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Review

Stem cell therapies for treating osteoarthritis: Prescient or premature?

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Higlights

- In recent years there has been unprecedented interest from commercial sectors, the public, veterinary clinicians and researchers in the use of stem cells as therapy for an array of diseases in companion animals
- Several therapeutic applications of stem cells are already employed in a clinical setting, in particular the use of mesenchymal stem cells to treat osteoarthritis in horses and dogs
- However, an assessment of the scientific literature highlights a marked disparity between the purported benefits of stem cell therapies and their proven abilities as defined by rigorously controlled scientific studies
- Thus, while the preliminary data generated from clinical trials in human patients are encouraging, current therapies on offer to veterinary patients are supported by very limited clinical data and the commercialisation of these therapies is perhaps premature
- This review introduces the three main types of stem cells relevant to veterinary applications – embryonic stem cells, induced pluripotent stem cells and mesenchymal stem cells – and draws together the research findings from in vitro and in vivo studies to give an overview of the current state-of-play of stem cell therapies for treating osteoarthritis in veterinary medicine
- The review concludes by discussing recent advances in tissue engineering, which is proposed as the future direction of effective stem cell-based therapies for osteoarthritis

Abstract

There has been unprecedented interest in recent years in the use of stem cells as therapy for an array of diseases in companion animals. Stem cells have already been deployed therapeutically in a number of clinical settings, in particular the use of mesenchymal stem cells to treat osteoarthritis in horses and dogs. However, an assessment of the scientific literature highlights a marked disparity between the purported benefits of stem cell therapies and their proven abilities as defined by rigorously controlled scientific studies.

Although preliminary data generated from clinical trials in human patients are encouraging, therapies currently available to treat animals are supported by very limited clinical evidence, and the commercialisation of these treatments may be premature. This review introduces the three main types of stem cells relevant to veterinary applications, namely, embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem cells, and draws together research findings from in vitro and in vivo studies to give an overview of current stem cell therapies for the treatment of osteoarthritis in animals. Recent advances in tissue engineering, which is proposed as the future direction of stem cell-based therapy for osteoarthritis, are also discussed.

Keywords: Mesenchymal stem cells; Induced pluripotent stem cells; Embryonic stem cells; Osteoarthritis; Tissue engineering

Introduction

Stem cell biology and the identification of veterinary applications comprise a rapidly developing field of research driven by the growing significance of cats and dogs as companion animals, the economic impact of the horse industry, and the recognition that many human diseases have counterparts in companion animals. Several commercial companies around the world offer stem cell preparations as regenerative therapy for osteoarthritis in dogs and horses, and in the treatment of ligament and tendon injuries in horses. A contributing factor to the proliferation of veterinary stem cell-based applications is that the use of such therapies in animals is not regulated in any of the countries in which they are offered. A further concern is the fact that the efficacy of these therapies is frequently not supported by published data.

We review below the main types of stem cells, with particular attention to mesenchymal stem cells (MSCs), which have been used most extensively in veterinary applications. The current main therapeutic use of stem cells in the treatment of osteoarthritis in animals will then be reviewed, and recent advances in tissue engineering, which uses a potent combination of biomaterials and stimulatory factors to deliver stem cells and to direct their differentiation, will be discussed. The latter likely represents a key process in the future application of effective stem cell-based therapies for companion animals, as it circumvents the current practice of delivering cell suspensions that fail to provide any long term engraftment of cells.

Types of stem cells

Generally speaking, stem cells can be divided into two main classes: embryonic and adult. Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst

stage embryo. Given that these cells will give rise to the embryo proper, they exhibit the unique property of pluripotency; that is, they have the potential to differentiate into all of the various cell types that constitute the adult individual (Fig. 1). A further defining feature of ESCs is that they are immortal and under in vitro culture conditions are capable of indefinite proliferation without undergoing differentiation, a property known as self-renewal.

Adult stem cells, by contrast, are found within the majority of tissues, where they play a role in tissue maintenance and repair. The adult stem cells that hold the greatest therapeutic potential, and so have attracted significant research interest, are the MSCs, also known as mesenchymal stromal cells (Fig. 1). A third category of stem cell, which is neither 'embryonic' nor 'adult', is the induced pluripotent stem cell (iPSC) (Fig. 1). These stem cells are unique in that they are essentially manufactured from adult, terminally differentiated, cells such as fibroblasts via a process of 're-programming'.

Stem cells as a therapeutic resource

Embryonic stem cells

Given their properties of self-renewal and pluripotency, ESCs offer the possibility of an unlimited, renewable source of cells that can be induced to differentiate into any cell of the body and so hold substantial appeal as a therapeutic resource. While ESCs from mice and humans have been characterised extensively, and the optimal conditions elucidated for their derivation and maintenance in vitro, the same cannot be said for ESCs derived from companion animals.

