Is there a role for stem cells in treating articular injury?

ABSTRACT

Articular cartilage is a specialized tissue with a high prevalence of injuries. The complex architecture of articular cartilage means that injuries are difficult to treat. The sequelae of such injuries include post-traumatic osteoarthritis. Current treatments include microfracture, microdrilling, osteochondral transplantation and matrix autologous chondral implantation. However, current surgical therapies have a number of disadvantages. Mesenchymal stem cells have been suggested as a potential alternative therapy, with a theoretical ability to regenerate articular cartilage. Research, although positive, is mainly limited to case series, in which the follow up is short to medium term. Stem cells may hold the answer to the age-old problem of articular cartilage injury but more robust evidence is required.

William Hunter wrote in 1742 that if one were to consult 'the standard surgical writers from Hippocrates to the present age, we shall find that an ulcerated Cartilage is universally allowed to be a very troublesome disease; that it admits of a Cure with more Difficulty than a carious Bone and that when destroyed, it is never recovered' (Hunter, 1742). Over 250 years later, Hunter's statement remains relatively unchallenged by medical progress.

This review discusses and evaluates the current evidence for using stem cells in treatment of articular cartilage injuries. The scale of the problem is hard to quantify. Two large-scale case series of arthroscopies, with more than 25 000 and 31 000 patients respectively, report a prevalence of cartilage injuries of 60% and 63% respectively (Curl et al, 1997; Widuchowski et al, 2007).

Evidence suggests that injury to articular cartilage can cause post-traumatic osteoarthritis, a risk reported to be as high as 75% (Schenker et al, 2014). Post-traumatic osteoarthritis accounts for some 12% of all cases of symptomatic osteoarthritis, approximately 5.6 million people in the United States (Brown et al, 2006).

Structure and properties of hyaline cartilage

The complex architecture of hyaline cartilage makes restoration problematic. Hyaline cartilage is a complex matrix, largely consisting of type II collagen, water and chondrocytes. Histologically, four zones are discernible, each characterized by the shape and density of the chondrocytes as well as the appearance of the associated collagen fibrils.

The collagen network bestows an ability to withstand compression by extruding water, which is then reabsorbed upon release. The cartilage returns to its previous shape. The chondrocyte has an almost anaerobic metabolism, being avascular and without a neural supply. Nutrition is received via a combination of diffusion and being channelled from subchondral bone (Minas, 2012; Goldring, 2013; Gracitelli et al, 2016) (*Figures 1* and *2*).

The regeneration of articular cartilage depends on whether the lesion extends into the subchondral bone. The fact that cartilage is relatively avascular means that articular cartilage cannot mount a tissue healing response while progenitor cells from the blood cannot access the cartilage surface. Adult chondrocytes do not have the ability to migrate and cannot regenerate (Minas, 2012; Goldring, 2013).

A cartilage defect which extends into subchondral bone ensures bleeding, clot formation and inflammation, which later mature into fibrocartilage. This fibrocartilage differs in terms of its material properties but is widely reported to improve patients' symptoms (Gracitelli et al, 2016).

Present surgical treatment

The creation of fibrocartilage forms the basis of microfracture and microdrilling surgical techniques. The procedure came to prominence in the 1960s and was latterly advocated by Steadman (Smith et al, 2005; Robert, 2011).

Both microfracture and microdrilling procedures can be performed arthroscopically. During microfracture an awl is advanced to the articular defect and used to create holes of between 2–4 mm width in the subchondral bone (Verdonk et al, 2015).

Osteochondral autologous transplantation or mosaicplasty involves the creation of a mosaic of transplanted autologous osteochondral tissue to fill a defect (Hannon et al, 2014). 'Plugs' of osteochondral tissue are harvested from areas of the joint surface which are less subject to weight-bearing pressure, such as the intercondylar notch of the knee or the margins of the patello-femoral joint. The procedure is reported as having a high rate of good to excellent results in the largest series but concerns remain (Bedi et al, 2010). Issues include the limitation of harvest tissue, donor site morbidity and the morphological considerations of the graft with regards to the recipient lesion site. Other post-implantation concerns include dead space between the graft and recipient tissue and graft subsidence (Bedi et al, 2010).

