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Full length article

Equine adult, fetal and ESC-tenocytes have differential migratory, proliferative and gene expression responses to factors upregulated in the injured tendon

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ABSTRACT

Tendon injuries are a common problem in humans and horses. There is a high re-injury rate in both species due to the poor regeneration of adult tendon and the resulting formation of scar tissue. In contrast, fetal tendon injuries undergo scarless regeneration, but the mechanisms which underpin this are poorly defined. It is also unclear if tendon cells derived from embryonic stem cells (ESCs) would aid tendon regeneration. In this study we determined the responses of adult, fetal and ESC-derived equine tenocytes to a range of cytokines, chemokines and growth factors that are upregulated following a tendon injury using both 2-dimensional (2D) and 3-dimensional (3D) in vitro wound models.

We demonstrated that in 2D proliferation assays, the responses of fetal and adult tenocytes to the factors tested are more similar to each other than to ESC-tenocytes. However, in 2D migration assays, fetal tenocytes have similarities to both adult and ESC-tenocytes. In 3D wound closure assays the response of fetal tenocytes also appears to be intermediary between adult and ESC-tenocytes. We further demonstrated that while TGFβ3 increases 3D gel contraction and wound healing by adult and fetal tenocytes, FGF2 results in a significant inhibition by adult cells.

In conclusion, our findings suggest that differential cellular responses to the factors upregulated following a tendon injury may be involved in determining if tendon repair or regeneration subsequently occurs. Understanding the mechanisms behind these responses is required to inform the development of cell-based therapies to improve tendon regeneration.

Introduction

Tendon injuries are common in both humans and horses. In humans, tendon injuries occur in professional and amateur athletes (Gurau et al., 2023) and the military (DeFoor et al., 2024), but also in workers and other people with a sedentary lifestyle (Steinmann et al., 2020). Tendon injuries therefore have a significant economic burden (Hopkins et al., 2016). In horses, tendon injuries account for up to 46 % of all limb injuries in Thoroughbred racehorses (Williams et al., 2001; Avella et al., 2009) and also occur in other horses taking part in a wide range of sporting activities (Williams et al., 2001; Inness and Morgan, 2015; Misheff, 2010; Singer et al., 2008; Murray et al., 2006; Murray et al., 2010). Tendon injuries are a leading cause of retirement (Lam et al., 2007) because up to 67 % of horses will re-injure upon their return to work (Dyson, 2004). In both horses and humans, high rates of tendon re-injury have been attributed to poor tissue regeneration and the formation of scar tissue during tendon repair (Nichols et al., 2019). The mechanisms behind scar tissue formation are largely unknown. However, horses provide a relevant, large animal model for human tendon injuries (Patterson-Kane et al., 2012; Zhang et al., 2022) and may be useful to determine the cellular processes underpinning repair.

In contrast to adult tendon injuries, fetal tendon injuries undergo

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scarless regeneration (Beredjiklian et al., 2003). This is intrinsic to the fetal cells, as fetal tendons transplanted into adults retain their regenerative capacity (Favata et al., 2006; Tang et al., 2014). We have previously derived equine embryonic stem cells (ESCs) (Guest and Allen, 2007; Li et al., 2006) that can differentiate into tenocytes in vivo (Guest et al., 2010) and in vitro (Barsby et al., 2014; Barsby and Guest, 2013). We demonstrated that ESC-tenocytes are more similar to fetal than adult tenocytes in their global gene expression profiles when cultured in a 3-dimensional (3D) collagen matrix, but still represent a unique population of cells (Paterson et al., 2020a). Many of the genes that are differentially expressed in adult and fetal tenocytes are involved in cell migration and inflammatory responses. These include genes encoding a range of cytokines, chemokines, growth factors and their receptors (Paterson et al., 2020a), and several of these factors are upregulated in the injured tendon.

