Proposed research project

Title: A Double-Blinded, Randomized-Control-Trial to Investigate the Effect of Pulsed Electromagnetic Field (PEMF) for Patients with Knee Osteoarthritis

Background:

Health care costs are increasing alarmingly, which will impose an overwhelming economic burden to an aging society like that of Hong Kong. For example, degenerative musculoskeletal disorders such as osteoarthritis (OA) present a grand challenge with its high prevalence (>40% in the elderly suffered from knee OA). OA is a debilitating progressive disease with typical symptoms such as acute pain causing loss of mobility Currently there is no cure for OA. Pharmacological treatment and new regenerative technologies such as stem cell therapy are actively being developed, but most of these options are very expensive per se, and side effects are not uncommon. Costeffectiveness is also a major consideration for devising new therapeutic modalities for OA.

PEMF treatment can modulate mitochondrial activities for muscle gain. Molecular processes are sensitive to the magnetic flux at extremely low frequency (<300 Hz), which are equivalent to the Earth's natural magnetic resonance (Schumann resonance). Applying magnetic fields at these low frequencies in the form of magnetic pulses, like a timely push to swing. The pulsing action will enhance the human magnetosensitive processes, which include the electron transport chain in mitochondria, calmodulin signalling and ion channels. The affected signal transduction pathways further influence the downstream myogenesis. (1) Therefore, a pulsed electromagnetic field (PEMF) may be a possible intervention to amplify molecular processes in favour of myogenesis. (2)

The therapeutic effects of PEMF in the field of orthopaedics have been recognized (4-6) and approved for clinical use for over the 40 years (3). The clinical evidences of the use of PEMF to treat knee OA has been summarized in Cochrane Reviews (7), suggesting that PEMF may provide moderate benefit for OA sufferers in terms of pain relief (8); but the improvements in function and quality of life (9) are not significant, probably due to persistent muscle weakness. Basic science studies revealed that PEMF can reduce cartilage degeneration (10), promote chondrogenesis in stem cells (11, 12), augment anabolic effect in OA chondrocytes (13) and cartilage explant (14), counteract inflammation (15), and improved subchondral bone quality (16, 17). However, large variations in treatment conditions still present major obstacles to optimize the therapeutic effects of PEMF, such as device parameters (PEMF frequency, amplitudes symmetry, field gradient) (18) and treatment regimen (duration of PEMF sessions, number of session and total treatment duration needed). Moreover, there is room for further improvements for PEMF treatment for OA patients with respect to function and quality of life. Our approach is unique in that we target muscle to act as a delivery system for regenerative agents that will promote healing, naturally.

Our study will examine the efficacy of PEMF treatment introduced at patient with Knee OA. Also, it helps to set up the treatment regime for the PEMF device to impose myogenic effects, including exposure time per session, the number of treatment sessions and the duration of treatment. To our best knowledge, this proposed study aims to apply PEMF as an intervention for patients with knee OA. In turn, it would be the first study to examine the effect of PEMF for patients with knee OA to relief in OA symptoms.

Objective:

To conduct a double-blinded, randomized controlled trial to investigate the effect of PEMF treatment on knee pain for patients with knee OA.

Hypothesis:

PEMF treatment may have a positive effect on increasing quadricep muscles strength. With the enhanced muscle strength and power, it may lead to relief in OA symptoms and improve knee functions.

Setting:

The study will be carried out in the Department of Orthopaedics and Traumatology at Prince of Wales Hospital.

Subject recruitment:

The study protocol will be in compliance with Declaration of Helsinki and ICH-GCP. Subjects will be recruited from the Department of Orthopaedics and Traumatology of Prince Wales Hospital in Hong Kong and patients who can meet all the inclusion criteria will be invited to participate in two sessions of assessments.

