Original article

Comparison of autologous bone marrow and adipose tissue derived mesenchymal stem cells, and platelet rich plasma, for treating surgically induced lesions of the equine superficial digital flexor tendon

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Abstract

Several therapies have been investigated for equine tendinopathies, but satisfactory long term results have not been consistently achieved and a better understanding of the healing mechanism elicited by regenerative therapies is needed. The aim of this study was to assess the separate effects of autologous bone marrow (BM) and adipose tissue (AT) derived mesenchymal stem cells (MSCs), and platelet rich plasma (PRP), for treating induced lesions in the superficial digital flexor tendon (SDFT) of horses. Lesions were created surgically in both SDFTs of the front limbs of 12 horses and were treated with BM-MSCs (six tendons), AT-MSCs (six tendons) or PRP (six tendons). The remaining six tendons received lactated Ringer's solution as control. Serial ultrasound assessment was performed prior to treatment and at 2, 6, 10, 20 and 45 weeks post-treatment. At 45 weeks, histopathology and gene expression analyses were performed. At week 6, tendon echogenicity score in tendons treated with BM-MSCs suggested earlier ultrasonographic improvement, while at week 10 all treatment groups reached the same level, which was superior to the control group. In treated tendons, collagen orientation score suggested better histopathology outcome. Gene expression was indicative of better tissue regeneration after all treatments, especially for BM-MSCs, as suggested by upregulated collagen type I, decorin, tenascin and matrix metalloproteinase III. Considering all findings, a clear beneficial effect was elicited by all treatments compared with the control group. Despite the absence of great differences between treatments tested, BM-MSCs resulted in a better outcome than PRP and AT-MSCs.

Keywords: Equine; Mesenchymal stem cells; Platelet rich plasma; Regenerative medicine; Tendon repair

Introduction

The superficial digital flexor tendon (SDFT) is subjected to large forces during athletic activity in horses (Dowling et al., 2000). Its limited regeneration potential makes tendon repair a slow process, resulting in the formation of scar tissue, which has inferior biomechanical properties and is prone to re-injury (Dahlgren, 2009; Dakin et al., 2014; Gulati et al., 2015). Several treatments for equine tendonitis have been investigated, but injured animals are rarely able to return to the same level of performance (Genovese et al., 1990; Nixon, 1990; Dehghan et al., 2007).

Regenerative medicine, including the intralesional use of mesenchymal stem cells (MSCs) and platelet rich plasma (PRP) is a promising approach for treating tendon injuries in horses (Bosch et al., 2010). Numerous studies have suggested potential therapeutic benefits of MSCs for the functional regeneration of tendons and ligaments (Godwin et al., 2012; Carvalho et al., 2013; Smith et al., 2013; Conze et al., 2014; Gulati et al., 2015). MSCs from different sources, such as bone marrow (BM) (Smith et al., 2013), adipose tissue (AT) (Conze et al., 2014) or umbilical cord blood (Van Loon et al., 2014), have to a certain extent shown efficacy in terms of reduction of the re-injury rate and improving outcome in both naturally occurring and experimentally induced lesions (Godwin et al., 2012; Martinello et al., 2013; Conze et al., 2014).

However, several questions about the effectiveness of MSCs and PRP remain unanswered. Previous reports on the use of regenerative products showing superior healing of equine tendinopathies have combined different products (Pacini et al., 2007; Del Bue et al., 2008; Carvalho et al., 2013; Smith et al., 2013), making it difficult to elucidate if MSCs are more or less effective than PRP or if their effects are additive

(Koch et al., 2009; Schnabel et al., 2013). The aim of this study was to separately assess the effectiveness of autologous BM-MSCs, AT-MSCs and PRP for treating induced injuries of the equine SDFT.

Material and methods

Animals

Twelve cross-breed geldings (H1-H12) aged 5-8 years were determined to be healthy and free of tendon injury, as shown by their history, clinical assessment and ultrasonographic exam. The project was approved by the Ethical Committee for Animal Experiments from the University of Zaragoza (project license PI36/07; date of approval 15 February 2008. The care and use of animals were performed in accordance with the Spanish Policy RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for scientific purposes.

