Histol Histopathol (2014) 29: 417-422
DOI: 10.14670/HH-29.10.417

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Treatments of the injured tendons in Veterinary Medicine: from scaffolds to adult stem cells

Marco Patruno and Tiziana Martinello
Department of Comparative Biomedicine and Food Science, Università di Padova, Agripolis, Italy

Summary. In order to treat frequently occurring conditions such as traumatic rupture or over-strain tendinopathies, the techniques of tissue engineering and cell-based therapies have become an accepted *modus operandi* since other available remedies appear to be ineffective in restoring the original structure and function of the injured tissue. However, the mechanisms accounting for the effectiveness of novel regenerative approaches in treating equine tendon and ligament injuries remain poorly characterised. In this review we summarize and discuss the most significant results of our research regarding bioscaffold technology for treating complete tendon tears and the use of adult stem cells for treating tendon lesions induced by over-strain.

Key words: Bioscaffold, Mesenchymal stem cells, Tendon injuries

Can the tendon repair itself?

The mechanisms which explain the effectiveness of regenerative approaches in treating equine tendon and ligament injuries remain poorly characterised. Tendons and ligaments are very specialized and metabolically slow tissues, characterized by an abundant extracellular matrix, low cellularity and regionally low vascularity, although furnishing optimised mechanical properties of strength and elasticity. Tendons are composed of low numbers of resident cells named tenocytes, which are a fibroblast population embedded within parallel oriented dense collagen fibre bundles and very few elastic fibres. When the tendon is damaged an acute inflammatory phase occurs and the increasing level of growth factors leads to the proliferative and the subsequent remodelling phase (Sharma and Maffulli, 2005). Tendon healing can occur via an extrinsic or intrinsic pathway (Bi et al., 2007). In the extrinsic pathway the abrasion repairs itself by invasion of cells from the surrounding sheath and synovium. By contrast, in the intrinsic pathway the repair mechanism mimics embryonic development, with fibroblasts migrating from the epitenon or endotenon to the site of defect where they are responsible for synthesizing collagen fibrils. Another peculiarity of tendinopathy is that it does not produce any real inflammation except in some special conditions, like paratendinopathy or in the first week of tendon repair (Nourissat et al., 2013). However, wounding also provokes the release of growth factors and cytokines from platelets, polymorphonuclear leukocytes, macrophages, and other inflammatory cells (Sharma and Maffulli, 2005); these growth factors induce neo-vascularization and chemotaxis of fibroblasts and tenocytes, and stimulate fibroblast and tenocyte proliferation as well as synthesis of collagen (Oakes, 2004). Also, matrix metalloproteinases (MMPs) are important regulators of extracellular matrix network remodelling and their levels are altered during tendon healing (Magnusson et al., 2010). MMPs may represent central contributors in the control of tendon healing and thus be one key to understanding mechanisms of stem

Offprint requests to: Marco Patruno, Department of Comparative Biomedicine and Food Science, Università di Padova, Agripolis, Viale dell'Università, 16, 35020 Legnaro (PD), Italy; e-mail: marco.pat@unipd.it

cell action as observed in several studies: in a rat flexor tendon laceration model, the expression of MMP-9 and MMP-13 peaked between the seventh and fourteenth days after the surgery. MMP-2, MMP- 3, and MMP- levels increased after the surgery and remained high until the twenty-eighth day (Oshiro et al., 2003; Vieira et al., 2013). These findings might suggest that MMP-9 and MMP-13 participate only in collagen degradation, whereas MMP-2, MMP- 3 and MMP-14 participate both in collagen degradation and in collagen remodelling.

Nowadays regenerative medicine has become an important field for treating frequently occurring conditions such as traumatic rupture and tendinopathies (Riley, 2008; Martinello et al., 2012; Siegel et al., 2012); this approach, together with the use of a cell-based therapy, aims to improve the quality of tendon healing, since currently available remedies often fail to restore the original structure and function of the injured tissue (Kuo et al., 2010; Delince and Ghafil, 2012; Sadoghi et al., 2013). When the tendon shows a partial laceration the tissue can repair naturally but the quality of healing is not comparable to the original since the deposited collagen fibres lack organization and a predominance of type III collagen expression occurs; this fact might represent a problem since tendons heal slowly and the consequences when they return to sport activities are significant.

