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Mesenchymal Stem Cells: The hope, the hype and the reality in the treatment of osteoarthritis

A knowledge synthesis of clinical research (2010-2016) emphasizing the safety and efficacy of stem cell treatment for osteoarthritis

Bone and Joint Health Strategic Clinical Network (BJH SCN) White Paper

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EXECUTIVE SUMMARY

Stem cells are drawing a significant amount of attention in the public, health care and academic realms. Riding the wave of hope and potential, patients are actively seeking stem cell therapy for a number of musculoskeletal conditions, such as degenerative conditions, in particular osteoarthritis (OA). Yet, the scientific evidence for effectiveness is inconclusive and recommendations for clinical application are variable. Further, there is increasing concern/focus on issues related to stem cell safety in this largely unregulated environment. To address these challenges we currently face regarding stem cell therapy for osteoarthritis, the purpose of the AHS BJH SCN Stem Cell Workshop is to engage a range of stakeholders in an evidence-based discussion to contribute to the realization of the potential of stem cells by generating recommendations to inform practice, research and/or policy regarding this emerging therapeutic approach. This white paper aims to provide the background and an overview of the current knowledge on stem cells, specifically mesenchymal stem cells (MSCs), in the treatment of OA to enable an evidence-based foundation for the workshop and the discussion that we will engage in to understand the topic of stem cells and generate ideas that will inform the path forward in Alberta.
We conducted a systematic search of the published literature to generate a knowledge synthesis regarding the efficacy and safety of MSC-based treatments for OA. This knowledge synthesis was focused on reviews published between 2010 and 2016 (up to May), which included human clinical trials focused on cartilage repair and symptom relief. The results of this work indicate that MSCs are sourced from a number of different areas within the body; in the treatment of OA, with the most common sources being bone marrow and adipose tissue. There are also a number of different approaches currently used to prepare MSC for delivery via injection and implantation - a surgical procedure that often also includes the use of matrices and/or scaffolds. In conjunction with MSC treatment, co-interventions are often used, such as microfracture sub-chondral drilling, debridement, and platelet rich plasma. Due to the significant variability associated with the MSC sources, current techniques and approaches being applied in the preparation and delivery of MSC therapies, and the number of different co-interventions used in conjunction with MSCs, it is challenging to make conclusive statements regarding the efficacy of MSCs. Broadly, based on the results clinical trials and expert opinion, MSC as a therapy for OA are considered safe, although there are areas of concern that require investigation, and there are indicators that they are efficacious (symptom relief and structural repair). However, although promising, these results are preliminary and not definitive. Advancing knowledge on MSC therapy requires that a number of issues are addressed. These include, but are not limited to, standardization of protocols, enhancing the rigor of study designs (which includes long term outcomes tracking), and determining patient characteristics that influence effectiveness. The realization of the potential of stem cells as a viable therapeutic option is also based on a determination of who is responsible for safety (e.g. Government, regulatory bodies) and effectiveness (e.g. researchers, clinicians, policy makers). Therefore, progress forward is a shared responsibility, central to which are the patients seeking and undertaking this form of treatment for their OA.

INTRODUCTION

The allure of stem cells to repair or regenerate tissues damaged by injury, disease, or developmental "missteps" has been increasingly promoted. According to mainstream media, such as the recent cover of Time magazine – "big things are on the horizon thanks to stem cells and it is not too far away!" That is the hope, however, it is not yet clear that our understanding of what we are calling stem cells, specifically mesenchymal stem cells , or more accurately, mesenchymal stromal cells (MSCs), has kept pace with the potential applications being lauded. Unfortunately, the popular press has created a hype that has overtaken the hope in some circumstances, and created unreasonable expectations regarding the successful application of this emerging therapeutic approach given our current scientific knowledge in this area. Although demand is increasing, there is ample data from animal studies but limited clinical data, and limited understanding of the underlying mechanisms of action, upon which to base clinical application in patient populations.

The hope of stem cells also appears to mean different things to different stakeholder groups. Many researchers feel we need to have a comprehensive understanding about the cells before we should attempt applications. Patients with painful, debilitating diseases such as osteoarthritis (OA) want effective treatment solutions. Clinicians see the needs of the patients and want to implement effective interventions, informed by clinical experience and what information is currently available. And finally, Governments have a vested interest in supporting both research and the implementation of clinically effective and cost effective procedures. Thus, a key question is how we can balance all of those needs in a timely and knowledgeable manner, and what each stakeholder group is responsible for in response to the various needs and demands, and achieving the goal of successful and safe treatments for OA, and determining if stem cell therapeutics has a place in this category.

THE PROBLEM:

Patients are actively seeking stem cell therapy for a number of musculoskeletal conditions, such as degenerative conditions, in particular osteoarthritis (provincially, nationally and internationally). Yet, the scientific evidence for effectiveness/efficacy is inconclusive and recommendations for clinical application are variable and largely unproven. Further, there is increasing focus on issues related to stem cell safety in this largely unregulated environment. Lastly, the challenge is also related to timeliness – the balance between time needed to undertake the necessary research to generate sufficient understanding of what 'success' looks like, and the current demands and pressing information needs of patients, clinicians and health policy makers. There are aso differences in approach in the sense that many clinics worldwide provide treatments without definitive evidence, while some research groups and companies are endeavouring to complete well-designed clinical trials. The latter take many years to complete.

ADDRESSING THE PROBLEM:

Purpose of the Workshop:

To address these challenges, the purpose of the AHS BJH SCN Stem Cell Workshop is to engage a range of stakeholders in an evidence-based discussion to contribute to the realization of the potential of stem cells by generating recommendations to inform practice, research and/or policy on this emerging therapeutic approach.

To achieve this, the theme of day 1 (Oct 27) of the workshop is *where are we now*, focusing on current knowledge and perspectives of different stakeholders. Applying these learnings, the theme of Day 2 (Oct 28) is *where we go from here*. Engaging participants in several activities, the aim will be to identify what is required to support access to safe and efficacious stem cell therapies for OA patients.

The first important and guiding principle of our work is how to ensure **safety** in a variety of environments (e.g. developing effective criteria or applications in private offices and clinics, or privately funded procedures in publicly funded infrastructure, such as operating rooms) using a variety of protocols (e.g. source of cells, isolation or enrichment parameters, number of cells to be used, etc). Secondarily, do we need to develop vehicles such as RCTs or comparative effectiveness trials to determine the optimal protocol(s) for stem cell use, and applications of stems – is there a greater benefit gained through their potential immuno-modulatory / anti-inflammatory function than tissue repair? Thirdly, how do we define success, and do we need to put in place province-wide systems to capture elements such as baseline data, appropriate tools to capture outcomes and adverse events to monitor safety and efficacy? While the workshop may not lead to the final answers for all of these issues, certainly the expectation is that after the Workshop we will have a better appreciation of where we are, what is needed, and potential routes to get to where we need to be.

Purpose of the White Paper

This white paper aims to provide the background and an overview of the current knowledge on stem cells in the treatment of OA to enable an evidence-based foundation for the workshop and the discussion that we will engage in to understand the topic of stem cells and generate ideas that will inform the path forward in Alberta.

BACKGROUND:

Osteoarthritis

Osteoarthritis (OA) is a chronic, progressive, and irreversible degenerative joint disease that affects over 4.6 million Canadians, with those numbers expected to double in the next 30 years (Arthritis Alliance of Canada 2011; Marshall et al. 2015). Although the mechanism(s) underlying the disease are not clearly delineated, multiple risk factors including age, sex, obesity, genetics, and joint trauma can likely contribute to its onset. Clinical symptoms include restricted range of motion, limited activity, neuropathic pain, depression, and sleep disorders. Biomechanically abnormal joint loading resulting from obesity, joint instability, or trauma can affect the bone, synovium, and muscle of the joint via progressive cartilage deterioration, subchondral bone remodeling, loss of joint space, marginal osteophytosis (boney growths), and loss of joint function. (Barry & Murphy 2013; Ham et al. 2015). In this context, a joint is an organ system, where damage to one component can lead to organ failure (see Frank et al. 2004; Loeser et al. 2012).

Three key structures affected by OA degeneration are 1) articular cartilage, 2) menisci and 3) subchondral bone. *Articular cartilage* (AC) is a stable hyaline tissue with no blood, lymphatic, or nerve supply. It contains only a single cell type, called chondrocytes, which remain suspended in the cartilage matrix and are responsible for synthesizing the AC components. Chondrocytes are suspended in a highly hydrated extracellular matrix composed of collagen fibers to provide tensile strength, proteoglycans for compressive strength, and molecules which contribute to the “toughness” of the tissue. Due to the avascular environment and low metabolic activity of chondrocytes, AC has limited capability for intrinsic repair (Guilak et al. 2004; Bauge & Boumediene 2015). *Menisci* (medial and lateral meniscus) are paired structures composed of semilunar fibrocartilage. They play an essential role in normal function of the knee by providing structural integrity and stability to the knee joint. Similar to AC, the natural healing capacity of meniscal tissue is limited to the vascular region of the tissue (Yu, Adesida, & Jomha 2015; Starke, Kopf, Petersen, & Becker 2009). *Subchondral bone* lies underneath the AC, providing it with support. When subjected to microfractures the subchondral bone can act to release undifferentiated (naïve) mesenchymal stem cells (MSCs) from the bone marrow tissue to repair chondral defects. However, this process mainly results in the formation of scar tissue or fibrocartilage. Fibrocartilage is poorly organized and has inferior mechanical and biochemical characteristics compared to normal hyaline cartilage. It eventually wears, leading to secondary OA. Without exposure to the subchondral bone, the AC has little access to undifferentiated cells that promote repair, but synovial fluid does contain mesenchymal stem cells which could potentially home to injured AC or menisci (de Souza et al. 2014; Ando et al. 2014) As a result of the lack of observed endogenous repair, acute trauma and/ or degradation of the AC is most often considered irreversible. (Bauge & Boumediene 2015).

Treatment of Articular Cartilage Lesions

There are a number of therapies applied to treat AC lesions or defects. Although effective to various extents, currently there is no cure for advanced OA. Broadly, current therapies can be divided into non-cell-based and cell based. Non-cell based (exogenous) therapies include interventions such as microfracture and mosaicplasty. A description of this approach and additional examples of intervention are provided in **Appendix 1.**

Cell based therapies have been developed as a way of therapeutically addressing the lack of effective innate or endogenous repair systems. These therapies aim to simulate biological restoration of lesions in the articular surface (Bauge & Boumediene 2015). These techniques involve local delivery of *ex vivo* preparations of cells with the objective of:

- Reducing degenerative changes associated with OA
- Healing AC lesions with tissue that has native AC biological and mechanical properties
- Enhancing current joint repair techniques

Cell based therapies can be divided into differentiated cell therapy (primarily chondrocytes) and progenitor cell-based therapy (primarily mesenchymal stem cells (MSC)) (Counsel, Bates, Boyd, & Connell 2015), which have emerged as a possible solution to both the limited source and differentiation obstacles associated with harvesting chondrocytes (Shimomura et al. 2015). A description of the non-stem cell (or chondrocyte)-based approaches is provided in Appendix 2.

Given the focus of the workshop, the remainder of this report is specific to mesenchymal stem cells (MSC).

Stem Cell Based Therapies broadly encompass any treatment of a disease or condition that utilizes the stem cell's ability to proliferate and differentiate. Stem cells are defined as undifferentiated or non-specialized cells that can replicate and differentiate into more than one type of cell with specialized functions (Barry & Murphy; Uzbas et al. 2015; Martin, De Boer, & Sensebe 2016).

Research utilizing stem cells for cartilage repair have focused on utilizing ***mesenchymal stem cells (MSCs)***, also labelled as bone marrow stromal cells, multipotential adult stem cells, human marrow stromal cells, or mesenchymal progenitors (Guilak et al. 2004). These adult stem cells are widely thought to have their origins in the mesoderm, however, recent studies have posited origins from the neural crest ectoderm. They are distinguished by their potential to differentiate into cartilage, bone, muscle, tendon, ligament, and fat (Pittenger et al. 1999). MSCs have the capacity for self-renewal and rapid proliferation, and are particularly attractive for treatments aimed at OA due to paracrine anti-inflammatory and immunomodulatory properties. Current therapies primarily utilize adult stem cells, given the ethical concerns related to harvesting of embryonic stem cells (Counsel 2015).

Point of interest

Attention in this area was provoked by the multipotent potential of MSCs, in particular towards chondrogeneiss and osteogenesis. Further, evidence indicates MSCs isolated from progressive OA joints are substantially limited in proliferation and differentiation potential (Barry & Murphy 2103) and have other altered features (Krawetz et al 2012; Harris et al 2013), pointing to a role 'healthy' MSCs could have in preventing joint degradation. (Barry & Murphy 2013). It is important to note, the differentiation deficiencies of OA MSCs can be reversed by culturing on mediums supplemented with epidermal growth factor; therefore use of autologous MSCs is still a viable option (Barry & Murphy 2013).

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has established **the following minimal set of standard criteria to provide a uniform characterization of such cells**:

(1) They must be plastic-adherent when maintained in standard culture conditions;

(2) they must express CD105, CD73, and CD90 and lack surface expression of CD45, CD34, CD14 (or CD11b), CD79a (or CD19), and HLA-DR;

(3) and they must be capable of differentiating to chondrocytes, osteoblasts, and adipocytes in vitro.

