Injectable Biological Treatments for Osteoarthritis of the Knee

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Abstract

» The increasing prevalence of knee osteoarthritis in an aging and active population necessitates the development of therapies designed to relieve symptoms, to delay the need for total joint replacement, and to potentially stimulate chondrocyte growth.

» Growth factor therapies such as platelet-rich plasma have been studied extensively for knee osteoarthritis, with the recent publication of clinical studies. Although the majority of studies conclude that platelet-rich plasma has the potential to provide symptomatic relief on a short-term basis, to our knowledge, long-term data are lacking.

» Growing interest in stem cell therapy for knee osteoarthritis has led to various studies utilizing mesenchymal stem cells from adipose, bone marrow, or peripheral blood sources. Although studies have shown excellent early clinical results, the ability to improve collection rates of mesenchymal stem cells and methods to direct mesenchymal stem cell differentiation to chondrocytes and to promote chondrogenesis remains a focus for future research.

» There are still many questions about platelet-rich plasma and its effectiveness for knee osteoarthritis. Differences in preparation technique make effective evaluation and comparison difficult.

» Bone marrow mesenchymal stem cells currently remain the only stem cell product that appears to be approved by the U.S. Food and Drug Administration (FDA) without any potential questions about use. Further clinical studies are still necessary to fully understand their role.

Osteoarthritis of the knee is one of the most prevalent diseases in the United States and is a common condition associated with pain and substantially impaired quality of life[1]. In addition to aging, an increase in sporting and physical activity across all age groups has contributed to an increased prevalence of articular cartilage disease[2]. Although osteoarthritis is becoming a more widespread problem, there remains a lack of recommended nonoperative treatment options.

A variety of oral medications are currently available that appear to be effective in the early disease stages, such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs). However, these do not prevent or reverse the

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underlying cartilage damage, nor do they provide lasting effects. Other options include injection therapies, which are usually reserved for patients not responding to oral medications. One popular choice of injection that has been widely used for the management of symptomatic knee osteoarthritis is corticosteroids. Despite their popularity, they have limited proven long-term effectiveness and can have adverse reactions for the patient[3]. Additionally, if changes in activity level, weight loss, pain relievers such as acetaminophen or NSAIDs, physical therapy, and corticosteroid injections become ineffective, viscosupplementation injections with hyaluronic acid may be an option. However, to our knowledge, the most recent research has not found viscosupplementation to be effective at reducing pain or improving function[4].

Developments in biological research have highlighted the importance of growth factors in maintenance of normal tissue structure and tissue lesion repair. New injectable therapies simply called "biologics" are now available, and some may suppress inflammation and may promote regenerative pathways. Biologics refer to natural products that are harvested and are used to supplement a medical process and/or the biology of healing. Although these may hold promise for the treatment of knee osteoarthritis, more high-quality and consistent studies are necessary. The three main categories of therapy currently available are endogenous growth factors (contained in commercially available platelet-rich plasma products), cells (mesenchymal stem cells derived from bone marrow and adipose tissue and embryonic cells from embryonic tissue), and amniotic or placental-derived tissues. This article will review these therapies, will discuss current U.S. Food and Drug Administration (FDA) guidelines regarding these products, and will discuss their potential to become recommended treatment.

Stem cell therapies are regulated by the FDA, which categorizes them as human cells, tissues, and cellular and tissue-based products regulated under Section 361 of the Public Health Service (PHS) Act. Title 21 of the Code of Federal Regulations (CFR)[5] defines 4 criteria to determine if products are considered low-risk and do not require preclinical animal trials or phased clinical trials prior to human treatment. The 4 criteria that a product needs to meet to be considered low-risk are: (1) the product has little manufacturing manipulation, (2) the product has an autologous nature with no systemic effect, (3) the product cannot be combined with other products, and (4) the product has to be utilized in a homologous way or in the same way as its original function. If the product does not comply with these criteria, then it is considered as a new drug requiring traditional preclinical animal trials, clinical trials, and strong regulatory oversight as listed in Section 351 of the PHS Act. On the basis of these guidelines, the only biologics currently available as injectable therapies are bone marrow aspirate and platelet-rich plasma.