Several authors have described the establishment of canine ESCs (Hatoya et al., 2006; Schneider et al., 2007; Hayes et al., 2008; Vaags et al., 2009; Wilcox et al., 2009); however,

there appear to be significant differences between the individual lines of canine ESCs in terms of morphology, demonstrable pluripotency (i.e. whether they can they differentiate into derivatives from all three embryonic germ layers), and their ability to be maintained in vitro. Similar problems have been encountered in ESCs isolated from the horse (Saito et al., 2002; Li et al., 2006) and cat (Yu et al., 2009). In part, these difficulties may be attributed to the differences in the patterns of embryonic development between species, but also to our lack of understanding of the specific culture conditions required for maintaining these cells in their pluripotent state (i.e. preventing them from differentiating). For example, the culture conditions required for mouse and human ESCs differ substantially, not least in the growth factors required for their survival and proliferation (Pera and Tam, 2010).

Since equine ESCs have become commercially available, their use has been demonstrated through effecting significant healing of experimentally induced lesions in the equine superficial digital flexor tendon (Watts et al., 2011). Currently, these ESCs are the only cells of this type of any species commercially available to veterinarians for therapeutic applications. However, it remains possible that the use of ESCs as a therapeutic resource is compromised by one of the very factors that, on initial consideration, would appear to be a compelling reason for their use, namely, their pluripotency.

The ability of ESCs to differentiate into any cell type when harnessed and driven to a specific end-point is the essence of their curative potential. However, if not tightly controlled, this property has the potential to create disease in the form of teratomas, tumours that develop from pluripotent cells and that contain cells derived from all three embryonic germ layers. Ensuring that transplanted ESCs differentiate along a specific pathway, and do not form

undesired tissue (e.g. bone at the articular surface of a joint), is one of the major challenges facing the use of ESCs in both humans and animals.

Induced pluripotent stem cells

The advent of induced pluripotent stem cells (iPSCs) has significantly advanced the prospects of stem cell-based therapies in humans and companion animals (Takahashi and Yamanaka, 2006). These stem cells are generated from adult, terminally differentiated cells, such as fibroblasts, following the introduction of four genes central to the regulation of pluripotency in mammalian cells, *Oct4, Sox2, cMyc* and *Klf4*. The induced expression of these pluripotency factors re-programmes differentiated cells into stem cells with properties very similar to those of ESCs.

iPSCs have been generated from a variety of companion and domestic animal species, including dogs (Shimada et al., 2010; Lee et al., 2011; Luo et al., 2011; Whitworth et al., 2012), horses (Nagy et al., 2011; Breton et al., 2013; Whitworth et al., 2014a), pigs (Esteban et al., 2009; Ezashi et al., 2009; Wu et al., 2009), sheep (Bao et al., 2011; Li et al., 2011; Liu et al., 2012; Sartori et al., 2012), cattle (Han et al., 2011; Sumer at al., 2011; Cao et al., 2012), and buffaloes (Deng et al., 2012).

Since iPSCs can be generated from nominated individuals (typically from a small skin biopsy) it is possible to produce iPSCs from individuals with a specific disease or genetic background. Thus, iPSCs can be used to model diseases in vitro, serve as a platform for drug screening and be manipulated with gene correction strategies for transplantation back into the donor. Furthermore, because iPSCs can be produced from a patient's own cells, they have the

potential to serve as a renewable source of cells for regenerative therapies as required by that individual.

However, several obstacles need to be overcome before iPSCs can be used safely in cell-based therapies. Traditionally, cells have been re-programmed using lentiviruses or retroviruses to deliver the re-programming factors as transgenes, resulting in both the backbone of the viral vector and the re-programming transgenes becoming incorporated into the recipient cells' genome. These insertions have the potential to cause mutations (Yu et al., 2007) and, in some instances, the transgenes encoding the re-programming factors may not be silenced, or may become reactivated, which can result in the formation of teratomas (Okita et al., 2007). Consequently, virally re-programmed cells are not suitable for clinical applications.

Several strategies have been developed to enable transient expression of the re-programming factors without them becoming integrated into the recipient cells' genome. These include the use of adenoviruses (Stadtfeld et al., 2008), Sendai virus (Fusaki et al., 2009), *piggyBac* transposons (Woltjen et al., 2009; Yusa et al., 2009) and episomal plasmids (Okita et al., 2008) to deliver the transgenes and, more recently, transducing the cells with synthetic modified messenger RNAs encoding the re-programming factors (Mandal and Rossi, 2013).

