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Repair of large osteochondritis dissecans lesions using a novel multilayered tissue engineered construct in an equine athlete.

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Title: Repair of large osteochondritis dissecans lesions using a multi-layered collagen-based osteochondral graft substitute in an equine athlete.

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Place where this case was operated:
University College Dublin Veterinary Hospital, Large Animal Surgery Service, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland.

Ethical approval and consent form:
As this was a true clinical case and not an experimental case, no ethical approval was required. Authorisation from the Irish Department of Agriculture, Food and the Marine was granted to use the multi-layer collagen-based osteochondral graft substitute on this specific case. The horse was permanently stamped “Out of the Food Chain”. The owner signed a consent form discharging the University College Dublin Veterinary Hospital and the Royal College of Surgeons in Ireland from any legal responsibilities.

Oral/poster presentation of this work:
This work has not been presented at any national or international meeting.

Conflict of Interest:
Tanya J Levingstone and Fergal J O’Brien hold IP with a commercial product of related composition to the collagen-based scaffolds used in this study.

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Osteochondral lesions, resulting from osteochondritis dissecans, are problematic to treat and present a significant challenge for clinicians. The aims of this study were to investigate the use of a scaffold-assisted microfracture approach, employing a novel multi-layered collagen-based osteochondral graft substitute in the treatment of severe osteochondritis dissecans of both lateral femoral trochlear ridges in an equine athlete, and to assess the potential of this novel scaffold to enhance repair of the osteochondral unit. A 15-month-old female filly presented with large osteochondritis dissecans lesions involving both femoral lateral trochlear ridges. After routine arthroscopic debridement and microfracture of the subchondral bone, multi-layered osteochondral defect repair scaffolds were implanted into the fragmentation beds in both left and right femoropatellar joints via mini-arthrotomy. Exploratory arthroscopy 5 months post-implantation revealed smooth cartilaginous repair tissue, contiguous with adjacent cartilage, covering the defect. At 22-month follow up, the filly had no signs of lameness and was exercising at her intended level. Radiographically, although still slightly flattened, the femoral trochlear ridges were smooth, with no evidence of osteoarthritis. Ultrasonographically the defects were filled with bone and covered with an overlying cartilaginous layer, with the trochlear ridge contour almost entirely restored. This report demonstrates the effective clinical use of this novel multi-layered osteochondral defect repair scaffold in the treatment of osteochondritis dissecans of an equine athlete. The successful repair achieved here using this novel scaffold in an equine patient with large bilateral lesions shows the potential for clinical translation in the treatment of human patients presenting with osteochondral defects.
1. Introduction

Repair of articular cartilage defects, occurring as a result of osteoarthritis, fracture, fragmentation, or surgical debridement of osteochondritis dissecans (OCD) lesions or subchondral bone cysts, represents a significant challenge for orthopaedic surgeons. Articular cartilage regeneration following injury is impaired by inherently poor vascular supply and the limited cellular content of hyaline cartilage (Nixon et al., 2011). Greater challenges present with larger defects and with subchondral bone involvement and currently no ‘gold standard’ technique exists for repair of such defects.

Osteochondritis dissecans (OCD), a disruption of endochondral ossification, is a common orthopaedic developmental disease in many species, including humans and horses, and results in separation and instability of the overlying articular cartilage. OCD affects 10 to 30% of the equine population, depending on breed and joint (Desjardin et al., 2014) and as such is a major concern in the horse industry (Jeffcott, 1996). In humans, OCD is less common, with prevalence estimated at 15 to 21 per 100,000 (Hughston et al., 1984), and defects most commonly occurring on the femoral condyles (Pascual-Garrido et al., 2009). Arthroscopic debridement of OCD lesions is the primary surgical strategy to facilitate healing in horses (Van Weeren and Jeffcott, 2013; McIlwraith, 2013; McIlwraith et al., 2015). Prognosis is inversely proportionate to the lesion’s size; with lesions in excess of 40 mm long carrying a 54% chance of achieving expected athletic performance with arthroscopic debridement alone (Foland et al., 1992). In order to enhance repair of OCD lesions in veterinary patients, similar approaches to those used in human surgery have been applied. Autologous osteochondral grafting (mosaicplasty) has been used successfully in the repair of deep osteochondral defects resulting from debridement of subchondral bone cysts (Bodó et al., 2004; Bodó et al., 2014). Autologous chondrocyte implantation has shown success
in the healing of experimentally created full-thickness cartilage lesions (1.5cm diameter) in the lateral trochlear ridge of horses with improved histologic scores at 8 weeks post-implantation (Nixon et al., 2011). However, debridement, with or without microfracture, remains the most commonly used treatment for OCD defects in horses for practical reasons (McIlwraith, 2013; McIlwraith et al., 2015; Frisbie et al., 1999).

