A Unique Case of Crossover Second Toe Syndrome treated by Autologous Peripheral Blood Stem Cells

by

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Abstract

There have been many clinical designations for crossover second toe deformity, including plantar plate tears, hammertoe, metatarsalgia, predislocation syndrome and metatarsophalangeal joint (MPJ) instability. However, the crossover second toe is a common and high problematic case of foot and ankle. Mostly, the issue is associated with some form of progressive bone metabolic degenerative condition as deforming arthritis that ends with a multitude of forefoot deformities. Although multiple theories have been suggested, the true underlying cause of the deformity is multifactorial and only some of these factors have been scientifically analyzed and clarified. This makes the situation more problematic as the procedure requires to treat associated bone deformities, tendinopathies and neuropathies all at the same time in order to achieve a good outcome. Anatomically and pathologically this condition is a result of few condition such as the aging process, the gradual deprivation of bone structure consequently of metabolic disorders and a chronic inflammatory state as result of a prolonged increasing of medial pull of the flexor apparatus effects in rupture or attenuation of the lateral collateral ligaments of the second MPJ complex with valgus tendency exacerbating the underlying problem and inflammation. A second co-deforming force is the dorsal pull of the long and short extensor tendons without opposition from the plantar plate resulting in a hammertoe deformity with dorsal contracture or dislocation of the toe at the MPJ. Therefore it's essential to consider soft tissue deformity together with osseous deformity in crossover second toes. Inevitably, those who treat the condition commonly note there is a long metatarsal associated with the deformity with presence of tear to the plantar soft tissue structures together with prolonged periods of high peak pressures during walk.
An adjunctive concern is due to hypermobility of the first ray linked with elevated pressures from plantar to the second metatarsal head region. The direct pressure coupled with adductus of

the hallux, which may cause dorsal displacement of the second toe, may be also be one of the basic causes of the crossover second toe.We reported a case of a 56-year-old woman presented to our formation with multiple joint osteo-arthritis with painful swelling on shoulders, bilateral knees, lower back, ankles with a visible bilateral cross over second toe condition. An MRI and X-ray were performed, and objectified a multiple osteoarthritis and grade 4 dual cross over second toe syndrome with deformity associated with hallux valgus, hallux rigidus, neuroma of the third intermetatarsal space with metatarsus sub-luxation. The patient received a treatment of autologous PB-SCs infusion during a period of 3 weeks, the results showed a complete recovery of cross over second toe condition on both left and right foot.

Introduction

The term “second crossover toe” was firstly introduced by Coughlin in 1987 to describe a multi-plane deformity at the MPJ [1,2]. Anatomically and mechanically, the crossover second toe deformity is a results from instability of the second MPJ in conjunction with further feebleness resulting from hallux abducto-valgus deformity and often gastrocnemius equinus. The instability occurs in the MPJ is also a event that is combined with a subluxation and/or dislocation in a dorsal and medial direction [2,3]. Chronicity of this state leads to plantar plate rupture and collateral ligament damage [2,3]. The presence of abducto-valgus deformity may increase the instability of the second MPJ that eventually determines to the crossover toe deformity [2,3]. This deformity may be associated with neuroma of the second intermetatarsal space [4,5]. The stabilizing structures of the 2nd MTP joint tend to decline under the presence of chronic inflammation or trauma [1-5]. Eventually, the disorder start enhancing a domino succession of events where each single component of the structure begin to fall apart. As lateral

collateral ligament collapses, it leads to a medial deviation of the second toe and 3 of the plantar plate and its flexor tendon attachments misplace converging medially. As consequence it develops a combination of eccentric forces that tend to generate extra-additional medial displacement, with an overwhelming pulling forces from extensor tendons [5-7].The plantar plate is the second structure to cede, there is an inclination with weight-bearing to move the proximal phalanx dorsally, with a consequent resistance force of both plantar plate and the intrinsic flexors (interossei and lumbricles) forcing the proximal phalanx back into a more resting position at the MTP joint.Hyperextension forces on the proximal phalanx play a crucial role on either stretching or weakening of the plate, leading to a general instability that end up to a dorsomedial subluxation of the proximal phalanx on the metatarsal head [5-7].Further, the presence of arthritic changes also may be fully highly influential to the degeneration of the structure and can be determined from radiographic evaluation and MRI, particularly those changes associated with systemic process such as rheumatoid arthritis and the inflammatory arthritis [3].The current case of a 56-year-old woman presented with multiple joint osteoarthritis with painful and swelling on shoulders, bilateral knees, lower back and ankles with a visible bilateral cross over second toe condition (Fig. 1-3)**.** As far as we know this the first case of a patient suffering of a crossover second toe deformity that has been successfully treated with autologous PB-SCs infusion during a period of 21 days.