Platelet-Rich Plasma

Platelet-rich plasma consists of autologous blood with a platelet concentration above the normal baseline level[6]. It is prepared via a two-stage centrifugation of peripherally drawn blood. The first centrifugation or "soft spin" separates the platelet-containing plasma from the red and white blood cells. By spinning at a relatively low 1,200 to 1,500 revolutions per minute (rpm), the platelets remain suspended as red and white blood cells are pulled to the bottom because of their larger size and mass. The second centrifugation or "hard spin" is at a much higher 4,000 to 7,000 rpm, creating the necessary force to further separate the plasma into platelet-rich and platelet-poor portions[7]. However, platelet-rich plasma configurations are highly heterogeneous; more than 40 commercially available preparation systems exist, which differ by centrifugation time, initial blood volume, and use of activating agents or techniques[7]. These various preparations can be characterized into one of two main categories based on their cellular composition: eukocyte-rich and leukocyte-poor. Leukocyte-rich, platelet-rich plasma is defined as having a leukocyte concentration above the physiologic baseline, and leukocyte-poor, platelet-rich plasma is defined as having a concentration below the baseline[8].

Platelet-rich plasma has emerged as a potential therapeutic modality for many conditions, including knee osteoarthritis[9]. Platelets contain a variety of growth factors and cytokines, which have been shown to stimulate extracellular matrix synthesis and chondrocyte proliferation, to promote bone remodeling and wound-healing, and to inhibit catabolic pathways in ex vivo and in vitro studies[8-11]. However, despite its rapidly growing popularity, platelet-rich plasma is still considered an experimental treatment, falling under the FDA's Title 21 CFR Part 1271[5], making it exempt from traditional regulatory pathways. It is not considered a human cell, tissue, and cellular and tissue-based product by the FDA and is not covered by the PHS Act. Although there are a multitude of studies comparing platelet-rich plasma with placebo and other knee osteoarthritis treatment modalities, such as hyaluronic acid viscosupplementation, the wide heterogeneity of preparation methods and injection frequency variance[6] has made it difficult to assess and compare them.

Patel et al.[12] illustrated the efficacy of leukocyte-poor, platelet-rich plasma (with a platelet count 3 times that of baseline) compared with a saline solution placebo injection for the treatment of early knee osteoarthritis (Ahlbäck grade I or II). A total of 78 patients with bilateral knee osteoarthritis (156 knees) were randomly divided into 1 of 3 cohorts (group A, single platelet-rich plasma injection; group B, 2 platelet-rich plasma injections 3 weeks apart; and group C, single saline solution injection), treated, and followed for 6 months. Patel et al. reported a significant difference ($p < 0.001$) in favor of both platelet-rich plasma injection groups

compared with the saline solution injection group using a visual analog scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and patient satisfaction scores at 6 months after treatment. Smith[13] also illustrated the efficacy of leukocyte-poor, platelet-rich plasma compared with a saline solution placebo. A total of 30 patients (30 knees) with knee osteoarthritis (Kellgren-Lawrence grade 2 or 3) were randomized into 2 cohorts (leukocyte-poor, platelet-rich plasma or saline solution) that received a series of 3 weekly injections. Smith reported a significantly greater improvement ($p < 0.001$) of WOMAC scores in the leukocyte-poor, platelet-rich plasma cohort throughout the study compared with the saline solution cohort. Twelve months after treatment, the leukocyte-poor platelet-rich plasma group had improved by 78% from their baseline WOMAC score, but the placebo group had improved by only 7%.