Equine models are currently recommended for preclinical assessment of new biomaterial-based strategies for cartilage repair as they provide the closest approximation to humans in terms of cartilage thickness (Malda et al., 2012). A number of experimental studies have thus been carried out using equine models to assess the regenerative potential of new biomaterials (Frisbie et al., 2008; Frisbie et al., 2009; Kon et al., 2010b; Nixon et al., 2011; Seo et al., 2013). Few studies have been carried out in horses to investigate the use of these biomaterial-based strategies for the repair of osteochondral defects resulting from injury or disease, such as OCD. In one case, Tsuzuki et al. (2013) showed fibrocartilaginous repair of an OCD lesion using a gelatin β-tricalcium phosphate sponge, impregnated with platelet-rich plasma, bone morphogenetic protein-2, mesenchymal stem cells. While the biomaterial-based solutions investigated to date have shown some potential, generally fibrocartilaginous tissue results and an urgent need remains for repair strategies that will facilitate long lasting repair of the osteochondral unit.

To meet this need a biocompatible, biomimetic and highly porous (> 95%) multi-layered collagen-based scaffold has been developed within the Tissue Engineering Research Group in the Royal College of Surgeons in Ireland (Gleeson et al., 2010; Levingstone et al., 2014). This novel construct mimics the inherent graduated structure of healthy osteochondral tissue: a bone layer composed of type I collagen and
hydroxyapatite (HA), with demonstrated osteogenic properties (David et al., 2015; Gleeson et al., 2010; Murphy et al., 2014), an intermediate layer composed of type I collagen and hyaluronic acid (HyA) and a cartilaginous region composed of type I collagen, type II collagen and HyA. The properties of the material are designed to provide the biological and biomechanical cues required to encourage infiltration of host cells from the bone marrow and to promote differentiation of these cells towards the required lineage in each region. The regenerative potential of this scaffold has been demonstrated in vitro and in vivo in both rabbits and goats (Levingstone et al., 2014; Levingstone et al., Under review a; Levingstone et al., Under review b).

This study describes the use of this novel, multi-layered osteochondral scaffold in the treatment of bilateral OCD of the lateral trochlear ridges in the femoropatellar joints of an Irish Sport Horse. The extent of the osteochondral lesions in this horse was such that the prognosis for future athleticism was poor. Loss of articular cartilage and destruction of subchondral bone architecture, resulting from the required curettage of the lesions, was expected to disrupt the contour of the trochlear ridges. The degree of disruption would likely have led to instability in the joint, resulting in osteoarthritis (McIlwraith, 2013). The aims of this study were to assess the potential of a scaffold-assisted microfracture approach, combining microfracture with implantation of a novel multi-layered collagen-based scaffold to enhance the repair of the osteochondral unit of both lateral femoral trochlear ridges in a horse affected by OCD.

2. Materials and methods

2.1 Case description

A 15-month-old female Irish Sport Horse presented to the University College Dublin Veterinary Hospital (UCDVH) with severe effusion of both femoropatellar (FP) joints.
Radiographic examination of the FP joints demonstrated marked intra-articular soft tissue swelling and the contour of the middle and proximal aspects of the lateral trochlear ridge (LTR) of the distal femur of both the left and right hind limbs was markedly irregular, with large areas of resorption deep in the subchondral bone. Non-detached osteochondral fragments were visible in these lesions. There was a small, mineralized fragment detached from the parent bone in the cranial aspect of the right FP joint (Figure 1). The proximodistal length and craniocaudal depth of the osteochondral lesions on the left side were 46 mm and 22 mm, respectively, and on the right side were 49 mm and 23 mm, respectively. Ultrasonographic (US) examination confirmed the presence of marked synovial effusion of the FP joints. The surface of the osteochondral unit was undulating, irregular in shape and thickness, and was deeply fissured. Non-detached osteochondral fragments were noted (Figure 2). The mediolateral measurement of the lesion was 20 mm for the left trochlea and 19 mm for the right trochlea. A free ossified fragment was found in the right FP joint. These changes were consistent with a diagnosis of OCD and were classified according to the International Cartilage Repair Society (ICRS) OCD lesion classification scale as grade IV (i.e. lesions with a complete discontinuity, with a dislocated fragment or a loose fragment within the bed; grade IV is the most severe grade) (Brittberg and Winalski, 2003).