Following a tendon injury there is an influx of cells, including immune cells and tenocytes, which secrete a range of factors that can influence cell behaviour (such as proliferation, migration, differentiation, gene expression). We have previously demonstrated that they can have differential effects on tenocytes representing different stages of development. For example, inflammatory cytokines are upregulated following a tendon injury (Morita et al., 2017) and have detrimental effects on adult tenocyte gene expression and ability to contract a collagen gel (Beaumont et al., 2023; Smith et al., 2023), whilst ESC-tenocytes are less responsive to the same cytokines (Smith et al., 2024; McClellan et al., 2019a). A range of growth factors are also upregulated following an injury (Wang and Li, 2023). Some of the proteins produced following fetal and adult tendon injuries differ (Ribitsch et al., 2021), but we hypothesise that the cellular response to these factors may also be different in tenocytes representing the different stages of development. Understanding how adult and fetal tenocytes respond to factors that are upregulated during adult tendon injury repair may help identify mechanisms that underpin regeneration and aid in developing stem cell-based therapies.

The aim of this study was to determine if the factors upregulated following a tendon injury have differential effects on adult, fetal and ESC-tenocytes in terms of cell proliferation, cell migration, wound healing and gene expression using 2D and 3D wound models.

Materials and methods

Study design

An overview of our experimental approach is provided in the graphical abstract. Initial experiments were performed to test the effect of 11 factors on the proliferation and migration of adult, fetal and ESC-tenocytes cultured individually (monoculture) in 2D. For five of these factors, we then performed 2D co-culture migration assays using combinations of fetal plus adult tenocytes, ESC-tenocytes plus adult tenocytes and ESC-tenocytes plus fetal tenocytes. We also measured the effects of these five factors on cell viability and wound closure in a monoculture 3D wound model. Based on these results, we then measured gene expression and cell distribution in our 3D monoculture models in response to TGFβ3 and FGF2.

Tenocyte isolation and culture

Tenocytes were isolated from the superficial digital flexor tendon of 10 adult Thoroughbred or Thoroughbred-type horses (aged 2–11, seven males and three females) that were euthanised for reasons unrelated to this study. Tenocytes were also isolated from the superficial digital flexor tendon of 4 fetal Thoroughbreds aged 271–321 days of gestation (one male and three females) that had undergone spontaneous abortion. All cells were isolated and used with the approval of the Royal Veterinary College Clinical Research Ethical Review Board (URN 2020 2017-2).

Tendon tissue was cut into small pieces and digested with 1 mg/mL type I collagenase from *Clostridium histolyticum* (Sigma-Aldrich, Dorset, UK) for 12–16 h at 37 °C to isolate cells. Tenocytes were cultured in tendon growth media; Dulbecco's modified Eagle's medium (DMEM) high glucose (4500 mg/mL), with 10 % fetal bovine serum (FBS), 1 % penicillin-streptomycin (P/S) and 2 mM L-glutamine (all Gibco, Thermo Fisher, Hemel Hempstead, UK). Tenocytes were cultured at 37 °C, 5 % CO_2 and passaged every 3–4 days with 0.25 % trypsin-EDTA (Sigma-Aldrich). All tenocytes were used at a maximum of passage 7 in the below experiments.

Labelling tenocytes with green fluorescent protein (GFP)

A lentiviral vector was used to generate adult and fetal tenocytes stably expressing GFP. HEK293T cells (RRID:CVCL_0063) were plated at a density of 1×10^5 cells per well of a 6 well plate in tendon growth media without P/S. 24 h after plating, the cells were transfected with 1 μg TRC2-pLKO-puro Turbo GFP (SHC203; Sigma-Aldrich), 750 ng psPAX2 and 250 ng pMD2.G per well, using FuGENE 6 transfection reagent (Promega, Hampshire, UK) according to the manufacturer's instructions. pMD2.G and psPAX2 were a gift from Didier Trono (Addgene plasmids #12259 and 12260; http://n2t.net/addgene:12259 http://n2t.net/addgene:12260; RRID: Addgene_12259 and 12260).

The packaging cell supernatant containing the lentivirus was collected 72 h post transfection, filtered through a 0.45 μm filter (Merck Millipore, Watford, UK) and used immediately to infect the target tenocytes in the presence of 10 μg/mL polybrene (Sigma-Aldrich). The tenocytes had been seeded at 1×10^5 cells per well of a 6-well plate 24 h before transduction. Following transduction, GFP positive tenocytes were selected with 4 μg/mL of puromycin (Sigma-Aldrich), which was optimised using an antibiotic kill curve on non-transduced cells.

