Malaysian Orthopaedic Journal 2015 Vol 9 No 3

The Current Role of Stem Cells in Orthopaedic Surgery

Maniar HH, MD, Tawari AA, MD, Suk M, MD, Horwitz DS, MD
Department of Orthopaedic Surgery, Geisinger Medical Center, Danville, USA

ABSTRACT
Basic science and experimental research on stem cells has increased exponentially in the last decade. Our present knowledge about stem cell biology is better than ever before. This new paradigm shift in research has been reflected in the field of orthopaedic surgery. Various experimental models have suggested a potential application of stem cells for different orthopaedic conditions, and early clinical results of stem cell use have been encouraging. These cells can be easily isolated, processed and made available for clinical use. From healing of bone defects caused by trauma, tumor or infection to cartilage defects, nerve, tendon and ligament healing, stem cell use has the potential to revolutionize orthopaedic practice. The purpose of this article is to orient a general orthopaedic surgeon towards the current use and clinical applications of stem cell based therapy in orthopaedics and to provide a complete overview of the clinical advances in this field.

Key Words:
Stem cell; orthopaedic surgery

INTRODUCTION
With an increased emphasis on evidence based medicine, there has been an increasing focus on the pathophysiology of orthopaedic injuries and disease processes, and their impact on overall outcome. Traditional treatment strategies are evolving to encompass tailored approaches that account for age, occupation and patient expectation. Newer strategies for management are routinely implemented and encouraged in an effort to improve patient outcomes. It is to achieve these objectives that regenerative medicine which utilizes the use of stem cells and tissue engineering has newly emerged.

Definitions:
Stem Cells: Undeveloped biological cells capable of proliferation, self-renewal, conversion to differentiated cells and regenerating tissues.

Totipotent cells: These are cells that can develop into all cell types in the human body and can also form extra embryonic and placental cells. The cells of the early stages of the embryo are the only totipotent cells and are not in clinical use due to ethical concerns.

Pluripotent cells: Cells that can develop into cells of all the three germ layers (endoderm, ectoderm or mesoderm). Cells of late stages of embryo after the blastocyst stage are pluripotent cells.

Multipotent cells: These are cells that can develop into more than one but not all germ layers. Adult stem cells and cord blood cells are multipotent cells.

Types of Stem Cells:
Embryonic Stem Cells (Pre-natal): These cells are obtained from the blastocyst stage of the embryo. Pluripotent in the truest sense, they have a capacity to form into any tissue of the body and multiply in an unlimited manner. This is predominantly due to the phenomenon of asymmetric division – production of one stem and one non-stem daughter cell. These properties, however, also make them prone to tumorigenesis. This and the necessity of harvest from embryos causes safety and ethical dilemma.

Adult Stem cells (Post-natal): These cells are obtained later in life after the embryonic stage. They are multipotent, undifferentiated cells located among specialized tissues with a primary function of their maintenance and repair. Mesenchymal stem cells (MSC), which originate from the mesoderm, are a type of adult stem cells that have a good potential to develop into adipocytes, chondrocytes, myoblasts and osteoblasts.

Sources of Stem cells:
Stem cells can be obtained from bone marrow, periosteum, adipose tissue, placenta, umbilical cord, blood, human amniotic fluid, dental pulp, synovial tissue, skin and skeletal muscle. Among these, bone marrow, adipose tissue and muscle derived MSCs are most commonly used as they are easily obtained and abundantly available.
Isolation of Stem cells:
Stem cells may be “unselected cells” obtained from autologous bone marrow after centrifugation or “selected” and enhanced in culture utilizing their affinity to tissue plastics. Cost involved, time to culture, risk of infection and loss of function in vitro are factors preventing regular clinical use of cultured MSCs. It should be noted that absolute number and the purity of cells obtained from cultures is higher, an important factor for clinical effect. The posterior iliac crest has been shown to have a higher yield for MSCs as compared to anterior in case of bone marrow aspiration.

Route of administration:
Stem cells may be directly applied into a lesion either surgically or via local injection with a suitable scaffold/carrier. MSCs may be taken through initial phases of differentiation, forming bone or cartilage precursors under laboratory conditions and then implanted into lesions. In addition, MSCs may be administered intravenously. Their ability to migrate systemically and colonize the bone marrow after a peripheral injection has been utilized for treatment of Osteogenesis Imperfecta.

