Nanofracturing: a new technique for bone marrow stimulation in equine cartilage repair

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Summary: Microfracture is the current standard in treatment of focal full-thickness cartilage lesions in horses, but clinical outcome may vary. Nanofracture is a novel technique that uses a commercially developed device to yield smaller diameter perforations with deeper penetration into the subchondral bone. Experimentally, in rabbits and sheep, nanofracture has been shown to result in superior repair compared to microfracture. The objective was to study the feasibility and preliminary outcome of nanofracture using a commercial device for treatment of cartilage defects in horses. Nanofracture was tested ex vivo in n = 2 cadaveric equine stifle joints and in vivo in n = 8 horses with experimental partial thickness cartilage defects in the medial femoral trochlear ridge. These were treated with an experimental biomaterial or nanofracture, and repair tissue was studied macroscopically (ICRS-I score) and microscopically (histological ICRS-II score and micro-CT) after 7 months. Both in cadaveric equine stifle joints and in vivo, the nanofracture device could readily be applied and allowed easy penetration of the subchondral bone. Repair tissue after 7 months was graded 'near-normal' macroscopically, while histologically, the abundant repair tissue proved mainly fibrocartilaginous in nature. Micro-CT revealed near-full restoration of mid-lesion cartilage layer thickness but altered subchondral bone microarchitecture. The in vivo study did not include a control group treated with conventional microfracture for comparison. To our knowledge, this is the first report on bone marrow stimulation using nanofracture as a potential method to enhance chondral defect repair in horses. In the in vivo study, no clinical adverse effects were observed, and promising good defect filling with fibrocartilaginous tissue was seen 7 months after treatment.

Keywords: Horse, bone marrow stimulation, nanofracture, cartilage repair

Introduction

Microfracture is the most frequently used technique to recruit bone marrow cells and growth factors for enhancement of endogenous articular cartilage repair (*McIlwraith* et al. 2010). While considered a current clinical standard of treatment for full-thickness cartilage defects in horses and humans, long-term clinical outcome in humans varies (*Goyal* et al. 2013). Variability in the amount and quality of repair tissue, along with the observation of significant subchondral bone changes after microfracture, has also been reported (*Frisbie* et al. 2006, *Minas* et al. 2009, *Mithoefer* et al. 2016).

The variable and limited penetration of the subchondral bone and the relatively large diameter of the microfracture awl are thought to contribute to surrounding subchondral bone compaction, intralesional osteophyte formation and suboptimal repair (*Cokelaere* et al. 2016, *Minas* et al. 2009, *Mithoefer* et al. 2016). To overcome the mechanical limitations and associated variable outcomes of microfracture, the nanofrac-

ture technique was recently developed (*Behrens* et al. 2011, *Benthien* et al. 2013). It provides for smaller diameter and deeper subchondral bone needle perforations with improved access to the bone marrow (*Behrens* et al. 2011, *Benthien* et al. 2013, *Cokelaere* et al. 2016).

Nanofracturing of osteochondral lesions in adult sheep has been shown to provide better repair tissue than microfracture, with improved restoration of articular cartilage architecture and greater type II collagen content (*Zedde* et al. 2016). This technical note reports on the feasibility and preliminary outcome of nanofracture for the treatment of partial thickness cartilage defects in the equine femoropatellar joint.

Materials and methods

Nanofracture device and technique

The nanofracture device (NanoFx®)ª consists of a reusable hand-held instrument and a single-use disposable 1 mm di-

ameter nitinol needle (PleuriStik™-titanium and nickel alloy)[a] to perform the subchondral needling. The handle[a] of the device comes either with a 15° angled tip or with an A-Curve tip. The A-curve tip was used in the current work.

The nanofracturing technique was previously described (*Behrens* et al. 2013) and is shown schematically in Figure 1 (a). It is performed in a systematic, spiral fashion starting from the periphery of the lesion. Hammer strikes on the handle advance the needle into the subchondral bone to a stop-controlled depth of 9 mm. Perforations are placed approximately 3 mm apart for an even distribution and to maintain adequate bone bridges between channels.