Several randomized controlled trials have been performed comparing the efficacy of platelet-rich plasma, either leukocyte-poor or leukocyte-rich, with that of hyaluronic acid. The studies involving leukocyte-poor, platelet-rich plasma demonstrated superior results compared with platelet-rich plasma treatment[14,15], but the leukocyte-rich, platelet-rich plasma studies have shown mixed results (Table I)[16-18]. Although the majority of these studies have shown improved outcomes with platelet-rich plasma compared with hyaluronic acid at a short-term follow-up, there is still insufficient evidence to support its use because of the heterogeneity of platelet-rich plasma preparations and injection frequencies tested.

Stem Cells

Stem cell therapy has emerged as another potential biological treatment option in the treatment of knee osteoarthritis. Stem cells are advantageous because they can mobilize during angiogenesis, can differentiate into specialized cell types, can proliferate and regenerate, and can release immune regulators and growth factors. Common mesenchymal stem cell sources currently available include bone marrow and adipose sources. Embryonic stem cells are not currently available. Amniotic or placental-derived tissues and cells will be discussed in the Tissues section.

Mesenchymal stem cells have been the most widely used stem cells in orthopaedic and non-orthopaedic applications over the last decade because of their potential to differentiate into multiple cell lines, including osteoblasts, fibroblasts, and chondrocytes. These can further differentiate into cells that make cartilage, tendon, muscle, and bone. Mesenchymal stem cells are characterized by several features including ease of isolation, high differentiation capabilities, colony expansion, powerful anti-inflammatory properties, and ability to localize to damaged tissue. Mesenchymal stem cells have also been

TABLE I Platelet-Rich Plasma Studies*

	Study				
	Cerza et al. (2012)[14]	Sánchez et al. (2012)[15]	Filardo et al. (2012)[16]	Filardo et al. (2015)[18]	Spaková et al. (2012)[17]
Platelet-rich plasma preparation	Leukocyte poor	Leukocyte poor	Leukocyte rich	Leukocyte rich	Leukocyte rich
No. of patients					
Total	120	176	109	192†	120
Platelet-rich plasma	60	89	54	94	60
Hyaluronic acid	60	87	55	89	60
Osteoarthritis grade	Kellgren-Lawrence 1, 2, and 3	Ahlbäck I, II, and III	Kellgren-Lawrence 0, 1, 2, and 3	Kellgren-Lawrence 0, 1, 2, and 3	Kellgren-Lawrence 1, 2, and 3
Outcome scores	WOMAC	WOMAC and Lequesne	IKDC, EuroQol visual analog scale, Tegner, and KOOS	IKDC, EuroQol visual analog scale, Tegner, and KOOS	WOMAC and 11-point pain intensity Numeric Rating Scale
Results	Significantly better outcomes in platelet-rich plasma group	Significantly better short-term (<24-wk) outcomes in platelet-rich plasma group	Platelet-rich plasma not superior to hyaluronic acid	Platelet-rich plasma not superior to hyaluronic acid	Significantly better outcomes in platelet-rich plasma group at follow-up of 3 and 6 mo

*IKDC = International Knee Documentation Committee, and KOOS = Knee injury and Osteoarthritis Outcome Score. †Nine patients were not included in the final analysis because of lack of complete data at final evaluation.

shown to secrete cytokines locally with paracrine effects on local tissue to control and modulate inflammation, to stimulate cell repair and proliferation, and to improve blood flow through the secretion of chemokines, cytokines, and growth factors[19]. As a result, mesenchymal stem cells have the potential to support chondrogenesis in an osteoarthritic joint and to prevent further cartilage loss while possibly regenerating new cartilage.

The advantages of adipose-derived mesenchymal stem cells include high prevalence and ease of harvest; however, several animal studies have shown inferior results when compared with bone marrow mesenchymal stem cells. More research is needed to determine the ideal source material for mesenchymal stem cells, which will likely depend in part on the procedure for which they are employed. It is not fully understood how many mesenchymal stem cells survive harvest to implantation and how long these cells may survive once placed in an osteoarthritic environment. However, locally secreted cytokines may lead to a healing environment that lasts beyond the duration of mesenchymal stem cell viability in the joint[20].

