

Orthopedic cellular therapy: An overview with focus on clinical trials

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Abstract

In this editorial, the authors tried to evaluate the present state of cellular therapy in orthopedic field. The topics the authors try to cover include not only the clinical trials but the various research areas as well. Both the

target diseases for cellular therapy and the target cells were reviewed. New methods to activate the cells were interesting to review. Most advanced clinical trials were also included because several of them have advanced to phase III clinical trials. In the orthopedic field, there are many diseases with a definite treatment gap at this time. Because cellular therapies can regenerate damaged tissues, there is a possibility for cellular therapies to become disease modifying drugs. It is not clear whether cellular therapies will become the standard of care in any of the orthopedic disorders, however the amount of research being performed and the number of clinical trials that are on-going make the authors believe that cellular therapies will become important treatment modalities within several years.

Key words: Orthopedics; Cellular therapy; Treatment gap; Disease modifying drugs; Standard of care

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Core tip: The use of cellular therapies for the treatment of orthopedic diseases is one of the pioneering developments in the history of medical research. Many papers have reported on basic research on cellular sources and methods to localize the cells. Although many review articles have been published, papers discussing clinical trial status were not always available. The authors attempted to review not only the research status of cellular therapy but the status of clinical trials which are on-going in the United States. We hope this editorial can help orthopedic surgeons in keeping up to date in their knowledge of clinical and research stage cellular therapy.

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INTRODUCTION

Orthopedic diseases are one of the earliest targets for cellular therapy. Cartilage repair was the first indication for cellular therapy and bone repair has been tried clinically with bone morphogenic protein (BMP). Mesenchymal stem cells (MSCs), embryonic stem cells, umbilical cord blood cells, primary cultured cells from specific sites and cell mediated gene therapies are the possible sources of cells for tissue repair. Quite naturally, cellular therapy became the one of the promising solutions in the regenerative medicine field. After reviewing papers, the authors came to believe that cellular therapy has great potential for becoming the standard of care in certain orthopedic disorders. We believe it is time for orthopedic surgeons to review the advancements of orthopedic cellular therapies within the scope of research and clinical trials.

HISTORY OF CELLULAR THERAPY AS TISSUE ENGINEERING

In 1994, one of the pioneering papers in orthopedic field was presented in the *New England Journal of Medicine*^[1]. Lars Peterson's group tried to regenerate cartilage tissue with autologous cartilage cells and showed evidence of regeneration in both animals and humans. Until that point, most orthopedic surgeons believed that cartilage tissue cannot regenerate. This article was considered as a breakthrough but raised many questions about the mechanism of action. Nevertheless, they showed clearly that cartilage tissue can be regenerated by infusing cartilage cells into the lesion site. That means the cartilage cells can adhere to the site and produce type II collagen and glycosaminoglycan at the damaged area.

As investigators' interests increase about tissue engineering with different sources of cells, many cells have been tried to show the regeneration potential for orthopedic disorders^[2,3]. Cell adhesion studies, mechanical stimulation and cytokine stimulation of cells have been reported to elucidate the mechanism of action^[4,5]. Stem cells have been most widely used^[6], and cell mediated gene therapies have reached phase III clinical trials^[7]. It is quite amazing that this new era of cell based on orthopedic therapies has developed into a burgeoning new industry^[8]. The relationship of United States spending to innovation of regeneration therapies has also been reported^[9]. Even though autologous chondrocyte transplantation was not successful commercially, the potential revenue generation of regenerative medicine using cellular therapy is becoming a more important economic issue. Allogeneic cells with mass production potential may provide a possible answer for the commercial success of cellular therapy.

TENDON REGENERATION

This new technology garnered popular interest

after the reporting of autologous platelet injection into the knee joint to help repair ligament damage after a sports injury in a professional football player. The fans witnessed the superstar playing in critical games within short period of time after the injury. This was very impressive debut of cellular therapy in the orthopedic arena. Autologous platelet injection is in phase I clinical trials in United States. Tendon injuries can be considered as a serious and unsolved condition because the damaged tendon heals slowly and restoring structural integrity can be sometimes difficult even with a surgical procedure. Mechanical stimulation is another important factor that influences the healing process of the tendons^[10].