ESC culture and 2D tenocyte differentiation

Three lines of equine ESCs (one male, two female) that had been previously derived and characterised (Guest and Allen, 2007; Paterson et al., 2020a; McClellan et al., 2019b) were used in this study with the approval of the Royal Veterinary College Clinical Research Ethical Review Board (URN 2020 2017–2). Undifferentiated ESCs were cultured on a feeder layer of mouse embryonic fibroblasts that had been mitotically inactivated using 10 μg/mL mitomycin C from *Streptomyces caespitosus* (Sigma-Aldrich) for 2 h at 37 °C. ESC base media consisted of DMEM/F12 containing 15 % FBS, 2 mM L-glutamine, 1 % non-essential amino acids, 1 mM sodium pyruvate, 0.1 mM 2-mercaptoethanol (all from Gibco, Thermo Fisher). For the growth of undifferentiated ESCs (ESC growth media), ESC base media was supplemented with 1000 Units/mL recombinant human leukaemia inhibitory factor (LIF; Peprotech, London, UK). Growth media was changed daily and the ESCs were passaged mechanically every 6–8 days in the presence of 2 μM Thiazovivin (Miltenyi Biotech, Woking, UK).

For differentiation into tenocytes in 2D culture, the ESCs were cultured for 14 days in the absence of feeders and in ESC base media supplemented with 20 ng/mL TGF-β3 (Peprotech) (ESC-differentiation media).

2D proliferation assays

Adult and fetal cells were passaged using trypsin-EDTA and seeded at 5000 cells/well of a 96-well plate. 24 h after seeding, media was replaced with tendon growth media containing a reduced FBS content of 2 %, with the addition of one of the 11 factors at three different doses (listed in Table 1), or the corresponding vehicle controls (phosphate buffered saline (PBS) or water at 1:100–1:1000). Cells were maintained under these conditions for a further 72 h prior to measuring cell numbers using the CyQUANT™ NF Cell Proliferation Assay (Thermo Fisher) according to the manufacturer's protocol.

ESC-tenocytes were produced as described above and passaged using TrypLE™ Select x10 (Thermo Fisher) prior to seeding at 5000 cells/well of a 96-well plate in normal ESC base media in the presence of 2 μM Thiazovivin. 24 h after seeding, media was replaced with ESC base

Table 1
Information on the eleven factors used in the study. Where human proteins have been used, the sequence similarity between the recombinant protein and the equine protein has been included.

Protein	Supplier	Catalogue number	Species	Dose (ng/ml)			Reason for selection	Expression in injured tendon	Effect on migration
				Low	Medium	High			
TGFβ3	Peprotech	100-36E	Human (100 %)	5	25	100	Differential effect on adult and fetal tenocyte migration (Paterson et al., 2020a)	(Miescher et al., 2023)	Differential effect on adult and fetal tenocytes (Paterson et al., 2020a)
FGF2	Peprotech	100-18B	Human (98 %)	10	20	50	Differential expression of receptors (Suppl. Fig. 1)	(Molloy et al., 2003)	Promotes skin fibroblast migration (Song et al., 2016)
PDGFBB	Peprotech	100-14B	Human (95 %)	5	30	50	Differential expression of receptors and gene (Suppl. Fig. 1)	(Molloy et al., 2003)	Promotes skin fibroblast migration (Li et al., 2004)
IGF1	Peprotech	100-11	Human (98 %)	150	300	500	Differential expression of receptors and gene (Suppl. Fig. 1)	(Dahlgren et al., 2005)	Promotes BMSC migration (Lin et al., 2020)
SDF1	Peprotech	300-28A	Human (97 %)	50	100	200	Differential expression of gene (Suppl. Fig. 1)	(Shimode et al., 2009)	Promotes MSC migration (Nakamura et al., 2013)
IL1β	Peprotech	200-01B	Human (80 %)	5	10	17	Differential expression of receptors and gene (Suppl. Fig. 1)	(Morita et al., 2017)	Promotes MSC migration (Chen et al., 2018)
TNFα	Peprotech	300-01A	Human (96 %)	10	20	100	Differential expression of receptors and gene (Suppl. Fig. 1)	(Morita et al., 2017)	Promotes fibroblast migration (Postlethwaite and Seyer, 1990)
IFNγ	R&D systems	1586-HG-025	Equine	10	20	100	Differential expression of receptors and gene (Suppl. Fig. 1)	(Morita et al., 2017)	Inhibits fibroblast migration (Adelmann-Grill et al., 1987)
IL8	Kingfisher Biotech	RP03123-005	Equine	1	10	100	Differential expression of gene (Suppl. Fig. 1)	(Morita et al., 2017)	Promotes fibroblast migration (Dunlevy and Couchman, 1995)
IL10	R&D systems	1605-IL-010/CF	Equine	10	25	50	Differential expression of gene (Suppl. Fig. 1)	(Morita et al., 2017)	Promotes tendon-derived stem cell migration (Deng et al., 2018)
FG9	Peprotech	100–23	Human (100 %)	5	20	100	Differential expression of receptors and gene (Suppl. Fig. 1)	(Mao et al., 2020)	Promotes lung fibroblast migration (Joannes et al., 2016)