Case Presentation

A 56-year-old female presented with multiple site deforming osteo-arthritis, six months previous the visit she received a completely bilateral knee prosthesis replacement. The patient was considerably overweight. The joint damages were visible on both ankles and feet with a bilateral second toes cross-over condition. The supportive ligaments were noticeably weak and

lead to failure of the joint to stabilize the toe. Physical evaluation identified severe pain on both ankles and knees during walking, there were moment she needs a support for moving, the area of knees and ankles showed swelling and tenderness, lower limbs muscle atrophy, contractures and deformities are present on both feet. The injured ankle's active and passive range of motion were evaluated and compared with the contralateral movements, flexion, extension, rotation were precisely evaluated. It was assessed the specific nature of the pain, including its quality, radiation, severity, and timing, as well as palliative and aggravating factors. In addition, the patient pointed out the most painful area and indicate where the pain radiates during walking. X-ray were performed, and reified a grade 3/4 dual cross over second toe syndrome with deformity associated with hallux valgus on right side, hallux rigidus both right and left (Fig. 1), neuroma of the 1stintermetatarsal space with metatarsus sub-luxation on both right and left. In addition there was arthritis process on phalanges joint on both feet (Fig. 2)

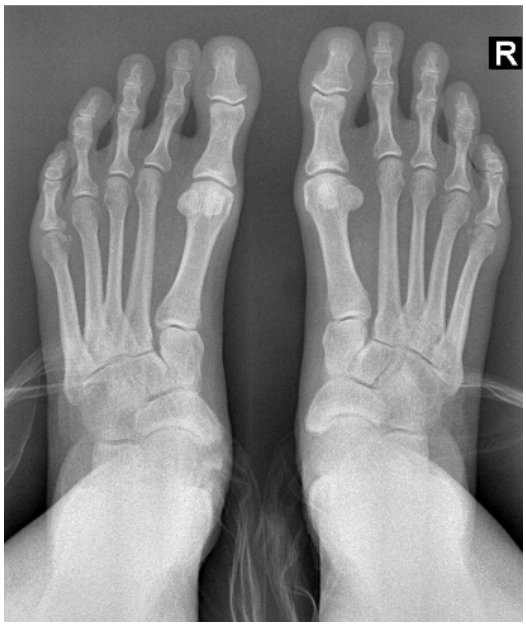
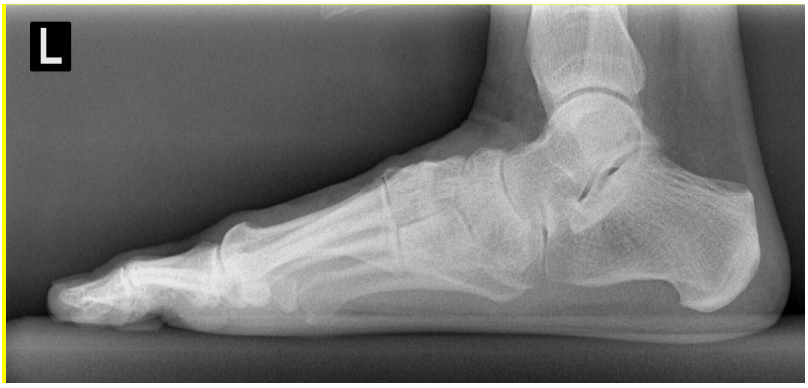


Figure 1. Bilateral metatarsal dislocation with deformities that end on 4th stage Crossover second toe syndrome. There is a visible valgus formation on R foot and arthritic process at metatarsal-phalangeal joint bilaterally. This type of condition and deformity is often associated with hallux

valgus, hallux rigidus, a hammertoe, or a neuroma. It has a peak incidence in women older than 50 years.

