

The Effect of High Voltage, High Frequency Pulsed Electric Field on Slain Ovine Cortical Bone

Hajarossadat Asgarifar, Adekunle Oloyede, Firuz Zare

School of Biomedical Engineering and Medical Physics, Faculty of Science and Engineering, Queensland University of Technology, Brisbane, Australia

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ABSTRACT

High power, high frequency pulsed electric fields known as pulsed power (PP) has been applied recently in biology and medicine. However, little attention has been paid to investigate the application of pulse power in musculoskeletal system and its possible effect on functional behavior and biomechanical properties of bone tissue. This paper presents the first research investigating whether or not PP can be applied safely on bone tissue as a stimuli and what will be the possible effect of these signals on the characteristics of cortical bone by comparing the mechanical properties of this type of bone pre and post expose to PP and in comparison with the control samples. A positive buck-boost converter was applied to generate adjustable high voltage, high frequency pulses (up to 500 V and 10 kHz). The functional behavior of bone in response to pulse power excitation was elucidated by applying compressive loading until failure. The stiffness, failure stress (strength) and the total fracture energy (bone toughness) were determined as a measure of the main bone characteristics. Furthermore, an ultrasonic technique was applied to determine and comprise bone elasticity before and after pulse power stimulation. The elastic property of cortical bone samples appeared to remain unchanged following exposure to pulse power excitation for all three orthogonal directions obtained from ultrasonic technique and similarly from the compression test. Nevertheless, the compressive strength and toughness of bone samples were increased when they were exposed to 66 h of high power pulsed electromagnetic field compared to the control samples. As the toughness and the strength of the cortical bone tissue are directly associated with the quality and integrity of the collagen matrix whereas its stiffness is primarily related to bone mineral content these overall results may address that although, the pulse power stimulation can influence the arrangement or the quality of the collagen network causing the bone strength and toughness augmentation, it apparently did not affect the mineral phase of the cortical bone material. The results also confirmed that the indirect application of high power pulsed electric field at 500 V and 10 kHz through capacitive coupling method was safe and did not destroy the bone tissue construction.

Key words: Cortical bone tissue, effect of electrical pulsed power, high voltage and high frequency pulsed electric field, mechanical properties of cortical bone, pulsed power

INTRODUCTION

Over the last few decades, electric and electromagnetic fields have achieved a significant role as both a stimulator and a therapeutic facility in biology and medicine. In particular, low magnitude, low frequency, pulsed electromagnetic field (PEMF) has shown significant positive effect on bone fracture healing and treatment of some bone disease such as non-union fracture healing, osteoporosis.^[1-5] A review of the advantages of PEMF and pulsed electric field stimulation on connective tissue in both animal and clinical studies and the observation of a lack of studies in the field of high power, high frequency electrical fields application, spurred interest in investigation of the possibility of applying pulsed power (PP) signals for stimulating bone.^[6-9]

PP systems convert low power, long-time input to high-power, short-time output. These systems generally store energy within an electrostatic field (i.e., capacitors) or magnetic field (i.e., inductors) over a comparatively long time and release it very quickly (in microseconds or less) which results in the delivery of larger amount of instantaneous power (several kilowatts) in a very short time, though the total energy is the same.^[10]

Bone is a complex tissue that has several functions dependent on both its composition and structure. Evaluating the behavior of bone in response to PP excitation requires assessing the functional properties of bone. The primary function of bone is to resist or bear loads applied to it through both internal and external forces. In addition, it should be tough enough to resist breakage and remain stiff.

Address for correspondence:

Mrs. Hajarossadat Asgarifar, 3/25, 2nd Street, Kooye Sepahan, Isfahan, Iran. E-mail: h_asgarifar@yahoo.com

Therefore, there is always a need to obtain information about bone strength, stiffness and toughness particularly when evaluating bone condition in health and diseases and investigating the effect of an external stimulus.

Bone primarily consists of cells (living component) ensconced in an extra cellular matrix (ECM). ECM is the composite material portion of cortical bone and consists of about 70% mineral (mostly hydroxyapatite), 22% organic matrix (more than 90% type I collagen and less than 10% non-collagens proteins) and 8% water by weight. ECM is the base substance of the functional and mechanical competence of bone and is in particular, the target of this study.