Indeed, horses stay out of work for a long time and the increased risk of re-injury or continuing defective problems when they do resume training is enhanced by the bad quality of the regenerate (scar tissue). Here, recent results obtained from our research group are reviewed; in particular, we discuss the use of bioscaffolds and experimental damage to be used as models for sport subjects.

Are scaffold materials effective for improving tendon regeneration after full thickness damage?

In order to overcome re-injury problems and improve the speed and quality of regeneration *in vivo*, different approaches are constantly under investigation, such as the use of locally-applied stem cells, even for cellularization of bioscaffolds, or the use of growth factors together with different kinds of scaffolds (Kuo et al., 2010; Martinello et al. 2013; Sadoghi et al. 2013;). Indeed, one of the approaches developed by our group was to recellularize natural scaffolds after a complete decellularization (Martinello et al. 2012); this method offers the unique opportunity of obtaining a scaffold with a hypo-immunogenic natural extracellular matrix structure and displays biomechanical properties very similar to the original tissue, providing optimised support for injected cells to be used in clinical applications and for research (Thaker and Sharma, 2012). Indeed, in the patient population that presents a complete tendon rupture, scaffolds represent complementary surgical tools that should be designed for several functions - transmitting physiological loads and acting as templates for cell proliferation and the deposition of extracellular matrix. Various natural and synthetic materials have been used for *in vitro* cell culture and *in vivo* tissue regeneration, although there is no current scaffolding material that simultaneously offers superior biocompatibility, bio-functionality, effective mechanical properties and tractability. One of the first successful natural materials employed was small intestine submucosa (SIS) which is a collagen matrix ready for graft purposes and its extracellular proteins confer the stability of the product. However, its use is now limited because of immunologic reactions and *in vivo* contracture (Badylak et al., 1998). More recently, three major categories of scaffolding materials have been employed: polyester, polysaccharides and collagen derivatives. The polyesters include polyglycolic acid (PGA), polylactic acid (PLGA) and their copolymers, which present good mechanical properties but do not support a high level of cell adhesion because of their hydrophobic nature (Wan et al., 2003). The polysaccharide, chitosan, has been used in an attempt to reg-enerate tendons, even if the mechanism that saccharides play in cell signalling and immune recognition has not been well elucidated. Collagen derivatives have been intensely investigated since tendon ECM is mainly composed by Type I collagen; however, despite its superior bio-functional properties and biocompatibility the mechanical characteristics are usually more limited. Recently, electrochemically aligned collagen (ELAC) matrices resulted in good collagen orientation and adherence of MSC, but this scaffold has now been overtaken by poly 1, 8 octanediolco-citrate (POC) scaffolds, which are novel elastomeric materials capable of supporting cells and which deliver incorporated growth factors through slow release upon scaffold degradation (Sharma et al., 2012). Another strategy in tendon repair is the use of allografts and autografts, such as the use of hamstring, patellar or quadriceps tendons for human anterior cruciate ligament reconstruction in which the biomechanical stability is also an important requirement (Macaulay et al., 2012). However, allografts might still induce an immunogenic response and donor-site morbidity might limit the applicability of autografts. To avoid these problems, decellularization of the donor tendon tissue is capable of removing the risk of antigenicity from resident tenocytes. Therefore, we think that the use of a natural decellularized scaffold from cadaveric tissue could represent a good tool to be used in full thickness tears; this kind of scaffold preserves the physiological and mechanical properties as well as the ECM proteins for attachment, migration and proliferation of cells (Gilbert et al., 2006) and reduces immunogenicity (Hudson et al., 2004). Several approaches have been employed to reach optimal decellularization, using a combination of physical, chemical, and enzymatic methods. All protocols should preserve the ECM and include methods to lyse the cell membrane, separate cells from the ECM and remove cellular debris from the tissue.

Decellularization/recellularization of scaffolds: looking for the right protocol.

