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TENOGENIC POTENTIAL OF TENDON-DERIVED MESENCHYMAL STEM CELLS ISOLATED *POST-MORTEM*: IMPACT OF CRYOPRESERVATION

Levoz Marine*

Department of Morphology and Pathology, Histology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Poirier Wilfried

Department of Infectious and Parasitic Diseases, Mycology-Parasitology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Piret Joëlle

Department of Morphology and Pathology, Histology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Javaux Justine

Department of Infectious and Parasitic Diseases, Immunology-Vaccinology, FARAH, Quartier Vallée 2, B43b, Uliège, 4000 Liège, Belgium

Dubois Axel

Department of Morphology and Pathology, Embryology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Toppets Vinciane

Department of Morphology and Pathology, Histology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Vandenhove Benoît

Department of Morphology and Pathology, Embryology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Gillet Laurent

Department of Infectious and Parasitic Diseases, Mycology-Parasitology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Grobet Luc

Department of Morphology and Pathology, Embryology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

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Kaux Jean-François

Physical Medicine Service and Department of Motility Sciences, GIGA, Avenue de l'Hopital 11, B34, Uliège, 4000 Liège, Belgium

Antoine Nadine

Department of Morphology and Pathology, Histology, FARAH, Quartier Vallée 2, B43a, Uliège, 4000 Liège, Belgium

Abstract. *In situ* injection of mesenchymal stem cells appears as a promising treatment of tendinopathies. Tendon-derived mesenchymal stem cells (TDSCs)[1] are widely studied and show a lot of interesting characteristics for clinical use.

The aim of this study is to confirm the tenogenic potential of cryopreserved TDSCs and to confirm their ability to produce type I and/or type III collagens fibers in culture.

Tendon-derived mesenchymal stem cells are harvested from the tendon no later than 72 h *post-mortem*. Their tenogenic potential has been assessed by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis of different genes involved in tenogenic differentiation. The stemness phenotype of TDSCs has been confirmed by flow cytometry. In order to demonstrate their capacity to synthesize type I and III collagen fibers, a stimulation with Transforming Growth Factor-β1(TGF-β1) has been performed in culture.

TDSCs comply with the expected phenotype of mesenchymal stem cells as defined by ISCT. All the samples and conditions analyzed by flow cytometry were positive for CD105, CD90, CD29, CD44 and negative for MHC-II. Their tenogenic potential is also confirmed by qRT-PCR. TDSCs are metabolically active cells, showing an ultrastructure reflecting high level of proteins synthesis. Moreover, they are able to synthetize collagen fibers under stimulation by TGF-β1.

Freshly harvested or cryopreserved TDSCs are involved in the tenogenic pathway and are able to produce an extracellular collagenous network. These properties make them suitable for banking and subsequent clinical use for contributing to tendon repair.

Keywords. Horse, Tendinopathy, Tenogenic differentiation, Stemness, Collagens production, Mesenchymal stem cells.

[1] Abbreviations: MSCs, mesenchymal stem cells; TDSCs, tendon-derived mesenchymal stem cells; ISCT, International Society for Cell and Gene Therapy; DMSO, dimethyl sulfoxide; SCX, scleraxis; EGR-1, early growth response-1; COL1A1, collagen type I alpha 1 chain; COL3A1, collagen type III alpha 1 chain; TNC, tenascin; DCN, decorin; TNMD, tenomodulin; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; DPBS, Dulbecco's phosphate buffered saline; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; PS, penicillin-streptomycin; P3, passage three; P10, passage ten; PI, propidium iodide; PBMCs, peripheral blood mononuclear cells; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TGF-β1, transforming growth factor – beta1; HSC, hematopoietic stem cell; ˆ ECM, extracellular matrix; TEM, transmission electron microscope.

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1. Introduction

Tendinopathies are the most frequent musculoskeletal pathologies in athletic horses. The tendons most commonly involved are the superficial digital flexor tendon and the suspensory ligament (Thorpe et al., 2010). The organization of type I collagen bundles within the extracellular matrix enables the tendon to transmit muscle contraction forces, dampening impact loads generated by locomotor and athletic activities. Repeated overload or degenerative conditions, such as inappropriate training surfaces or program, chronic inflammation or micro-trauma induce accumulation of matrix "microdamage" leading to clinical injury (O'Brien et al., 2021).

