Current trends of stem cell-based approaches for knee osteoarthritis

B Kristjánsson¹, S Honsawek¹²*

Abstract

Introduction

Osteoarthritis (OA) is the most prevalent joint disorder resulting in cartilage destruction, subchondral sclerosis, osteophyte formation, and synovitis. Knee osteoarthritis presents a great clinical challenge to clinicians due to the limited inherent repair capacity of avascular, aneural articular cartilage. Articular cartilage defects are increasingly common among the elderly, causing severe pain, joint stiffness, reduced motion, disability, and deformity among affected patients. Although cartilage defects are typical features of OA, current treatments can barely restore the full function of native cartilage. Recent studies have provided new perspectives for cartilage engineering using multipotent mesenchymal stem cells (MSCs). Growing knowledge on the biology of MSCs has provided new insights into their clinical applications, particularly for OA. MSCs isolated from a variety of adult tissues including the bone marrow, have the capacity to differentiate into different cell types such as bone and cartilage and have therefore attracted scientific interest as possible therapeutic tools and hold promise for cartilage regeneration. At present, regenerative medicine offers the exciting potential for treating osteoarthritis. In this review, we highlight recent researches that address critical challenges of stem cell based therapies for OA.

Furthermore, we provide innovative studies that have been carried out in the potential treatments for knee osteoarthritis, including stem cells and tissue engineering.

Conclusion

The utility of stem cells for the treatment of osteoarthritis is increasing in clinical researches. Modes of delivery of stem cells to the articular cartilage differ from direct injection to implantation with composite scaffolds. Currently, there is limited evidence of direct clinical benefit and randomized, prospective studies for these innovations should be conducted to validate the safety and efficacy of cartilage regeneration.

Introduction

Osteoarthritis (OA) is a debilitating degenerative joint disease leading to pain, stiffness, swelling, crepitus, and disability in the elderly¹. The joints most often affected are the knee, hip, and hand. It is characterized by progressive deterioration of articular cartilage, osteophyte formation, subchondral sclerosis, subchondral bone cysts, and synovitis². Cell therapy by surgically implanting autologous chondrocytes has been used to regenerate cartilage damage for over two decades, the repair process is slow and often insufficient due to the poor self-renewal and regeneration abilities of the chondrocytes³. Other less invasive and reasonable methods have been suggested such as the use of stem cells. Mesenchymal stem cells have shown chondrogenic potential in vitro and might therefore provide an alternative treatment of damaged cartilage⁴,5,6.

Mesenchymal stem cells (MSCs) are pluripotent progenitor cells that can differentiate into cells of the mesodermal lineage. They are capable of establishing colonies from a single cell referred to as colony-forming fibroblast units. MSCs can be found in various tissues and organs but reside mainly within the bone marrow. Bone marrow-derived mesenchymal stem cells (BMSCs) are therefore a widely used and well-studied cell line. Synovial MSCs can be found in most tissues of the synovial joints in mammals. It is likely that they play a role in providing a reservoir of repair cells that can be activated for growth, repair or remodelling. However, MSCs found in cartilage appear to lack the ability of functional repair since it is well known that cartilage fails to regenerate following injury. A significantly greater number of MSCs can be recovered from the affected joints of OA or rheumatoid arthritis patients as well as those of ligament injury compared with that from healthy joints. The number of MSCs also increases with the severity of the disease and one hypothesis suggests that they originate in the degrading synovium⁷. The aim of this review was to discuss current trends of stem cell-based approaches for knee osteoarthritis.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

MSCs for cartilage repair: Availability and safety

Obtaining cells for tissue engineering can be a major technical issue. It is possible to use allogeneic cells from donors but this is not common clinical

*Corresponding author
Email: Sittisak.H@chula.ac.th

¹ Department of Biochemistry, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand
² Department of Orthopaedics, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand
³ All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.

