Level V Evidence

The Role of Stem Cells and Tissue Engineering in Orthopaedic Sports Medicine: Current Evidence and Future Directions

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Abstract: The use of stem cell therapies for the treatment of orthopaedic injuries continues to advance. The purpose of this review was to provide an update of the current role and future directions of stem cell strategies in sports medicine. The application of cell-based treatments in the sports medicine arena has expanded in recent years. Promising preclinical results have led to translation of these novel therapies into the clinical setting. Early well-designed comparative clinical studies have also shown positive outcomes. Despite significant advances in this arena, there remains a need for additional high-powered and well-designed clinical trials to confirm the safety and efficacy of treatment.

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Stem-cell and cell-based therapies represent a novel approach to the repair and regeneration of various musculoskeletal tissues such as tendon, cartilage, and muscle. The ability to intervene at the molecular level to readjust the pathologic cascade has generated great interest among physicians and researchers across the numerous fields that treat sports medicine injuries. As a result, the application of cell-based treatments in the sports medicine arena has expanded in recent years. Although the majority of advancements have been at the preclinical level, the delivery of stem cell–based therapies for orthopaedic applications continues to progress with the translation of novel therapies into the clinical setting. Early well-designed comparative clinical studies have shown positive outcomes. Despite significant advancements in this arena, there remains a need for additional high-powered, well-designed clinical trials to confirm the safety and efficacy of treatment. This review is meant to summarize the current evidence and future considerations of stem cell therapy for sports injury applications.

Current State of Stem Cell–Based Therapy in Sports Medicine

The management and treatment of orthopaedic injuries has improved greatly over the past 2 decades with the advent of minimally invasive operative techniques and sophisticated rehabilitation augmented by the always-increasing knowledge of biomechanics and tissue engineering. Despite this progress, scientists and orthopaedic surgeons continue to struggle with the limited healing capacity of damaged structures such as torn cruciate ligaments, articular cartilage defects, tendon ruptures, and meniscus tears. Therapeutic approaches that address the underlying pathophysiologic characteristics of these disorders at the cellular and molecular level are quickly becoming a clinically applicable reality.

Tissue engineering and regenerative medicine are rapidly evolving fields that focus on creating living tissue to repair, replace, or improve diseased tissue. The main goal of tissue engineering is to construct biomaterials that are capable of integrating bioactive molecules (e.g., growth factors) or cells, or both. Tissues can be synthesized by both in vitro and in vivo techniques and can be

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Table 1. The 4 Critical Components of Tissue Engineering

Production: Stem cell progenitors and precursors
Structure: Conduction matrices or scaffolds, or both, to promote cell attachment and growth
Induction: Cellular proliferation and differentiation using cytokines, growth factors, and signaling proteins
Acclimation: Applying mechanical or biomechanical force stimulation to engineered constructs

made to resemble components of virtually every mammalian organ system. No matter what techniques are used or which organ system is mimicked, tissue engineering requires 4 critical components: the production of stem cell progenitor and precursor cells; conduction matrices or scaffolds to promote cell attachment and cell growth; induction using signaling proteins, cytokines, and growth factors to stimulate cellular proliferation differentiation; and mechanical and biomechanical force stimulation, such as shear or strain stress, to acclimate the engineered tissue (Table 1).[1]

The "production" component involves the isolation and expansion of cellular precursors that may include single or multiple cell types at various levels of maturity ranging from embryonic to fully mature cells. These cells include stem cells and the various progenitor cells that they become. Stem cells are responsible for the development and regeneration of tissues and organs. Biochemical and biomechanical signals trigger the proliferation and differentiation of stem cells during early development and regeneration after injury or disease. Stem cells may be of postnatal or embryonic origin. Postnatal stem cells have been found in many tissues throughout the body such as skin, muscle, bone marrow, and brain, liver. The use of stem cell therapy in sports medicine is still in development, but considerable progress has been made in this arena. The stage has been set for more practical research initiatives closer to delivery for use in everyday practice. Currently, the majority of stem cell research is dominated by small animal models with scaffolding and gene transfer techniques, but the generalizability of these findings and their application to humans remains questionable and potentially challenging.[1]

Many tissue-engineering techniques applied to small animal models have shown promise with respect to preserving the meniscus. Although this progress is of note given the meniscus' innate function as a shock absorber and cushion between the joint space of articular cartilage, the applicability of the results beyond the small animal model remains questionable because of low external validity.