Mesenchymal stem cells (MSCs) use is emerging as a promising therapeutic option for the treatment of tendinopathies. Capable of self- renewal and multilineage differentiation, they also play an important role in regulating the inflammatory microenvironment through secretion of pro- and anti-inflammatory cytokines (Ortved, 2018).

Recently, mesenchymal stem cells were isolated from *post-mortem* equine tendons (TDSCs) (Shikh Alsook et al., 2015), meeting the criteria established by the International Society for Cell and Gene Therapy (ISCT) (Saeedi et al., 2019).

Easily available without invasive procedure, these TDSCs can be used to establish cell banks. These offer many assets for regenerative medicine. Use of such cells from frozen stocks allows (i) to reduce considerably pre-treatment time, moreover, allowing convenient pre-injection microbiological safety tests or *in vitro* stimulation, (ii) to search for the best available donors, *e.g.* regarding age, sex, or other factors that may influence donor compatibility. It is noteworthy that, similarly to other stem cells, the younger the donors, the more effective the proliferation and differentiation potentials of TDSCs (Webb et al., 2016).

Establishing a cryobank of TDSCs for clinical use requires use of an optimal cryopreservation process allowing recovery of the higher achievable yield in live and biologically unchanged cells after thawing. The use of glycerol, as cryoprotectant, is employed routinely in our laboratory as a surrogate for dimethyl sulfoxide (DMSO) and it is demonstrated that glycerol preserves the characteristics of mesenchymal stem cells (Zhang et al., 2020).

Cryopreserved stem cells need to keep the specific genes expression pattern which prefigures their tenogenic potential. Scleraxis *(SCX)*, a transcription factor specific for the tendon and ligament lineages, is expressed at early stages of differentiation. To promote *SCX* expression, another transcription factor is required, Early Growth Response 1 (*EGR- 1*). It boosts tenogenesis from stem cells. Both are involved in the regulation of type I (*COL1A1*) and type III (*COL3A1*) collagen gene transcription directly implicated in network upgrowth in developing tendons (Gaut and Duprez, 2016). Other genes encoding matrix proteins, such as tenascin (*TNC*), tenomodulin (*TNMD*) and decorin (*DCN*) are also transcribed at later stages of differentiation (Donderwinkel et al., 2022).

The aim of this study is twofold, (i) to confirm the tenogenic potential of cryopreserved TDSCs by quantitative reverse transcription- polymerase chain reaction (qRT-PCR) analysis of the different genes

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involved in tenogenic differentiation and (ii) to demonstrate the capacity of TDSCs to synthesize type I and III collagen fibers *in vitro*.

2. Materials and methods

2.1. TDSCS ISOLATION, CULTURE AND CRYOPRESERVATION

TDSCs were isolated from the equine superficial digital flexor tendon within 72 h after death. The chosen horses were not affected by any apparent musculoskeletal disease and were sampled *post-mortem* in an age range from birth to four years old. The three horses used in our study were aged six, three and ten months respectively. The first two were euthanized and the third died of colon torsion. TDSCs were prepared as described in a previous study (Shikh Alsook et al., 2015). Briefly, tendons were harvested and cut into small fragments. The samples were digested with 0,2 % collagenase in Dulbecco's phosphate buffered saline (DPBS) for 18 h. The filtered solution containing cells was centrifuged and the cells were transferred to a T-flask containing 88 % of Dulbecco's modified Eagle medium (DMEM) low glucose with L-glutamine and with sodium pyruvate (Biowest, USA) + 10 % heat inactivated fetal bovine serum (FBS) (Biowest, USA) + 1 % penicillin (10,000UI/ml)-streptomycin (10,000 μg/ml) (PS) solution (Gibco, ThermoFisher Scientific, USA) + 1 % amphotericin B 100× (Biowest, USA). Cells were passaged upon reaching confluence. TDSCs were detached with trypsin-EDTA 1× diluted in PBS (Biowest, USA), the cells were resuspended in a larger volume of culture medium and cultured in a T-flask.

