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Research Article

L-PRF in Osteoarthritis treatment: Results of a Pilot Study

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Abstract

Osteoarthritis (OA) is the most common chronic musculoskeletal disorder and is the most frequent single cause of disability in older adults [1]. OA is a chronic disease progressively involving the entire joint. Progression involves capsule-bursa inflammation, synovial fluid modifications, cartilage erosions and osteochondral inflammatory deteriorations leading to bone erosion and distortion [1]. Early OA defines the initial cascade of events that trigger the disease and lead to the full-blown OA. The disease progression can sometimes last for years being quite often neglected or mistreated with palliative medications. Joint resident MSCs has always been a target for our research into and treatment of OA [1,2,3]. Recently L-PRF (leukocyte and platelet-rich fibrin), showed promising properties in connective tissue regeneration and, for this reason, is now widely applied in chronic wound healing and jawbone growth [4,5,6]. After centrifugation, L-PRF membranes hold vital platelets, leukocytes and various peripheral blood cells [7,8]. As a result, we exposed these membranes to a thermic shock aimed to increase the pool of HSPs. The final product was named supercharged L-PRF. Supercharged L-PRF components -membranes and hyper-acute serum- were used in knee OA patients in a small preliminary comparative study. 20 consecutive patients were randomly divided into 2 groups: 10 patients treated with supercharged L-PRF and 10 with PRP+HA (PRP+ Hyaluronic acid). The primary outcome of this study was to induce persistent pain relief and recovery of motility. This article reports supercharged L-PRF preliminary experience in degenerative OA treatment.

Keywords: Regenerative medicine; PRF, Resident MSCs; HSPs; Osteoarthritis

Introduction

Degenerative Osteoarthritis (OA) is the most common chronic musculoskeletal disorder. OA is a leading cause of disability and increasing source of societal cost in older adults [9]. OA can affect all joints; however knee, spine, hip and hands are most frequent [1]. OA risk factors encompass obesity, sedentary lifestyle, chronic postural defects, bone density, occupational injury, trauma and genetic predisposition [1]. Chronic joint pain, stiffness and impairment lead to disability that is the most common final consequence of OA. In the UK, NICE

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osteoarthritis guidelines show that about 8.5 million people have painful joints due to OA. OA is more common in women and people in older age; X-ray studies show that at least 50% of people older than 65 have evidence of OA with or without symptoms [10]. Likewise, in the United State osteoarthritis is the most common joint disorder [11].

The number of people affected by symptomatic OA is likely to increase shortly due to the aging of the population and the obesity epidemic [12]. OA has traditionally been defined as a degenerative chronic disease centered on cartilage degeneration [13,14]. Coherently, OA classification criteria require the presence of radiographic bony changes, joint space narrowing and osteophyte formation as per Kellgren and Lawrence (K&L) standard [15]. Only recently, early OA physiopathology shed new light on the cascade of events progressively involving all joint tissues at the advanced stage of the disease [13,14].

OA physiopathology and early OA presentation

The prevalent physiopathology interpretation of OA describes that synovitis may be induced by a mechanical injury that generates micro-fissures and micro cartilage lesions and fragments. These cartilage debrides and microcrystals released into the synovial fluid are phagocyte by synovium macrophages (Type A cells). Type A cells generate and maintain the inflammation of the synovium membrane releasing pro-inflammatory interleukins and proteases which, in turn, diffuse through the synovial fluid into the cartilage generating further degradation. This results in progressive cartilage erosion and fragmentation, sustaining and promoting the vicious circle by producing additional inflammation [1,14]. Nonetheless, some authors suggest that synovium inflammation may be primary to the other joint structural changes and a key factor to induce early OA. Deviations in joint biomechanics and chronic postural defects certainly play a key role in early OA induction. Excessive or abnormal joint loading and motility disbalance may stimulate Substance P (SP) secretion. SP is a neuropeptide secreted by sensory nerve fibers in the synovium, acting as a neurotransmitter and as a neuromodulator [16,17]. Substance P stimulates Type A cells to produce pro-inflammatory factors and proteases which ultimately promote early OA development [17]. SP mediates interactions between neurons and immune cells promoting monocyte activation and migration to sites of inflammation, thereby modulating immune cell proliferation and cytokine production [18]. Furthermore, the breakdown of cartilage-ECM and proteins-matrix exposure generates the activation of Type A cells and the release of proinflammatory cytokines as TNFα, IL-1 and IL-6. These cytokines cause chondrocytes to further release metalloproteinase (MMPs) preventing type II collagen production and promoting chondrocytes apoptosis.