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Autologous chondrocyte implantation

The idea of implanting chondrocytes into the defect (autologous chondral implantation) was suggested at the beginning of the 20th century, but it was not realized until Grande's work on the knee joints of rabbits (Grande et al, 1989).

In 1994, Brittberg advanced the technique, describing autologous chondral implantation in human subjects. The process involves the harvest of chondrocytes from a non-articular part of the knee during arthroscopy. The chondrocytes undergo isolation and laboratory culture for up to 21 days (Brittberg et al, 1994). The patient then undergoes an arthrotomy in which the chondral lesion is excised without involvement of subchondral tissue and chondrocytes implanted. The lesion is subsequently covered by periosteal flap from the proximal tibia (Brittberg et al, 1994). At a 2-year follow up, 14 out of 16 patients with femoral condylar implants had good to excellent results while biopsies of 11 of 15 femoral implants demonstrated an appearance of hyaline cartilage.

The process has now evolved to its third generation, known as matrix autologous chondral implantation (Bartlett et al, 2005; Vijayan et al, 2012). Matrix autologous chondral implantation is also the only cell-based therapy approved by the Food and Drug Administration.

The process of matrix autologous chondral implantation remains imperfect. The proliferation potential of chondrocytes decreases with advancing patient age. A low yield of chondrocytes is also described – from 1 g of chondrocytes, less than 22% will undergo successful isolation (Nazempour and van Wie, 2016). Those chondrocytes undergoing expansion in an in-vitro matrix are also subject to the process of de-differentiation, which occurs when a chondrocyte reverts from a specialized function to a simpler state. In the case of chondrocytes, this is characterized by the production of type I collagen rather than the type II which distinguishes hyaline cartilage (Nazempour and van Wie, 2016) (*Figure 3*).

Controversy remains about whether it is more effective to perform microfracture or the matrix autologous chondral implantation procedure. Microfracture can provide good, initial clinical outcomes in those patients with smaller articular cartilage lesions but the tissue is composed of fibrocartilage rather than hyaline cartilage. Matrix autologous chondral implantation is a two-stage procedure, with the potential for donor site morbidity following tissue harvest (Smith et al, 2005).

A Cochrane review examined the efficacy of mosaicplasty, microfracture and allograft transplantation in isolated articular cartilage defects in the knee. Primary outcomes examined included knee function and quality of life scores while secondary outcomes included pain and activity level. The review discovered three trials comparing mosaicplasty to microfracture (Gracitelli et al, 2016). There were no trials examining allograft transplantation or microdrilling. The review concluded that the evidence is of low quality and insufficient to draw conclusions about whether mosaicplasty should be favoured over microfracture (Gracitelli et al, 2016).

Articular injuries are thus common, with a well-established risk of post-traumatic osteoarthritis with a

Figure 1. Structural layers of articular cartilage. From Kelc et al (2013).

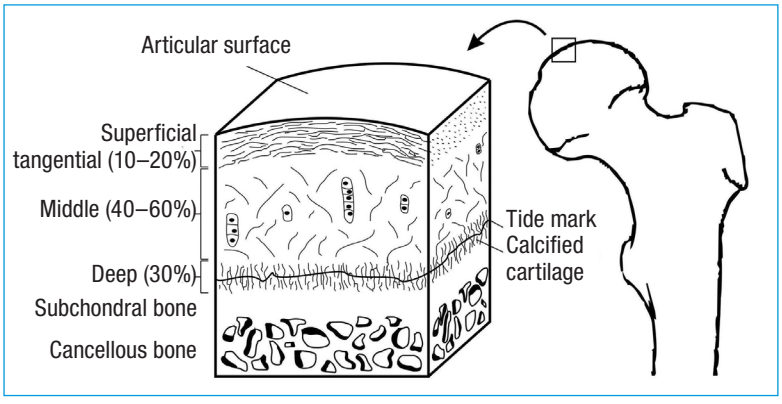


Figure 2. Partial and full thickness defects of articular cartilage. From Kelc et al (2013).