Ex vivo study

With owner consent, two fresh cadaver stifle joints obtained from n = 2 adult horses were used to test the feasibility of nanofracture for penetration of equine subchondral bone. In each joint, 6 partial- and full-thickness 6–8 mm cartilage defects in both medial and lateral femoral trochlear ridges and condyles were created via arthrotomy, and nanofracture of the defect beds was undertaken. Number of attempted perforations, success or failure to penetrate the subchondral bone, as well as any special observations were recorded.

In vivo prospective experimental study

The study protocol was approved by the institutional ethical and animal welfare committee of the National University of Costa Rica (approval number FCSA-EMV-CBA-001-2013). Eight healthy Criollo breed horses (mean age 7.1 years; range 5–9; mean weight 319 kg; range 275–375) were used. All were sound at walk and at trot on a straight line (subjective lameness evaluation) and free from radiographic evidence of stifle joint pathology. They were housed in individual box stalls and fed a daily maintenance ration of 0.5 kg concentrate with unlimited hay and water.

By blind draw from an envelope prior to surgery, one limb of each animal was randomly assigned to nanofracture, as a control treatment for a contralaterally applied experimental biomaterial (*Both* et al., manuscript in preparation). Surgery was performed under general anaesthesia, with peri- and post-operative analgesia provided by phenylbutazone (2.2 mg/kg bwt PO BID). One partial-thickness chondral defect (1 mm depth) was created via mini-arthrotomy in the mid-medial trochlear ridge of each femoropatellar joint by the same surgeon (SC) using a 7-mm diameter biopsy punch. For nanofracture, each cartilage lesion was treated with three perforations distributed across the 7-mm defect. Defects were flushed with saline to remove any remaining debris. Incisions were routinely closed, and horses were returned to their stalls immediately after surgery.

Post-operative care and monitoring

Post-operatively, horses received antibiotics for 5 days (pro-caine penicillin[b] 15,000 IU/kg bwt IM SID and gentamicin[c]

6.6 mg/kg bwt IV SID) and non-steroidal anti-inflammatory drugs (phenylbutazone[d] 2.2 mg/kg bwt PO BID) for 10 days. The animals were monitored daily at walk for lameness, rectal temperature, heart rate and respiratory rate; haematology and serum biochemistry were checked 1, 3 and 6 months post-operatively. During 3 months, horses were boxrested with daily handwalking (15–20 min twice daily) from 3 weeks post-operatively onwards. From 3 months post-operatively, horses were turned out at pasture until the end of the experiment at 7 months. At this timepoint, horses were sound at walk and trot on a straight line (subjective gait evaluation) and humanely euthanised.

Repair tissue assessment: Macroscopic and microscopic scoring

Osteochondral plugs were obtained and fixed in formalin for histopathologic and micro-CT analysis. The ICRS-I Visual Assessment Scale (*Van den Borne* et al. 2007) (Table 1) was used for macroscopic scoring of cartilage repair. Images were coded and scored by three blinded observers. Histologic assessment was based on Haematoxylin-Eosin (HE), Safranin-O and Alcian Blue (AB) stained slides obtained from one border of the defect as well as mid-defect. Scores were assigned using the ICRS-II scoring system (*Mainil-Varlet* et al. 2010) (Table 2) by three blinded observers; polarised light microscopy was not performed.

Micro-CT

The samples were dried and manually positioned in the μCT system[e]. Specific settings included voltage of 90 kV, current of 200 μA, field of view (FOV) of 20 mm and scan time of 4.5 minutes. These settings were equal for all samples and created 512 μCT images per sample. Fiji imageJ software[f] was used to edit μCT images for further analyses, with manual selection of regions of interest (ROI) at the level of the defect for subsequent cartilage thickness determination.

Statistical analysis

Interobserver reliability of macroscopic and microscopic repair tissue scoring was assessed with statistical software (SPSS 25.0)[g] using Cronbach's alpha statistic, with significance set at p < 0.05.