In our recent work (Martinello et al., 2012) we published a decellularization protocol optimised for a clinically-relevant tendons section (two cm length of the flexor tendons from the human hand). This methodology treated the tendon with a hypotonic Tris buffer containing 0.1% (w/v) EDTA and two proteases inhibitor for 2 h at 37°C. Subsequently, the tendons were placed in 0.1% SDS (w/v) in hypotonic buffer for 5 h at 37°C with agitation. Finally, the tendons were rinsed and incubated in DNase. After washings, tendon samples were stored in PBS with antibiotic solution and sterilized under UV light overnight (Martinello et al., 2012). In the case of larger tendons, the protocol had to be modified by adding freeze-thaw cycles and extending the incubation time at room temperature in order to achieve satisfactory levels of decellularization. The option to obtain a cell-free, native tendon structure that resembles the human tendons holds great promise, not only for current research use, but also raises the possibility of xenogeneic transplantations when tendons derived from different species, but with similar structure and composition, could be used. In addition, initiating a tendon regeneration process requires the identification and isolation of appropriate cell types that should be able to proliferate and sustain growth over the healing process while maintaining physiological integrity of the graft. Previous studies suggested that fibroblasts, tenocytes or adipose-derived MSC might be used to repopulate scaffolds, although some technical problems remain to be solved for all of these different cell types (Chen et al., 2009; Angelidis et al., 2010; Woon et al., 2011). There is controversy as to which cell types are most suitable for recellularizing scaffolds. Some researchers (Gulotta et al., 2009) do not believe that MSCs have a therapeutic effect in tendon healing, based on experimental evidence where MSC did not improve structure, composition, or strength of the tendon because of the lack of growth factor present in the construct. However, the delivery of cells in other scaffolds, such as a fibrin matrix, may not be a suitable substrate for MSC differentiation under *in vivo* conditions. It is a fundamental principle to obtain an even cell seeding throughout the tissue scaffold. Static techniques (surface seeding or injection) or dynamic techniques (movement of the cell solution) have been established to improve the seeding efficiency and distribution in the scaffolds but this was not always successful (Thevenot et al., 2008). A simple method we have developed utilises a pre-treatment with a collagen solution which at 37°C forms a gel in order to help the cell dissemination in the core of the tendon. For the future, we believe that the combination of MSC (possibly pre-committed towards a tenogenic fate) with a natural scaffold or with a special connective tissue isolated from marine invertebrates and able to release growth factors (unpublished data) will be optimal for producing biomechanically robust constructs before treating tendon injuries *in vivo*.

Does Veterinary Medicine need stem cells for tendon healing?

Regarding the use of adult stem cells used in veterinary medicine for treating tendon injuries, we note that it is becoming a very popular method, especially in association with other classic therapies. The use of scaffolds has gained less attention since the most common tendon injury in the horse results only in disruption of the central portion of the superficial digital flexor tendon (SDFT), and therefore there is no real need to insert a scaffold material because the surrounding 'intact' tendon tissue can act as biomechanical splint and scaffold. Usually the most commonly-used cells are MSCs, which are directly injected into the horse tendon lesion where there is a proposed ideal environment of natural collagen fibres and local growth factors.

Mesenchymal stem cells have been sourced from a number of tissues, since many tissues possess a niche of multipotent progenitor cells in their stromal compartment, such as the equine bone marrow (Fortier et al., 1998) or equine and dog adipose tissue (Vidal et al., 2007; Martinello et al., 2011). Alternative and promising sources of MSC are represented by peripheral blood (Koerner et al., 2006; Martinello et al., 2010), synovial membrane (Koga et al., 2008) and umbilical cord blood or matrix (Corradetti et al., 2011) although further studies have to confirm these results, especially in equine clinical practice. Some studies have explored the use of adipose-derived stem cells (ADSCs) in the treatment of tendon disorders (de Mattos Carvalho et al., 2011) demonstrating that these cells induced a better fiber organization and diminished the inflammatory infiltrate in a model of tendinosis in horses; moreover, immunohistochemical analysis showed an increased expression of type-I collagen in the treated group compared to the controls. These data suggest that regeneration of tendons (by type-I collagen production) can be initiated by these cells improving, therefore, the classic repair response (characterized by an high expression of type-III collagen production).