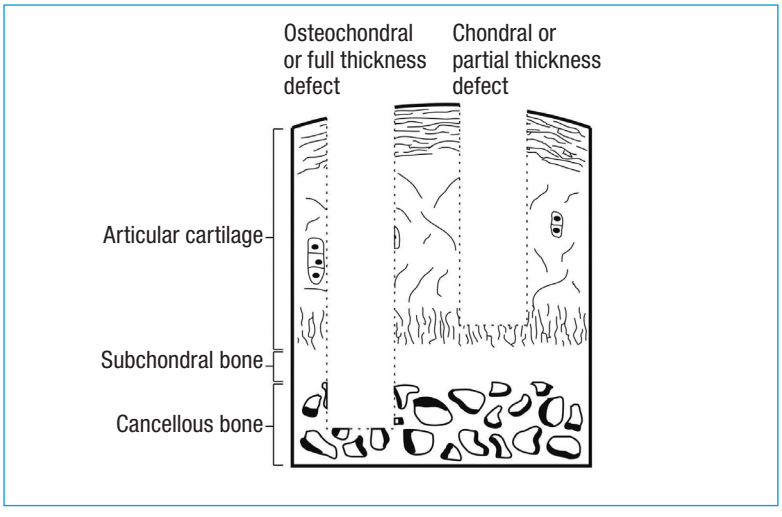
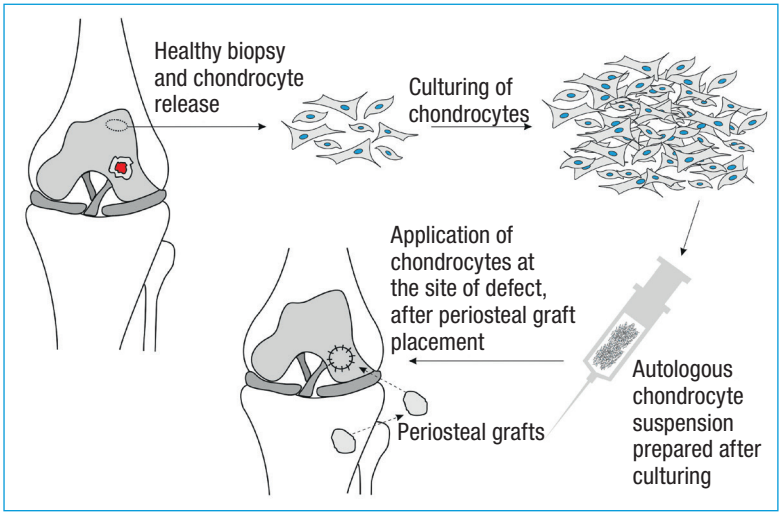


Figure 3. Schematic diagram showing the different stages involved in the process of autologous chondrocyte implantation. From Kelc et al (2013).



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significant disease burden. Given that surgical techniques for the treatment of articular injury remain imperfect, the search for a more effective therapy remains pertinent.

Introduction to stem cells

Mesenchymal stem cells have been acclaimed as just such a therapy. Mesenchymal stem cells are strictly defined as those cells fulfilling a number of criteria as suggested by the International Society of Cellular Therapy. These properties include an ability to adhere to plastic, the expression of a number of cell markers including CD105, CD73 and CD90 while undergoing multilineage differentiation with an ability to self-renew (Ikebe and Suzuki, 2014; Mirza and Oussedik, 2017).

Mesenchymal stem cells are abundant in multiple different tissues and can be harvested with relative ease without the limitation of any donor site morbidity, as per matrix autologous chondral implantation. Bone marrow, periosteum and adipose tissue are the most common sources, but they can also be found in the muscle, brain and kidney (Murray et al, 2014).