The quality and spatial arrangements of bone constituents determine its functional characteristics and can be influenced by different factors such as mechanical environment, diseases, aging and other internal or external stimuli. The mineralized collagen fibrils form the main structure of ECM and determine the mechanical properties of bone at nanoscale level. The structural quality of this matrix pertains to both quality and orientation of its collagen fibrils.^[11] The integrity and the quality of the collagen matrix have a direct impact on the toughness and strength of cortical bone tissue while it has no considerable effect on bone stiffness.^[12] If collagen composition is altered (in quality or orientation) or denatures (e.g., by heating over 160°C) cortical bone toughness and strength will be changed.^[13]

Although, the bone strength has a direct correlation with the increase of the mineralization, the ultimate strength of the cortical bone tissue does not have such a deep association with the mineral content as does the Young's modulus.^[14]

Pulse power technology has been used variously in biology and medicine, especially at intercellular scale. Some of its established/demonstrated applications are controlling the ion transport processes across membranes, prevention of biofouling, bacterial decontamination of water and liquid food, delivery of chemotherapeutic drugs into tumor cells, gene therapy, transdermal drug delivery, programmed cell death which can be used for cancer treatment and intracellular electro manipulation for gene transfer into cell nuclei.^[15] However, no published work has reported its utilization in skeletal system for stimulation purposes and its possible effect on functional behavior and biomechanical properties of bone tissue. Along this way, before animal or clinical study, assurance of the safe application of high power signals on bone tissue is necessary to prevent any thermal effect or extra loading which can disturb the quality of the bone composite material. The motivation for this research was to explore the safe and controlled application of PP on bone tissue as a proof-of-concept for potential in future clinical application. This study was aimed to investigate if PP can be applied safely on bone tissue and how the functional

behavior of cortical bone will be influenced by PP stimulation. For this purpose, a compressive test and an ultrasonic velocity measurement were conducted to determine whether or not PP can affect the biomechanical properties of cortical bone. Using small-sized samples in these methods can increase the effect of the pulse electric field on specimens.

THE THEORETICAL CONSIDERATION

Ultrasound Velocity Measurement

According to the theory of small amplitude elastic wave propagation in anisotropic solids,^[16,17] the rate at which the shear or longitudinal waves travel through a solid matter is dependent upon its elastic property and density. A longitudinal wave is generated when the transmitter vibrates in the same direction as wave propagation. If the transmitter vibrates in a perpendicular direction to the wave propagation, shear waves are produced.

Both longitudinal and shear waves can propagate in two modes inside the bone based on specimen geometry and wavelength of the waves (velocity/frequency). If the cross-sectional dimension of the specimen is greater than the ultrasound wavelength, the wave does not reach the sample boundaries. This leads bulk wave propagation. The second case, where the characteristic specimen dimensions are smaller than the wavelength, is known as bar wave propagation. In this case, the ultrasound wave propagates as a complex bar wave, consisting of both shear and longitudinal waves and the entire specimen cross section is excited by the passing wave.^[18,19]

For bulk wave propagation, velocity is given by:^[20]

$$v = \sqrt{\frac{K + \frac{4}{3}G}{\rho}} \quad (1)$$

Where ρ is density, K is bulk modulus and G is shear modulus which for isotropic material are defined by Young's modulus (E) and Poisson's ratio (ν) as:

$$K = \frac{E}{3(1-2\nu)} \quad (2)$$

$$G = \frac{E}{2(1+\nu)} \quad (3)$$

For bar wave propagation, the velocity can be defined directly by the Young's modulus and density given as:^[20,21]

$$v = \sqrt{\frac{E}{\rho}} \quad (4)$$

Therefore, if the density of bone samples and the ultrasound velocity are specified, the young's modulus is determined as:

$$E = \rho v^2 \quad (5)$$

To determine ultrasound wave velocity the time in which the wave pass through the specimen is measured by the substitution method. In this method, the difference in ultrasound transit time with and without a sample in the position gives the time delay.

MATERIALS AND METHODS

Sample Preparation

Fresh tibia was obtained from a slain ovine animal within 24 h of slaughter. The surrounding soft tissue was removed from the bone and the tibia was wrapped in a 0.15 M physiological saline soaked cloth and stored at -20°C until required for testing. Prior to sample treatment, the tibia was thawed for 1 h. Parallel-side cubic specimens were prepared from cortical diaphysis of ovine tibia. Creating flat parallel surfaces is crucial for accurate compressive testing, determination of ultrasound velocity and bone elasticity. Hence, the cubic specimens were cut using a linear high precision saw (Isomet 5000) while the bone was kept moist. Their physical dimensions were then measured using a precise digital caliper. Table 1 provides the average size of 10 specimens prepared for each experiment separately.