2.2 Scaffold fabrication

Multi-layered scaffold sheets, 60 mm x 60 mm x 20 mm, were fabricated using a unique iterative layering fabrication method (Levingstone et al., 2014). The multi-layered scaffold consisted of a bone layer containing type I collagen (Col1) [Southern Lights Biomaterials, Napier, New Zealand] and hydroxyapatite (HA) [Plasma Biotal, UK], an
intermediate layer, consisting of Col1 and hyaluronic acid sodium salt derived from *Streptococcus equi* (HyA) [Contipro, Dolní Dobrouč, Czech Republic] and a cartilage layer, consisting of Col1, type II collagen (Col2) [porcine type II collagen, Symatese, Chaponost, France] and HyA. Scaffolds were freeze-dried as previously described in order to produce a multi-layered scaffold with seamless layer integration (Levingstone *et al.*, 2014; Gleeson *et al.*, 2009). Following freeze-drying the scaffold was cross-linked using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC)/N-hydroxysuccinimide (NHS) (Sigma–Aldrich, Arklow, Ireland) at a concentration of 6 mM EDAC g⁻¹ of collagen, and a 5:2 M ratio of EDAC:NHS for 2 hours at room temperature (Haugh *et al.* 2011). Scaffolds were then dehydrothermally (DHT) cross-linked in a vacuum oven (VacuCell, MMM, Germany) at 105°C and a pressure of 50 mTorr for 24 hours to generate cross-links through a condensation reaction and also to sterilise the scaffolds.

### 2.3 Surgical procedure

The horse was anaesthetized and placed in dorsal recumbency. A routine arthroscopic approach to the left FP joint was made between the middle and lateral patellotibial ligaments (McIlwraith *et al.*, 2015). The instrument portal was made through stab incision lateral to the lateral patellotibial ligament. The cartilage covering the middle and proximal parts of the LTR was undulating, irregular and deeply fissured with non-detached but unstable fragments (Figure 3). The cartilage lesions were debrided and the subchondral bone was curetted until solid, bleeding bone was encountered. Microfracture of the subchondral bone was carried out with a 30° microfracture pick (Sontec Instruments, Colorado, USA) and a mallet, as previously described (Frisbie *et al.*, 1999). Debris and small fragments were then lavaged from the FP joint with the aid
of a third portal created in the suprapatellar pouch with an 11 mm laparoscopic cannula
(Vinardell et al., 2008; McNally et al., 2011). A second surgical team performed an
identical procedure concurrently on the right FP joint. As the defects were extensive
and the prognosis for athleticism was poor, a tissue engineering approach was applied
in this horse. To enable scaffold implantation a small arthrotomy (50 mm) was created
directly over the LTR of each stifle (lateral to the lateral patellotibial ligament). Self-
retaining retractors were placed to expose the fragmentation bed (Figure 4). A sterile
foil template was press-fitted into the fragmentation bed and the sterilized multi-layered
scaffold sheets were cut to match the size and shape of the templates. The scaffolds
were then soaked in sterile saline and pressed gently into the defect. The left scaffold
was secured with a combination of fibrin glue (Tisseel, Baxter, Dublin, Ireland) and
cyanoacrylate glue (Histoacryl, B Braun Medical, Dublin, Ireland) (Figure 4). The
scaffold in the right defect was retained using a press-fit approach. Closure of the
arthrotomy was performed in five layers and the arthroscopy portals were closed in one
layer. After closure a combination of morphine [Morphine Sulphate, Mercury Pharma]
10 mg, bupivacaine [Marcain, AstraZeneca] 200 mg and gentamicin [Gentaject,
Franklin] 500 mg was injected intra-articularly. Sterile adhesive dressings were placed
prior to recovery from anesthesia.

2.4 Post-operative evolution
The Anderson Sling system [Charles D. Anderson, Care for Disabled Animals, Potter
Valley, CA] was used in recovery to support the horse until fully conscious and weight
bearing on all limbs, reducing stress on the scaffolds (Taylor et al., 2005). Perioperative
antibiotics (Procaine penicillin [Depocillin, Interchem] 22,000 IU/kg IM q 12 hours
and gentamicin sulphate [Gentaject, Franklin] 6.6 mg/kg IV q 24 hours) were continued
for five days. Non-steroidal anti-inflammatory (phenylbutazone [Phenylarthrite, Vetoquinol] 2.2 mg/kg IV q 12 hours) was continued for five days on a tapering dose. The horse was discharged from the hospital on day eight post-operatively, with recommendations to remove the skin sutures on day 12-14 post-surgery and to restrict exercise to a large box stall for the first 6 weeks, then to walk the horse in hand for 6 weeks and finally to turn her out to a paddock for the following 3 months.

