Supplement

Regenerative Cells for the Management of Osteoarthritis and Joint Disorders: A Concise Literature Review

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Abstract

As the global population ages, the prevalence of osteoarthritis (OA) and joint disorders represent a major cause of disability and a significant public health burden. As current approaches for the management of OA focus on slowing the progression of disease, without repairing the underlying damage, novel treatments are necessary to improve outcomes. Over the past decade, autologous cell-based therapies using regenerative cells from fat or bone marrow have become a major focus of research into new approaches for the treatment of osteoarthritis and joint disorders. This review is intended to summarize findings in existing literature and identify gaps in knowledge that should be addressed in order to advance the field. We acknowledge that some findings may appear inconsistent, but show that apparent inconsistency in the literature may be attributable to variation in source of cells, stage of disease, method of delivery, follow-up time, evaluation method, and a number of other idiosyncrasies of individual studies. Still, a number of themes emerge from the data and some broader conclusions may be drawn that can be used to guide future studies. Ultimately, we conclude that there is overwhelming evidence demonstrating the safety of the autologous cell-based therapies. Furthermore, the data support the claim that regenerative cells are capable of reversing progression of OA. Regenerative cells, and especially those from adipose tissue, represent a promising new approach for the treatment of OA. Future work should include appropriate controls, and focus on answering questions related to dose required, appropriate delivery vehicle, and the impact of multiple treatments. Additionally, future studies should look at short and long-term effects of the treatments, and use functional as well as radiologic methods to evaluate efficacy.

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Osteoarthritis (OA) is the most common musculoskeletal disorder and is the major cause of disability among elderly individuals in developed nations.[1] Current Center for Disease Control estimates state that nearly one-quarter of adults (22.7%) in the United States suffer from physician-diagnosed arthritis. That percentage is higher among obese individuals (31.2%) and individuals over the age of 65 (49.7%). With current trends in global population demographics toward an ever-increasing ratio of elderly to young people, degenerative diseases, including osteoarthritis, are increasingly a global health concern and should be treated as a priority.[2,3]

Current conventional approaches to the management of OA are entirely targeted at minimizing pain and further joint damage by reducing inflammation while strengthening and protecting the joint from physical insult.[4,5] These approaches do nothing to address the underlying problem of cartilage degeneration in the joint.[6] Because of this, there is increasing interest in regenerative approaches to the treatment of OA. Cell-based approaches using stem cells are particularly appealing because they have the potential to treat multiple aspects of the disease state. OA is an inflammatory disease that is coupled to degradation of the extracellular matrix

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and failure to regenerate chondrocyte by a depleted cellular reservoir. Stem cells are capable of immune modulation, matrix remodeling, and replenishing the cellular reservoir by self-renewal and differentiation. The ability of cell-based therapies to treat OA by multiple mechanisms makes them a particularly exciting new approach.

While a number of studies appear to confirm that these treatments are safe, there has yet to be a large-scale clinical trial assessing the efficacy of these approaches relative to controls.[7] In fact, published studies seem to report varying degrees of efficacy. That being said, we have observed that many apparent discrepancies in the literature may be attributable to inconsistent methods of treatment and evaluation. These inconsistencies include, but are not limited to, the patient population, the source of the regenerative cells, the manner in which they are harvested, how they are delivered to the site of injury, the number of cells used in the treatment, whether or not these cells are expanded in vitro, the vehicle of delivery, the time elapsed at follow up, and the approaches utilized to evaluate improvement.

These inconsistencies represent major unanswered questions in designing effective regenerative medicine treatments to OA and other joint disorders, and the major obstacle to the wide-spread clinical implementation of new approaches. In this review, we endeavor to digest the current literature on the treatment of OA with regenerative cells in order to provide a better understanding of challenges and opportunities, and to identify areas where future work should be focused to advance the field.

