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Review

# Cartilage defect repair in horses: Current strategies and recent developments in regenerative medicine of the equine joint with emphasis on the surgical approach

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ABSTRACT

Chondral and osteochondral lesions due to injury or other pathology are highly prevalent conditions in horses (and humans) and commonly result in the development of osteoarthritis and progression of joint deterioration. Regenerative medicine of articular cartilage is an emerging clinical treatment option for patients with articular cartilage injury or disease. Functional articular cartilage restoration, however, remains a major challenge, but the field is progressing rapidly and there is an increasing body of supportive clinical and scientific evidence. This review gives an overview of the established and emerging surgical techniques employed for cartilage repair in horses. Through a growing insight in surgical cartilage repair possibilities, surgeons might be more stimulated to explore novel techniques in a clinical setting.



## Introduction

The principal aim of the treatment of joint problems in the horse focuses on pain relief and minimisation of progression of joint deterioration, thus delaying the onset of osteoarthritis. However, this is palliative only, providing temporary relief but no cure, as the structural integrity of the articular cartilage surface, and potentially the underlying bone, is damaged. In fact, healing or functional repair of osteochondral lesions is still a major challenge in orthopaedic surgery. As early as 1743 William Hunter stated: *"From Hippocrates down 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 carious bone; and that, when destroyed, it is not recovered"* (Hunter, 1743) (Fig. 1).

Hyaline cartilage is an avascular, aneural and highly organised tissue that generally forms (inferior) repair tissue. There are three mechanisms for cartilage repair: extrinsic, intrinsic and matrix flow. Extrinsic repair is seen in full-thickness cartilage lesions that extend through the tidemark. Mesenchymal elements from the subchondral bone migrate to the defect and fill it with repair tissue (Hunziker and Rosenberg, 1996; Hunziker, 1999; Hurtig et al., 1988). Small full-thickness lesions of 5 mm or less tend to heal by intrinsic repair (from within the cartilage). This implies proliferation of injured or exposed chondrocytes that produce repair tissue, whereas matrix flow, which relies upon migration of the cartilage margins from the perimeter towards the centre of the defect, plays a role in the repair of all types of lesions, but is most important in smaller defects (Convery et al., 1972; Hurtig et al., 1988). In all cases, the resulting repair tissue consists of type I collagen containing fibrocartilage with inferior structural and functional properties compared to native type II collagen found in hyaline cartilage (Fig. 1). This repair tissue does not integrate well with the surrounding hyaline cartilage and has limited longevity (Mankin, 1974; Hurtig et al., 1988; Desjardins and Hurtig, 1990; Gomoll and Minas, 2014).

Ideally, techniques for cartilage repair should generate a repair tissue with a structure and function approximating native articular cartilage, which integrates well with the adjacent cartilage and bone. Stimulation of the natural repair process is theoretically the best method to achieve this, but it should be realised that for larger defects some form of (temporary) filling, such as implantation of articular grafts, will probably always be necessary.

Regeneration of articular cartilage is challenging in man, but more so in the horse. Equine joints share many features with those of humans (Malda et al., 2012) and the equine model is now well accepted for orthopaedic research (McIlwraith et al., 2011a). However, in a practical sense clinical conditions are more challenging in the horse whilst economic feasibility is often less. In some joints, like the carpus and fetlock, equine cartilage is thinner, precluding the use of several techniques that would be relevant in humans (Lee et al., 2014). Also, the necessity of immediate weight-bearing postoperatively and the often substantial subchondral bone involvement in the horse needs to be considered before simply applying

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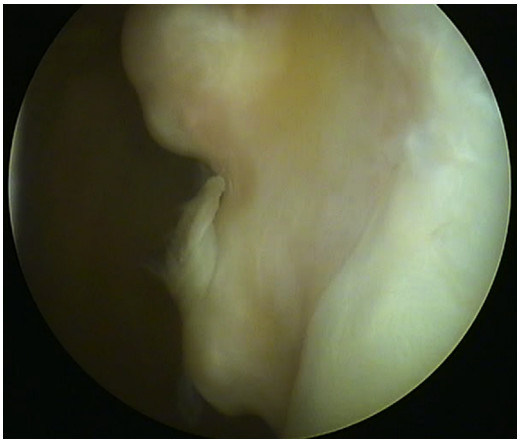

**Fig. 1.** Arthroscopic image of a fibrocartilaginous repaired osteochondral defect in the stifle of an adult horse 1 year after surgical debridement.

techniques used in humans. In a surgical-technical sense, many modern approaches for cartilage repair, like cell and tissue engineered transplantation techniques, add complexity to the surgery, and often preclude the use of arthroscopy.

This review focuses on surgical cartilage repair. While articular cartilage cannot be restored in isolation, the joint should be seen as an organ (Samuels et al., 2008), in which homeostasis plays a crucial role in the success of any intervention. Therefore, supplementary medical care and appropriate rehabilitation are highly likely to be necessary as the whole joint should be treated to achieve the best functional and structural outcome.

## Surgical techniques for articular cartilage repair

Surgical approaches to treat joint disorders characterised by tissue damage have been grouped into three categories (Hunziker, 2002; Hunziker et al., 2015): palliative (arthroscopic debridement and lavage), reparative (marrow stimulation techniques) and restorative (osteochondral grafting, autologous chondrocyte implantation or ACI).

## Palliative treatments: Cartilage debridement and lavage

Surgical debridement and joint lavage are the first-line palliative treatments, which, together with removal of free bodies in the joint and limited excision of osteophytes, have been shown to decrease pain and improve joint function (McIlwraith et al., 2005).