Likewise, a loss of "protein expression profile" in culture is also a challenge in the human application of stem cell—based therapy. In fact, the struggle of moving stem cell concepts as applied to tendon pathologic conditions to clinical practice resides in this loss. Because tendon and ligament injuries represent up to 50% of musculoskeletal conditions, creating techniques to offset this loss in culture is critical. Potential offset methods in development in various clinical studies include administration of several growth factors and application of cocondition media and coculture media.[2,3]

Despite the aforementioned challenges to the practical use of stem cell—based therapy, progress is on the horizon. The academic arena is longing for steps closer to human application, which can be achieved by investing in experiments transitioning from small to large animal models and clinical studies.[4,5] The current state of cell-based therapy applications to sports medicine remains in its infancy, but the promising potential of progressive research can lay the groundwork for clinical applications that can transcend patient recovery.

Cell-Based Therapy for Cartilage Repair

Many studies in the past have focused on cell-based therapies for meniscal repair as an indirect way to protect cartilage, but more recent studies directed at addressing cartilage repair and regeneration have surfaced. Koh et al.[6] evaluated the use of adipose stem cells in knee osteoarthritis by injecting stromal mesenchymal tissue into the knee and measuring various clinical outcome scores as well as performing direct observation through arthroscopy. Results indicated improvement in cartilage architecture and function, with overall promising results when applied to the elderly. Another study also directed toward the elderly evaluated outcomes when stem cells were delivered intra-articularly and stimulated from the bone marrow simultaneously for osteochondral lesions of the talus. The injection delivered with marrow stimulation showed the best results for patients older than 50 years on the American Orthopaedic Foot and Ankle Society and Roles and Maudsley scores.[7] A recent prospective randomized controlled trial evaluated the delivery of stem cells to the knee and the optimal medium in 56 knees in 56 patients with unicompartmental osteoarthritis and genu varum. Patients underwent microfracture and medial opening wedge high tibial osteotomy. Intra-articular injection of cultured bone marrow—derived mesenchymal stem cells (MSCs) exhibited improvement in clinical outcomes (Lysholm, Tegner, and International Knee Documentation Committee scores) and defect healing and repair (magnetic resonance observation of cartilage repair tissue [MOCART] scores) in varus knees with cartilage defects after microfracture and high-tibial osteotomy.[8]

The current status of animal models and clinical trials was recently explored by Anderson et al.[4] They conducted a systematic review evaluating the current role of stem cell therapy in animal and clinical models for knee cartilage repair. Small and large animal models have shown some promise, but human clinical trials have yet to show substantial results, with most of the studies made up of low-level case studies. The authors

of these studies concluded that the future direction of stem cell treatment for knee cartilage suggests a move toward joint work between researchers, basic scientists, and bioethicists to develop safety and efficacy protocols that are transparent for effective long-term follow-up. Nonetheless, more trials are being conducted, but clinical implications have yet to be implied.[4]

With most current studies that look at stem cell applications for cartilage repair remaining in the preclinical phase, long-term questions regarding the appropriate conditions, delivery methods, dosing, and treatment indications are still unanswered.[9] Cell therapy for cartilage repair seems intuitive because of the homogeneous make up of cells, but the application is more complex. More research in the application of cell-based therapy to cartilage regeneration and repair is needed to further understand the conundrum of cartilage preservation.

Cell-Based Therapy for Knee Tendon Healing and Repair

Cell-based therapies directed at tendon healing are centered on environmental considerations for the cells to differentiate. Regarding the loss of protein expression profile in culture, attempts to mitigate this loss were facilitated with the administration of several growth factors, application of cocondition media, and coculture media.[2,3]

Preclinical studies have begun to glean which environment may prove fruitful for mesenchymal cells. Huang et al.[10] evaluated rat mesenchymal cells under hypoxic conditions to evaluate if healing was enhanced when these cells were transplanted into an injured Achilles tendon. Biomechanical testing, histologic analysis, and immunohistochemical evaluation were performed and showed increased healing capacity compared with normoxic stem cells. This was shown by an ultimate failure load in hypoxic mesenchymal stem cells that was greater than that in untreated normoxic mesenchymal stem cells at 2 and 4 weeks. This was subsequently confirmed with histologic analysis and immunohistochemical evaluation at 2 and 4 weeks, and 5-bromo-2-deoxyuridine labeling of MSCs before injection confirmed the incorporation and retention of transplanted cells at the rupture site.[10]

Recently, Kraus et al.[11] designed an animal study using a medium of stem cells and fibroblast growth factor to improve Achilles tendon rupture. Immunohistochemical patterns were promising when orchestrated in vivo but failed to show relevance when mimicked in a biomechanical model that yielded subpar results. Tendon applications with platelet-rich plasma (PRP) have exhibited strong healing potential as well. In a randomized single-blinded control trial, patients who received PRP during anterior cruciate ligament reconstruction experienced better healing of the patellar tendon harvest site when observed on magnetic resonance imaging (MRI) 6 months postoperatively, but there was no difference observed in knee function and isokinetic testing and knee function questionnaires.[12] Stem cell therapy directed toward tendon healing and repair lies in understanding the environment in which these cells can thrive and function appropriately.