METHODS

The literature search for this manuscript utilized PubMed and Google Scholar search engines. The search was conducted in May 2016 and repeated in December 2016. With the exception of a number of articles used to give historical context to understanding of adult stem cells and the treatment of OA, as well as the prevalence of OA in society, articles published within the last decade were given greater weight in evaluating relevance. For clinical data to be included in our evaluation of the current literature the latter need only specify that the study aimed to use some kind of regenerative cells for the treatment of OA. We did not disregard any studies based on the type or stage of OA, nor the type of regenerative cells being used. Keywords used for the searches included: "Osteoarthritis," "Regenerative Medicine," "Stem Cells," "Stem Cell Clinical Trials," "Adult Stem Cells," "Evaluating Osteoarthritis," "Osteoarthritis Treatment," "Stromal Vascular Fraction," "Mesenchymal Stem Cell," and a number of combinations and variations on the terms listed here.

Current Approaches to the Treatment of Osteoarthritis

Standard Therapy

Currently, the Osteoarthritis Research Society International (OARSI) guidelines for the management of OA center around protecting the joint from physical insult while treating inflammation in order to reduce pain.[4] Nowhere in the guidelines are approaches designed to repair damaged cartilage discussed. Indeed, dozens of interventions are described in the literature. However, the improvements resulting from conventional therapies are generally unexceptional, and few approaches aim to repair damaged cartilage.[5,6]

Autologous Chondrocyte Transplantation

Over the past 2 decades, a number of groups have reported success repairing OA damage by autologous chondrocyte transplantation, either using expanded cells seeded onto a scaffold or by grafting cartilage from a second surgical site.[8-11] While these approaches represent a significant step forward in the field, as they represent a shift in treatment paradigm from managing symptoms to addressing underlying pathology, they come with significant drawbacks and limitations.[12] From a functional standpoint, these approaches may result in donor site morbidities. Additionally, there may be imperfections in the orientation of cartilage plugs and formation of fibrocartilage.[12-15] These may result in the formation of sites of stress concentration that may reduce cartilage longevity.[16,17] From a practical standpoint, the need for a second procedure increases risks, costs, and inconvenience for patients.[9,18] Moreover, while suitable for certain cases involving focal defects, diffuse OA has been an exclusion criteria in studies evaluating the efficacy of chondrocyte transplantation.[8,10,11,19] Taken together, these limitations necessitate the development of a new treatment paradigm.

Mesenchymal Stem Cells

Over the past decade a number of groups have reported promising results utilizing autologous mesenchymal stem cells (MSCs) for the treatment of OA. These are especially exciting developments because direct injection of MSCs could avoid surgeries and the associated risks and side effects. Moreover, the ability of stem cells to differentiate into chondrocytes in vivo and to modulate inflammation, make them especially promising therapeutic agents.[20,21]

Mesenchymal stem cells were originally isolated from bone marrow as progenitor cells for the various stromal elements.[22] We now understand their role in development, the multilineage potential of adult MSCs, their paracrine effects, and their presence in most tissues of

the body.[23,24] Due to their ability to modulate inflammation and regenerate damaged cartilage, these cells are especially exciting.

A consensus is emerging in the fields of regenerative medicine and orthopedics that MSCs from fat and bone marrow are capable of regenerating cartilage in vivo. Indeed, intra-articular injection of regenerative cells is the only approach shown to reverse damage resulting from diffuse OA.[25] Still, there is debate in the field as to whether symptom relief is primarily due to tissue regeneration or to the anti-inflammatory effects of MSC therapies. Even when tissue regeneration is observed, it is unclear whether the injected cells are directly responsible for tissue formation, or indirectly by recruiting and stimulating cells via paracrine effects of secreted cytokines.[26,27] Regardless of the mechanism, results of pilot studies using MSCs report promising results. In addition to improved cartilage, patients with OA treated with MSCs show improvements in pain, stiffness, range of motion, and functional tests.