Study design

In 24 tendons (both forelimbs of 12 horses), lesions were induced as described below and randomly divided into four batches. Each batch of six tendons was assigned to one treatment (BM-MSCS, AT-MSCs or PRP) or control (lactated Ringer's solution, LRS). H1-H6 received BM-MSCs in one tendon and H7-H12 received AT-MSCs in one tendon. In the other tendon, H1-H3 and H7-H9 received PRP, whereas H4-H6 and H10-H12 received LRS as a control treatment. The different treatments were administered 1 week after the lesion induction. Clinical and ultrasonographic parameters were recorded throughout the study in weeks 1 (pre-treatment), 2, 6, 10, 20 and 45, after which animals were euthanased. Subsequently, histological and gene expression analyses of tendons were performed (See Appendix: Supplementary Material 1).

101

102 *Autologous BM-MSCs, AT-MSCs and PRP*

103 Procedures for preparation of MSCs were carried out as described by Ranera et al.
104 (2011). BM was aseptically aspirated from the sternum of horses H1-H6, layered over
105 Lymphoprep (Atom) and centrifuged at 300 *g* for 20 min. Nucleated cells were harvested and
106 suspended in basal medium, consisting of low glucose Dulbecco's modified Eagle's medium
107 (DMEM) supplemented with 10% foetal bovine serum, 1% glutamine and 1%
108 streptomycin/penicillin (Sigma-Aldrich). Adipose tissue was aseptically collected from the
109 supra-gluteal subcutaneous area of horses H7-H12. The stromal vascular fraction was isolated
110 by digestion with 0.01% collagenase type I (Sigma-Aldrich) for 30 min at 37 ºC with
111 continuous shaking. Cells were suspended in the basal medium described above. BM and AT
112 derived cells were expanded at 37 ºC in 5% CO_2 until the third passage and then characterised
113 as MSCs by their immunophenotype and their tri-lineage differentiation potential using
114 methodology and markers described previously (Ranera et al., 2011). Subsequently, cells
115 were cryopreserved and thawed for expansion 7 days before their in vivo use.

116

117 Autologous PRP was obtained by using the double centrifugation tube method
118 (Arguelles et al., 2006). Peripheral blood was collected in citrated tubes from horses H1-H3
119 and H7-H9, centrifuged at 120 *g* for 5 min and the 50% fraction closest to the buffy coat was
120 collected and centrifuged again at 240 *g* for 5 min. Subsequently, the lower 25% fraction was
121 collected and used for treatment. Platelet and white blood cell (WBC) counts were determined
122 using a flow cytometry haematology system (LaserCyte Dx, IDEXX Laboratories).

123

124 *Surgically induced injury*

Horses were sedated with 0.04 mg/kg IV romifidine (Sedivet, Boehringer-Ingelheim) and 0.02 mg/kg IV butorphanol (Torbugesic, Pfizer). Anaesthesia was induced with 2.2 mg/kg IV ketamine (Imalgene, Merial) and 0.05 mg/kg IV diazepam (Valium, Roche), and maintained using a triple-drip, consisting of 15 mg romifidine, 500 mg ketamine and 25 g guaifenesin (Glicefar, DragPharma) mixed in 500 mL of 5% glucose solution at 2 mL/kg/h. Lesions of approximately 5 cm length were mechanically induced in the SDFT of both forelimbs of each animal, approximately at 18 cm distal to the accessory bone, using a controlled motor rotor and a 4 mm drill through a small longitudinal incision into the core of the SDFT (Cadby et al., 2013). The lesion was created in the central tendon area (maximal injury zone, MIZ) (Fig. 1). The incisions in the paratenon and the skin were closed in a routine fashion. Twice daily, 22 mg/kg IM procaine benzylpenicillin (Depocillin, Merck-Sharp) and once daily 6.6 mg/kg IV gentamicin (Gentavex, SP Veterinaria) were administered for 3 days. A two-layer bandage was applied and changed daily for 7 days. Pre-operative and post-operative analgesia were provided with oral phenylbutazone (EqZona, Calier) at 2.2 mg/kg twice daily for 3 days. Animals still presenting signs of pain received butorphanol at 0.05 mg/kg IV every 4 h. After surgery, horses were box-rested for 2 weeks and then daily hand-walked for 10 min until week 6. Subsequently, they were placed in small paddocks for restricted exercise and 10 weeks after lesion induction were allowed unrestricted exercise in bigger paddocks until the end of the study.