Finally, cartilage degradation intensifies [19,20]. Therefore, early OA means the failure of synovium macrophages (Type A cells) and chondrocytes to maintain the homeostasis between synthesis and degradation of the ECM components. Clinical data showed that synovium inflammation was existing in early OA well before the development of significant

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radiologic changes. MRI and USS, as well as diagnostic arthroscopy, suggested that even at the very beginning, before detectable cartilage degeneration has occurred, OA was already an inflammatory synovium disease in progress [15]. Some MRI studies have indicated that synovium inflammation is an essential parameter of OA at the early stages. MRI quite often showed thickening of capsule bursa tissue that leads to cartilage degeneration and OA disease advancement [21,22,23].

In the beginning, joint pain is caused by inflammatory changes to the joint capsule, synovium membrane, tendons and ligaments; structures where there is a significant innervation. Further, Substance P was found to be secreted by sensory nerve and was consistently identified in the synovium, synovial fluid and, even isolated in the infrapatellar fat pad IFP [18,24]. Early OA is clinically described by recurrent joint pain together with no or minimal changes detectable by X-ray imaging. Only MRI and USS can identify the initial signs of early OA in the synovium membrane thickening and also some superficial cartilage erosions [25,26,27]. These criteria and approaches facilitate early identification of degenerative OA and this expedites early therapeutic interventions to stop and reverse the normal disease advancement [1,27].

Joint Resident MSCs

Current literature reports several types of MSCs enclosed in the joint structure. The most studied are SM-MSCs, IFP-MSCs, P-MSCs (Periostium-MSCs), E-MSCs (Endosteum-MSCs) and Subchondral BM-MSCs [28; 29; 30]. Joint resident MSCs mostly reside in pericytes position spreading together with the microvasculature. SM-MSCs lie in the SM intima and subintima. They are mostly grouped in the enthesis such as the synovium and the capsule insertion to the periosteum. Likewise, SM-MSCs are in tendons and ligaments close to the bone junctions [3,31].

IFP-MSCs have been recognized in the Hoffa's pad and it was supposed they may reside in adipose tissue other joints, probably located close to the bursa-capsule [29]. Periosteum P-MSCs and endosteum E-MSCs are very well known stem cells for the fundamental role they play during bone fracture repair and endochondral ossification [30]. Subchondral BM-MSCs reside in the bone medullary cavities, including the mineralized bone matrix and the adipose marrow [30]. Subchondral bone marrow is a highly vascularized tissue providing a reservoir of MSCs and progenitor cells. In the subchondral bone marrow, MSCs support local homeostasis and promote cartilage regeneration in particular conditions [32].

In the trabecular bone, the bone marrow preserves a heterogeneous population of various multipotent mesenchymal stromal cells (BM-MSCs). They provide progenitor cells for differentiation of osteochondral and other mesenchyme cell lineages [33].

Joint Resident MSCs a therapeutic target

Resident MSCs are heterogeneous stem cells population. MSCs expression varies in different tissues because they are subjected to the influence of the tissue-specific microenvironment to accomplish their biological role. Tissue-specific MSCs

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populations include both multipotent and progenitor cells. They deliver local and exclusive control on tissue regeneration and immune response modulation through paracrine effects [34]. Caplan AI identified resident MSCs as pericytes wrapping the connective tissue microvasculature [35]. Afterward, Sacchetti recognized that MSCs-CD146+ were cells enveloping micro-vessels in the bone marrow [36].

The MSCs-pericytes location is regulated by several local mediators however, the PDGF receptor is currently considered the master component. PDGF activation promotes chemotactic and mitogen stimulus for MSCs and facilitates connections between endothelial cells and MSCs [35,37]. The therapeutic stimulation of resident MSCs in degenerative OA has been our research target for or over the last 20 years. Specifically, the aim of the study was the activation of joint resident MSCs through stimulation of the extracellular microenvironment (ECM). For this purpose, we used small fractions of nucleic acids- polydeoxyribonucleotides (PDRN) - and a specific set of Heat Shock Proteins (HSPs) with a glycerol scaffold [2,3].