Bone marrow-derived stem cells are typically harvested, in a minimally invasive manner, from the posterior superior iliac crest. Multiple other sites including the anterior superior iliac crest have been described, but the posterior superior iliac crest generally produces the highest stem cell yield[21-23]. Mesenchymal stem cells are more concentrated and have increased proliferation potential in younger patients. Choudhery et al.[24] investigated the expansion and differentiation potential in patients who were younger (<30 years), middle-aged (35 to 50 years), and older (>60 years). Mesenchymal stem cells from older patients had less viability, proliferation, and differentiation potential than those from younger patients. In addition to age-dependent variables affecting stem cell concentration, technique-dependent variables such as strong aspiration with small-volume, 10-mL syringes appear to maximize stem cell concentration[21-23,25-29].

Alternatively, adipose-derived stem cells are harvested by liposuction. However, compared with bone marrow aspirate, FDA guidelines consider the use of adipose tissue for bone and joint disorders to be non-homologous, which essentially means that adipose tissue injected in the knee does not serve the same function of adipose tissue elsewhere in the body. In addition, the processing of adipose-derived stem cells, as defined by the FDA, "alters the original relevant characteristic of adipose tissue relating to the tissue's utility for reconstruction, repair or replacement"[30] and is considered more than minimal manipulation, requiring the developers to abide by Section 351 of the PHS Act by initiating an Investigational New Drug Program[22,31]. Confusion remains as many clinics continue to provide adipose stem cell treatment, claiming that their processing is minimal manipulation, with only the most egregious offenders being sent warning letters by the FDA and some treatment centers being closed[32]. An open forum set by the FDA to discuss these issues and provide clarity for clinicians and industry was planned for early 2017.

Over the past decade, multiple human trials have been published demonstrating the efficacy of mesenchymal stem cell injections into patients with osteoarthritis. Davatchi et al.[33] performed a single intra-articular injection of bone marrow stem cells in 4 patients with knee osteoarthritis. There were improvements in clinical outcome scores and physical parameters such as the number of stairs that the patient was able to climb and walking time. Similarly, Emadedin et al.[34] reported that 6 patients with knee osteoarthritis, treated with a single injection of bone marrow stem cells, had improvements in pain and functional status of the knee up to 6 months after the injection. Magnetic resonance imaging (MRI) analysis prior to mesenchymal stem cell therapy and at a 6-month follow-up showed an increase in cartilage thickness and decreases in subchondral edema in 50% of patients in that study. Orozco et al.[35] followed 12 patients treated with bone marrow stem cell injection for knee osteoarthritis. At a 12-month follow-up, patients reported significant improvements ($p < 0.001$) in clinical outcomes and MRI showed a decrease in the size of lesions with poor cartilage, as defined on T2 relaxation measurements[35].

Koh et al.[36] performed adipose-derived stem cell injection with arthroscopic lavage in 30 elderly patients with knee osteoarthritis. "Second-look" arthroscopy was performed in 16 patients. All patients showed significant improvement ($p < 0.05$) in clinical outcomes at a 24-month follow-up, and only 5 patients demonstrated worsening of the Kellgren-Lawrence grade[36]. On second-look arthroscopy, 88% of patients had improved or maintained cartilage status from the time of adipose mesenchymal stem cell injection. Jo et al.[37] performed adipose-derived stem cell injection in 18 patients with low, medium, and high dosages of stem cells (dosage correlated with the cell count within 3 mL of saline solution). Patients in the low and medium-dosage groups did not show clinical improvement at 6 months; however, the high-dosage group had significant improvement ($p = 0.003$) in outcomes. There were no changes in Kellgren-Lawrence grade throughout the study period in any group. MRI demonstrated decreases in the size of chondral defects on the tibia and femur in the high-dosage group, with no changes present in the low-dosage or medium-dosage groups.

Many of these preliminary studies evaluating the efficacy of bone marrow and adipose mesenchymal stem cells as well as safety appear promising and safe[38] but have small patient numbers and lack long-term follow-up. Several randomized controlled trials are currently ongoing to confirm these preliminary findings[28].