After three (P3) and ten (P10) passages, freshly isolated TDSCs were either used for experiments or stored in a cryopreservation solution containing 20 % glycerol BP229–1 (Thermo Fisher Scientific, USA) diluted in DMEM +1 % PS + 1 % amphotericin B and stored, after a slow cooling method (using Mister Frosty©), in liquid nitrogen until use. Before subsequent use, cryopreserved cells were thawed and rinsed in DMEM heated to 37 °C and resuspended in culture medium.

2.2. FLOW CYTOMETRY

Flow cytometry analysis using specific markers of stemness was performed on TDSCs harvested at P3 and P10 before and after 30 days of cryopreservation.

Briefly, TDSCs were detached using TrypLE Express Enzyme 1× (Thermo Fisher, USA) for 5 min at 37 °C. Collected cells were centrifugated at 300 *g* for 5 min. Cell pellets were resuspended and stored for 15 min at 4 °C in a solution containing FACS buffer (PBS + 0,5 % bovine serum albumin (BSA) (Roth, Deutschland) + 2 mM EDTA +0,01 % sodium azide (Thermo Fisher Scientific, USA)) supplemented with 2 % equine serum (Thermo Fisher Scientific, USA). After centrifugation and removal of blocking solution, TDSCs were incubated for 30 min at 4 °C in 100 μl of a solution with antibodies of interest. Table 1 summarises the specificities of each of the antibodies and the dilutions at which they were used.

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Table 1
Specific antibodies and dilutions used.[1]

Antibody	Clone	Host species	Target species	Fluorophore	Company	Dilution
CD105	SN6	Mouse	Human	Alexa 647	Southern Biotech	1:100
CD29	TS2/16	Mouse	Human	PE	Bio Legend	1:50
CD90	eBio5E10	Mouse	Human	PE-Cy7	Invitrogen	1:500
CD44	CVS20	Mouse	Horse	PE	Bio Rad	1:200
MHC-II	CVS18	Mouse	Horse	FITC	Bio Rad	1:100

To assess the viability of TDSCs, and evaluate cells death, at the end of incubation, cells were washed with FACS buffer and resuspended in FACS buffer containing propidium iodide (PI) as a viability marker and diluted at 1/2000 (Invitrogen, USA).

At the same time, peripheral blood mononuclear cells (PBMCs) were labelled with the same panel of antibodies following the same experimental protocol. These cells were used as controls to confirm antibody specificity.

Finally, the cells were analyzed using a four lasers BD LSRFortessa™

X-20 flow cytometer (BD Biosciences, Heidelberg, Germany) and FlowJo® software (Treestar, Ashland, OR, USA). Suppliers of all reagents for staining are specified in Table 1.

2.3. RNA EXTRACTION AND RT-QPCR

Total RNA was extracted from TDSCs at P3 and P10 before (freshly isolated cells) or after (cryopreserved cells) cryopreservation using ROTI©-Zol RNA solution (Roth, Deutschland). RNA was purified and subsequently treated with DNAse using the NucleoSpin® RNA kit (Macherey-Nagel, Deutschland), following to the manufacturer's protocol. A total of 1000 ng of the recovered RNA was reverse transcribed with oligoDT primers using Superscript IV Reverse Transcriptase kit (Invitrogen, California), according to the protocol supplied by the manufacturer. cDNA samples were diluted 10-fold and used as template for qPCR.

qPCR reactions were performed with PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, USA) and primers sequences are listed in Table 2. qPCR reactions were carried out on a CFX96 Real-Time System (Bio-Rad, USA) with the following thermal cycling program: one run of 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 30 s. Melting curve analysis (95 °C for 15 s and stepwise annealing from 65 to 95 with 1 °C increments) was performed immediately after amplification. The mRNA expression of TDSCs genes was calculated using the ΔCt method using *GAPDH* as a reference gene to normalize the expression levels of candidate genes (Cui et al., 2015). These analyses were carried out on TDSCs isolated from three tendons taken from different horses. An mRNA extraction was also performed on three mature tendon tissues.