More recently, we started to investigate and apply Platelet-rich fibrin (PRF) to treat degenerative OA. Specifically, we used L-PRF as biostimulator of resident MSCs. Unlike PRP, PRF does not require any biochemical activators like anticoagulants or thrombin for fibrin polymerization. The preparation of PRF begins with the immediate centrifugation of the patient's venous blood collected in normal glass tubes. Choukroun et al. first described the process in 2000 [38].

PRF is an autologous biomaterial, made of a strong fibrin matrix that variably contains:

- High concentration of vital and non-vital: platelets, leucocytes and circulating MSCs
- Variable pool of cytokines
- An elevated concentration of long releasing growth factors (GFs). These include platelet-derived growth factor (PDGF A-B), vascular endothelial growth factor (VEGF), transforming growth factor (TGF β -1,2), insulin-like growth factor (IGF-I), epidermal growth factor (EGF); connective tissue growth factor (CTGF); bone morphogenetic protein 2 (BMP-2) [39; 40]
- An elevated concentration of fibrin, fibronectin, vitronectin, and thrombospondin

A variable pool of heat shock proteins HSPs not yet completely studied.

Tissue regeneration is a complex sequence involving stem cell activation and differentiation promoted by ECM modifications. This process is mediated by an extensive range of biological-immunological events and, different molecules as cytokines, GFs and other tissue mediators play a significant role.

Although in vitro it is recognized that cells of different lineages generate these molecules, in vivo the right sequence and details of events leading to regeneration are not well known. Platelets such as leukocytes and fibroblasts play a key role as an autologous source of growth factors

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[6,41]. Since their activation, platelets secrete multiple GFs including Platelet-derived growth factor (PDGF); Vascular endothelial growth factor (VEGF); Transforming growth factor beta-1 (TGF-b1); Epidermal growth factor (EGF); Basic fibroblast growth factor (FGF-ß) and Insulin-like growth factor-1 (IGF-1) [42].

Autologous platelet concentrates are widely used in clinical practice as a bioactive component to decrease inflammation and increase the speed of connective tissue healing [43]. Specific types of PRF with different biological properties can be generated by distinct centrifuge characteristics and adjustments to methods of centrifugation [40]. Centrifuge stability, frequency of vibrations and the temperature developed in the tubes determine the properties and quality of the final PRF. Specifically, the centrifuge characteristics impact the making of L-PRF clot and this, in turn, determines cell survival pools, fibrin architecture and also the cytokines, GFs and HSPs contained in its components: membrane and hyper-acute serum [40].

L-PRF membrane shows a strongly polymerized thick fibrin matrix and includes various blood cells trapped in the fibrin net. A large number of these cells appear to be alive and with a normal shape [44]. After centrifugation, a fibrin clot is made through the activation of autologous thrombin. Three distinct layers can be seen in the tube: red blood corpuscles RBCs at the bottom of the tube, platelet-poor plasma PPP on the top of the tube, and the PRF clot in the middle of the tube. The clot contains a great amount of exudate, which is full of cytokines, GFs and HSPs. This exudate named hyper-acute serum can be pressed out and collected by gentle compression of the clot to obtain PRF membranes. Hyper-acute serum squeezed out from the PRF clot has a massive cell proliferative effect on different connective cell lineages such as bone marrow mesenchyme stem cells (BM-MSCs), osteoblasts and chondroblasts cells [45, 46]. After removing the hyper-acute serum fraction, the remaining PRF membrane is an adhesive and biodegradable fibrin scaffold. The membrane surface and fibrin network structure facilitate cell migration and cell interactions. Moreover, the PRF membrane has the property of slowly releasing bioactive molecules that enable migration, adhesion and proliferation of resident MSCs [43,47,48]. In vitro, the L-PRF membrane maintains scaffolding features and slowly releases GFs for at least 7 days.

During centrifugation, the fibrinogen transforms to fibrin polymerizing to a three-dimensional structure. This fibrin net entraps platelets and leukocytes on the surface and inside of the PRF membrane. Further, centrifuge activated platelets and leukocytes produce a massive pool of GFs, cytokines and HSPs [49]. Platelet generated GFs are commonly used in tissue regeneration; one of the best known is the PDGF, which enhances MSCs adhesion and proliferation. [43].