The prepared specimens were labeled according to their site and segregated randomly into two groups labeled as PP-exposed samples, which were exposed to PP and the control specimens. All samples were then refrigerated at 4°C in 0.15 M physiological saline solution for immediate experimentation and subsequently stored at -20°C for later testing.

Experimental Procedure

Density Measurement

It has been established that there is a direct correlation between bone density and its strength and stiffness.^[22] It is necessary to measure cortical bone specimen density, to calculate Young's modulus from ultrasonic technique. For cortical bone, the material density can be measured by the wet weight divided by the specimen volume, which is the function of both porosity and mineral content of bone. Because there is no marrow space in cortical bone, its apparent density is the same as its material density.^[23]

True volumetric density of cortical bone samples can be derived via micro computed tomography (micro-CT) utilizing Scanco micro-CT40 scanner. The calibration

phantom was performed to convert Hounsfield numbers into volumetric density. It has been suggested (particularly for *in vivo* studies) that bone mineral density (BMD) obtained from micro-CT data can be substituted into Eq. (5) and combined with ultrasound velocity to find bone stiffness.^[20]

$$E = \text{BMD} \cdot v^2 \quad (6)$$

Pulse Power Stimulation of Bone Samples

A positive buck boost converter for generating PP signals was applied in this research.^[24] The output pulse parameters (magnitude, frequency and duty cycle) were controlled using a programmed microcontroller. A TMS320F28335 Digital Signal Controller (Texas Instruments) was used and programmed to control the output pulse parameters. The output signal was voltage pulses up to 500 V at 10 kHz, which can be adjusted by three potentiometers controlling pulse magnitude, frequency and duty cycle manually. The PP signals were delivered through two wire leads attached to two series of metal screws [Figure 1]. Application of millimeter-sized samples and screws with small contact cross-section increase the electric field intensity applied to the bone specimens. Since the direct connection of screws and cables with bone provides very low impedance, a significant current can pass through the bone, leading to potential drying and burning. Therefore, screws were covered by electrical isolation tape to change the characteristics of the bone from a resistive load to a capacitive load. The pulsed electric field was applied to the bone samples through capacitive coupling method. In this case, the conduction current is reduced due to high resistance of the configuration which has a direct impact on thermal effect. On the other hand, the electric field effect on the bone structure was increased as a consequence of creating more capacitive coupling across the bone insulated by tape. The cortical bone specimens in the PP-exposed group were placed in radial direction between isolated screws for stimulation. They were exposed to a high voltage, high frequency pulsed electric field for 66 h. During this period PP-exposed samples keep moist seeping the saline slowly using syringe with small needle. Specimens from the control group were placed in similar environmental condition as the PP-exposed group but they were not exposed to the pulse power field, to consider any possible effect of environment on the results. Both control and treated samples were kept moist with 0.15M physiological saline during the experiment.

Ultrasound Velocity Measurement

High precision measurement of ultrasound velocity was conducted using a high frequency pulser-receiver (Panametrics PR5800), water tank containing two matched 5 MHz, 12.5 mm diameter ultrasound transducers; a transmitter and a receiver. They were highly damped to

Table 1: Mean values \pm SD for the specimen's dimensions

Direction	Mean (mm)	SD
Longitudinal	10.3	0.05
Tangential	3.21	0.16
Radial	1.91	0.26

