**March , 2019**

**ORTHOPEDICELL CLINICAL PROTOCOL**

**KNEE OSTEOARTHRITIS**

**Background:**

Osteoarthritis, especially of the knees and elbows, effects tens of thousands of people in the US. This condition has many causes, including routine osteoarthritis associated with advancing age, as well as previous injury, obesity, and other factors, which result in loss of articular cartilage leading to the significant pain due to “bone-on-bone” finding on imaging. There are many treatments on the market, ranging from the most aggressive being total knee replacement with a prosthetic joint, to non-invasive treatments, most of which are based on very little if any science, and offer variable benefit in most patients.

The body has a number of electrical fields as evidenced by the EKG. EEG, and the EMG. Bioelectric stimulation is a growing field that takes advantage of manipulating electrical fields using non-invasive micro-current stimulation of the target tissue at very precise frequencies. Decades of research have led to identification of the precise frequency that is the signature of up to 13 pro-regenerative proteins in the body. Stimulation of that precise signal can induce the controlled expression and upregulation of the protein, each of which has been shown to be beneficial in organ regeneration. This includes multiple growth factors such as IGF, HGF, PDGF, EGF, HGF, and VEGF, and a protein that is reduced with age called Klotho. The stimulator used is a 510K approved programmable device that delivers each of the signals at specific duration and strength for that protein, and in any sequence selected. This strategy much more closely mimics the multiple proteins involved in native tissue and organ repair.

OrthoCell is an approach to the treatment of orthopedic problems including osteoarthritis, that is based primarily on use of bioelectric stimulation to deliver a combination of precise non-invasive microcurrent signals to the knee joint to upregulate the local tissue expression of six pro-regenerative target proteins that drive native stem cell recruitment, proliferation, and differentiation, to achieve significant pain relief and regeneration of cartilage1-9. It has been used in many patients for a variety of clinical conditions and proven safe, including now being evaluated as an anti-cancer therapy. All subjects in this study will receive bioelectric stimulation (BES) delivered by a Mettler stimulator which has FDA 510K approval for the indications of pain relief, improved blood supply, improved muscle function, and improved wound healing. The stimulator is connected to patch electrodes on the sides of the knee. Each patient will receive bioelectric stimulation treatments twice/week for 30 minutes for 8 weeks.

In addition, patients will receive a Prizm knee wrap that provides additional microcurrent bioelectric stimulation. Prizm Medical, Inc. develops and manufactures electrotherapy devices for therapeutic pain management, increased blood circulation, improve range of motion, and retardation of disuse atrophy (muscle wasting). Their products include mesh wraps that contain microelectrodes and are connected to a small light weight stimulator worn on the ankle at home for 30 minutes daily to provide additional bioelectric stimulation to enhance clinical benefit. The stimulators are pulsed direct current neuromuscular and TENS pain relief stimulators. It also has 510K approval to treat acute and chronic injuries, sources of pain, increase circulation while improving range of motion, and to minimize soft tissue atrophy and injury and has been used in over 250,000 patients with documented benefit. The wrap is to be worn for 30 minutes daily to deliver more bioelectric stimulation and benefit.

A second pro-regenerative agent is Platelet Rich Fibrin(PRF), which is an non-manipulated acellular preparation that contains a large number of stem cell growth factors that have a ten-fold longer half-life than platelet rich plasma23-32. It is obtained by drawing a small aliquot of the patient’s blood which is then processed in the clinic via a centrifuge (provided by the sponsor) and injected into the knee. PRF has been used for a large number of indications, and is considered safe for this indication and type of delivery.

**Study Description:**

This is a prospective, non-randomized, open label, consecutive series pilot study to evaluate the safety and feasibility of OrthoStim treatment regimen to relieve the pain and disability of significant osteoarthritis of the knee. The treatment regimen is detailed below.

The study, including protocol and consent form, will have been approved without stipulations by the Institutional Review Board at Hoag Hospital as meeting safe and good clinical practice before any subject will be enrolled.