Tissues

Studies on allogeneic matrices from amniotic or placental-derived tissues

have found clinical utility in patients for treating ocular wounds, skin ulcers, burns, and wounds. These matrices possess antibacterial, anti-inflammatory, antiadhesive, antiangiogenic, and immunomodulatory properties, which make them ideal candidates for use in tissue regeneration therapies. One of the most promising aspects of the amniotic membrane and chorionic membrane, from a tissue engineering and regenerative medicine perspective, is the readily available and developmentally juvenile stem cells found within the extracellular matrix of these membranes. In a pilot study, Vines et al. followed 6 patients for 12 months after intra-articular injection with a cryogenically preserved amniotic suspension allograft consisting of particulated human amniotic membrane and human amniotic fluid-derived cells (ReNu; NuTechMedical). No significant injection reactions were noted, and there was no significant effect of the amniotic cells on blood cell counts, lymphocyte subsets, or inflammatory markers. The study demonstrated the feasibility of a single intra-articular injection of amniotic cells for the treatment of knee osteoarthritis[39].

Companies are marketing these products derived from perinatal products as regulated by PHS Act 361. Multiple FDA letters have suggested that these products do not meet the autologous and homologous criteria required to avoid preclinical studies[31,40].

Discussion

There are still many questions about platelet-rich plasma and its effectiveness for knee osteoarthritis. Differences in preparation technique make effective evaluation and comparison difficult. Both DeLong et al.[41] and Mishra et al.[42] have proposed systems that classify platelet-rich plasma preparation methods by activation mechanism, platelet number, and/or cell content. This will ultimately help to standardize platelet-rich plasma preparation and aid in comparison of clinical trials and outcomes obtained following platelet-rich plasma injections, providing the first step toward demonstrating the efficacy of platelet-rich plasma for the treatment of knee osteoarthritis.

Bone marrow mesenchymal stem cells currently remain the only stem cell product that appears to be approved by the FDA without any potential questions about use. Optimal cell number determination for appropriate clinical effect and long-term clinical studies are still necessary to fully understand their role and when they should be instituted in the overall algorithm of management of the patient with knee osteoarthritis. Use of adipose stem cells is complicated by warning letters from the FDA suggesting orthopaedic use to be improper. The 2017 FDA open forum meeting will undoubtedly provide more regulatory insight and guidelines on the orthopaedic use of these biologics.

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References

1. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, Gabriel S, Hirsch R, Hochberg MC, Hunder GG, Jordan JM, Katz JN, Kremers HM, Wolfe F; National Arthritis Data Workgroup. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum. 2008 Jan; 58(1):26-35.

2. Görmeli G, Görmeli CA, Ataoglu B, Çolak C, Aslantürk O, Ertem K. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. Knee Surg Sports Traumatol Arthrosc. 2015 Aug 2. [Epub ahead of print].

3. MacMahon PJ, Eustace SJ, Kavanagh EC. Injectable corticosteroid and local anesthetic preparations: a review for radiologists. Radiology. 2009 Sep;252(3):647-61.

4. American Association of Orthopaedic Surgeons. Treatment of osteoarthritis of the knee: evidence-based guideline. 2nd edition. 2013. http://www.aaos.org/research/guidelines/TreatmentofOsteoarthritisoftheKneeGuideline.pdf. Accessed 2016 Oct 19.

5. Human cells, tissues, and cellular and tissue-based products, 21 C.F.R. §1271.10(a). 2016.

6. Hall MP, Band PA, Meislin RJ, Jazrawi LM, Cardone DA. Platelet-rich plasma: current concepts and application in sports medicine. J Am Acad Orthop Surg. 2009 Oct;17(10):602-8.

7. Hsu WK, Mishra A, Rodeo SR, Fu F, Terry MA, Randelli P, Canale ST, Kelly FB. Platelet-rich plasma in orthopaedic applications: evidence-based recommendations for treatment. J Am Acad Orthop Surg. 2013 Dec;21(12):739-48.

