Review Article

Mesenchymal Stem Cells and Orthopaedics (A Current Concept)

Abstract

Orthopaedics tissues, such as bone, cartilage, and tendon, involve cells that are difficult to culture and grow *in vitro* for reconstruction of damaged tissues. A small number of cells called stem cells have the ability to self-renew and differentiate into connective tissue lineages, involving bone, cartilage, tendon and ligaments**.** Recent development in stem cell research has led to an exciting effort in applying stem cells for orthopaedics tissue regeneration. This review summarizes recent findings regarding the potential clinical use of Mesenchymal Stem Cells (MSCs) in Nonunions, Osteogenesis imperfect, Human Cartilage defects, Osteoarthritis and Rheumatoid arthritis to provide a better understanding of the issue engineering with stem cell research, as well as the potential therapeutic purpose of these cells in orthopedic surgery.

Keywords: Mesenchymal stem cells, Orthopaedics, Clinical trial

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*** Amin Razi, MD; * Mohammad-Taghi Peivandi, MD; * Ali Birjandinejad, MD;**
*** Ali Parsaa, MD; **Sara Amel Farzad, MD; ***Maryam Hosseini Hasanabady**

*Department of Orthopaedics, Mashhad University of Medical Sciences, Emdadi Kamyab Hospital, Mashhad, Iran,
**Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
***Department of Biology, Science and Research Branch, Islamic Azad University, Mashhad, Iran

Corresponding Author:
Mohammad-Taghi Peivandi, MD
E-mail:
PeivandiMT@mums.ac.ir

Introduction

In the United States, tendon, ligament, and joint capsular injuries report for 45% of the 32 million musculoskeletal injuries each year[1]. and more than half a million patients receive bone defect repairs, with a cost higher than $2.5 billion[2]. A lot of studies were published about the noticeable shortcomings, limitations, and complications of modern clinical treatments for bone repair and regeneration and other disease connected with orthopaedic. Regenerative medicine (RM) refers to a field in the health sciences that helps to improve, enhance and re-build organ-specific repair mechanisms to reconstitute organ structure and function[3] in order to restore or establish normal function. RM is so extensive that subdividing the field into clusters to bring together scientists with overlapping backgrounds is important. Today, RM has advanced to be of interest in orthopaedics. This technique is a considerable hope was set on RM to develop substitute therapies for huge bone defects, cartilage damage, and atrophic tendon ruptures during the last decade[4]. Stem cell (SC), scaffold, and growth factors are essentially important in RM and tissue engineering. Stem cells originated at the end of the 19th century as a theoretical theorize to account for the ability of certain tissues (blood, skin, etc.) to self-renew for the lifetime of an organism even though they are involved of short-lived cells[5]. However, clinical operation of this cells requires more time .This review focuses specifically on application of MSCs and scaffolds in RM for orthopaedic indications. We discuss the history of SCs, their use in preclinical trials and clinical trials and current approaches in orthopaedic History of SC beginning by Till and McCulloch (1961) who while subjecting the mice with lethal doses of radiation pursued by injection of bone marrow cells found that these cells formed clumps due to cells cloned from them that was the main reason of survival of the mice[6].