A total of 110 subjects and 20 matched non-Knee OA subjects will be recruited and follow up for 8 weeks. They will undergo 1:1 randomization into two study arms:

1. PEMF treatment
2. Control (Sham treatment)

Inclusion criteria:

1. Age 50 or above
2. Unilateral knee OA with Kellgren-Lawrence grade 2-3 by X-ray
3. Sedentary lifestyle (Tegner activity level ≤ 3)
4. No acute knee injuries in past 12 months
5. No muscle strain in past 12 months
6. No degenerative joint diseases in other joints except the involved knee

Exclusion criteria:

1. Age smaller than 50 years old
2. Experienced any concomitant bone fracture, ACL injury, major meniscus injury
3. Any rheumatological diseases
4. Metal implants that would cause interference on MRI
5. Previous contralateral knee injury
6. Recent knee injections (prior 3 months)
7. Physical inability to undertake testing procedures
8. Pregnancy or possibility of pregnancy

Methods:

Intervention

The intervention will be held at Prince of Wales Hospital twice a week. Participants in the intervention group will be exposed to PEMF treatment by a PEMF device (Quantum Tx, Singapore). The active pulse electromagnetic field device does not produce heat or cause any sensation to the tissue which allows the participants to be blinded to the treatment. Participants in the control group will receive a sham exposure with same PEMF device. Involved leg will be exposed to PEMF or sham treatment for 10 minutes per session, and the treatment regime will run biweekly for 8 weeks, summing up 16 sessions of PEMF or sham exposure in total. According to our pilot study in Singapore, such an arrangement establishes a cross-feeding scenario whereby muscle recovery is fully supported and systemic myokine release is optimized. The procedure of PEMF treatment is shown as follows:

- The subject will be seated at a 90 degrees position on a chair.
- The solenoids of PEMF device will be adjusted to be over the thigh (quadriceps and hamstring).
- The options of the appliance will be adjusted to 1 mT, 15Hz on one leg for 10 minutes.

Outcome measures:

Self-reported knee functions

The subjective functional outcome will be evaluated by the The Knee injury and Osteoarthritis Outcome Score (KOOS),International Physical Activity Questionnaire (IPAQ), and Tegner activity score:

1) The Knee injury and Osteoarthritis Outcome Score (KOOS)

   It contains 42 items with 5 subscapes: pain, other symptoms, function in daily living (ADL), function in sport and recreation (Sport/Rec), and knee-related quality of life (QOL). It allows us to obtain information on how the patient's activities of daily living and overall quality of life is affected by the knee condition.

2) International Physical Activity Questionnaire (IPAQ)

   The level of physical activities during the past 7 days will be evaluated with a validated Chinese version of the quantitative physical activity questionnaire.

3) Tegner activity score

   This is an activity level scale from 1 (low activity) to 10 (high activity).

4) Short Form-36 questionnaires (SF-36)

   It will be used to evaluate Health-Related Quality of Life.

Muscle strength assessment by hand-held dynamometer

Hand-held dynamometer microFET2 (Hoggan Scientific, Salt Lake City UT, USA) will be used to assess lower limb strength and power. Assessment of isometric muscle strength and power will be performed with the participants in a seated position to assess knee extensors and knee flexors. All tests will involve maximal voluntary isometric contractions. Instructions provided to participants for all trials will be 'at the count of three, push/pull as hard and as fast as you can and hold that contraction until I say relax' in Cantonese. Each test will last between three to five seconds and ended after a steady maximal force was produced by the subject. Constant verbal encouragement will be provided throughout the testing. Both limbs will be assessed to record side-to-side difference. Two trials were recorded for each muscle group.

Posture assessment

Patients with knee OA demonstrated postural deficits during double leg squat on their affected and unaffected sides when compared with healthy controls (19). The centre of pressure (COP) excursion range in the anterior-posterior direction during squat can be used to monitor postural changes related to functional impairment in knee OA (20). In brief, the subjects will start with feet shoulder-width apart and arms outstretched and parallel to the ground on a pressure sensor mat (I-Scan, Tekscan, Boston, Massachusetts), then they will be instructed to squat down as low as possible and then rise to the upright position with their preferred pace while keeping their arms parallel to the ground. COP will be determined by the pressure sensor and presented as percentage of the longitudinal foot.