Follow up US was performed at 8 days, 5 months and 22 months postoperatively. Follow up radiographic examination was performed at 5 and 22 months postoperatively. A radiographic scoring system, devised by Sparks et al., (2011) was used to evaluate radiographs at 5 and 22 months. This system assessed the postoperative radiographic appearance of the subchondral filling (scale: 0 = No evidence of prior OCD to 3 = Worsening/progression of the subchondral lucencies) and subchondral bone contour (scale: 0 = smooth, contiguous to 4 = free floating mineralized bodies).

3. Results

3.1 Post-operative outcome

The horse recovered uneventfully from anesthesia using the Anderson Sling system. Moderate effusion of the FP joints was observed at suture removal 12 days post-operatively. The incisions were intact at this time. Eight days later, both arthrotomy sites began discharging clear serous fluid and dehisced fully over the next 2 days. The horse was treated with trimethoprim-sulphadiazine [Noroprim granules, Norbrook] (30 mg/kg q 12 hours per os) for 2 weeks from the onset of dehiscence. The wounds were cleaned daily with dilute chlorohexidine solution and had fully healed 7 weeks later. No fever or signs of lameness developed during this period. Exercise was restricted to box rest during the entire management of this complication (total of 10 weeks). Once
wound healing was achieved, a walking programme was introduced consisting of five minutes walking in hand daily for 1 week with the duration of daily walks increased by five minutes per week. This was continued for 6 weeks. The horse was then turned out in a small paddock until undergoing a second bilateral FP arthroscopy. Twenty-two months after scaffold implantation the horse was sound and in full training: ridden exercise under saddle at walk, trot, canter and jumping. There was evidence of mild joint effusion and small scars were present at the arthrotomy sites.

### 3.2 Diagnostic imaging

Ultrasonographic examination performed eight days postoperatively showed the subchondral bone to have a concave contour, representing the area of the LTR that had been debrided (Figure 2). These defects were filled with homogeneous echogenic material, which was determined, based on previous experience, to be due to the presence of the scaffolds. The scaffold was well fixated and in line with joint surface on the left side, and in line with the joint surface, but was partially dislodged distolaterally on the right side. On ultrasonographic examination, 5 months postoperatively the defects in the LTRs resulting from the curettage were still evident; however, the defects were noticeably shallower. The defects were smooth and filled with mildly echogenic heterogeneous material. Ultrasonography, 22 months postoperatively, showed mild effusion of the left FP joint with some synovial proliferation. The subchondral defects, created by the surgical debridement, into which the scaffolds had been positioned, were no longer evident. These areas were instead, filled with bone as evidenced by a mildly undulating hyperechogenic line, contiguous with adjacent normal subchondral bone. Repair of the overlying cartilaginous layer was also evident. While this cartilage layer was thinner than the surrounding cartilage tissue,
the echogenicity was largely similar to that of the adjacent normal hyaline cartilage. Some areas of increased echogenicity were observed within the repair tissue indicating that some tissue remodeling may still be ongoing. Radiographic examination performed at 5 months demonstrated modest reduction in intra-articular soft tissue swelling. The subchondral lucencies within the LTRs were reduced in size but still apparent. Lucent areas were less well defined by a rim of sclerosis. The proximal and distal limits of the defects were smooth and round. Evaluation of the radiographs of both left and right sides showed Grade 2 subchondral filling (i.e. <50% resolution of lucencies) and Grade 2 subchondral contour both (i.e. mild/moderate irregularities). The length (proximodistal) and depth (craniocaudal) of the osteochondral lesions at 5 months in the left were 46 mm and 17 mm, and for the right were 40 mm and 18 mm, respectively. Radiographic examination performed 22 months postoperatively showed no evidence of osteoarthritis and no free bodies. The LTRs were smooth, although with a flatter appearance than normal. Repair of the subchondral bone was evident; however, a small area of incomplete healing was seen to persist on the left side. Assessment of the radiographs at 22 months postoperatively (Figure 1) showed Grade 1 subchondral filling (i.e. >50% resolution of subchondral lucencies) and Grade 1 subchondral contour (i.e. flattened). The length (proximodistal) and depth (craniocaudal) of the osteochondral lesions at 22 months in the left were 33 mm and 17 mm, and for the right were 35 mm and 17 mm, respectively.