Re-programming strategy aside, the acquisition of pluripotency poses the same double-edged sword for the clinical use of iPSCs as it does for ESCs. Specifically, given their pluripotency, iPSCs carry the same risk of tumourigenesis as ESCs when transplanted. This problem could be circumvented by fully differentiating the cells before transplantation and

then identifying and destroying any residual pluripotent cells from the graft. Although several methods have been developed, the efficacy of these to prevent the formation of teratomas in vivo remains unproven (Wu and Hochedlinger, 2011).

Mesenchymal stem cells

MSCs are multipotent cells capable of differentiating into bone, cartilage and adipose tissue (Pittenger et al., 1999). Recent studies have demonstrated that they are also able to form pancreatic islet cells (Santos et al., 2010), hepatocytes (Lee et al., 2004; Ong et al., 2006) and neurons (Woodbury et al., 2000) in vitro. In humans, MSCs are typically isolated from adult bone marrow and adipose tissue (Kern et al., 2006; Bieback et al., 2008) but they have also been successfully collected from placenta (In't Anker et al., 2004), umbilical cord blood (Kern et al., 2006) and amniotic fluid (In't Anker et al., 2004; Tsai et al., 2004) in addition to various fetal tissues, such as kidney (Al-Awqati and Oliver, 2002), pancreas (Hu et al., 2003), liver (Campagnoli et al., 2001; In't Anker et al., 2003) as well as lung and spleen (In't Anker et al., 2003).

Many studies have described the isolation of MSCs from a range of canine tissues, including adipose tissue (Neupane et al., 2008; Vieira et al., 2010; Kisiel et al., 2012; Reich et al., 2012; Takemitsu et al., 2012), bone marrow (Kisiel et al., 2012; Reich et al., 2012; Takemitsu et al., 2012), muscle (Kisiel et al., 2012), periosteum (Kisiel et al., 2012) and synovium (Zhang et al., 2013). Similarly diverse sources have been identified for equine MSCs (Hegewald et al., 2004; Koch et al., 2007; Crovace et al., 2010; Raabe et al., 2011; Ranera et al., 2012; Burk et al., 2013; Barberini et al., 2014; De Schauwer et al., 2014), as well as caprine (Murphy et al., 2003), ovine (Jager et al., 2006; Mrozik et al., 2010) and porcine (Wang et al., 2008; Cao et al., 2011; Miernik and Karasinski, 2012).

An important feature of MSCs that has enhanced their clinical appeal is that they are thought to be immune privileged, most likely due to an absence of MHC class II expression (DiMarino et al., 2013). In domestic species, both canine and equine MSCs appear to lack expression of MHC class II (Wood et al., 2012; Barberini et al., 2014; De Schauwer et al., 2014). In contrast, Schnabel et al. (2014) found equine bone marrow-derived MSCs to have marked heterogeneity in their expression of MHC class II, and that MSCs initially identified as being MHC class II negative can potentially upregulate MHC class II expression when placed into an environment of active inflammation.

MSCs are themselves immunomodulatory and are potent regulators of inflammation, seemingly through their active suppression of both the innate and adaptive immune systems (Griffin et al., 2010). MSCs have been shown to regulate the proliferation and function of a variety of immune cells, including B and T lymphocytes, natural killer cells, neutrophils and dendritic cells (Griffin et al., 2010). Equine MSCs express transforming growth factor (TGF)-β and hepatocyte growth factor (HGF), both of which suppress T lymphocyte proliferation (De Schauwer et al., 2014). MSCs also secrete a range of anti-fibrotic, anti-apoptotic, bactericidal and pro-angiogenic factors (DiMarino et al., 2013). This relative lack of immunogenicity, in addition to their immunomodulatory and anti-inflammatory properties, have made MSCs an ideal choice for allogeneic, 'off-the-shelf', stem cell therapies for diseases with an inflammatory, or immune-mediated, component including osteoarthritis.

However, a common feature of MSCs, irrespective of species-of-origin, is that they represent a very small fraction of the cells isolated from both bone marrow and adipose tissue (0.001-0.01% and 0.05%, respectively) for human tissues (Kern et al., 2006; Bieback et al.,

2008). Also, in both humans and dogs, the quality and quantity of MSCs that can be collected decline with increasing age of the donor (Stolzing et al., 2008; Zhou et al., 2008; Kisiel et al., 2012; Guercio et al., 2013; Zhang et al., 2013) which has the potential to be problematic for regenerative therapies, such as those targeting osteoarthritis, where the majority of patients are aged. In contrast, human placental and fetal-derived MSCs have superior proliferative and differentiative abilities compared to MSCs from adult tissues (In't Anker et al., 2004; Gotherstrom et al., 2005; Guillot et al., 2007). It remains to be determined whether this comparison holds true for MSCs collected from domestic species.