Chronic injury of the tendon is also challenge for orthopedic surgeons because there are not many transplantable tissues available. To regenerate the tendon and ligaments, MSCs and gene therapy approaches have been reported^[11]. Adipose derived stem cells have been tried for primary tendon repair^[12] and biomechanical and immunological evaluations have also been performed after treatment. Autologous adipose derived stem cells are sufficient in number to heal or regenerate the damaged tendons. Dosage effects on cellular responses and cytokine profiles have been reported^[13]. Interestingly, the lower dose of cells proved to be more effective in improving functional properties. We believe that different tissues will show different cell numbers are optimal for maximum efficacy. Skeletal muscle cells and bone marrow derived stromal cells were also used to compare the differentiation capabilities into the tendon^[14].

BONE REGENERATION

In 2008, Lee *et al.*^[15] published a review paper about cell therapy for bone engineering. They covered the issues such as sources of stem cells, scaffolds, gene therapy and clinical applications in nonunion, tumors, osteonecrosis, revision arthroplasties, and spine fusion. They concluded that there exist opportunities to translate MSC technologies into clinical treatments even though challenges remain. To overcome the challenges, cell sources have been evaluated in terms of the ability to scale up manufacturing procedures under current good manufacturing practice (cGMP) guidelines^[16]. The biological characteristics of peripheral blood cell derived MSCs were also evaluated to determine an adequate number of cells to regenerate bone^[17]. For use of cells in human, identifying a sustainable source of cells that can be manufactured in sufficient quantities is important for commercial success.

Apoptotic resistance, proliferation kinetics, cellular senescence, and karyotype analysis were performed to compare the characteristics of peripheral blood and bone marrow derived MSCs. The influence of hormones on osteogenic differentiation of MSCs was also evaluated^[18]. 17- β estradiol showed both osteogenic and adipogenic stimulatory effects *in vitro*. Estrogen

stimulated osteogenesis through both estrogen receptor (ER) α and β and stimulated adipogenesis through ER β ^[19]. Dexamethasone supplementation to expand the MSCs was also evaluated and it was shown that a low concentration rather than the physiological concentration facilitated osteogenic proliferation^[20]. Other cell types besides bone marrow derived MSCs, such as umbilical cord-derived MSCs (UCB-MSCs), adipose-derived stem cells (ADSCs), muscle-derived stem cells and dental pulp derived stem cells were also evaluated^[21]. A gene therapy approach also showed osteogenic potential with *BMP-2* gene^[22] transfected chondrocytes. We believe that the chondrocytes can induce bone formation through the endochondral ossification process.

Methods for modulating endochondral ossification with multi-potent stromal cells were also reported by Gawlitta *et al.*^[23]. In this paper, potent modulators of endochondral bone formation including oxygen tension and mechanical stimuli were reviewed. We believe that autocrine stimulation of chondrocytes with BMP-2 protein production within the cell can also modulate the endochondral ossification. Cell adhesion is a very important issue to consider in determining the mechanism of action of how the cells can generate tissue. Cell adhesion to scaffolds with extracellular matrix proteins was reviewed^[24]. Extracellular matrix proteins can be an anchor for the cells to adhere in bone and cartilage damaged sites. Alkaline phosphatase, osteonectin, BMP-2 and Runx2 expression were used to evaluate the efficacy of bone regeneration^[25]. With these parameters, bone marrow MSCs showed a better capacity for osteogenic differentiation than unrestricted somatic stem cells and adipose MSCs.

Cellular interaction between two different cell types is very interesting topic for differentiation and proliferation. Zachos *et al.*^[26] cultivated MSCs together with programmable cells of monocytic origin (PCMO) to test whether co-cultures promote the osteogenic differentiation process. They showed that PCMO obviously promote osteogenic differentiation of MSCs *in vitro*. Mixed cell therapy can be another way to address the problem of providing greater cell numbers. A dynamic 3-D culture system was evaluated to assess the effect on proliferation and differentiation of MSCs^[27]. The authors observed the increased ingrowth and osteogenic differentiation in 3-D dynamically cultured human MSCs. They explained this phenomenon by generation of fluid shear stress and enhanced mass transport to the interior of the scaffold mimicking the native microenvironment of bone cells. Red light emitted from a light-emitting diode was also evaluated for its effect on MSCs^[28]. They concluded that noncoherent red light can promote proliferation but cannot induce osteogenic differentiation of MSCs. Low level laser irradiation was also tried for MSC proliferation^[29], and the authors concluded that low-level laser irradiation might lead to reduction in healing times and potentially reduce risks of failure.