Mechanism of action:
In addition to differentiation into bone, muscle, cartilage, ligament or tendon cells, MSCs also have a paracrine effect whereby they secrete growth factors and cytokines such as bone morphogenic proteins (BMPs), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF). These play an important role in angiogenesis, repair, cell survival and proliferation. MSCs also have the ability to migrate to the site tissue injury to modulate an inflammatory response. Genetically modified MSCs for long term release of growth factors are being currently developed.

Role of Mesenchymal Stem Cells in Orthopaedic Surgery:
MSCs have an ability to develop into any mesodermal tissue. Thus, they can be prompted to form precursor cells to develop into tissues including bone, cartilage, muscle, tendon, and ligament. The use of stem cells for various orthopaedic challenges is outlined below.

Trauma and bone defects:
Nonunion/Delayed union and bone defects following trauma, tumor or infection are challenging aspects of orthopaedic surgery that may require biologic augmentation for optimum healing. Autologous cancellous graft is the current ‘gold standard’, but limited supply and donor site morbidity limit their use. Allografts and bone graft substitutes are routinely used to augment bone healing. However, poor graft integration and osteonecrosis of the graft remain primary issues with this method. Bone marrow aspirates that contain stem cells in a proportion of 1:10,000 to 1:1,000,000 of nucleated cells have been successfully used to enhance healing of non-unions. Tissue engineering, involving the use of stem cells with scaffolds such as hydroxyapatite (HA), demineralized bone matrix (DBM) and tri-calcium phosphate (TCP), have been studied and found to be useful for bridging bone defects. Due to absence of an extracellular matrix to grow on, MSCs alone have not proven to be beneficial for filling defects caused by simple/aneurysmal bone cysts. Healing rates, are however, enhanced when these are used in conjunction with scaffolds.

Spine and peripheral nerve surgery:
Spine Fusion:
Neen et al, in a prospective study, showed that unselected stem cells used with HA scaffolds had similar healing rates as autologous grafting; thereby preventing donor site morbidity. Similar results were obtained by Gan et al using β-TCP.

Intervertebral Disc Degeneration:
Intervertebral disc degeneration is one of the most common causes of backache in a young productive population. Despite the high prevalence there is no treatment available which reverses the primary pathology. Animal experiments have shown increased proteoglycan content and maintenance of disc height with percutaneous stem cell injections. Clinical trials are in progress to evaluate these results in humans with positive interim results.

Spinal cord and peripheral nerve injuries:
Spinal cord and peripheral nerve injuries have a significant impact on quality of life of affected individuals. Animal studies have highlighted some positive effects of MSC use via intrathecal and local administration, however, the response seen in clinical studies is mixed. In an animal study, Tamaki et al, demonstrated that muscle derived MSCs aided in successful regeneration of a crushed peripheral nerve. Further prospective clinical studies are necessary to establish the role of MSCs in managing these patients.

Articular cartilage:
Articular cartilage is a highly specialized tissue with a poor intrinsic capacity to repair itself. The goal of any cartilage procedure is to restore its integrity so that it can withstand the wear and tear of daily activity.

Focal cartilage damage:
Since Pridie introduced subchondral drilling in the late 1950s, various procedures such as microfracture and abrasionplasty have been developed to recruit MSCs from adjacent bone marrow to proliferate into chondrocytes. Unfortunately, these procedures result in the formation of an inferior quality nonhyaline cartilage. Data on use of MSCs with suitable scaffolds in cartilage healing is mostly based on animal studies, with a few human case series showing improved healing and better function after autologous MSC implantation techniques.
Osteoarthritis (OA):
Due to their role in inhibiting the catabolic activity of matrix metalloproteinases (MMP), MSCs have been shown to have a beneficial effect in OA \(^\text{18}\). In a recent study, Sato et al showed that guinea pigs with age related OA treated with MSC laden hyaluronic injections had better cartilage regeneration with higher type II collagen and lower MMP content \(^\text{19}\). Except for a few case series which show some clinical improvement there is a paucity of trials with human subjects which study the effect of MSCs on OA \(^\text{20}\).

High tibial Osteotomy (HTO) and Arthroplasty:
In a randomized control trial, Dallari et al showed that lyophilized bone chips treated as grafts with platelet gels and MSCs had higher rate of osteointegration in HTOs \(^\text{21}\). With appropriate use of nanotechnology to make optimum implant surfaces, MSCs have a great potential to revolutionize joint replacement surgery by facilitating osteointegration. Three dimensional scaffolds with MSCs may be used in the future to form autologous osteochondral grafts suitable for a ‘biologic’ arthroplasty \(^\text{22}\).