Competing interests: None declared.
practice due to the risk of rejection or disease transmission\(^9\). The use of autologous cells is considered safe because there is neither an immunological reaction nor the risk of disease transmission. However, using autologous cells also has limitations; availability may be scarce and it is important to select a tissue which results in minimal morbidity for the patient\(^9\).

Autologous BMSCs are widely used because they can be easily obtained causing minimum morbidity and can be collected without producing tissue defects\(^10\). The yield obtained from bone-marrow can be quite low and cells are usually expanded in vitro. Another good source for MSCs is adipose tissue. The number of cells that can be harvested from adipose tissue has been estimated to be up to 1,000 times greater than that from bone-marrow making it a good source for stem cells\(^11\). Obtaining adipose-derived mesenchymal stem cells (AMSCs) is relatively simple with the use of liposuction where fat-pads are the major harvest sites. Like the harvesting of BMSCs, this technique causes minimal morbidity and is considered a safe method.

Most reports on the use of MSCs to treat cartilage defects focus on the use of BMSCs and a small number investigating AMSCs.

Synovium derived mesenchymal stem cells (SMSCs) have been suggested for cartilage repair since some in vitro and animal studies have shown SMSCs to have a greater chondrogenic potential than BMSCs\(^12,13\). So far, there have been no clinical trials for this cell source in humans and harvesting SMSCs requires the use of arthroscopy which is more invasive than obtaining BMSCs\(^14\).

**BMSCs in the treatment of osteoarthritis**

The first report of using BMSCs to treat osteoarthritis was documented by Wakitani et al. in 2002\(^15\). The study consisted of 24 patients with knee osteoarthritis who underwent a high tibial osteotomy. Twelve of these patients received autologous BMSC transplantations and the other 12 served as a control group. BMSCs were harvested from the iliac crest and expanded in culture. During the high tibial osteotomy, the knee joint was opened using the parapatellar medial approach. They observed the medial femoral condyle and medial tibial plateau; in all cases the articular cartilage on the medial femoral condyle was lost as well as the subchondral bone being eburnated.

The mean number of 1.3 x 10\(^7\) BMSCs was introduced in a gel-cell composite consisting of 2.0 ml of 0.25% type I acid soluble collagen from the porcine tendon put onto a collagen sheet and gelatin. This gel-cell composite was applied to the abraded area and covered with collagen sheets. They were able to obtain samples of repair tissue and observe the transplants through arthroscopy in the following two surgeries when the pins and staples were removed. Interestingly, it was observed that the defects were covered with white soft tissues and some hyaline cartilage-like tissues in the cell-transplanted group. This was not the case for the cell-free group where white material with an irregular surface could be evident and in some areas underlying bone was visible.