In an attempt to circumvent the decreased capacity for proliferation and differentiation of adult MSCs, we and others (Lian et al., 2010; Chen et al., 2012; Whitworth et al., 2014b) have produced MSCs from iPSCs. In humans, these iPSC-derived MSCs resemble primary, tissue-sourced MSCs in terms of their immunophenotype and in their ability to differentiate into the mesodermal derivatives of cartilage, bone and adipose tissue (Lian et al., 2010; Chen et al., 2012). Using the methodology of Chen et al. (2012), we have generated canine iPSC-derived MSCs that are highly proliferative and readily differentiate along the osteogenic, chondrogenic and adipogenic pathways (Whitworth et al., 2014b). The ability to efficiently derive large numbers of highly proliferative MSCs from iPSCs represents an important step towards being able to source sufficient cells of high osteogenic and chondrogenic ability for MSC-based therapies in both veterinary and human medicine.

In summary, because of their strong propensity to form cartilage, relative ease of harvest from adult tissues, immune privileged status and anti-inflammatory effects, MSCs have been the focus of stem cell-based therapies targeting cartilage repair in veterinary and human patients with osteoarthritis.

Can mesenchymal stem cells effect cartilage repair in animals with osteoarthritis?

Many studies have been performed on a variety of animal species testing the efficacy of MSCs at repairing damaged cartilage in both naturally occurring and induced models of osteoarthritis. While space limitations prevent us from being able to discuss each published study, the salient points from each are summarised in Tables 1 and 2. However, it is important to consider key observations that can be extracted from the literature.

Mesenchymal stem cells do not engraft into cartilage defects

Current commercial MSC-based therapies for the treatment of osteoarthritis involve injecting a suspension of MSCs into the joint space. A study of the literature makes it apparent that MSCs introduced in this fashion do not engraft into the endogenous cartilage and directly affect repair. Desando et al. (2013) injected labelled MSCs into the stifle joints of rabbits with mild osteoarthritis. Joints were assessed at 3 and 20 days post-MSC transplant and, while labelled MSCs were detected in the synovial membrane and medial meniscus none, were seen in the cartilage. Similar engraftment of MSCs into the meniscus, but not the cartilage, was obtained by Hatsushika et al. (2013), also in rabbits.

Using a goat model of osteoarthritis, Murphy et al. (2003) found that injected MSCs had engrafted in high numbers into the synovium, fat pad and lateral meniscus, but not the damaged cartilage. Further studies in mice (ter Huurne et al., 2012), rats (Horie et al., 2009) and miniature pigs (Pei et al., 2013) also describe a lack of engraftment of MSCs into cartilage defects. In a recent study in guinea pigs, a small number of labelled MSCs were detected within the osteoarthritic cartilage at 1 week after transplantation; however, by 5 weeks, the cells had disappeared (Sato et al., 2012). A lack of engraftment has also been

observed in a horse model; McIlwraith et al. (2011) found no significant improvement in the healing of osteochondral defects in the stifle joints of horses treated with bone marrow-derived MSCs compared to controls, except that MSC-treated joints showed an increase in the 'firmness' of the repair tissue, which was shown to have significantly greater levels of aggrecan, a component of articular cartilage.

Mesenchymal stem cells may retard the progression of osteoarthritic lesions in the short term

Whereas MSCs do not engraft within osteoarthritic lesions they do appear to have chondroprotective benefits, retarding the progression of cartilage destruction by reducing inflammation. In a rabbit model of osteoarthritis, joints treated with MSCs had reduced expression of tumour necrosis factor (TNF)-α, which is an inflammatory cytokine, and matrix metalloproteinase (MMP)-1 which degrades proteoglycans in cartilage (Desando et al., 2013). In a mouse model of traumatic osteoarthritis, MSCs failed to induce regeneration of new cartilage, but they did mitigate the development of osteoarthritis during the 2 month study period (Diekman et al., 2013). In the horse, Frisbie et al. (2009) similarly observed no significant effects of MSCs on the repair of osteoarthritic lesions but did note reduced inflammation in the joints of horses treated with MSCs, further highlighting the anti-inflammatory properties of MSCs (Frisbie et al., 2009).