It is important to note, some common MSC subpopulations of bone marrow stem cells (BMSCs) and adipose derived MSCs (AMSCs) do not fall under this definition as they are non-adherent to plastic. (Kristjansson & Honsawek 2014)

Sources of MSCs

MSCs were first isolated from bone marrow, as a cell class separate from hematopoietic stem cells and are naturally found in many tissues. For cartilage repair techniques, the most common sources include adipose tissue (subcutaneous and infrapatellar fat pad), autologous bone marrow, and synovial fluid; less commonly used were peripheral blood and periosteum sources (Counsel 2015). Preclinical trials have exploited a wider variety of tissues including skeletal muscle, amniotic fluid, synovial membrane, and dental pulp (Counsel et al. 2015; Xu-et al. 2015; discussed in Hart 2014). Other natural sources include umbilical cord blood, endometrium, and placenta (Counsel et al. 2015).

Although there are common characteristics between the cells from each source, significant differences exist. These include: expression of cell surface markers, immunomodulatory activity, abundance and ease of harvest, proliferation and differentiation potential, as well as potential tissue-specific epigenetic alterations that can influence cell activities (Xu et al. 2015, Perez-Camp et al 2015; discussed in Hart 2014). Cell populations from different sources seem to be 'inclined' towards certain lineages. For example, cells derived from bone marrow appear to undergo osteogenesis effectively, while those from synovial fluid or synovial membranes tend to undergo chondrogenesis (Ando et al. 2014; discussed in Hart 2014). These differences influence the sources potential utility in stem cell therapy. Disadvantages and advantages associated with the most common cartilage repair sources are outlined in **Appendix 3**.

Preparation of MSCs

Once tissue has been harvested, there are two main methods of preparing cells before their application as a stem cell therapy (see Martin et al. 2016 for more discussion).

Concentrated - The aspirate (or extract) of fluid, connective tissue, and cells is separated into the mononuclear cells (the aspirate) and the extraneous tissues (Chahla et al. 2016). This can be done via collagenase digestion (for adipose derived cells) or centrifuging (bone marrow derived cells) (Filardo et al. 2016). Depending on the tissue source, the resulting aspirate concentrate can have, in addition to MSCs, a variety of growth factors, immune cells, leukocytes, and others (Counsel et al. 2015; Filardo et al. 2016). In concentrates, the cells are "free" or modified but not extensively manipulated.

Use of concentrated aspirates, such as stromal vascular fraction (SVF) and bone marrow concentrate (BMC) have advantages of negligible time lag between extraction and implantation, as there are devices that can distill (or concentrate) aspirate down to a reasonable volume to ensure same-day harvesting and implantation procedures (Counsel et al. 2015). This minimizes risks of contamination during cell culture, and reduces cost and logistical challenges of treatment (Counsel et al. 2015). The non-MSC components of the concentrate have also been speculated to have anabolic and anti-inflammatory effects, which could further promote positive outcomes (Chahla et al. 2016). Further, this technique is also not considered a pharmacological intervention in most jurisdictions, and therefore does not currently require regulatory approval.

The disadvantages of such concentrated preparations lie in the variability of the number of MSCs in the preparations, as well as the environment of the concentrate (Filardo et al. 2013; Wolfstadt et al. 2015). MSC counts vary widely between patients, therefore standardization of concentrate injections is currently limited (Counsel et al. 2015). The number of cells used for treatment is also limited to what can be extracted (Feisst, Meidinger, & Locke 2015). In addition, there is concern the autologous microenvironment of the concentrate can be influenced by local pro- and anti-inflammatory small molecules, decreasing the healing potential of the extracted MSC (Ham et al. 2015; Woldstadt et al. 2015). This would be particularly problematic in patients with risk factors for decreased number and quality of MSCs (ex: obesity, elevated age, OA), as they may not benefit from significant clinical outcomes (Wolfstadt et al. 2015).

Expanded - Expansion is an amplification process that allows for the isolation of a homologous sample of MSC, and as well as obtaining the cell numbers thought necessary for therapeutic advantages (Counsel et al. 2015; Feisst et al. 2015). Expansion occurs in a culture medium with or without exogenous serum or non-serum supplements.

Once expanded, MSCs can usually be injected directly, or undergo activation toward chondrogenesis to further increase the healing potential (Bauge & Boumediene 2015; Filardo et al. 2013). Activation has been achieved by the addition of growth factors or cytokines, and culturing in hypoxic conditions (Bauge & Boumediene 2015; Filardo et al. 2013; Adesida et al. 2012). Addition of a variety of cytokines has been shown to overcome the low chondrogenic potential of adipose derived stem cells (ADSC) in some studies (Filardo et al. 2013; Ham 2015).

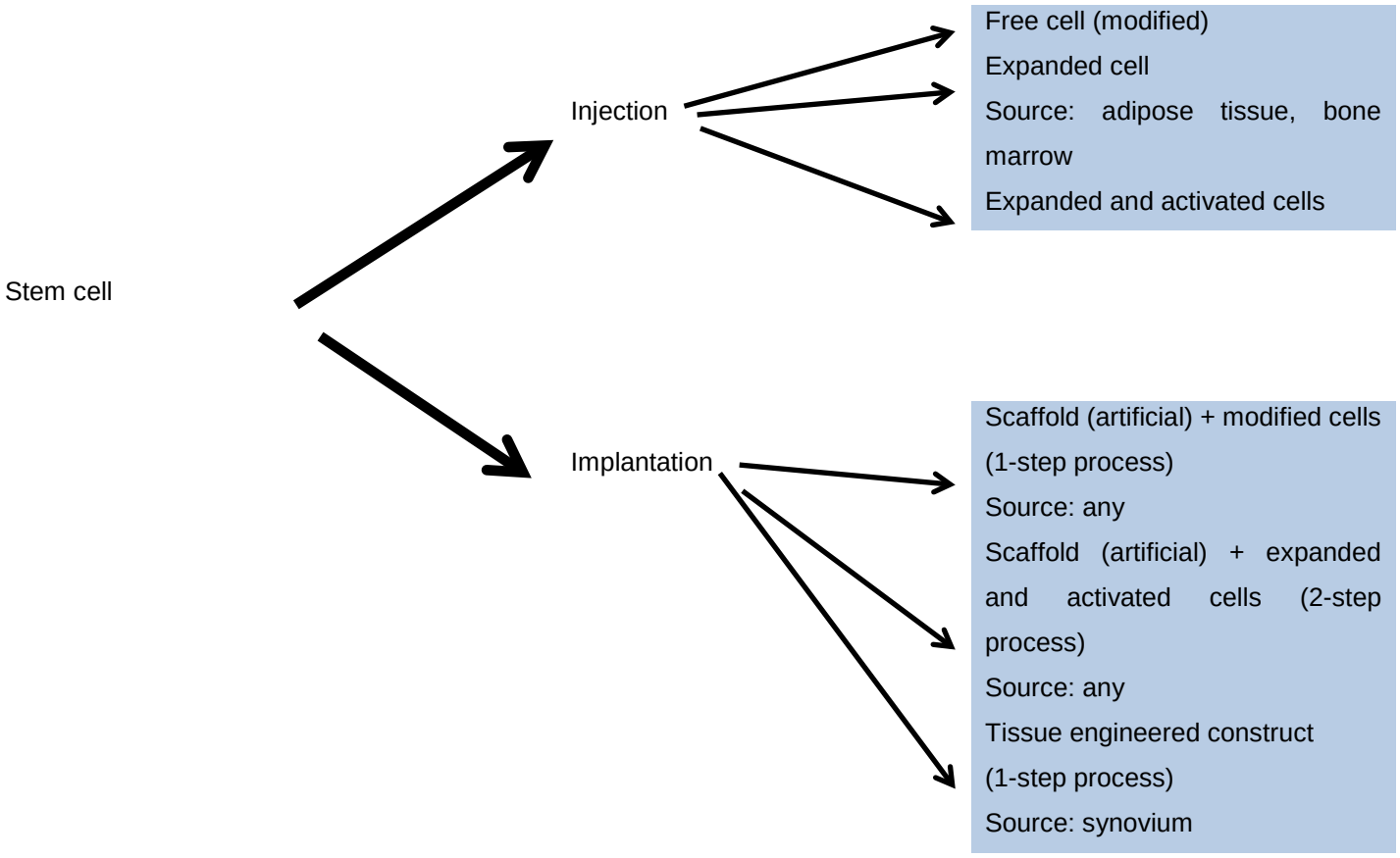
Expanding, or culturing, of the concentrated aspirate is advantageous because MSCs are rare under normal conditions and the aspirate mixture includes non-MSC cell populations (Wong et al. 2013).

Techniques for Delivery of MSCs

There are a number of techniques used to deliver prepared MSCs to the affected area, however, they can be broadly categorized into injection (non-surgical procedure) and implantation (surgical procedure). (Filardo et al. 2013) – see **Figure 2**.

Injection - This may involve injection of stem cells directly into the intra-articular synovial fluid compartment, either immediately after extraction (concentrated form) or after expansion/amplification (2-4 weeks post extraction). The medium is usually hyaluronic acid, a major component of synovial fluid, and a molecule already in use via injections for treatment of OA (Barry & Murphy 2013) or co-supplementation using commercial preparations. Injections can be direct delivery of MSCs in hyaluronic acid, or in an attempt to improve the therapeutic impact of the transplanted MSCs, be mixed with growth factors and cytokines, most commonly platelet-rich plasma (PRP) (Ham 2015). PRP is an enriched autologous sources of chondrogenic growth factors including TGF-β and platelet-derived growth factor (Ham 2015). Intra-articular injections have the advantages of easy application, limited surgical time, short recovery time, low cost, and low risk to the patient (Filardo et al. 2013; Kristjansson & Honsawek 2014). However, a key finding remains that only a small percentage of the MSC populations injected into the joint actually remain at the site of injury (discussed in Hart 2014). The imprecise site delivery and poor localization of MSC (discussed in Hart 2014; Filardo et al. 2013; Hart 2014) have led to the innovation of implantation techniques.

Figure 2 – Variations in delivery mechanisms, MSC preparations and sources used



Implantation - is a surgical procedure during which modified stem cells (amplified or expanded) are placed directly on or within the defect site. Implantation procedures can be done in a one or two step approach. In one step procedures, the harvested cells are mechanically dissociated, and embedded in a scaffold, and implanted in one surgical stage (Filardo et al. 2013). Two step procedures involve harvesting cells and then ex vivo expansion and possibly amplification (Filardo et al. 2013). The processed cells are then seeded into a scaffold matrix and implanted in a second surgical procedure (Filardo et al. 2013).

Implantation of MSC can be carried with or without a *scaffold* (Barry & Murphy 2013). *Scaffolds* were innovated in order to provide a 3-D structure for easy handling of the cell culture and to prevent chondrocyte leakage from the implantation site (Filardo et al. 2013). A scaffold is a structure made of a biocompatible biologic or synthetic material (Barry & Murphy 2013). It is used as a mechanism to aid stem cell adhesion, differentiation, proliferation, as well as provide a provisional three-dimensional matrix to promote tissue formation (Bornes, Adesida, & Jomha 2014; Bauge & Boumediene 2015). They are a

challenging engineering feat as they should be biodegradable, permeable, reproducible, non-cytotoxic, and temporary (Bentley et al. 2013). The first matrices introduced into clinical practice were hyaluronic acid or collagen based (Filardo et al. 2013). These ingredients are already components of hyaline cartilage, and therefore are potentially able to integrate readily (Filardo et al. 2013). Additional information regarding scaffolds is provided in **Appendix 4**. Current research with hydrogels, polymers, biomimetic scaffolds and nanomaterials provide exciting new possibilities for optimising the cell construct and the repair response.

Implantation can be achieved *scaffold-free* using a 'one step repair technique' by direct injection of suspended free MSCs, or by mixing the cells with cytokines or growth factors, such as a platelet gel, platelet-rich fibrin glue, collagen powder, or hyaluronic acid gel sponges. The implantation is then secured by a periosteal or collagen cover (Counsel et al. 2015). However, these scaffold-free approaches have been met with challenges. Due to its unique matrix organization, AC has anti-adhesive properties that present challenges for integration of implanted tissues. In addition, animal studies have shown the suture track used to secure the patch to the surrounding cartilage can triggered subsequent degradation of the margin between implant and adjacent AC (Shimomura et al. 2015). Further, the implanted cells do not contain an extra-cellular matrix to assemble the implanted cells in the same highly organized manner as in the native cartilage (Shimomura et al. 2015). To address these issues, some researchers have developed scaffold-free 3D Tissue Engineered Constructs (TEC). These are monolayer cultures of MSCs exposed to a medium that increases collagen synthesis to create a sheet-like structure. This matrix contracts to form TEC derived from MSCs. With or without chondrogenic stimulation, the organized TEC can be implanted into a defect without a covering, and with higher integration potential (Shimomura et al. 2015).

Point of interest

Scaffolds *without* cells (or cell free scaffolds)

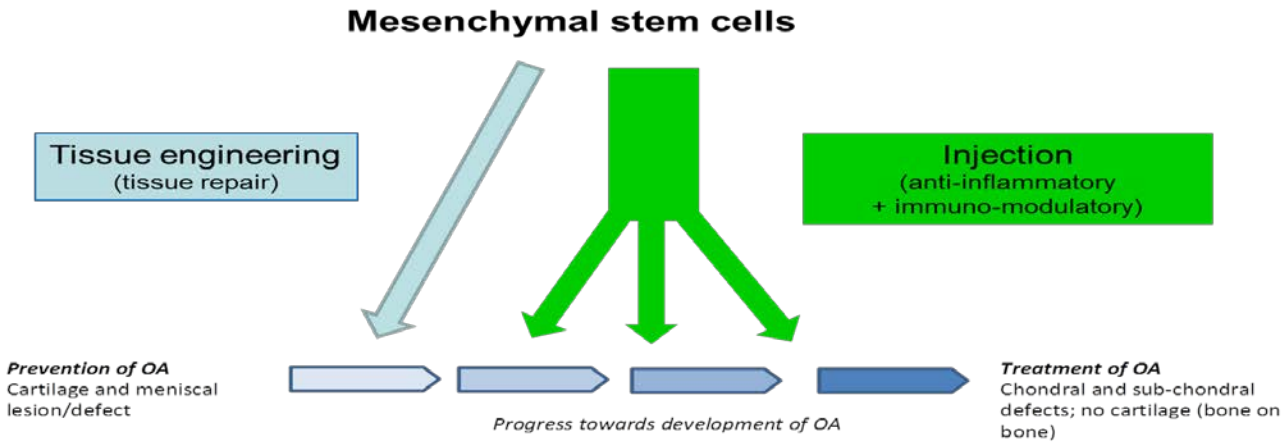
A new technique gaining interest involves triggering 'in situ' cartilage repair by implantation of various biomaterials without cells that subsequent promote stem cell chondrogenesis (Filardo et al. 2013). This approach is based on the function of scaffolds as not just carriers of cells, but also that they possess the ability to stimulate chondral or osteochondral regeneration (Kon et al. 2015). An example of this is autologous matrix-induced chondrogenesis (AMIC), combines microfracture with the implantation of a collagen bilayer matrix to stabilize the resulting blood clot. AMIC "plus" procedures add a platelet-rich plasma (PRP) gel to further enhance the healing response (Filardo et al. 2013).