Cell-Based Therapies for Meniscal Repair

Cell-based therapy directed toward meniscus repair has manifested in many forms, e.g., intra-articular injection in large animal models and humans, direct surgical injection, or through coupling of PRP with human chondrocytes through scaffolding vehicles. Hatsushika et al.[13] conducted a large animal study in 10 skeletally mature pigs receiving unilateral intra-articular injections of 50 million MSCs 2 weeks after bilateral resection of the anterior half of the medial meniscus. The pigs received MSC injections at 0, 2, and 4 weeks and MRI evaluation at 2, 4, 8, 12, and 16 weeks. Macroscopic and histologic evaluation was also performed on the regenerated menisci at 16 weeks. It was shown that meniscal regeneration was significantly better in the MSC versus the control group on histologic and MRI evaluation because of increased staining with Safranin O, larger sections of type I and type II collagen, and significantly lower T2 signal intensity in the MSC group from 2 weeks through 16 weeks. Articular cartilage on the medial femoral condyle was found to be significantly better preserved macroscopically in the MSC knees versus the control knees in all 7 recipients based on the International Cartilage Repair Society score for macroscopic observation and MRI, and articular cartilage in the MSC group was found to be better preserved at all time points and had significantly better MRI scores from 8 weeks onward.

Kwak et al.[14] showed that human chondrocytes can be delivered with pretreated PRP and a mesh poly lactic-co-glycolic acid scaffold. This combination manifested histologically as increased cell attachment and healing of the meniscus in vivo, and it improved the seeding efficiency 2-fold. The delivery of stem cells by injection is also starting to be understood. Vangsness et al.[15] designed a randomized double-blind controlled trial of 55 patients receiving intra-articular injections of human MSCs with partial meniscectomies. They reported improved pain as well as an increased volume of the meniscus seen on MRI among cohorts. Injections were well tolerated, with many adverse events that were no different from those after intra-articular injection. There were no shifts in immunologic parameters from baseline. MRI was used to calculate meniscal volume with significant change set at a 15% increase from baseline. At 12 months, the proportion of patients

meeting the criteria of greater than 15% improvement in MRI-based meniscal volume was significant in both group A and the overall group comparison. Clinical outcome measures were also significantly improved, with 100-mm visual analog scale scores through 2 years and improved Lysholm knee scale total scores relative to baseline at all time points.[15] Cell-based therapy directed at meniscus repair can present an opportunity to preserve the knee cartilage and its surrounding tissue through novel understanding of optimal delivery and indications. The current status is promising but more work lies ahead.

Evaluating Stem Cell and Tissue Engineering Studies

When assessing the quality of stem cell and tissue engineering publications, the design and evidence level of the study and recording of adverse events ought to be considered. Several systematic reviews have evaluated studies on Level I-IV investigations and grouped them based on setting: in vitro, in vivo, in vitro and in vivo, preclinical, and clinical.[16] Inclusion criteria considered were measures of functional and clinical outcomes, including the use of pain scores and minimum follow-up periods.[9] Pastides et al.[17] performed a systematic review of stem cell therapy for human cartilage defects and compared study size, patient age, lesion location, size, severity, and stem cell harvest and implantation technique. When comparing functional outcomes, pre- and postoperative scores, postoperative rehabilitation, duration of follow-up, and measurement modalities such as MRI or arthroscopy were included.

Regarding the evaluation of safety, Lalu et al.[18] performed a systematic review on the adverse effects of intravascularly delivered mesenchymal stromal cells studied in clinical trials in adult, pediatric, and mixed adult and pediatric populations. Adverse events were grouped according to immediacy of the event, such as acute infusion toxicity or fever, occurrence of organ system complication, infection, and longer-term adverse effects such as death and malignancy. Also to be considered was whether expected adverse events were listed and defined in the methods section and if follow-up frequency and duration were stated in the publication.

Conclusions

Our overview of the role that stem cell and tissue engineering therapy plays in the evolving treatment of sports injuries represents a promising step forward in the field of orthopaedics. Our understanding of the application of stem cells, cytokines, growth factors, and PRP, along with various tissue engineering modalities, continues to expand. Like many advancements, challenges exist in our search for evidence of their efficacy as innovations remain in preclinical or early clinical phases, with a focus on understanding the optimal environments and hybrid combinations required for successful and safe application. The current role is inconclusive, but the future direction should focus on establishing an ethical threshold that is effective and obtainable for future researchers to partake in more high-level studies within the clinical setting. High research and development costs, in combination with the current regulatory environment, present a challenge to high-quality evidence-based study.[19] Going forward, these direct and indirect barriers must be dissolved so as to not dissuade future scientists and clinicians from effectively designing and implementing high-quality clinical trials for sports medicine. As research at the cellular level continues to expand, the opportunity for growth is limitless, with cell-based applications and tissue engineering potentially setting the stage for how sports medicine is practiced today and in the future.