Moreover, using ASCs during repair of rabbit Achilles tendons, some researchers (Uysal et al., 2012) found a better strength in the experimental groups than in the control group. Type-I collagen, FGF and VEGF levels were statistically higher in the experimental group. At the same follow up, 10% of labeled ASCs were detected, suggesting that 10% of ASCs may become tenocytes, acting as direct regenerative actors. Similar results were reached with the use of bone marrow MSC in sheep (Crovace et al., 2010). Taken together, these studies report that, when implanted, BM-MSCs and ASCs engraft into the tendon and improve tendon architecture (Uysal et al., 2012). Other important requirements to catch up with human regenerative medicine will be to perform unambiguous surface marker analysis on MSCs isolated from all species of

veterinary interest and to evaluate their applicability in an allogeneic setting for regenerative purposes. For instance, MSCs derived from equine bone marrow express surface markers such as CD44, CD29 and CD90, but not CD45, CD14 and other endothelial or epithelial markers (Radcliffe et al., 2010), whereas MSCs isolated from equine blood also expressed CD13 (Martinello et al., 2010); the expression of markers was even different for MSCs isolated from equine adipose tissue (Vidal et al., 2007). This fact probably reflects the different techniques used during the analysis and the unavailability of species-specific antibodies. Regarding the allogeneic implantation of MSCs, recent results indicate the absence of inflammation elicited by their injection (Chong et al., 2007; Guest et al., 2008; Carrade et al., 2011) and therefore it is likely that in veterinary medicine allogeneic transplantation will become accepted clinical practice providing the legislation allows it. In our recent work (Martinello et al., 2013) we used sheep as experimental models since large animals are considered better models for human tendinopathy than laboratory animal equivalents. Thus, for instance, the SDFT of large animals is functionally equivalent to the human Achilles tendon and suffers from similar pathologies (in naturally exercising animals, such as the horse). In the recent past, clinical follow-up and ultrasonographic examinations revealed the favourable outcomes of MSC injections in sport horses (Smith et al., 2008; Godwin et al., 2011; Renzi et al., 2013); good fibre alignment, echogenicity scores and also a decreased re-injury rate were observed compared to conventional medical treatments (reviewed nicely by Brehm et al., 2012). However, relevant outcomes should also include histological, immunohistochemical and molecular analysis that are essential to elucidate the effects of treatments on matrix composition. PRP therapy is another popular treatment method, which administers high numbers of autologous platelets for the purpose of releasing their growth factor stores in physiologically relevant ratios, which are critical to tissue regeneration, cellular recruitment, and angiogenesis, although often they induce scar tissue rather than "true" regeneration. In recent years this method has raised considerable interest but also wide criticism, as observed in the literature (Maffulli and Del Buono, 2012). PRP preparations are highly variable and there is no scientific evidence for consistent effects when they are administered by local injection (Fisher and Mauck, 2013). The main aim of our research was to compare the effectiveness of three different protocols designed to promote tendon healing, testing the combination of blood-derived MSC and PRP to explore the putative synergistic action of these treatments. In our tests the consequences of the injection of MSC, PRP or a mixture of both were evaluated at 30 and 120 days from the induction of experimental lesions obtained using the bacterial collagenase-1A into the digital flexor tendons of sheep. Our results suggested there was a beneficial effect of MSCs due to improvement of structural organization and matrix composition, as assessed by higher expression of collagen I and COMP proteins and the lower expression of collagen III. In contrast, these observations were not detected when PRP was used alone or in combination with MSCs. Therefore, the expected synergistic effect of MSC/PRP did not occur and this might be a consequence of several reasons: i) MSC did not react well with the several PRP growth factors (some proinflammatory cytokines and some angiogenic factors); ii) they follow independent and opposing pathways and behaviours; iii) our protocols were probably not optimal for stimulating the synergic performances between the two treatments. By contrast, using mice as an experimental model, Chen et al. (2012) found a synergist effect when using PRP and adult stem cells and this fact shows that many variables are still to be regulated before consistent responses can be obtained and explained.