Supercharged L-PRF

Following some basic biological principles that bring to the activation of residential MSCs [1,2,3,28,32,33,34,46] and knowing that several vital blood cells remain trapped in the PRF membrane fibrin network; we have started preparing thermic stressed L-PRF membranes to

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generate a greater amount of HSPs. A specific thermic algorithm showed that membranes subjected to low-high temperature shock release a large pool of HSPs. This PRF preparation aims to activate SM-MSCs in joints affected by OA.

The resulting product, named 'supercharged L-PRF' when used in a procedure, enhances the stimulation of SM-MSCs with a pool of HSPs. These are slowly released by the treated membranes when applied on the surface of capsule-bursa tissues. Supercharged L-PRF is applied through a minimally invasive surgical procedure. The surgical access takes advantage of trauma-induced hypoxia which is a well-known tissue regeneration master activator [2;3]. Hypoxia works by stimulating the activation of local MSCs-pericytes to promote neo-vessels angiogenesis [50;51]. Specifically, local hypoxia and lower pH are optimal conditions for MSCs exosomes best performance. MCSs exosomes carry miRNAs, HSPs and GFs which have shown antiapoptotic and anti-inflammatory effects and are capable to support neovascularization [52,53]. Furthermore, trauma-induced hypoxia facilitates tissue regeneration increasing the local secretion of Hypoxia-inducible factor-1α (HIF-1α), HSPs families 90,70,40,27 and other small HSPs members. All these mediators work in a team to stimulate and coordinate the biological cascade of events which convey to the local tissue healing and regeneration [54,55,56].

Materials and Methods

From December 2019 to March 2020, we consecutively treated a group of 20 patients affected by unilateral knee degenerative OA with symptoms lasting more than 6 months. All knees were affected by degenerative OA involving mostly the internal compartment. 11 out of 20 patients were female and 9 males. 12 patients were staged Kellgren and Lawrence Scale (K&L) 3 and 8 patients staged as 2. Average BMI 29, the average age was 63 range 52-75. Patients were not under any other treatments for OA in the last month besides occasional pain killers. Criteria of exclusion: patients treated with long release corticosteroids infiltration over the last month; patients on immune suppressor or chemotherapy treatments; INR over 2; acute rheumatoid arthritis and rheumatoid immune diseases; local/general infections; patients with previous joint replacement, low platelets (<100.000) or low fibrinogen, oncologic patients.

Patients were randomly divided into two cohorts of 10. Both groups had 6 patients K&L stage 3 and 4 KL stage 2. In the last 3 days before treatments, patients did not take any painkillers.

- Group 1 was treated with USS-guided infiltration of PRP+ Hyaluronic acid (HA): 8 ml PRP and 2 ml HA single dose. PRP was made with 60 ml patient's blood 1203 rpm for 10 minutes at 22°C. HA, Sinovial HL 2 ml, hybrid composition low and high molecular weight.
- Group 2 was treated with supercharged L-PRF. L-PRF prepared with 9 ml patient's fresh blood in glass-coated plastic tubes, immediately centrifuged at 2700 rpm for 12 minutes. The Hyper-acute serum was immediately infiltrated in the capsule-bursa

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tissue under USS guidance. The membrane was under thermic stress treatment, low-high temperature, for 60 minutes. Then it was sectioned in 3 parts and surgically implanted on the capsule-bursa tissue. The membrane implants were located in 3 different painful areas of the joint.

Treatments were provided in ambulatory care facilities and under local anesthetic. This pilot study was conducted following the Code of Ethics of the World Medical Association [57] and approved by the Ethical-Scientific Committee of the Villa Aurora Hospital, Foligno (Italy) where the treatments have been performed. All the patients gave informed consent to the procedures of supercharged L-PRF and PRP+ Hyaluronic acid (HA) and to be randomly selected for one of the procedures. Patients agreed to participate in the study using their blood to extract the L-PRF or PRP and to have their data collected for the scope of this research. Completely anonymized demographic and clinical data have been prospectively recorded into an electronic database and duly analyzed.

- No adverse reactions were detected; specifically, for group 1 no episodes of blood hypertension, diarrhea, rash or proteinuria.
- Paracetamol 3 gr TDS for 3 days and Ibuprofen 400 mg if needed were prescribed.
- Primary outcome measure: pain relief

Pain measured with Visual analogue scale (VAS). VAS was completed by the patient before the treatment and during the face to face follow up at 7 and 30 days; 3 and 6 months follow up were in remote consultation due to the COVID pandemic.