References

1. Gates CB, Karthikeyan T, Fu F, Huard J. Regenerative medicine for the musculoskeletal system based on muscle-derived stem cells. *J Am Acad Orthop Surg* 2008;16:68-76.
2. Paschos NK, Brown WE, Eswaramoorthy R, Hu JC, Athanasiou KA. Advances in tissue engineering through stem cell-based co-culture. *J Tissue Eng Regen Med*, in press, available online 3 February, 2014. doi:10.1002/term.1870.
3. Holladay C, Abbah SA, O'Dowd C, Pandit A, Zeugolis DI. Preferential tendon stem cell response to growth factor supplementation. *J Tissue Eng Regen Med*, in press, available online 29 January, 2014. doi:10.1002/term.1852.
4. Anderson JA, Little D, Toth AP, et al. Stem cell therapies for knee cartilage repair: The current status of preclinical and clinical studies. *Am J Sports Med* 2014;42:2253-2261.
5. Deponti D, Giancamillo AD, Scotti C, Peretti GM, Martin I. Animal models for meniscus repair and regeneration. *J Tissue Eng Regen Med*, in press, available online 27 May 2013. doi:10.1002/term.1760.
6. 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* in press, available online 11 December, 2013. doi:10.1007/s00167-013-2807-2.
7. Kim YS, Park EH, Kim YC, Koh YG. Clinical outcomes of mesenchymal stem cell injection with arthroscopic treatment in older patients with osteochondral lesions of the talus. *Am J Sports Med* 2013;41:1090-1099.
8. Wong KL, Lee KB, Tai BC, Law P, Lee EH, Hui JH. Injectable cultured bone marrow-derived mesenchymal stem cells in varus knees with cartilage defects undergoing high tibial osteotomy: A prospective, randomized controlled clinical trial with 2 years' follow-up. *Arthroscopy* 2013;29:2020-2028.
9. Filardo G, Madry H, Jelic M, Roffi A, Cucchiarini M, Kon E. Mesenchymal stem cells for the treatment of cartilage lesions: From preclinical findings to clinical

application in orthopaedics. *Knee Surg Sports Traumatol Arthrosc* 2013;21:1717-1729.
10. Huang TF, Yew TL, Chiang ER, et al. Mesenchymal stem cells from a hypoxic culture improve and engraft Achilles tendon repair. *Am J Sports Med* 2013;41:1117-1125.
11. Kraus TM, Imhoff FB, Wexel G, et al. Stem cells and basic fibroblast growth factor failed to improve tendon healing: An in vivo study using lentiviral gene transfer in a rat model. *J Bone Joint Surg Am* 2014;96:761-769.
12. Rahnama R, Wang M, Dang AC, Kim HT, Kuo AC. Cytotoxicity of local anesthetics on human mesenchymal stem cells. *J Bone Joint Surg Am* 2013;95:132-137.
13. Hatsushika D, Muneta T, Nakamura T, et al. Repetitive allogeneic intraarticular injections of synovial mesenchymal stem cells promote meniscus regeneration in a porcine massive meniscus defect model. *Osteoarthritis Cartilage* 2014;22:941-950.
14. Kwak HS, Nam J, Lee JH, Kim HJ, Yoo JJ. Meniscal repair in vivo using human chondrocyte-seeded PLGA mesh scaffold pretreated with platelet-rich plasma. *J Tissue Eng Regen Med*, in press, available online 19 June, 2014. doi:10.1002/term.1938.
15. Vangsness CT Jr, Farr J II, Boyd J, Dellaero DT, Mills CR, LeRoux-Williams M. Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial meniscectomy: A randomized, double-blind, controlled study. *J Bone Joint Surg Am* 2014;96:90-98.
16. Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Marcacci M. Platelet-rich plasma: Why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. *Knee Surg Sports Traumatol Arthrosc* in press, available online 26 November, 2013. doi:10.1007/s00167-013-2743-1.
17. Pastides P, Chimutengwende-Gordon M, Maffulli N, Khan W. Stem cell therapy for human cartilage defects: A systematic review. *Osteoarthritis Cartilage* 2013;21:646-654.
18. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): A systematic review and meta-analysis of clinical trials. *PLoS One* 2012;7:e47559.
19. Martin I, Smith T, Wendt D. Bioreactor-based roadmap for the translation of tissue engineering strategies into clinical products. *Trends Biotechnol* 2009;27:495-502.