Later studies defined SC potential for differentiation into definite cell types without senescence, and called as SCs. SCs are defined by two functional properties: an obviously unlimited capacity for self-renewal and the ability to develop multiple mature cell types (multi potentiality)[7]. SCs necessarily present in all normal tissues and defined, in general, as resting cells (not actively proliferating) that are being in small numbers in normal tissues[8]. During injury, a SC self-renews – undergoes cell division and gives rise to two cells[9]. One of them (daughter cell) proliferates symmetrically, often for many cell divisions, to produce a plenty of progeny referred to as progenitors. These progenitors subsequently differentiate to form a mature tissue. In comparison, the second cell returns to the original resting state of the mother cell until a new activating signal or event occurs[10]. SCs are a certain population of cells that form the source of tissues. SCs can be further divided into two major groups: the first group is embryonic SCs (ESCs), that together with the totipotent zygote present a cell population able to give rise to a multitude of cell types and tissues[11]. The second type of SCs is adult SCs (ASCs), that locate in adult tissues and give rise to differentiated, tissue-specialized cells [12]. Many of scientist consider the zygote to be the totipotent cell because it is able to differentiate into any cell type[13]. ASCs can be achieved from different sources such as bone marrow, peripheral blood, umbilical cord, fetal liver, neural tissue and probability to use in autologous therapy[14]. MSCs are pluripotent progenitor cell that divide many times and progeny finally gives rise to skeletal tissues: cartilage, bone, tendon, ligament, connective tissue[15]. MSCs was in first diagnosed by Friedenstein et al. as a population of mononuclear, fibroblast-like tissue culture adherent cells capable of colony formation[16]. In normal tissue, the number of MSCs is low and declines with age, e.g. the number of MSCs shows at the most 0.001% to 0.01% of the original mononuclear cells of bone marrow and this population is heterogeneous[17]. MSCs have been defined over the expression of various CD markers (CD34, CD45, CD14, CD11b, CD19, and HLA-DR negative and STRO-1, CD29, CD73, CD90, CD105, CD106, CD166, CD146, and CD44 positive)[18]. MSCs separated from the adult sources involving brain, skin, heart, kidneys and liver[19], synovial membrane[20], peripheral blood[21], umbilical cord blood[22], adipose tissue[23], deciduous teeth[24], dental pulp[25], bone marrow[26] and amniotic fluid[27] through an almost simple protocol that mainly relies on their ability to adhere to plastic in tissue culture . MSCs can be grown for many generations in the laboratory and still retain a permanent morphology and normal chromosome complement[28].

Migration

MSCs have migratory capacity. When MSCs transplanted systemically have ability to transport to sites of physical damage or injuries. Chemokine receptors and their ligands and adhesion molecules have a main role in migration process[28]. After acute injury (hours to days), MSCs can regulate or balance local and systemic inflammatory responses (local and systemic) by producing immunosuppressive factors, such as transforming growth factor β, prostaglandin E2 and indoleamine 2,3-dioxygenase 1 (commonly known as IDO). In next stage or intermediate periods (from days to weeks), MSCs can contribute to the repair process by differentiating into chondrocytes and osteoblasts. MSCs have a considerable benefit when introduced late (from weeks to months), but at least one instance in which late delivery of MSCs can be beneficial is delayed union or non-union of bone[29].

Differentiation

Differentiation of MSCs toward other tissue cell types including muscle, tendon/ligament, and stromal tissue[30]. has been reported. Moreover, the regeneration ability of MSCs to non-mesenchymal lineages, such as cardiac,

neuronal, and skin tissues, has been proved[17]. Identification of MSCs differentiation pathways will be critical in the design of three-dimensional culture systems and bioreactors for automated bioprocessing through mathematical models used to systems biology and network science. Specially, the Wnt signaling pathway and transforming growth factor-beta (TGF-beta)/bone morphogenetic protein (BMP) signaling pathways are well known to modulate in MSCs the molecular differentiation into cartilage and bone. In addition, it is demonstrated that physical factors can also participate in the regulation of MSC differentiation[31]. Fibroblast growth factor (FGF)-2, has been shown to promote cell proliferation and to maintain the MSC population in an extended undifferentiated state[32]. Moreover, two pathways, centered on FGF-2, and platelet-derived growth factor (PDGF), that proved to be main in the growth and essential in the differentiation of MSCs[33].

Scaffold

In the middle of 1980s, RM has continued to evolve as an exciting and multidisciplinary field aiming to expand biological substitutes to repair, replace or regenerate damaged tissues. SC and scaffolds are generally indicated as the RM triad, the key components of RM. Scaffolds, structural support, prepare an essential niche for cell attachment and subsequent tissue development[34]. Ideally, a scaffold in RM should have four characteristics: 1) high porosity, and a high surface-area to volume ratio, with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste, 2) holding appropriate surface properties promoting cell adhesion, proliferation and differentiation, 3) sufficient mechanical properties and any in vivo stresses, 4) biocompatibility[35]. Nowadays, 3D scaffolds is superior to the 2D scaffolds for cell proliferation and the cell growth in the 3D scaffolds continues for longer time periods than that of 2D scaffolds[36]. Scaffolds for RM fall into two general categories: natural and synthetic. Natural scaffolds including chitosan, small intestinal sub mucosa, collagens, renal capsule matrix, and silk fibers. In contrast, synthetic scaffolds have been derived from polymeric materials such as poly-L-lactic acid, modified poly (DL-lactide-co-glycolide) (PLGA) or polyglycolic acid (PGA)[37].In addition, several of the interested scaffold in the RM are macroporous calcium phosphate ceramics, particularly HA, tricalcium phosphate (TCP) or biphasic mixtures (BCP) that have been widely used for scaffolding cells[38].This materials are appealing materials for RM because they can homogeneously suspend cells while allowing rapid diffusion of nutrients and metabolites[39]. Chitosan-based scaffolds in perfusion bioreactors and knitting PLGA nanofibres around a PLGA scaffold are examples of both instances[40]. Both natural and synthetic scaffolds are reported as increasing extracellular matrix (ECM) deposition over controls.