Avascular Necrosis:
Avascular Necrosis (AVN) of the head of femur is one of the most debilitating disorders in young patients. It is characterized by a decreased blood supply to the bone and associated increase in intraosseous pressure. The integrity of the subchondral plate is one of the most important deciding factor between head preserving (core decompression, bone grafting, femoral osteotomies) or head sacrificing (hip resurfacing/arthroplasty) procedures. Stem cells have angiogenic and osteogenic properties. Early stages of AVN are amenable to treatment with stem cell concentrate injection combined with routine retrograde procedures such as core decompression. Bone marrow aspirates administered after core decompression have been shown to be beneficial in AVN. Stem cells were isolated and used in a study by Rastogi et al where 60 hips in early stages of AVN were randomized to be treated either with core decompression and bone marrow injection or with core decompression and injection of isolated stem cells \(^\text{23}\). Two year follow up showed a better functional outcome and better radiographic healing in the stem cell group.

Wound Healing:
Although not typical in orthopaedic practice, poorly healing wounds are commonly encountered in treating patients with risk factors such as diabetes or open fractures. MSC treatment of acute and chronic wounds results in accelerated wound closure with increased epithelialization, granulation tissue formation and angiogenesis \(^\text{24}\).

Bone-Tendon interface and Tendon Healing:
Numerous commonly employed surgical procedures such as anterior cruciate ligament reconstruction, rotator cuff repair or retrocalcaneal bursa excisions depend on optimum healing of the bone-tendon interface. Fibrovascular scar formed during healing possesses inferior biochemical and mechanical properties. MSCs have been shown to promote early healing of the bone tendon interface by increasing the proportion of Sharpey’s fibers. MSCs used with bone morphogenetic protein 2 (BMP-2) are associated with improved biomechanical properties of the bone tendon interface including stiffness and maximal load. A recent study by Adams et al showed that rats with Achilles tendon tear treated with stem cell-bearing sutures have higher failure strength and better histological properties \(^\text{25}\). Unselected MSCs were used for ultrasound-guided injections in a case series by Pascual-Garrido et al for chronic patellar tendinopathy with good clinical results \(^\text{26}\).

Paediatric Orthopaedics:
Osteogenesis Imperfecta (OI):
This is a heterogenous group of diseases with abnormality of type I collagen primarily leading to increased susceptibility to fractures, slow growth and loss of bone mass. Systemic infusion of allogenic MSCs by Horwitz et al in six children with OI showed improvement in bone mass and bone growth acceleration \(^\text{27}\).

Physseal injuries:
Bone bridge formation is an adverse complication following traumatic, infectious or other insult on the physis, leading to angular and/or longitudinal deformities. In a pig study, Planka et al showed that MSCs with scaffolds used in physseal defects differentiated into chondrocytes forming hyaline cartilage and prevented bony bridge formation \(^\text{28}\). Currently, there are no clinical studies to support this.

Osteoporosis:
In spite of tremendous advances in drug therapy, osteoporosis plays a significant role in overall health of geriatric patients. Increasing age is associated with decrease in number and function of osteoblasts and osteoprogenitor cells. Systemic infusion of MSCs has failed to promote bone formation due to their inability to migrate to the surface of the bone, a critical step for bone formation. In an animal study, Guan et al used MSCs modified to express certain surface proteins which enabled them to migrate to the periosteum leading to increased trabecular bone formation and bone mass \(^\text{29}\). Concepts such as these are positive steps towards utilizing MSCs for a generalized disease like osteoporosis.