Stem cells therapies have broad potential applicability in the treatment of OA, being applied across the therapeutic spectrum of OA – from prevention by treating chondral lesions and defects resulting from injury or trauma, to treatment of osteochondral defects or lesions that have progressed to the degenerative stage of the disease (see **Figure 3** below). There has been a proliferation of research

activity investigating a range of stem cells sources, preparations, and delivery mechanisms in the treatment of articular cartilage across this tissue damage continuum.

Our goal in the next section is to provide an overview of the current scientific knowledge regarding the safety and efficacy of MSC therapy for chondral and OA defects.

Figure 3 – From prevention to disease treatment – approaches to treating chondral defects and degenerative changes related to OA



KNOWLEDGE SYNTHESIS:

There has been a proliferation of research activity investigating a range of stem cells sources, preparations, and delivery mechanisms in the treatment of articular cartilage across the tissue damage continuum. For example, currently there are 17 open clinical trials underway around the world specifically focused stem cell therapy for osteoarthritis (**Appendix 5).**

We conducted a systematic search of the published literature to generate a knowledge synthesis regarding the efficacy and safety of MSC-based treatments for OA. The search was focused on reviews (systematic reviews, comprehensive reviews, clinical reviews, and/or meta-analysis) published between 2006 and 2016 (Sept to May) in the English language which discussed clinical studies on humans for the treatment of cartilage lesions (chondral and/or osteochondral defects and/or lesions) with mesenchymal stem cells (MSCs). The methods used in the generating the knowledge synthesis are described in further detail in **Appendix 6 and Appendix 7**.

Our search resulted in the identification of 19 reviews that met our inclusion criteria (listed in **Appendix 8**). The reviews were published between 2013 and 2016. Ten of the reviews were systematic reviews. Two reviews included a meta-analysis. The reviews were authored by international group of scientists

from Europe, Asia, and North America. More than half were published from European research institutions (11/19), predominantly from Italy. Two were published by Canadian research teams, one of which is co-authored by scientists based at the University of Alberta (Adetola Adesida and Nadr Jomha).

There were a total of 261 studies cited in the reviews, 67 of which were cited multiple times (see **Appendix 9**). The types of clinical studies included in the reviews were case reports, case series, comparative trials (e.g. case control, cohort studies, controlled studies), and RCTs (see **Appendix 10**). It is important to note that more than half of the clinical studies (11/19) included in these reviews are considered low level evidence (level IV and V): case reports and case series. RCTs, considered the "gold standard" in determining efficacy, represented only 10% of the body of knowledge which currently informs currently conclusions regarding efficacy/ effectiveness and safety.

The **MSC source** was reported in all but one review. The most common sources of the MSC were bone marrow (bone marrow concentrate and bone marrow aspirate concentrate) and adipose tissue, cited in 11/19 and 10/19 reviews, respectively. Stromal vascular fraction (SVF), which is derived from adipose tissue, was specifically addressed in three of the nineteen reviews. Less common MSC sources include: peripheral blood (5/19), synovial fluid (2/19), umbilical cord (2/19), and amniotic fluid (1/19).

A number of different **approaches for preparing MSC** were described. This included use of non-modified (or non-expanded) MSCs (7/19); expanded MSC (where expansion took place from 3-4 hours to 3 to 5 weeks) (9/19); expanded and cultured (6/19); expanded and centrifuged (1/19); centrifuged only (1/19); and expanded, cultured, stimulated and minimally processed with centrifuge (1/19).

As described earlier, **the MSC delivery mechanisms** currently used in research and in clinical settings are broadly categorized as injection and implantation through surgical procedures. Twelve reviews included studies using *injections*. The most common sources of MSC used in injections are bone marrow aspirate concentrate (BMAC), adipose tissue and peripheral blood. Several reviews also include studies where BMAC injections were combined with hyaluronic acid or various serums. *Implantation* was used in studies cited in twelve reviews; application of a variety of scaffolds was discussed in all twelve of these reviews. It is worth noting that for one step scaffold approaches, bone marrow concentrate has been the main choice, whereas expanded MSCs are preferred for two-step approaches (Kon et al. 2015). Implantation of MSC-seeded matrices, which are also available in a range of biomaterials such as collagen, fibrin glue and platelet rich fibrin glue, were described in six reviews . Four reviews included studies that used the scaffold-free implantation method.

Most study designs included a ***co-intervention***(s) – interventions used in conjunction with the specific stem cell approach. There are numerous co-interventions being used. The most common are microfracture, sub-chondral drilling, debridement, and platelet rich plasma (PRP). Those cited less commonly include hyaluronic acid, albumin and serum, osteophyte removal, and surgical interventions such as ACL repair and high tibial osteotomy (HTO).

Effectiveness of the interventions was determined through observation and measurement of structural outcomes of tissue repair and clinical outcomes based on function and symptom relief. ***Measurement tools*** used to evaluate ***structural outcomes*** are provided in **Table 1**. Specific features of tissue repair that were assessed were implant stability, defect filling, integration with border zones, cartilage thickness, regeneration of cartilage or that hyaline-like features were exhibited (rather than the presence of fibrocartilage). Consistently measured ***clinical outcomes*** were: pain, quality of life, and physical functioning (which includes, walking, activities of daily living, sport activities). These were measured using a broad range of outcome measures, listed in **Table 1**, with additional descriptive information provided in **Appendix 11**. Other outcomes of interest were joint function, range of motion, and safety. The review by Peeters et al. (2013) focused specifically on safety, where "Severe Adverse Events (SAE)" were defined as death, neoplasms, infections, pulmonary embolisms, anaphylactic shock and haematological neoplasms. Patient satisfaction was assessed less frequently.

Table 1 – Measurement tools used in clinical studies of MSC interventions for osteoarthritis

Measurement tools for structural outcomes	**Measurement tools for patient reported or clinical outcomes**	
Magnetic Resonance Imaging (MRI) Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score Arthroscopy (macroscopic evaluation) International Cartilage Repair Society (ICRS) scoring system (used in arthroscopy evaluation) Radiographs Biopsy X-rays T2 mapping (measure of cartilage repair)	*(most commonly used)* Tegner Activity Score Lysholm Knee Score (10/19), Visual Analog Scale (VAS) (10/19), Hospital for Special Surgeries knee scoring system (HSS) (7/19) Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (6/19) *(less commonly used):* Knee Injury and Osteoarthritis Outcome Score (KOOS) SF-36 International Knee Documentation Committee (IKDC)	*(less commonly used con't):* American Orthopedic Foot and Ankle Score (AOFAS) MARX rating scale Stanmore-Bentley Functional Rating System Roles and Maudsley Score Function Rating Index Lequesne Index Osteoarthritis Outcome Score (OAOS) SF12, SF26 (Functional and mental components) LEFS NPRS

Outcomes

The interpretation of the results of the clinical studies included the 20 reviews, and by extension the reviews included in this white paper, is challenging due to the significant variability associated with the MSC sources, current techniques and approaches being applied in the clinical and research settings for preparation and delivery of MSC therapies, and the variety of co-interventions used in conjunction with MSCs.

Efficacy

With a broad brush stroke, clinical trials and expertise suggests that stem cell therapies are effective in symptomatic relief related to chondral defects and defects, or lesions resulting from degenerative processes leading to osteoarthritis. There is variability in terms of what was observed and/or measured as a significant improvement and variability in the methods of comparison to traditional or standard care procedures (e.g. microfracture) (Chahla et al. 2016; Gopal et al. 2014; Kon et al 2014). However, pain reduction and increased function are the most frequently reported clinical outcomes. In terms of tissue repair, several of the reviews report successful repair and integration. Again, there was extensive variability in the frequency of such results.

Factors identified as important in modulating the benefits of stem cell therapies include:

- age (younger patients tend to have better outcomes),
- gender (males tend to have a better outcomes compared to their female counterparts)
- BMI (lower BMI is associated with better outcomes),
- lesion or defect size (better repair associated with smaller lesion size for focal lesions)
- stage of OA (earlier stages of OA, mild to moderate, correlated with better outcomes)

Negative outcomes which have been reported and identified in the reviews include: increased cartilage thickness, generation of fibrocartilage, worsening of outcomes 24 and/or 48 months post intervention, and adverse events, which were primarily pain, discomfort, and swelling and related to injections. These were factors directly or indirectly related to the procedure and stem cell product administered (Peeters et al. 2013; Wolfstsadt et al. 2015). Specific events reported that may be related to the MSC procedures include infection following bone marrow aspirate (one event), pulmonary embolism occurring 2 weeks post injection of bone marrow aspirate (one event) (Peeters et al. 2013).

The potential of stem cell efficacy is evident. However, the consensus of the scientific community at this time is that although promising, the findings are preliminary and inconclusive. There is overwhelming need for phase 2 and 3 trials. Further, t there is need for standardization of processing approaches, detailed protocols, and standardized outcomes assessment.

Safety

Although negative outcomes have been reported, as noted above, these were not determined to be at a level of risk to patients that precludes the therapeutic viability of MSCs in the treatment of chondral and OA lesions. Peeters et al. (2013), authors of a systematic review focused on stem cell safety, conclude: "…based on current literature review, we conclude that application of cultured stem cells in joints appears to be safe". Perceived safety is also based on the fact that comparatively, more recent approaches and techniques for MSC application are less invasive than traditional or standard care approaches. For example, they do not involve the donor site morbidity requirements of autologous chondrocyte implantation (ACI), which may lead to additional risks related to the donor site. However, areas flagged as requiring consideration (specific attention) to ensure safety include procedure-related complications, device-related complications, and re-operation rate (Fischer & Kisser 2016).

There appears to be a general consensus that stem cell therapies are safe, however, several authors of the reviews state that adverse event reporting and safety specific outcomes in published work is either under-reported or poorly reported (inconsistent or incomplete). Further, the issues of regulation, specific to cell processing and procedures, was not directly addressed or discussed in the reviews. The exception being a comment by Pak et al. (2016), regarding the impact of regulatory requirement and approvals on use of culturally expanded adipose derived stem cells in clinical settings (Pak et al. 2016): *"…due to regulatory issues, only [adipose derived stem cells (ADSCs)] in the form of [stromal vascular fraction] are currently allowed for clinical uses in humans. Culture-expanded ADSCs, although more convenient, require clinical trials for a regulatory approval prior to uses in clinical settings."*

CURRENT AND CONTINUING ISSUES

Of primary concern is patient safety. Controlling risk while using MSCs demands regulation of their isolation and manipulation, the process of application, and mitigation of any long term side effects. All of these require evidence and quality assurance, particularly GMP manufacturing facilities. Certainly, most of the literature would suggest safety in the short term, except for a small number of deaths reported in the USA that are currently being investigated. The long term safety has yet to be fully determined.

Another key linking issue is regarding balancing the needs and demands of the stakeholders in a timely manner to ensure safety. Some of these disconnect between current needs and the prolonged timeline for validation of MSC applications has led to the development of a "cottage industry" regarding the use of MSC injections for patients who pay out of pocket for the procedures. The growth of this cottage industry in a mainly unregulated manner, is now appearing on government radars to ensure patients are not at risk. To move ahead, we have to balance risk with needs, but certainly mitigating risk is critical.

The second big issue is that of MSC application effectiveness. The term effectiveness is complex, as it depends on the definition of success in their use from the patient perspective (e.g. independent living, quality of life, decreased disability, less pain, etc), the clinical perspective (e.g. structural repair of

damaged cartilage, mensci, etc in addition to symptom management), and the government perspective (e.g. decreased use of the health care system, a return to employment, etc)

Moving forward will require perseverance, as well as stewardship in the research community, combined with efficient exchange of valid information based on rigorous science and clinical experience.

MOVING FORWARD – FUTURE DIRECTIONS

Analysis of the reviews included in this white paper indicates there are a number of details that need to be addressed in future research and practice in order to clarify the efficacy and safety of MSC therapy for treatment of cartilage repair. The following aims to summarize the main recommendations from the reviews authors:

MSC handling and preparation

- Researchers and clinicians should clearly define how they identified and controlled the composition of the cells utilized for treatment
- The optimal MSC source tissue (adipose, bone marrow etc.) for cartilage repair treatments has yet to be identified. Clinical studies focusing on comparing the different MSC sources must be undertaken.
- The optimal cell dose, defined as cell count and/ or concentration, must be defined. Preliminary research points to a dose-response relationship, although only one study has investigated this issue (Jo et al. 2014). Every study should provide clear information regarding their MSC concentrations in order to allow comparisons between studies by way of meta-analysis.
- MSC quality is not reported in most studies on cell-based products. Quality can be influence by dedifferentiation of chondrocytes during cultivation. Acceptable standards, preferable at an international level, regarding MSC cultured products must be delineated to ensure efficacy and patient safety.
- The total population doubling index and conditions of isolation (e.g oxygen tension) must be documented.
- Processing within GMP facilitates has many advantages. However, regulated manufacturing requires appropriate resourcing and infrastructure – all of which is significant cost. To that end, centralized manufacturing may be a better option as a national approach (e.g. The UK Cell Therapy Catapult) may better serve all of the stakeholders.