Results

From December 2019 to March 2020, we consecutively treated a group of 20 patients affected by unilateral knee degenerative OA with symptoms lasting more than 6 months. Group 1 (10 patients) was treated with a single intraarticular infiltration of PRP 8 ml + HA 2 ml. Group 2 (10 patients) was treated in a single session on 3 different joint areas with supercharged L-PRF (hyperacute serum + membrane). Hyperacute serum was infiltrated into the capsule-bursa tissue and membrane applied on the same tissue surface. Patients self-assessed joint pain using the VAS score in a standing position.

The Baseline pain (day 0) was measured just before procedures. In the last 3 days before treatments, patients did not take any pain killers. Before treatment, the average VAS pain score was 7.5 for group 1 and 8 for group 2; both starting scores were considered "severe pain" and unified in graph A. Group 2 VAS score follow up showed better pain control and more persistent pain relief in short-and-mid-term than group 1.

After 3 months the pain restarted to increase in both groups. However, the pain-free interval was much more consistent and longstanding in group 2 than 1. No complications such as infection, collection and adverse reactions were reported in either groups.

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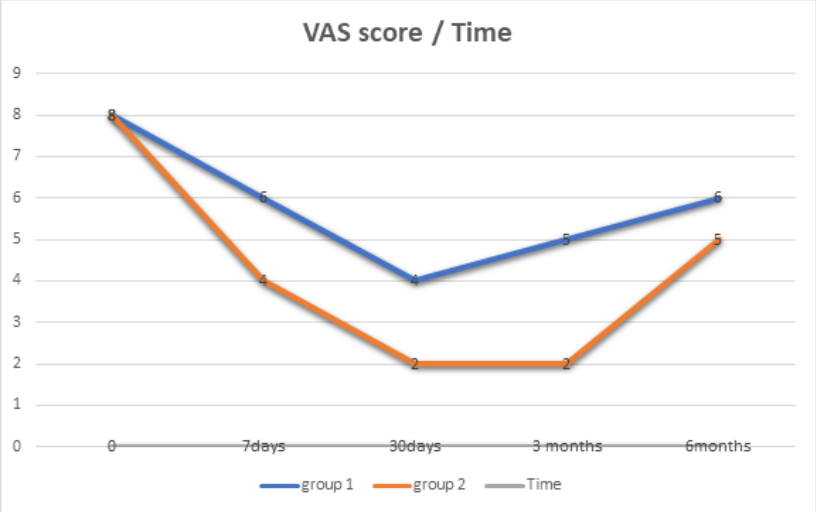

Discussion

Recently, the physiopathology of early OA has shed new light on processes facilitating the disease progression. Specifically highlighted is the crucial role played by the synovium membrane and synovial fluid to catalyze OA advancement together with articular cartilage degeneration [1]. Joint pain is mostly generated by changes to non-cartilaginous components such as the joint capsule, synovium membrane, tendons and ligaments where there is a significant innervation [1; 18; 24]. These structures might be considered the primary therapeutic target not only for pain relief but also for regenerative medicine treatments. Since 2012 the author published research and procedures to define and stimulate joint resident MSCs. Multiple pieces of evidence have shown that resident MSCs are the master regulators of the local regenerative microenvironment [28;34]. Our study aimed to demonstrate OA symptoms control through the stimulation of resident MSCs, the recovery of local cell homeostasis and finally tissue repair. We examined an individual medication that contained polydeoxyribonucleotides (PDRN), a specific set of Heat Shock Proteins (HSPs) and a glycerol scaffold. The HSPs were derived from the patient's blood through a process of thermic stress [2;3]. This product named Gel repair ® has shown to be a biological activator able to stimulate the resident MSCs located in the capsule and synovium tissue [2;3]. An observational clinical trial with 3 years follow-up indicated a persistent improvement of symptoms together with a radiologic recovery of joints affected by degenerative OA. Persistent pain relief and increased joint mobility were observed in almost 80% of treated patients and imaging quite often showed a downgrading of the degenerative OA [2]. Coherent with the same biological principles, we have been using thermic stressed L-PRF membranes to produce a wide pool of HSPs. The resulting product, called 'supercharged L-PRF' aims to activate SM-MSCs (synovium Membrane - MSCs) located in the capsule-bursa tissue. The HSPs slowly released by the membrane together with the hyperacute-serum seem to induce persistent analgesia in knee OA (K&L stage II-III) as shown in this pilot study. Our clinical experience revealed that PRP alone is an extremely diffusible