8. Riboh JC, Saltzman BM, Yanke AB, Fortier L, Cole BJ. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. Am J Sports Med. 2016 Mar;44(3):792-800. Epub 2015 Apr 29.

9. Zhu Y, Yuan M, Meng HY, Wang AY, Guo QY, Wang Y, Peng J. Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: a review. Osteoarthritis Cartilage. 2013 Nov;21(11): 1627-37. Epub 2013 Aug 7.

10. Kabiri A, Esfandiari E, Esmaeili A, Hashemibeni B, Pourazar A, Mardani M. Platelet-rich plasma application in chondrogenesis. Adv Biomed Res. 2014 Jun 25; 3:138.

11. Sundman EA, Cole BJ, Karas V, Della Valle C, Tetreault MW, Mohammed HO, Fortier LA. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. Am J Sports Med. 2014 Jan;42(1): 35-41. Epub 2013 Nov 5.

12. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. Am J Sports Med. 2013 Feb;41 (2):356-64. Epub 2013 Jan 8.

13. Smith PA. Intra-articular autologous conditioned plasma injections provide safe and efficacious treatment for knee osteoarthritis: an FDA-sanctioned, randomized, double-blind, placebo-controlled clinical trial. Am J Sports Med. 2016 Apr;44(4):884-91. Epub 2016 Feb 1.

14. Cerza F, Carnì S, Carcangiu A, Di Vavo I, Schiavilla V, Pecora A, De Biasi G, Ciuffreda M. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. Am J Sports Med. 2012 Dec;40(12):2822-7. Epub 2012 Oct 25.

15. Sánchez M, Fiz N, Azofra J, Usabiaga J, Aduriz Recalde E, Garcia Gutierrez A, Albillos J, Gárate R, Aguirre JJ, Padilla S, Orive G, Anitua E. A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. Arthroscopy. 2012 Aug;28(8):1070-8.

16. Filardo G, Kon E, Di Martino A, Di Matteo B, Merli ML, Cenacchi A, Fornasari PM, Marcacci M. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. BMC Musculoskelet Disord. 2012 Nov 23;13:229.

17. Spaková T, Rosocha J, Lacko M, Harvanová D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid.

Am J Phys Med Rehabil. 2012 May;91(5):411-7. Epub 2012 Apr 20.

18. Filardo G, Di Matteo B, Di Martino A, Merli ML, Cenacchi A, Fornasari P, Marcacci M, Kon E. Platelet-rich plasma intra-articular knee injections show no superiority versus viscosupplementation: a randomized controlled trial. Am J Sports Med. 2015 Jul;43(7): 1575-82. Epub 2015 May 7.

19. Stoddart MJ. Mesenchymal stem cells as a source of repair cytokines: mesenchymal stem cells as the conductor. J Am Acad Orthop Surg. 2015 Jul;23(7):452-3. Epub 2015 Jun 3.

20. Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol. 2006 Apr;24(4):150-4. Epub 2006 Feb 20.

21. Hyer CF, Berlet GC, Bussewitz BW, Hankins T, Ziegler HL, Philbin TM. Quantitative assessment of the yield of osteoblastic connective tissue progenitors in bone marrow aspirate from the iliac crest, tibia, and calcaneus. J Bone Joint Surg Am. 2013 Jul 17;95(14):1312-6.

22. Narbona-Carceles J, Vaquero J, Suárez-Sancho S, Forriol F, Fernández-Santos ME. Bone marrow mesenchymal stem cell aspirates from alternative sources: is the knee as good as the iliac crest? Injury. 2014 Oct;45(Suppl 4):S42-7.

23. Pierini M, Di Bella C, Dozza B, Frisoni T, Martella E, Bellotti C, Remondini D, Lucarelli E, Giannini S, Donati D. The posterior iliac crest outperforms the anterior iliac crest when obtaining mesenchymal stem cells from bone marrow. J Bone Joint Surg Am. 2013 Jun 19;95 (12):1101-7.

24. Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. J Transl Med. 2014 Jan 7;12:8.

