process following a full thickness cartilage defect in an attempt to identify at what specific point the healing processes diverge between the two mice strains.

Methods: Two groups of MRL/MpJ and C57BL/6 male mice (5 weeks of age; n = 6 per time point) were used in the study. A custom made depth controlled needle (26 gauge) was used to introduce a full thickness cartilage defect into the femoral groove of the left knee. At 4 and 6 weeks post-surgery, the respective groups were euthanized and the legs were dissected out and fixed in 10% NBF. Using a 9.4 T magnet and a quadrature coil, samples underwent ex vivo magnetic resonance imaging using a RARE sequence (Repetition time = 2000 ms; Echo time = 7.6 ms; FOV =1.92; Matrix = 256). After imaging, samples were decalcified using 10% EDTA, embedded in paraffin, and sectioned at 7 um thickness. Sections were then stained with Safranin-O and immunohistochemistry (IHC) was performed using the anti-mouse Ly-6A/E (also known as Stem Cell Antigen- 1; Sca-1).

Results: MRI scans at 4 weeks showed higher signal intensity at the cartilage compared to those scanned at 6 weeks (Fig1). There were no observable differences between strains at each time point. Safranin-O staining of the C57 sections at the 4 weeks, showed lower proteoglycan content than the MRL at the same time point. Defects were observable in C57 mice at all time points, but were not observed in MRL mice (Fig2). However, tissues within the MRL defect were not identical with the surrounding tissues, in specific regards to proteoglycan content, matrix structure, and chondrocyte orientation. Also of interest, it was observed that Sca-1 positive cells were enriched within the C57 defect, but were not found within the MRL defect (Fig2).

Conclusion: In this study, we sought to characterize the early stages of endogenous cartilage repair that occurs in MRL/MpJ mice using both a non-invasive imaging technique (MRI) and tradition histological methods. Interestingly, MRI scans revealed that at 4 weeks post injury, there were no observable differences between C57 and MRL mice, though the Safranin-O stains did show increased cellularity in MRL samples at the defect compared to C57. Furthermore, the scarcity of Sca-1+ cells in MRL samples was surprising as the Safranin-o stains had revealed the superior healing and increased cellularity of the defect compared to the C57's. A possible explanation could be that the recruitment of Sca-1 cells in the MRL's occurs much earlier than the time points used in this study. This would explain the lack of Sca-1 cells as well as the increased cellularity and structural organization at the defects of the MRL's compared to the C57's. Future efforts will be focused on earlier time points than what was used in the present study.

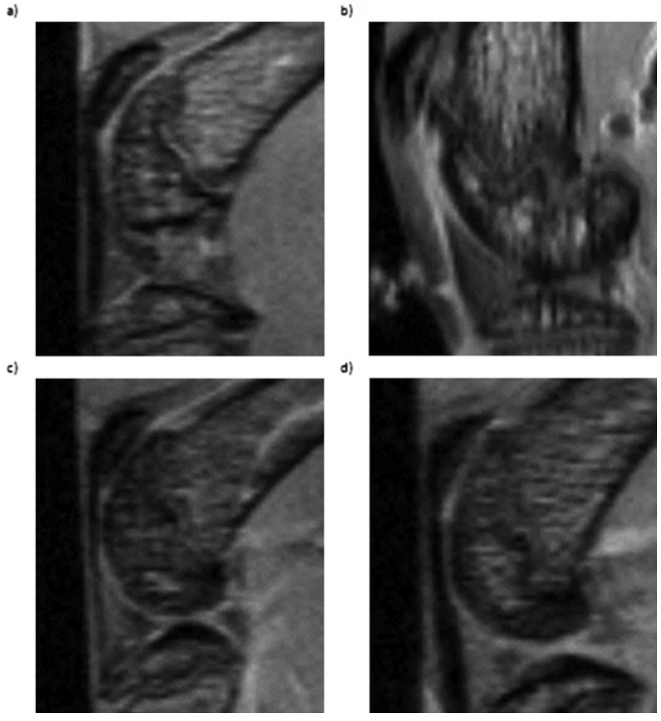

Figure 1. Imaging of a) C57 and b) MRL legs 4 weeks post injury as well as the c) C57 and d) MRL legs 6 weeks post injury.