Results

Ex vivo study

The needle penetrated the subchondral bone with relative ease in all joint locations; of 20 perforations attempted, 19 were immediately successful, with only the first giving slippage of the device during hammer strikes. Blood appeared from each of the channels upon removal of the device. There was no qualitative difference in ease of subchondral bone penetration in partial vs. full thickness lesions. The needle could be re-used up to 10 times without breakage.

In vivo study

Technical performance

Nanofracture proved easy to perform in an experimental setting (Fig 1b, c) and was consistently associated with visible bleeding from all 24 perforations. A small depression left by the device in the adjacent cartilage and minimal cartilage debris surrounding perforations were observed in 2 out of 8 defects.

Post-operative monitoring

Over the 7 months follow-up, 7 out of 8 horses recovered without any complications. Two months postoperatively, one horse died due to an unrelated traumatic head injury. In the remaining horses, no lameness of a degree higher than 1/5 on the AAEP scale was observed at any time, and routine clinical, haematology and biochemistry parameters remained within normal physiologic limits.
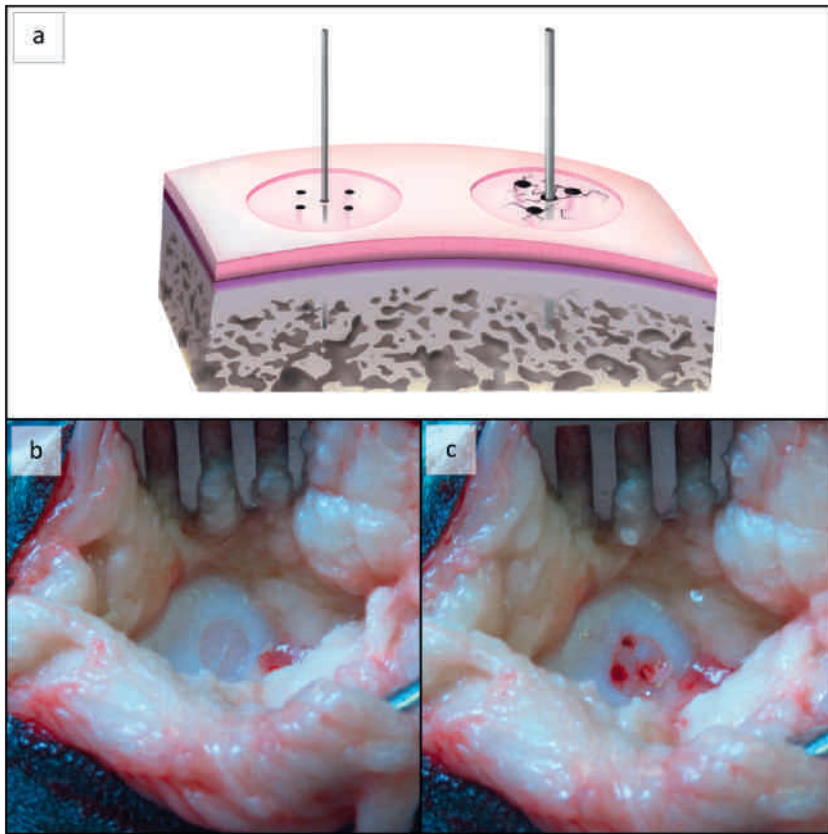
Fig 1   Concept and technical performance of nanofracturing.
a) Schematic diagram depicting nanofracture (left) compared to microfracture (right).
b) The 7-mm diameter, 1-mm deep experimental chondral defect in vivo after debridement.
c) The same defect immediately after nanofracture
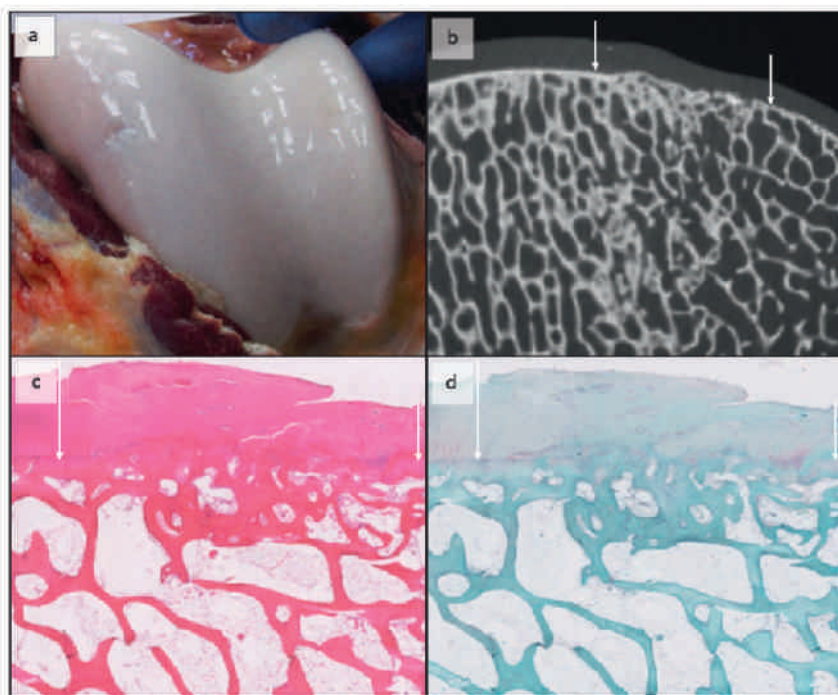
Fig 2   Outcome of nanofracture treatment of 7-mm diameter, 1-mm deep experimental chondral defects after 7 months. All images are from the same joint and are representative of outcome in all n = 7 horses.
a) Macroscopic appearance of the medial trochlear ridge of the femur showing the defect.
b) Micro-CT image (sagittal view) of the defect (between white arrows) and underlying subchondral bone.
c) Haematoxyllin-Eosin stained photomicrograph (magnification 10 ×) of defect repair.
d) Safranin-O stained photomicrograph of the same defect (magnification 10 ×).