Application of MSCs in Orthopedics

The most interesting properties of MSCs are easily isolated from the bone marrow (BM)[41], immunologically tolerated as an allogeneic transplant[42], and multilineage potential have led to acute investigation as a cell-based therapeutic[43]. Nowadays, a variety of studies initially focused not only on their characterization, but also on their using in the treatment of several diseases[44]. MSCs have a great potential for therapy involving their unique characteristics has been demonstrated in various in vivo disease models and has shown supportive results for probable clinical use[45]. MSCs, derived from ASCs, are probably the most engaging SCs for orthopedic applications because of their potential to differentiate to both bone and cartilage[17]. Recently, MSCs are now being explored in clinical, trials for various conditions, including orthopedic injuries, graft versus host disease (GVHD) following bone marrow transplantation (BMT), autoimmune diseases, cardiovascular diseases, and liver diseases. In

addition, MSCs modified to overexpress antitumor genes has provided prospects for use as anticancer therapy in clinical settings[46].

Bone Fracture Non-union

Bone ability to fracture repair may be compromised by the size and location of the bone defects, and by connected vascular or soft tissue injuries[38]. In general, bone fractures that fail to repair even after 6-8 months of therapy are regarded as non-union[47]. Fracture non-union occurs in about 15 percent of patients of complex trauma as a result of mechanical factors, as seen in comminuted fractures with multiple bone fragments; infection, as seen with bacterial contamination of the injury site or a patient's basic viral diseases; smoking and other tobacco- related or drug-related toxins; and endocrine disorders, such as type 2 diabetes mellitus, obesity, osteopenia and osteoporosis[29]. In contrast with cardiac repair studies that applied a populations of MSCs, a single cell type, MSCs, has been used in studies of bone repair[48]. Cell-based therapies, especially SC strategies, for fracture repair in cases of nonunion are currently receiving noticeable attention. The use of MSCs for fracture repair has been tried successfully. Various MSCs have osteogenic potential and, as mentioned, are present in bone morrow and other tissues. These cells can be obtained by bone morrow aspiration of the iliac crest[38]. Mechanisms can help to enhance bone repair occurs involves directly providing MSCs for osteogenic differentiation and bone formation, as well as enhanced osteoinductivity of the biomaterial by the release of osteogenic growth factors and stimulation of the migration and differentiation of host osteoprogenitors. Preclinical trials with MSCs have confirm effective in advancing bone repair in various scenarios, involving- critical-size femoral defects, cranio- maxillofacial deformities, and spinal fusions . The number and concentration of this cells and were transplanted for the treatment of nonunion assessed by Hernigou et al. They exhibited that autologous bone-marrow grafting by injection is an useful and safe method for the treatment of an atrophic tibial diaphyseal non-union. However, this method efficacy develops to be related to the number of cells in the graft, and the number of available cells in bone marrow aspirated from the iliac crest appears to be less than optimal in the absence of concentration[49]. Nowadays, two techniques have been employed in the preclinical and clinical protocols while managing the critical defects. In the first protocols used of directly SCs injection at the lesion site and in other they were expanded ex vivo before being implanted. Scientist concluded that both the approaches were equally correct in principle but will require further studies to demonstrate unambiguously their efficacy in such conditions[50,51]. In recent multi centric study in Italy ,France ,Spain and Germany 108 patients with nonunion of long bones fractures involved and three method of surgery randomly performed ,they compare the efficacy of autologous mesenchymal stromal cell versus iliac crest autograft to enhance bone healing .this clinical trial start at 2017 and to be continued . The characteristics and number of the extracted cells require further studies.