media but containing only 2 % FBS with the addition of one of the 11 factors or vehicle controls. Cells were maintained for 72 h prior to measuring cell numbers as above.

As a positive control, cells were cultured in normal serum levels (10 % FBS for adult and fetal tenocytes, 15 % FBS for ESC-tenocytes) and cell numbers measured as described. As a negative control mitomycin C treated tenocytes were utilised. 10 μg/mL of mitomycin C was applied for 2 h at 37 °C, 24 h post-seeding to ensure that no proliferation occurred over the subsequent 72 h.

Monoculture 2D migration assays

Prior to cell seeding, 24-well plates were coated with rat tail collagen I (Sigma, 122–20) at 5 μg/cm^2 for 2 h at 37 °C. After coating, the collagen was removed, and wells were washed with 0.5 mL PBS prior to adding Ibidi culture-inserts to each well (Thistle Scientific, Rugby, UK). Subsequently, 2.8×10^4 of mitotically inactivated (using mitomycin C as described above) adult, fetal and ESC-tenocytes were seeded in each chamber of the Ibidi culture-inserts (5.6×10^4 cells/insert) in 70 μL of either tendon growth media (for adult and fetal tenocytes) or ESC base media containing 15 % FBS and 2 μM thiazovivin (for ESC-tenocytes). After 24 h (i.e. day 1), inserts were removed and each well was briefly washed with 0.5 mL PBS prior to adding 0.5 mL of fresh corresponding 2 % FBS media containing one of the proteins at a specific concentration (Table 1) or vehicle control (PBS or water at 1:100–1:1000) for 72 h (i.e. days 1–4). We also assessed migration in low (2 %) versus high (10 % for adult and fetal tenocytes or 15 % for ESC-tenocytes) FBS media for all cell types. Images of the same field of view were acquired every 20 mins with a ZenCELL microscope (LabLogic, Sheffield, UK) and the change in open wound area (i.e. percentage of the image without cells) was calculated with TScratch software (Gebäck et al., 2009) to provide an index of tenocyte migration over the 72 h stimulation period.