Adipose Stromal Vascular Fraction

In recent years, as scientists and clinicians have worked to develop regenerative medicine therapies that could comply with Food and Drug Administration standards for "minimal manipulation," applications of the stromal vascular fraction (SVF) have gained popularity. During this time, available literature on SVF applications has increased exponentially. In most cases, the SVF is isolated in a closed, disposable system from adipose tissue obtained via suction-assisted liposuction of the abdomen. The SVF is then injected with no further processing directly to the site of injury.[28] This is done during a single out-patient visit. Aside from avoiding regulatory hurdles, SVF has been a popular alternative to culture-expanded stem cells, in part, because the SVF is known to contain a high frequency of adipose tissue-derived MSCs. While there is unanimous agreement that the SVF does contain stem cells, the reported frequency of stem cells relative to total mononuclear cells in the SVF varies from roughly 0.02% to nearly 7%.[7,27] Regardless of the frequency of MSCs in the SVF, therapies utilizing the SVF for the treatment of OA have shown substantial promise. In Garza et al, the authors report nearly 4 times greater relief in pain intensity relative to reported improvements using vsico-supplementation.[7] The second remarkable finding from this study was that relief from symptoms of OA became greater with time, rather than diminishing as we see with other approaches. These results are supported by similar clinical observations in other studies.[25,29-33] The observation that pain relief and functionality increases over time lends further legitimacy to claims that these therapies reversed underlying cartilage damage.

Challenges and Opportunities for Adipose Regenerative Cells for the Treatment of Osteoarthritis

While promising, clinical trials have been insufficient in scope to draw broad conclusions about the efficacy of a particular therapy in order to shift standard practices for the treatment of OA. While nearly all reports in literature cite improvements, studies universally lack adequate controls, and vary widely in approach. Table 1 lists some of the idiosyncrasies that vary from study to study making it difficult to compare results.

The potential of adipose tissues to repair tissues damaged by degenerative disease is clear, as is the safety of the procedure.[34] However, a number of clinical trials mention similar limitations regarding our understanding of the proper use of adipose-derived MSCs and the SVF in the clinic. In order to move the field toward evidence-based applications of adipose cells for regenerative therapies in OA, we must address gaps in knowledge as they pertain to several broad areas outlined below that are important for developing efficacious treatments.

Required Cellular Dose

In published studies there appears to be a positive correlation between the number of cells used for treatment and the magnitude of the observed positive effect.[29,32] In Jo et al, the authors conclude that 1×10^8 nucleated cells from the SVF are necessary for consistently positive results. They did not observe significant improvement on all parameters at lower doses.[32] Similarly, Koh et al noted that effect size was proportional to dose of stem cells in culture-expanded adipose-derived stem cells.[29] In contrast to Jo's finding, Garza et al and Fodor et al, using average cell counts of 4.8×10^7 and 1.4×10^7, respectively, consistently observed substantive improvement in patient pain and function.[7,33] However, neither of these studies reported new cartilage formation. One study that failed to observe substantial improvements in objective measures suggested that this was perhaps due to the relatively low dose applied in their study.[35] While there is some evidence of deleterious side effects at high doses in rodent models, no similar tolerance limit has been evidenced in humans.[36] In fact, meta-analysis of studies totaling 844 cases found no serious safety concerns.[34] Based on current evidence, it appears that adipose regenerative cells should be applied at relatively high doses to achieve optimal outcomes and that these doses will be safe for patients. Still, more studies are required to determine minimum effective dose and maximum tolerated dose.

The Number and Timing of Injections

A major appeal of using the SVF is convenience; during a single visit, a patient can undergo lipoaspiration and

the SVF can be isolated while the patient is being prepared for intra-articular injection.[37] A single treatment, however, may not be adequate for all disease states, and different approaches should be considered for different conditions. Though there has been minimal literature published on repeated administration of regenerative cells for degenerative disease, strong rational exists based on observations from in vivo studies. First, most studies seem to indicate that stem cells injected into a damaged microenvironment fail to persist.[38] Still, many of these studies seem to suggest that cell therapies are still capable of creating lasting changes to the microenvironment through paracrine effects.[39] This could explain why repeated injections enhances survival of injected stem cells in ischemia models.[40] This suggests that repeated treatments may create an environment that is more hospitable for injected cells. Randomized controlled trials (RCTs) comparing the efficacy of a single treatment vs multiple treatments within a fixed period of time are necessary to determine the best approach for the treatment of OA. The number and timing of treatments may be especially important in cases of severe OA where damage to the extracellular environment may negatively impact the regenerative potential of adipose-derived stem cells used for an initial therapy.[38,41,42]