Study design

- Most studies report on the combined use of MSC therapy with a co-intervention (microfracture, Platelet Rich Plasma (PRP) injection etc). It is impossible to distinguish the effects of MSC therapy without proper control and reporting of co-interventions, or isolation of the treatment.
- There is a need for clinical imaging investigations to determine where MSCs localize after intraarticular injection in order to clarify MSC interactions with the structures of the in vivo environment.
- The most effective timing and frequency of injection is unknown.
- Investigations comparing scaffolding techniques (materials, placement, cell-free etc). should be undertaken.
- Culturing environments, including supplementary growth factors, should be clearly defined to develop optimal procedure for chondrogenic potential.
- Determination of optimal stem cells from any source has yet to be determined, with more recent investigations ranging from 1.0×10^7 to 1.0×10^8.
- Lack of comprehensive follow up puts patients at risk for unforeseen long term side effects. Designs that track patient outcomes long term (over 2 years) are needed.
- Studies should have comprehensive study designs to investigate the effect of placebo on pain and function outcomes.

Participant Characteristics

- Cartilage lesions characteristics (size, location, cause etc.) must be fully defined and controlled.
- Study sample characteristics, including those that have been linked to efficacy of treatment such as obesity, age, stage of OA, must be fully disclosed and statistically controlled in further investigations.
- Details regarding post-operative medication use and rehabilitation programs must be considered when reporting outcomes.

Outcomes

- Heterogeneity in study outcomes is preventing comparative analysis of investigations. A consensus on the primary clinical, safety, and repair outcomes and appropriate methods to evaluate those outcomes is required to ensure consistency and efficacy.
- Investigations should include evaluations of the biomechanical properties and composition of the regenerated cartilage
- ‘n’ of the patient sample has to be sufficiently large to capture and characterize subsets of patients (e.g.: respondents and non-respondents).

Safety

- Perhaps the matter of most urgency surrounding MSC therapy is the need to establish certification around cell preparation, culturing, and delivery procedures. Currently, there is concern around the spontaneous transformation of MSCs into unwanted tissue, both during the culture process and in vivo from aspirate injections.
- Use of expanded MSCs likely provides the clearest path forward for standardization of the culture quality and composition. However, ex vivo culture confers risk of contamination, cellular transformation, and premature differentiation of cells. Any expanded protocols should comply with good manufacturing practice (GMP) guidelines, requiring a clearly defined and document procedure, which can add a regulatory burden to clinical investigation.
- A consensus on the definition of 'adverse events' should be found to ensure these incidents are documented. Future studies must include adequate methods to collect adverse events including patient diaries, clinical assessments, imaging, and or arthroscopy.

In summary, the quality of investigation around MSC therapy for treatment of cartilage lesions must be elevated. All authors agreed there is a great need for well-conducted, multi-center randomized controlled trials with systematic, long term follow up.

CONCLUSIONS:

Current studies appear to confirm the potential of MSC use in joint injury repair and in OA specifically. Thus, the hope remains, that is the reality. However, limitation or progress in the field depends, in large part, on standardization of protocols for use of MSC and long term outcomes assessment in a largely unregulated industry. This is complicated by the fact that most patients pay out of pocket for these private services in Alberta and other domains. Thus, there is likely a need for certification of facilities, validation of protocols, and formation of long term databases to ensure patient safety and procedure effectiveness. In addition, continued work in advancing our knowledge in this area is need which is dependent on active participation of funding bodies, including government agencies, to investment in large clinical trials and the establishment and commissioning of centralized GMP facilities in Alberta (or nationally). The realization of the potential of stem cells as a viable therapeutic option is based on a determination of who is responsible for safety (e.g. Government, regulatory bodies) and effectiveness (e.g. researchers, clinicians, policy makers). Therefore progress forward is a shared responsibility, central to which are the patients seeking and undertaking this form of treatment.

THE WORKSHOP: LESSONS LEARNED AND NEXT STEPS

The following is a summary of the discussions, keynote talks, and short perspective talks that took place at the BJH SCN workshop on stem cells for osteoarthritis treatment, which took place on October 27 and 28, 2016 in Calgary Alberta.

The issue of stem cells and their application for the treatment of osteoarthritis is complex. This complexity results from the biology of the stem cell itself (heterogeneity, incomplete characterization, differences in function between in vitro and in-vivo), the numerous options currently experimented with regard to the different sources that can be used, and then, how the stem cells are extracted or harvested, processed or prepared, and delivered to the patient. The complexity is then further driven by the need for decision making – at the patient, clinical practice and the policy levels – in an environment where patients are actively seeking such a treatment even given the limitations of our current knowledge regarding efficacy. Thus, there is need for better communication with patients and providers who council patients to consider stem cell therapy, more research evidence to define the cells and their efficacy, standardization of protocols used, generation of databases to monitor safety and efficacy, and optimizing patient outcomes.

The hope of stem cell use to treat osteoarthritis, currently a non-curable disease, is an emerging area of research and clinical application with intensive focus from around the world on generating solid evidence and knowledge about stem cells and their function in treating and/or repairing damage joint tissues. The blunt reality is that currently (late 2016), we simply do not know conclusively if stem cells are an effective OA treatment. And, if the observed effects reported to date are as a result of the stem cell itself (and its characteristics), placebo effects, and/or related factors or co-factors. In fact, there is a greatly deal of uncertainty if it is the stem cells at all that are the mechanism driving any observable effect.

Closely associated with the issue of efficacy is safety. Although research and clinical experience suggests that autologous stem cell applications are safe, it remains a concern. Thus, questions of safety continue to arise with respect to the use of autologous or allogenic stem cells, and their various sources (e.g. bone marrow, adipose tissue etc). Such questions are also impacted by the various procedures used in the extraction, processing/preparation, and delivery of the stem cells as in more instances it is not known whether there is 1 or a million stem cells being re-administered to the patient. Furthermore, the quality of the environments where stem cells are prepared and delivered requires attention. Although facilities, such as those currently run by Calgary Lab Services and the GMP facility associated with the University of Alberta, follow international standards, other clinical settings are a potential concern due to the relatively unregulated nature of such facilities.

The above issues were presented and discussed by workshop participants in order to identify how we may proceed in Alberta given the emergent and complex nature of this treatment approach. We elaborate on these issues on the following pages:

1. *Access to stem cell treatments*

Currently stem cell treatments for OA are not publicly funded. As such, patients who choose this treatment option pay for it privately. These raised the question of whether it should be offered through the public system and if so, what are the implications, including funding. Other key questions that require further consideration in determining access include: first, who is mostly likely to benefit from stem cell treatments and when should stem cell treatment be considered as a treatment option. This is related to stage of the disease, co-morbidities (e.g. obesity, diabetes) which impact the stem cell function, age etc. Secondly, who (what providers) should be delivering stem cells.

The above is of course depends on the core question of whether or not stem cells should be made available as a treatment option in Alberta today, given the current health care environment and state of knowledge on efficacy. The position of the workshop participants (representing multi-stakeholders) was not clear and nor did they settle on one conclusive option. There was a distinct opinion that we should not be offering stem cells as a treatment option at this time, in private or public settings, as we simply do not know enough about stem cells and their potential efficacy. This was balanced by the opinion that given that they are generally perceived to be safe and patients are seeking out this treatment, and will travel outside of Alberta to receive it, we should make it available in the private settings, but with a focus on standardization to ensure safety, and data collection to monitor both safety and efficacy (which is limited in interpretation due to the unblinded nature of the treatment). Thus, if made available in certified facilities, it should be monitored for an extended period of time with standardized outcomes.

Although consensus was not sought through the workshop, it was evident that there was mixed opinions expressed by the stakeholders present regarding current access to stem cell treatment for OA in Alberta.

2. *Standardization*

If stem cells are to be delivered in Alberta, now or in the future, a key issue that arose was how to standardize the processes to ensure safe (and effective) delivery. Standardization refers to how to define procedures to ensure consistency and alignment with evidence and or best practice. Discussions pointed to the fact that standardization needs to occur at several levels or points: the facilitates where stem cells are prepared (extraction, processing and delivery mechanisms), the provider delivering the treatment, and the site where the processing and/or treatment is being provided. Development of such standardizations should involve regulatory bodies (e.g. the College of Physicians and Surgeons), federal bodies (Health Canada), and a multi-stakeholder working group to contribute to such guideline development.

3. *Generating evidence of efficacy*

Our knowledge in this area is advancing, however, the evidence regarding mechanism of action and efficacy is currently inconclusive. As such, ongoing research is needed. Specifically, it was recognized

that Phase 3 and 4 randomized trials are required. Alberta may be a potential site for a trial, informed by an international advisory panel; however, given the work already underway and the relations that were initiated through the workshop, there was also consideration of Alberta making a meaningful contribution to the work of experts in the field and/or well established programs. Another approach which was discussed, and mentioned above, was the development of a provincial database to capture standardized outcomes within clinics delivering this treatment in Alberta as a first step. Thus, one responsibility of being certified, could be the requirement to participate in such databases.

4. *Communication tools to support decision making*

There is a clear need for credible and comprehensive information on this emerging treatment for OA that will inform decision making. The appetite for such communication tools was quite strong, but the audience for such communications is diverse. The key stakeholders identified through the workshop include: patients, front line Health Professionals (Family Physicians, Physiotherapists, etc), providers (delivering stem cells or addressing questions from patients about stem cells), policy makers, and regulators. The communication vehicles and access points may vary from one stakeholder group to the next, however, it was recommended that all need to inform the recipient regarding the current state of knowledge, the complexity associated with this treatment approach, and potential risks. Thus, appropriate communication tools, frequently updated, would assist in managing expectations of patients, and be a trusted source of information for health care professionals.

5. *Collaborative relationships*

Given that the issue of stem cells as a potential health care intervention for OA impacts and is influenced by various stakeholders, there was recognition that forging relationships to enable safe and effective delivery of stem cells is critical. Such relationships may involve the private and public health care sectors in the province; clinical practices and academia to ensure appropriate interpretation of results and their implications; as well as researchers to allow for efficient use of research resources required to perform the required research and trials. Given that stem cells as a potential treatment for OA are not isolated to Alberta, we may also consider partnerships with national and international stakeholders external to Alberta who are already doing work in this area. The bottom line is that partnerships, both locally and internationally, are beneficial to enable appropriateness and efficiency of efforts put forward, to enhance the rate of progress, and to avoid redundancy in efforts.

Action items

The BJH SCN led the initiation of this multi-stakeholder discussion on stem cell treatment for OA in Alberta. It now has an instrumental role in facilitating action around these three topics. It is imperative that we maintain the momentum generated at the workshop and ensure that there is productive and proactive action on these key issues. Therefore, we recognize it is our responsible for enabling the movement

forward. Given our provincial leadership role, we are well positioned to enable and facilitate cross-provincial collaborations of the necessary stakeholders to move forward.

Based on the rich discussion and emergent ideas and recommendations, several areas requiring attention to ensure we proactively move forward in Alberta on the issue of stem cells for OA were identified. One of the key outputs of the workshop is the identification of three priority areas that need immediate action:

1. Establish the efficacy of stem cells for the treatment of osteoarthritis.
2. Develop appropriate communication tools and strategies to ensure that the right information is accessible to inform decision making by patients, providers and policy makers
3. Standardization of practice for safe delivery of stem cells for OA

These priority areas will be addressed by implementation committees. Each will be tasked with identifying specific recommendations and engaging in the required activities to progress the work in that area. Deliverables may include, but are not limited to, one or more of the following:

- Peer reviewed publications
- Knowledge syntheses/literature reviews
- Research project grants proposals/applications
- Outcomes database development
- Guidelines
- Progress reports (biannual)

Our goal is to initiate these committees between January and March 2017.

The Scientific Director (DH) and Assistant Scientific Director (AKR) of the BJH SCN will provide oversight to each of the committees and to ensure that the groups remain on track in terms of objectives, timelines and deliverables. The committees will report directly to the BJH SCN Scientific Office.

GLOSSARY

Autologous –derived from the same individual (e.g: autologous cells are harvested and implanted from the same patient)

Allogenic -derived from the same species as the recipient but not genetically identical (ex: allogenic aspirate)

Aspirate - the mixture of cells and fluid that is the result of tissue extraction.

Aspirate Concentrate - an aspirate's mononuclear cell layer, that is isolated by centrifuge (Chahla et al. 2016). The use of aspirate concentrate involves mesenchymal cells that are minimally manipulated—no ex vivo culturing or expanding takes place (Wolfstadt et al. 2015). The exact number or proportion of MSCs in the concentrate is unknown at time of administration (Wolfstadt et al. 2015). Procedures that use aspirate concentrate do not require regulatory approval in most jurisdictions, including Canada and the USA (Wolfstadt et al. 2015).

Chondral defect - localized area of damage in articular cartilage

Derived cells (ex: adipose derived MSCs) - stem cells that have been isolated via centrifuge from the tissue aspirate, then cultured, and expanded. *Ex vivo* culturing and expanded MSCs would fall under biological drugs or pharmacological treatments by some regulatory agencies.

Endogenous – material that produced or synthesized within the patient

Exogenous – material that is introduced from or produced outside of the patient.