Additional conditions and symptoms included fatigue, sleeping issues, nocturia, constipation, gastro-esophageal reflux (GERD), chronic urinary tract, and weight gain. She was taking Arthrotec (a combination of diclofenac and misoprostol), nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce pain and inflammation. Against the advice of her rheumatologist, she had recently discontinued taking prednisone due to significant side effects. Lab tests to identify



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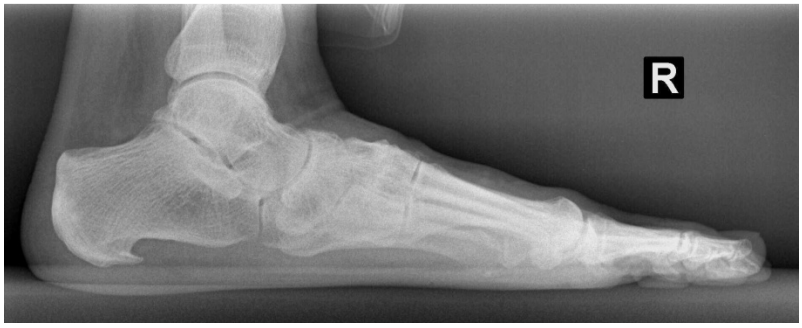

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Figure 2. Arthritis can affect any joint in the body. This x-ray shows an MTP big toe joint affected by arthritis underlying imbalances and to direct treatment were ordered. Treatment included dietary, nutritional, hormonal, and mind/body support.

Imaging and Lab Tests

Clinical examination performed in her hometown (MRI-Xray) were sufficient for an accurate diagnosis, however additional laboratory test were also performed:

Normal CBC count;

Lipid and Liver profile: high Cholesterol 6.27 mM/L (normal range 3.37-5.18 mM/L);

Hormon profile: Estradiol high 53.26 pg/mL (normal range post menop. 0-50), low Testosterone

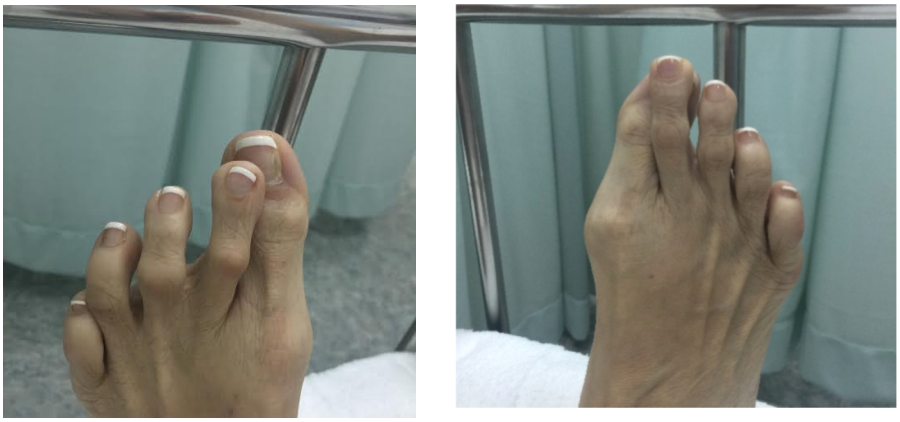

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Figure 3. The presence of crossover second toe deformity on both A) left and B) right feet. The condition is a derivation of mutltiple factore locally and distally. Locally there is a lack of compensatory forces derived from the different tendons of the area which is consequently to a gradual degenerative arthritic process that eventually erode cartilage, bone and damages tendons;

distally on of the main issues is due to degeneration of ankle and knee structure that cause a deviation of correct posture. Determining a a deviant force pressure on feet.