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biostimulator and, contrary to in vitro evidence, does not have regenerative effects due to ephemeral tissue stimulation. Hyaluronic acid (HA) has been widely used in the last two decades to increase the persistence of PRP in the infiltration site. PRP+HA attempts to combine PRP anti-inflammatory and tissue regeneration properties with viscosupplementation. Although the effectiveness of this procedure is well recognized in literature, it has not been proven that PRP persists in the joint cavity longer if combined with HA rather than infiltrated alone. In the last few years, joint resident MSCs were found in the capsule bursa tissue, at the insertion of ligaments and tendons, in the subchondral bone marrow, in the Hoffa's fat pad and in the periosteum and endosteum [1]. There has not been consistent evidence of MCSs in the joint cavity that is for synovial fluid. Therefore, to be effective, joint regenerative treatments should target resident MSCs in their specific tissue location. Joint pain was considered the primary outcome of this study. For this reason, we used the VAS score to simply evaluate this basic parameter. In a future trial design, questionnaires such as KOOS will give us more complete information on mobility, daily activities, disability and pain. Imaging as MRI or USS 3D would implement long term information about joint structure recovery that may be expected with supercharged L-PRF procedure.

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References

1. Di Nicola V. Degenerative osteoarthritis a reversible chronic disease. Regen Ther. 2020;15:149-60.
2. Di Nicola V, Di Nicola R. Self-repair in degenerative joint disease. Curr Aging Sci. 2012;5(3):273-87.
3. Di Nicola V, Pierpaoli W. Biological baseline of joint self-repair procedures. Curr Aging Sci. 2013;6(2):206-14.
4. Pinto NR, Ubilla M, Zamora Y, Del Rio V, Dohan Ehrenfest DM, Quirynen M. Leucocyte-and platelet-rich fibrin (L-PRF) as a regenerative medicine strategy for the treatment of refractory leg ulcers: a prospective cohort study. Platelets. 2018;29(5):468-75.
5. Bakhtiar H, Esmaeili S, Tabatabayi SF, Ellini MR, Nekoofar MH, Dummer PM. Second-generation platelet concentrate (platelet-rich fibrin) as a scaffold in regenerative endodontics: a case series. J Endod. 2017;43(3):401-8.
6. Saluja H, Dehane V, Mahindra U. Platelet-Rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. Ann Maxillofac Surg. 2011;1(1):53.
7. Dhurat R, Sukesh MS. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. J Cutan Aesthet Surg. 2014;7(4):189.
8. Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang BS, Nally M, Lanata N, Wang HL, Quirynen M. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte-and platelet-rich fibrin (L-PRF) clot and membrane. Platelets. 2018;29(2):171-84.
9. DJ Hunter, S Bierma-Zeinstra. Osteoarthritis. The Lancet 2019; 393, issue 10182, 1745-59
10. https://www.nice.org.uk/guidance/cg177/documents/osteoarthritis-update-final-scope2.
11. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, Kington RS, Lane NE, Nevitt MC, Zhang Y, Sowers M. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med. 2000;133(8):635-46.

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DOI: https://doi.org/10.37191/Mapsci-2582-385X-3(1)-048

