred at the femoral neck and the vertebra[14], the next study was the study of the effect of the electromyostimulation at the left quadriceps as the site of stimulation on the mechanical properties of vertebrae as the unstimulated bones.

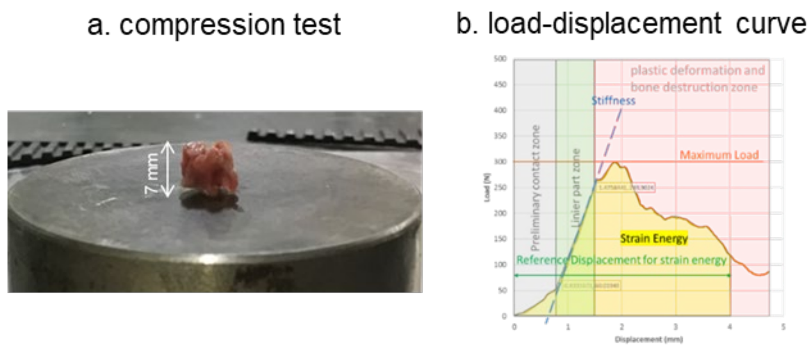

Figure 3. Compression test calculation to obtain the maximum load, the maximum displacement, the toughness (strain energy) and the stiffness.

Effect of Electromyostimulation on Mechanical Properties and Microarchitectual Properties of Lumbar Vertebrae

The purpose of this study was to investigate the effect of the electromyostimulation on the mechanical properties of vertebrae as the trabecular bones which were beyond the stimulation place. Most of the experimental procedures were performed as the same as the previous experimental procedures, which were on the long bones' diaphysis. The different thing was the investigation objects which were the lumber vertebra number 2 (L2), 3 (L3), 4 (L4), and 5 (L5). To identifying the mechanical properties, the compression test was chosen based on the daily loading environment at the vertebrae (figure 3a). Moreover, the μCT scanning was used to analyze the microarchitecture of the vertebrae as the comparator by analyzing the mineral content and the bone structure.

As well as prediction, interesting results were shown by mechanical testing and μCT scanning. Compression test results show that the RdPT increases the L2 stiffness and decreases the L4 stiffness (figure 4). On the other hand, no significant changing in the mechanical properties of bones was observed in the L3 or the L5 after the stimulation. Moreover, microstructure identification showed that both stimulations the PrPT and the RdPT, influenced the L2 in microstructural level by reducing the BMC (figure 5), although no remarkable differences existed among these groups when were identified with μCT images (figure 6).

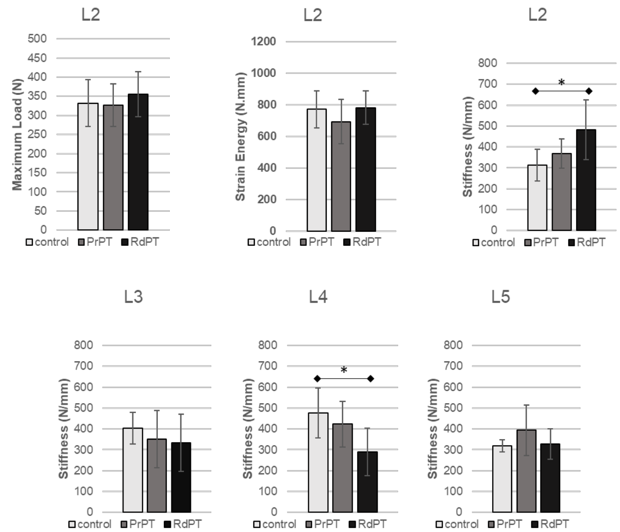

Figure 4. The L2 and the L4 stiffness were influenced significantly by RdPT.

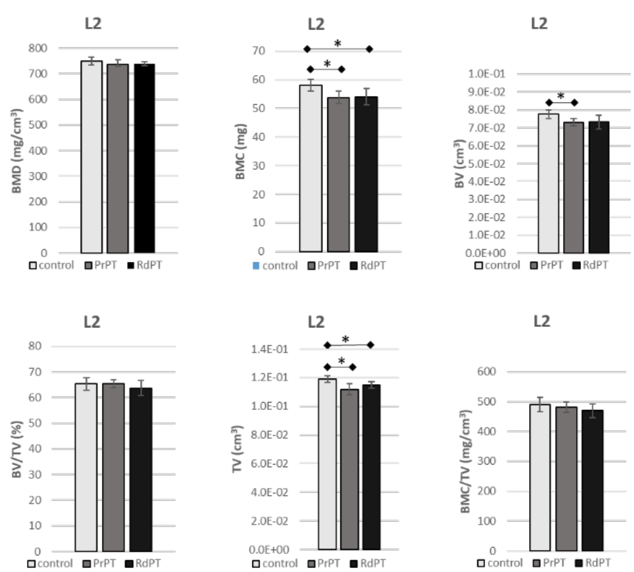

Figure 5. The L2 mineral were influenced by PrPT and RdPT.