Muscular dystrophies:
These are group of conditions wherein muscle fibers are replaced by fibrotic and adipose tissues, due to genetic mutations in several muscle proteins, which are essential for normal muscle function. There is no cure for these patients and treatment is focused on comfort care, respiratory assistance and delaying loss of function. Local and systemic transplantation of well differentiated myoblasts is associated
| No | Type of MSC                                                                 | Study Type                        | Pathology                                      | No of Patients | Results                                                   | Reference                                | Follow up |
|----|-----------------------------------------------------------------------------|-----------------------------------|-----------------------------------------------|----------------|-----------------------------------------------------------|------------------------------------------|-----------|
| 1  | Cultured stem cells obtained from skin fibroblasts and modulated to grow collagen producing cells | Level I - Randomized Control Trial | Patellar tendinopathy                         | 46 patients, 60 patellar tendons                | Improvement in pain and function.            | Clarke AW et al., [30] 2011               | 6 months |
| 2  | Cultured cells differentiated into osteoblasts injected with fibrin          | Level II - Randomized Control Trial with no blinding | Fracture healing rate and safety             | 64 patients, 51 hips                            | Acceleration of fracture healing and safe    | Kim SJ et al., [31] 2009                 | 2 months |
| 3  | Uncultured mononuclear cell instillation obtained from bone marrow instilled following core decompression | Level II – Prospective comparative study | Avascular Necrosis of head of femur          | 40 patients, 24 hips                            | Better clinical scores and radiological outcomes | Sen RK et al., [32] 2012                | 2 years |
| 4  | Uncultured cell instillation obtained from bone marrow and implanted with core decompression | Level II – Prospective comparative study | Avascular Necrosis of head of femur          | 40 patients, 20 cases and 20 controls           | Improvement in pain and progression to collapse | Gangji V et al., [33] 2011               | 5 years |
| 5  | Cultured cells transplanted to area surrounding injury                      | Level III – Case Control study     | Complete and Chronic Cervical Spinal cord injury | 40 patients, 20 cases and 20 controls           | Improvement in neurological function at 6 months in 10/20 patients in treatment group | Dai G et al., [34] 2013                  | 6 months |
| 6  | Cultured bone marrow derived stem cells                                     | Level III – Prospective comparative | Articular cartilage repair                   | 72 patients                                     | No significant improvement as compared to autologous chondrocyte implantation except for one stage procedure. | Nejadnik H et al., [17] 2010             | 24 months |
| 7  | Uncultured bone marrow derived stem cells with hyaluronic acid membrane scaffold with platelet rich fibrin | Level III – Prospective comparative | Osteochondral lesions of Knee                | 20 patients                                     | No significant improvement as compared to autologous cartilage implantation except for one stage procedure. | Buda R et al., [35] 2010                | 2 years |
| 8  | Uncultured mononuclear cell concentrate obtained from peripheral blood and reinfused through arteriography | Level IV – Case Series             | Chronic Spinal Cord Injuries                 | 26/39 (66.7%) patients                        | 26/39 (66.7%) patients showed recovery of somatosensory evoked potentials | Cristante AF et al., [36] 2009           | 2.5 years |
| 9  | Uncultured cells obtained from skin                                          | Level IV -Case Series               | Knee cartilage repair                        | 52 patients                                     | Improvement in Knee scores                  | Skowronski J et al., [37] 2012          | 6 years |
| 10 | Cultured – Collagen producing stem cells obtained from skin                 | Level IV – Case series             | Lateral Epicondylitis                        | 12 patients                                     | Improvement in elbow scores and chondrocyte showing features of healing | Connell D et al., [38] 2009             | 6 months |
| 11 | Uncultured cells implanted arthroscopically with collagen powder or Hyaluronic acid scaffolds with platelet gel | Level IV -Therapeutic Study        | Osteochondral lesions of talus               | 48 patients                                     | Improvement in Ankle Score. Histologically, tissue in various degrees of remodeling but none was entirely hyaline cartilage | Giannini S et al., [39] 2009           | Minimum 2 years, Mean 29 months |
| 12 | Uncultured mononuclear cells obtained from bone marrow aspiration with collagen sponge scaffold | Level IV -Case Series               | Filling of Bone defects. (Trauma and Tumor)  | 10 patients                                     | Healing of all bone defects                 | Jager M et al., [40] 2009               | Variable (maximum - 18 months) |

Table I: Recent (within 5 years) clinical studies (minimum 10 patients) utilizing stem cell use in orthopaedic surgery, sorted by levels of evidence
with poor cell survival, limited migration from injection site and immune rejection. In a phase I clinical trial, Torrente et al showed that muscle derived stem cells with specific surface markers were safely transplanted in eight boys with no side effects 27. Genetically modified MSCs are being developed for potential use of these cells in Muscular dystrophies.

Summary:
Regenerative medicine with the use of stem cells is expected to revolutionize patient treatment. Their utilization for bone tissue engineering with appropriate scaffolds provides us with exciting opportunities for research and development. Efforts must be made to ensure safe, economical, efficient and effective introduction of stem cells for regular clinical use. Well-developed, randomized, prospective, clinical studies that build on the existing animal data will provide us with much needed information for the correct application of this field of science to well documented needs of orthopaedic surgeons. (Table I summarizes all recent clinical studies utilizing stem cell use in orthopaedic surgery organized by levels of evidence)