A preliminary trial of autologous adipose-derived

stem cells trial from elderly patient with osteoporosis provided interesting observations that bear watching for future study^[30]. The authors used a collagen I hydrogel scaffold with ADSCs and showed osteogenic potency. In rabbit model, Fu *et al.*^[31] showed enhanced bone formation and demonstrated successful posterolateral spine fusion by using a combination of MSCs with low dose rh-BMP-2 proteins. The BMP protein has already reached the clinic for dental use and ADSCs are in phase II clinical trials now. One of the most interesting clinical trials in orthopedic field was reported in Japan^[32]. The authors injected MSCs mixed with β -tricalcium phosphate (β -TCP) in 10 patients with idiopathic osteonecrosis of the femoral head. All procedures were successful and some young patients with extensive necrotic lesions demonstrated good bone regeneration with amelioration of pain. They performed this procedure with a vascularized iliac bone graft. It is not clear that the regeneration happens only with MSCs because the effect of β -TCP and the vascularized bone graft cannot be clearly ruled out. A multiplex rehabilitation program^[33] also helped the patients to achieve significant improvements in physical function and pain. In addition to this non-life-threatening disorder, MSCs were used for treating malignant bone tumors^[34]. The authors injected the MSCs to the host-to-allograft bone junction after bone tumor resection in 92 patients. They found no increase in the local cancer recurrence rate in patients after an average follow-up period of 15.4 years. MSCs were also used clinically to ameliorate the host vs graft rejection phenomenon.

CARTILAGE REGENERATION

Osteoarthritis (OA) is a disease of aging. The patient population is so large that it is not easy to calculate how many patients are there in the world. But in the United States alone, more than 500000 patients undergo total knee replacement arthroplasty every year. The course of OA is so long that each patient has to have customized treatment according to their stage of OA. Nevertheless, the currently available treatments until do not adequately cover each patient's need. There is a definite need for optional treatments in moderate and severe OA. In addition, there is no disease modifying treatment for OA currently available. This treatment gap opens a large avenue for cellular therapy. To achieve tissue regeneration with cells, the mechanism of action is to make the basic elements of cartilage within the damaged area. Cytokines produced by the cells can be useful to improve the healing by augmenting the body's own regenerative potential^[35]. A lot of preclinical research and numerous clinical trials have been reported for cartilage repair^[36].

As previously mentioned, autologous chondrocyte implantation was the first treatment to regenerate cartilage. A recent paper pointed out that the OA is a rising global burden among musculoskeletal diseases^[37].

They explore the challenges associated with cartilage repair using cell-based therapies. The cell-based therapies also allow the versatility of using scaffolds and growth factors, or gene therapy^[38]. They pointed out the challenges in identifying the optimal source of stem cells, along with the conditions that enhance expansion and chondrogenesis. Brady *et al.*^[39] studied Transforming growth factor- β 3 (TGF- β 3) and BMP-2 for their potential to generate type II collagen and proteoglycan. They concluded that these growth factors can initiate chondrogenesis. Warsat *et al.*^[40] also showed that TGF- β enhances the integrin α 2 β 1-mediated attachment of MSCs to type I collagen. Interestingly, TGF- β 1 and rhGDF-5 showed different responses to human MSCs^[41]. We believe that TGF- β 1 exhibits different responses depending on its concentration in accordance with its bimodal mode of action. Intra-articular injection of FGF-18 is currently in phase II clinical trials.

Small molecules that can modulate chondrogenesis were also reviewed^[42]. ERK1/2 inhibitor promoted chondrogenesis of MSCs. The influence of ascorbic acid and collagen matrix was also evaluated^[43]. An immunogenicity study of MSCs reported that chondrogenic differentiation may increase the immunogenicity of MSCs by leading to stimulation of dendritic cells. The up-regulated expression of B7 molecules on the chondrogenic-differentiated MSCs may be responsible for this event^[44]. The longevity of cells was also evaluated^[45] and showed that the chondrogenic potential of MSCs declines with age. Synovium-derived stem cells were also evaluated for chondrogenic potential^[46]. The combination of hypoxia, FGF-2 and extracellular matrix contribute to the highest expansion rate. They indicated that the three-dimensional micro-environment for *ex vivo* expansion can be optimized to provide high-quality stem cells for cartilage repair.