SD – Standard deviation

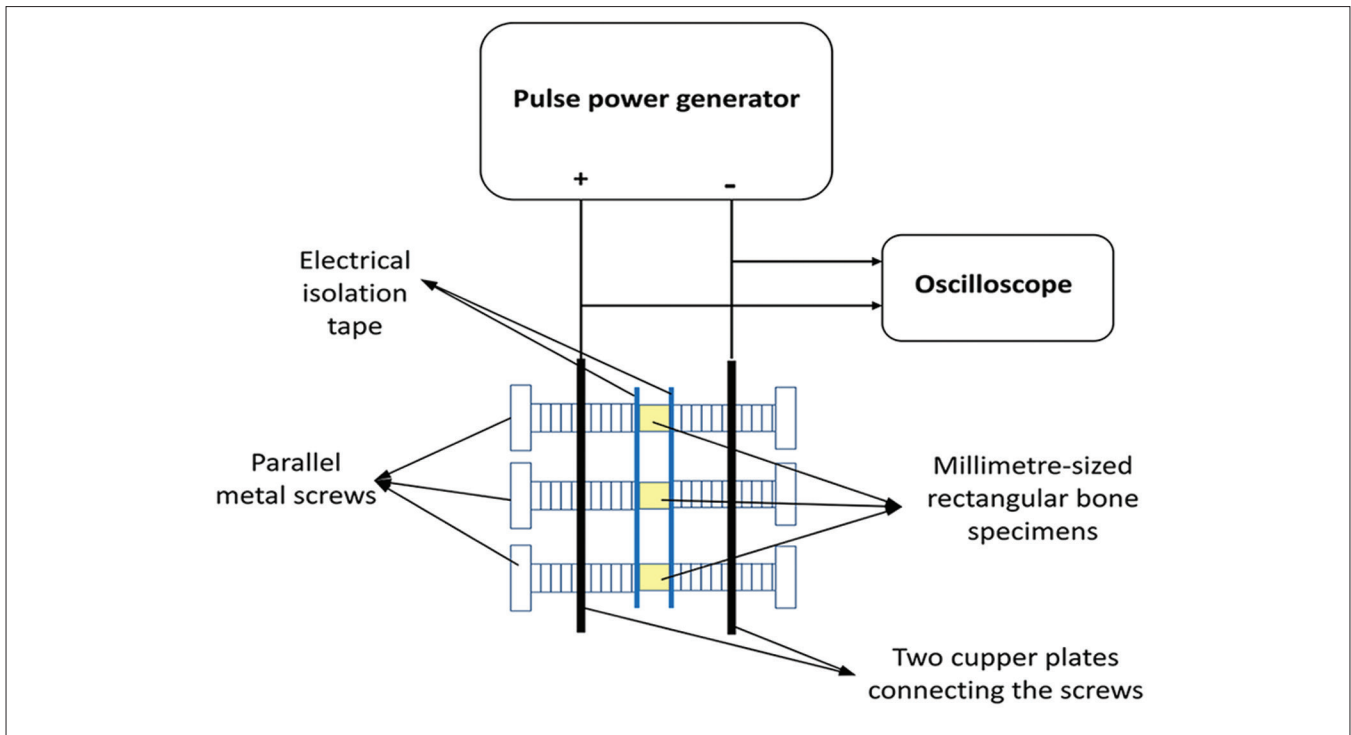


Figure 1: Sketch of experimental set-up for pulsed power stimulation of millimetre-sized cortical bone samples

provide short pulses [Figure 2] and a 100 MHz PC-housed digitization card (NI PCI5122).

The water tank was filled with warm water to above the face of the upper transmitting transducer and the water temperature was measured and recorded. Existence of any air bubbles on the faces of both transducers was checked regularly and if present, wiped away. The cables were connected between computer and pulser-receiver in their appropriate locations and the initial setting on the pulser-receiver was carried out. Ultrasound waves produced by the transducers were monitored and recorded in “Lab view Signal Express” software.

The “substitution” method was applied to calculate the ultrasound velocity. In this method, the difference in ultrasound transit time with and without, a sample in position was measured and recorded. The cortical bone specimen, whose density and dimensions were measured previously, was then placed on top of the downer transducer. The difference transit time of the ultrasound wave through the sample (dt), the water temperature ($T^{\circ}\text{C}$) and sample thickness (D) in each direction were applied to determine the ultrasound velocity in water (V_o) and through the sample (V_s) as:^[25]

$$V_o = 1405.03 + 4.624T - 0.0383T^2 \quad (7)$$

$$V_s = \frac{V_o}{1 - dt \left(\frac{V_o}{D} \right)} \quad (8)$$

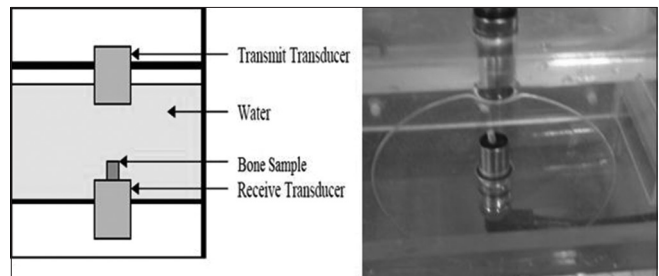


Figure 2: Ultrasound velocity measurement set up in water tank

Ultrasound velocity was measured 5 times in longitudinal, tangential and radial directions before and after pulse power excitation. The average of the measurements was used for calculation. Since the lateral dimension of cortical bone samples were small (compared with ultrasound wavelength), this study assumed that the bar wave was propagated through the sample and therefore the straightforward Eq. (5) was used to calculate Young’s modulus of bone samples.