2D co-culture migration assays

Ibidi culture-inserts were set up on collagen coated 24-well plates as described above. 2.8×10^4 cells (not mitotically inactivated) were then plated in each side of the chamber. In this experiment the following combinations of cells were used: unlabelled ESC-tenocytes (produced as described above and seeded in 70 μL of ESC-base media with 2 μM Thiazovivin) plus adult tenocytes labelled with GFP; ESC-tenocytes plus fetal cells labelled with GFP; and adult tenocytes labelled with GFP plus unlabelled fetal tenocytes. i.e. 5.6×10^4 cells were seeded per insert in total made up of a 50:50 ratio of the two cell types being investigated. Adult and fetal cells were seeded in 70 μL tendon growth media. Cells were then left to attach for 24 h prior to removing the inserts, washing in PBS and adding 0.5 mL of media containing 2 % serum to each well (day 0). Where ESC-tenocytes were present, the media used was ESC base media (2 % serum). Where no ESC-tenocytes were present, the media used was tendon growth media (2 % serum). The following conditions were tested: 2 % serum alone, high serum (positive control, 10 % used for tendon growth media, 15 % used for ESC base media), 5 ng/mL TGFB3, 10 ng/mL FGF2, 150 ng/mL IGF1, 5 ng/mL PDGFBB, and 10 ng/mL IL10. Images were taken at day 0, day 1 and day 2 on a EVOS FL (Thermo Fisher). Wound closure for these experiments was measured using the Image J plugin Wound_healing_size_tool (Suarez-Arnedo et al., 2020).

3D culture and wound model

3D culture was carried out as previously described (Barsby et al., 2014); 6-well plates were coated with silicone (Sylgard 184 Silicone elastomer; Corning) and three pairs of 0.2 mm diameter minutien pins (InterFocus Fine Science Tools, Cambridge, UK) were placed 15 mm apart. For monoculture, cells (fetal or adult tenocytes, or small colony pieces of undifferentiated ESCs) were suspended at 4×10^5 cells/mL in a mixture of two parts corresponding growth media and eight parts PureCol (Bovine collagen type 1; Advanced Biomatrix, Carlsbad, USA) at pH 7.2–7.6. Immediately, 200 μL of collagen-cell suspension was pipetted between each pair of minutien pins prior to incubation at 37 °C for 60 to 90 min until the collagen constructs had set.

For constructs containing a wound, the method was adapted and a third minutien pin was initially placed at the midpoint of each pair of minutien pins. On top of this, an inverted 200 μL pipette tip (with the pipette receiving end cut off to allow the plate lid to fit) was positioned. The collagen mixture was then pipetted around and between the end pair of minutien pins, passing around either side of the central, inverted tip and the collagen was set as described above.

Following setting, the parafilm was removed and growth media was added to the constructs and they were cultured for 72 h at 37 °C before the central tip and minutien pin were removed to leave a core lesion in

the "wounded" constructs. At this point, media was replaced with growth media alone or growth media containing one of either 10 ng/mL FGF2, 5 ng/mL TGFβ3, 10 ng/mL IL10, 5 ng/mL PDGFBB, or 150 ng/mL IGF1. Media (with or without factors) was replaced every three to four days and the constructs cultured for up to 11 further days (i.e. a total of 14 days of culture). The growth media used was either ESC base media for the ESCs or tendon growth media for the fetal/adult tenocytes. 2 % and 7.5 % serum concentrations were initially tested, but collagen constructs containing fetal tenocytes and ESCs failed to contract in 2 % serum, therefore 7.5 % was taken forward for further experiments.

The 3D constructs were imaged daily, and contraction and wound closure (wound height and width) was measured using ImageJ (National Institutes of Health, USA). All data are shown as a percentage of the day 3 value. Cell viability, gene expression and histological analyses were performed as described below.

PrestoBlue cell viability assay in the 3D wound model

Cell viability after 14 days of 3D wound monoculture (and no wound control) was measured using PrestoBlue™ (Thermo Fisher) according to the manufacturer's instructions in monocultured fetal, adult or ESC-tenocytes in the presence or absence of 10 ng/mL FGF2, 5 ng/mL TGFβ3, 10 ng/mL IL10, 5 ng/mL PDGFBB, or 150 ng/mL IGF1. Briefly, for each well of a 6-well plate that contained three gels, the media was replaced with 2.7 mL of fresh media and 300 μL of PrestoBlue™ reagent and incubated for 4 h at 37 °C. Subsequently, 100 μL of this supernatant was transferred to a 96-well plate and analysed on a TECAN plate reader (Infinite M Plex, Tecan, Switzerland) at excitation 560 nM, emission 590 nM. Media plus PrestoBlue™ without cell culture incubation served as the background control. Values are expressed as a fold change relative to the control (i.e. no factors added).