12. Zhang Y, Jordan JM. Epidemiology of osteoarthritis. Clin Geriatr Med. 2010 Aug;26(3):355-69. Erratum in: Clin Geriatr Med. 2013;29(2):ix. PMID: 20699159; PMCID: PMC2920533.
13. Dieppe P. Developments in osteoarthritis. Rheumatology 2011; 50:245e7.
14. Martel-Pelletier J, Pelletier JP. Is osteoarthritis a disease involving only cartilage or other articular tissues? Jt Dis Relat Surg. 2010;21(1):002-14.
15. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. Ther Adv Musculoskelet Dis. 2013;5(2):77-94.
16. Sutton S, Clutterbuck A, Harris P, Gent T, Freeman S, Foster N, Barrett-Jolley R, Mobasheri A. The contribution of the synovium, synovium derived inflammatory cytokines and neuropeptides to the pathogenesis of osteoarthritis. Vet J. 2009;179(1):10-24.
17. Vina ER, Kwoh CK. Epidemiology of osteoarthritis: literature update. Curr Opin Rheumatol. 2018;30(2):160.
18. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothoulakis C, Dana R. Neuropeptide substance P and the immune response. Cell Mol Life Sci. 2016;73(22):4249-64.
19. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, Ding C. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis Cartilage. 2010;18(11):1441-7.
20. Buckwalter JA, Mankin HJ, Grodzinsky AJ. Articular cartilage and osteoarthritis. Instructional Course Lectures. J Am Acad Orthop Surg. 2005;54:465.
21. Krasnokutsky S, Belitskaya-Lévy I, Bencardino J, Samuels J, Attur M, Regatte R, Rosenthal P, Greenberg J, Schweitzer M, Abramson SB, Rybak L. Quantitative magnetic resonance imaging evidence of synovium proliferation is associated with radiographic severity of knee osteoarthritis. Arthritis Rheum. 2011;63(10):2983-91.
22. Roemer FW, Guermazi A, Felson DT, Niu J, Nevitt MC, Crema MD, Lynch JA, Lewis CE, Torner J, Zhang Y. Presence of MRI-detected joint effusion and synovitis increases the risk of cartilage loss in knees without osteoarthritis at 30-month follow-up: the MOST study. Ann Rheum Dis. 2011;70(10):1804-9.
23. Di Nicola V, Di Pietrantonio M. MRI in degenerative joint disease (DJD): a proposal for imaging standardization in regenerative medicine. SM Musculoskelet Disord. 2016;1(2):10.
24. Mora JC, Przkora R, Cruz-Almeida Y. Knee osteoarthritis: pathophysiology and current treatment modalities. J Pain Res. 2018;11:2189.
25. Luyten FP, Denti M, Filardo G, Kon E, Engebretsen L. Definition and classification of early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc. 2012;20(3):401-6.
26. Kaeley GS, Bakewell C, Deodhar A. The importance of ultrasound in identifying and differentiating patients with early inflammatory arthritis: a narrative review. Arthritis Res Ther. 2020;22(1):1-0.
27. Chiba D, Nakamura T, Fu FH. Early osteoarthritis—definition, pathogenesis, diagnosis, management and prevention: Management. Ann Joint. 2019;4(1).
28. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovium membrane. Arthritis Rheum. 2001;44(8):1928-42.
29. Kouroupis D, Bowles AC, Willman MA, Orfei CP, Colombini A, Best TM, Kaplan LD, Correa D. Infrapatellar fat pad-derived MSC response to inflammation and fibrosis induces an immunomodulatory phenotype involving CD10-mediated Substance P degradation. Sci Rep. 2019;9(1):1-6.
30. Knight MN, Hankenson KD. Mesenchymal stem cells in bone regeneration. Adv Wound Care. 2013;2(6):306-16.
31. Russell T, Watad A, Bridgewood C, Khan A, Millner P, Loughenbury P. FRI0520 The human enthesis contains populations of Mesenchymal stem cells with distinct functional characteristics. Annals of the Rheumatic Diseases 2019;78:955
32. S.Tarafder, C.H.Lee. In Situ Tissue Regeneration: Host Cell Recruitment and Biomaterial Design 2016; Ch. 14; Pages 253-273.
33. Säämänen AM, Arokoski JP, Jurvelin JS, Kiviranta I. The structure and regenerative capacity of synovium joint tissues. In Regenerative medicine and biomaterials for the repair of connective tissues. Woodhead Publishing. 2010;1-38.
34. Principle of Regenerative Medicine Ch 15. 3rd ed. Elsevier Inc;2019.
35. Caplan AI. All MSCs are pericytes?. Cell stem cell. 2008;3(3):229-30.