3.3 Exploratory arthroscopy

The horse was represented to UCDVH 5 months postoperatively due to mild persistent effusion of the femoropatellar joints. The horse did not exhibit lameness at the walk or trot. Small scars (20 mm x 10 mm) were present at site of the previous arthrotomies.
The FP joints were explored arthroscopically under general anaesthesia as described earlier. Repair tissue within the graft implantation sites was visualized arthroscopically (Figure 3) and scored using the International Cartilage Repair Society (ICRS) cartilage repair assessment tool as shown in Table 1 (Brittberg and Peterson, 1998; Peterson et al., 2000). The sites of the scaffold implantation were covered by immature cartilage that was contiguous with the adjacent normal cartilage along the entire perimeter (Figure 3). The cartilage was smooth although the repair tissue maintained a lower profile than the trochlear ridge proximally and distally. An area of proliferative synovitis/granuloma covered the joint capsule at the site of the arthrotomies. This was more marked in the left femoropatellar joint. Macroscopic assessment of repair tissue using the ICRS scoring system resulted in a score of 10/12 for each defect site, placing them in the Grade II (nearly normal) category. Exploration of the entirety of the joint demonstrated an absence of debris or fragments. The arthroscopy portals were closed and sterile adhesive dressings were placed prior to recovery. The filly recovered uneventfully from anesthesia using the Wilderjans rope recovery system (Niimura del Barrio et al., Under review).

### 3.4 Synovial fluid analysis

Synovial fluid analysis 5 months postoperatively showed that synovial fluid from both joints was subjectively less viscous than normal synovial fluid with nucleated cell count from the left and right femoropatellar joints found to be normal (0.3x10⁹ cells/L) and mildly elevated (3.6x10⁹ cells/L), respectively. The percentage neutrophils from the left and right femoropatellar joints were 7% and 12%, respectively. The total protein from the left and right FP joints was 8 g/L and 12 g/L, respectively and synovial fluid serum amyloid A (SAA) levels was less than 5 μg/mL for both joints. Percentage neutrophils,
total protein and synovial fluid SAA levels were normal in both joints (Stack et al., Under review).

4. Discussion

This study demonstrates the successful use of a multi-layered collagen-based scaffold in the treatment of large OCD lesions in both femoral trochlear ridges of an equine athlete. The results show the potential of this scaffold to promote repair of bone and cartilage within the defect sites, leading to restoration of the joint surface. Exploratory second-look arthroscopy, 5 months post scaffold placement, revealed that the defects were covered with immature cartilage, contiguous with the surrounding cartilage, although the profile of repair tissue was slightly below the normal joint surface. At 22 months post-implantation, the horse was undergoing a full athletic regimen. Radiographic and US examinations 22 months postoperatively confirmed filling of the subchondral bone defects with new bone and restoration of the integrity of the joint surface.

*Osteochondritis dissecans*, a manifestation of osteochondrosis (OC), is the most common cause of lameness and the most common indication for surgery in the equine stifle joint (van Weeren, 2012). It occurs due to derangement of the normal endochondral ossification process and results in irregular, thickened cartilage, necrosis of the underlying subchondral bone, and often, fissuring and fragmentation of the affected area of cartilage (Laverty and Girard, 2013; Sparks et al., 2011). The origin is still unclear, however it is commonly accepted that there are a number of contributing factors including dietary imbalance, biomechanical factors, genetic susceptibility, declining metabolic rate in the young adult and physiological factors; such as growth, conformation and hormonal imbalance (Desjardin et al., 2014; Erickson et al., 2013;
Laverty and Girard, 2013; Van Weeren, 2012). Clinical signs develop when the joint surface is breached by the dissecting lesion or when a fragment completely detaches, leading to synovitis, varying degrees of lameness and development of osteoarthritis (Desjardin et al., 2014). The horse presented in this study had no history of trauma and was free from OCD in the other predilection sites. The specific aetiology in this horse is undefined, as is often the case.