Similar results were described by Murphy et al. (2003) in the goat. At 20 weeks after the experimental group had received the MSC treatment, both controls and MSC-treated animals showed significant osteoarthritic lesions; however, the degree of cartilage destruction, osteophyte formation and subchondral sclerosis were reduced in 4/6 MSC-treated animals (Murphy et al., 2003). Again, this points to an anti-inflammatory, rather than regenerative, effect of the MSCs on the cartilage. An important additional point to note with

this study, and others that have involved a meniscectomy, is that while MSCs do not engraft within the cartilage, they do engraft within the meniscus, where they contribute to the development of new meniscal tissue (Horie et al., 2012). It is this ability to regenerate new meniscus that is at least partly responsible for the improved condition of the cartilage lesions in the MSC-treated groups, as shown by the study by Murphy et al. (2003) in the goat where the two MSC-treated animals that did not show a significant improvement in the cartilage lesions compared to controls also failed to grow new menisci.

The timing of MSC treatment with respect to the onset of osteoarthritis also seems to be a key factor in the degree to which MSCs modulate the progression of cartilage destruction. In a mouse model of osteoarthritis, lesions were induced by injection of collagenase into the joint (ter Huurne et al., 2012). If MSCs were injected 7 days after the collagenase treatment, cartilage destruction was reduced by 35% compared to controls. However, if the delivery of MSCs was delayed until 14 days after the collagenase injection, there was no difference in cartilage lesions between the MSC-treated and control groups (ter Huurne et al., 2012).

Given that MSCs do not engraft within the cartilage and appear to reside only transiently within the synovium, fat pad and menisci (Murphy et al., 2003; ter Huurne et al., 2012; Desando et al., 2013), these anti-inflammatory effects would reasonably be expected to be short-lived. This is borne out in a study of dogs with osteoarthritis secondary to hip dysplasia, which were injected with autologous MSCs (Vilar et al., 2013). The dogs showed improved lameness up to 6 months after the injection of MSCs into the coxofemoral joint; however, at around 10 months after treatment, the dogs began showing signs of regression in lameness scores (Vilar et al., 2013).

A similar short-lived effect has been observed in the horse. Wilke et al. (2007) applied bone marrow-derived MSCs to induced full-thickness cartilage lesions at the femoropatellar articulation in adult horses. At 30 days post-MSC transplant, lesions that had been exposed to the MSCs had significantly better healing scores than controls, an effect that can probably be attributed to reduced inflammation. However, this observation was short-lived, since at 8 months post-transplantation there were no histological, immunocytochemical or biochemical differences between the MSC-treated joints and the controls.

Chondrogenic effects may be observed when scaffolds and pro-chondrogenic molecules are combined with mesenchymal stem cells

While MSCs injected as a free suspension into the joint do not engraft into the cartilage, MSCs that are incorporated into support structures such as scaffolds, or combined with pro-chondrogenic molecules, such as hyaluronic acid (HA), appear to engraft and so contribute more significantly to cartilage repair. HA is an important component of the extracellular matrix of cartilage that has been shown, both in vitro and in vivo, to encourage the differentiation of MSCs into chondrocytes and to downregulate the expression of factors that degrade the cartilage matrix (Grigolo et al., 2009).

Sato et al. (2012), using a guinea pig model of spontaneous OA, injected MSCs, MSCs and HA, or saline, into the affected joints. Guinea pigs that received MSCs alone showed no improvement in the damaged cartilage as compared to saline-injected controls, while guinea pigs that received MSCs in addition to HA showed some improvement in cartilage quality. In contrast, in a rabbit model of osteoarthritis, Kim et al. (2012) noted histological improvements of equal quality in the cartilage of rabbits treated with HA and MSCs, and those treated with HA alone. Similarly, Grigolo et al. (2009) also found that rabbits in which

HA-containing scaffolds were placed over the lesion showed signs of cartilage regeneration, albeit to a lesser degree than those rabbits that received the HA-containing scaffolds seeded with MSCs.

Thus, most studies using MSCs in an attempt to repair damaged cartilage have involved injecting a suspension of MSCs into a joint space; at best this serves to retard the progression of osteoarthritis, most likely through paracrine anti-inflammatory effects, but does not generate any long-term physical repair of the cartilage, almost certainly because the MSCs fail to engraft. In contrast, studies in which the MSCs are spatially supported by a matrix (Fig. 2) show more promising results, especially when they are combined with biomaterials, such as HA, that support chondrogenesis. This, in essence, is tissue engineering: the combination of cells and biomaterials with a supporting matrix, or scaffold, in an attempt to regenerate damaged or diseased tissues.

Tissue engineering

Articular cartilage is a complex tissue consisting of four spatially distinct regions, each of which is characterised by a particular distribution of cells within an extracellular matrix of specific composition which, in turn, confer unique mechanical properties (Nguyen et al., 2011). Given this complexity in structure, it is perhaps naïve to suppose that an injection of freely suspended cells into the joint space will recapitulate this complex tissue. Scaffolds, hydrogels and other matrices aim to organise the cells into a three-dimensional pattern that closely replicates that found in the endogenous tissue. In the case of cartilage, the goal is to induce MSCs to form the four distinct layers of articular cartilage and to mimic their mechanical properties.