Clinical evaluations before and after surgeries were performed using the Hospital for Special Surgery knee-rating scale. Both groups showed significant improvements in pain, function, and muscle strength. However, no difference was observed between the cell-transplanted group and cell-free group. Although patients showed no improvement in the quality of life in either group, BMSCs were able to produce cartilage-like tissues in in vivo transplants. In 2011, Saw et al. investigated the quality of articular cartilage regeneration after arthroscopic subchondral drilling\(^16\). They postoperatively injected 5 patients with autologous peripheral blood progenitor cells (PBPCs) in combination with hyaluronic acid (HA)
Table 1: Summary of studies where MSCs were used to treat knee osteoarthritis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number of patients</th>
<th>Delivery system</th>
<th>Number of cells</th>
<th>Follow-up time</th>
<th>Control group</th>
<th>Stem cell origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees (^\text{15})</td>
<td>2002</td>
<td>12</td>
<td>Surgery, implantation, cell sheets, gel - cell composite</td>
<td>(1.3 \times 10^7)</td>
<td>28-95 weeks</td>
<td>12 cell free controls</td>
<td>Autologous BMSCs from iliac crest</td>
</tr>
<tr>
<td>Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells (^\text{22})</td>
<td>2008</td>
<td>1</td>
<td>Three intra-articular injection</td>
<td>(2.2 \times 10^7)</td>
<td>3 months</td>
<td>None</td>
<td>Autologous BMSCs from iliac crest</td>
</tr>
<tr>
<td>Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells (^\text{22})</td>
<td>2008</td>
<td>1</td>
<td>Three timely spaced intra-articular injections</td>
<td>(4.6 \times 10^7)</td>
<td>3 months</td>
<td>None</td>
<td>Autologous BMSCs from posterior superior iliac spine</td>
</tr>
<tr>
<td>Osteochondral lesions of the knee: a new one-step repair technique with bone marrow-derived cells (^\text{23})</td>
<td>2010</td>
<td>20</td>
<td>Surgery, implantation hyaluronic acid membrane scaffold</td>
<td>N/A, 2 ml of bone-marrow concentrate</td>
<td>24 months</td>
<td>None</td>
<td>Autologous BMSCs from iliac crest</td>
</tr>
<tr>
<td>Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients (^\text{24})</td>
<td>2011</td>
<td>4</td>
<td>Single intra-articular injection</td>
<td>(8.0 \times 10^6 - 9.0 \times 10^6)</td>
<td>12 months</td>
<td>None</td>
<td>Autologous BMSCs</td>
</tr>
<tr>
<td>Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis (^\text{21})</td>
<td>2012</td>
<td>25</td>
<td>Single intra-articular injection</td>
<td>(1.2 \times 10^6)</td>
<td>12 months</td>
<td>25 cell free controls</td>
<td>Autologous AMSCs from infrapatellar fat pad</td>
</tr>
<tr>
<td>Infrapatellar fat pad injections improve symptoms of knee osteoarthritis (^\text{21})</td>
<td>2012</td>
<td>6</td>
<td>Single intra-articular injection</td>
<td>(2.0 \times 10^7 - 2.4 \times 10^7)</td>
<td>12 months</td>
<td>None</td>
<td>Autologous BMSCs from iliac crest</td>
</tr>
<tr>
<td>Mesenchymal stem cell injections improve symptoms of knee osteoarthritis (^\text{24})</td>
<td>2013</td>
<td>18</td>
<td>Single intra-articular injection</td>
<td>(1.2 \times 10^6)</td>
<td>24 months</td>
<td>None</td>
<td>Autologous AMSCs from infrapatellar fat pad</td>
</tr>
<tr>
<td>Treatment of knee osteoarthritis with autologous mesenchymal stem cells: A pilot study (^\text{22})</td>
<td>2013</td>
<td>12</td>
<td>Single intra-articular injection</td>
<td>(4.0 \times 10^7)</td>
<td>12 months</td>
<td>None</td>
<td>Autologous BMSCs from iliac crest</td>
</tr>
</tbody>
</table>

for improving the regeneration of cartilage. The patients received the first injection one week after the surgery followed by 4 more injections at weekly intervals. They performed a second-look arthroscopy which confirmed articular cartilage regeneration and histologic sections stained positive suggesting the formation of hyaline cartilage, both results are consistent with previous findings\(^\text{15,17}\). In addition, they also performed histologic and magnetic resonance imaging (MRI) studies of articular cartilage regeneration in

Licensee OAPL (UK) 2013. Creative Commons Attribution License (CC-BY)

patients treated with or without PBPCs and HA after arthroscopic subchondral drilling. The histologic and MRI scores of the intervention group were significantly better than those of the control group. It was concluded that treatment by regularly injecting PBPCs and HA after surgery improved the quality of articular cartilage repair. Recently, less invasive procedures have been suggested by injecting PBPCs directly into the knee joint after performing subchondral microdrilling combined with an injection of growth factors and hyaluronic acid which has been proved as being beneficial for cartilage health and repair.