Treatment

Horses received 20 x 10^6 BM-MSCs (BM treatment) or AT-MSCs (AT treatment) suspended in 7 mL LRS, 7 mL PRP (PRP treatment) or 7 mL LRS (control) in assigned tendons 1 week after the injury induction, according to the distribution described above. Horses were sedated as described above and a high palmar nerve block was performed. The

designated treatment or LRS was administered intralesionally using an 18 G 40 mm needle. The volume was equally distributed into the core of the lesion and perilesionally 1-2 cm proximally and distally to the core lesion (Watts et al., 2014). Administration was carried out by single injection and needle redirection through the site of injury under ultrasonographic guidance.

Clinical assessment

Daily recordings were made of duration of recumbency, body temperature, heart and respiratory rates, and intestinal motility during the first week after lesion induction. Surgical wounds were examined daily for any sign of infection. Tendon palpation was carried out to detect oedema, inflammation and pain. Lameness was assessed visually whilst horses were hand walked and trotted along a straight line on a hard surface on week 1 (walked only), 2, 6, 10, 20 and 45.

Ultrasonographic evaluation

Ultrasonography was performed using a 7.5 MHz linear transductor (HDI-3000, ATL). Five transverse and five longitudinal images were obtained along the MIZ region (at approximately 2 cm intervals) at each time point. The ultrasonographic parameters fibre pattern score (FPS, scores 0-3), tendon echogenicity score (TES: score 0-3) and cross sectional area (CSA, %) were assessed by a clinician blinded for the treatments (Genovese et al., 1986; Rantanen et al., 2003). Values for each parameter were assigned as the sum of the scores obtained from 10 images.

Real time quantitative PCR

At week 45, horses were sedated as described above and euthanased with sodium pentobarbital (Euthasol, Esteve) at 200 mg/kg IV. Samples from the zones Z1 (off-lesion control) from one tendon of each horse (n = 12) and MIZ (Fig. 1) from each SDFT (n = 24) were collected and RNA was isolated using Trizol (Qiagen), chloroform and isopropanol (Chou et al., 2013). Genomic DNA was extracted using the DNAse Turbo kit (Ambion) and cDNA was synthesised using the SuperScript II System (Life Technologies). The expression of genes encoding molecules related to extracellular matrix (ECM) production, tissue healing and remodelling were analysed (Table 1). Reactions were performed with the Fast SYBR Green Master Mix and the StepOne Real Time PCR System device (Applied Biosystems). The normalisation factor was calculated from two housekeeping genes (GAPDH and B2M) (Kolm et al., 2006). Gene expression levels were determined by the comparative Ct method.

Histopathology

Eighteen histological preparations were evaluated from the MIZ of each tendon; six from the proximal region, six from the distal region and six from the central area (Fig. 1). Each sample was longitudinally embedded in a paraffin block. Histological sections were prepared, stained with haematoxylin-eosin and examined under a light microscope. The histological parameters tenocyte morphology (TM), vascularity (V), ground substance (GS), collagen orientation (CO) and cell number (CN) were assessed by a single blinded operator and scores obtained from each preparation were summed using a semi-quantitative assessment (Maffulli et al., 2008).

Statistical analysis

Statistical analysis was performed using SPSS 19.0 (IBM). Evolution of ultrasonographic parameters was studied using two-way analysis of variance (ANOVA)

repeated measures and Bonferroni post-hoc testing. Differences between groups for histological parameters were analysed using one-way ANOVA and Duncan post-hoc testing. Cronbach's α coefficient was calculated to assess the intra-observer agreement in ultrasonographic and histopathological evaluations (see Appendix: Supplementary Material 2). Differences between treatments and off-lesion control samples (Z1) for gene expression were analysed with the Mann-Whitney test. Since each animal received two different substances, not all observations were independent. Therefore, both tendons of each animal were compared using a paired Student's *t* test for the following BM versus PRP (animals H1-H3), BM versus LRS (animals H4-H6), AT versus PRP (animals H7-H9) and AT versus LRS (animals H10-H12). Significances obtained from paired tests coincided with those obtained from ANOVA analyses. Significant results shown in figures correspond to ANOVA analyses comparing all groups. The level of significance was set at $P < 0.05$.

Results

Characterisation of BM-MSCs, AT-MSCs and PRP

Cells obtained from BM and AT from all donors were successfully characterised as MSCs by surface marker pattern and tri-lineage differentiation (data not shown). The mean ± standard deviation (SD) platelet and WBC counts in PRP were $263.3 \times 10^3 \pm 99.9 \times 10^3$ and $8.9 \times 10^3 \pm 2.5 \times 10^3$ cells/µL, respectively.