Mechanical Testing

After 66 h pulse power excitation, both control and PP-exposed samples were subjected to compressive loading until fracture. The millimeter-sized samples were placed on a flat platen attached to an Instron testing machine (model 5944, 2 kN load cell). The compressive test was performed in displacement control at the extension rate of 0.1 mm/min until complete failure occurred and the load was measured from the load cell. The results were recorded as load and

displacement data and converted to stress and strain data (using the cross-section area and length of the samples) for farther analysis.

RESULTS

Samples Density

In this study, cortical bone density was measured using: (1) The conventional method (wet weight/specimen volume) and (2) micro-CT before and after pulse power excitation. Micro-CT data provides BMD of the specimens. No significant variation (using two-tail paired Wilcoxon signed rank test [a non-parametric paired test]) was found in cortical bone density measurement from both methods due to pulse power stimulation. Table 2 presents the results of the two methods in mean value (MV) ± standard deviations (SD). The density obtained from micro-CT was used in further calculations.

Elastic Modulus

The results of ultrasound velocity through the sample and its equivalent elastic modulus in three directions ([1] longitudinal, [2] radial and [3] tangential) showed that although the elastic property of cortical bone was different in three main orthogonal directions (due to anisotropic nature of bone), similar variation was found between the elasticity of the control and PP-exposed samples. The two-tail paired Wilcoxon signed rank test (a non-parametric paired test) compared the ultrasound velocity and Young's modulus variation in each group (control and PP-exposed) before and after pulse power excitation. All differences were considered significant at the value $P < 0.05$ (95% confidence). Tables 3 and 4 present the values of ultrasound velocities and Young's modulus of the cortical bone samples in PP-exposed and control groups before and after PP excitation respectively. The mean ultrasound velocity passing through the samples did not change significantly in both the control and the PP-exposed group after pulse power excitation compared with the initial measurement ($P > 0.05$). In addition, no significant variation in elastic properties of the cortical bone specimens of both groups was found, after application of high power pulses compared to those before stimulation.

Figure 3 compares the total average elastic modulus of control and PP-exposed samples, before and after excitation, obtained from the ultrasonic technique. The graph highlights that the elastic modulus of both normal and treated samples (without and with PP exposure) appeared to remain unchanged.

The comparison of the cortical BMD, also showed [Table 2] that the bone mineral content remained unchanged in both the control and PP-exposed samples after 66 h pulse power excitation. This outcome also confirms the results of comparison of Young's modulus of the control and

Table 2: Mean density ± SD for cortical bone specimens before and after PP excitation

Density measurement method (g/cm ³)	Before PP excitation	After PP excitation	P value
Micro computed tomography	1.148±0.049	1.165±0.06	0.437 (>0.05)
Weight	2.030±0.019	2.036±0.03	0.5625 (>0.05)

The mean density of the samples did not change significantly pre and post PP excitation ($P > 0.05$). SD – Standard deviation; PP – Pulsed power

Table 3: Mean value ± SD for ultrasound velocity and Young's modulus of PP-exposed samples before and after PP excitation in longitudinal, radial and tangential directions respectively

Parameter	PP-exposed samples before excitation	PP-exposed samples after excitation	P value
V1 (m/s)	4032±355.6	4562±137.1	0.1772
V2 (m/s)	3774±391.3	3810±111.7	0.8644
V3 (m/s)	3937±153.9	4191±303.7	0.7545
E1 (GPa)	18.63±2.656	22.17±1.476	0.1127
E2 (GPa)	16.36±2.873	17.06±1.079	0.6938
E3 (GPa)	17.72±1.519	20.75±3.431	0.5041

SD – Standard deviation; PP – Pulsed power

Table 4: Mean value ± SD for ultrasound velocity and Young's modulus of control samples before and after PP excitation period in longitudinal, radial and tangential directions respectively

Parameter	Control group before excitation	Control group after excitation	P value
V1 (m/s)	4158±29.88	4191±152.1	0.3271
V2 (m/s)	3491±322.9	3713±24.66	0.4346
V3 (m/s)	3757±145.8	3917±103.4	0.2492
E1 (GPa)	19.96±1.383	21.10±0.1579	0.3664
E2 (GPa)	14.05±1.825	15.79±1.443	0.4011
E3 (GPa)	16.91±1.536	18.52±1.137	0.3557

SD – Standard deviation; PP – Pulsed power

PP-exposed samples [Figure 4]. Illustrates the average amount of BMD of the control samples compared with that of the samples exposed to pulse power.

The combination of these outcomes, along with the elasticity measurement results demonstrate that pulse power stimulation does not apparently affect the mineral phase structure in cortical bone. As stated previously, the mineral content is the predominant factor in bone stiffness and has a significant correlation with Young's modulus of bone.^[14,26,27] Obviously, because the bone cells were dead they cannot influence the mineralization process in bone tissue.