The goal of debridement of full-thickness cartilage defects is to create a contained lesion by removing loose cartilage and bone together with any other degenerated tissue in order to stimulate spontaneous repair. The depth of the debridement and the angles of the lesion affect the formation of repair tissue in the defect (Rudd et al., 1987). Open or arthroscopically assisted manual curettage gives the best quality of debridement (Drobnic et al., 2010) (Fig. 2a, 2b, 2c). Debridement should be thorough, but not overly aggressive. The amount of residual bone after debridement, especially in the carpus, is an important prognostic factor for return to athletic activity (McIlwraith et al., 1987). Removal of the calcified cartilage layer while retaining the subchondral bone plate provides an optimal effect, whereas leaving the calcified cartilage layer in place markedly restricts healing (Frisbie et al., 2006). In practice, the debridement includes removal of all the dull or yellowish tissue down to the bone, extending laterally to the normal cartilage (Frisbie et al., 2006; Drobnic et al., 2010). To retain congruency with the opposing joint surface, it is advisable to keep as much as possible of the subchondral bone plate intact. The remaining bone in the defect should be viable and crumbly, brownish bone should be removed. Minor bleeding from the subchondral bone can be managed using higher fluid pressure or by local application of epinephrine and thrombin, fibrin sealant or haemostatic sponges (Brittberg, 2008).

An interesting recent observation was the use of hyperosmotic saline irrigation solutions (600 mOsm) that, compared to normal saline (300 mOsm), seemed to be chondroprotective and may help in reducing unintentional cartilage injury during articular reconstructive surgery as well as promoting integrative cartilage repair (Eltawil et al., 2015).

Full-thickness cartilage defects need debriding, but there is debate about partial-thickness defects (Baumgaertner et al., 1990). It is not yet possible to truly restore hyaline cartilage, and consensus favours chondroplasty or trimming of the articular surface with removal of protruding strands or fibrillation by use of mechanical debridement with automated synovial abrasers in cases of partial thickness articular cartilage erosion (Ryan et al., 2003; McIlwraith et al., 2005). Debridement of the articular surface aims to reduce shedding of cartilage-derived debris into the synovial environment. Mechanical debridement or shaving can be frustrating because fibrillation may persist and leave behind unviable cartilage or the iatrogenic removal of healthy cartilage may occur (Hunziker and Quin, 2003). Thermal chondroplasty is another technique for smoothing the articular surface. This needs to be used with caution, however,

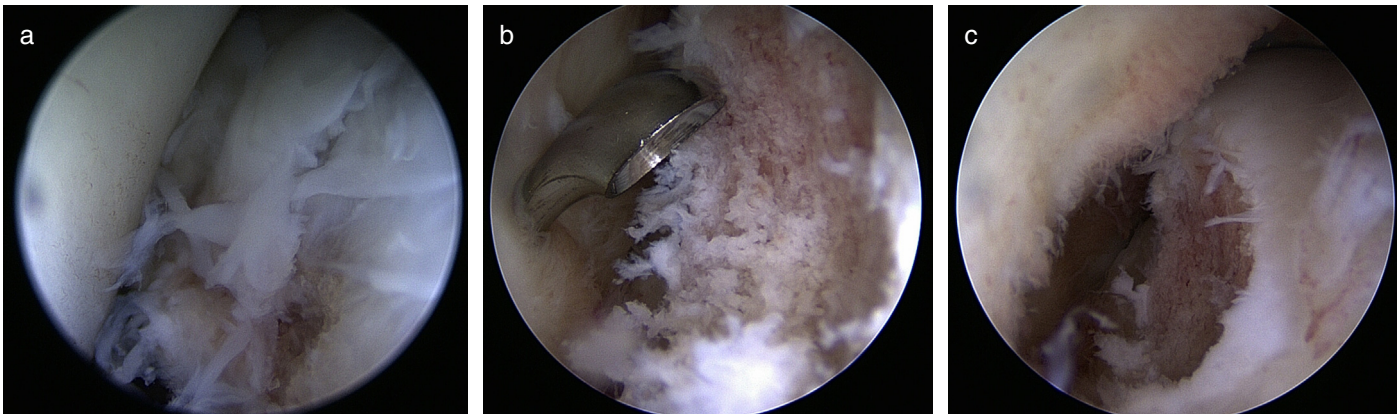


**Fig. 2.** Debridement of an osteochondral lesion in the fetlock of an adult horse: (a) severe cartilage (subchondral bone) damage on the sagittal ridge of the third metacarpal bone, (b) curettage with removal of the abnormal cartilage/subchondral bone, and (c) final version of the debrided osteochondral defect.

asexcessive matrix debridement or chondrocyte death can easily result (Ryan et al., 2003; Lubowitz, 2006).

In osteochondritis dissecans (OCD) arthroscopic debridement of lesions in the femoropatellar joint is recommended in horses >11 months of age where lesions are more than 2 cm in length or >5 mm in depth. In the tarsocrural joint, if clinical signs are present, surgery is recommended in all predilection sites, preferably after 5 months of age (McIlwraith, 2013). The overall success rate of surgical treatment of OCD lesions at the distal intermediate ridge of the tibia varied from 74 to 87% (McIlwraith et al., 1991; McIlwraith, 2013). In a recent retrospective study on outcome of arthroscopic debridement of OCD lesions on the lateral femoral trochlear ridge in mainly Warmbloods, 65% of horses returned to their intended use, which was comparable with previous studies (Foland et al., 1992; McIlwraith, 2013). The length of the lesion was significantly associated with the prognosis in horses with lesions <2 cm in length having 78% return to function, against 54% for lesions >4 cm (McIlwraith, 2013; UpRichard et al., 2013). Also in subchondral cystic lesions of the medial femoral condyle the amount of cartilage surface damage is an important prognostic factor. In a study of 150 cases, 70% of horses with <15 mm of surface debridement returned to their previous use compared with 30% of horses with >15 mm defects (Sandler et al., 2002). In another study, breed differences were reported with Quarter horses having poorer results after debridement compared with Thoroughbreds and Arabians (Howard et al., 1995). There is also an age effect. After arthroscopic debridement of a subchondral cystic lesion of the medial femoral condyle 64% of horses aged 0–3 years returned to soundness, against only 34% of horses older than 3 years (Smith et al., 2005). Retention of the cartilage surface over the cystic lesions is important (Sandler et al., 2002), and there is an association between debridement of medial femoral condylar defects and medial meniscus or meniscal ligamentous injury (Hendrix et al., 2010).