In this investigation, it seems that electromyostimulation, especially RdPT, at the left quadriceps could influence the mechanical properties and the microarchitecture of the lumbar vertebra, but it was depended the vertebra locations. It is possible that nerve plays an important role in this adaptation. It is possible the stimulation at left quadriceps generates the nerves signals which were contributed to changing the mechanical properties and the microarchitecture of the vertebrae. Moreover, it has been known that the bone mechanical properties do not only depend on the bone mineral density (BMD) but also the mineral-collagen ratio and the enzymatic crosslinking[15,16]. The stiffness increasing at L2 suggests the RdPT induced the neural signal to promote the enzymatic cross-linking, despite the BMD didn't change because of this stimulation.

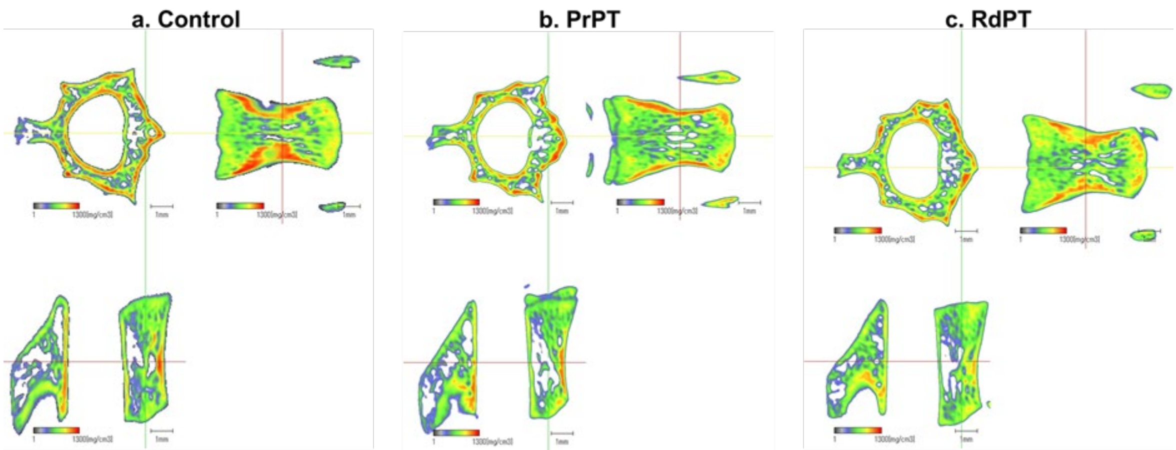

Figure 6. No remarkable difference of μCT images of typical L2 among the Control group, the PrPT group, and the RdPT group.

Conclusion

The investigation of the possibility of an electromyostimulation to stimulate the mechanical properties and the structure of bones beyond the stimulated site, shows that the RdPT electromyostimulation (but not the PrPT electromyostimulation) has capability to influence the mechanical properties of bones beyond the stimulation area, but it is limited with type of bone, which is mostly trabecular bone, and depended on the trabecular bone's location in the nervous system.

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令和　元年　8月　1日

学位論文審査報告書（甲）

1．学位論文題目（外国語の場合は和訳を付けること。）

STUDY ON ELECTROMYOSTIMULATION INFLUENCING MECHANICAL AND MICROSTRUCTURAL PROPERTIES OF BONES BEYOND THE STIMULATED SITE

（刺激箇所を超えて骨の力学的及び微細構造的特性に影響を与える電気的筋刺激の研究）

2．論文提出者　(1) 所　　属　機械科学　専攻

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3．審査結果の要旨（600～650字）

当該学位論文に関し、令和元年８月１日に第１回学位論文審査委員会を開催し、提出された学位論文および関連資料について詳細に審査した。同日に行われた口頭発表の後、第２回学位論文審査委員会を開催し、慎重に協議した結果、以下の通り判定した。

本研究は、骨粗鬆症の新たな予防法として電気的筋刺激に着目し、その骨への刺激効果について刺激箇所を超えた全身的範囲において調査することを目的として行われた。ラットの大腿四頭筋に対し電気刺激を与え、刺激箇所の大腿骨以外の遠方の骨（上腕骨、尺骨ー橈骨、腰椎、脛骨）における刺激効果を力学的特性と構造特性の観点より調べ、同刺激の有効性が腰椎にまで及ぶことを明らかにしている。なお同刺激は、腰椎の骨密度を変化させないことから、骨コラーゲンの架橋状態に影響を与えているという重要な学術的知見を得ている。加えて、一般的な周期的刺激と神経伝達パルスを模したランダム刺激を比較し、後者のみがこの遠隔的な刺激効果を有することも明らかにし、神経伝達機構を利用した骨代謝制御という新しい学術的課題を提案している。

以上のように、本研究は、電気的筋刺激が持つ遠隔的骨刺激効果の有効範囲を力学的・組織構造学的観点から明らかにし、同刺激法を臨床応用するための重要な知見を見出している。このことから、本論文は生体医工学における学術的価値が高く、博士（学術）に値するものと判定した。

4．審査結果　(1) 判　　定（いずれかに○印）　合　格　・　不合格

(2) 授与学位　博　士（　学術　）