The mechanism by which mesenchymal stem cells cause cartilage regeneration is not understood. However, mesenchymal stem cells have been noted to have similar actions to what Caplan and Dennis (2006) refer to as 'trophic mediators'. These actions include immunosuppression and the inhibition of scar forming tissue. Other actions include the stimulation of differentiation, mitosis and angiogenesis (Caplan et al, 2006). Aggarwal and Pittenger (2005) found that when mesenchymal stem cells were cultured together with refined innate immune cells, the mesenchymal stem cells inhibit the production of pro-inflammatory cytokines such as tumour necrosis factor (TNF) alpha. Simultaneously the production of cytokines, such as IL-10, which can suppress the immune response, is promoted. Besides the effect on the innate immune system, mesenchymal stem cells can also act on the acquired immune system reducing the number of regulatory T cells and diminishing the secretion of interferon gamma in natural killer T cells. A variety of different mechanisms govern T cell suppression including cell–cell contact, the release of soluble factors and promotion of regulatory T cells (Haddad and Saldanha-Araujo, 2014). Another theory postulated is that the mesenchymal stem cells may act on subchondral bone, forming the primary repair cartilage (Pelttari et al, 2008).

Mesenchymal stem cells and tissue engineering

The administration of mesenchymal stem cell therapy involves a number of steps:

1. The cell harvest
2. Further refinement, preparation and culturing of the cells to engineer the articular tissue substitute
3. Final delivery to the lesion of interest.

In surgical delivery, the procedure is normally a two-stage intervention. The mesenchymal stem cells are harvested, then cultured or concentrated. Two main culture preparations used were Dulbecco modified Eagle medium and alpha minimum essential medium (Reissis et al, 2016). Hyaline cartilage was found to be regenerated more commonly in those studies using Dulbecco modified Eagle medium. After this the cells are placed within a scaffold biomatrix and applied to the osteochondral defect with some authors favouring the use of an overlying sutured periosteal patch (Reissis et al, 2016).

Injection therapy has a number of benefits including its less invasive nature, as well as the ability to be performed in an outpatient setting in a single visit. The cells can be harvested in advance, followed by culture or concentration. In a de novo fashion, the mesenchymal stem cell can be immediately transplanted.

The optimal preparation of stem cells involves a number of essential components. These include the mesenchymal stem cells themselves, bioactive factors, a three-dimensional carrier matrix and an optimal physical environment (Kessler and Grande, 2008; Ahmed and Hincke, 2014). Kessler and Grande (2008) described the three-dimensional carrier matrix according to whether it is protein-, carbohydrate- or hydrogel-based. It has also been classified according to whether it is natural or artificial material such as polylactic and polyglycolic acid (Getgood et al, 2009).

Protein-based scaffolds include gelatin, fibrin and collagen while carbohydrate-based scaffolds include alginate, hyaluronan or agarose among others. A more recent scaffold includes the hydrogel family, a hydrophilic polymer that has both liquid and solid properties and can maintain a three-dimensional structure. The use of a scaffold aims to recreate the extracellular matrix in which chondrocytes reside, as well as providing the architecture to allow regeneration (Getgood et al, 2009).

The structures of the matrices are chosen for a number of different properties ranging from previous uses in drug delivery (agarose and alginate), promoting the stabilization of cartilage (hyaluronate) to its low immunogenicity and the maintenance of the chondrocytic phenotype in vivo (collagen) (Kessler and Grande, 2008; Ahmed and Hincke, 2014). At present the evidence does not identify a single optimum scaffold. Bioactive factors include those factors which promote the realization of chondrogenic potential. Such factors include, among others, bone morphogenetic proteins, transforming-growth factor beta, insulin-like growth factor and platelet-derived growth factor.

Bone morphogenetic protein promotes chondrogenesis. Transforming-growth factor beta increases the expression of collagen II and increases the amount of glycosaminoglycans. Insulin-like growth factor increases chondrocytic mitosis and cell differentiation (Nerem, 2007; Kessler and Grande, 2008). Physical factors also have a positive influence on

chondrogenic potential with an increase in hydrostatic pressure shown to increase collagen II and aggrecan production (Smith et al, 2004; Hu and Athanasiou, 2006). Others have discovered positive effects with the use of electromagnetic fields and slide contact (Guilak et al, 2009; Ahmed and Hincke, 2014).