Critically, debridement of full-thickness cartilage lesions results in fibrocartilage and imperfect hyaline repair tissue. A permanent irregularity of the subchondral bone plate frequently persists, as has been demonstrated on long-term follow-up radiographs (McIlwraith et al., 2014). Especially in cases of larger lesions, such as advanced OCD cases of the stifle, development of osteoarthritis and persistent lameness may ensue. While debridement remains the first step to stimulate and start cartilage repair, additional reparative procedures should thus be considered.

## Reparative techniques: Bone marrow stimulation techniques (BSTs)

Bone marrow stimulation techniques are among the first-line surgical treatment options for symptomatic articular cartilage defects. BSTs are based on the penetration of the subchondral bone plate at the base of the cartilage defect, evoking an endogenous repair response. Bleeding from the bone marrow under the subchondral plate will bring a low number of pluripotent stem cells and growth factors to the defect that are embedded in the blood-clot that forms at the base of the defect and are crucial for the early stages of repair (Frisbie et al., 1999). The procedure is also said to result in improved anchorage of the repair tissue to the underlying subchondral bone and to some extent to the surrounding cartilage (Steadman et al., 2010).

Surgically, penetration of the subchondral bone spaces can be accomplished with small drill bits (subchondral drilling), awls (microfracture) or more aggressively by generalised or limited abrasion with burs (abrasion arthroplasty). Abrasion arthroplasty involves removal of a uniform layer of residual cartilage and a superficial layer of dead subchondral bone until mild bleeding is induced. Positive clinical results have been reported for OC lesions in the hock and stifle, but no comparative studies have been published (Scott et al., 2004). A more aggressive version of abrasion arthroplasty is called spongialisation and involves complete removal of the subchondral bone plate. Although this may be beneficial in cases with advanced cartilage and subchondral bone damage (Ficat et al., 1979), it is preferable to preserve the integrity of the subchondral bone plate and limit the use of this technique to exceptional cases.

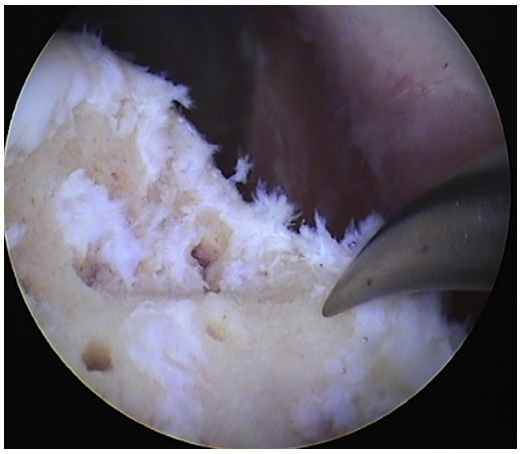

**Fig. 3.** Microfracturing (with an awl) of an osteochondral defect after debridement in the stifle of an adult horse.

Subchondral drilling or forage was proposed in humans for the treatment of osteochondrosis dissecans and for knee osteoarthritis (Smillie, 1957; Pridie, 1959). Controlled horse studies using subchondral drilling (1-mm diameter 1-cm deep holes) showed better healing compared to untreated defects in partial-thickness defects in the third carpal bone (Shamis et al., 1989). Another study revealed a greater surface coverage with more fibrocartilaginous tissue and better attachment to the underlying tissue (Vachon et al., 1986). Subchondral drilling also has been used in the sclerotic rim of subchondral cystic lesions, but led to complications such as cyst enlargement or development of additional cysts or intralesional osteophytes (Howard et al., 1995; McIlwraith et al., 2005).

Arthroscopic microfracturing or micropicking of lesions in humans, aiming at recruitment of MSCs, was introduced in the early 1980s (Steadman et al., 1997) (Fig. 3). This technique is simple, cost-effective and forms a basis for further treatments. Before microfracturing, removal of the calcified cartilage while retaining the integrity of the subchondral bone plate is necessary. Through various handheld angled tapered awls or picks, microfractures are made in the subchondral bone perpendicular to the surface, starting at the periphery and progressing towards the centre of the defect. Microfracture holes are placed 3–5 mm apart to cover the entire debrided area in a cartilage defect, including the perimeter, as this encourages tissue healing at the junction of the residual cartilage and the new repair tissue. The microfracture awls should penetrate 2 to 4 mm into the subchondral bone to optimise the access to the bone marrow. The rough surface that is created facilitates anchorage of the repair tissue.