Materials that can promote cartilage regeneration were also interesting topics of study^[47]. Biomimetic composites such as biomaterial scaffolds, nano-fibrous scaffolds and hydrogels were reviewed. The interactions of these materials with embryonic stem cells, ADSCs, MSCs and progenitor stem cells were reported. The effects of chondroitin sulfate-coated nano-topographies on cell characteristics and chondrogenic differentiation on human MSCs were also investigated^[48]. This study demonstrated the sensitivity of MSC differentiation to surface nano-topography and highlighted the importance of incorporating topographical design in scaffolds for cartilage tissue engineering. Mineralized collagen was also reviewed for its influence on MSC proliferation^[49]. They concluded that the integration of transplanted cells and MSC associated matrix synthesis encourages the use of MSC loaded mineralized collagen for tissue engineering. A similar report was also published by Ragety *et al.*^[50]. They reported that cell attachment and distribution were improved on chitosan coated with type II collagen. A study of the effect of growth factors on the proliferation of MSCs encapsulated in a hydrogel scaffold was also reviewed and TGF- β 3 was

the most potent for maintaining the cell phenotype^[51]. Interestingly, an induced pluripotent stem cell approach without a scaffold showed enhanced chondrogenesis^[52]. The authors used electroporation-mediated transfer of SOX trio genes (*SOX-5*, *SOX-6*, and *SOX-9*) to enhance the chondrogenesis of MSCs.

Cellular therapy for the treatment of cartilage lesions is the most advanced in terms of clinical trials^[53]. However, the authors emphasized the need for a randomized study to evaluate the advantages and disadvantages. They also emphasized the need for long-term follow up. Arthroscopic injection of MSCs was evaluated in an animal model^[54]. Additionally single stage arthroscopic human cartilage repair procedures were evaluated in 30 patients^[55]. The surgical procedure involved debridement of the lesion, micro-fracture and application of concentrated bone marrow aspirate concentric cells with hyaluronic acid and fibrin gel under CO₂ insufflation. Clinical outcome showed significant benefit but the effect of cells only should be evaluated. The efficacy of cellular therapy can be augmented by combining it with multiple injections, arthroscopic injection and with minor surgery. A cell mediated gene therapy with irradiated TGF- β 1 transfected chondrocytes and normal chondrocytes (Invossa™) was evaluated in a placebo-controlled, randomized clinical trial in patients with Kellgren and Lawrence grade III OA of the knee with statistically significant improvement seen in pain (visual analog scale) and function (International Knee Documentation Committee subjective knee score)^[56].

SCAFFOLD AS A CARRIER

Scaffolds have been used for orthopedic disorders for long time. Porous coating of implants for the ingrowth of osteoid tissue is one example. They serve not only as the 3D structural support but also as an artificial extracellular environment to regulate stem cell behavior^[57]. Biomaterials with various physical, mechanical and chemical properties can be designed to control MSCs' development for regeneration. Murphy *et al.*^[58] compared several substances such as allografts, demineralized bone matrix, collagen and various forms of calcium phosphate for cellular proliferation. They concluded that biochemical and structural properties of biomaterials play in cellular function, potentially enhancing or diminishing the efficacy of the overall therapy. Autologous chondrocytes implanted into a scaffold (NeoCart™) is in phase III clinical trials. Small molecules have been impregnated to a poly (lactic-co-glycolic acid) scaffold to promote chondrogenesis^[59].

The cell/matrix/ceramic constructs showed immediate *in vivo* bone formation^[60]. For bone reconstruction surgeries, large defect area were filled with newly formed bone. In these cases, this technology may be a solution in consideration of the improved MSCs' proliferation and differentiation capacities. Magnetic nanoparticles (MNP) have been applied to aid the development and translation of orthopedic therapies

from research to the clinic^[61]. Characterization of cell localization and associated tissue regeneration can be enhanced, particularly for *in vivo* applications. MNPs have been shown to have the potential to stimulate differentiation of stem cells for orthopedic applications. Hydroxyapatite (HA)-containing composite nanofibers with MSCs were evaluated for osteogenic potential^[62]. They showed that the introduction of HA could induce MSCs to differentiate into osteoblasts. Moreover, 3D poly (3-hydroxybutyrate-co-3-hydroxyvalerated)/HA scaffolds made from aligned and random-oriented nanofibers were implanted into critical-sized rabbit radius defects and exhibited significant effects on the repair of cortical bone defects. A report on scaffold based management of osteochondral lesions of the human ankle was also reviewed^[63]. They concluded the regenerative surgical approach with scaffold-based procedures is emerging as a potential therapeutic option for the treatment of chondral lesions of the ankle. However, they concluded that well-designed studies are lacking, and randomized long-term trials are necessary to confirm the potential of this approach.