Concluding remarks

In summary, our and other recent studies indicate that MSC obtained from different sources are safe and have the potential to enhance functional recovery in equine injuries, although it will be necessary to increase the number of clinical and experimental cases in a long-term follow-up period (this would be necessary also for PRP studies), in order to evaluate the incidence of re-injury and analyse the histological and molecular parameters of the healed tissues. Moreover, we can also state that (1) decellularised scaffolds are a potentially useful clinical tool; (2) MSCs are safer and appear to have beneficial effects; and (3), in our opinion, PRP and MSC treatments should not be considered synergistic.

Aknowledgements. We would like to thank Prof. A. Rowlerson and Prof. R. Smith for improving the quality of the manuscript.

References

Angelidis I.K., Thorfinn J., Connolly I.D., Lindsey D., Pham H.M. and Chang J. (2010). Tissue engineering of flexor tendons: the effect of a tissue bioreactor on adipoderived stem cell-seeded and fibroblast-seeded tendon constructs. J. Hand Surg. 35, 1466-1472.

Badylak S.F., Record R., Lindberg K., Hodde J. and Park K. (1998). Small intestinal submucosa: a substrate for *in vitro* cell growth. J. Biomat. Sc. Pol. Edition. 9, 863-878.

Bi Y., Ehirchiou D., Kilts T.M., Inkson C.A., Embree M.C., Sonoyama W., Li L., Leet A.I., Seo B.M., Zhang L., Shi S. and Young M.F. (2007). Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat. Med. 13, 1219-1227.

Brehm W., Burk J., Delling U., Gittel C. and Ribitsch I. (2012). Stem cell-based tissue engineering in veterinary orthopaedics. Cell Tissue Res. 347, 677-688.

Carrade D.D., Owens S.D., Galuppo L.D., Vidal M.A., Ferraro G.L., Librach F., Buerchler S., Friedman M.S., Walker N.J. and Borjesson D.L. (2011). Clinicopathologic findings following intra-articular injection of autologous and allogeneic placentally derived equine

mesenchymal stem cells in horses. Cytotherapy 13, 419-430.

Chen X., Zou X.H., Yin G.L. and Ouyang H.W. (2009). Tendon tissue engineering with mesenchymal stem cells and biografts: an option for large tendon defects? Front. Biosci. (Schol Ed) 1, 23-32.

Chen L., Dong S.W., Liu J.P., Tao X., Tang K.L. and Xu J.Z. (2012). Synergy of tendon stem cells and platelet-rich plasma in tendon healing. J. Orthop. Res. 30, 991-997.

Chong A.K., Ang A.D., Goh J.C., Hui J.H., Lim A.Y., Lee E.H. and Lim B.H. (2007). Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit achilles tendon model. J. Bone Joint Surg. Am. 89, 74-81.

Corradetti B., Lange-Consiglio A., Barucca M., Cremonesi F. and Bizzaro D. (2011). Size-sieved subpopulations of mesenchymal stem cells from intervascular and perivascular equine umbilical cord matrix. Cell Prolif. 44, 330-342.

Crovace A., Lacitignola L., Rossi G. and Francioso E. (2010). Histological and immunohistochemical evaluation of autologous cultured bone marrow mesenchymal stem cells and bone marrow mononucleated cells in collagenase-induced tendinitis of equine superficial digital flexor tendon. Vet Med Int. 2010, 250978.

Delince P. and Ghafil D. (2012). Anterior cruciate ligament tears: conservative or surgical treatment? A critical review of the literature. Knee Surg. Sports Traumatol. Arthrosc, 20, 48-49.

de Mattos Carvalho A., Alves, A.L.G., de Oliveira P.G.G., Luis Emiliano Cisneros Álvarez L.E.C., Amorim R.L., Hussni C.A. and Elenice Deffune E. (2011). Isolation and immunophenotypic characterization of mesenchymal stem cells derived from equine species adipose tissue. J. Equine Vet. Sci. 31, 26-34.

Fisher M.B. and Mauck R.L. (2013). Tissue engineering and regenerative medicine: recent innovations and the transition to translation. Tissue Eng. part B. 19, 1-13.