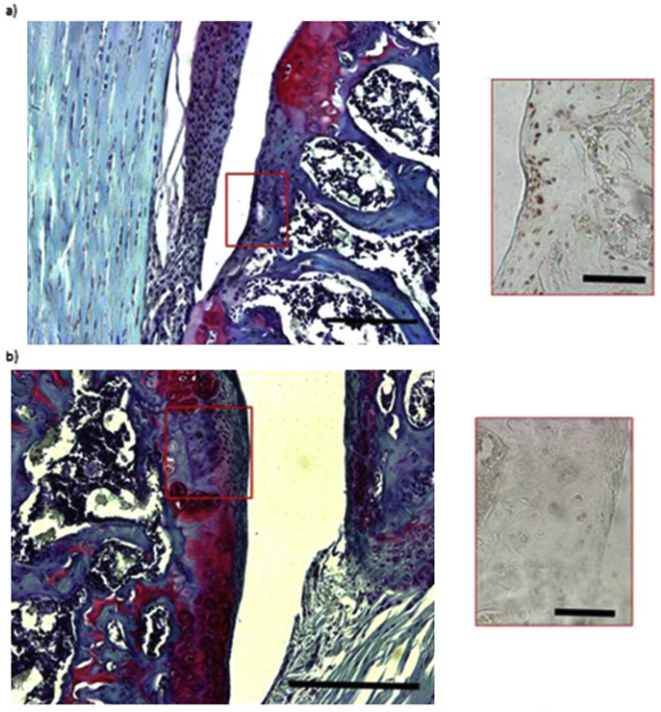

Figure 2. 4 weeks post injury. Safranin-O stain of a) 057 and b) MRL with the respective Sca-1 IHC on the right.

212

MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE AND PLASMA RICH IN GROWTH FACTORS IN DEGENERATIVE JOINT DISSEASE IN DOGS

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A. Purpose: The aim of this study was to compare the effect of adipose mesenchymal stem cells (aMSC) (Dog Stem®) and Plasma rich in growth factors (PRGF) (PRGF-Endoret®) in the treatment of osteoarthritis disease (OA) in dogs.

B. Methods: The study was performed in 50 dogs with elbow, knee or hip degenerative ostheoartritis (documented by X-Rays and clinical findings).

The inclusion criteria was the following, healthy animals weighting more than 20 kg, with presence of OA in one of these joints.

Animals were randomly assigned to one of these two study groups:

- PRGF: one intraarticular injection (2cc) of autologous PRGF obtained by Anitua's method. Citrate tubes centrifugates at 460G during 8 minutes (n=23).
- aMSC: one intraarticular injection (2cc containing 30 millions of mesenchymal stem cells) of autologous adipose mesenchymal stem cells obtained by Stem Cells method. The adipose tissue was obtained by open byopsy and cultivated in Stem Cells Laboratories (Belgium) (n=25).

Dogs were evaluated the day before the tratment was applied (basal) and one, three and six months after intraarticular infiltrations. The primary outcome variable was OA degree (assesed by the Bioarth Ostheoarthritis Scale: radiographic findings (mild, moderate or severe), owners questionnaire for functional limitation (measurement scale: 0 to 23 points) and animal joint movement (measurement scale: 0 to 7 points). Additional measures included the visual analogic scale (VAS), performed by the owner and the veterinarian to assess pain relief

improvement, owner satisfaction questionnaire (which included six questions about patients response to the treatment and life quality) and all adverse effects to treatments recorded (Aes).
Results were analyzed by the SPSS 20.0 program. The nonparametric Kruskal-Wallis and Mann-Whitney tests were used to compare non-categorical variables and crosstabs with contingency coefficient to evaluate the categorical ones.
C. Results: A total of 42 males and 8 females, with mean age of 74±39 (8-135) months and mean weigth of 36,6±11,9 (20-66,2) kg were included in the study. No differences were appreciated between groups in these variables.
Mild OA was present in 3 animals from the PRGF group, 3 and 10 animals of the PRGF and aMSC groups respectively, presented moderate OA, and 17 animals presented severe OA in both groups. OA degree radiologically evaluated did not vary within groups, and there was no improvement in time. However, in the rest of variables (functional limitation, Joint movement, Joint flexion and extension degree, owner's and veterinarians VAS, Joint movement range, muscular atrophy and patient's life quality) a clear improvement has been seen since the first month of study in both groups, maintaining up to six months, although the aMSC group obtained better results at 6 months than the PRGF group in Joint movement (p=0,022), flexion degree (p=0,0034), extension degree (p=0,02), owners VAS (p=0,044), veterinarians VAS (p=0,00) and range of movement (p=0,003). There were no adverse effects present during the study.
D. Conclusions: OA is one of the most common causes of lameness in dogs. In this study both PRGF and aMSc treatments have demonstrated to improve functional limitation, joint movement and pain feeling even without radiological improvement and with absence of adverse effects. Both AMSC and PRGF therapy are new means of treatment and create big hopes to overcome cartilage regeneration, maintaining or improving joint function and structure.