Static postural conditions include stand on double leg with open (DO) and close eyes (DC) for 30s and stand on one leg with open eyes (SO) for 10s. For DC condition, visual input will be eliminated by wearing a blindfold. During double leg stance, each subject will be asked to stand barefoot in a comfortable position (shoulder width apart) on the force-platform while positioning the arms on their sides. To prevent falls during the testing conditions, one of the experimenters stand beside patients. During single leg stance condition, the subjects will be asked to remain as motionless as possible while standing on the involved leg on the center of the platform, keep their eyes open and maintain the arms relaxed at their sides. In these cases, the failed trial will not be used in the data analysis. The patients will perform three trials of each condition. The conditions will be separated from each other by a 1 min rest time to minimize fatigue.

Dynamic postural control will be evaluated using the task of transitioning from double-leg to single-leg standing. The patients will be instructed to stand on a force plate with the load distributed as evenly as possible between the two legs and to keep looking straight ahead, with their feet shoulder-width apart and their arms folded across the chest. Then, the patients perform the transition from double-leg to single-leg standing as fast as possible after a verbal cue and will be asked to maintain single-leg standing as stably as possible for at least 5 s. The more affected limb will be tested in the single leg standing task. All patients will practice the task three times. Data collection starts when they become familiar with the task. Each patient will be allowed to rest at any time to prevent the effects of fatigue. Trials in which the patients fail to maintain single leg standing for at least 1 s will be excluded from the analysis. Three successful trials perform by each patient will be analyzed.

Chair stand test

While seated in a custom-built chair allowing only the bare feet to exert force on a force plate (Tekscan, U.S.), the participant will perform trials of six STS cycles as fast as possible. The

seat height is 120% tibia length. The inter-feet distance is the width of the shoulders, the knee angle is 90˚-100˚, and the arms are placed across the chest. At least three approved trials will be performed after task familiarization. A trial will be approved if the knees are fully extended while standing, the back touches the backrest while seated, and the feet and hands retain their positions throughout the trial. To ensure maximal performance, an additional trial will be performed if the last approved trial is the fastest. The completion time will be calculated as the time-period from the 1st vertical ground reaction force peak of the first repetition to the first peak of the 6th repetition. Thus, it will reflect the time of five repetitions. The rate of force development will be determined as the mean slope of the rising force of the first force peak of each of the 2nd to the 6th repetition in the interval of 30–70% exerted peak force. Results are reported as the mean of the two fastest approved trials in body weight per second (BW/s).

Biochemical Assays

Blood samples will be taken under non-fasting conditions. Serum / plasma obtained will be immediately stored at -80°C until analysis. Serum 25(OH) Vit-D assay: Serum 25(OH)Vit-D levels will be measured by commercial 25(OH) Vitamin D ELISA kit (Abcam ab213966) according to the manufacturer's instruction, providing the quantitative determination of 25(OH) Vitamin D3 and 25(OH) Vitamin D2. Sensitivity: 1.98 ng/ml (Range: 0.5 ng/ml - 1010 ng/ml).

Range of metabolites will also be evaluated. Human serum was vortexed with 500 μL of acetonitrile with internal standard (25 ng/mL 25-hydroxyvitamin D3-d3 and 24R,25-dihydroxyvitamin D3-d6, 10 ng/mL 3-epi-25-hydroxyvitamin D3-d3, 5ng/mL 1α,25-dihydroxyvitamin D3) for 1 minutes and then stood in 4oC for 30 minutes. Proteins were precipitated and spun down by centrifugation for 15 min at 14,000×g. The supernatant was transferred into 2-mL tubes containing 400 μL 0.4 M potassium hydrogen phosphate (K2HPO4) and vortexed for 30 seconds followed by the addition of 500 μL methyl t-butyl ether (MTBE) for liquid–liquid extraction. The upper organic layer was transferred into 2-mL tubes for drying under the stream of nitrogen gas. The deried extract was derivatizated with 500 μL 0.15 mg/mL 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) in acetonitrile at room temperature for 1 h and then stored overnight at +4 °C. The derivatized extract was dried under the stream of nitrogen gas and then redissolved with 30 μL methanol. The resulting solution was subjected to UPLC-Qtrap/MS