Nguyen et al. (2011) encapsulated mouse MSCs into polyethylene glycol (PEG)-based hydrogels into which they incorporated various biomaterials to generate three distinct layers of different compositions. After 6 weeks in culture, they produced a cartilage product that possessed the spatially varying mechanical and biochemical properties of endogenous cartilage (Nguyen et al., 2011). This is an exciting development, because it holds the promise of using MSCs to engineer a cartilage that can anchor to the native subchondral bone via the calcified zone, provide a smooth articular surface at the superficial zone and withstand the normal compression and shearing forces present within the joint.

An in vivo study using a rat model of osteoarthritis showed that when human ESCs were encapsulated into hydrogels incorporating HA they were able to repair full-thickness osteochondral defects with newly generated hyaline cartilage that was fully integrated with the surrounding native cartilage (Toh et al., 2010). In a similar study in the dog, canine MSCs seeded into bilayered scaffolds that induced separate (but integrated) layers of cartilage and bone development, were effective at repairing a large osteochondral defect in the articular surface of the femoral condyles (Yang et al., 2011).

A recent study in the horse demonstrated the efficacy of MSCs, used in combination with scaffolds and bioactive molecules, in regenerating osteochondral defects in a large animal model. Seo et al. (2013) impregnated gelatin/β-tricalcium phosphate (GT) sponges with bone marrow-derived MSCs and bone morphogenetic protein (BMP)-2, which stimulates osteogenesis, and inserted them into a full-thickness osteochondral defect in the lateral trochlear ridge of the talus. On top of this sponge, they then placed a second GT sponge loaded with chondrocytes, bone marrow-derived MSCs and platelet-rich plasma. After 4 months post-transplantation, a statistically significant repair in the osteochondral

defects was observed in the treatment group as compared to the controls that received the GT sponges only. Specifically, the size of the defect was significantly reduced and, most importantly, a greater proportion of the repair tissue was hyaline cartilage than was observed in the controls.

Conclusions

Because of their chondrogenic ability, anti-inflammatory effects and lack of tumourigenicity, MSCs are an attractive prospect for stem cell-based therapies aimed at cartilage repair. Although the injection of MSC suspensions into the joint appears to retard the progression of osteoarthritis, at least in the short term, it is necessary to facilitate the engraftment of the new cartilage to the native cartilage and bone in order to affect significant physical repair of the damaged cartilage. Recent advances in tissue engineering offer the most promising results to date and are, at this stage, the most likely means of achieving significant, long-term, cartilage repair in patients with osteoarthritis. An important caveat, however, is that any instability or incongruity within the joint that was the impetus for the development of osteoarthritis in the first place must be addressed if repair of the damaged cartilage is to be maintained long-term.

Conflict of interest statement

Neither of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Figure legends

Fig.1. Schematic illustration of stem cell types of clinical relevance. Induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) are pluripotent, enabling them to give rise to all three germ layers of the embryo. Mesenchymal stem cells (MSCs), in contrast, are multipotent and predominantly differentiate into mesodermal derivatives including bone, cartilage, fat and muscle. It should be noted, however, that MSCs can also give rise to some derivatives of ectoderm (neural tissue) and endoderm (cells of liver and pancreas).

Fig. 2. Schematic illustration of tissue engineering. Mesenchymal stem cells (MSCs) spatially supported by a matrix exhibit better engraftment within endogenous cartilage and offer a greater prospect of regenerating damaged cartilage.

Table 1 Summary of studies evaluating the therapeutic potential of mesenchymal stem cells in animals with spontaneous osteoarthritis.

Study	Species	Joint affected	Treatment	Evaluation methods	Study period	Results
Vilar et al., 2013	Dog Experimental (*n*=8) Control (*n*=5)	Hip; secondary to hip dysplasia	3 x 10^7 Autologous AD-MSCs	Gait analysis using a force plate	6 months	Significant increase in peak vertical force and vertical impulse in treated dogs vs. controls
Black et al., 2008	Dog Experimental	Elbow	Autologous stromal vascular fraction containing some putative	Subjective assessments of lameness, pain, ROM	6 months	Subjective assessment that all dogs had improved lameness, pain