Studies on the effects of direct injection of MSCs without any surgical procedures have also shown promising results in the treatment of knee osteoarthritis. The extensive literature on stem cell isolation, chondrogenic differentiation, and composite scaffold design has empowered researchers and clinicians to consider the possibility of using stem cells to modify the progression of OA and using tissue engineering to resurface an entire osteoarthritic joint surface and facilitate osteochondral integration (Figure 1).

**Direct single intra-articular injections: BMSCs**

In 2011, Davatchi et al. reported their results on the direct injection of BMSCs into the knees of patients suffering from osteoarthritis.

All four patients in this study were over 50 years old and suffering from moderate to severe knee osteoarthritis due to obesity. BMSCs were obtained from 30 ml of bone-marrow blood from the patients and expanded in culture; they were confirmed as MSCs by immunophenotyping. The mean volume of 5.5 ml containing 8.0-9.0 x 10^6 cells was injected into one knee of each patient. Although improvement was observed in 3 out of 4 patients it was minor and the researchers concluded the results as encouraging, but not excellent. Another similar report from 2012 describes 6 female volunteers who needed total joint replacement (TJR) surgery but received a BMSCs injection instead. In this study, they obtained 50 ml of bone-marrow blood and expanded in culture, likewise they confirmed their cell population as MSCs by immunophenotyping. They injected 20.0-24.0 x 10^6 BMSCs intra-articularly into the knees of the volunteers. MRI demonstrated an increased cartilage thickness in 3 out of 6 patients and patients reported a reduction of pain as well as improvement in walking distance for the first 6 months then slightly reducing for the following 6 months. Taken together, these studies were both promising and encouraging but not fully satisfactory as a standard treatment for knee osteoarthritis.

More promising results with intra-articular injections of autologous BMSCs alone were produced in 2013 by Orozco et al. Their study consisted of 12 patients with osteoarthritic knee pain who failed conservative treatment and 9 out of 12 had already undergone previous surgery. Bone-marrow was extracted from the iliac crest for MSC isolation. Cells were expanded in culture and confirmed as MSCs by immunophenotyping. After 3 weeks of cell culture, cells were harvested and injected into the patients. The patients received an 8.0 ml injection of 40.0 x 10^6 cells which was considerably larger number than in previous comparable studies.

Clinical outcomes were followed for one year by evaluating pain, disability, quality of life as well as measuring articular cartilage quality through MRI. By 3 months, pain was significantly reduced with additional progress in the 9 months to follow and was significant at all-time points observed. Patients showed rapid and progressive improvement of the Lequesne index that approached 65-78% after 1 year.

Patients were also satisfied with the treatment and 11 out of 12 reported lasting pain relief throughout the study period. MRI also showed a significant increase and improvement in cartilage quantity and quality in 11 out of 12 patients.

Furthermore, it was demonstrated that the feasibility and safety of the treatment reached up to 78% of treatment results with 100% being a perfect treatment. It compared favourably with the conventional treatments producing considerably better results.

Additionally, it compares well with other invasive methods such as TJR surgery since it is simple and does not require hospitalization or surgery, resulting in over-all lower costs. Their results were considerably better than in the other studies mentioned previously. One of the reasons might be that they injected approximately 2-4 times more MSCs than in the other two studies.

**Direct single intra-articular injections: AMSCs**

Although the main focus has been on the use of BMSCs, some researchers have chosen to use AMSCs as an alternative. This is attributed to the abundance of available adipose tissue for cell harvesting and the higher yield obtained from each gram of tissue. In 2012 and 2013, Koh et al. published two papers on the same study which revolved around the use of AMSCs for the treatment of osteoarthritis. This study recruited 18 patients who received an injection of AMSCs to the knee.

The adipose tissue was harvested from the inner side of the infrapatellar fat pad via a skin incision after arthroscopic debridement. Interestingly, they did not culture the cells but directly isolated them from the fat tissue by centrifuging the tissue sample. They did not perform immunophenotyping to confirm their cell population as MSCs, but simply counted them with a haemocytometer and presumed their cell population consisted of MSCs. Since this was a quick process, they were able to inject the cells back into the patients on the same day as they were harvested. They did not receive the same yield of cells from each patient and the injected cells ranged from 0.3 x 10^6 to 2.7 x 10^6 in number.