Macroscopic assessment of repair tissue

Defects were still readily recognizable (Fig 2a) but showed good degrees of filling and integration with adjacent tissue (ICRS scores ranging from 2–4, table 1). Macroscopic ICRS-1 scoring after 7 months showed good inter-observer reliability (Cronbach's alpha 0.86; p < 0.001). Median overall visual assessment score for nanofracture treated defects was 9 (interquartile range, IQR 7–10), denoting 'near-normal' cartilage (Table 1). Degree of defect repair received the highest sub-scores (median 3, IQR 2.25–4), followed by integration to border zone (median 3, IQR 2–3).

Histopathologic analysis

Histopathologic assessment (interobserver reliability) using the ICRS-II scoring system showed an acceptable Cronbach's alpha of 0.78 (p < 0.001), where > 0.8 is rated as 'good'. After 7 months, defects demonstrated ample but mainly fibro-cartilaginous repair tissue, with adequate integration to adjacent tissue, but limited matrix staining and surface restoration (Fig 2c, d). Total ICRS-II score was 48 % ± 10 % (mean ± SD), where 100 % denotes normal cartilage; 'basal integration' received the highest sub-scores (67 % ± 9 %).

Micro-CT analysis

Mean cartilage layer thickness mid-defect was 97.6 ± 7.7 % (mean ± SD) of adjacent cartilage thickness. Micro-CT showed mild subchondral bone disruption surrounding nanofracture channels in all treated defects 7 months post-operatively (Fig 2b).

