

## MicroCT Comparison of two different marrow stimulation techniques in the Horse

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**INTRODUCTION:** Articular cartilage defects in diarthrodial joints are common and contribute to the development of osteoarthritis (OA), a major public health issue. Articular cartilage is an avascular, hypocellular tissue with a very low reparative capacity. Reconstructive surgical treatments that aim to stimulate cartilage repair in response to cartilage/chondral defects include the widely performed marrow stimulation techniques (MST), including the microfracture surgery (MFX) technique. During MF surgery, an awl or needle is used to perforate the subchondral bone plate, which allows for the recruitment of autologous bone marrow-derived mesenchymal stem cells (MSCs) into cartilage defects. MSCs differentiate within the chondral defect and lay down an extracellular fibrocartilaginous matrix that contributes to the remodeling of the defect. This fibrocartilage repair tissue is suboptimal, and several reports have demonstrated that MF surgery promotes significant bone changes that compromise joint integrity. Previous studies have demonstrated a higher failure rate of ACI after MST due to delamination, degeneration of the graft tissue overlying an intralesional osteophyte or subchondral cyst formation, which can occur with abrasion arthroplasty, drilling, or microfracture. Specifically, MFX has been associated with structural alterations of the subchondral bone, including bone cysts and intralesional osteophytes.<sup>2</sup> There is growing evidence that small diameter (1 mm) holes, deeper than 9 mm, may preserve the underlying bone in comparison to more traditionally larger (2 mm) and shallower (2-4 mm) holes<sup>3</sup>. This study aimed to evaluate the impact of two different methods of MST in critically sized chondral defects in an equine model. This study increased the cohort size of previous work.

**METHODS:** Two 15mm diameter chondral defects were created on the lateral trochlear facet in eight skeletally mature thoroughbred horses; one proximal and one distal, with a 10mm distance in between. Both defects were treated with MFX using a standard 45° Steadman awl in one defect and a needle marrow access device (SmartShot®) in the other. Horses were euthanized 12 months postoperatively. The stifles were harvested for macroscopic evaluation, Raman spectroscopy, and specimens were analyzed using microCT and histological analysis. Bone segmentation of microCT data was performed using AI machine learning segmentation, and cortical and trabecular separation were performed using the Buie method. Adjustments for age, weight, sex, and defect location were performed during the statistical analysis of microCT results. Defects were defined for analysis purposes using a cylinder of 16mm diameter and 10mm depth.

**RESULTS:** MicroCT analysis revealed the presence of intra-lesional osteophytes in all lesions, consistent with previously reported histological findings. Qualitative evaluation/intralesional finding counts revealed a significant reduction in intralesional bridging ( $p = 0.016$ ), the total number of intralesional osteophytes ( $p = 0.007$ ), and the total number of intralesional findings ( $p = 0.010$ ) in SmartShot-treated lesions compared to traditional microfracture awls. Quantitative analysis of Micro-CT showed significant differences between SmartShot and awl-produced microfracture defects in connective density ( $p = 0.0001$ ). No statistical differences were observed between the two groups for the structure model index, trabecular thickness, bone volume/trabecular volume, trabecular separation, and trabecular number.

**DISCUSSION:** The present study directly compared bony changes that occurred post-MST using two different methods of marrow stimulation: awl-based microfracture and needle puncture (SmartShot®), finding that there are significant measurable reductions in bony changes, in particular intra-lesional osteophytes, in SmartShot treated samples. Previous studies have highlighted the variability in MST, from the technique itself to patient outcomes. There is considerable intra- and inter-surgeon variability with MST, which likely contributes to the variable cartilage fill grade (18 to 95 %). While the focus of the present study was not on improving the repeatability of MST, the results did support the use of SmartShot® technology, which could offer greater control and uniformity of hole depth across surgeons. Our findings from the equine model indicated that this needle-puncture device best preserved the underlying subchondral bone relative to other marrow access approaches. This may relate to the degree of bony compaction occurring with marrow access, as the Steadman awl approach, which consolidated the bone the most, induced the most significant bone damage with marrow stimulation. The finer needle puncture device is designed to better preserve subchondral bone compared to legacy methods like microfracture, K-wire drilling, and powered picks.

**CLINICAL RELEVANCE:** This study was built on previous literature suggesting that small diameter and deeper MST holes may reduce subchondral bone abnormalities and ultimately improve cartilage repair. While a few studies have compared tool sizing for MST, none have jointly investigated tool sizing and the method of hole creation. Our results suggest that a repeatable needle-puncture device (SmartShot® or a similar device) is best to preserve the subchondral bone and minimize aberrant remodelling after MST. Awl MFX appears to cause more damage to the underlying subchondral bone, which SmartShot® MST mitigates.

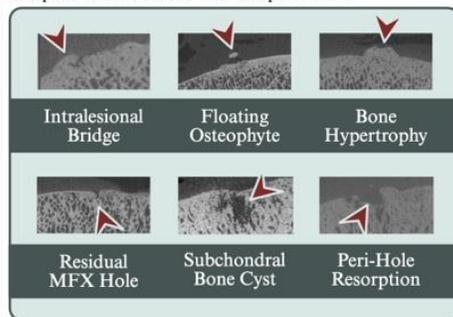
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### GRAPHICS:

**Figure 1 (below):** examples of 6 lesion types identified on qualitative evaluation: intralesional bridging, floating osteophytes, and bone hypertrophy were included in "total osteophytes" while residual microfracture holes, subchondral bone cysts, and peri-hole resorption were included in "total resorptive lesions"



**Figure 2 (right):** 2A: comparison of quantitative bone analysis values for which no statistical significance was found 2B: comparison of connective density values, the quantitative bone analysis value for which statistical significance was found. Increased connective density correlates to increased bone stiffness and an increase in intralesional osteophytes. 2C: comparison of qualitative bone analysis values for which no statistical significance was found 2D: comparison of qualitative values for which statistical significance was found: defects treated with traditional microfracture awl had an increased prevalence of intralesional bridging, total osteophytes, and total intralesional findings when compared to SmartShot treated defects