Currently, surgical removal of OCD fragments, curettage of necrotic bone and cartilage alone, or in conjunction with microfracture of the subchondral bone, remains the recommended clinical approach for treatment of OCD defects in horses (Foland et al., 1992; Frisbie et al., 1999; McIlwraith, 2013; McIlwraith et al., 2015). Microfracture alone has shown clinical success; however, often repair tissue is composed of fibrocartilage rather than hyaline cartilage and can break down over time (Miller et al., 2004; Steadman et al., 2003). In the case presented here, the OCD lesions were large with extensive lytic lesions in the subchondral bone and thus, the prognosis for athleticism using standard approaches was poor (Foland et al., 1992; Sparks et al., 2011). Debridement, with or without microfracture, would likely have resulted in joint instability and osteoarthritis as a sequel (McIlwraith and Nixon, 1996). The large size of the lesion precluded use of osteochondral grafting procedures, such as mosaicoplasty (Bodó et al, 2014), as the osteochondral tissue required to fill the defects could not have been harvested without substantial donor site morbidity. Autologous cartilage implantation (ACI) (Frisbie et al, 2008; Nixon et al, 2011) was deemed inappropriate due to the extent of diseased subchondral bone requiring debridement, and the requirement for an additional surgery to harvest cartilage for expansion. Thus in this study, a scaffold-assisted microfracture approach was utilised. The technique involved curettage of diseased cartilage and bone, combined with microfracture of the
subchondral bone, followed by implantation of a novel multi-layer osteochondral defect repair scaffold. Similar enhanced microfracture procedures have shown promise in the repair of chondral and osteochondral lesions in humans (Anders et al., 2013; Dhillander et al., 2012; Gille et al., 2010; Kon et al., 2014; Kusano et al., 2012). Thus, we hypothesised that the use of this approach would lead to enhanced regenerative responses within the defect sites. To the authors’ knowledge, this is the first time this scaffold-assisted microfracture approach has been used to treat OCD lesions of the distal femoral trochlear ridges in horses. Additionally, this is the first use of this multi-layered osteochondral defect repair scaffold in the treatment of disease in a veterinary patient.

The multi-layered scaffold employed in this case has been designed for osteochondral defect repair, and specifically for use as an off-the-shelf, cell-free biomaterial for press-fit implantation into an osteochondral defect site. The use of microfracture here serves to enable cells, most notably stem cells, from the bone marrow to populate the defect. The scaffold was seen to fill with blood on implantation demonstrating that the hydrophilic nature and porous scaffold architecture enables infiltration of blood and cells from the host bone marrow through the scaffold’s seamlessly integrated multi-layered structure (Figure 4). Clot formation within the defect also results in improved retention of the scaffold within the defect site (Frisbie et al., 1999). The incorporated extracellular matrix macromolecules, and scaffold biostructural and biomechanical properties have been previously shown to direct the differentiation of mesenchymal stem cells (MSC) to produce bone, calcified cartilage and cartilage within the requisite regions of the defect site, with restoration of the anatomical tidemark and to result in joint regeneration (Levingstone et al., Under review a; Levingstone et al., Under review b). The scaffold demonstrated numerous advantages, including sufficient mechanical
strength, durability and flexibility to withstand surgical handling. It was easily cut to shape and sculpted to conform well to the debridement bed, so much so that in the right limb a press-fit implantation could be achieved.

Successful clinical results were demonstrated using both press-fit and gluing fixation methods. Fixation poses a significant challenge for biomaterials in articular cartilage repair. Suturing has proved successful but is limited by technical challenges and the long surgery times required. Other potential fixation approaches include polydioxanone (PDS) staples (Frisbie et al., 2009) and PDS pins (Nixon et al., 2004; Sparks et al., 2011). The scaffold employed here, once hydrated, becomes compliant with reduced mechanical strength, although still sufficient for surgical handling, this poses challenges for retention of staples, pins or sutures. Press-fit implantation offers advantages, such as reduced surgical time and avoidance of the need to include additional materials, and has been used successfully in small focal lesions (Levingstone et al., Under Review a; Levingstone et al., Under Review b); however larger lesions present greater challenges. In this case, ultrasound confirmed persistence of the scaffold within both defect sites eight days postoperatively. Incomplete fixation was observed on the right side with some detachment evident. However, despite this, the bulk of the scaffold remained in situ. Tissue glues, such as fibrin glue and cyanoacrylate glue, have demonstrated some success in scaffold fixation (Ayan et al 2007; Patel et al., 2010). The gluing approach employed here, using a combination of fibrin and cyanoacrylate glue proved more successful, with scaffold fixation observed ultrasonographically eight days postoperatively. This approach is thus recommended for fixation of this multi-layered scaffold in large defects in the future. Recovery from anaesthesia was an important consideration to ensure protection of the scaffold in the initial postoperative phase. Use of the Anderson sling recovery system resulted in optimal recovery with
minimal flexion of the stifle joints. Furthermore, the use of the sling resulted in less
tension and trauma to the laterally placed arthrotomy suture lines.