Osteoarthritis – broad area of cartilage breakdown or degeneration; may also involve underlying subchondral bone

Osteochondral defect – localized area of damage that includes the cartilage and underlying bone.

Stromal Vascular Fraction (SVF) - the heterogeneous mixture of mesenchymal cells separate from the mature adipocytes that are collected when lipo-aspriate is digested with collagenase. SVF is separated and cultured to isolate adipose mesenchymal stem cells.

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Appendix 1 – Description of non-cell based (exogenous) therapies

To overcome the lack of blood supply and source of undifferentiated cells, surgical techniques have included debridement, microfracture and bone marrow stimulation, and abrasion of the subchondral bone plate. These mechanisms are thought to trigger the innate biological repair systems and can lead to influx of chondroprogenitor cells (Counsel). However, these approaches tend to result in the formation of fibrocartilaginous repair tissue. While this can decrease pain and morbidity with proper rehabilitation in the short and intermediate term, it does not appear to lead to the differentiation of cells into the chondrocyte lineage, and therefore, has little efficacy as a long term solution (Guilak 2004). Other mechanical approaches have involved osteochondral grafting (mosaicplasty) and high tibial osteotomy (HTO). (Guilak 2004 & Bauge & Wong 2013). Osteochondral grafts involve transplantation from a tissue donor (allografts), or from the patient's own body (autografts) of healthy cartilage and underlying subchondral bone are also options that have been used clinically. However, they have met with challenges of integration of the implanted graft and host cartilage, limited availability of non-weight-bearing grafts, as well as evidence for long-term efficacy (Guilak 2004, Hangody 2003). High tibial osteotomy (HTO) involves shortening of the tibia, and effectively leads to an unload of the medial compartment to allow some easing of stresses on articular cartilage (Wong 2013). This approach has shown to be an effective clinical intervention, however, is not a long term solution (Wong 2013).

The main limitation: These therapies do not replace cartilage lesions with tissue that replicates the biological, biomechanical, and physical structure of native AC (Filardo 2013). Further, the long term efficacy of non-cell based therapies continues to be unproven (Guilak- 2004).

List of non-cell based therapies for treatment of AC defects/lesions

- Microfracture
- Abrasion chondroplasty
- Adjacent tissue stimulation
- Mosaicplasty
- Graft implantation
- Rigid fixation (osteo-chondral fracture)
- Partial and full joint replacement
- High tibial osteotomy
- Weight loss
- Exercise (prescribed)
- Cell-free scaffolds (e.g. autologous matrix-induced chondrogenesis (AMIC)

Appendix 2 – Description of non-stem cell therapies (chondrocytes)

Most recent advances in non-stem cell based therapies involve implantation of a 3-D biomaterial to exploit and enhance innate cartilage repair systems with an in an 'in situ' strategy. This takes the form of autologous chondrocyte implantations (ACI) using mature chondrocytes (Counsel, 2015). Mature chondrocytes can be quiescent within the AC matrix, analogous to osteocytes in bone. During limb development, chondrocytes form the cartilage in the joint space (Goldring 2012) or undergo chondrocyte hypertrophy, ultimately being calcified and reabsorbed by bone. (Goldring 2012). Chondrocytes used in autologous chondrocyte implantations are sourced from healthy cartilage at non-weight bearing, unaffected parts of the joint (Filardo 2013). Target areas for extraction include the non-weight bearing area of the intercondylar notch or the femoral condyle superior ridge). (Bauge 2015) The cartilage can be obtained from the patient themselves (autograft) or from a donor (allograft), although autologous chondrocytes are preferred due to decreased risk of immunogenic response or disease transfer (Bauge 2015). autologous chondrocyte implantation therapies utilize mature, cultured autologous chondrocytes suspend in an injection suspension and covered with periosteal flap (ACI-P) as in first generation ACI, or collagen membrane (ACI-C) as in second generation ACI. In third generation techniques, chondrocytes are seeded into a collagen membrane (matrix-induced autologous chondrocyte implantation, MACI) and implanted into cartilage lesions. (Counsel 2015). Since introduction of MACI into clinical practice in 1998 (Filardo 2013), a wide variety of study populations, technical procedure, surgical approach, and matrix compositions have been seen in the research literature. (Filardo 2013). The potential for ACI is limited by the availability of healthy cartilage donor sites. (Bauge 2015). There have also been challenges in cell culturing: monolayer chondrocyte cultures often switch to production of fibrocartilage collagen (collagen I) than the preferred hyaline cartilage collagen (collagen II) (Bauge 2015). Scaffolding techniques can limit the number of cells that can be transplanted successfully (Bulman 2013). Further, there are links to poor outcomes due to obesity, smoking history, and age. (Bentley 2013). There is a paucity of literature comparing stem cell treatment to ACI (Counsel 2013).

The main limitations: Limited donor tissue availability for transplantation, morbidity at the donor site; differentiation leading to hypertrophy of implanted chondrocytes, and de-differentiation during the expansion phase leading to the formation of fibrocartilage (Jayasuriya 2015).

List of types of chondrocyte based delivery techniques and methods

- Autologous chondrocyte implantation (ACI)
- (*first generation/traditional ACI*): ACI + periosteal flap (ACI-P)
- *second generation/traditional ACI*): ACI+collagen flap (ACI-C)
- *(third generation):* Matrix-induced autologous chondrocyte implantation (MACI)

Appendix 3 – Advantages and disadvantages of different sources of MSCs

Cell Source	Advantages	Disadvantages	Quantity/ Prevalence
Bone Marrow	High chondrogenic potential Relative ease of collection	High variability in MSC number MSC numbers and quality decline with age	5-50 x 10^6 cells in 5 mL
Adipose Tissue	Ease of Harvest Large amount of tissue can be extracted Limited donor site morbidity	MSC numbers decline with obesity Lower chondrogenic potential	5 x 10^4- 2x10 5 cells in 1g
Synovial Membrane	Highest chondrogenic potential Lowest osteogenic potential among MSCs	Limited number	?

Appendix 4: Additional information about scaffolds

Scaffolds have been created from numerous materials. In recent years, there has been the development of a variety of biomaterial matrices that vary by fixation technique, treatment in bioreactors, and composition of scaffold (Filardo 2013). A number of natural materials have emerged for scaffolds such as agarose, alginate, fibrin glue and chitosan (Filardo 2013). Synthetic scaffolds are commonly made up of polylactides, including polylactic and polyglycolic acid (Filardo 2013). The opportunity to innovate regarding the composition and physical forms (ex: fibers, meshes, gels) of these scaffolds has led to a number of patented products including Haylograft C, Bioseed c, and NeoCart to name a few (Filardo 2013). While 3-D scaffolds for ACI (autologous chondrocyte implantation) have been in used for some time (as early as 2001), scaffolds seeded with MSCs have had less rigorous analysis (Bauge 2015), but this is an active area of research. In the treatment of osteochondral articular defects, specific biphasic scaffolds have been developed to meet the challenge of guiding the growth of the bone and articular cartilage involved in the defect (Filardo 2013). Biphasic scaffolds are in their clinical infancy, however, they are somewhat revolutionary in their attempt to treat the entire osteochondral unit as an integrated composite.

Appendix 5: List of Clinical Trials Currently in Progress

(sources: clinicaltrials.gov; http://apps.who.int/trialsearch/)

Public Title	Study Design	Country	PI (Key Contact)	Condition	Intervention	Primary Outcome
Mesenchymal Stem Cells in Knee Cartilage Injuries	Non-Randomized, Case Control	Jordan	Abdallah Awidi, MD;Mahasen S Najjar, MD;Hiba Khalil, PhD	Knee OA	Biological: Autologous Mesenchymal Stem Cells	Therapeutic Benefits
UCMSC Transplantation in the Treatment of Cartilage Damage	Non-Randomized, Case Control	China	Xuetao Pei, M.D.,Ph.D	OA	Biological: umbilical cord mesenchymal stem cells;Device: Hyaluronic acid	KOOS
Autologous Stem cells, Chondrocytes Or the Two?	Single centre RCT	United Kingdom	Not Indicated	Knee Cartilage Defects	Autologous chondrocytes and BMSCs combined in an injection in two stages; each the patient will receive a total cell population of between 1 and 20 million cells	Modified Lysholm, pre-operative score
Autologous Transplantation of Mesenchymal Stem Cells (MSCs) and Scaffold in Full-thickness Articular Cartilage	Case Series	Iran	Hamid gourabi, PhD;Mohamadreza Baghaban Eslaminejad, PhD;Leila Taghiyar, Msc	Knee OA	Biological: Bone marrow derived mesenchymal stem cells	Knee cartilage defects
Evaluation of mesenchymal stem cells in the treatment of hip cartilage lesions post arthroscopic microfracture – prospective case series data collection	Not Stated	Australia	Dr Julien Freitag	Hip OA	Autologous adipose derived mesenchymal stem cells (200 × 10*6 MSCs) injected and arthroscopic microfracture	Hip Injury and Osteoarthritis Outcome Score; Numerical Pain Rating Scale (NPRS)
Articular Cartilage Resurfacing With Mesenchymal Stem Cells In Osteoarthritis Of Knee Joint	Case Series	Iran	Hamid Gourabi, PhD;Mohammadreza Baghban Eslami Nejad, PhD;Mohssen Emadeddin, MD;Nasser Aghdami, MD,PhD	OA	Biological: Mesenchymal Injection	Pain relief

Regenerative Medicine of Articular Cartilage: Characterization and Comparison of Chondrogenic Potential and Immunomodulatory Adult Mesenchymal Stem Cells	Case Series	France	Dr Ronan Guillou;Claire Vinatier;Claire Vinatier	Knee OA	Procedure: arthroplasty	chondrogenic markers
Transplantation of Bone Marrow Stem Cells Stimulated by Proteins Scaffold to Heal Defects Articular Cartilage of the Knee	Case Series	France	Michel Assor, MD;Shimon Slavin, MD;Michel Assor, MD;Michel Assor, MD	Knee OA	Procedure: Transplantation of Bone Marrow Stem Cells Activated in Knee Arthrosis	International Knee Score;IKS, International Knee Score
Transplantation of Bone Marrow Derived Mesenchymal Stem Cells in Affected Knee Osteoarthritis by Rheumatoid Arthritis	Case Control	Iran	Hamid Gourabi, PhD;Nasser Aghdami, MD,PhD;Mohsen Emadedin, MD;Farhad gharibdoost, MD;Soraya Shadmanfar, MD	Rheumatoid Arthritis	Biological: mesenchymal cell transplantation;Biological: placebo	pain;physical activity;walking distance
Synovium Brushing to Augmented Microfracture for Improved Cartilage Repair	RCT	United Kingdom	Dennis G McGonagle, MB BcH BAO;Dennis G McGonagle, FRCPI PhD;Owen R Wall, MB ChB	Knee OA	Device: Arthroscopic synovial brushing;Procedure: Microfracture	The mean change in the number of MSCs present in the knee pre- and post-microfracture/microfracture plus arthroscopic synovial brushing.
Adult Stem Cell Therapy for Repairing Articular Cartilage in Gonarthrosis	Case Control	Spain	Robert Soler, MD	Knee OA	Other: Autologous MSC knee implantation	Feasibility of autologous bone marrow mesenchymal stem cells (MSC) knee articular infiltration.;Safety of autologous bone marrow mesenchymal stem cells (MSC) knee articular infiltration.
Human Umbilical Cord Mesenchymal Stem Cell Transplantation in Articular Cartilage Defect	Case Series	China	Ping J Chen, Professor	OA	Biological: Human umbilical cord mesenchymal stem cells	Severity of adverse events
Autologous Bone Marrow Mesenchymal Stem Cells Transplantation for Articular Cartilage Defects Repair	Case Series	Brazil	Paulo Brofman, PhD;Paulo Brofman, PhD;Alexandra Senegaglia, PhD	OA	Procedure: Bone marrow aspiration	Change in WOMAC (Western Ontário and MacMaster Universities)score

Evaluation of mesenchymal stem cells in the treatment of hip osteoarthritis – prospective case series data collection	Not Indicated	Australia	Dr Julien Freitag	OA	Intra-articular injection of 20 million Autologous adipose derived mesenchymal stem cells at 0 and 6 months (total of 40million cells).	Hip Injury and Osteoarthritis Outcome Score - Numerical Pain Rating Scale (NPRS); MRI quantitative data
Evaluation of mesenchymal stem cells in the treatment of knee osteoarthritis – A randomised Controlled Trial	Not Indicated	Australia	Dr Julien Freitag	OA	Autologous adipose derived mesenchymal stem cells in 3 Treatments: Group 1. Single intra-articular injecton of 100million stem cells at 0months. Group 2. Intra-articular injection of 100million stem cells at 0 and 6 months (total of 200million cells) Group 3. Control Group - conservative management.	KOOS; Numerical Pain Rating Scale (NPRS);MRI quantitative data
Evaluation of mesenchymal stem cells in the treatment of knee osteoarthritis – prospective case series data collection	Not Indicated	Australia	Dr Julien Freitag	OA	Intra-articular injection of 100 million autologous adipose derived mesenchymal stem cells at 0 and 6 months (total of 200million cells).	KOOS; Numerical Pain Rating Scale (NPRS);MRI quantitative data
Clinical Trial to Compare ReJoinTM to Sodium Hyaluronate Injection for Knee Osteoarthritis Cartilage Defects	Case Crossover	China	You Wang;You Wang	Knee OA	Biological: ReJoinTM;Drug: Sodium Hyaluronate	WOMAC scores
The Use of Autologous Bone Marrow Mesenchymal Stem Cells in the Treatment of Articular Cartilage Defects	Case Series	Egypt	Hazem M Atta, Ph.D	OA	Bone marrow mesenchymal stem cell implantation	Improvement in Clinical Scores and Radiological images
Intra-Articular Autologous Bone Marrow Mesenchymal Stem Cells Transplantation to Treat Mild to Moderate Osteoarthritis	RCT	Malaysia	Dr Ya Mohammad Hassan Shukur	OA	Hyaluronic Acid & Autologous bone marrow-derived mesenchymal stem cells	MRI Visual Analog Score; IKDC Subjective Knee Evaluation Form (2000); Change from baseline in progression of osteoarthritis at 12 months by plain radiograph (X-ray)
Mesenchymal Stem Cells Enhanced With PRP Versus PRP In OA Knee	Double-Blind RCT	India	Aditya K Aggarwal	Knee OA	100 million Bone marrow mesenchimal stem cells vs. Platelet Rich plasma (PRGF)	Pain relief Functional Outcome