UMBILICAL CORD BLOOD CELLS AS RESOURCES

Cord blood banking has become very popular in many countries including the US. Theoretically, everybody can reserve their potential future personalized medicine in a bank. Cord-blood-derived stem cells have been proven clinically useful for numerous diseases as have been MSCs^[64]. MSCs in cord-blood heralds cord blood as an untapped resource for nonhematopoietic stem cell-based therapeutic strategies. Cord blood MSCs were compared with bone marrow-derived MSCs for the repair of segmental bone defects in a rabbit model^[65]. This study showed that cord-blood MSCs have similar biological characteristics and osteogenic capacity as bone marrow-derived MSCs. They concluded cord blood-derived MSCs can be used as a new source of seeding cells for bone regeneration. An additional study of osteogenesis *in vivo* evaluated seed cells with human cord blood cells for bone tissue engineering^[66]. They showed that cord blood MSCs loaded with the scaffold displayed the capacity for human osteogenic differentiation leading to osteogenesis *in vivo*. Human cord blood MSCs also exhibited an immature nucleus pulposus cell phenotype in a laminin-rich pseudo-three-dimensional culture system^[67].

FUTURE OF CELLULAR THERAPY IN ORTHOPEDICS

Recently, one of the interesting topics in biology is CRISPR/Cas9 as a versatile tool for engineering biology^[68]. By custom designed single gene modification, many genetic disorders can be treated. In addition, with iPS technology, reprogramming cells to switch their fate is

Table 1 Summary of cellular therapy for regenerative medicine

Target tissue	Source of cell	Ref.
Tendon	Autologous platelet	Sakabe <i>et al</i> ^[10] , 2011
	Mesenchymal stem cell	Hoffmann <i>et al</i> ^[11] , 2007
	Adipose-derived stem cell	Uysal <i>et al</i> ^[12] , 2012
	Stromal cell	Sassoon <i>et al</i> ^[14] , 2012
Bone	Peripheral blood-derived stem cell	Fu <i>et al</i> ^[17] , 2012
	Multi-potent stromal cell	Gawlitta <i>et al</i> ^[23] , 2010
	Adipose-derived stem cell	Jiang <i>et al</i> ^[30] , 2014
	Cord blood-derived stem cell	Fan <i>et al</i> ^[30] , 2012
Cartilage	Autologous cartilage cell	Brittberg <i>et al</i> ^[1] , 1994
	Mesenchymal stem cell	Vilquin <i>et al</i> ^[36] , 2006
	Synovium-derived stem cell	Li <i>et al</i> ^[46] , 2011
	Allogeneic chondrocyte	Ha <i>et al</i> ^[56] , 2012

possible^[69]. This paper reviewed landmark developments in cell reprogramming and technical developments on the horizon with significant promise for biomedical applications. Pluripotent stem cells can be directly generated from fibroblasts of the patient by gene transfer technology^[70]. The number of gene therapy clinical trials has increased dramatically worldwide since 2012^[71]. The basic knowledge gained by cellular differentiation research is enormous right now. The authors believe that by combining several technologies, there is hope for the near future in treating many orthopedic single gene mutation diseases that were previously untreatable.

CONCLUSION

The first human clinical trial of a cellular therapy was performed for an orthopedic disorder. Two of the major indications of cellular therapy are bone and cartilage repair. Most advanced clinical trials of cellular therapies are being performed for orthopedic disorders. The results of several clinical trials have been reported and showed initial indication of efficacy. Even though it is not clear whether cellular therapy can become a standard of care, the data are being generated to evaluate the efficacy of this technology (Table 1). Because there exists a definite treatment gap in some orthopedic disorders, the authors believe that cellular therapy can attain the status as a standard of care within several years especially when supplemented with procedures that improve the efficacy of these treatments.

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