Serum myokine evaluation

Phlebotomy (5ml) will be performed on the day before PEMF treatment, and at 2-, 3-, 6- and 12- months after commencement of treatment. The serum will be prepared by centrifugation and kept in a -80o freezer until use. Quantitative analysis for myokines and proteins related to muscle metabolism will be performed by Human Myokine Magnetic Bead Panel (Millipore) with Bioplex-200 bead-based suspension assay system (LKSIHS core facilities), or enzyme-linked immunosorbent assay (ELISA). These include Brain-derived neurotrophic factor (BDNF), Fibroblast growth factor-21 (FGF-21), Interleukin-6 (IL-6), IL-15, Irisin, Myostatin (MSTN)/GDF8, Insulin-like growth factor 1 (IGF-1), FGF-2, IL-8, Follistatin, Musclin, Myonectin, Decorin, Meteorinlike, Osteopontin, Secreted protein acidic and rich in cysteine (SPARC), Klotho, Procollagen type III N-terminal peptide (P3NP), and C- terminal of troponin T1 (TNNT1).

Visual Analogue Scale

The subjective measurement for chronic and acute pain will be recorded be Visual Analogue Scale (VAS). VAS is consisted of a 10-cm line which represent the continuum between "pain less" and "worst pain from 0 cm to 10 cm. Subject will be ask to draw a mark of it before and after the PEMF treatment.

Study design

Randomization and blinding

Participants will be randomized into 1:1 allocation. Blocked randomization will be undertaken based on the computer-generated allocation sequence, whereby 110 participants would be divided into blocks of 10 for 2 groups, Thereafter, the participants will use their key card to complete the assigned treatment regime, without knowing if the machine is delivering active or sham PEMF. Outcome assessors and trial administrators are blinded for the group allocation.

Follow up time points

Subjects with OA knee will be recruited into this randomized controlled trial. Baseline assessments including KOOS, IPAQ, muscle strength, posture control and serum markers will be taken before PEMF treatments, and same set of measurements will be monitored at 1- and 2- months after the commencement of PEMF, and at 3-, 6- and 12- months after finish the PEMF intervention if possible.

Sample size calculation:

KOOS Score will be employed as primary outcome for sample size estimation. The minimal clinically important change has been determined as 10 for KOOS (21). A repeated ANOVA will be used to compare KOOS scores, IPAQ, muscle strength, posture control and serum markers before PEMF treatment and at 1-, 2-months after the commencement of PEMF treatment, and at 3-, 6- and 12- months after finish the intervention. When the sample size is 34 per groups with 6 repeated measurements, a one-way analysis of variance will have 95% power to detect at the 0.05 level and change of 10 with a standard deviation of 15 KOOS score (22). An additional 35% will be added to account for possible attrition. Thus, 110 (55x2) subjects will be recruited in total.

Data processing and analysis:

The data obtained in the tests will be analysed using Statistical Package for Social Science (SPSS) (IBM SPSS ver.26). For parametric data, paired sample t test will be used. Wilcoxon signed rank test will be used to analyse the data sets if they are non-parametric. The alpha level will be set at 0.05 for all statistical tests.

Potential pitfall and contingency plan:

Patients that are potentially intolerable for magnetic low-field magnetic stimulation or trigger discomfort will not be included in our study during screening. If the patient record did not indicate any potential adverse reaction to the PEMF treatment and experience such a situation during the intervention, the patient would be immediately removed from the study in their best interest to safeguard their health. All staff operating the PEMF would have thorough training on the operation of the machine and familiarize themselves with the troubleshooting scheme. Study personnel would indicate and explain to patients during recruitment about the intervention duration and frequency to stress the importance of compliance, accompanied with frequent reminders to participants. In the case of patient non-compliance, study personnel

would remove the patient from the study only when multiple failed attempts to contact and convince the patient occurs.

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