Clinical assessment

Health status was satisfactory in all animals at the daily checks. At the time of treatment, lesion areas were similar amongst horses and the peritendinous reaction was mild, with a slight fibrous reaction, in all animals. Five weeks post-surgery, no lameness was observed and skin wounds had healed completely. Irregular skin thickening was detected at

the site of injury, and the tendons appeared to be sensitive and thickened. No systemic adverse effects were noticed in any horse.

Ultrasonography

All ultrasonographic scores progressively decreased throughout the study, including those in the control group. At week 6, only the BM-treatment led to a significant reduction in TES score with respect to baseline. At week 10, significant reductions in FPS and TES were seen with BM-treatment, AT-treatment and PRP-treatment, and in CSA with AT-treatment and PRP-treatment. Twenty weeks after induction of lesions, significant reductions of all scores of all three parameters compared with baseline data were observed in all treatment groups. Moreover, TES was also significantly reduced in the control tendons. By week 45, all scores were significantly reduced in all groups, except for CSA in the control group (Figs. 2 and 3).

Histopathology

The treatment groups had lower score results than the control group, which had the highest values for all parameters. CO score was significantly lower for tendons in the BM-treatment and PRP-treatment groups compared with the control group. Tendons in the PRP-treatment also had significantly lower GS scores than the control group (Figs. 4 and 5).

Gene expression

Significant upregulation of collagen type I (COL1A1), decorin (DCN), tenascin (TNC) and matrix metalloproteinase III (MMP-3) over Z1 was found in tendons in the BM-treatment group. COL1A1, TNC, MMP-3 plus collagen type III (COL3A1), aggrecan (ACAN) and tenomodulin (TNMD) were significantly overexpressed in

tendons in the PRP-treatment group. BM-treatment resulted in the highest expression of COL1A1, DCN and MMP-3. Tendons in the PRP-treatment group expressed the highest values of TNC and TNMD, whilst there was no significant difference in expression of scleraxis (SCX). Control tendons had the highest expression of COL3A1, ACAN and COMP. AT-treatment did not elicit significant differences compared to Z1 or control tendons (Fig. 6).

Discussion

The aim of this study was to evaluate the effect of intralesional injections with autologous BM-MSC, AT-MSCs or PRP on surgically induced SDFT lesions, through ultrasonographic monitoring, histopathology and assessment of gene expression after 45 weeks. Mechanically induced lesions more closely resemble naturally occurring injuries in terms of histological findings (Cadby et al., 2013) and provide a more standardised and limited lesion than those induced enzymatically (Birch et al., 1998; Watts et al., 2012). Despite the usefulness of experimental models of tendinopathy, some differences to naturally occurring injuries, related to the aetiology and pathophysiology of the lesions, must be considered (Cadby et al., 2013). Therapy with BM-MSCs is associated with better histological results in naturally occurring equine tendinopathies (Smith et al., 2013) than in induced injuries (Caniglia et al., 2012), which might be due to differences in pathogenesis.

In similar studies, therapies have been administered 2-4 weeks after induction of lesions (Caniglia et al., 2012; Carvalho et al., 2013; Conze et al., 2014). In the current study, treatment was performed 1 week after injury because the optimal time of administration of MSCs appears to be during the subacute phase of repair, when

inflammation has decreased and there is limited formation of scar tissue (Koch et al., 2009). In tendon lesions, the subacute stage starts a few days after injury, reaching its maximum level at approximately 3 weeks, with fibroblast infiltration and high production of collagen type III, making the tissue prone to re-injury (Fackelman, 1973). In the present study, treatments were performed at the beginning of the subacute phase, with aim to influence this early stage of scar formation.

A limitation of this study was that both forelimbs of each horse received different treatments and, therefore, not all observations were independent. The study was designed to minimise the number of experimental animals. Furthermore, to reduce possible interference, MSCs from different sources were not administered to the same animal.

The methodology for obtaining PRP was chosen based on its simplicity and safety (Arguelles et al., 2008). The platelet and WBC counts in PRP were in agreement with those previously described with this technique (Arguelles et al., 2006).

The clinical assessment did not reveal differences between treatments, in agreement with the study by Watts et al. (2011). No adverse reactions were noted after the intra-lesional administration of different products.