Toughness, Strength and Stiffness in Compression

The area under the stress-strain curves and the ultimate stress present the total fracture work and the compressive strength respectively. The total strain failure energy which is measured as the area under the stress-strain curve until

complete fracture is an indication of sample toughness. The elastic moduli of the specimens were also determined from the slope of elastic linear portion of stress-strain graphs.

To be more conservative Mann-Whitney test (a unpaired non-parametric test) was applied to compare toughness, strength and stiffness between PP-exposed samples and the control ones. $P < 0.05$ was considered to be significant. Table 5 presents the MVs \pm SD of fracture energy, ultimate compressive stress and Young's modulus for both groups. The results showed that the strength and toughness of the samples exposed to PP electric field were higher compared with those of the control samples ($P < 0.05$). In contrast, the mean Elastic modulus did not change significantly in both the control and the PP-exposed groups ($P > 0.05$).

The graph bars of the three basic mechanical properties of bone samples confirm the overall above results. As it can be seen from [Figure 5a], the samples which were

stimulated by a high voltage, high frequency pulsed electric field can absorb much larger amounts of energy before fracture. It suggests that these specimens become tougher compared with the samples not exposed to pulse power. The PP-exposed samples showed also higher compressive strength compared to the control samples [Figure 5b].

The comparison of the average Young's modulus of the samples exposed to pulse power with that of the control samples, also supports the results of the ultrasonic method that showed no significant effect on the cortical bone stiffness due to pulse power stimulation. Figure 6 presents the comparison of average compressive elastic modulus of the cortical bone samples with and without pulse power excitation.

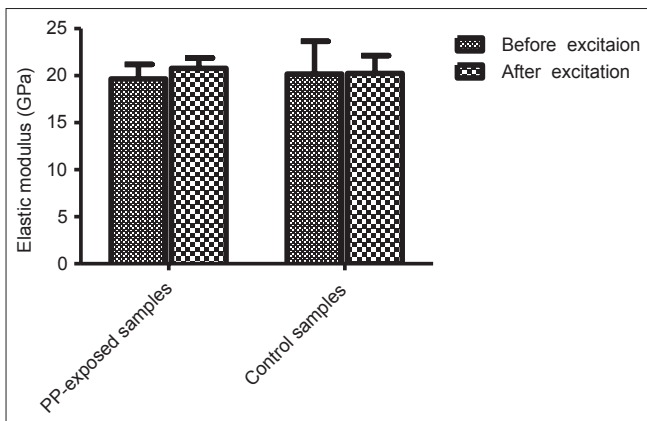


Figure 3: The average elastic modulus of the normal specimens compared with that of the samples exposed to pulse power obtained from ultrasonic technique. The graph shows that the elastic modulus of both normal and treated samples (without and with pulsed power exposure) remains unchanged

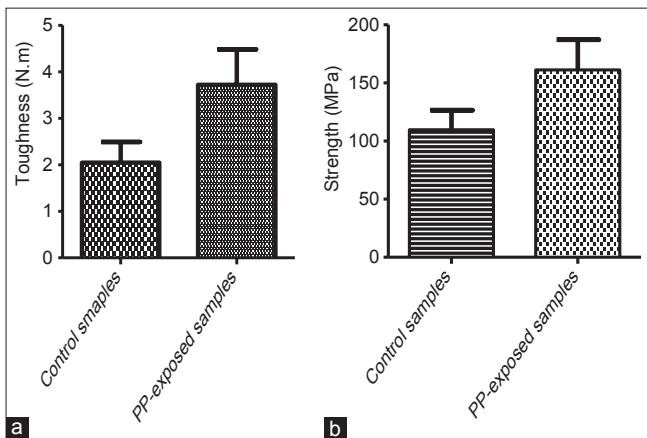


Figure 5: The average strength and total fracture energy absorption of the samples exposed to pulse power compared with those parameters of the control samples. Samples exposed to pulse power became stronger and tougher compared with the control samples