Clinical outcomes were evaluated before treatment and in the following...
two years after treatment. Overall, the treatment was a success and there was no major complication. The data showed a significant reduction of pain and an increased quality of life for all patients. A positive correlation was found between the number of cells injected and pain improvement.

Furthermore, MRI images taken before and after treatment confirmed that the whole-organ MRI score had increased significantly and the improvement was also correlated with the number of cells injected. They concluded that AMSCs were a valid cell source for treating cartilage damage.

Their method is also simple and cost effective with cells being harvested and re-injected into the patient on the same day resulting in reduced costs from cell expansion and from the fact that no hospitalization is required. The weakness of their study was that they did not confirm their population as MSCs. Therefore, the cell population might consist of more cell types such as adipocytes. The effectiveness of intra-articular delivery of MSCs in the knee has already been investigated in a number of clinical trials (Table 1).

**Current clinical trials**

Currently, a number of clinical trials are underway in the treatment of cartilage damage with MSCs. As of November 2013 the total of 22 clinical trials on osteoarthritis treatment with MSCs are listed on the National Library of Medicine website. Most of these studies are on knee OA with others focusing on hip or ankle OA. They mainly revolve around the use of expanded autologous MSCs derived either from bone marrow or adipose tissues, although some trials use allogenic or non-culture expanded MSCs. Most researchers focus on the use of intra-articular injections without the use of scaffolds or major surgeries since injections are more cost effective, cause little morbidity and are a desirable way of treatment if they are successful. Since optimal dose-studies have not been carried out yet the ideal dose of MSCs is unknown and doses in the current trials ranges from $1.0 \times 10^7$ to $1.0 \times 10^9$. These studies will further help in determining what tissues are good sources of viable MSCs for cartilage repair; what the optimal cellular dose should be as well as demonstrating if a single injection is sufficient or multiple injections might be required for satisfying results.

**Conclusion**

The promising results from the studies described in this review demonstrate that there are alternative means to treat moderate to late stage OA. The traditional major surgeries used to treat the condition are both expensive and come with risks. The less invasive methods described here have shown good results but the development of the treatment is ongoing.

Better outcomes were obtained with higher numbers of MSCs injected but the optimum dose still remains to be decided. Interestingly, all the studies in the last few years have focused on a single injection hoping it would provide a permanent relieve instead of multiple timed injections. The results from these single injection studies showed that there was an improvement, but in some cases that improvement was reduced over time suggesting multiple or even regular injections of MSCs into the joints might be necessary.

A notable weakness of most of the studies presented in this review is the absence of control groups. The ultimate solution would be a single injection of MSCs alone or in combination of growth factors, which would fully regenerate articular cartilage damage and result in a lasting tissue and eliminating the pain which follows the condition.

In order to achieve such a dream solution, a number of studies are needed with satisfying and consistent results as well as determining all factors of the treatment such as the required cellular dose, vehicles used to deliver and if any external factors are needed. Further prospective clinical researches of large scale randomized control trials will be warranted to fully determine the effectiveness of stem cell and tissue engineering treatment for osteoarthritis.

**Acknowledgement**

This work was supported by Ratchadapiseksomphoth Fund (RA55/22), Faculty of Medicine, Chulalongkorn University, and the Thailand Research Fund (DBG4980017). The authors would like to thank Thomas Mabey for kindly reviewing the manuscript.

**Abbreviations list**

AMSCs, adipose-derived mesenchymal stem cells; BMSCs, Bone marrow-derived mesenchymal stem cells; HA, hyaluronic acid; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; OA, Osteoarthritis; PBPCs, peripheral blood progenitor cells; SMSCs, Synovium derived mesenchymal stem cells; TJR, total joint replacement.

**References**