REFERENCES
1. Mafi R, Hindocha S, Mafi P, Griffin M, Khan WS. Sources of adult mesenchymal stem cells applicable for musculoskeletal applications - a systematic review of the literature. *Open Orthop J.* 2011; 5 Suppl 2: 242-8.
2. Shostak S. (Re)defining stem cells. *Bioessays.* 2006; 28: 301-308.
3. Shahriyari L, Komarova NL. Symmetric vs. asymmetric stem cell divisions: an adaptation against cancer? *PLoS One.* 2013; 8: e76195.
4. Aldahmash A, Atteya M, Elsaafadi M, Al-Nbaheen M, Al-Mubarak HA, Vishnubalaji R, et al. Teratoma formation in immunocompetent mice after syngeneic and allogeneic implantation of germline capable mouse embryonic stem cells. *Asian Pac J Cancer Prev.* 2013; 14: 5705-5711.
5. Hernigou P, Poignard A, Manicom O, Mathieu G, Rouard H. The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone. *J Bone Joint Surg Br.* 2005; 87: 896-902.
6. Pierini M, Di Bella C, Dozza B, Frisoni T, Martella E, Bellotti C, et al. The posterior iliac crest outperforms the anterior iliac crest when obtaining mesenchymal stem cells from bone marrow. *J Bone Joint Surg Am.* 2013; 95: 1101-1107.(Level IV)
7. Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, et al. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood.* 2001; 97: 1227-31.(Level III)
8. Giuliani N, Lisignoli G, Magnani M, Racano C, Bolzoni M, Dalla Palma B, et al. New insights into osteogenic and chondrogenic differentiation of human bone marrow mesenchymal stem cells and their potential clinical applications for bone regeneration in pediatric orthopaedics. *Stem Cells Int.* 2013; 2013: 312501.(Level V)
9. Reichert JC, Cipitria A, Epari DR, Saifzadeh S, Krishnakanth P, Berner A, et al. A tissue engineering solution for segmental defect regeneration in load-bearing long bones. *Sci Transl Med.* 2012; 4: 141.
10. Wright JG, Yandow S, Donaldson S, Marley L, Simple Bone Cyst Trial Group. A randomized clinical trial comparing intralesional bone marrow and steroid injections for simple bone cysts. *J Bone Joint Surg Am.* 2008; 90: 722-730.(Level I)
11. Neen D, Noyes D, Shaw M, Gwilym S, Fairlie N, Birch N. Healos and bone marrow aspirate used for lumbar spine fusion: a case controlled study comparing healos with autograft. *Spine (Phila Pa 1976).* 2006; 31: E636-40.(Level III)
12. Gan Y, Dai K, Zhang P, Tang T, Zhu Z, Lu J. The clinical use of enriched bone marrow stem cells combined with porous betatricalcium phosphate in posterior spinal fusion. *Biomaterials.* 2008; 29: 3973-3982.(Level II/III)
13. Miyamoto T, Muneta T, Tabuchi T, Matsumoto K, Saito H, Tsuji K, et al. Intradiscal transplantation of synovial mesenchymal stem cells prevents intervertebral disc degeneration through suppression of matrix metalloproteinase-related genes in nucleus pulposus cells in rabbits. *Arthritis Res Ther.* 2010; 12: R206.
14. Mesoblast. Intervertebral Disc Repair. Available at: http://www.mesoblast.com/products/orthopedic-diseases-of-the-spine/intervertebral-disc-repair. Accessed May 1st, 2014.
15. Park JH, Kim DY, Sung IY, Choi GH, Jeon MH, Kim KK, et al. Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans. *Neurosurgery*. 2012; 70: 1238-47; discussion 1247. (Level IV)

16. Tamaki T, Hirata M, Soeda S, Nakajima N, Saito K, Nakazato K, et al. Preferential and comprehensive reconstitution of severely damaged sciatic nerve using murine skeletal muscle-derived multipotent stem cells. *PLoS One*. 2014; 9: e91257.

17. Nejadnik H, Hui JH, Peng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med*. 2010; 38: 1110-1116. (Level III)

18. Lozito TP, Tuan RS. Mesenchymal stem cells inhibit both endogenous and exogenous MMPs via secreted TIMPs. *J Cell Physiol*. 2011; 226: 385-396.

19. Sato M, Uchida K, Nakajima H, Miyazaki T, Guerrero AR, Watanabe S, et al. Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. *Arthritis Res Ther*. 2012; 14: R31.

20. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician*. 2008; 11: 343-353. (Level IV)

21. Dallari D, Savarino L, Stagni C, Cenni E, Cenacchi A, Fornasari PM, et al. Enhanced tibial osteotomy healing with use of bone grafts supplemented with platelet gel or platelet gel and bone marrow stromal cells. *J Bone Joint Surg Am*. 2007; 89: 2413-2420. (Level I/II)

22. Maclaine SE, McNamara LE, Bennett AJ, Dalby M, Meek RM. Developments in stem cells: implications for future joint replacements. *Proc Inst Mech Eng H*. 2013; 227: 275-83. (Level V)

23. Rastogi S, Sankineani SR, Nag HL, Mohanty S, Shivanand G, Marimuthu K, et al. Intratraumatic autologous mesenchymal stem cells in management of osteonecrosis of femur: a preliminary study. *Musculoskelet Surg*. 2013; 97: 223-8. (Level III)

24. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells*. 2007; 25: 2648-2659.

25. Adams SB Jr, Thorpe MA, Parks BG, Aghazarian G, Allen E, Schon LC. Stem cell-bearing suture improves Achilles tendon healing in a rat model. *Foot Ankle Int*. 2014; 35: 293-299.

26. Pascual-Garrido C, Rolan A, Makino A. Treatment of chronic patellar tendinopathy with autologous bone marrow stem cells: a 5-year followup. *Stem Cells Int*. 2012; 2012: 953510. (Level IV)

27. Planka L, Srnec R, Rauser P, Stary D, Filova E, Jancar J, et al. Nanotechnology and mesenchymal stem cells with chondrocytes in prevention of partial growth plate arrest in pigs. *Biomed Pap M ed Fac Univ Palacky Olomouc Czech Repub*. 2012; 156: 128-34.

28. Guan M, Yao W, Liu R, Lam KS, Nolta J, Jia J, et al. Directing mesenchymal stem cells to bone to augment bone formation and increase bone mass. *Nat Med*. 2012; 18: 456-462.

29. Sen RK, Tripathy SK, Aggarwal S, Marwaha N, Sharma RR, Khandelwal N. Early results of core decompression and autologous bone marrow mononuclear cells instillation in femoral head osteonecrosis: a randomized control study. *J Arthroplasty*. 2012; 27(5): 679-86. (Level II)

30. Gangji V, De Maertelaer V, Hauzeur JP. Autologous bone marrow cell implantation in the treatment of non-traumatic osteonecrosis of the femoral head: Five year follow-up of a prospective controlled study. *BMC Musculoskelet Disord*. 2009; 10: 20. (Level II)

31. Clarke AW, Alyas F, Morris T, Robertson CJ, Bell J, Connell DA. Skin-derived tenocyte-like cells for the treatment of patellar tendinopathy. *Am J Sports Med*. 2011; 39(3): 614-23. (Level I)

32. Guan M, Yao W, Liu R, Lam KS, Nolta J, Jia J, et al. Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. *Cell Transplant*. 2007; 16: 563-577. (Level IV)

33. Kim SJ, Shin YW, Yang KH, Kim SB, Yoo MJ, et al. A multi-center, randomized, clinical study to compare the effect and safety of autologous cultured osteoblast(Ossron) injection to treat fractures. *BMC Musculoskelet Disord*. 2009; 10: 20. (Level II)
36. Cristante AF, Barros-Filho TE, Tatsui N, Mendrone A, Caldas JG, Camargo A, Alexandre A, Teixeira WG, Oliveira RP, Marcon RM. Stem cells in the treatment of chronic spinal cord injury: evaluation of somatosensitive evoked potentials in 39 patients. *Spinal Cord.* 2009 Oct; 47(10): 733-8. (Level IV)

37. Skowroński J, Skowroński R, Rutka M. Cartilage lesions of the knee treated with blood mesenchymal stem cells - results. *Ortop Traumatol Rehabil.* 2012; 14(6): 569-77. (Level IV)

38. Connell D, Datir A, Alyas F, Curtis M. Treatment of lateral epicondylitis using skin-derived tenocyte-like cells. *Br J Sports Med.* 2009; 43(4): 293-8. (Level IV)

39. Giannini S, Buda R, Vannini F, Cavallo M, Grigolo B. One-step bone marrow-derived cell transplantation in talar osteochondral lesions. *Clin Orthop Relat Res.* 2009; 467(12): 3307-20. (Level IV)

40. Jäger M, Jelinek EM, Wess KM, Scharfstädt A, Jacobson M, Kevy SV, Krauspe R. Bone marrow concentrate: a novel strategy for bone defect treatment. *Curr Stem Cell Res Ther.* 2009; 4(1): 34-43. (Level IV)