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Table 2
Equine specific primer pairs.[2]

Gene Name		Primer sequence (5′ → 3′)	Product size (bp)	Reference	Genbank accession number	% of efficience
GAPDH	For:	GTGTCCCCACCCCTAACG	131	(Yang et al., 2019)	NM_001163856.1	92
	Rev:	AGTGTAGCCCAGGATGCC				
COL1A1	For:	GAGAGCATGACCGACGGATT	404	This study	XM_023652710.1	99
	Rev:	CAGACAGGGCCAATGTCGAT				
COL3A1	For:	CAAAGGAGAGCCAGGAGCAC	98	(Martinez et al., 2022)	AF 117954.1	100
	Rev:	CTCCAGGCGAACCATCTTTG				
SCX	For:	TACCTGGGTTTTCTTCTGGTCACT	51	(Doll et al., 2021)	NM_001105150.1	102
	Rev:	TATCAAAGACACAAGATGCCAGC				
TNC	For:	GGGCGGCCTGGAAATG	70	(Reed and Johnson, 2014)	XM_023628752.1	102
	Rev:	CAGGCTCTAACTCCTGGATGATG				
DCN	For:	TCTATGTGCGCTCTGCCATT	149	This study	XM_005606467.3	100
	Rev:	ACACTGCAGTTAGGTTTCCAGTA				
EGR-1	For:	GGACATGCTCACCTCTAGCC	109	This study	XM_001502553.5	95
	Rev:	ACATTCTGGAGAACCGAGGC				
TNMD	For:	GACCCCATGCTGGATGAGAG	153	This study	NM_001081822.1	123
	Rev:	GCAAGGCATGATGACACGAC				

2.4. COLLAGEN PRODUCTION

2.4.1. LIGHT MICROSCOPY

TDSCs at P3 and P10, both before and after cryopreservation, were distributed in 6-wells plates and cultured up to confluence. At this point, collagen stimulation medium was added to three wells and culture medium into the three remaining controls wells. The stimulation medium was composed of a solution of culture medium with 10 ng/ml Transforming Growth Factor β1 (TGF-β1) (Human TGF-beta 1 Recombinant Protein, *HEK293 cells*, PeproTech®). Cells were cultured for seven days until fibrils could be observed under inverted optical microscope. At this time, TDSCs were fixed with formol, stained with "Sirius Red/Fast Green Collagen Staining Kit" (Chondrex, USA) and analyzed qualitatively by microscopy.

2.4.2. TRANSMISSION ELECTRON MICROSCOPY

TGF-β1 1 stimulated TDSCs were mechanically detached from the plate and centrifugate. The cell pellet was fixed with 2,5 % glutaraldehyde solution in 0.1 M sodium cacodylate buffer (pH 7,4, 2 h at 4 °C) and postfixed with 1 % osmium tetroxide 2 h at room temperature. The sample was dehydrated in graded ethanol solutions, infiltrated and embedded in EPON 812 (Fluka) according to standard procedure. Briefly, ultra-thin sections (70–90 nm) were collected on copper grids (200 mesh) and contrasted with 2 % (*w*/*v*) alcoholic uranyl acetate and Reynolds lead citrate before examination with an EM910 transmission electron microscope (TEM) (Zeiss). The images were captured using Jenoptik Germany ProgRes Capture Pro 2.5.

2.5. STATISTICAL ANALYSES

Statistical analyses for the qRT-PCR results were performed using R software (R4.4.1 The R project for statistical computing). To determine significance, ANOVA was carried out and a $P \leq 0,05$ was considered significant.

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3. Results

3.1. TENDON-DERIVED MESENCHYMAL STEM CELLS PHENOTYPE

Flow cytometry data were analyzed concerning the expression of four MSCs specific cell surface molecules (*i.e.* CD105, CD90, CD29 and CD44) and one hematopoietic stem cell (HSC) marker, which is not expected to be expressed by MSCs (MHC-II).