Clinical trial results of microfracture in equine joint disease have not been published yet, but several experimental studies have been described. Frisbie et al. (1999) showed improvement in the quantity of repair tissue and type II collagen content and earlier bone remodelling at 4 and 12 months after microfracture of full-thickness defects through the calcified cartilage, but not penetrating the subchondral bone plate. The repair tissue was characterised superficially by fibrous tissue with deeper a mixture of fibrocartilage and hyaline cartilage. The difference between treated and control sites had been

established at or before 4 months with no change between 4 and 12 months. At 12 months, degeneration in repair tissue was noted in the femoral condyles compared to the radial carpal bones, probably due to biomechanical instability caused by enzymatic degradation of the repair tissue. In this and another study it appeared that thorough and complete removal of the calcified cartilage improves overall repair tissue attachment (Frisbie et al., 1999, 2006). In a further study type II collagen gene expression was increased within 8 weeks of microfracture, whereas aggrecan expression appeared uninfluenced (Frisbie et al., 2003).

The microfracture technique does however also have some drawbacks compared to drilling. In a mature rabbit model, cartilage repair was impaired due to compaction and fracturing of bone around microfracture holes compared to drilling with cooled irrigation that permitted clearance of bone from the holes with improved access to the bone marrow. Shearing and crushing of adjacent bone with microfracture caused more osteocyte death than heat by drilling (Chen et al., 2009). In a rabbit study on the effect of microfracture and drill hole depth (6 mm vs. 2 mm) and hole type (microfracture versus drill), deeper drilling was more beneficial for cartilage repair and no difference in quantity and quality of repair was observed between microfracture and drilling to 2 mm depth (Chen et al., 2011). However, subchondral drilling caused longer lasting damage to the subchondral microarchitecture, with more formation of subchondral cysts and intralesional osteophytes in a sheep model (Orth et al., 2012). Use of smaller drill holes induces less subchondral bone disturbance and is more effective for osteochondral repair (Eldracher et al., 2014). A sheep study showed that the use of small-diameter awls of 1 mm instead of larger awls decreased the extent of subchondral bone damage and improved articular cartilage repair (Orth et al., 2015). Nanofracturing or subchondral needling represents an innovative technique that overcomes the shortcomings of microfracturing with a better bone marrow access (Benthien and Behrens, 2015; Zedde et al., 2015).

Microfracture studies in humans have shown that the repair tissue is a form of fibrocartilage at best and that the clinical outcome deteriorates after 2 years of follow-up, with inconclusive durability and possible treatment failure beyond 5 years (Kreuz et al., 2006; Mithoefer et al., 2009; Goyal et al., 2013). Subchondral bone alterations such as subchondral cystic lesions or intralesional osteophytes were also seen in up to one-third of patients treated with microfracture (Kreuz et al., 2006; Mithoefer et al., 2009).

Microfracture can be performed alone, or in combination with other reparative procedures to improve healing. Autologous matrix-induced chondrogenesis (AMIC) is single-stage technique that is a combination of microfracture and a scaffold or biomembrane that is sutured or glued into the cartilage defect. The implanted matrix is thought to stabilise the resulting blood clot and prevent loss of cells (Benthien and Behrens, 2011). Enhanced marrow stimulation and formation of more hyaline-like cartilage have been achieved when using scaffolds like collagen type I/III porcine membrane (Chondrogide) or a chitosan-based liquid scaffold (BST-Cargel) (Benthien and Behrens, 2011; Stanish et al., 2013; Bark et al., 2014). Further biologic enhancement of microfracturing or AMIC in human and animal studies has been achieved by delivery of growth factors (platelet rich plasma, autologous serum), hyaluronic acid or even pulse electro-magnetic fields (Mirza et al., 2015; McIlwraith et al., 2011b; Doral et al., 2012; Makris et al., 2015, Karakaplan et al., 2015; Marmotti et al., 2015).

The field of tissue regeneration and marrow stimulation is constantly evolving. Further well-designed studies in horses (and humans) are needed to determine the long-term efficacy and define the usefulness of each technique and its specific clinical indications compared to other cartilage repair techniques (Bert, 2015; Makris et al., 2015).

## Restorative techniques: Transplantation procedures (grafting)

### *Tissue-based transplantation procedures*

#### *Cartilage reattachment*

Salvage and reattachment of large OCD flap lesion using multiple resorbable polydioxanone pins (>2 cm) has been described by Nixon et al. (2004). The OCD flap should be still in situ within the original defect, with some residual continuity along at least 50% of the perimeter with normal surrounding cartilage. The surface of the OCD flap must be smooth and congruent with minimal fibrillation and the flap should not be entirely mineralised. The method leads to rapid resolution of joint effusion and lameness and the reconstitution of the subchondral surface. In a study in 27 horses, long-term follow-up with a mean duration of 15.6 months revealed an overall success rate for intended athletic use of 95%. Radiographic improvement started within weeks of surgery and many lesions resolved radiographically within 3 to 6 months of surgery with better reformation of the subchondral bone contour than following cartilage flap removal (Sparks et al., 2011b).

Subchondral cystic lesions (type 1) of the medial femoral condyle in three horses have been treated successfully by polydioxanone pin reattachment of the overlying cartilage and injection of the cystic cavity with bone marrow aspirate concentrate or allogeneic chondrocytes (Sparks et al., 2011a).

#### *Osteochondral autografting and allografting*

Larger defects that involve subchondral bone require osteochondral transplantation. Osteochondral autografts and allografts have the advantage of immediate reconstruction of the articular surface by transfer of mature intact hyaline cartilage and the underlying subchondral bone (Hangody and Fules, 2003). These resurfacing techniques have been employed successfully in humans since the mid-1990s (Hangody et al., 1997). The success is mainly dependent on graft chondrocyte viability and mechanical stability of the host–graft interface (Pallante et al., 2012a).