2.64 ng/dl (normal range female 20-120 ng/dl), low IGF-< 25 ng/ml (normal range >40 yo 50-250 ng/ml), low Vitamin D 11 ng/ml (normal range 50-150 ng/ml);

Immunity and Inflammation: TNFalpha high 10.6 pg/ml (normal range <8), Il 6 high 7.8 pg/ml (normal range <7).

Telomere analysis by RT-PCR CFX 96 (Biorad) has been performed at that time and the results showed normal activity of telomerase and long telomere inside cells 12.5 Kb /chromosome and 287.5 Kb/cells that increased after the treatment to 16.3 Kb and 376.8 (normal range 1.70 to 24.83 kb).

Materials and Methods

Treatment

Patient's education is the key of an appropriate treatment [8]. An understanding of what caused the problem is essential to prevent its future occurrence. While the inflammatory response is essential to the healing process, limitation of its intensity, duration and pain is the goal of early treatment. Conventional management of these issues is mainly based on management inflammatory processes and the first-line therapies include rest, corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs) with immobilizing splint [8-10]. Surgical intervention, though considered effective and curative in nearly all cases [11] is the only solution left in those case who do not respond to more conservative management [12-13]. In the present case the treatment was quite innovative and in combination with a plaster immobilization in association

with active movements every other day and a low glycemic index diet as recommended in several edited studies [14-15]. We enrolled the participant during early 2016. The eligible patient was a 56 year old woman having chosen stem cell therapy rather than conventional treatment (for reasons unrelated to the study), so it was possible to assess the association between changes in lifestyle and changes in telomere length without potentially confounding treatments. There were not specific inclusion criteria the protocol for this study was approved by the TTU institutional review board. The patient provided written informed consent.

Interventions

Patient underwent to basic life style change intervention and a course of 15 injection of autologous PB-SCs, 1 injection per time, isolated and incubated in our facility. Injection were performed by using a micro-needle (JBP Japan), partly locally and bilaterally under medial malleoli, on Tibialis Anterior tendon, on Extensor Allucis longus and brevis, on 1stinterosseus and on the insertion of Tibialis Posterior tendon. The patient received a course of supportive medication to enhance and promote mobilization of stem cells from bone marrow niches into circulatory system (Table 1). A physician and two nurses were on site during procedures, after the first 6 months a new meeting is required and patient could continue to meet on their own for two times per month for the duration of the study.

Peripheral blood mononuclear cells 35 mL blood was drawn from the participant into heparin tubes at baseline. The samples were anonymized so that laboratory technicians were unaware of treatment group and collection time. PB-SCs were isolated within 1 h with Ficoll

Table 1

Support medication

MEDICINE	TIME	Ml

Vit B (Cenexi SAS France)	5	2
Vitamin D (HauptPharmaLivron France)	4	1
Hemax 4.000 I.U. (Biosidus SA Argenetina)	3	2
Vit B (Cenexi SAS France)	2	5
	15	10
GF2 (Laenex Lab. Japan)	20	2
GF1 (Lucchini Lab Swiss)	1	2
Hepatin (BTO Pharma.co. LTD Korea)	1	5
Hydrocortisone (Hsinchu Taiwan)	1	2
Vitamin C (Cenexi SAS France)	7	5ml
GH implant	1	8mg
Vit K (Rotexmedica Germany)	4	10mg/1ml
Testosterone (Subcutaneous)	1	100 mg

gradient (Ficoll-Paque PLUS, GE Healthcare, Pittsburgh, PA, USA) with ratio 1:2 (1 portion Ficoll, 2 portions blood) . Viable PB-SCs were counted with the Trypan blue exclusion method (0·4% Trypan blue stain, Invitrogen, Grand Island, NY, USA) of 10^7/mL. Primary PB-SCs were cultured in 25-T flasks (Nunc-thermo scientific USA) with 5 ml SFM medium (Gibco Technologies USA) and incubated at 37°C-05% CO2 for 48 hours. PB-SCs (attached ones) were harvested by using 2 ml trypsin-EDTA and collected in 2 mL patient's serum. Viable cells were counted using the Trypan blue exclusion method to obtain 10^6 cells/ml. After centrifugation at 5024 g in a refrigerated microcentrifuge (Silfradent, Bologna, Italy) at 4°C for 15 min, cells/pellets at the bottom was aspirated off.