Fortier L.A., Nixon A.J., Williams J. and Cable C.S. (1998). Isolation and chondrocytic differentiation of equine bone marrow-derived mesenchymal stem cells. Am. J. Vet. Res. 59, 1182-1187.

Gilbert W.T., Sellaro T.L. and Badylak S.F. (2006). Decellularization of tissues and organs. Biomat. 27, 3675-3683.

Godwin E.E., Young N.J., Dudhia J., Beamish I.C. and Smith R.K. (2011). Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. Equine Vet. J. 44, 25-32.

Guest D.J., Smith M.R. and Allen W.R. (2008). Monitoring the fate of autologous and allogeneic mesenchymal progenitor cells injected into the superficial digital flexor tendon of horses: preliminary study. Equine Vet. J. 40, 178-181.

Gulotta L.V., Kovacevic D., Ehteshami J.R., Dagher E., Packer J.D. and Rodeo S.A. (2009). Application of bone marrow derived mesenchymal stem cells in a Rotator cuff repair model. Am. J. Sports Med. 37, 2126-2133.

Hudson T.W., Zawko S., Deister C., Lundy S., Hu C.Y., Lee K. and Schmidt C.E. (2004). Optimized acellular nerve graft is immunologically tolerated and supports regeneration. Tissue Eng. 10, 1641-1651.

Koerner J., Nesic D., Romero J.D., Brehm W., Mainil-Varlet P. and Grogan S.P. (2006). Equine peripheral blood-derived progenitors in comparison to bone marrow-derived mesenchymal stem cells. Stem Cells 24, 1613-1619.

Koga H., Muneta T., Nagase T., Nimura A., Ju Y.J., Mochizuki T. and Sekiya I. (2008). Comparison of mesenchymal tissues-derived stem cells for *in vivo* chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res. 333, 207-215.

Kuo C.K, Marturano J.E. and Tuan R.S. (2010). Novel strategies in tendon and ligament tissue engineering: Advanced biomaterials and regeneration motifs. Sports Med. Arthrosc. Rehabil. Ther. Technol. 2, 20-33.

Macaulay A.A., Perfetti D.C. and Levine W.N. (2012). Anterior cruciate ligament graft choices. Sports Health 4, 63-68.

Maffulli N. and Del Buono A. (2012). Platelet plasma rich products in musculoskeletal medicine: any evidence? Surgeon. 10, 148-150.

Magnusson S.P., Langberg H. and Kjaer M. (2010). The pathogenesis of tendinopathy: balancing the response to loading. Nat. Rev. Rheumatol. 6, 262-268.

Martinello T., Bronzini I., Maccatrozzo L., Iacopetti I., Sampaolesi M., Mascarello F. and Patruno M. (2010). Cryopreservation does not affect the stem characteristics of multipotent cells isolated from equine peripheral blood. Tissue Eng. C. 16, 771-781.

Martinello T., Bronzini I., Maccatrozzo L., Mollo A., Sampaolesi M., Mascarello F., Decaminada M. and Patruno M. (2011). Canine adipose-derived-mesenchymal stem cells do not lose stem features after a long-term cryopreservation. Res. Vet. Sci. 91, 18-24.

Martinello T., Bronzini I., Volpin A., Vindigni V., Maccatrozzo L., Caporale G., Bassetto F. and Patruno M. (2012). Successful recellularization of human tendon scaffolds using adipose-derived mesenchymal stem cells and collagen gel. J. Tissue Eng. Regen. (in press).

Martinello T., Bronzini I., Perazzi A., Testoni S., De Benedictis G.M., Negro A., Caporale G., Mascarello F., Iacopetti I. and Patruno M. (2013). Effects of *in vivo* applications of peripheral blood-derived mesenchymal stromal cells (PB-MSCs) and platlet-rich plasma (PRP) on experimentally injured deep digital flexor tendons of sheep. J. Orthop. Res. 2, 306-314.

Nourissat G., Houard X., Sellam J., Duprez D. and Berenbaum F. (2013). Use of autologous growth factors in aging tendon and chronic tendinopathy. Front Biosci Jun 1;5:911-21.