Discussion

In the experiments reported here, we found that nanofracture using a commercially available device is a feasible and ready-to-use technique for bone marrow stimulated cartilage repair in horses. Some mechanical limitations of microfracture may be overcome with nanofracture, potentially enabling surgeons to create more standardised smaller and deeper holes into the

Table 1   International Cartilage Repair Society (ICRS)-I scoring system for visual assessment of cartilage repair tissue (*Van den Borne* et al. 2007)

Cartilage repair assessment ICRS	Points
Degree of defect repair	
In level with surrounding cartilage	4
75 % repair of defect depth	3
50 % repair of defect depth	2
25 % repair of defect depth	1
0 % repair of defect depth	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border < 1 mm	3
¾ of graft integrated, ¼ with notable border > 1 mm width	2
½ of graft integrated with surrounding cartilage, ½ with a notable border > 1 mm	1
From no contact to ¼ of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures of cracks	2
Several, small or few but large fissures	1
Total degeneration of grafted area	0
Overall repair assessment	
Grade I: normal	12
Grade II: nearly normal	8–11
Grade III: abnormal	4–7
Grade IV: severely abnormal	1–3

Table 2   International Cartilage Repair Society (ICRS)-II scoring system for histological assessment of cartilage repair tissue (*Mainil-Varlet* et al. 2010)

Histological Parameter	Score
1. Tissue morphology (viewed under polarized light)	0 %: Full-thickness collagen fibers 100 %: Normal cartilage birefringence
2. Matrix staining (metachromasia)	0 %: No standing 100 %: Full metachromasia
3. Cell morphology	0 %: No round/oval cells 100 %: Mostly round/oval cells
4. Chondrocyte clustering (4 or more grouped cells)	0 %: Present 100 %: Absent
5. Surface architecture	0 %: Delamination, or mayor irregularity 100 %: Smooth surface
6. Basal integration	0 %: No integration 100 %: Complete integration
7. Formation of tidemark	0 %: No calcification front 100 %: Tidemark
8. Subchondral bone abnormalities	0 %: Abnormal 100 %: Normal marrow
9. Inflammation	0 %: Present 100 %: Absent
10. Abnormal calcification/ ossification	0 %: Present 100 %: Absent
11. Vascularization (within the repaired tissue)	0 %: Present 100 %: Absent
12. Surface/Superficial assessment	0 %: Total loss or complete disruption 100 %: Resembles intact articular cartilage
13. Mid/deep zone assessment	0 %: Fibrous tissue 100 %: Normal hyaline cartilage
14. Overall assessment	0 %: Bad (fibrous tissue) 100 %: Good (hyaline cartilage)

subchondral bone. Compared to the microfracture technique, the nanofracturing technique differs as it causes less subchondral bone disturbance and increased access to the subchondral bone marrow; which makes it possible to decrease the distance between holes (*Behrens* et al. 2013, *Zedde* et al. 2016).

Marrow-stimulation procedures have not only shown improved defect filling in full-thickness cartilage defects, but also in partial-thickness defects compared to untreated defects, due to access to the subchondral bone marrow (*Shamis* et al. 1989).

In humans and horses, repair tissue quality and outcome of microfracture treatment is known to deteriorate over time (*Goyal* et al. 2013, *McIlwraith* et al. 2010, *Mithoefer* et al. 2016). Subchondral bone alterations after microfracture in horses and in sheep have been reported (*Frisbie* et al. 2006, *McIlwraith* et al. 2010, *Zedde* et al. 2016), and cystic lesions or intralesional osteophytes may be seen in up to one-third of human patients and also in horses and sheep treated with microfracture (*Kreuz* et al. 2006, *McIlwraith* et al. 2010, *Zedde* et al. 2016). In light of this, the use of clinical standard microfracture awls is being debated, and it appears that every effort should be made to minimize bone injury from marrow-stimulation procedures (*Cokelaere* et al. 2016, *Zedde* et al. 2016).