Ultrasonographic monitoring revealed faster lesion improvement in treatment groups versus the control group. Reduction in CSA, which is the parameter most closely related to the quality of the healing process (Rantanen et al., 2003), was significant only in treated groups at the end of the experiment. BM-treatment produced the most rapid

ultrasonographic improvement (significant difference at 6 weeks), followed by PRP-treatment (significant difference at 10 weeks). These findings suggest that MSCs and PRP provided a quicker repair, enhancing the reparative process and reducing the degeneration of the tendon fibres. This effect was seen after all treatments, but was most marked for BM-treatment, and is broadly in line with different studies on biological regenerative products (Pacini et al., 2007; Del Bue et al., 2008; Carvalho et al., 2011; Caniglia et al., 2012; Godwin et al., 2012; Marfe et al., 2012).

Whereas histopathology did not show great variation, there were a few distinct differences between the treated groups and the control group, with the latter showing poorer regeneration. Furthermore, BM-treatment and PRP-treatment were associated with a better histopathological outcome than AT-treatment. Our findings are in agreement with those from a previous study reporting significant decreases in vascularity and CO (Smith et al., 2013). It is hypothesised that larger differences in histopathology results could have been identified at earlier stages, as shown by ultrasonography. However, since the mean time needed for complete lesion recovery is 36-48 weeks (Fortier and Smith, 2008; Dakin et al., 2014) and since most studies have been conducted over shorter periods, the study duration of 45 weeks was considered appropriate to provide long term results.

COL1A1 and COMP are indicators of tendon matrix synthesis (Smith et al., 1997, 2002a, b; Oshiro et al., 2003; Ritty and Herzog, 2003; Sodersten et al., 2005) and upregulation of COL1A1 after BM-treatment and PRP-treatment might indicate a reparative process. In contrast, there was no significant upregulation of COMP after PRP-treatment and in control tendons; similar or higher COMP expression in control

tendons compared with MSC-treatment or PRP-treatment has been reported previously (Schnabel et al., 2009; Carvalho et al., 2013). DCN appears to be important for tendon ECM production (Dowling et al., 2000; Thomopoulos et al., 2003) and collagen fibrillogenesis (Scott, 1996; Sodersten et al., 2005), so its upregulation after all treatments, especially in tendons receiving BM-treatment, is consistent with its biological function.

Tissue produced after tendon injury is rich in COL3A1 and has a disorganised fibre pattern, which can persist for up to 14 months after lesion formation (Goodship et al., 1994; Dowling et al., 2000). In our study, COL3A1 expression was upregulated in all injured tendons, but the highest expression was found in control tendons. ACAN and COL3A1 overexpression are associated with chronic tendinopathy (Corps et al., 2006) and are considered to be adaptive responses of the tendon to changes in biomechanical loads (Thomopoulos et al., 2003). Therefore, the high ACAN upregulation found in control tendons might be related with the chondroid metaplastic degeneration with disorganised fibre pattern seen in this group. The upregulation of ACAN and COL3A1 was also significant in tendons receiving PRP-treatment, but not in those receiving BM-treatment or AT-treatment.

The significant upregulation of MMP-3 and TNC in the BM-treatment and PRP-treatment groups may indicate enhanced remodelling, since MMP-3 is related to changes in collagen fibre alignment during the remodelling phase and TNC modulates the binding of cells to ECM components (Chiquet-Ehrismann and Chiquet, 2003; Jones et al., 2006). BM-treatment and PRP-treatment led to significant upregulation of ECM synthesis and markers indicative of remodelling, whilst AT-treatment did not elicit

significant changes in gene expression. However, PRP-treatment also resulted in a significant increase in ACAN and COL3A1, suggesting poorer healing than in BM-treated tendons.

Conclusions

BM-treatment and PRP-treatment produced similar results, with enhanced tendon gene expression, echogenicity and histopathological parameters compared to AT-treatment. However, PRP-treatment resulted in higher COL3A1 and ACAN expression, suggesting less regeneration, which might be reflected in lower tendon functionality. Although all the treatments showed a similar beneficial effect compared to the control group, our data suggest that BM-MSCs might provide better tendon healing.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of paper.

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Appendix: Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi: ...'

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Table 1

Primers used for gene expression by RT-qPCR.