Relevant cells were first defined based on their forward and sideward scatter. The analysis of the markers was carried out only on the population of live cells that were not labelled with PI. It should be noted that the percentage of dead cells in the studied cell populations was on average 2–3 %, which appears very low.

High expression levels were observed for MSC-specific markers. This important expression is representative for all the labels in all the conditions, while, as expected, the major histocompatibility complex II was very weakly expressed (on average 2 %). TDSCs maintained obviously the phenotype of mesenchymal stem cells (Fig. 1).

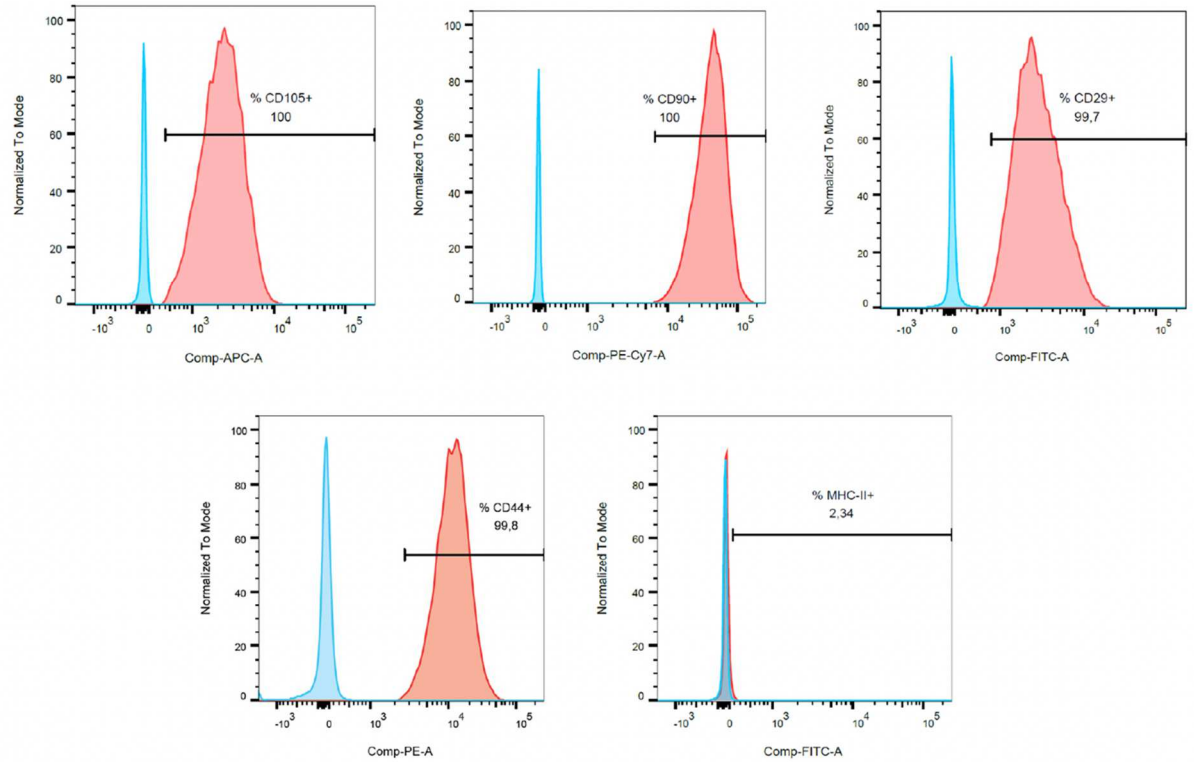

Fig. 1. Tendon-derived mesenchymal stem cells phenotype. The blue histograms represent non marked cells, the red histograms show the cell surface marker staining. Results are presented after normalization of the percentage of interest cells. TDSCs have the same phenotype at passage 3 and 10 and for each passage after cryopreservation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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3.2. NUMBER OF PASSAGES AND CRYOPRESERVATION DO NOT IMPACT EXPRESSION LEVELS IN TDSCS

The tenogenic potential of TDSCs was assessed by measuring the mRNA expression of seven genes (*COL1A1*, *COL3A1*, *SCX*, *TNC*, *DCN*, *EGR-1*, *TNMD*) using RT-qPCR after total RNA extraction under different conditions (Fig. 2 & S1). Except for the *SCX* gene, which was significantly more overexpressed at P3 compared to P10 in freshly isolated cells, all other genes exhibited the same expression profile across the different conditions studied. Expression of the gene encoding tenomodulin (*TNMD)* was not detectable except for adult tendon tissue.