Our study represents the first evaluation of nanofracture for partial thickness chondral defect treatment in horses. The technique could be applied with ease. Surgery was via mini-arthrotomy for chondral defect creation, but nanofracturing would have been possible arthroscopically. Clinical outcome 7 months post-operatively was good, with no lameness seen in any horse. Defects demonstrated excellent filling and a 'near normal cartilage' macroscopic score, while histologic assessment revealed intermediate quality of repair tissue. Previous work showed benefit of removal of the calcified cartilage layer in horses to enhance basal integration of repair tissue after microfracture (*Frisbie* et al. 2006, *McIlwraith* et al. 2010). However, histopathology showed better basal integration after nanofracture of chondral defects in the medial femoral trochlear ridge in the current study, subjectively compared with caution to that historically reported for microfracture in the weightbearing aspect of the medial femoral condyle when the calcified cartilage layer was retained (*Frisbie* et al. 1999, *McIlwraith* et al. 2010); in fact, despite the retention of deeper cartilage layers, 'basal integration' was the highest scoring criterion in this study.

Micro-CT demonstrated subtly altered subchondral bone microarchitecture underlying the defects 7 months post-nanofracture. It is difficult to compare the extent of bone disturbance to that previously reported after microfracture in equine stifle joints (*Frisbie* et al. 1999): In the current study, defects were partial thickness and outcome was assessed 7 months post-operatively, not 4 or 12 months, while in the study by *Frisbie* et al. (1999), micro-CT was not performed. Micro-CT findings highlight that even with the small-diameter nanofracture needle, subchondral bone changes are detectable 7 months post-operatively and may be underestimated with histopathology alone. The absence of any associated clinical signs, while encouraging, does not rule out possible clinical relevance if follow-up had been prolonged to one to two years or even longer (*Goyal* et al. 2013, *Kreuz* et al. 2006). Results of using nanofracture in this study can be summarised as ample defect filling with better basal integration than surface restoration, and minor disturbance of subchondral bone architecture. Although this outcome is largely similar to what was seen after microfracture in horses (*Frisbie* et al. 1999, *McIlwraith* et al. 2010), the reduced subchondral bone disturbance from smaller diameter penetrations with nanofracture is an inherent advantage of the technique. Several limitations to the work reported here must be noted: First and foremost, our work represents a preliminary investigation of nanofracture as a procedure for bone marrow stimulated cartilage repair in horses. As the contralateral medial femoral trochlear ridges were treated with an experimental biomaterial (the subject of a human pre-clinical study), we were unable to directly compare nanofracture to conventional microfracture. Second, we did not include a control group without any treatment in this study; as this was not the goal of our study. Previous research has also been shown that in general partial-thickness cartilage defects do not heal spontaneously and progression to further cartilage and subsequent joint injury is possible (*Cokelaere* et al. 2016, *Guermazi* et al. 2017, *Hunziker* et al. 1999), *Shamis* et al. 1989). Given known topographic variation in osteochondral tissue properties, no extrapolation of results to other joints, other regions within the stifle joint, or to other defect depths is warranted. Furthermore, detailed longitudinal monitoring (e.g., motion analysis or imaging) was not available, and outcome was assessed at a single time point.

In conclusion, this is the first report, to our knowledge, on the technical performance and intermediate-long term outcome of nanofracture for experimentally created chondral defects on the medial trochlea of the femur in horses. Whether nanofracture results in superior cartilage repair with less subchondral bone disturbance compared to conventional microfracture should be addressed in a randomised experimental trial prior to its clinical application in equine patients.

Manufacturers' addresses

[a] NanoFx®, Nanofracture, Arthrosurface, MA, USA
[b] Brocacef Groep NV, Maarssen, the Netherlands
[c] Kepro BV, Deventer, the Netherlands
[d] Lisan, San José, Costa Rica
[e] μ-CT 80, ScancoMedicalAG, Switzerland
[f] https://imagej.net/Fiji/Downloads
[g] IBM SPSS statistics 25.0, IBM corp., Armonk, NY, USA

Authorship

manuscript preparation. All authors gave their final approval of the manuscript.

Source of Funding

Competing interests

The authors have declared no competing interests.

Ethical Animal Research

The protocol and study described was pre-approved by the institutional ethical and animal welfare committee of the National University of Costa Rica (approval number FCSA-EMV-CBA-001–2013).

Owner informed consent

Obtained in writing at admission of the horses that were euthanised and used for the ex vivo study.

Data accessibility statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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