Gene	Accession number [a]	Primer sequence (5'-3')	Amplicon size (bp)
GAPDH	NM_001163856	F: GGCAAGTTCCATGGCACAGT	128
		R: CACAACATATTCAGCACCAGCAT	
B2M	NM_001082502.2	F: TCGTCCTGCTCGGGCTACT	102
		R: ATTCTCTGCTGGGTGACGTGA	
COL1A1	AF034691	F: ACACAGAGGTTTCAGTGGTTTGG	89
		R: CACCATGGCTACCAGGTTCAC	
COL3A1	XM_001917620	F: GGAGGATGGTTGCACTAAACA	139
		R: GTCCACACCGAATTCTTGATC	
COMP	AF325902	F: GGCGACGCGCAAATAGA	111
		R: GCCATTGAAGGCCGTGTAA	
ACAN	AF019756	F: CTACGACGCCATCTGCTACA	96
		R: ACCGTCTGGATGGTGATGTC	
SCX	AB254030.1	F: AACCAGAGAAAGTTGAGCAAGGA	111
		R: TGTGCCCGAGTCAGGTCC	
TNC	XM_001916622	F: TGTGTTCCTGAGACGCAAAA	75
		R: TCCCAAACCCAGTAGCAT	
MMP3	NM_001082495	F: TGATGTGACTGGCATTCAATCC	112
		R: ATCGCACATGGCTAGTGTTCCA	
TNMD	Watts et al. 2012	F: AAGACTTTGAGGAGGATGGTGAAG	60
		R: CCACCCACTGCTCGTTTTG	
DCN	AF038127	F: AAGTACATCCAGGTTGTCTACCTTCATAA	73
		R: CAGGTGGGCAGAAGTCATTAGATC	

[a] GenBank accession numbers of sequences used for primers design.

F, forward; R, reverse; bp, length of the amplicon in base pairs; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; B2M, β-2 microglobulin; COL1A1, collagen type I; COL3A1, collagen type III; COMP, cartilage oligomeric protein; ACAN, aggrecan; SCX, scleraxis; TNC, tenascin; MMP3, matrix metalloproteinase 3; TNMD, tenomodulin; DCN, decorin.

Figure legends

Fig. 1. Assignment of zones in the superficial digital flexor tendon (SDFT) in this study. Zone 1 was used as an off-lesion control for gene expression analysis. Zone 3 is the maximal injury zone (MIZ) and corresponds to the area of the lesion. The MIZ is subdivided into proximal, central and distal areas. Samples were collected from these areas for histopathology and gene expression analysis. Zones 2 and 4 are transition zones, while zone 5 is an off-lesion area; these zones were not used in the study.

Fig. 2. Results of serial ultrasonographic assessment using the scales of Genovese et al. (1986) and Rantanen et al. (2003). Mean ± standard deviation (SD; $n = 6$) of each parameter assessed at each time-point (weeks) for each treatment. BM-MSCs, bone marrow derived mesenchymal stem cells; AT-MSCs, adipose tissue derived mesenchymal stem cells; PRP, platelet rich plasma; CTRL, control (administration of lactated Ringer's solution). (A) Mean ± SD of tendon echogenicity score (TES); (B) Mean ± SD of fibre pattern score (FPS); (C) Mean ± SD of cross sectional area percentage (CSA%). Significant differences between each time point and the pre-treatment value are presented for each parameter (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

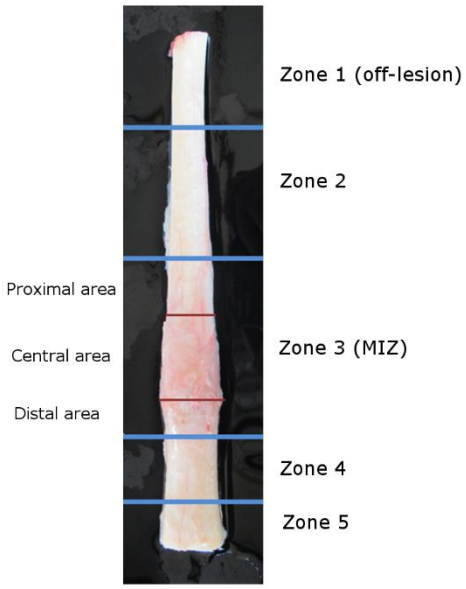
Fig. 3. Ultrasonographic evolution over the 45 week study period. Typical examples of transverse and longitudinal ultrasonographic images obtained from a tendon treated with bone marrow derived mesenchymal stem cells (BM-MSCs) (animal H3) at different times throughout the study.