Osteochondral autologous transfer (OAT) and mosaicplasty focus on transferring one large cylindrical osteochondral graft (dowel/shell graft) or multiple smaller cylindrical grafts from minimally weight-bearing regions in regions with greater weight-bearing (Lynch et al., 2015). Mosaicplasty, which uses a number of small-diameter cylindrical heterotopic osteochondral grafts placed in mosaic-like fashion, has gained popularity, also in horses, as it enables the relatively easy reconstruction of a relatively congruous joint surface (Hangody and Fules, 2003; Bodo et al., 2014). However, donor site availability and joint congruency remain major concerns, limiting the use of osteochondral autograft transfer. Allografts, harvested from available cadaveric donors, overcome these limitations and have the advantage of orthotopic transfer of the harvested tissue (Bugbee et al., 2015; De Caro et al., 2015).

In humans, a fresh osteochondral allograft transplantation (mega-OAT) can be used in large osteochondral defects or if another cartilage repair procedure has failed (Sherman et al., 2014). Fresh osteochondral allografts stored at 37 °C or room temperature have the highest chondrocyte viability (Pallante et al., 2012b). Host response to the allogenous tissue, mainly to the bone, can affect cartilage viability. As chondrocytes are tightly embedded in the extracellular matrix, immunogenicity seems to be low and seems to have minimal impact on graft survival (Hurtig et al., 2001; Bugbee et al., 2015; Smith et al., 2015). Currently, shell osteochondral allografts have the subchondral bone plate as the only bony component that allows secure graft fixation while minimising potential immunogenicity (Smith et al., 2015).

Long-term follow-up (up to 10 years) in humans shows good clinical and functional outcomes with osteochondral grafting (Hangody and Fules, 2003; De Caro et al., 2015; Lynch et al., 2015).

Mosaic arthroplasty has been used successfully clinically and experimentally in horses with subchondral cystic lesions in the stifle, fetlock and tarsal joints (Bodo et al., 2000, 2004, 2014; Janicek et al., 2010). Osteochondral grafts are harvested from a less weight-bearing region and transferred to a prepared cylindrical hole into the defect, using arthrotomy and/or arthroscopy. Good matching of donor and recipient sites with respect to cartilage thickness, biochemical and physical properties is necessary. In a stifle matching study, grafts from the trochlear groove and axial aspect of the lateral trochlear ridge were the closest match for the medial femoral condyle, whereas grafts from the trochlear groove and axial aspect of the medial trochlear ridge matched closest with the lateral femoral condyle (Changoor et al., 2006). Mosaic arthroplasty of other locations, like the third carpal bone, yielded mixed results. In those locations, tissue matching was not possible, hence the transplants needed to adapt to their new biomechanical environment (Hurtig et al., 2001). Overall, the use of the technique in horses is limited due to the technical difficulties in harvesting and insertion, especially when using arthroscopy.

*Particulated cartilage grafts*

Particulated autografts and off-the-shelf allografts are constructs on the basis of minced cartilage placed in a scaffold or fibrin glue (Albrecht et al., 1983; Makris et al., 2015). Mechanical mincing allows chondrocytes to escape from their surrounding extracellular matrix, migrate to surrounding tissues and form a new hyaline-like cartilage tissue matrix (Albrecht et al., 1983; Riboh et al., 2015). The procedure is easy to perform and can be done in a minimally invasive single-stage procedure.

The cartilage autograft implantation system (CAIS) involves mechanical fragmentation of arthroscopically harvested cartilage from a low-load-bearing region. Cartilage fragments are subsequently placed on a synthetic, absorbable scaffold using fibrin glue and fixed with synthetic, absorbable staples into the defect (without microfracture). Good long-term (12 months) healing has been demonstrated in an equine model (Frisbie et al., 2009). Based on the same principles, minced allografts of juvenile articular cartilage have been used, as the gene expression pattern in juvenile cartilage is more favourable for regeneration and there is increased proliferative potential in juvenile compared to adult chondrocytes (Riboh et al., 2015; Yanke et al., 2015).

BioCartilage is a dehydrated micronised allograft cartilage scaffold that is mixed into a paste with platelet-rich plasma (PRP) implanted over a microfractured defect. Studies in humans reported more hyaline-like tissue compared to microfracture alone (Abrams et al., 2013). In a recent equine study comparing BioCartilage to microfracture alone, better repair-host integration, base integration and collagen type II 13 months postoperatively were observed in chondral defects treated with BioCartilage compared to microfracture alone (Cole et al., 2015).

*Cell-based transplantation procedures*

*Chondrocyte-based strategies*

Autologous chondrocyte implantation (ACI) was first reported in humans in 1994 for the treatment of focal chondral injury in the tibiofemoral and patellofemoral joints (Brittberg et al., 1994). Clinical outcome in long-term human studies has been reported as good to excellent for treatment of symptomatic full-thickness chondral or osteochondral lesions in the femoral condyle, with 80% to 90% return to pain-free function (Brittberg, 2008; Peterson et al., 2010). In the original first-generation ACI-technique, a small biopsy of healthy articular cartilage was arthroscopically harvested from a low-weight-bearing location of the diseased joint. A few hundred thousands of chondrocytes were isolated, expanded to generate more than 10 million cells and subsequently re-implanted into the defect, which was then covered by a periosteal flap sutured to the margins (Brittberg et al., 1994). Disadvantages of the technique are that the delivery of the cells requires an arthrotomy and harvesting and placement of a periosteal flap is technically demanding, with postoperative graft delamination, tissue hypertrophy, graft failure and adhesions as possible complications. In subsequent modifications of the technique, collagen-membranes have been used instead of a periosteal flap (second generation ACI), as well as cell-delivery in a variety of three-dimensional scaffolds with different methods of fixation (third generation ACI or MACI for matrix-induced autologous chondrocyte implantation) (Kon et al., 2013; Gobbi et al., 2015).