**Target Number Enrolled**: 20

**Number of Enrolling Sites**: TBD

**PROTOCOL:**

**Inclusion Criteria:**

1. Age 40-80 yrs of age
2. Subjects must be in good health with a BMI < 35.
3. Must have significant osteoarthritis by imaging within 3 months of treatment
4. Willing to be present for the required treatment/study visits.
5. Willing to complete the required survey of the level of pain and limitation of mobility at the specified intervals before after the end of treatment
6. Willing and able to give informed consent and follow study instructions and requirements.
7. Must speak, read, and understand English

**Exclusion Criteria:**

1. Patients who have a planned arthroscopic procedure on the knee intended for treatment of osteoarthritis in the next 3 months.
2. History of bleeding disorder
3. Able to have any anti-platelet or anticoagulant medication including aspirin, Plavix, warfarin, or other oral anticoagulant discontinued for at least 3 days prior to the procedure
4. Allergic to lidocaine, epinephrine, cephalosporins, penicillins or chlorhexidine gluconate
5. Individuals with diminished decision-making capacity
6. Current Smoking and use of other tobacco products.
7. Pregnancy or current breast feeding for females

**Treatment Regimen:**

 **Bioelectric Stimulation:**

 All patients will receive a series of bioelectric stimulation treatments to the treated knee via a set of surface electrodes placed on the both sides of the knee, and connected to a Mettler 510 K approved desk top stimulator for 30 minutes twice/week for one month, then once/week for the next 8 weeks delivered in the outpatient clinic.

 In addition, patients will be given a knee wrap to use a minimum of 30 minutes/day, which includes embedded electrodes that will be connected to a small wearable stimulator to provide additional bioelectric stimulation at home.

 **Specific proteins to be stimulated:**

VEGF, PDGF, IGF, HGF, and KLOTHO

 **Platelet Rich Fibrin**:

 All patients will also receive intraarticular injection of Platelet Rich Fibrin (PRF), which is obtained by drawing a small sample of their own blood which is then placed in a centrifuge on site to yield the amount of PRF needed(3-5 ml). The PRF will be drawn into a syringe and injected directly into the knee joint at the start of the study and at months 1,2, and 3 after study initiation.

**Screening:**

Any subject with qualifying osteoarthritis, pain, and reduced mobility who meet all the Inclusion and none of the Exclusion criteria, will be eligible for participation. Each potential subject will have a brief history and examination performed by the Investigator or designee, and if acceptable, will be provided with an overview of the study and offered an opportunity to review the Consent Form.

If they choose to participate, and sign the Consent form, they will be enrolled in the study.

**Screening Test of Bioelectric Stimulation:**

All patients enrolled in the study will have a screening test to assure the safety and tolerability of the BES treatment regimen on the opposite knee before use in the study. This will be done by placement of patch electrodes on both sides of the non-involved knee and connected to a Mettler bioelectric stimulation signal generator that has been previously tested and proven to be capable of delivering the required current. The stimulator will be turned on and run for up to a 20 minute period of escalating micro-currents to a peak of 1.0 volt to test tolerability of the stimulation in each patient. If there is no significant skin irritation or pain or adverse effect at the end of the test period of bioelectric stimulation, the patient will be eligible for participation in the study.

**Disability Survey/Range of Motion Assessment:**

All eligible subjects will complete a baseline survey to record the amount of pain and disability before starting treatment. This survey will be repeated at the end of the 12 week treatment period, and then at 1 and 3 months of follow up. In addition to the subjective survey, the range of motion of the effected knee will be measured by a technician trained in this assessment tool. The survey and range of motion should be supervised by an assistant not directly involved in the conduct of the study and ideally not an employee of the Clinic to avoid bias of data.

**Sponsor Provided Equipment**

All treatment-related components will be provided to the clinic including:

Mettler 740 bioelectric stimulator

Patch electrodes

Prizm knee wrap with embedded electrodes and wearable stimulator

Low speed PRF centrifuge for separation and retrieval of the patient’s fibrin.

**Treatment Schedule**:

**Bioelectric Stimulation:** Mettler stimulator

 Duration of Each Treatment: 30 minutes

 Frequency of Treatments: once/week for four weeks, then once/week for8 weeks

 Total Treatment Period: 12 weeks

**Bioelectric Stimulator**: Prizm wearable wrap

 Duration of Treatment: 30 minutes

 Frequency of Treatment: daily for 3 months

**Platelet Rich Plasma**:

Frequency of Treatment: Once/month X 4, including baseline, months 1, 2, 3

Patients will have the Platelet Rich Fibrin(PRF) delivered via direct injection into the treated knee.