Telomere Length Measurement by Quantitative PCR

Real-time telomere quantitative PCR (qPCR) was used for the telomere length measurements. The methods is well established and has been shown to be reliable process to measure the telomere length in previous scientific studies and in basic research [25,26]. Lymphocytes were collected from fresh blood by venipuncture into vacutainer blood tubes (Silfradentheparine). The blood was diluted blood 1:1 with HBSS and gently invert to mix Overlay diluted blood onto 1/3 volume of Ficoll-Paque and centrifuged 400 g for 30 min at 20degC. Lymphocyte layer were moved to a fresh tube using a Pasteur pipette and diluted with 3x volume HBSS at room temperature the whole was then centrifuged at 180 g for 10 minutes. Once supernatant was discarded the cell pellet were re-suspended with in HBSS. Cell count and viability test was performed by using haemocytometer and trypan blue. The real-time qPCR method described by O'Callaghan and Fenech was used based on the Cawthon procedure for relative measurement of telomere length (TL) with a modification that considered the use of an oligomer standard to quantify a TL.Power SYBR I mastermix (Applied Biosystems, #4367396). Contains AmpliTaq Gold DNA polymerase, dNTPs, SYBR I Green Dye, opitimised buffers and passive reference dye (ROX). For this study, the β-globin gene (hgb) was used as the single copy reference. Real time kinetic quantitative PCR determines the fractional cycle (Ct) number at which the well's accumulating fluorescence crosses a set threshold of 40 that is several standard deviations above baseline fluorescence. A standard curve is established by dilution of known quantities of a synthesized 84 mer oligonucleotide containing only TTAGGG repeats. The number of repeats in each standard is calculated using standard techniques, the oligomer standard is 84 bp in length (TTAGGG repeated 14 times), with a molecular weight (MW) of 26667.2, the weight of telomere standard is: 2.6667 × 104/6.02 × 1023 = 0.44 × 10-19g. The highest concentration standard (TEL STD A) has 60 pg of telomere oligomer (60×10^{-12}g) per reaction,

thus there are $60 \times 10^{-12}/0.44 \times 10^{-19} = 1.36 \times 10^{9}$ molecules of oligomer in TEL STD A. The quantity of telomere sequence in TEL STD A is calculated based on the formula: $1.36 \times 10^{9} \times 84$ (oligomer length) = 1.18×10^{8} kb of telomere sequence in TEL STD A. A standard curve has been obtained by a calculation based on aTLqPCR assay on serial dilutions of TEL STD A (10^{-1} [1.18×10^{8}] through to 10^{-6} [1.18×10^{3}] dilution). In order to keep a constant 20 ng of total DNA per reaction tube plasmid DNA (pBR322) was supplemented to each standard. The information of telomeric sequence per sample in kb was measured by using the standard curve.

Discussion

There is some disagreement regarding the true etiology of crossover second toe syndrome, however is frequently described as a gradual degenerative condition where the foot structure tend to deform under a steady dynamic antithetic and non-compensatory forces belong to the structure itself. Haddad was the first to suggest a staging system, starting from synovitis and mild medial deviation at metatarsal joint, dorsomedial deviation (subluxation) at the metatarsal joint, the overlapping of the Hallux and complete dislocation at the metatarsal joint synovitis. This process is accompanied by a constant inflammatory process around the tendon sheaths, fascia plantaris and nerves together with a bone degenerative processes such as osteoarthritis and rheumatoid arthritis; this concept is supported in this condition by the resolution of the symptoms in the majority of cases with the use of anti-inflammatory therapies, such as oral NSAID or peritendinous corticosteroid injections and eventually surgical intervention [16]. The preferred option is always to proceed with non-invasive procedures however in case of mild-severe deformities the option is soft tissue procedures or a shortening osteotomy of the second metatarsal, the distal oblique metatarsal osteotomy, known as the Weil osteotomy [17,18].