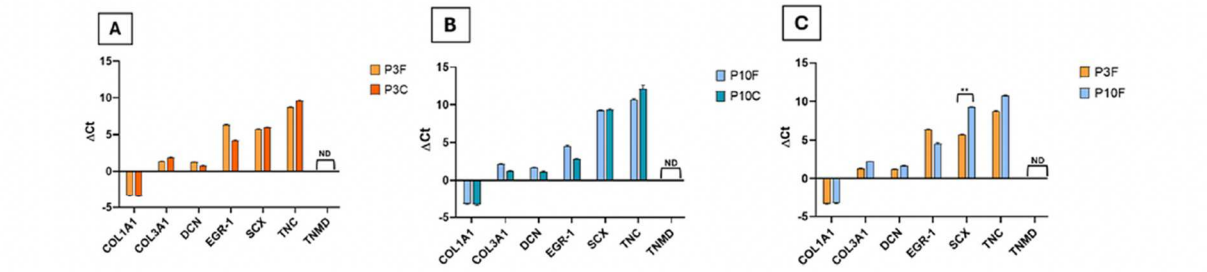

Fig. 2. Specific gene expression of tendon-derived mesenchymal stem cells tenogenic potential. The mRNA expression was assessed by RT-qPCR after total RNA extraction from TDSCs on (A) P3 and (B) P10 passages before and after cryopreservation, and (C) the mRNA expression level of freshly isolated cells was also compared between P3 and P10 ($n = 3 +$ SD; ANOVA; * $P < 0{,}01$ ND, undetected Ct > 45). Complete names and accession numbers of studied genes are listed in Table 2. Ct, threshold cycle; P3F, passage three freshly isolated cells; P10F, passage ten freshly isolated cells; P3C, passage three cryopreserved cells; P10C, passage three cryopreserved cells.

3.3. CONFIRMING THE ABILITY OF TDSCS TO PRODUCE COLLAGEN *IN VITRO*

3.3.1. LIGHT MICROSCOPY

As an anticipated use in regenerative medicine, TDSCs would be injected into a lesion site composed of a disorganized extracellular matrix. In order to mimic such environmental conditions, it seems important to assess their capacity to produce collagenous fibrillar proteins *in vitro*, after stimulation with Transforming Growth Factor β1 for 7 days. The same assay should be performed before and after cryopreservation to monitor influence of cryobanking on TDSCs therapeutic abilities.

To highlight this collagen production, cell evolution was monitored in culture. Fibrillar structures appeared from P3 to P10, both for freshly isolated and cryopreserved TDSCs.

Staining with Sirius Red-Fast Green in the culture wells shown red fibrillar collagen-like proteins in all the conditions studied. Compared with control, stimulated cells produced more collagen proteins (Fig. 3).

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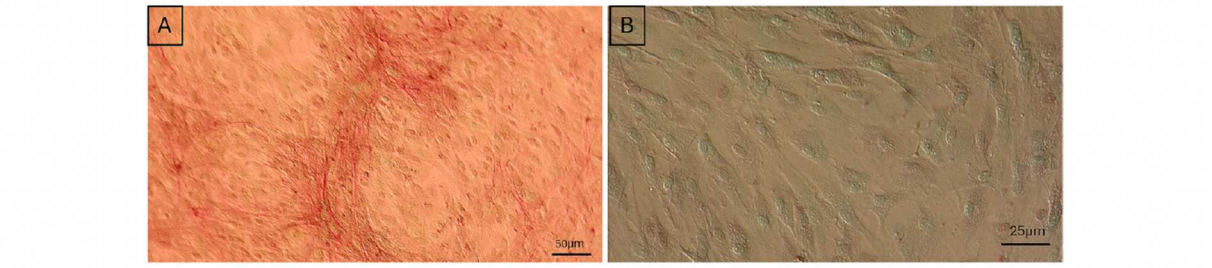