**Pause/Stopping Rules**:

Treatment will be paused for any complaint by the patient of significant or intolerable pain or discomfort, or local adverse effect. The patient will be allowed to resume treatment after a minimum of seven days at the investigator discretion.

**Follow Up Evaluations**:

Each patient will have a follow up visit at month 1, 2, and 3 post study initiation at the clinic to complete the self-assessment survey and have range of motion and pain assessed a designated individual trained in this survey.

Each subject will also have a brief interview inquiring about any adverse effects noted by the subject since enrolling in the study.

**Grading Survey and Range of Motion**:
Both the subject and the investigator will independently grade the level of response to treatment as Mild, Good, or Very Good. The results of all 20 enrolled subjects will be collated by the clinic and submitted to the sponsor within two weeks of study completion.

**End Points**:

**Primary End Point**: All changes will be a comparison from baseline to after 6 and 12 weeks of treatment. End Points include

1. Safety of bioelectric stimulation and intraarticular injection of PRF and Regenerative Fluid

**Secondary Endpoints**:

1. Pain reduction
2. Improved range of motion of the treated knee
3. Change in Quality of Life and Patient Satisfaction

**Additional Subjects**: Additional subjects may be enrolled into the study if approved by the local IRB Committee, the Principal Investigator, and the Sponsor.

**References:**

**Bioelectric Stimulation**

1. [Hunckler J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Hunckler%20J%5BAuthor%5D&cauthor=true&cauthor_uid=28461755), [de Mel A](https://www.ncbi.nlm.nih.gov/pubmed/?term=de%20Mel%20A%5BAuthor%5D&cauthor=true&cauthor_uid=28461755). [Hunckler J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Hunckler%20J%5BAuthor%5D&cauthor=true&cauthor_uid=28461755)1, [de Mel A](https://www.ncbi.nlm.nih.gov/pubmed/?term=de%20Mel%20A%5BAuthor%5D&cauthor=true&cauthor_uid=28461755)1. A current affair: electrotherapy in wound healing. [J Multidiscip Healthc.](https://www.ncbi.nlm.nih.gov/pubmed?otool=umnbmlib&term=bioelectric+stimulation+current+affair) 2017 Apr 20;10:179-194.
2. Birmingham k, Gradinaru V, Ludwig K, Famm K. Bioelectronic medicines: A research roadmap. Nat Rev Drug Discovery. 2014;13(6):399-407
3. McLaughlin KA, Levin M. Bioelectric signaling in regeneration: Mechanisms of ionic controls of growth and form. Dev Biol. 2018 Jan 15;433(2):177-189
4. [Jacob J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Jacob%20J%5BAuthor%5D&cauthor=true&cauthor_uid=29497465)1, [More N](https://www.ncbi.nlm.nih.gov/pubmed/?term=More%20N%5BAuthor%5D&cauthor=true&cauthor_uid=29497465)1, [Kalia K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kalia%20K%5BAuthor%5D&cauthor=true&cauthor_uid=29497465)1, [Kapusetti G](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kapusetti%20G%5BAuthor%5D&cauthor=true&cauthor_uid=29497465)1. Piezoelectric smart biomaterials for bone and cartilage tissue engineering. [Inflamm Regen.](https://www.ncbi.nlm.nih.gov/pubmed/29497465) 2018 Feb 27;38:2. doi: 10.1186/s41232-018-0059-8. eCollection 2018.
5. Reddi AH. Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. Tissue Eng. 2000; 6(4):351–9.

# Hoare JI, Rajnicek AM, McCaig CD, Barker RN, Wilson HM. Electric fields are novel determinants of human macrophage functions. J Leukoc Biol. 2016;99(6):1141–1151.