	(*n*=14) Control (*n*=0)		AD-MSCs			and ROM at 6 months
Black et al., 2007	Dog Experimental (*n*=21) Control (*n*=0)	Hip; secondary to hip dysplasia	Autologous stromal vascular fraction containing some putative AD-MSCs	Subjective assessments of lameness, pain, ROM	3 months	Subjective assessment that all dogs had improved lameness, pain and ROM at 3 months
Sato et al., 2012	Guinea pig (Hartley strain) Experiment 1 (*n*=15) Experiment 2 (*n*=15) Control 1 (*n*=15) Control 2 (*n*=15)	Stifle	7 x 10^6 Human BM-MSCs suspended in PBS (Experiment 1) or HA (Experiment 2)	Macroscopic, histological and immunohistochemical analyses	5 weeks	Partial cartilage repair in the experimental group receiving the BM-MSCs with HA, but not in the other groups

Exp, experimental; Cont, control; AD-MSCs, adipose-derived MSCs; BM-MSCs, bone marrow-derived MSCs; PBS, phosphate buffered saline; HA, hyaluronic acid; ROM, range of motion.

916 **Table 2 Summary of studies evaluating the therapeutic potential of mesenchymal stem cells in animals with experimentally-induced**
917 **osteoarthritis**
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Study	Species	Type of lesion	Treatment	Evaluation methods	Study period	Results
Diekman et al., 2013	Mouse C57BL/6 Experimental (*n*=8) Control (*n*=8)	Post-traumatic OA via osteochondral fracture of tibial plateau	1 x 10^4 BM-MSCs	Histological analysis and micro-computed tomography	2 months	BM-MSCs mitigated development of post-traumatic OA during 2 month study period
Pei et al., 2013	Miniature pig Experimental (*n*=10) Control (*n*=3)	Partial thickness cartilage defects on medial femoral condyle	Unspecified number of allogeneic S-MSCs	Histological and immunohisto-chemical analyses	3 months	Tissue positive for GAG and collagen II immunostaining present in experimental, but not control, pigs. However, no engraftment of injected S-MSCs detected in repair tissue
Desando et al., 2013	Rabbit Experimental (*n*=48) Control (*n*=24)	Mild OA via transection of cranial cruciate ligament	2 x 10^6 (*n* = 24) or 6 x 10^6 (*n* = 24) Autologous AD-MSCs	Macroscopic, histological and immunohisto-chemical analyses	6 months	AD-MSCs retarded progression of OA. No engraftment of AD-MSCs into endogenous cartilage
Lee et al., 2013	Rabbit Experimental (*n*=27) Control (*n*=27)	Osteochondral defect in femoral trochlear groove	4 x 10^6 autologous S-MSCs	Macroscopic, histological and immunohisto-chemical analyses	6 months	Hyaline cartilage repair in both controls that received platelet-rich plasma gel and experimental animals that received platelet-rich plasma gels and S-MSCs
Seo et al., 2013	Horse (*n* = 6) Contralateral joint used as control	Full thickness osteochondral defect in the lateral trochlear ridge of the talus	5 x 10^4 and 5 x 10^6 Autologous BM-MSCs seeded into sponges containing various chondro- and osteo-inductive molecules	Macroscopic, histological, radiographic, immunohisto-chemical and quantitative CT analyses	4 months	Osteochondral regeneration was observed in lesions treated with BM-MSC-sponge constructs, but not in joints that received sponges alone
ter Huurne et	Mouse	Collagenase-	2 x 10^4	Histological and	6 weeks	When AD-MSCs were injected 7 days after collagenase treatment,

al., 2012	C57BL/6 Unspecified numbers of control and experimental mice	induced OA of stifle	Allogeneic AD-MSCs	immunohisto-chemical analyses		cartilage destruction was retarded compared to controls. In contrast, when AD-MSCs were injected 14 days after collagenase treatment, no effects were seen compared to controls. At 5 days after injection, no AD-MSCs could be detected
Al Faqeh et al., 2012	Sheep Experimental (*n*=12) Control (*n*=4)	OA induced via medial meniscectomy and transection of cranial cruciate ligament	1 x 10^7 Autologous BM-MSCs	Macroscopic and histological analyses	6 weeks	Cartilage destruction was retarded in animals that received BM-MSCs as compared to controls
Kim et al., 2012	Rabbit Experimental (*n*=15) Control (*n*=3)	Osteochondral defect in medial femoral condyles	Injection of HA (*n* = 3) or 1 x 10^6 allogeneic BM-MSCs (*n* = 3) or 1 x 10^6 allogeneic BM-MSCs and HA (*n* = 9)	Macroscopic and histological analyses	7 weeks	Animals treated with HA or HA and BM-MSCs showed statistically significant improvements in the healing of defects as compared to controls. There was no significant difference in defect healing between the HA and the HA and BM-MSC groups
McIlwraith et al., 2011	Horse (*n* = 10) Contralateral joint used as control	Osteochondral defect created in medial femorotibial joint followed by microfracture	2 x 10^7 Autologous BM- MSCs	Clinical, histological, immunohisto-chemical, radiographic and MRI analyses	12 months	No significant clinical improvement in the joints treated with BM-MSCs as compared to controls; however, BM-MSC-treated joints showed an increase in the firmness of the repair tissue
Toghraie et al., 2011	Rabbit Experimental (*n*=10) Control (*n*=10)	OA induced via transection of cranial cruciate ligament	1 x 10^6 Allogeneic AD-MSCs	Radiographic and histological analyses	5 months	Rabbits that received AD-MSCs showed less cartilage degeneration, osteophyte formation and subchondral sclerosis than controls
Yang et al., 2011	Dog Experimental (*n*=16) Control (*n*=8)	Osteochondral defect in femoral condyles	1 x 10^6 Allogeneic, chondro-genically-stimulated	Macroscopic, histological, histochemical, biomechanical and micro-CT	6 months	Osteochondral defects of the dogs that received the scaffolds seeded with chondrogenically-stimulated BM-MSCs showed significantly better cartilage repair than controls