Fig. 4. Results of semi-quantitative histopathological assessment based on the classifications of Movin and Bonar (Maffulli et al., 2008). Mean ± standard deviation (SD; $n = 6$) score of each parameter for each treatment. BM-MSCs, bone marrow derived mesenchymal stem cells; AT-MSCs, adipose tissue derived mesenchymal stem cells; PRP, platelet rich plasma; CTRL, control (administered lactated Ringer's solution). An asterisk (*) indicates a significant difference between different treatments and control ($P < 0.05$).

Fig. 5. Histological sections showing different qualities of the tendon healing process. (A, D) Good quality of the repaired tendon, with well-oriented collagen fibres parallel to each other, normal morphology of tenocytes, avascularity and low cellularity (A, scale bar = 100 µm; D, scale bar = 400 µm). (B, E) Tendon scar of medium quality, with disoriented collagen fibre bundles, high cellularity, moderate vascularisation and heterogeneous fibroblastic morphology (scale bar = 100 µm). (C, F) Tendon scar of poor quality, with disorientated non-parallel collagen fibres, high cellularity, chondroid cell morphology, high vascularity and a considerable amount of ground substance filling the spaces between fibres can be observed (C, scale bar = 100 µm; F, scale bar = 200 µm).

Fig. 6. Results of gene expression analysis. Mean ± standard error (SE) of mRNA relative expression from the zone off-lesion Z1 ($n = 12$) and MIZ from groups treated with bone marrow derived mesenchymal stem cells (BM-MSCs; $n = 6$), Adipose tissue derived mesenchymal stem cells (AT-MSCs; $n = 6$) and platelet rich protein (PRP; $n = 6$), and the control group (CTRL; $n = 6$) for genes encoding different markers related to the tendon healing process. Significant differences between treatments with respect to

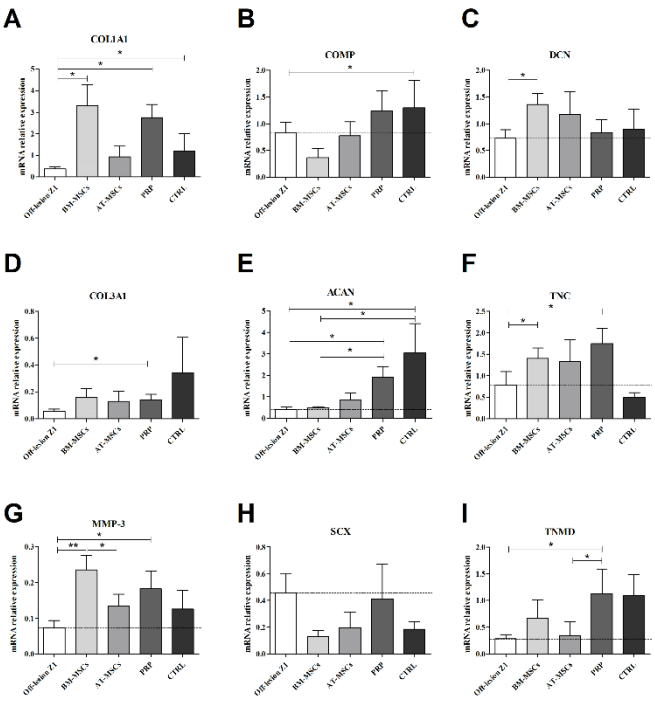
654 expression in the controls and the off-lesion Z1 are presented for each gene. COL1A1,
655 collagen type I; COMP, cartilage oligomeric protein; DCN, decorin; COL3A1, collagen
656 type III; ACAN, aggrecan; TNC, tenascin; MMP3, matrix metalloproteinase 3; SCX,
657 scleraxis; TNMD, tenomodulin; MIZ, maximal injury zone; Z1, zone 1 off-lesion. * $P <$
658 0.05; ** $P < 0.01$.
659