Here, we describe an autologous PB-SC–based strategy for clinical application (Fig. 4). Transplantation of hPB-SCs, which include both pluripotent and multipotent stem cells such as neural stem cells, mesenchymal stem cells, hematopoietic stem cells, embryonic like stem cells and progenitor NK cells in clinical application, have been the subject of other recent reviews [19,20]. The current case is a very unique case where this condition has been adjusted through e series of autologous PB-SCs micro injection either directly on pain site or distally. We claim that thanks to immune-modulatory properties, regenerative ability and anti-inflammatory effects of stem cells present in peripheral blood, the affected area eased showing an improvement either on mobility or pain and therefore in tendon contractility. The idea behind these improvements in such short time may be related to stem cells ability to trigger the repair process by homing to the damaged part of the tissue and carrying out regeneration. Cell therapy renovates lost tissues, in this case newly osteoblasts and tenocytes replace the dead ones and their progenitors. The immediately phase after their introduction it's their modulating activity within their micro-environment by introducing other cell types able to restore missing proteins and growth factors to an otherwise deficient environment which are responsible for cell proliferation, cytoprotection, and angiogenesis, recovering the lost tissue functions [21]. A further intriguingly result that came out from this study is the increase of telomere length after the stem cell treatment. Telomere analysis by RT-PCR performed before treatment, telomere length 12.5 Kb/single cell chromosome and 287.5 Kb/totality cells, show a rise after the treatment respectively of 16.3 Kb and 376.8 Kb (normal range 1.70 to 24.83 kb). As a well-accepted concept, telomere length diminishes with time leading to karyotype instability, senescence, apoptosis, or oncogenic transformation of somatic cells affecting quality life and expectancy [22,23]. Yet, senescence *per se* it's a process that have been associated with metabolic

degenerative disorders, such as cardiovascular disease, diabetes, atherosclerosis, bone/cartilage degeneration and cancer. Thus, the major challenge is to determine the link between senescent cells and age-related tissue functionality, trying to point out a possible correlation in terms of cause-effect condition [24-26]. Absolutely in this case, the use of autologous hPB-SCs resulted in an interesting benefit effects on reassessing the crossover second toe deformity, reducing pain and associated discomforts and augmenting telomere length (Fig. 5).

Conclusion

This study is a part of a major and larger ongoing project with hPB-SCs and degenerative condition and telomere we are well aware that more data and evidences are needed to confirm and establish a more solid and valuable procedure.

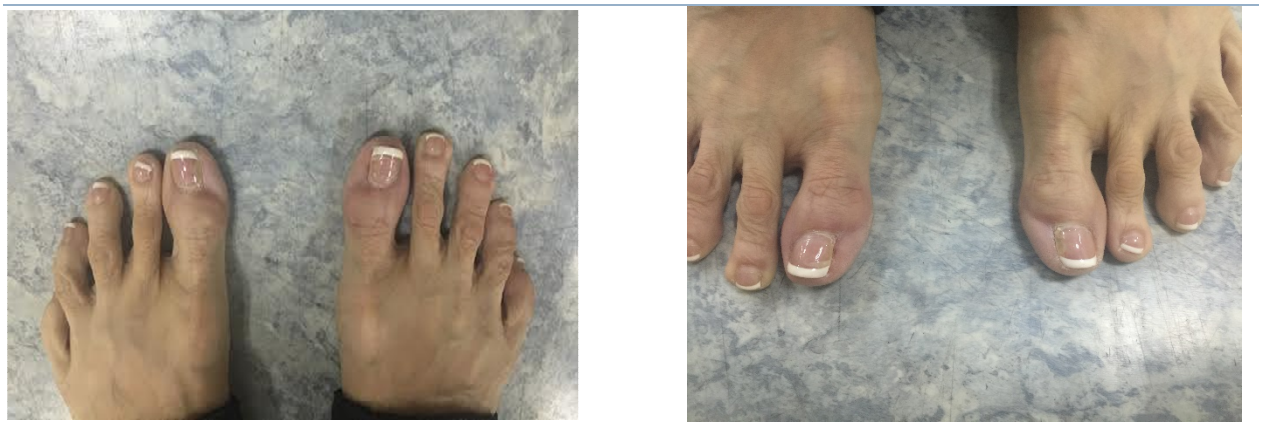

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*Figure 4***.** A) During the course and B) after the course of autologous PB-SCs injected directly on site, the crossover second toe tended to normalize. It seems that the area was completely decontracted and without major pain or discomforts.