25. Hernigou J, Picard L, Alves A, Silvera J, Homma Y, Hernigou P. Understanding bone safety zones during bone marrow aspiration from the iliac crest: the sector rule. Int Orthop. 2014 Nov;38(11):2377-84. Epub 2014 May 3.

26. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions influence of the number and concentration of progenitor cells. J Bone Joint Surg Am. 2005 Jul;87(7):1430-7.

27. Hernigou P, Flouzat Lachaniette CH, Delambre J, Zilber S, Duffiet P, Chevallier N, Rouard H. Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: a case-controlled study. Int Orthop. 2014 Sep;38(9):1811-8. Epub 2014 Jun 7.

28. Hernigou P, Homma Y, Flouzat Lachaniette CH, Poignard A, Allain J, Chevallier N, Rouard H. Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. Int Orthop. 2013 Nov;37(11):2279-87. Epub 2013 Jul 24.

29. Hernigou P, Poignard A, Zilber S, Rouard H. Cell therapy of hip osteonecrosis with autologous bone marrow grafting. Indian J Orthop. 2009 Jan;43(1):40-5.

30. U.S. Food & Drug Administration. Tissue Reference Group. http://www.fda.gov/BiologicsBloodVaccines/TissueTissueProducts/RegulationofTissues/ucm152857.htm. Accessed 2016 Oct 19.

31. U.S. Food & Drug Administration. Osiris Therapeutics, Inc. 2013. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/ComplianceActivities/Enforcement/UntitledLetters/ucm371540.htm. Accessed 2016 Nov 1.

32. Griffin DL, O'Donnell L. Adipose tissue regulation in the United States. 2013 Apr. http://c.ymcdn.com/sites/www.celltherapysociety.org/resource/resmgr/uploads/files/Telegraft/2013/Vol.%2020%20No.%202%20(April)/April_2013_Telegraft_FAT_regulations_article_FINAL.pdf. Accessed 2016 Oct 19.

33. Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. Int J Rheum Dis. 2011 May;14(2):211-5. Epub 2011 Mar 4.

34. Emadedin M, Aghdami N, Taghiyar L, Fazeli R, Moghadasali R, Jahangir S, Farjad R, Baghaban Eslaminejad M. Intra-articular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis. Arch Iran Med. 2012 Jul;15(7):422-8.

35. Orozco L, Munar A, Soler R, Alberca M, Soler F, Huguet M, Sentís J, Sánchez A, García-Sancho J. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. Transplantation. 2013 Jun 27;95(12): 1535-41.

36. Koh YG, Choi YJ, Kwon SK, Kim YS, Yeo JE. Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2015 May;23(5): 1308-16. Epub 2013 Dec 11.

37. Jo CH, Lee YG, Shin WH, Kim H, Chai JW, Jeong EC, Kim JE, Shim H, Shin JS, Shin IS, Ra JC, Oh S, Yoon KS. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. Stem Cells. 2014 May;32(5): 1254-66.

38. Centeno CJ, Bashir J. Safety and regulatory issues regarding stem cell therapies: one clinic's perspective. PM&R. 2015 Apr;7(4)(Suppl):S4-7.

39. Vines JB, Aliprantis AO, Gomoll AH, Farr J. Cryopreserved amniotic suspension for the treatment of knee osteoarthritis. J Knee Surg. 2016 Aug;29(6):443-50. Epub 2015 Dec 18.

40. Department of Human & Health Services. 2015. http://www.fda.gov/downloads/BiologicsBloodVaccines/Guidance-ComplianceRegulatoryInformation/ComplianceActivities/En-forcement/UntitledLetters/UCM452862.pdf. Accessed 2016 Oct 19.

41. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy. 2012 Jul;28(7):998-1009. Epub 2012 Jun 29.

42. Mishra A, Harmon K, Woodall J, Vieira A. Sports medicine applications of platelet rich plasma. Curr Pharm Biotechnol. 2012 Jun;13(7): 1185-95. Epub 2011 Jul 12.