Osteogenesis imperfecta

Features of osteogenesis imperfect (OI) are: osteoporosis, low strength, severe bone fragility and skeletal malformations caused by various mutations in structure and type I collagen[52]. Affects 1 in 15,000 births and currently has no treatment[53]. Common treatments for OI: non-surgical treatments including physiotherapy, rehabilitation, casting and splinting. surgical treatments including interamedullary nailing, spinal and basilar impression surgery) and pharmacological management (drugs to increase bone density and decrease the chance of fractures, for example bisphosphonates or growth hormone, depending on the type of OI)[54,55]. Nowadays, SCs have been indicated as an alternative and new OI treatment[56]. A number of studies

have exhibited the beneficial effects of MSCs for OI. Horwitz et al. investigation describes clinical achievement of the first children to undergo allogeneic bone marrow transplantation for severe OI, a genetic disorder represented by defective type I collagen, osteopenia, bone fragility, severe bony deformities, and growth retardation[57]. Westgren M et al. investigate on prenatal mesenchymal stem cells (MSC) transplantation and they hypothesize that it is safe and effective in treatment of OI but require multidisciplinary working to develop guidelines[58]. Mesenchymal cells to treat six children who had undergone standard bone marrow transplantation for severe OI. Five of six patients showed an acceleration of growth velocity during the first 6 month postinfusion[59]. Le Blanc et al. used adult human leukocyte antigen (HLA)-matched MSC in SC therapies of OI. These findings exhibited that allogeneic fetal MSC can engraft and differentiate into bone in a human fetus even when the recipient is immunocompetent and HLA-incompatible [60].

Cartilage

Cartilage has limited capacity for regeneration once it is injured because of the lack of blood supply. This tissue after injuries repaired with fibrous tissue which have not efficiency of normal hyaline cartilage[61]. Therefore, different treatment system developed for cartilage repair but this systems has many complications, such as chondrocyte dedifferentiation during expansion in vitro, easy suspension of injected cells, and impaction of injected cells in cell sap[62]. Recently, most treatment technique focus on use of SCs and other source of cells to obtain sufficient quantities necessary for tissue regeneration[63]. At the beginning of this, new systems by using assorted scaffolds and cell sources to induce chondrocyte regeneration have emerged[64]. MSCs have obtained applied in repair of cartilage tissue due to various causes, such as the ability to differentiate into connective tissue, such as hyaline cartilage, and the separation from various tissues such as bone marrow, fat tissue, and umbilical cord[36]. In the last two decades, valuable investigations have been made to evaluate MSC potential in repair of cartilage defects in animal models and human.

Humans Cartilage defects

Wakitani et al. study determined the effectiveness of autologous BM stromal cell transplantation for the repair of articular cartilage defects in the patellae of female and male. Six months after transplantation, pain and walking ability of patients had improved notably and for the next years, improvement has persisted in effect and both patients have been satisfied with the result[65]. In a similar work, Kuroda et al. used of autologous BMSC, that were embedded within a collagen scaffold. Seven months after transplantation, result exposed the defect to be covered with a new smooth tissue. Next year after transplantation, the clinical symptoms had improved significantly[66]. In the next investigation of Wakitani et al., autologous culture-expanded bone marrow mesenchymal cell (BMMC) transplantation into nine full-thickness articular cartilage defects of the patello-femoral joints in the knees of three patients. This outcome also showed that clinical symptoms had improved and the improvements have been remained over the follow-up periods[67]. Although, past work reported various factors to direct SC differentiation lineage, but our knowledge is little about how nature orchestrates the MSC differentiation and bone morphogenesis during skeleton development and bone regeneration. Lee et al. proposed a novel, new technique without scaffold for cartilage repair in the human knee that combines arthroscopic micro fracture and outpatient intra-articular injections of autologous bone marrow-derived MSCs and hyaluronic acid (HA). After 24.5 months, improvements have been maintained in physical component score and visual analogue pain scores in both treatment groups[68]. MSCs is an effective