Acknowledgment and Conflict of Interest

All the authors have actively participated to groundwork, research and tuition of this current work. There is no conflict of interest to declare.

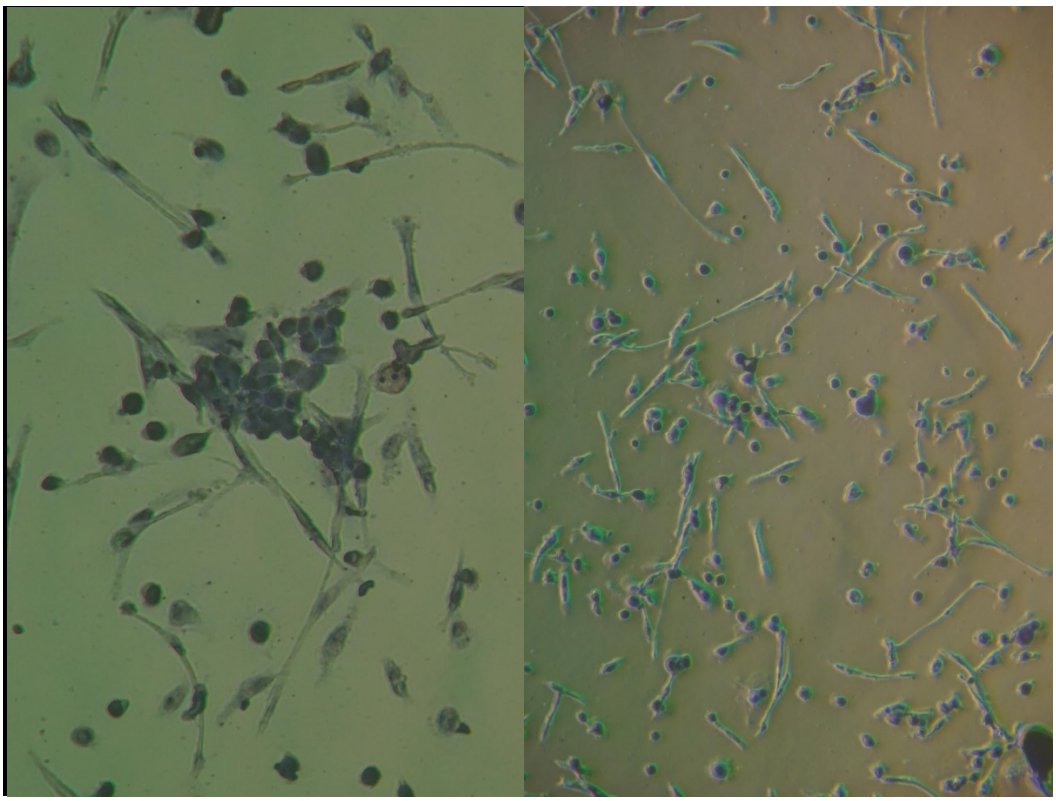

Figure 5. Left) hPB-SCs stained by Giemsa 5 days under microscope 200x; Right) hPB-SCs under microscope 5 days culture in SFM.

References

DiDomenico LA, Rollandini J (2014). *A closer look at tendon transfers for crossover hammertoe*. Podiatry Today. 27(6).

Thomas JL, Blitch EL, Chaney DM, Dinucci KA, Eickmeier K, Rubin LG, Stapp MD, V. Vanore JV (2009). *Diagnosis and treatment of forefoot disorders. section 1: digital deformities*. J Foot Ankle Surg. 48(2):230-272.

Nery C, Coughlin MJ, Baumfeld D, Raduana F, Szejnfeld Manna T, Catenaa F (2015). *Rev Bras Ortopedia.* 50 (6):720–728.

Karlock LG (2003). *Second metatarsophalangeal joint fusion: a new technique for crossover hammertoe deformity*. A preliminary report. J Foot Ankle Surg. 42:178 –182.