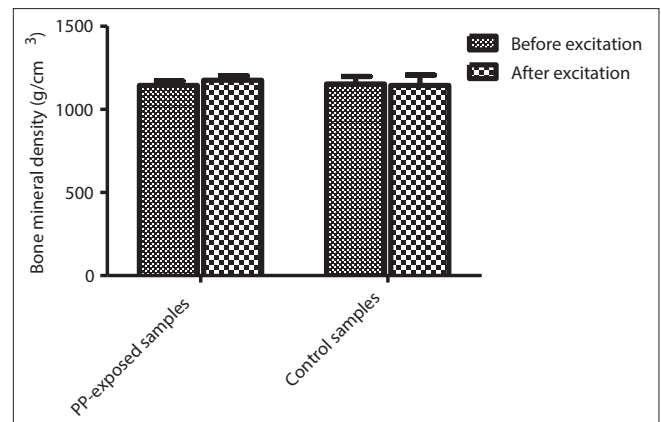


Figure 4: The comparison of the average bone mineral density (BMD) of control and pulsed power (PP)-exposed samples before and after pulse power excitation. The comparison of the cortical BMD, showed that the bone mineral content remained unchanged in both the control and PP-exposed samples after 66 h pulse power excitation

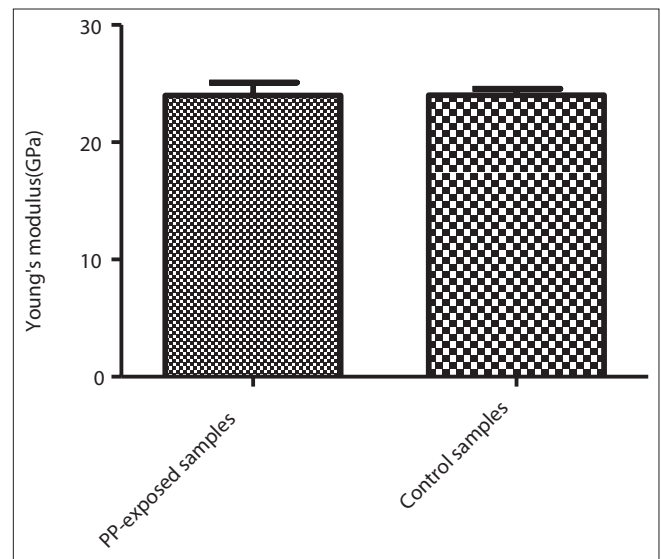


Figure 6: Comparison of Young's modulus of the samples exposed to pulse power with those of the control samples. The stiffness of samples exposed to pulse power remain unchanged compared with the control samples

Table 5: Mean value \pm SD for strength and toughness and stiffness of the control samples compared with those of the PP-exposed samples

Parameter	Control samples (unexposed to PP)	Samples exposed to PP for 66 h	P value
Strength (MPa)	109.79 \pm 37.54	161.25 \pm 58.198	0.042 (<0.05)
Toughness (N.m)	2.0489 \pm 0.995	3.7237 \pm 1.71	0.047 (<0.05)
Stiffness (GPa)	24.01 \pm 1.241	23.98 \pm 2.484	0.989 (>0.05)

SD – Standard deviation; PP – Pulsed power

DISCUSSION AND CONCLUSIONS

Low-power electromagnetic fields have been applied during the last 40 years as a stimulation for osteogenesis and a useful treatment for some chronic musculoskeletal disorders like non-union bone fractures.^[28,29] Nevertheless, the behavior of bone in response to high voltage and high frequency electromagnetic fields (PP) has been poorly explored. Applying this type of electrical stimulation on live bone firstly requires the identification and introduction of controlled parameters and a safe method for applying pulse power to bone tissue, which requires investigating its effect on the fundamental physical properties of bone structure. This study provides the first step in this direction.

The main aim of this research was to investigate the feasibility of the safe and controlled application of pulse power on bone tissue and how the functional properties of bone are influenced by pulse power stimulation. In the other words, whether or not pulse power can be applied safely and controllably on bone tissue and what is the reliable method for this application and explore if PP excitation can affect the basic mechanical properties of bone or not and in which manner.

This study investigated the effect of a high power, high frequency pulsed electric field with 500 V at 10 kHz frequency on the cortical bone material elasticity using an ultrasonic technique. Performing the experiments in two parallel groups, with and without pulse power application, but in a similarly controlled environmental condition, is likely to omit the possible influence of the other issues (e.g., environmental condition) on the bone material elasticity. There appeared to be no significant changes in ultrasound velocity passing through the samples and bone density and therefore their elasticity for both groups before and after pulse power excitation.

This work also evaluated the variation in compressive strength and toughness of cortical bone samples due to pulse power exposure. The results demonstrated that the ultimate compressive stress and the total fracture energy of the cortical samples increased after 66 h of PP stimulation [Figure 5]. These findings confirmed that indirect application of high power pulsed electric field at 500 V and 10 kHz through capacitive coupling method

accompanying with continuous hydration of the bone samples appeared to be safe and controlled with no destructive effect on bone structure.