Treatment of Osteoarthritis by Intra-articular Injection of Bone Marrow Mesenchymal Stem Cells With Platelet Rich Plasma	Case Control	Spain	José Lamo-Espinosa, MD	Knee OA	100 million Bone marrow mesenchimal stem cells; Platelet Rich plasma (PRGF);HTO with microfracture; Transplantation of adipose derived stem cell	VAS, KOOS (1,3,6,12 months), WOMAC, SF-36, EuroQuol-5D, Lequesne Index, Femorotibial distance, Serious and Non-serious adverse events, VAS (1,3,6,12 months)
Clinical Outcomes of Open Wedge High Tibial Osteotomy With Autologous Bone Marrow or Adipose-derived Stem Cell Therapy	RCT	Korea	Dongsik Chae	OA	HTO with microfracture; Transplantation of bone marrow stem cell; Transplantation of adipose derived stem cell	Knee score MRI Arthroscopic finding
Clinical Trial of Allogenic Adipose Tissue-Derived Mesenchymal Progenitor Cells Therapy for Knee Osteoarthritis	RCT	China	Chunde Bao, M.D. & Ph.D	OA	Mesenchymal progenitor cells	WOMAC Score; Recording of Adverse Events and Serious Adverse Events; VAS Score; SF-36; The volume of articular cartilage; WORMS Score
Treatment of Osteoarthritis With the Stromal Vascular Fraction of Abdominal Adipose Tissue - a Pilot Study	Case Series	Den.	Not Indicated	OA	injection with the stromal vascular fraction (SVF) of adipose tissue	Adverse events; KOOS; Numeric Rating Scale for Assessment of Pain Intensity
Human Autologous MSCs for the Treatment of Mid to Late Stage Knee OA	Case Series	Canada	Jas Chahal	OA	3 doses of MSC: 1 x 10^6 MSCs; 10 x 10^6 MSCs; 50 x 10^6 MSCs	Safety as determined by the occurrence of local and systemic adverse events and/or serious adverse events; KOOS; Marx Activity Scale; Short-Form 36; WORMS; Gadolinium-enhanced MRI; T2 Mapping; Cartilage oligomeric matrix protein (COMP); Hyaluronic acid (HA); C-terminal telopeptide of type II collagen (CTXII); Types I and II collagen cleavage (C1,2C); Type II collagen cleavage (C2C); IL-6/TNFα/IL-15

Autologous Adipose-Derived Stromal Cells Delivered Intra-articularly in Patients With Osteoarthritis.	Case Series	United Kingdom	Sharon McQuillan, MD	OA	Intra articular infusion of AD-SVF	Visual Analogue Scale (VAS); Quality of life scores; Reduction in analgesics; Number of adverse events reported; x-ray, sonogram, or MRI imaging of affected joint compared to baseline.
HyaloFAST Trial for Repair of Articular Cartilage in the Knee	RCT	Hungry, Italy	Not Indicated	Cartilage Defects	Superiority of Hyalofast® with Bone Marrow Aspirate Concentrate (BMAC) vs. Microfracture	KOOS;IKDC;MRI MOCART Score; Evaluator Global Assessment; Adverse Events (AE) from baseline to 2 year follow up

Appendix 6: Description of the methods used to generate the knowledge synthesis

We searched the research and clinical literature to determine the current evidence specific to the efficacy and safety of MSC-based treatments for osteoarthritis, with a particular interest in knee OA. As our goal was to identify the current state of knowledge, the search was focused on published reviews (systematic reviews, comprehensive reviews, clinical reviews, and/or meta-analysis) published between 2006 and 2016 (Sept. and May) in the English language which discussed clinical studies on humans for the treatment of cartilage lesions (chondral and/or osteochondral defects and/or lesions) with cell based therapies, specifically mesenchymal stem cells (MSCs).

Reviews matching our criteria were identified through a systematic search of 6 databases: Embase, Medline, and Cochrane Database of Systematic Reviews (through OVID), as well as CINHAL, Web of Science, and Sport Discus. The search was conducted between May and August 2016 and supported by a University of Calgary Health Sciences librarian and the AHS Knowledge Team librarian. Keywords and subject headings [MeSH] used for each database are provided in the table below. In addition, reference lists of included articles were reviewed to identify any articles that may have been missed. Articles recommended by members of the workshop planning committee were also gathered and screened for inclusion.

Data extracted from each review included: geographic location of authorship team, year of publication, type of review, level(s) of evidence of sited studies, source of MSC, preparation methods, delivery techniques, co-interventions, outcome measurement tools, outcomes reported, and safety issues identified. Relevant data were extracted and collected in a unique database with the consensus of all co-authors.

In total 695 studies were identified from all sources based on the search terms. An additional 9 resources were found through independent searches and recommendations. In the abstract and title screening, 625 studies in total were excluded: 17 duplicates, 605 were not review articles, and 3 were excluded when the full text could not be accessed. 70 studies remained, and another their titles and abstracts were screened in detail. 50 articles were excluded: 1 was a conference poster presentation, 4 had exclusively a preclinical focus, 3 were not review articles, 6 did not focus on cell therapies, and 28 did not complete a comprehensive review of the literature. 28 full text articles were left. Upon further scrutiny, 8 were deemed to have not evaluated the current clinical studies on the topic. 20 full text records were included in the final analysis.

Appendix 7: Keywords and MeSH terms used in the literature search

Database	Keywords	Subject Headings
Medline	“mesenchymal stem cell*” or mesenchymal stromal cell*” or "adipose tissue-derived stem cell*" or "bone marrow-derived stem cell*" or “scaffold*” AND "osteoarthritis" AND "review" or "systematic review*" or "pubmed" or "embase" or "ovid" or "medline" or "meta analy*"	Mesenchymal Stromal Cells Osteoarthritis, Hip/ or exp Osteoarthritis/ or exp Osteoarthritis, Spine/ or exp Osteoarthritis, Knee/ Practice Guideline/ or exp Guideline/ Practice Guidelines as Topic/ Tissue Engineering/ or exp Stem Cells/ or exp Tissue Scaffolds/
Embase	“mesenchymal stem cell*” or mesenchymal stromal cell*” or "adipose tissue-derived stem cell*" or "bone marrow-derived stem cell*" or “scaffold*” AND "osteoarthritis" AND "review" or "systematic review*" or "pubmed" or "embase" or "ovid" or "medline" or "meta analy	mesenchymal stem cell/ adipose derived stem cell/ bone marrow cell/ or exp hematopoietic stem cell transplantation/ or exp mesenchymal stem cell/ "Western Ontario and McMaster Universities Osteoarthritis Index"/ or exp knee osteoarthritis/ or exp "Knee Injury and Osteoarthritis Outcome Score"/ or exp hip osteoarthritis/ or exp osteoarthritis/ "review"/ "systematic review"/
Cochrane Database of Systematic Reviews	“mesenchymal stem cell*” or mesenchymal stromal cell*” or "adipose tissue-derived stem cell*" or "bone marrow-derived stem cell*" or “scaffold*” AND "osteoarthritis" AND "review" or "systematic review*" or "pubmed" or "embase" or "ovid" or "medline" or "meta analy*"	
CINHAL	“mesenchymal stem cell*” or mesenchymal stromal cell*” or "adipose tissue-derived stem cell*" or "bone marrow-derived stem cell*" or “scaffold*” AND "osteoarthritis" AND "review" or "systematic review*" or "pubmed" or "embase" or "ovid" or "medline" or "meta analy*"	
Web of Science	“mesenchymal stem cell*” or mesenchymal stromal cell*” or "adipose tissue-derived stem cell*" or "bone marrow-derived stem cell*" or “scaffold*” AND "osteoarthritis" AND "review" or "systematic review*" or "pubmed" or "embase" or "ovid" or "medline" or "meta analy*"	
Sport Discus	“mesenchymal stem cell*” or mesenchymal stromal cell*” or "adipose tissue-derived stem cell*" or "bone marrow-derived stem cell*" or “scaffold*” AND "osteoarthritis" AND "review" or "systematic review*" or "pubmed" or "embase" or "ovid" or "medline" or "meta analy*"	

Appendix 8: List of 19 reviews included in the knowledge synthesis

Author	Year	Title	Publication Reference	Country
Bornes, T. D., Adesida, A. B. and Jomha, N. M.	2014	Mesenchymal stem cells in the treatment of traumatic articular cartilage defects: a comprehensive review	Arthritis Res Ther. 2014;16(5):432.	Canada
Chahla, J., Dean, C. S., Moatshe, G., Pascual-Garrido, C., Serra Cruz, R. and LaPrade, R. F.	2016	Concentrated Bone Marrow Aspirate for the Treatment of Chondral Injuries and Osteoarthritis of the Knee: A Systematic Review of Outcomes	Orthop J Sports Med. 2016 Jan 13;4(1)	United States of America
Counsel, P. D., Bates, D., Boyd, R. and Connell, D. A.	2015	Cell therapy in joint disorders	Sports Health. 2015;7(1):27-37	Australia
Deng, Z., Jin, J., Zhao, J. and Xu, H.	2015	Cartilage Defect Treatments: With or without Cells? Mesenchymal Stem Cells or Chondrocytes? Traditional or Matrix-Assisted? A Systematic Review and Meta-Analyses	Stem Cells Int. 2016; 14 pages	China
Filardo, G., Madry, H., Jelic, M., Roffi, A., Cucchiarini, M. and Kon, E.	2013	Mesenchymal stem cells for the treatment of cartilage lesions: from preclinical findings to clinical application in orthopaedics	Knee Surg Sports Traumatol Arthrosc. 2013 Aug;21(8):1717-29	Italy
Filardo, G., Perdisa, F., Roffi, A., Marcacci, M. and Kon, E.	2016	Stem cells in articular cartilage regeneration	Journal of Orthopaedic Surgery and Research2016;11:42	Italy
Fischer, S. and Kisser, A.	2016	Single-step scaffold-based cartilage repair in the knee: A systematic review	J Orthop. 2016 Jun 25;13(4):246-53	Austria
Gopal, K., Amirhamed, H. A. and Kamarul, T.	2014	Advances of human bone marrow-derived mesenchymal stem cells in the treatment of cartilage defects: A systematic review	Exp Biol Med (Maywood) June 2014 vol. 239 no. 6 663-669	Malaysia
Kon, E., Roffi, A., Filardo, G., Tesei, G. and Marcacci, M.	2015	Scaffold-based cartilage treatments: with or without cells? A systematic review of preclinical and clinical evidence	Arthroscopy. 2015 Apr;31(4):767-75	Italy
Pak, J., Lee, J. H., Kartolo, W. A. and Lee, S. H.	2016	Cartilage Regeneration in Human with Adipose Tissue-Derived Stem Cells: Current Status in Clinical Implications	BioMed Research International Volume 2016, Article ID 4702674, 12 pages	Republic of Korea
Papalia, R., Franceschi, F., Balzani, L. D., D'Adamio, S., Maffulli, N. and Denaro, V.	2013	Scaffolds for partial meniscal replacement: An updated systematic review	British Med Bull. 2013;107:19-40	Italy

Pastides, P., Chimutengwende-Gordon, M., Maffulli, N. and Khan, W.	2013	Stem cell therapy for human cartilage defects: A systematic review	Osteoarthritis Cartilage. 2013 May;21(5):646-54	United Kingdom
Peeters, C. M., Leijs, M. J., Reijman, M., van Osch, G. J. and Bos, P. K.	2013	Safety of intra-articular cell-therapy with culture-expanded stem cells in humans: a systematic literature review	Osteoarthritis Cartilage. 2013 Oct;21(10):1465-73	The Netherlands
Perdisa, F., Gostynska, N., Roffi, A., Filardo, G., Marcacci, M. and Kon, E.	2015	Adipose-Derived Mesenchymal Stem Cells for the Treatment of Articular Cartilage: A Systematic Review on Preclinical and Clinical Evidence	Stem Cells International Volume 2015 (2015) Article ID 597652, 13 pages	Italy
Author	Year	Title	Publication Reference	Country
Reissis, D., Tang, Q. O., Cooper, N. C., Carasco, C. F., Gamie, Z., Mantalaris, A. and Tsiridis, E.	2016	Current clinical evidence for the use of mesenchymal stem cells in articular cartilage repair	Expert Opin Biol Ther. 2016;16(4):535-57	United Kingdom
Rodriguez-Merchan, E. C.	2014	Intra-articular injections of mesenchymal stem cells for knee osteoarthritis	Am J Orthop (Belle Mead NJ). 2014 Dec;43(12)	Spain
Shimomura, K., Ando, W., Moriguchi, Y., Sugita, N., Yasui, Y., Koizumi, K., Fujie, H., Hart, D.A., Yoshikawa, H. and Nakamura, N.	2015	Next Generation Mesenchymal Stem Cell (MSC)–Based Cartilage Repair Using Scaffold-Free Tissue Engineered Constructs Generated with Synovial Mesenchymal Stem Cells	Cartilage. 2015 Apr;6(2 Suppl):13S-29S	Japan
Veronesi, F., Giavaresi, G., Tschon, M., Borsari, V., Nicoli Aldini, N. and Fini, M.	2013	Clinical use of bone marrow, bone marrow concentrate, and expanded bone marrow mesenchymal stem cells in cartilage disease	Stem Cells Dev. 2013 Jan 15;22(2):181-92	Italy
Wolfstadt, J. I., Cole, B. J., Ogilvie-Harris, D. J., Viswanathan, S. and Chahal, J.	2015	Current concepts: the role of mesenchymal stem cells in the management of knee osteoarthritis	Sports Health. 2015 Jan;7(1):38-44	Canada
Xu, S., Liu, H., Xie, Y., Sang, L., Liu, J. and Chen, B.	2015	Effect of mesenchymal stromal cells for articular cartilage degeneration treatment: a meta-analysis	Cytotherapy. 2015 Oct;17(10):1342-52	China