treatment modality for improvement in the knee injury and osteoarthritis outcome score (KOOS), Lysholm score, visual analog scale, and KOOS pain scale in patients with ICRS grade 3 or 4 lesions for up to 6 years. Nowadays, gene therapy could be an encouraging strategy for efficient treatment of cartilage defects. In many studies, MSC- mediated gene delivery has been applied for this patient using a variety of chondrogenic growth factors[69,70]. The application of gene transfer to defected cartilage was beignet by Evans and co-workers to treat arthritis. This treatment can be concluded by either direct vector administration to cells located at or surrounding the defects, or by transplantation of genetically modified chondrogenic cells into the defect[69]. OA is well suited to local, intra-articular gene therapy. The synovium and the cartilage are possible intra-articular sites of gene transfer. Interestingly, when MSCs transplanted to synovial and meniscal surfaces, this is demonstrated that MSCs served an orchestrated role as opposed to supplying the direct building blocks of regeneration. In general MSCs transplantation has good results in cartilage defect but need technical improvement.

Osteoarthritis (OA)

Osteoarthritis (OA) is the most common chronic condition of the joints involves the erosion of articular cartilage, inflammation of synovial membrane, and resorption of the underlying subchondral bone affecting 80% of old people. Current treatments for OA are largely limited to analgesics and anti-inflammatory drugs that only provide symptom relief[71]. A number of studies have determined application of MSCs for patients with severe OA. In addition, a more generic approach to current treatment methods revolves around some combination of non-pharmacological and pharmacological treatment modalities[72]. Wakitani et al. transplanted cells to repair human articular cartilage defects in osteoarthritic knee joints. After forty-two weeks, metachromasia was detected in almost all areas of the sampled tissue and hyaline cartilage-like tissue was partly observed[67]. Centeno et al. has effectively used the MSCs to regenerate the cartilage and damaged knee meniscus. After five months of post-injection, the patient had statistically significant cartilage and meniscus growth[71]. In Emadedin and coworker indicated the potential of intra-articular injection of MSCs has been evaluated in six OA patients. They found no local or systemic adverse events after a year. Walking distance and functional status of the knee improved up to six months post-injection, after that pain appeared to be slightly increased and patients' walking abilities lightly decreased[72]. In addition intra-articular SC injections[73,74] and surgical SC transplantation for osteoarthritis have been considered so far using bone marrow-derived MSCs and adipose-derived SCs[75]. Osiris Therapeutics, Inc. (Columbia, MD, USA), in a phase I/II trial conducted intra-articular administration of allogeneic BMSCs in patients with OA significantly reduced pain in comparison with the placebo group. Interestingly, this effect was observed even in patients receiving a low dose (50 million cells) as well as in patients receiving a high dose (150 million cells). However, Magnetic resonance imaging (MRI) result of the treated knee showed wide variability in the meniscus volume between the cell- treated and the control groups of patients[76-77]. In general, despite the tendency for cell infusion, long-term results are not clear and high quality clinical trials would be needed.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an inflammatory arthritis that affects nearly 1% of the world's adults. It is detected by symmetric polyarticular inflammation of the synovium, usually affect small joints of the hands (MCP and PIP), wrists and feet[78]. Pain and stiffness are a common symptom of this inflammatory, and can lead to progressive joint damage resulting in deformities and loss of function.

Persistent inflammation causes to erosive joint damage and functional impairment in the vast majority of patients. Early detection and treatment can affect symptomatic course, inhibit the progresion of joint Destruction or retard progression of erosive disease. In contrast, late diagnosis or no treatment, inflammation will cause to joint damages and bone destruction specially within the two years of disease onset.

Conclusion

In summary, MSCs provide exciting and promising strategies for repair of bone, curtilage, tendon and other tissues. Our knowledge of MSCs in the biological process of tissue regeneration continues to grow. In future, advanced studies are necessary to achieve both efficacy and safety in patients. In addition, the clinical application of MSCs will likely require in vitro expansion prior to therapeutic use.

The important point in this regard is the extraction method and the number of cells. Also, the acquired cells should be considered in terms of biomarkers and differentiation power, so in the treatment of the non union , the results are better, but in knee osteoarthritis it is not recommended and further high-level evidence studies are needed.

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