A number of studies have been carried out in horses in the past two decades. Initial trials of arthroscopically resurfacing 12 mm articular cartilage defects with allogeneic chondrocytes secured in a fibrin matrix showed an improved filling of the defect with significantly more aggrecan and 62% more type II collagen at 8 months, compared to empty defects (Hendrickson et al., 1994). As fibrin is difficult to attach in the graft site, methods using tissue-engineering approaches with collagen scaffolds were developed, but did not provide satisfactory improvement in repair of 15 mm cartilage defects (Sams and Nixon, 1995; Sams et al., 1995). Addition of anabolic growth factors (especially IGF-1) showed enhanced cartilage repair through the stimulation of matrix synthesis by the transplanted chondrocytes, resulting in substantially improved histologic appearance after 8 months, over controls. However, biochemical and biomechanical characteristics of the repair tissue were not significantly improved (Fortier et al., 2002). A retrospective study on treatment of subchondral cystic lesions of the medial femoral condyle in 49 mature horses with this combination showed a successful long-term clinical outcome in 74% of cases (Ortved et al., 2012). Articular chondrocytes genetically modified to express high levels of IGF-1 or bone morphogenetic protein (BMP)-7 have also been shown to improve the quality of repair tissue (Hidaka et al., 2003; Ortved et al., 2014). ACI has been proven effective for both short- and long-term repair of large partial-thickness and full-thickness cartilage defects in horses (Litzke et al., 2004; Nixon et al., 2011). However, due to the complexity of the whole procedure, the different approaches using ACI seem to be unfeasible or at least very impractical for the majority of equine surgical practices. Concern about uneven distribution of the cells in the defect and cell-leakage with injection of expanded chondrocyte suspension underneath a membrane led to further improvement of the ACI technique (Berta et al., 2015; Makris et al., 2015).

Matrix-induced autologous chondrocyte implantation (MACI) is a scaffold-plus-cell-based cartilage repair technique and has been performed in a few controlled horse studies as well. Results at 12 and 18 months of a modified MACI technique with autologous chondrocytes cultured on a collagen membrane implanted with PDS/PGA staples into 15 mm defects of the medial trochlear ridge of the femur showed effective repair compared with the collagen membrane alone and to empty defects (Frisbie et al., 2008). A recent 6-month study in horses using the original MACI implant, secured with fibrin into 15 mm femoral trochlear ridge defects, showed MACI yielded improved arthroscopic second-look, gross healing, and composite histologic scores, compared to spontaneously healing empty defects (Nixon et al., 2015). Biomechanically, the neo-tissue had similar compressive and frictional properties to native tissue, with inferior shear properties (Griffin et al., 2015).

With respect to cell source, the use of autologous versus allogeneic cells for transplantation is still under debate, related to risks of OA development due to the biopsy or immunologic reactions respectively (Hunziker et al., 2015). Donor chondrocyte viability is important for the success of chondrocyte transplantation and when using allogeneic cells, development of a banked source of equine

chondrocytes is an integral part of the approach. Foals of 3 to 12 months old and young adult horses are better donors than neonatal foals (Nixon et al., 1992). Results of an in vitro study using rat cells support the potential use of allogeneic chondrocytes in OA and cartilage defects. In this study, the lack of evident immunogenicity, despite exposure to a pro-inflammatory environment, coupled with the immunomodulatory ability suggested that these cells have the potential to evade the host immune system and suppress inflammation, thus potentially facilitating the resolution of OA induced inflammation and cartilage regeneration (Lohan et al., 2015).

*Stem cell-based strategies*

As chondrocyte based-strategies have been hampered by difficulties in achieving a high cell density and in maintaining their differentiation state, chondroprogenitor cells and mesenchymal stromal/stem cells (MSCs) have been shown to be a potential alternative cell source (Fortier et al., 1998; McCarthy et al., 2012; Frisbie et al., 2015; Madeira et al., 2015). Several commercial stem cell-based therapeutic options to treat joint disease are now available for equine practitioners. Currently bone marrow (BM-MSCs) or bone marrow concentrate (BMC) and adipose tissue (AT-MSCs) are the main sources of MSCs (Gutierrez-Nibeyro, 2011). Peripheral blood, umbilical cord blood, induced pluripotent stem cells (iPSCs) and synovial fluid and membrane are other potential chondrogenic cell sources (Koch et al., 2007; Berg et al., 2009; De Schauwer et al., 2011; Klontzas et al., 2015; Makris et al., 2015; Prado et al., 2015; Spaas et al., 2015; Williams et al., 2015).

Bone marrow aspirate is harvested from the sternum or ilium and adipose tissue is generally harvested from the tail head region (Taylor and Clegg, 2011; Adams et al., 2012) with aspirates from the ilium producing significantly better results than other sources in terms of tissue production, when matrix production of the cells is assessed following chondrogenic differentiation (Kisiday et al., 2013). Long-term cryopreservation of MSCs is possible, without loss of their proliferative and differentiation potential (Marquez-Curtis et al., 2015). MSCs can be grafted into a lesion using a scaffold, injected as bone marrow concentrate, or injected intra-articularly either alone or in combination with other products such as hyaluronan or platelet-rich plasma (Schnabel et al., 2013). A technique using a fibrin scaffold to retain MSCs within cartilage defects has been developed. Disadvantages with this technique are the need for preparation of autologous MSCs and the requirement of specialised equipment for arthroscopic implantation of MSC scaffolds (Frisbie and Stewart, 2011). The debate on the best cell type to be used is still ongoing. It remains unclear whether equine allogeneic MSCs incite an immune response, especially if administered repeatedly (Schnabel et al., 2013). Pigott et al. (2013) showed only a moderate inflammatory response with intra-articular injection, while Pezzanite et al. (2015) showed strong in vivo antibody responses to allogeneic BM-MSCs and cross-reactivity with MHC-types other than that of the donor. This can limit the clinical effectiveness of repeated injections, apart from generating untoward inflammatory responses.