The Proper Use of Co-Stimulators

Often stem cells are injected with platelet-rich plasma (PRP) in order to activate those cells, as platelets are known to contain a rich pool of growth factors in their α-granules that may accelerate chrondrogenesis in osteoarthritic knees.[43] However, there are conflicting reports showing that platelets may release some factors that have negative effects on the OA joint.[44] Still, it is often used as a costimulator to promote regenerative activity. In other studies, Ringer's solution or phosphate buffered saline are used as the delivery vehicle. It is still unclear what effect this has on the efficacy of treatment overall or the regenerative cells specifically. If it is true that PRP activates regenerative cells, it may be possible to pretreat cells with PRP regardless of the vehicle to be used. Recently, cultured stem cells were treated with steroids in order to enhance their immunomodulatory function in vivo in an animal model.[45] It is unclear what effect this type of treatment would have on therapies in humans, and treatments such as this are likely to receive substantially more regulatory scrutiny. However, they may also serve as a template for future studies seeking to promote maximal regenerative activity in stem cell therapies, or to avoid off-target effects of certain pharmaceuticals. Related questions must be asked with regard to combinations of cell types/subtypes. Especially, does the complex milieu of cells in the SVF promote regeneration or would a purified population be more efficacious?

Determination of Appropriate Cell Subtype(s)

Studies have demonstrated the efficacy of both culture-expanded cells and freshly isolated SVF nucleated cells in the treatment of OA. These populations of cells look very different as exhibited by marked shifts in phenotype after passages.[46] To reduce costs associated with expanding cells in accordance with good manufacturing practices, and to eliminate certain regulatory hurdles, it is preferable to use freshly isolated stromal vascular fraction for cell-based therapies, all other conditions being equal. However, as the fresh vs culture-expanded cells contain different cell subtypes, it is unclear whether one is more efficacious than the other or whether each is optimal for a specific set of circumstances. This is important to consider, because recent evidence suggests that specific environmental factors may have different effects on bone marrow mesenchymal stem cells (BM-MSCs) vs ASCs.[47] Moreover, a number of recent studies have begun to compare specific therapeutic properties of stem cells isolated from various tissues. For instance, it has been compellingly demonstrated that bone marrow vs adipose-derived stem cells possess very different angiogenic, osteogenic, and immunomodulatory properties.[47-49] Although no studies directly compare clinical effectiveness of the 2 populations, in vitro studies suggest that SVF is potentially applicable for many of the same applications as culture-expanded ASCs or BM-MSCs.[50,51] Therefore, for the time being, in lieu of RCTs comparing the effectiveness of SVF vs culture-expanded ASCs for treating OA under specific conditions, we conclude that the significant advantages in terms of availability and ease of isolation of SVF make it a preferable approach for immediate development.

The Stage of Disease to Select for MSC Transplantation

Several clinical studies have reported that degree of improvement seems to be negatively correlated with the severity of the disease state.[29,35] The authors suggest that this could be because SVF is primarily preventative rather than curative. This explanation is inconsistent with findings that intra-articular injection of autologous SVF results in objective cartilage improvement.[32] Given the evidence that SVF injection results in regeneration of hyaline cartilage, it seems more likely that in advanced disease states, the extracellular environment is such that normal function of regenerative cells is inhibited. This idea is supported by a large body of evidence describing the effects of a "damaged" microenvironment on stem cell behavior.[52-54] As the field matures it will be interesting to observe whether larger doses, multiple treatments, or appropriate use of costimulators can overcome the challenges posed by severe OA.