1. Rouabhia M, Park H, Meng S, Derbali H, Zhang Z. Electrical stimulation promotes wound healing by enhancing dermal fibroblast activity and promoting myofibroblast transdifferentiation. PLoS One. 2013; 8(8):e71660.
2. Gurgen SG, Sayin O, Cetin F, Tuc Yucel A. Transcutaneous electrical nerve stimulation (TENS) accelerates cutaneous wound healing and inhibits pro-inflammatory cytokines. Inflammation. 2014;37(3):775–784.
3. Payne S, Furness J, Stebbing M. Bioelectric neuromodulation for gastrointestinal disorders: effectiveness and mechanisms.[Nat Rev Gastroenterol Hepatol.](https://www.ncbi.nlm.nih.gov/pubmed/30390018) 2018 Nov 2. doi: 10.1038/s41575-018-0078-6. [Epub ahead of print]
4. Pastor J, Moe OW. Treating systemic Klotho deficiency. Am J Nephrol. 2019 Apr 12;49(5):410-412
5. Cheikhi A, Barchowsky A, Sahu A, Shinde SN, Franti M, Ambrosio F. [Klotho: An elephant in aging research.](https://www.ncbi.nlm.nih.gov/pubmed/30843026) J Gerontol A Biol Sci Med Sci. 2019 Mar 7.
6. Olausson H, Mencke R, Hillebrtands JL. Tissue expression and source of circulating αKlotho. [Bone.](https://www.ncbi.nlm.nih.gov/pubmed/28323144) 2017 Jul;100:19-35

**Autologous Stem Cells**

1. Centeno C, Sheinkop M, Dodson E, Stemper I, Williams C, Hyzy M, Ichim T, Freeman M. [A specific protocol of autologous bone marrow concentrate and platelet products versus exercise therapy for symptomatic knee osteoarthritis: a randomized controlled trial with 2 year follow-up.](https://www.ncbi.nlm.nih.gov/pubmed/30545387) J Transl Med. 2018 Dec 13;16(1):355.
2. Centeno CJ, Al-Sayegh H, Freeman MD, Smith J, Murrell WD, Bubnov R. [A multi-center analysis of adverse events among two thousand, three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions.](https://www.ncbi.nlm.nih.gov/pubmed/27026621) Int Orthop. 2016 Aug;40(8):1755-1765.
3. Centeno CJ, Al-Sayegh H, Freeman MD, Smith J, Murrell WD, Bubnov R. [A multi-center analysis of adverse events among two thousand, three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions.](https://www.ncbi.nlm.nih.gov/pubmed/27026621) Int Orthop. 2016 Aug;40(8):1755-1765.
4. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. [Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells.](https://www.ncbi.nlm.nih.gov/pubmed/18786777) Med Hypotheses. 2008 Dec;71(6):900-8.

**Placental Derived Fluid**

1. Magatti, M, Marta, T, et al. Isolation, culture, and phenotypic characterization of Mesenchymal Stromal Cells(MSCs) from the amniotic membrane of human term placenta. Mesenchymal Stem Cells: Methods and Protocols (2016):233-244.
2. Pozzobon, M, Piccoli M, De Coppi, P. Sources of mesenchymal stem cells: current and future clinical use. Mesenchymal Stem Cell: Basics and Clinical Application II. Springer Berlin Press, 2012: 267-286
3. Doran, M, Michael, R, Young, M. Mesenchymal Stromal Cells and Repair of Cartilage Tissue. Mesenchymal Cell Therapy. Humana Press, 2013:145-60.
4. Young, M, Doran M. Mesenchymal Stem Cell Therapies for Bone and Tendon Conditions. Mesenchymal Stem Cell Therapy. Humana Press, 2013:117-144.
5. Longo UG, Loppini M, Berton A, La Verde L, Khan WS, Denaro V. [Stem cells from umbilical cord and placenta for musculoskeletal tissue engineering.](https://www.ncbi.nlm.nih.gov/pubmed/22563663) Curr Stem Cell Res Ther. 2012 Jul;7(4):272-81.
6. Preitschopf A, Zwickl H, Li K, Lubec G, Joo G, Rosner M, Hengstschläger M, Mikula M. [Chondrogenic differentiation of amniotic fluid stem cells and their potential for regenerative therapy.](https://www.ncbi.nlm.nih.gov/pubmed/22869300) Stem Cell Rev. 2012 Dec;8(4):1267-74.
7. Haack-Sorenson, M, Ekblond, A, Kastrup, J. Cryopreservation and Revival of Human Mesenchymal Stromal Cells. Mesenchymal Stem Cells: Methods and Protocols. 2016:357-374.
8. Wu, T, Liu, Y, Zhang, Y, Tse H, Lian Q. Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. 2014 Cell Transplantation;9(5):424-431.
9. Hodgkinson, C, Conrad P. Emerging concepts in paracrine mechanics in regenerative cardiovascular biology. Circulation Research. 2016;118(1):95-107
10. Genecchi, M. Danielli, M, Malpasso, M, Ciuffreda, D. Paracrine mechanisms in mesenchymal stem cell tissue repair. Mesenchymal Stem Cells: Methods and Protocols. Springer Berlin Press. 2016;123-146.