To date, the published literature on the outcome of cartilage repair using MSCs in the horse is limited. Efficacy for the treatment of equine osteoarthritis (OA) and cartilage injuries seems more favourable for bone marrow-derived cells than adipose-derived cells (Wilke et al., 2007; Frisbie et al., 2009; Fortier et al., 2010; Ferris et al., 2014). BM-MSCs fibrin grafts in a 15 mm full-thickness cartilage defect model in the lateral femoral trochlear ridge improved the early healing response, but did not significantly enhance the long-term histologic appearance or biochemical composition (Wilke et al., 2007). A significant improvement was demonstrated in macroscopic and histologic scoring at 3 and 8 months for the defects treated by microfracture and grafted with BMC compared to microfracture alone in a similar model (Fortier et al., 2010). Delayed direct intra-articular injection of BM-MSCs in hyaluronan (HA) for the treatment of microfractured full-thickness medial femoral condylar cartilage defects led to increased firmness of repair tissue at 12 months, as well as increased aggrecan content (McIlwraith et al., 2011a). In a clinical follow-up of 33 horses with stifle injuries receiving an intra-articular injection of BM-MSCs following arthroscopic surgery, Ferris et al. (2014) showed clinical improvement with better ability to return to work compared to surgery alone and improved results for horses with meniscal injuries; joint flare post injection was 9%. An equine cartilage explant study demonstrated that chondrogenic priming of allogeneic peripheral blood MSCs (PB-MSCs) resulted in significantly enhanced and homogenous MSC adhesion and incorporation into lesions compared to unprimed cells. Interestingly, mechanical loading negatively affected the results and a lower stem cell density ($0.5 \times 10^6$) yielded better results than a higher one ($1.0 \times 10^6$) (Spaas et al., 2015).

Use of high numbers of stem cells alone for regenerative purposes can provoke unwanted side-effects. Formation of significant amounts of bone in the repair tissue may result when using an autologous platelet enhanced fibrin (APEF) scaffold combined with BM-MSCs (Goodrich et al., 2013). In the study by Fortier et al. (2010) this effect was not observed with bone marrow concentrate, which contained a much lower number of MSCs.

Articular cartilage progenitor cells, present in developing and adult normal and osteoarthritic articular cartilage, synovium and adipose tissue, demonstrate a multipotent differentiation capacity similar to that of BM-MSCs (Jayasuriya and Chen, 2015). In contrast with BM-MSCs, these cells retain their chondrogenic potential following extensive in vitro expansion and have limited risk for repair tissue mineralisation in vivo (McCarthy et al., 2012; Frisbie et al., 2015).

Co-culture of chondrocytes or even peripheral blood derived mononuclear cells (PBMCs) and stem cells is known to enhance MSC migration, chondrogenic differentiation and cartilage matrix production and seems to have greater promise for cartilage repair than MSCs alone (Wu et al., 2011; De Windt et al., 2015; Hopper et al., 2015a,b). PBMCs can acquire an MSC-like phenotype and have also shown to attract native chondrocytes from the diseased tissue to aid in cartilage repair (Hopper et al., 2015c). The therapeutic application of stem cells in joint disease holds great promise, but there remain many unanswered questions, warranting experimental and clinical studies.

*Tissue-engineering strategies: The use of seeded/unseeded scaffolds of various types and 3D-bioprinting*

Although the previously described techniques, like ACI and MACI, improve clinical outcome, they are not the definitive answer for (osteo-)chondral defect repair. None of the techniques result in the restoration of hyaline cartilage that is functionally equal to the original tissue and hence long-term prognosis is still guarded. There is thus an ongoing quest for novel techniques that are more efficacious. Tissue-engineering approaches are potentially promising, as they allow avoidance of donor site morbidity, host immune responses, and disease transmission (Seo et al., 2015b; Smith and Grande, 2015). Cells, 3D-scaffolds and growth factors are the three main components in tissue-engineering. Scaffolds, in different physical forms like fibres, meshes and gels, can be derived from biological or synthetic materials or combinations and can be functionalised with growth factors (such as BMP-2, TGF-β1, PRP) and/or mimicking peptides (Barnewitz et al., 2006; Seo et al., 2015a). Scaffolds can be used seeded with chondrocytes or stem/progenitor cells or unseeded, as scaffolds are not only a carrier system, but may also have an intrinsic regenerative ability (Kon et al., 2015; Seo et al. 2015b). They temporarily fill the defect and are ideally degraded and replaced simultaneously in a synchronised process by neo-tissue (Seo et al. 2015b; Smith and Grande, 2015).

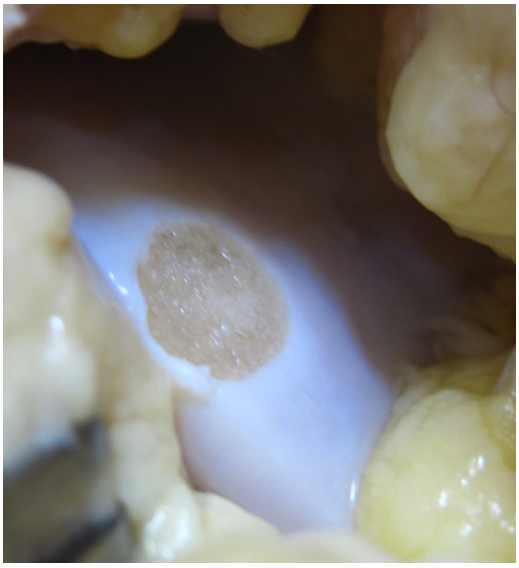

**Fig. 4.** Perioperative view of an implanted decellularised scaffold in an osteochondral defect (11 mm × 10 mm) of the medial femoral trochlear ridge in an adult horse (experimental study).