Fig. 3. Collagen production by tendon-derived mesenchymal stem cells. (A) Sirius Red – Fast Green stained TDSCs after 7 days in TGF-β1 stimulation medium and (B) Sirius Red – Fast Green stained TDSCs after 7 days in classic culture medium without stimulation. Collagen proteins are stained in red, other proteins are marked in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3.2. TRANSMISSION ELECTRON MICROSCOPY

TGF-β1-stimulated TDSCs were analyzed by transmission electron microscopy from P3 to P10, both for freshly isolated and cryopreserved TDSCs. Electron microscopy reveals characteristics of high metabolic activity. Indeed, TDSCs exhibit a decondensed nucleus and their cytoplasm contains a well-developed rough endoplasmic reticulum with particularly dilated cisternae. Primary and secondary lysosomes are also present. Cells were surrounded by an extracellular matrix composed of a large fibrillar network in close contact with the TDSCs membranes, as shown in Fig. 4c. These intercellular fibrillar structures displayed no particular organization and were devoid of striation.

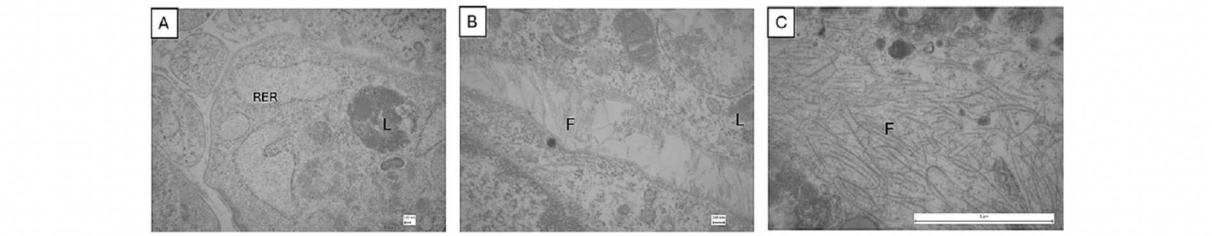

Fig. 4. Collagen production by tendon-derived mesenchymal stem cells highlighted by TEM. Ultrathin sections of TDSCs. (A) Abundance of rough endoplasmic reticulum (RER) with dilated cisternae. Primary and secondary lysosomes (L) are also present. (B—C) Extracellular matrix composed of fibrillar network (F). Scale bars: A = 100 nm; B = 100 nm; C = 1 µm.

4. Discussion

The aim of this study was to evaluate *in vitro* the therapeutic potential of tendon-derived mesenchymal stem cells (TDSCs) for treatment of tendinopathies. This potential was assessed under fresh tissue derivation and after specific freezing conditions and at different cell culture times.

Flow cytometry analysis showed that TDSCs retained their mesenchymal stem cell phenotype in culture, *i.e.* expression of specific markers in accordance with the criteria required by the International Society for Cell and Gene Therapy (ISCT). During the culture of both freshly isolated and cryopreserved cells, this phenotype is retained at least until passage 10.

For use in tendon regenerative medicine, TDSCs must demonstrate their ability to engage in a tenogenic differentiation pathway. Quantitative PCR studies on TDSCs revealed the expression of *SCX* and *EGR-1* transcription factors. These factors are implicated in the induction of tenogenesis and drive

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the expression of tenogenic extracellular matrix (ECM) components, such as collagen type I and III, decorin and tenomodulin (Gaut and Duprez, 2016).

The freezing process may have an impact on the expression of the *Early Growth Response-1* (*EGR-1*) gene. Although not significant, there is a trend towards increased expression of the *EGR-1* gene after the thawing procedure. Increased expression of EGR-1, a transcription factor, positively influences the expression of genes involved in tenogenic differentiation. The use of glycerol as a cryoprotectant probably attenuated the deleterious effects of freezing, explaining the non-significant differences in *EGR-1* expression under our deep-freeze conditions (Bahsoun et al., 2019; Periasamy et al., 2015).