Appendix 9 – List of clinical trials cited in 19 reviews

Author	# of times cited	Cited by	Year	Title
Kasemkijawatt-ana	8	Chahla et al. 2016 Filardo et al 2013 Filardo et al. 2016 Pastides et al. 2013 Peeters et al. 2013 Reissis et al. 2016 Veronesi et al. 2012 Bornes et al. 2014	2011	Autologous bone marrow mesenchymal stem cells implantation for cartilage defects: two cases report
Nejadnik	7	Deng et al. 2015 Filardo et al 2013 Filardo et al. 2016 Gopal et al. 2014 Reissis et al. 2016 Xu et al. 2015 Bornes et al. 2014	2010	Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study.
Wakitani	7	Bauge & Boumediene 2015 Bornes et al. 2014 Filardo et al 2013 Filardo et al. 2016 Pastides et al. 2013 Veronesi et al. 2012, Bauge & Boumediene 2015	2007	Repair of articular cartilage defects in the patellofemoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five patients
Buda	6	Filardo et al. 2016 Pastides et al. 2013 Reissis et al. 2016 Veronesi et al. 2012 Bornes et al. 2014, Filardo et al 2013	2010	Osteochondral lesions of the knee: a new one-step repair technique with bone-marrow-derived cells.
Giannini	6	Filardo et al. 2016 Reissis et al. 2016 Veronesi et al. 2012 Bornes et al. 2014 Pastides et al. 2013, Filardo et al. 2013	2010	Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: from open field autologous chondrocyte to bone-marrow-derived cell transplatation
Gigante	6	Bornes et al. 2014 Filardo et al. 2016 Reissis et al. 2016 Veronesi et al. 2012 Filardo et al 2013	2011	Use of collagen scaffold and autologous bone marrow concentrate as a one-step cartilage repair in the knee: histological results of second-look biopsies at 1 year follow-up.
Koh	6	Filardo et al. 2016 Pak et al. 2016 Perdisa et al. 2015 Rodriguez- Merchan 2016 Wolfstadt et al. 2015 Counsel et al. 2015	2013	Mesenchymal stem cell injections improve symptoms of knee osteoarthritis.

Centeno	5	Reissis et al. 2016 Veronesi et al. 2012 Wolfstadt et al. 2015 Bauge & Boumediene 2015	2008	Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologousmesenchymal stem cells,
Jo	5	Reissis et al. 2016 Filardo et al. 2016 Perdisa et al. 2015 Rodriguez- Merchan 2016 Pak et al. 2016	2014	Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial
Koh	5	Pak et al. 2016 Perdisa et al. 2015 Rodriguez- Merchan 2016 Filardo et al. 2016	2012	Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis
Koh	5	Pak et al. 2016 Perdisa et al. 2015 Xu et al. 2015 Filardo et al. 2016	2014	Comparative outcomes of open-wedge high tibial osteotomy with platelet-rich plasma alone or in combination with mesenchymal stem cell treatment: a prospective study.
Kuroda	5	Filardo et al 2013 Filardo et al. 2016 Pastides et al. 2013 Veronesi et al. 2012 Bornes et al. 2014	2007	Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells
Lee KB	5	Deng et al. 2015 Filardo et al. 2016 Peeters et al. 2013 Reissis et al. 2016 Counsel et al. 2015	2012	A novel, minimally invasive technique of cartilage repair in the human knee using arthroscopic microfracture and injections of mesenchymal stem cells and hyaluronic acid—a prospective comparative study on safety and short-term efficacy
Varma	5	Filardo et al. 2016 Rodriguez- Merchan 2016 Xu et al. 2015 Filardo et al 2013	2010	The new avenues in the management of osteoarthritis of knee-stem cells
Wakitani	5	Bauge & Boumediene 2015 Filardo et al. 2016 Reissis et al. 2016 Veronesi et al. 2012	2004	Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports
Wakitani	5	Counsel et al. 2015 Gopal et al. 2014 Veronesi et al. 2012 Wolfstadt et al. 2015 Bauge & Boumediene 2015	2002	Human autologous culture expanded bone marrow-mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees
Emadedin	4	Filardo et al. 2016 Peeters et al 2013 Wolfstadt et al. 2015 Bauge & Boumediene 2015	2012	Intra-articular injection of autologous mesenchymal stem cells in six patients with knee Osteoarthritis

Giannini	4	Filardo et al. 2016 Pastides et al. 2013 Veronesi et al. 2012 Bornes et al. 2014	2009	One-step bone marrow derived cell transplantation in talar osteochondral lesions
Koh	4	Pak et al. 2016 Perdisa et al. 2015 Rodriguez- Merchan 2016 Filardo et al. 2016	2015	Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis
Orozco	4	Reissis et al. 2016 Rodriguez- Merchan 2016 Wolfstadt et al. 2015 Bauge & Boumediene 2015	2013	Treatment of knee osteoarthritis with autologous mesenchymal stem cells: two-year follow-up results
Orozco	4	Reissis et al. 2016 Rodriguez- Merchan 2016 Wolfstadt et al. 2015 Bauge & Boumediene 2015	2013	Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study
Pak	4	Filardo et al. 2016 Pak et al. 2016 Perdisa et al. 2015 Filardo et al 2013	2011	Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose- tissue-derived stem cells: a case series
Saw KY	4	Filardo et al. 2016 Rodriguez- Merchan 2016 Xu et al. 2015 Counsel et al. 2015	2013	Articular cartilage regeneration with autologous peripheral blood stem cells versus hyaluronic acid: a randomized controlled trial.
Wakitani	4	Filardo et al. 2016 Reissis et al. 2016 Xu et al. 2015 Filardo et al 2013	2002	Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees.
Wakitani	4	Peeters et al. 2013 Reissis et al. 2016 Veronesi et al. 2012 Fischer & Kisser 2016	2011	Safety of autologous bone marrowderived mesenchymal stem cell transplantation for cartilage repair in 41 patients with 45 joints followed for up to 11 years and 5 months
Wong	3	Reissis et al. 2016 Rodriguez- Merchan 2016 Filardo et al. 2016	2013	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.
Bartlett	2	Kon et al. 2015 Deng et al. 2015	2005	Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee. A prospective, randomised study
Basad	2	Kon et al. 2015 Deng et al. 2015	2010	Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study

Davatchi	3	Reissis et al. 2016 Bauge & Boumediene 2015 Filardo et al. 2016	2011	Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients
Gobbi	3	Filardo et al. 2016 Rodriguez- Merchan 2016 Chahla et al. 2016	2014	One-step surgery with multipotent stem cells for the treatment of large full-thickness chondral defects of the knee.
Kim YS	3	Perdisa et al. 2015 Filardo et al. 2016 Pak et al. 2016	2015	Mesenchymal stem cell implantation in osteoarthritic knees: is fibrin glue effective as a scaffold?
Koh	3	Pak et al. 2016 Reissis et al. 2016 Filardo et al. 2016	2014	Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees
Kon/ Gobbi/ Filardo/ Marcacci/ Zaffagnini--	2	Kon et al. 2015 Deng et al. 2015	2009	Arthroscopic second-generation autologous chondrocyte implantation compared with microfracture for chondral lesions of the knee: prospective nonrandomized study at 5 years
Kuroda	3	Gopal et al. 2014 Reissis et al. 2016, Bauge & Boumediene 2015	2007	Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells
Panagopoulos	2	Kon et al. 2015 Deng et al. 2015	2012	Autologous chondrocyte implantation for knee cartilage injuries: moderate functional outcome and performance in patients with high-impact activities
Saw	3	Counsel et al. 2015 Filardo et al. 2016 Bornes et al. 2014	2011	Articular cartilage regeneration with autologous peripheral blood progenitor cells and hyaluronic acid after arthroscopic subchondral drilling: a report of 5 cases with histology.
Showron (skowronski)–	3	Counsel et al. 2015 Reissis et al. 2016 Chahla et al. 2016	2013	Osteochondral lesions of the knee reconstructed with mesenchymal stem cells—results
Zeifang	3	Deng et al. 2015 Kon et al. 2015 Counsel et al. 2015	2010	Autologous chondrocyte implantation using the original periosteum-cover technique versus matrix-associated autologous chondrocyte implantation: a randomized clinical trial
Adachi	2	Reissis et al. 2016 Filardo et al 2013	2005	Transplant of mesenchymal stem cells and hydroxyapatite ceramics to treat severe osteochondral damage after septic arthritis of the knee.
Bul	2	Perdisa et al. 2015 Pak et al. 2016	2013	Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study
Bulgheroni	1	Papailia et al. 2013	2009	Follow-up of collagen meniscus implant patients: clinical, radiological, and magnetic resonance imaging results at 5 years
Cole	2	Kon et al. 2015 Deng et al. 2015	2011	Outcomes after a singlestage procedure for cell-based cartilage repair: a prospective clinical safety trial with 2-year follow-up

Crawford	2	Kon et al. 2015 Deng et al. 2015	2010	Neo- Cart, an autologous cartilage tissue implant, compared with microfracture for treatment of distal femoral cartilage lesions: an FDA phase-II prospective, randomized clinical trial after two years,"
Davatchi	2	Rodriguez- Merchan 2016, Filardo et al 2013	2011	Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients
Emadedin	2	Reissis et al. 2016 Filardo et al 2013	2012	Intraarticular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis
Ferruzzi	1	Kon et al. 2015	2008	Autologous chondrocyte implantation in the knee joint: Open compared with arthroscopic technique. Comparison at a minimum follow-up of five years
Gigante	2	Filardo et al. 2016 Chahla et al. 2016	2012	Arthroscopic knee cartilage repair with covered microfracture and bone marrow concentrate.
Gobbi	2	Chahla et al. 2016 Bornes et al. 2014	2011	One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions: results at 2-year follow-up
Gobbi. Nakamura	2	Filardo et al. 2016 Deng et al. 2015	2014	Matrix-induced autologous chondrocyte implantation versus multipotent stem cells for the treatment of large patellofemoral chondral lesions: a nonrandomized prospective trial
Haleem	2	Gopal et al. 2014 Filardo et al 2013	2010	The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results
Kim YS	2	Filardo et al. 2016 Counsel et al. 2015	2013	Clinical outcomes of mesenchymal stem cell injection with arthroscopic treatment in older patients with osteochondral lesions of the talus.
Kon	1	Deng et al. 2015	2011	Articular cartilage treatment in high-level male soccer players: a prospective comparative study of arthroscopic second-generation autologous chondrocyte implantation versus microfracture
Linke	1	Pastides et al. 2013	2007	Replacement of the meniscus with a collagen implant (CMI).
Macmuil	1	Deng et al. 2015,	2012	The role of autologous chondrocyte implantation in the treatment of symptomatic chondromalacia patellae
Manfredin	2	Kon et al. 2015 Deng et al. 2015	2007	Autologous chondrocyte implantation: a comparison between an open periosteal-covered and an arthroscopic matrix-guided technique
Michalek	2	Pak et al. 2016 Filardo et al. 2016	2015	Autologous adipose tissue-derived stromal vascular fraction cells application in patients with osteoarthritis
Nejadnik H	2	Pastides et al. 2013 Counsel et al. 2015	2010	Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study.