660

661 Figure 1.

662



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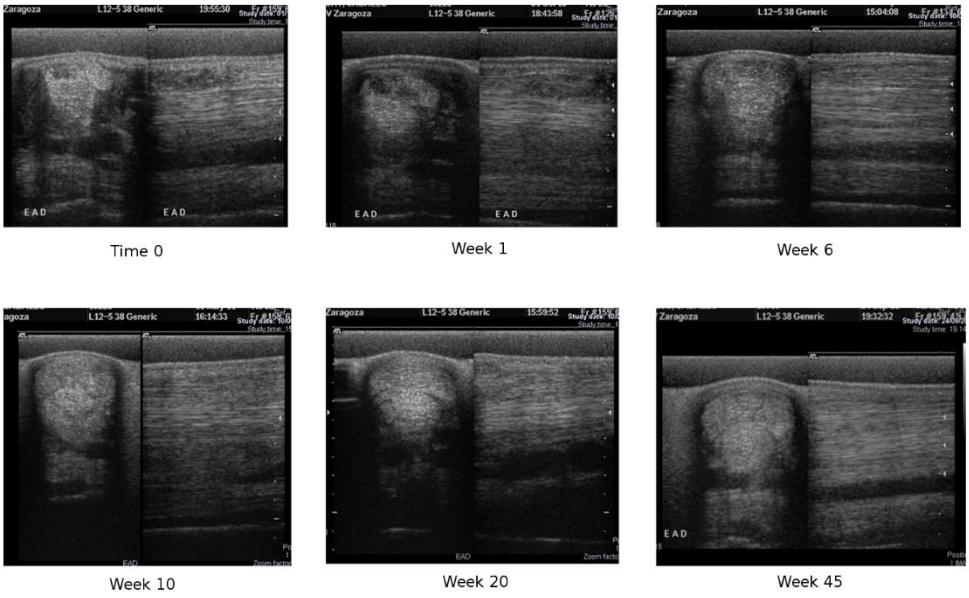

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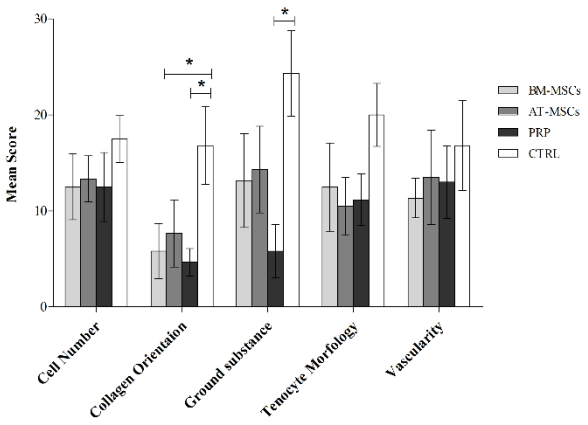

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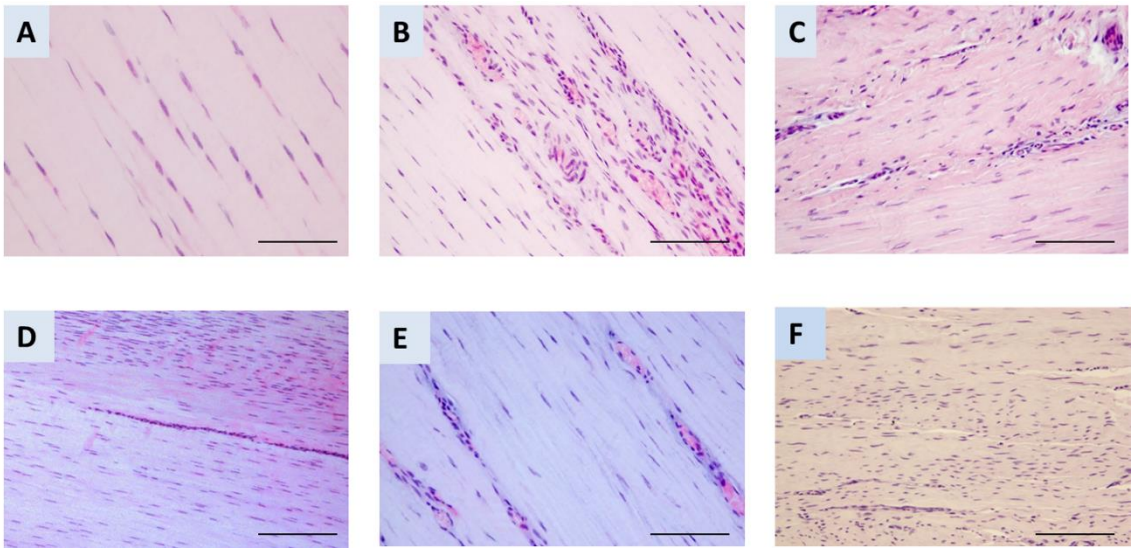

Figure 5