			BM-MSCs seeded into decellularised cartilage and cancellous bone matrix scaffolds	analyses		
Matsumoto et al., 2009	Rat Experimental (*n*=48) Control (*n*=12)	Mono-iodoacetate-induced OA of stifle joint	2.5×10^5 BMP4-transduced and 2.5×10^5 Flt1-transduced allogeneic M-MSCs	Macroscopic, histological and immunohisto-chemical analyses	4 months	Rats that received non-transduced M-MSCs showed marked OA comparable to that observed in controls. However, rats that received BMP4- and Flt1-transduced M-MSCs repaired defects with hyaline cartilage
Frisbie et al., 2009	Horse Experimental (*n*=8) Control (*n*=8)	Osteochondral fragment created in middle carpal joint via arthroscopy	$5.6\text{-}15 \times 10^6$ Autologous BM- MSCs	Clinical, histological, pathological and radiographic analyses	70 days	No significant differences between experimental and control animals
Grigolo et al., 2009	Rabbit Experimental (*n*=32) Control (*n*=18)	OA induced via transection of cranial cruciate ligament	2×10^6 Autologous BM- MSCs seeded onto HA-based scaffolds	Macroscopic, histological and immunohisto-chemical analyses	6 months	Rabbits that received BM- MSCs seeded onto HA-based scaffolds formed hyaline-like cartilage within the defects to a more significant degree than the controls which received scaffolds alone
Koga et al., 2008	Rabbit Experimental (*n*=24) Control (*n*=12)	Osteochondral defect created in trochlear groove of femur	1×10^7 Allogeneic S-MSCs placed directly into defect (*n* = 12) or injected into joint (*n* = 12)	Macroscopic, histological and immunohisto-chemical analyses	6 months	Cartilage regeneration was observed in animals in which the S-MSCs were placed directly into the defect. In contrast, animals that received S-MSCs via injection into the joint showed no improvement compared to controls
Lee et al., 2007	Miniature pig Experimental (*n*=18) Control (*n*=9)	Partial thickness articular cartilage defect of medial	7×10^6 Autologous BM-MSCs and HA	Macroscopic and histological analyses	3 months	Improved cartilage healing in pigs that received BM-MSCs and HA as compared to controls that received saline or HA alone

		femoral condyle				
Wilke et al., 2007	Horse (n = 6) Contralateral joint used as control	Osteochondral plug removed from femoropatellar joint	1.2 x 10^7 Autologous BM-MSCs	Macroscopic, histological and immunohistochemical analyses	8 months	No significant differences between BM-MSC-treated and control joints
Agung et al., 2006	Rat Experimental (n=16) Control (n=16)	OA induced via transection of cranial cruciate ligament, medial meniscus and femoral condylar cartilage injured with surgical blade	1 x 10^6 (n = 8) or 1 x 10^7 (n = 8) Allogeneic BM-MSCs	Histological and immunohisto-chemical analyses	1 month	Rats that received BM-MSCs showed no significant evidence of cartilage repair
Murphy et al., 2003	Goat Experimental (n=15) Control (n=9)	OA induced via transection of cranial cruciate ligament and medial meniscus	1 x 10^7 Allogeneic BM-MSCs and HA	Histological and histochemical analyses	5 months	Goats that received BM-MSCs showed regeneration of the meniscus which helped retard the progression of the OA

AD-MSCs, adipose-derived MSCs; BM-MSCs, bone marrow-derived MSCs; M-MSCs, muscle-derived MSCs; S-MSCs, synovium-derived MSCs; PBS, phosphate buffered saline; OA, osteoarthritis; HA, hyaluronic acid; GAG, glycosaminoglycans; BMP, bone morphogenetic protein.

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