Contrary to what is observed for *EGR-1* expression, a significant decrease in *scleraxis* (*SCX*) expression is observed after ten passages. *SCX* is present in tenogenic precursors cells and in tenocytes during tendon development (Sakabe et al., 2018). It is commonly accepted that *EGR-1 rather than SCX* is a master regulatory gene of tendon differentiation. Yet *SCX* remains a unique marker for monitoring early tendon development (Gaut and Duprez, 2016). As described by Webb and colleagues (2016), *SCX* expression levels are high in freshly isolated TDSCs, but decrease after several passages, remarkably after passage four. This may be linked to the significant decrease observed in *SCX* expression from the fetal stage to adulthood.

SCX expression is also essential for the expression of tenomodulin (*TNMD*), a crucial marker of differentiated tenocytes (Gaut and Duprez, 2016; Li et al., 2015). Regardless of the experimental conditions, and despite the expression of *SCX* gene, *TNMD* expression was undetectable by qRT-PCR, whereas the genes coding for other matrix components (decorin, tenascin, collagens) were expressed under all conditions.

Based on the gene expression profile, we can suggest that TDSCs (i) are well engaged in the tenogenic differentiation pathway, (ii) are at a tendon progenitor stem cell stage in agreement with Donderwinkel et al. (2022), (iii) express genes required for the synthesis of the early matrix constituents and (iv) do not express the gene coding for tenomodulin, a marker expressed at later differentiation stages.

After stimulation by TGF-β1, known to stimulate the production of collagen I and III in particular, freshly or cryopreserved TDSCs are able to synthesize fibrous collagen proteins from passage 3 until passage 10 at high level. Ultrastructural studies of TDSCs revealed metabolically active cells with extensive development of organelles involved in protein synthesis. A significant fibrillar network is present in the extracellular matrix. The absence of striation suggests that these fibrils are III or V collagen type. The precise nature of these fibrillar proteins will be studied in following immunocytochemical approaches and western-blot analysis.

In conclusion, the results obtained in this study confirm that under our experimental conditions, both freshly harvested or cryopreserved TDSCs are committed to the tenogenic pathway and are capable of synthesizing and producing an extracellular collagenous network. These two properties are not visibly impacted by culture passages until P10. These abilities are essentials to build up TDSCs banks requiring generation, amplification and cryopreservation of large quantities of cells.

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The next step will be to demonstrate, through a clinical study, that these cells, when injected at the injury site, promote effective and long- term tendon repair. Preliminary results obtained in a few horses treated for tendinopathy are promising. Follow-up of treated horses will be necessary to ensure that no relapses occur in the long term, as is often the case with current treatments. A sufficient sample size of treated horses will also be essential to confirm the safety and efficacy of the TDSCs-based treatment. Confirming the clinical results would make possible to envisage a clinical study in humans, the horse being considered as the model of choice for human tendon pathologies (Smith et al., 2023).

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CRediT authorship contribution statement

Levoz Marine: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Poirier Wilfried:** Writing – review & editing, Validation, Software, Resources, Formal analysis, Conceptualization. **Piret Joelle:** ¨ Resources, Investigation. **Javaux Justine:** Resources, Investigation. **Dubois Axel:** Writing – review & editing, Validation, Resources, Conceptualization. **Toppets Vinciane:** Visualization, Conceptualization. **Vandenhove Benoît:** Resources, Methodology, Investigation. **Gillet Laurent:** Writing – review & editing, Resources, Funding acquisition. **Grobet Luc:** Writing – review & editing, Funding acquisition, Conceptualization. **Kaux Jean-François:** Writing – review & editing, Conceptualization. **Antoine Nadine:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Informed consent and patient details

An informed consent document was signed by the owners of the 3 horses whose tendons were harvested at necropsy to generate the tendon-derived mesenchymal stem cells studied in this article. They can be provided on request. A blank specimen will be provided.

Declaration of competing interest

Authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2025.105722.

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Data availability

The data that support the findings of this study are openly available in Levoz Data at https://doi.org/10.6084/m9.figshare.28512461 - reference number [28512461].

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