On US examination at 5 months postoperatively, the defects were filled with slightly
echogenic material. During exploratory arthroscopic surgery performed the following
the day this was confirmed to be immature cartilage, with ICRS macroscopic scores
placing it in the near normal category. Synovial fluid analysed 5 months postoperatively
was normal in the left femoropatellar joint and showed signs of mild inflammation in
the right. The reason for mild synovitis in the right femoropatellar joint is unclear but
may be related to the arthrotomy site dehiscence and chronic synovial proliferation.
The mild increase in nucleated cell count may also reflect the normal inflammatory
process involved in healing of osteochondral defects. The lack of marked inflammatory
response at any time during the study demonstrates the biocompatibility and safety of
the scaffold. Modest radiographic healing was observed with the debridement bed
slowly filling with new bone over time. Due to the size of the lesions in this case, it was
not expected that the trochlear ridges would appear radiographically normal at any time
point (McIlwraith and Nixon, 1996), thus the aim here was to improve the trochlear
function and offer the horse an improved athletic prognosis. Healing was evidenced by
the loss of the sclerotic rim demarcating the abnormal from normal subchondral bone;
the new bone formation and the uniformity of the subchondral bone. In addition, there
was no evidence of osteoarthritis.

Ultrasound performed 22 months postoperatively revealed replacement of the deeper
layers of the scaffolds with bone, and cartilage superficially. Whilst the original profile
of the trochlear ridges had not been fully achieved, the articular surface of the LTRs
was resurfaced and functional. These results demonstrate the benefits of the
osteoinductive properties of the base layer of the scaffold provided by the hydroxyapatite component and the chondrogenic properties of the top layer provided by the type II collagen and hyaluronic acid. Notably, the cartilage and bone healing observed here was achieved in the absence of additional chondrogenic or osteogenic growth factors or cells. A more complete evaluation of the quality of the cartilage repair and expected long term clinical function could have been made if the tissues were biopsied during the second surgery (Krishnan et al., 2008). However, this was not possible due to ethical considerations. While the cartilage covering the healed subchondral bone was functional at 22 months post-surgery, the long term durability of the repaired cartilage remains unknown. At 22 months postoperatively, the horse was performing athletically at its intended level, with no evidence of lameness. The reason for the persistence of some effusion and synovial hypertrophy in the left femoropatellar joint is not clear, but is likely to be related to the synovial proliferation and granuloma formation at the arthrotomy site. The use of an arthrotomy was necessary in order to provide sufficient accessibility to place the scaffolds, however, this resulted in some side issues as highlighted by the dehiscence of both arthrotomy sites; a recognised complication of arthrotomy in the horse (Trotter et al., 1983; Pascoe et al., 1980). Dehiscence was partial in this case with the multiple layer closure used enabling the joint capsule to remain intact and preventing development of synovial communication or herniation; however, permanent scarring of the area resulted. While arthroscopic implantation of this scaffold is not currently possible, it is likely that use of arthroscopic methods would have reduced the likelihood of these complications occurring. Arthroscopy has been shown to minimise trauma to surrounding tissues, decrease muscular deficits, decrease chances of wound infection and improve cosmetic outcomes (Jameson et al., 2011; McIlwraith et al., 2015; Small, 1993).
While equine models are recommended for preclinical assessment of new tissue engineering strategies for cartilage repair, few studies have been carried out in horses using tissue engineering strategies for the repair of cartilage defects resulting from injury or disease. This study provided the opportunity to treat a veterinary patient, while also providing important information for clinical translation. The promising results in this study demonstrate that the use of this novel multi-layered osteochondral defect repair scaffold in this scaffold-assisted microfracture procedure is an effective approach to promote enhanced filling of osteochondral defects, following curettage, in the treatment of OCD.

5 Conclusions

This case study investigated the use of a scaffold-assisted microfracture approach, employing a novel multi-layered collagen-based scaffold, designed for osteochondral defect repair in humans, in the treatment of large *osteocondritis dissecans* (OCD) of both lateral femoral trochlear ridges in a horse. Clinical follow-up at 5 and 22 months revealed almost complete filling of the subchondral bone defect, and restoration of a smooth articular surface to the trochlear ridges. These promising results demonstrate the effective clinical use of this novel multi-layered osteochondral defect repair scaffold in the promotion of enhanced filling of osteochondral defects following debridement in OCD. The successful functional repair of the complete osteochondral unit achieved here in an equine athlete with large bilateral lesions shows potential for clinical translation to human patients presenting with large osteochondral defects.