Scaffolds may be based on the natural extracellular matrix (ECM) of a variety of tissues, which are decellularised (Fig. 4) and otherwise processed before being implanted. These scaffolds are thought to be well prepared for tissue engineering, as their natural ECM provides a unique, tissue-specific 3D environment containing both structural and functional molecules which, in interaction with the resident cells, determine tissue homeostasis (Benders et al., 2013). Chondrogenic potency of multipotent stromal cells, more than chondrocytes, on equine cartilage-derived matrix (CDM) scaffolds has been demonstrated in vitro (Benders et al., 2014). However, there are several issues that still need to be addressed when using cartilage tissue-engineering. These questions consist of (1) the recapitulation of the zonal structure of natural cartilage by these scaffolds; (2) which tissue might form the ideal basis for this type of scaffolds (collagen type II vs. type I); and (3) how to generate a neo-tissue with biphasic character (cartilage and bone), while minimising the risk of overgrowth of the latter (Klein et al., 2009).

Artificial scaffolds have the advantage that the composition is more reproducible, that immune-related problems are less likely to occur, and that regulatory approval will be easier and quicker to obtain. Recently, the use of artificial scaffolds seeded with extracellular vesicles rather than cells has been proposed, which is an exciting option as the biological effect may mimic that of cells whilst avoiding the regulatory problems associated with the use of living (allogeneic) cells (Malda et al., 2016). All scaffold-based approaches need extensive *in vitro* testing and, depending on their concept, functionalisation may require adding extra components, such as enzymes, cytokines and growth factors, that can already naturally be present in ECM.

Hydrogels prepared from natural and synthetic polymers are usually the principal component of scaffolds, as they are biocompatible and can exhibit comparable swelling and lubricating behaviour as articular cartilage, whilst offering an excellent environment for chondrocytes or stem cells (Spiller et al., 2011; Martins et al., 2014; Seo et al., 2015b; Vilela et al., 2015). Several types of hydrogels have been used for cartilage tissue engineering or are being developed (Schuurman et al., 2013; Levett et al., 2014; Moreira Teixeira et al., 2014). A great disadvantage of hydrogels is their low intrinsic stiffness, which is far below the stiffness of natural cartilage. There are various ways to address this problem, such as photo crosslinking, the use of chemicals, or the fabrication of a hybrid scaffold in which the hydrogel is combined with much stiffer materials such as poly(ε)caprolactone (PCL) or other fibrous materials (Schuurman et al., 2011; Wang et al., 2014; Seo et al., 2015a; Visser et al., 2015). Another item is fine-tuning of the in vivo degradation rate of the material, allowing for the gradual and simultaneous replacement of the scaffold by newly formed repair tissue.

Biofabrication is a rapidly developing field in regenerative medicine that holds promise for tissue engineering of osteochondral defects (Di Bella et al., 2015; Groll et al., 2016). In particular three-dimensional bioprinting, one of the main approaches in biofabrication, allows for the fabrication of biphasic, i.e. osseous and cartilaginous, constructs through the manipulation of scaffold composition and cell type (Levato et al., 2014; Seo et al., 2015b). It also allows for the mimicking of the zonal structure of cartilage and potentially larger anatomical structures (Visser et al., 2013). The research is slowly passing from the *in vitro* phase and the testing in small laboratory species to application in large animal models, of which the horse has been designed as one of the best models for cartilage repair (McIlwraith et al., 2011a). Significant breakthroughs are to be expected in the coming years.

## Conclusions

This review focuses on the current and future (surgical) cartilage regeneration strategies with their advantages, limitations and outcomes. Simple debridement and marrow stimulation techniques remain the mainstay in equine joint surgery. These techniques are simple and cost-effective, but do not result in the regeneration of hyaline cartilage. More advanced techniques aiming at improved anatomic and functional restoration have been used in horses, often in an experimental setting. These include cartilage reattachment, osteochondral grafting, chondrocyte-based and stem cell-based strategies. Outcomes in the short or medium term have been favourable in some cases, but the complexity and sometimes the invasive nature of those procedures, together with the high costs, are serious drawbacks for routine clinical use. Long-term functional outcome, which is essential in the equine industry, is as yet uncertain and many of the described techniques will still result in a more or less inferior fibrocartilaginous repair tissue and subchondral bone changes at the defect site, as is the case with the currently clinically used approaches. For this reason, there is still a large and thus far unmet need to develop novel tissue engineering techniques and to address the fundamental requirements of successful cartilage healing and joint function, in horses as well as in humans. To eventually reach this goal, well-designed, prospective, randomised controlled (pre-)clinical trials are crucial to compare novel technologies to current 'gold standard' clinical approaches. In this way, treatment algorithms can be formulated for horses, as in humans, to help guide the decision to treat the cartilage defect in the best possible way. The harsh biomechanical environment of the equine joint is a complicating factor that should be taken into account. Additionally the design of effective rehabilitation protocols is paramount, as these will certainly affect outcome of any cartilage repair strategy. The ultimate goal of achieving functional and durable healing of articular cartilage may still seem a long way off, but progress is certainly being made and we are getting closer in making this concept into a clinical reality.

## Conflict of interest statement

None 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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