The aim of engineering artificial cartilage is to recreate a tissue which mirrors the defect itself, and can become well integrated. The tissue is thus prevented from being subject to normal shear forces experienced within the joint. Furthermore the substitute aims to recreate the biphasic nature of articular cartilage with an 80% fluid phase and 10–20% solid phase of collagen II chondrocytes (Ahmed and Hincke, 2014). The material's stability allows for the resettlement of cartilage, while also being biodegradable. The material must also permit chondrocyte proliferation, interaction and differentiation.

Bone marrow mesenchymal stem cell preparation

Bone marrow cells are the most widely studied, owing to their extensive investigation in vitro and in vivo. Bone marrow-derived mesenchymal stem cells can also be easily cultured without losing chondrogenic potential (Wakitani et al, 2002; Reissis et al, 2016).

Bone marrow-derived mesenchymal stem cells are most commonly harvested from the bone marrow of the iliac crest although isolation is also feasible from the tibia and femur (Cox et al, 2011; Murray et al, 2014).

The harvested cells undergo a process of centrifugation, which separates the aspirate into mononuclear, fat and blood cell layers. The isolated mononuclear layer is subsequently cultured onto plastic and the cells, of which the mesenchymal stem cells predominate, undergo proliferation.

The cells are delivered to the site of interest by systemic infusion or with the use of scaffolds, following a period of growth (Murray et al, 2014).

Adipose-derived stem cell preparation

Adipose tissue is heterogenous in terms of cell composition, including adipocytes, periadipocytes and fibroblasts. The discovery of a high concentration of pluripotent stromal cells in the stromal vascular fraction of adipose tissue has led to a focus on the stromal vascular fraction particularly. Schäffler and Buchler (2007) describe a protocol for the preparation of adipose-derived stem cells. The harvest occurs by surgical resection or lipoaspiration. The cells are then subject to an enzymatic digest with collagenase. Following the removal of erythrocytes, the cells undergo expansion and plating.

Although the process of mesenchymal stem cell production has been demonstrated to be safe and feasible, the protocols for isolation and expansion of harvested cells show wide heterogeneity, which in turn affects the quality and efficacy of the prepared therapy (Ikebe and Suzuki, 2014).

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Clinical evidence for stem cells

The clinical evidence for the benefit of mesenchymal stem cells in articular cartilage defects is limited.

Perdisa and colleagues (2015) performed a systematic review examining preclinical and clinical evidence surrounding adipose-derived stem cells. The review discovered 28 animal model studies and 11 clinical studies, nine of which are case series. The follow up is short to medium term, with no study covering more than 30 months in follow up. The majority of studies recorded clinical outcomes with some recording radiological and arthroscopic outcomes. One study by Jo et al (2014) described histological outcomes with formation of hyaline cartilage in a group of patients given a high dose of adipose-derived stem cells.

The review includes two comparative studies. Koh and Choi (2012) compare a study group of those injected with adipose-derived stem cells to those injected with both adipose-derived stem cells and platelet-rich plasma. At mean follow up of 16.4 months, an improvement in clinical outcomes is recorded in the study group. However, there is no significant difference between the two groups.

Stem cells *vs* present surgical treatment

The evidence also remains ambivalent as to whether the use of mesenchymal stem cells is more efficacious than existing treatment. Nejadnik et al (2010) investigated this question, comparing the clinical outcomes of those treated with first generation autologous chondral implantation to those treated with cartilage repair using bone marrow-derived mesenchymal stem cells. A total of 72 patients matched by age and lesion site were recruited and followed up for 2 years. Results did not demonstrate a difference in clinical outcomes between the two cohorts, with the exception of physical role functioning over time and quality of life on the SF 36. However, the study established a greater improvement in men than women over the course of the study and also demonstrated better scores in those younger than 45 years of age (Nejadnik et al, 2010). Giannini et al (2010) reported similar results in a study evaluating a one-step approach to treatment of osteochondral lesions of the talar dome. The study compared three cohorts, an open autologous chondral implantation group, a closed group and those treated with bone marrow-derived mesenchymal stem cells. An improvement in mean American Orthopaedic Foot Ankle Society score, with histological changes suggestive of hyaline cartilage, was reported.