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Figure 1. Lateromedial radiographs of left and right femoropatellar joints before (A, B) and 22 months after arthroscopic debridement and scaffold placement (C, D). The contour of the lateral trochlear ridges of the distal femur of both the left and right hind
limbs were markedly irregular, with non-detached osteochondral fragments overlying large areas of cystic resorption deep in the subchondral bone (Lesion borders highlighted with white *). There was a small, free mineralized fragment in the cranial aspect of the right femoropatellar joint (white arrowhead). At 22 months postoperatively there is Grade 1 subchondral filling of the subchondral lucencies (i.e. >50% resolution) and the subchondral contour is smooth although slightly flattened (Grade 1) (black arrows).

Figure 2. Ultrasonographic images of the left and right lateral trochlear ridges in both longitudinal (column 1 and 3) and transverse planes (column 2 and 4). Preoperatively (top row), the surface of the osteochondral unit was undulating, irregular in shape and thickness (white +), and was deeply fissured. At 8 days post implantation (middle row), the scaffold (white *) is in situ in the left limb and largely in situ in the right limb with
a small area of detachment distolaterally. At 22 months (the bottom row) the subchondral defects, created by the surgical debridement, into which the scaffolds had been positioned, were no longer evident. These areas were instead, filled with bone as evidenced by a mildly undulating hyperechogenic line (white arrows), contiguous with adjacent normal subchondral bone. Proximal is to the right in longitudinal views. Lateral is to the right in transverse views.

Figure 3. Arthroscopic images of the left (A, C) and right (B, D) femoropatellar joints showing the lateral femoral trochlea (LTR) before debridement (top row) and during second-look arthroscopy five months later (bottom row). In A and B the cartilage covering the middle and proximal parts of the LTR was undulating, irregular and deeply
fissured (black arrowheads) with evidence of non-detached osteochondral fragments (OCD). In C and D repair tissue within the graft implantation sites scored 10/12 (nearly normal) according to the International Cartilage Repair Society (ICRS) cartilage repair assessment tool. The patella (P) is visible above the LTR. Black * mark the junction between normal and repair cartilage.

Figure 4. The scaffolds were soaked in sterile saline and pressed gently into the defect via arthrotomy (A). The left scaffold was secured with a combination of fibrin glue (Tisseel, Baxter, Dublin, Ireland) and cyanoacrylate glue (Histoacryl, B Braun Medical, Dublin, Ireland) (B, C, D). Note the scaffolds becoming blood soaked on implantation (C and D).
Table 1: International Cartilage Repair Society (ICRS) cartilage repair assessment tool (Brittberg and Peterson, 1998; Peterson et al., 2000). This tool is used by surgeons to evaluate the macroscopic appearance of cartilage repair tissue following interventions such as ACI, subchondral drilling and microfracture and rates cartilage repair tissue as Grade IV (severely abnormal), Grade III (abnormal), Grade II (nearly normal) or Grade I (normal) based, on the degree of defect repair, degree of integration and macroscopic appearance.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Degree of Defect Repair</strong></td>
<td></td>
</tr>
<tr>
<td>Level with surrounding cartilage</td>
<td>4</td>
</tr>
<tr>
<td>75% repair of defect depth</td>
<td>3</td>
</tr>
<tr>
<td>50% repair of defect depth</td>
<td>2</td>
</tr>
<tr>
<td>25% repair of defect depth</td>
<td>1</td>
</tr>
<tr>
<td>0% repair of defect depth</td>
<td>0</td>
</tr>
<tr>
<td><strong>Integration to Border Zone</strong></td>
<td></td>
</tr>
<tr>
<td>Complete integration with surrounding cartilage</td>
<td>4</td>
</tr>
<tr>
<td>Demarcating border &lt; 1mm</td>
<td>3</td>
</tr>
<tr>
<td>3/4 of graft integrated, 1/4 with a notable border &gt;1mm width</td>
<td>2</td>
</tr>
<tr>
<td>1/2 of graft integrated with surrounding cartilage,1/2 with a notable border &gt; 1mm</td>
<td>1</td>
</tr>
<tr>
<td>From no contact to 1/4 of graft integrated with surrounding cartilage</td>
<td>0</td>
</tr>
<tr>
<td><strong>Macroscopic Appearance</strong></td>
<td></td>
</tr>
<tr>
<td>Intact smooth surface</td>
<td>4</td>
</tr>
<tr>
<td>Fibrillated surface</td>
<td>3</td>
</tr>
<tr>
<td>Small, scattered fissures or cracks</td>
<td>2</td>
</tr>
<tr>
<td>Several, small or few but large fissures</td>
<td>1</td>
</tr>
<tr>
<td>Total degeneration of grafted area</td>
<td>0</td>
</tr>
<tr>
<td>Overall Score</td>
<td>Grade I</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>normal</td>
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<tr>
<td>Score</td>
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