Pascarella	2	Veronesi et al. 2012	2010	Treatment of articular cartilage lesions of the knee joint using a modified AMIC technique
Reguzzoni	1	Pak et al. 2016	2005	Histology and ultrastructure of a tissue-engineered collagen meniscus before and after implantation
Rodkey	1	Pastides et al. 2013	2005	Histology and ultrastructure of a tissue-engineered collagen meniscus before and after implantation
Rodkey	1	Pak et al. 2016	2003	Short-term evaluation of collagen meniscus implants by MRI and morphological analysis
Spencer	1	Papailia et al. 2013	2012	Meniscal scaffolds: early experience and review of the literature.
Stone	1	Papailia et al. 2013	1997	Regeneration of meniscal cartilage with use of a collagen scaffold. Analysis of preliminary data
Verdonk	1	Papailia et al. 2013	2012	Successful treatment of painful irreparable partial meniscal defects with a polyurethane scaffold: two-year safety and clinical outcomes.
Welsch	2	Kon et al. 2015	2010	Evaluation of cartilage repair tissue after matrix-associated autologous chondrocyte transplantation using a hyaluronic-based or a collagen-based scaffold with morphological MOCART scoring and biochemical T2 mapping: Preliminary results.
Zaffagnini	1	Pastides et al. 2013	2011	Prospective long-term outcomes of the medial collagen meniscus implant versus partial medial meniscectomy: a minimum 10-year follow-up study
Zaffagnini/ Kon/ Filardo/ Marcacci/ Busacca	1	Papailia et al. 2013	2012	Arthroscopic collagen meniscus implantation for partial lateral meniscal defects: a 2-year minimum follow-up study
Anders	1	Fischer & Kisser 2016	2013	A randomized, controlled trial comparing autologous matrix-induced chondrogenesis (AMIC) to microfracture: analysis of 1- and 2-year follow-up data of 2 centers
Bhattacharya	1	Xu et al. 2015	2010	Clinical Use of Amniotic Fluid in Osteoarthritis: A Source of Cell Therapy
Buda	1	Filardo et al. 2016	2015	Treatment of hemophilic ankle arthropathy with one-step arthroscopic bone marrow-derived cells transplantation
Buda	1	Filardo et al. 2016	2016	“Onestep” bone marrow-derived cells transplantation and joint debridement for osteochondral lesions of the talus in ankle osteoarthritis: clinical and radiological outcomes at 36 months
Buda	1	Filardo et al. 2016	2013	One-step bone marrow-derived cell transplantation in talarosteochondral lesions: mid-term results
Buda	1	Filardo et al. 2016	2015	Regenerative treatment in osteochondral lesions of the talus: autologous chondrocyte implantation versus one-step bone marrow derived cells transplantation
Buda	1	Filardo et al. 2016	2013	One-step arthroscopic technique for the treatment of osteochondral lesions of the knee with bone-marrow-derived cells: three years results

		Filardo et al. 2016		
Bui	1		2014	Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study
		Filardo et al. 2016		
Cadossi	1		2014	Bone marrow-derived cells and biophysical stimulation for talar osteochondral lesions: a randomized controlled study
Centeno	1	Peeters et al. 2013	2010	Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique
Centeno	2	Veronesi et al. 2012 Chahla et al. 2016	2008	Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells
Dallari	1	Xu et al. 2015	2007	Enhanced tibial osteotomy healing with use of bone grafts supplemented with platelet gel or platelet gel and bone marrow stromal cells
Davatchi	1	Filardo et al. 2016	2016	Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients
Efe	1	Papailia et al. 2013	2012	The safety and short-term efficacy of a novel polyurethane meniscal scaffold for the treatment of segmental medial meniscus deficiency
Enea	1	Chahla et al. 2016	2015	One-step cartilage repair in the knee: collagen-covered microfracture and autologous bone marrow concentrate. A pilot study.
Enea	1	Chahla et al. 2016	2013	Single-stage cartilage repair in the knee with microfracture covered with a resorbable polymer-based matrix and autologous bone marrow concentrate.
Ferruzzi	1	Deng et al. 2015	2008	Autologous chondrocyte implantation in the knee joint: open compared with arthroscopic technique,"
Filardo	1	Chahla et al. 2016	2013	Mesenchymal stem cells for the treatment of cartilage lesions: from preclinical findings to clinical application in orthopaedics.
Fu	1	Filardo et al. 2016	2014	Repair of large fullthickness cartilage defect by activating endogenous peripheral blood stem cells and autologous periosteum flap transplantation combined with patellofemoral realignment
Giannini	1	Bornes et al. 2014	2013	One-step repair in talar osteochondral lesions: 4-year clinical results and t2-mapping capability in outcome prediction
Giannini	1	Filardo et al 2013	2009	One-step bone marrow-derived cell transplantation in talar-osteochondral lesions
Gobbi	1	Pastides et al. 2013	2011	One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions.
Gudas	1	Counsel et al. 2015	2005	A prospective randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint in young athletes,

Gudas	1	Deng et al. 2015	2009	A prospective, randomized clinical study of osteochondral autologous transplantation versus microfracture for the treatment of osteochondritis dissecans in the knee joint in children,"
Jame EW	1	Chahla et al. 2016	2015	Repair of a complete radial tear in the midbody of the medial meniscus using a novel crisscross suture transtibial tunnel surgical technique: a case report
Kim JD	1	Chahla et al. 2016	2014	Clinical outcome of autologous bone marrow aspirates concentrate (BMAC) injection in degenerative arthritis of the knee.
Kim YS	1	Chahla et al. 2016	2015	Mesenchymal stem cell implantation in knee osteoarthritis: an assessment of the factors influencing clinical
Kim YS	1	Filardo et al. 2016	2014	Does an injection of a stromal vascular fraction containing adipose-derived mesenchymal stem cells influence the outcomes of marrow stimulation in osteochondral lesions of the talus? A clinical and magnetic resonance imaging study
Kim YS	1	Filardo et al. 2016	2016	Assessment of clinical and MRI outcomes after mesenchymal stem cell implantation in patients with knee osteoarthritis: a prospective study
Kim YS	1	Filardo et al. 2016	2015	Mesenchymal stem cell implantation in knee osteoarthritis: an assessment of the factors influencing clinical outcomes
Kobayashi	1	Counsel et al. 2015	2007	A novel cell delivery system using magnetically labelled mesenchymal stem cells and an external magnetic device for clinical cartilage repair
Kogo	1	Bauge & Boumediene 2015	2009	Mesenchymal stem cell-based therapy for cartilage repair: a review
Koh YG	1	Filardo et al. 2016	2016	Adipose-derived mesenchymal stem cells with microfracture versus microfracture alone: 2-year follow-up of a prospective randomized trial
Kon	1	Kon et al. 2015	2011	Articular cartilage treatment in high-level male soccer players: A prospective comparative study of arthroscopic second-generation autologous chondrocyte implantation versus microfracture
Marquass	1	Bornes et al. 2014	2011	Matrix-associatedimplantation of predifferentiated mesenchymal stem cells versus articular chondrocytes: in vivo results of cartilage repair after 1 year
Matsumoto	1	Pastides et al. 2013	2010	Articular cartilage repair with autologous bone marrow mesenchymal cells
Monllau	1	Papailia et al. 2013	2011	Outcome after partial medial meniscus substitution with the collagen meniscal implant at a minimum of 10 years' follow-up
Pak	1	Filardo et al. 2016	2011	Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series

Pak	1	Perdisa et al. 2015 Pak et al. 2016	2013	“Safety reporting on implantation of autologous adipose tissue-derived stemcellswith platelet-rich plasma into human articular joints
Pak	2	Perdisa et al. 2015 Pak et al. 2016	2013	A novel biological approach to treat chondromalacia patellae
Pak	1	Pak et al. 2016	2014	Regenerative repair of damaged meniscus with autologous adipose tissue-derived stem cells
Pascual-Garrido	1	Chahla et al. 2016	2011	Treatment of chronic patellar tendinopathy with autologous bone marrow stem cells: a 5-year-followup
Piontek	1	Chahla et al. 2016	2012	All-arthroscopic technique of biological meniscal tear therapy with collagen matrix.
Rich	1	Filardo et al. 2016	2015	Treatment of knee osteoarthritis with autologous expanded bone marrow mesenchymal stem cells: 50 cases clinical and MRI results at one year follow-up
Richter	1	Filardo et al. 2016	2013	Matrix-associated stem cell transplantation (MAST) in chondral defects of foot and ankle is effective
Rodkey	1	Papailia et al. 2013	1999	A clinical study of collagen meniscus implants to restore the injured meniscus
Sekiya	1	Filardo et al. 2016	2015	Arthroscopic transplantation of synovial stem cells improves clinical outcomes in knees with cartilage defects
Shino	1	Veronesi et al. 2012	1993	Deterioration of patellofemoral articular surfaces after anterior cruciate ligament reconstruction
Shive	1	Fischer & Kisser 2016	2014	BST-CarGel1 treatment maintains cartilage repair superiority over microfracture at 5 years in a multicenter randomized controlled trial
Showron	1	Chahla et al. 2016	2013	Large cartilage lesions of the knee treated with bone marrow concentrate and collagen membrane
Skoronski	1	Filardo et al. 2016	2013	Large cartilage lesions of the knee treated with bone marrow concentrate and collagen membrane—results
Skowronski	1	Filardo et al. 2016	2012	Cartilage lesions of the knee treated with blood mesenchymal stem cells—results
Skowronski	1	Filardo et al. 2016	2013	Osteochondral lesions of the knee reconstructed with mesenchymal stem cells—results
Slynarski	1	Veronesi et al. 2012	2006	Fresh bone marrow and periosteum transplantation for cartilage defects of the knee
Stanish	1	Fischer & Kisser 2016	2013	Novel scaffold-based BST-CarGel treatment results in superior cartilage repair compared with microfracture in a randomized controlled trial
Teo	1	Peeters et al. 2013	2013	Cell-based therapy improves function in adolescents and young adults with patellar osteochondritis dissecans

Turajane	1	Filardo et al. 2016	2013	Combination of intraarticular autologous activated peripheral blood stem cells with growth factor addition/ preservation and hyaluronic acid in conjunction with arthroscopic microdrilling mesenchymal cell stimulation Improves quality of life and regenerates articular cartilage in early osteoarthritic knee disease
Vangsness	1	Xu et al. 2015	2014	Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial meniscectomy: a randomized, double-blind, controlled study
Vega	1	Filardo et al. 2016	2015	Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: a randomized controlled trial
Visna	1	Kon et al. 2015	2004	Treatment of deep cartilage defects of the knee using autologous chondrograft transplantation and by abrasive
Wakitani	1	Fischer & Kisser 2016	2006	Serum keratan sulfate is a promising marker of early articular cartilage breakdown
Wakitani	1	Pastides et al. 2013	2008	Present status of and future direction for articular cartilage repair.
Wakitani	1	Veronesi et al. 2012	1994	Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage
Werner	1	Chahla et al. 2016	2003	Joint preserving surgery for osteonecrosis and osteochondral defects after chemotherapy in childhood.
Wong	1	Xu et al. 2015	2013	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
Yamasaki	1	Deng et al. 2015	2014	Cartilage repair with autologous bonemarrow mesenchymal stem cell transplantation: review of preclinical and clinical studies
Zaffagnini/ Kon	1	Papailia et al. 2013	2007	Arthroscopic collagen meniscus implant results at 6 to 8 years follow up

Appendix 10 – Levels of evidence of the clinical trials included in the 19 reviews

Author	No of articles included	Case reports (level of evidence: IV)	Case series (level of evidence: IV)	Comparative trials (prospective; cohort) (level of evidence: II and III)	RCTs (Level I)
Bornes	14	2	9	2	1
Chahla	11	1	4	3	0
Counsel	9	0	0	5	0
Deng	4	0	0	4	0
Filadro 2013	18	7	6	5	
Filardo 2016	60	9	31	13	7
Fischer	3	0	0 *excluded those with less than 50 people	1	2
Gopal	6	1	3	2	0
Kon	7	0	0	?	?
Pak	13	2	5	5	1
Pastida	11	1	7	3	0
Peeters	8	0	5	3	0
Perdisa	11	1	7	3	0
Reisis	35	6	18	8	2
Rodriguez-Merchant	10	0	2	6	2
Veronesi	15	n/a	n/a	n/a	n/a
Wolfstadt	7	0	5	2	0
Xia	7	0	0	1	6
Xu	12	0	0	8	4
TOTAL	**261**	**30**	**102**	**72**	**25**

Appendix 11 – Description of outcomes measures used to assess clinical and functional outcomes of stem cell therapy

Name	Description	No. of reviews
Tegner Activity Scale (TAS)	Standardized method for grading and work and sporting activities	11
Lysholm Knee Score (LKS)	Assess chondral disorders; Standardized method for grading and work and sporting activities	10
Visual Analog Scale (VAS)	10cm line with boundaries at 0 and 10cm	10
Hospital for Special Surgery Knee Scoring System (HSS)	Scoring chart for knee function from 0-100, with 100 being the best function	7
Western Ontario and McMaster Universities Arthritis Index (WOMAC)	Set of standardized questionnaires used to evaluate pain, stiffness, and physical functioning of the joints of patients with hip and/or knee OA	6
Knee injury and Osteoarthritis Outcome Score (KOOS)	Assess the patient's opinion about their knee and associated problem, including (5 subscales): Pain, other Symptoms, Function in daily living (ADL), Function in sport and recreation (Sport/Rec) and knee related Quality of life (QOL)	5
SF-36	Assess patient report health status based on 8 sections: physical functioning; pain; general health perceptions; physical role functioning; emotional role functioning; social role functioning; mental health	5
International Knee Document Committee (IKDC) - Subjective Knee	Designed to detect improvement or deterioration in symptoms, function, and sports activities due to knee impairment	4

Evaluation Form		
American Orthopedic Foot and Ankle Score (AOFAS)	Clinician-based score that measures outcomes in four different anatomic regions of the foot: the ankle-hindfoot, midfoot, metatarsophalangeal-interphalangeal for the hallux and lesser toes	4
Marx Knee PRO Tool	Reporting tool that focuses on 4 frequency of 4 activities within the past 12 months: running, deceleration, cutting, and pivoting	3
Stanmore-Bentley Functional Rating system or Stanmore Functional Rating System	Functional rating scale based on pain and level of activity	2
Modified Cincinnati Rating System (MCS)	Knee rating scale with a maximum score of 100 rating pain, giving away, swelling, walking ability, stair walking, running, jumping/ twisting, and overall activity	2
Roles - Maudsley Score	Subjective pain score from 1 (excellent) to 4 (poor)	2
Function Rating Index	Instrument specifically designed to measure subjective perception of function and pain of spinal and neck musculoskeletal system	2
Lequesne Index of Severity for Knee Disease (ISK)	Indices looking at pain, maximum distance walked, and activities of daily living for people with osteoarthritis of the knee	1
SF-12, SF-26	Shorter form(s) of the SF- 36: Assess patient report health status based on 8 sections: physical functioning; pain; general health perceptions; physical role functioning; emotional role functioning; social role functioning; mental health	1
Lower Extremity Functional	Measure patients initial function, ongoing	1

Scale (LEFS) (OA specific)	progress, and outcomes for osteoarthritis	
Numerical Pain Rating Scale (NPRS)	A generic unidimensional measure of pain intensity in adults. On a scale of 0-10, with 0 being no pain at all and 10 being the worst pain imaginable	1