Oakes B.W. (2004). Orthopaedic tissue engineering: from laboratory to the clinic. Med. J. Aust. 180 (5 Suppl), S35-38.

Oshiro W., Lou J., Xing X., Tu Y. and Manske P.R. (2003). Flexor tendon healing in the rat: a histologic and gene expression study. J. Hand Surg. Am. 28, 814-823.

Radcliffe C.H., Flaminio M.J. and Fortier L.A. (2010). Temporal analysis of equine bone marrow aspirate during establishment of putative mesenchymal progenitor cell populations. Stem Cells Dev. 19, 269-282.

Renzi S., Riccò S., Dotti S., Sesso L., Grolli S., Cornali M., Carlin S., Patruno M., Cinotti S. and Ferrari M. (2013). Autologous bone marrow mesenchymal stromal cells for regeneration of injured equine ligaments and tendons: A clinical report. Res. Vet. Sci. 95, 272-277.

Riley G. (2008). Tendinopathy: from basic science to treatment. Nat. Clin. Pract. Rheumatol. 4, 82-89.

Sadoghi P., Rosso C., Valderrabano V., Leithner A. and Vavken P. (2013). The role of platelets in the treatment of Achilles tendon injuries. J. Orthop. Res. 31, 111-118.

Sharma A.K., Bury M.I., Fuller N.J., Rozkiewicz D.I., Hota P.V., Kollhoff D.M., Webber M.J., Tapaskar N., Meisner J.W., Lariviere P.J., Destefano S., Wang D., Ameer G.A. and Cheng E.Y. (2012). Growth factor release from a chemically modified elastomeric poly (1,8-octanediol-co-citrate) thin film promotes angiogenesis *in vivo*. J.

Biomed. Mat. Res. 100, 561-570.

Sharma P. and Maffulli N. (2005). Tendon injury and tendinopathy: healing and repair. J. Bone Joint Surg. Am. 87, 187-202.

Siegel L., Vandenakker-Albanese C. and Siegel D. (2012). Anterior cruciate ligament injuries: anatomy, physiology, biomechanics, and management. Clin. J. Sport. Med. 2012; 22: 349-355.

Smith R.K. (2008). Mesenchymal stem cell therapy for equine tendinopathy. Disabil. Rehabil. 30, 1752-1758.

Thaker H. and Sharma A.K. (2012). Engaging stem cells for customized tendon regeneration. Stem Cells Int. 2012, 1-12.

Thevenot P., Nair A., Dey J., Yang J. and Tang L. (2008). Method to analyze three-dimensional cell distribution and infiltration in degradable scaffolds. Tissue Eng. 14, 319-331.

Uysal C.A., Tobita M., Hyakusoku H. and Mizuno H. (2012). Adipose-derived stem cells enhance primary tendon repair: biomechanical and immunohistochemical evaluation. J. Plast. Reconstr. Aesthet. Surg. 65, 1712-1719.

Vidal M.A., Kilroy G.E., Lopez M.J., Johnson J.R., Moore R.M. and Gimble J.M. (2007). Characterization of equine adipose tissue-derived stromal cells: adipogenic and osteogenic capacity and comparison with bone marrow-derived mesenchymal stromal cells. Vet. Surg. 36, 613-622.

Vieira C.P., De Aro A.A., Da Ré Guerra F., De Oliveira L.P., De Almeida Mdos S. and Rosa Pimentel E. (2013). Inflammatory process induced by carrageenan in adjacent tissue triggers the acute inflammation in deep digital flexor tendon of rats. Anat. Rec. 296, 1187-1195.

Wan Y.Q., Chen W., Yang J., Bei J. and Wang S. (2003). Biodegradable poly(L-lactide)-poly(ethylene glycol) multiblock copolymer: synthesis and evaluation of cell affinity. Biomaterials 24, 2195-2203.

Woon C.Y.L., Pridgen B.C., Kraus A., Bari S., Pham H. and Chang J. (2011). Optimization of human tendon tissue engineering: peracetic acid oxidation for enhanced reseeding of acellularized intrasynovial tendon. Plast. Recon. Surg. 127, 1107-1117.

Accepted November 7, 2013