Coughlin MJ Schenck, Jr., RC Shurnas, PJ, Bloome DM (2002). *Concurrent interdigital neuroma and MTP joint instability: long-term results of treatment.* Foot Ankle Int. 23:1018-1025.

Kaz AJ, Coughlin MJ. (2007). *Crossover second toe: Demographics, etiology, and radiographic assessment*. Foot Ankle Int. 28:1223-1237.

Deland JT, Sung IH (2000). *The medial crossover toe: a cadaveric dissection*. *Foot and Ankle Int.* 21(5):375-8.

Higgs PE, Mackinnon SE (1995). *Repetitive motion injuries*. Ann Rev Med. 46:1–16.

Hanlon DP, Luellen JR (1999). *Intersection syndrome: A case report and review of the literature*. J Emergency Med. 17:969–971.

Brinks A., Koes BW., Volkers AC., Verhaar JA., Bierma-Zeinstra SM (2010). *Adverse effects of extra-articular corticosteroid injections*: A systematic review. *BMC* Musculoskel *Dis.* 11:206.

Grundberg AB (1985). *Reagan DS. Pathologic anatomy of the forearm: Intersection syndrome*. J Hand Surg. 10(2):299–302.

Williams JG (1977). *Surgical management of traumatic noninfective tenosynovitis of the wrist extensors*. J Bone Joint Surg [Br]. 59–B(4):408–410.

Brunton LM., Wilgis EF (2010). *A survey to determine current practice patterns in the surgical treatment of advanced thumb carpometacarpal osteoarthrosis*. Hand.5(4):415–422.

The Diabetes and Nutrition Study: Group of the European Association for the Study of Diabetes (EASD) (2000). *Recommendations for the nutritional management of patients with diabetes mellitus*. Eur J Clin Nutr. 54:353–355.

Radulian G, Rusu E, Dragomir A, Posea M (2009). *Metabolic effects of low glycaemic index diets.* Nutr J.8:5

Haddad SL, Sabbagh RC, Resch S, Myerson B, Myerson MS (1999). *Results of flexor-to-extensor and extensor brevis tendon transfer for correction of the crossover second toe deformity*. Foot Ankle Int. 20(12):781-8.

Myerson M, Jung HG (2005). *The role of toe flexor-to-extensor transfer in correcting metatarsophalangealjoint instability of the second toe*. Foot Ankle Int. 26:675-9.

Coughlin, MJ; Mann RA: Lesser toe deformities. In: Coughlin, MJ; Mann, RA. Surgery of the Foot and Ankle. *St. Louis, Mosby* 1993 pp. 372-85.

Gargiulo C, Pham VH, Nguyen TH, Nguyen CDK, Phuc PV, Abe K, Flores V, Shiffman M (2015). Isolation and characterization of multipotent and pluripotent stem cells from human peripheral blood. *J fStem Cells Disc.* 5:1-16.

Fehlings MG, Vawda R (2011). *Cellular treatments for spinal cord injury: the time is right for clinical trials*. Neurotherapeutics. 8(4):704–720.

Goldman SA (2011). *Progenitor cell-based treatment of the pediatric myelin disorders*. Arch Neurology. 68(7):848–856.

Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM (1994). *Evidence for a mitotic clock in human hematopoietic stem cells: Loss of telomeric DNA with age*. Proc Natl Acad Sci USA. 91:9857.

Shammas MA (2011). *Telomeres, lifestyle, cancer, and aging*. Curr Opin Clin Nutr Metab Care. 14(1): 28–34.

Correia-Melo C, Hewitt G, Passos JF (2014). *Telomeres, oxidative stress and inflammatory factors: partners in cellular senescence?* Longevity Healthspan.3: 1.

Gray JW, Collins C (2000). *Genome changes and gene expression in human solid tumors*. Carcinogenesis.21:443–452.

Buxton J, Suderman M, Pappas JJ, Borghol N, McArdle W, Blakemore AI, Hertzman C, Power C, Szyf M, Pembrey M. (2014). *Human leukocyte telomere length is associated with DNA methylation levels in multiple subtelomeric and imprinted loci*. Sci Rep. 4:4954.