More recently Koh et al (2016) investigated two randomized groups, 80 patients in total, comparing microfracture alone to microfracture with adipose-derived stem cells. Clinical, radiological, histological and

KEY POINTS

- Articular cartilage is a specialized tissue which cannot be regenerated after injury.
- There is a significant prevalence of cartilage injuries which can lead to post-traumatic arthritis.
- Present surgical therapy, including microfracture, mosaicplasty and autologous chondrocyte implantation, has imperfect results and potential downsides.
- Mesenchymal stem cells are multipotential cells, with the ability to form bone, muscle, fat and cartilage.
- Evidence for the therapeutic effects of mesenchymal stem cell is positive but studies rely upon small patient cohorts and short-term follow up. Well-designed, robust randomized controlled trials are required to fully evaluate the benefits.

arthroscopic outcomes were evaluated. Knee injury and Osteoarthritis Outcome Score pain and symptom subscores significantly improved, but there was no difference in other clinical outcomes. There were no statistically significant intergroup differences at arthroscopy or the 57 knees, which were evaluated histologically. The study is of a higher level of evidence but the follow up remains short term with a mean follow-up of 27.4 months (Koh et al, 2016). The study also suffers with clinical improvements being ascribed to a post-hoc analysis rather than the outcomes that were to be analysed in the a priori analysis. It is also unclear whether the therapeutic benefit is in relation to the microfracture, the mesenchymal stem cells or a combination of the two.

Ethical concerns regarding mesenchymal stem cells

Stem cell therapies pose an ethical dilemma. In the face of a lack of robust evidence, commercial interests keen to exploit mesenchymal stem cell therapies – and patients – for financial gain have exaggerated the benefits of stem cells.

Such elements have taken advantage of the legislative confusion in the United States. A landmark case concerned the question of whether processed autologous stem cells are a biological drug, subjecting mesenchymal stem cell therapy to federal regulation by the Food and Drug Administration, or classified under 'the practice of medicine' which is not (Sipp, 2013). The courts eventually found in favour of the Food and Drug Administration and presently the Food and Drug Administration has only approved one haematopoietic stem cell progenitor cell product for use in haematological malignancy (Food and Drug Administration, 2015).

Meanwhile the European Medicines Agency has tightly controlled mesenchymal stem cell therapies using the advanced therapy medicinal products legislation (Hanna et al, 2016). Advanced therapy medicinal products are defined as a group of cell therapies undergoing minimal manipulation before use in a clinical application. As of 2015, only five advanced therapy medicinal products have received market authorization (Hanna et al, 2016).

A fear remains that the hype surrounding mesenchymal stem cell therapy could exploit vulnerable patients and lead to commercial interests inflating the case for the use of stem cells.

Conclusions

Surgery for articular cartilage injury is imperfect. Microfracture and microdrilling produce good clinical outcomes but cannot restore articular cartilage and work better in smaller lesions.

Mosaicplasty and autologous cartilage implantations are compromised by donor site morbidity as well as staged surgical interventions in the case of autologous chondral implantation.

Mesenchymal stem cells offer much, from ease of harvest and preparation to implantation without surgery. Early results are encouraging but are based upon small case series with short-term follow up and higher-level studies with poor design.

Thus many caveats remain. At present there is no high level evidence to determine which cells are optimal for harvest, nor which process of refinement, preparation and tissue engineering is best. It is unknown whether stem cell therapy is comparable to or better than existing therapies.

There is a well-founded concern that commercial interests might exaggerate the benefits of stem cells, using direct to consumer marketing and flouting legislation, exploiting vulnerable patients.

Significant questions remain as to whether mesenchymal stem cell therapy can genuinely regenerate articular cartilage tissue, and thus William Hunter's challenge to find a cure for ulcerated cartilage remains, as yet, unsolved. **BJHM**

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