213
CARTILAGE REPAIR IN AN OSTEOCHONDRAL DEFECT IN A RABBIT MODEL

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Purpose: Despite the fact that mesenchymal stem cells (MSC) offer clinical potential for osteoarthritis applications, retaining sufficient numbers of functional MSC at the site of injury for optimal repair still continues to be a major challenge. One method of overcoming this limitation is to create an artificial extracellular matrix or scaffold to hold the cells in place. Previous research suggests that biomaterials possessing an elastic modulus between 2-50MPa are suitable for functional cartilage repair. To this end, the main aim of this study was to examine the effect of scaffold mechanical properties on cartilage repair in a rabbit model in vivo.
Methods: Two three-dimensional (3D) scaffold structures fabricated from different biomaterials were selected; a 55/45 wt% polyethylene oxide terephthalate/polybutylene terephthalate (PEOT/PBT) scaffold created by 3D fiber deposition with a compressive modulus of 3.6 MPa and a bilayered PGA/PLGA+CaS composite construct (Trufit™) with a compressive modulus of 50 MPa. Using scanning electron microscopy, the 3D architecture of the scaffolds was visualized and the porosities measured using volume displacement. Upon characterization of rabbit MSC morphology, growth kinetics and tri-lineage differentiation potential, the optimal cell seeding density and attachment conditions were evaluated. Cartilage repair was examined in a 3x3x3 mm osteochondral defect in male White New Zealand rabbits in accordance with ethical guidelines and approval, with 3 groups, empty defect (n=3), empty scaffold (n=6) and MSC seeded scaffold (n=6). After 6 weeks, tissue repair was assessed using toluidine blue staining to evaluate tissue morphology and a modified ICRS scoring system using 3-blinded reviewers to grade cartilage repair.
Results: The 3D architecture of the scaffolds was comparable with structures previously used for cartilage repair, with porosities of 76% measured for the PEOT/PBT scaffold and a porosity gradient from 63% to 97% observed for the bilayered Trufit™ construct. Rabbit MSC were shown to have a fibroblastic morphology and were capable of osteogenic, adipogenic and chondrogenic differentiation. Optimal cell attachment was observed for 1 million cells/scaffold in combination with 50μg/ml fibronectin. There was evidence of repair in the empty defect, however, although not significant, cartilage regeneration was improved and degenerative changes were reduced in the presence of the scaffolds (Fig 1 and 2). In terms of scoring, no statistical difference was observed for both scaffolds, in terms of thickness of repair tissue or integration with native tissue. Seeding the PEOT/PBT scaffolds with MSC appears to produce lower scores for degenerative changes in the repair tissue and adjacent tissue when compared to the empty, contralateral control with no cells (Fig 2). In contrast, seeding the Trufit™ scaffold with MSC does not appear to improve degenerative affects. Moreover, there are bone cysts visible in the subchondral bone.
Conclusions: In summary, two scaffolds with mechanical properties at both ends of the materials property spectrum were analyzed. Although, both scaffolds revealed interesting, albeit different results, neither construct produced an optimal result. Thereby, suggesting that cartilage repair is a multifactorial problem, which is not modulated by mechanical properties alone.

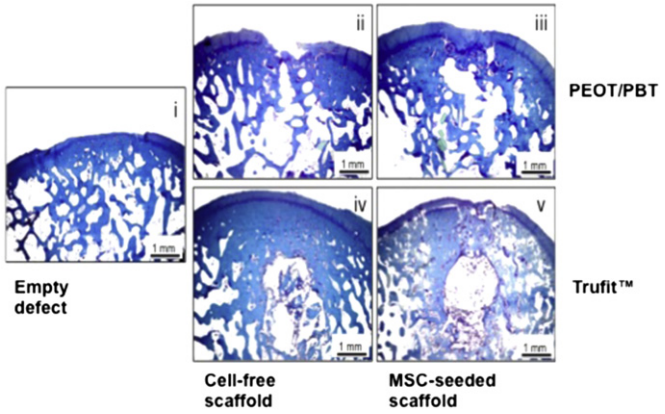

Figure 1. Representative images showing toluidine blue staining in osteochondral defect.

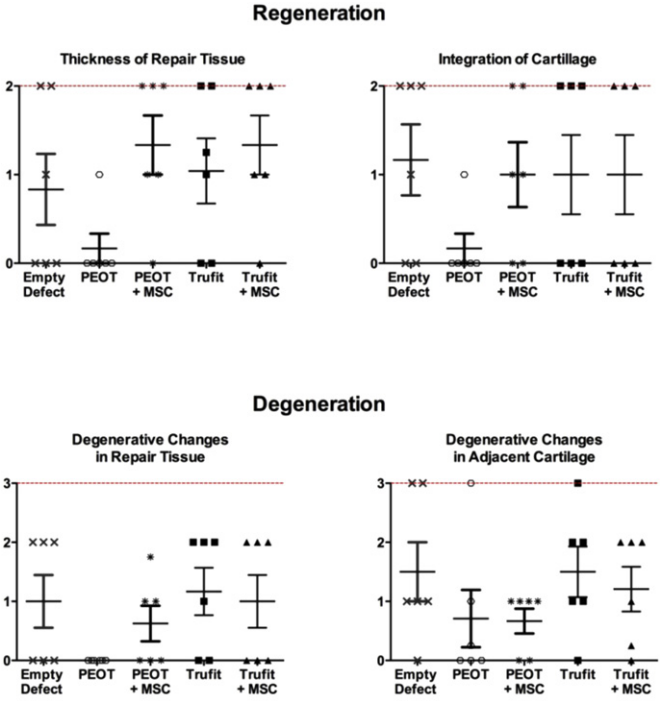

Figure 2. Histological scoring for **(A)** Regeneration **(B)** Degeneration, Red line indicates highest score possible.