Evaluation of Treatment Efficacy

As described in Table 1, studies described in the literature use a variety of measures to evaluate efficacy. Most

commonly, studies employ a combination of ordinal variables describing pain and joint function. This can make interpreting results especially difficult. First, because it is inherently difficult to compare ordinal variables from different scales. And second, because one or both of these measures may be significantly impacted by a placebo effect. In fact, one meta-analysis of OA treatments (> 40,000 patients) attributed greater that 75 % of therapeutic effects was attributed to placebo effect.[55] Although this study did not include any regenerative approaches, the confound is generalizable. Additionally, the extent to which a placebo effect may impact results may be further affected by the timing of the follow-up evaluation, as well as any number of idiosyncrasies of the clinician performing the procedure and/or follow-up evaluation. Moreover, to the best of our knowledge, only a single study discussed the confound of multiple follow-up measures diverging such that one measure indicated reduced symptoms while a second measure indicated an increasingly symptomatic joint following treatment.[33] To address these confounds, it is desirable that studies include objective measurement of joint structure by imaging. For OA treatment evaluation and diagnosis, a shift is occurring from the use of X-ray to more sensitive magnetic resonance imaging (MRI).[56] Still, more advanced techniques and more nuanced interpretation of results may improve diagnosis and treatment evaluation. For instance, to increase resolution, clinicians may consider using smaller step sizes and greater magnetic field.[33] Even with highly resolved images, techniques such as whole organ MRI score (WORMS), evaluate multiple features of the joint and may fail to adequately encompass disease stage-specific changes to the anatomy of the joint and site-specific cartilage regeneration with treatment.[57] Future work in this area should make an effort to identify relationships between in-depth evaluation of joint anatomy by high-resolution MRI and joint pain/function. This will enable more objective evaluation of treatment efficacy in the long term.

DISCUSSION

Here we summarize what is known about the treatment of OA with regenerative cells and the substantial knowledge gaps that still exist. Rapid advances in tissue engineering and an evolving regulatory environment that may make new stem cell approaches more feasible may drastically change the treatment of OA in the coming decade. So much so that the use of SVF, which was the focus of this review, may be eclipsed by other even more promising options. Still, the knowledge gaps and questions proposed here are fundamental to SVF therapies and to stem cell therapies more broadly. This review provides a framework for evaluating the efficacy of existing stem cell therapies and considerations for the development of novel approaches.

CONCLUSIONS

Mesenchymal stem cells are capable of superior results in the treatment of osteoarthritis relative to conventional approaches. This is likely due to their unique ability to repair the underlying cartilage damage that causes joint pain in those affected. Due to its abundance, ease of obtainment, relatively high concentration of MSCs, and safety, the stromal vascular fraction represents the most promising source of regenerative cells for the treatment of OA. Current literature suggests that intra-articular injection of freshly isolated SVF reliably provides pain relief, improved function, and new cartilage formation in diseased joints when a sufficient number of cells is delivered. Studies in human patients have consistently shown that MSC and SVF injections are safe and effective. Still, it is important that groups undertake large-scale clinical trials in order to better evaluate the improvement of cell-based therapies over current approaches and to optimize treatment parameters. Future work should be targeted at delineating the different factors in the SVF that are the most beneficial and most effective for minimizing the effects of OA and optimizing the number and timing of dosages, as well as the method of administration for various disease states. Overall, regenerative cells for the treatment of OA offer the promise of reversing damage that previous methods have fallen short of. Though the treatment of OA is not typically the responsibility of aesthetic or reconstructive surgeons, the isolation and application of SVF from abdominal fat offers an opportunity for meaningful collaboration among aesthetic surgeons with other specialties. Additionally, as Fodor and Paulseth point out, studies of SVF for the treatment of OA provide evidence of safety for any number of applications more directly relevant to aesthetic and reconstructive surgery.[33]

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