**Amniotic Fluid**

1. Gholizadeh-Ghalehaziz S, Farahzadi R, Fathi E, Pashaiasl M. [A Mini Overview of Isolation, Characterization and Application of Amniotic Fluid Stem Cells.](https://www.ncbi.nlm.nih.gov/pubmed/26634059) Int J Stem Cells. 2015 Nov;8(2):115-20.
2. Duerr RA, Ackermann J, Gomoll AH. [Amniotic-Derived Treatments and Formulations.](https://www.ncbi.nlm.nih.gov/pubmed/30466722)

Clin Sports Med. 2019 Jan;38(1):45-59.

 **Platelet Rich Plasma**

1. Miron RJ, Bishara M, Choukroun Plateler Rich Fibrin J.Dent Today. 2017 Apr;36(4):74-80.
2. Abd El Raouf M, Wang X, Miron R et al. Injectable Platelet Rich Fibrin using low speed centrifugation improves cartilage regeneration when compared to Platelet Rich Plasma. Platelets. 2017 Dec 14:1-9.
3. Ghanaati S, Herrera-Vizcaino C, Lorenz J, Miron RJ, Sader R. Fifteen years of Platelet Rich Fibrin(PRF) in dentistry and oromaxillofacial surgery: How high is the scientific evidence? J Oral Implantol. 2018 Jun 5. doi: 10.1563/aaid-joi-D-17-00179
4. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. [Effects of an injectable platelet-rich fibrin on osteoblast behavior and bone tissue formation in comparison to platelet-rich plasma.](https://www.ncbi.nlm.nih.gov/pubmed/28351189) Platelets. 2018 Jan;29(1):48-55.
5. [Autologous liquid platelet rich fibrin: A novel drug delivery system.](https://www.ncbi.nlm.nih.gov/pubmed/29772345)

Miron RJ, Zhang Y. Acta Biomater. 2018 Jul 15;75:35-51

1. Wend S, Kubesch A, Zender N, Dias A, Miron RJ, Ghanaati S et al. Reduction of centrifugal force influences cell number and growth factor release with injectable PRF-based matrices. J Mater Sci Mater Med. 2017 Oct 25;28(12):188
2. Fu CJ et al. [Evaluation of platelet-rich plasma and fibrin matrix to assist in healing and repair of rotator cuff injuries: a systematic review and meta-analysis.](https://www.ncbi.nlm.nih.gov/pubmed/26928856)Clin Rehabil. (2017)
3. Wong CC et al. [Platelet-Rich Fibrin Facilitates Rabbit Meniscal Repair by Promoting Meniscocytes Proliferation, Migration, and Extracellular Matrix Synthesis.](https://www.ncbi.nlm.nih.gov/pubmed/28783120) Int J Mol Sci. (2017)
4. Bai MY, Chuang MH, Lin MF, Tang SL, Wong CC, Chan WP. [Relationships of Age and Sex with Cytokine Content and Distribution in Human Platelet FibrinGels.](https://www.ncbi.nlm.nih.gov/pubmed/30006555) Sci Rep. 2018 Jul 13;8(1):10642. doi: 10.1038/s41598-018-28376-z
5. Varela HA, Souza JCM, Nascimento RM, Araújo RF Jr, Vasconcelos RC, Cavalcante RS, Guedes PM, Araújo AA. [Injectable platelet rich fibrin: cell content, morphological, and protein characterization.](https://www.ncbi.nlm.nih.gov/pubmed/30003342) Clin Oral Investig. 2018 Jul 12. doi: 10.1007/s00784-018-2555-2.

Prizm Wrap