Furthermore, the results suggest that the effect of pulse power excitation could be increased by pulsed electric field intensity enhancement using electrodes with small cross section (applying small screws).

The toughness and the strength of the cortical bone tissue are directly associated with the quality and integrity of the collagen matrix while its stiffness is primarily related to bone mineral content^[12,13,27] so that reorganization of collagen fibrils causes the maintenance of the mechanical properties of bone tissue including its strength, although BMD was decreased. On the other hand, the orientation of collagen fibrils can be affected by several factors such as an electromagnetic field exposure. This effect was used as a most common method for collagen fibril alignment in the synthesis of scaffolds that mimic the aligned collagen fibrils in very regular tissue like tendon and ligament or as an aligned sheets in bone and corneal tissue.^[30,31]

The total results from two series of experiments performed in this study implied that pulse power stimulation appeared to increase the bone strength and toughness [Figure 5]. Although the mechanism by which pulse power stimulation has increased the strength and toughness of the cortical bone samples is not fully understood, it can be inferred that pulse power stimulation influenced the arrangement or the quality of collagen fibrils (e.g., the crosslink density between the collagen molecules) leading to changes in mechanical properties in the small samples. Nevertheless, pulse power exposure apparently did not change the elastic properties of cortical bone samples and their mineral content which is the main factor in bone stiffness and rigidity [Figures 3,4 and 6].

To author's knowledge, this study was the first research investigating the effect of high voltage, high frequency PEMF on basic mechanical properties of cortical bone.

Understanding the mechanism by which pulse power stimulation influences changes in bone structure requires further research. For example microscopic transmission electron microscope with higher resolution capability, micro-CT with ability to quantify systematic variation in bone tissue microstructure, polarized light microscopy that can determine the reorganization of collagen fibrils or histological analysis with appropriate collagen staining, could be applied to clarify the mechanism behind the characteristics of cortical bone that has been exposed to PP. Although sheep bone is reported to be structurally and hormonally similar to the human bone and also is readily available as well as widely applied in orthopedic research, for future research, it is suggested pulse power stimulation be tested on a larger number of human bones before *in vivo* and clinical studies,

for more confidence. Other factors that may affect the results which need to be considered in this study include bone type, gender and age of the donor, more isolated controlable environmental conditions and other pulse power parameters such as pulse width and application of current pulse instead of voltage pulse. In addition, for an actual evaluation of structural and functional behavior of bone and its real response to pulse power stimulation, it is proposed to investigate the application of pulse power on live bone (the cell bones or *in vivo* study) in future researches. Furthermore, in this study, pulse electric field was applied in one bone crosswise direction (radial direction) and in mechanical testing (compression), only in longitudinal direction. Nevertheless, due to the anisotropic and inhomogeneous nature of cortical bone tissue, it responds differently to different direction loading pattern. Hence, consideration of the other directions of pulse power excitation and mechanical loading would be required for a more complete assessment.

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BIOGRAPHIES

Hajarossadat Asgarifar received her BSc (Eng) degree in Electronic Engineering from Isfahan University of Technology (IUT), Isfahan, Iran and her MSc degree in Biomedical Engineering from Queensland University of Technology (QUT), Brisbane, Australia. Her current research interest is in the area of medical instrumentation and biomedical engineering while she has been working on design and manufacturing of FPGA and CPLD boards.

E-mail: h_asgarifar@yahoo.com

Adekunle Oloyede received his PhD from Imperial College, London, his MSc from Cranfield Institute of Technology, and his BSc in Mechanical Engineering with Honors from University of Lagos. He is now a professor at school of Biomedical Engineering and Medical Physics, QUT. His main areas of expertise are articular cartilage biomechanics, soft tissue biomechanics, materials and biomaterials (permanent scaffold), experimental, mathematical and finite element modelling

of biomechanical systems. He has published over 100 papers in these areas and is a world renown expert in the biomechanics of articular cartilage.

E-mail: k.loyede@qut.edu.au

Firuz Zare received his BSc (Eng) degree in Electronic Engineering, his MSc degree in Power Engineering and his PhD degree in Power Electronics. His main research interests include Pulsed Power, Power Electronics Topologies and Control, Pulse Width, Modulation Techniques and Renewable energy Systems. He has published over 135 journal and conference papers and technical reports in the area of Power Electronics. He has spent several years in industry as a team leader and development engineer where he was involved in different electronics and power electronics projects and was associate prof. at school of Engineering System, QUT.

E-mail: f.zare@qut.edu.au