Citation: Di Nicola V. L-PRF in Osteoarthritis treatment: Results of a Pilot study. J Regen Biol Med. 2020;3(1):1-12.
DOI: https://doi.org/10.37191/Mapsci-2582-385X-3(1)-048

36. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell. 2007;131(2):324-36.
37. Gerhardt H, Semb H. Pericytes: gatekeepers in tumor cell metastasis? J Mol Med. 2008;86(2):135-44.
38. Theys T, Van Hoylandt A, Broeckx CE, Van Gerven L, Jonkergouw J, Quirynen M, van Loon J. Plasma-rich fibrin in neurosurgery: a feasibility study. Acta Neurochirurgica. 2018;160(8):1497-503.
39. Dhurat R, Sukesh MS. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. J Cutan Aesthet. 2014;7(4):189.
40. Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang BS, Nally M, Lanata N, Wang HL, Quirynen M. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte-and platelet-rich fibrin (L-PRF) clot and membrane. PLT. 2018;29(2):171-84.
41. Düregger K, Gäble A, Eblenkamp M. Development and evaluation of a spray applicator for platelet-rich plasma. Colloids Surf B Biointerfaces. 2018;171:214-23.
42. Naik B, Karunakar P, Jayadev M, Marshal VR. Role of Platelet rich fibrin in wound healing: A critical review. J Conserv Dent. 2013;16(4):284.
43. Kardos D, Hornyák I, Simon M, Hinsenkamp A, Marschall B, Várdai R, Kállay-Menyhárd A, Pinke B, Mészáros L, Kuten O, Nehrer S. Biological and mechanical properties of platelet-rich fibrin membranes after thermal manipulation and preparation in a single-syringe closed system. Int J Mol. 2018;19(11):3433.
44. Madurantakam P, Yoganarasimha S, Hasan FK. Characterization of leukocyte-platelet rich fibrin, a novel biomaterial. J Vis Exp. 2015:e53221.
45. Simon M, Major B, Vácz G, Kuten O, Hornyák I, Hinsenkamp A et al. The Effects of Hyperacute Serum on the Elements of the Human Subchondral Bone Marrow Niche. Stem Cells Int. 2018; 2018:4854619
46. Kuten O, Simon M, Hornyák I, De Luna-Preitschopf A, Nehrer S, Lacza Z. The effects of hyperacute serum on adipogenesis and cell proliferation of mesenchymal stromal cells. Tissue Eng Part A. 2018;24(11-12):1011-21.
47. Di Liddo R, Bertalot T, Borean A, Pirola I, Argentoni A, Schrenk S, Cenzi C, Capelli S, Conconi MT, Parnigotto PP. Leucocyte and Platelet-rich Fibrin: a carrier of autologous multipotent cells for regenerative medicine. J Cell Mol Med. 2018;22(3):1840-54.
48. Aulino P, Costa A, Chiaravalloti E, Perniconi B, Adamo S, Coletti D, Marrelli M, Tatullo M, Teodori L. Muscle extracellular matrix scaffold is a multipotent environment. Int J Med Sci.2015;12(4):336.
49. Varela HA, Souza JC, Nascimento RM, Araújo RF, Vasconcelos RC, Cavalcante RS, Guedes PM, Araújo AA. Injectable platelet rich fibrin: cell content, morphological, and protein characterization. Clin Oral Investig.2019;23(3):1309-18.
50. Tandara AA, Mustoe TA. Oxygen in wound healing—more than a nutrient. World J Surg. 2004;28(3):294-300.
51. Knighton DR, Silver IA, Hunt TK. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. Surg. 1981;90(2):262.
52. Lai RC, Yeo RW, Lim SK. Mesenchymal stem cell exosomes. Semin Cell Dev Biol. 2015 Apr; 40:82-8.
53. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. Mol Ther. 2015;23(5):812-23.
54. Hong WX, Hu MS, Esquivel M, Liang GY, Rennert RC, McArdle A, Paik KJ, Duscher D, Gurtner GC, Lorenz HP, Longaker MT. The Role of Hypoxia-Inducible Factor in Wound Healing. Adv Wound Care (New Rochelle). 2014;3(5):390-399.24804159; PMCID: PMC4005494.
55. Atalay M, Oksala N, Lappalainen J, Laaksonen DE, Sen CK, Roy S. Heat shock proteins in diabetes and wound healing. Curr Protein Pept Sci. 2009;10(1):85-95.
56. Guo J, Chang C, Li W. The role of secreted heat shock protein-90 (Hsp90) in wound healing-how could it shape future therapeutics? Expert Rev Protiomic. 2017;14(8):665-75.
57. World Medical Association. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects.2020.

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