Interleukin 6 ELISA

3D monoculture wound models (and no wound controls) were set up as described above using either adult, fetal or ESC-tenocytes in the presence and absence of either 5 ng/mL TGFβ3 or 10 ng/mL FGF2 (7.5 % serum). Conditioned media was collected at day 7 and stored at −70 °C until use. IL6 was measured using an equine IL6 ELISA kit (R&D systems, US) according to the manufacturer's instructions and measured on a TECAN plate reader at 450 nm with background correction at 540 nm. A seven-point standard curve with four-parameter logistic regression was used to calculate absolute IL6 concentrations (pg/mL). This ELISA has been previously validated to detect equine IL6 (Beaumont et al., 2023; Smith et al., 2024).

RNA extraction, cDNA synthesis and qPCR

3D monocultures with or without a wound and containing either ESC tenocytes, adult tenocytes or fetal tenocytes were set up as described above. In constructs containing a wound this was performed under control media conditions, or growth media supplemented with either 5 ng/mL TGFβ3 or 10 ng/mL FGF2 (7.5 % serum). After 7 days, nine collagen constructs per condition were harvested into TRI Reagent™ (Sigma-Aldrich). RNA was isolated using a RNeasy mini kit (Qiagen, Manchester, UK). Genomic DNA was removed using the DNA-free™ DNA removal kit (Thermo Fisher) and RNA concentration measured using a DeNovix DS-11 Spectrophotometer (DeNovix, Wilmington, USA). 1 μg of RNA was reverse transcribed using a SensiFAST™ cDNA Synthesis Kit (Bioline, London, UK). 20 ng of cDNA was then used in qPCR reactions using SYBR Green containing supermix (Bioline) and equine primers as listed in Supplementary Table 1. qPCR was performed on a C1000 Touch Thermal Cycler (BioRad, Hertforshire, UK). Cycling parameters were 95 °C for 10 min, followed by 45 cycles of 95 °C (15 s), 60 °C (15 s) and 72 °C (15 s). Following this, a melt curve was generated with readings taken every 1 °C from 65 °C to 95 °C. Gene expression was normalised to the *18 s rRNA* housekeeping gene using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Histological analysis of the 3D wound models

Monoculture 3D gels were harvested after 7 days and fixed in 3 % paraformaldehyde for 60 min at room temperature. Fixed gels were then immediately embedded in CellPath OCT compound (Thermo Fisher) on cork discs and snap frozen in liquid nitrogen-cooled isopentane (Thermo Fisher). Longitudinal sections of 11 μm thick were cut using a Bright OTF5000 cryostat (Bright Instruments, Huntingdon, UK), fixed for 10 min at room temperature in 100 % acetone (Thermo Fisher) and stored at −20 °C. Sections were used in H&E (ab245880, Abcam, Cambridge, UK) staining according to the manufacturer's instructions. Images were captured using a Nikon Eclipse Ti2 series microscope (Nikon, Surrey, UK).

Statistical analysis

All statistical analysis was performed with SPSS (version 28.0; IBM, UK, SPSS (RRID:SCR_002865)). An independent *t*-test was utilised to compare two means. When greater than two means were compared, a one-way ANOVA with Tukey *post-hoc* was utilised. Normality was examined with the Shapiro–Wilk test and the distribution of variances was determined with Levene's test of homogeneity. If these assumptions were violated, data was log-transformed prior to analysis. If this did not result in a normal distribution of the data, the non-parametric Kruskal–Wallis test was performed followed by Dunn's pairwise comparisons adjusted with a Bonferroni correction. If log transformation did not result in equal variance of the data, the Welch's ANOVA with a Games Howell *post-hoc* was employed. Variables measured over time were analysed with a one-way repeated factor ANOVA. If the assumption of sphericity was violated, a greenhouse–geisser correction was applied. When two or more groups were analysed over time, a two-way or three-way mixed factor ANOVA with a Bonferroni post hoc correction for multiple comparisons was employed. In all cases $p < 0.05$ was considered statistically significant. Lastly, for the 2D co-culture migration experiments, Cohen's d effect sizes (ES) examined the magnitude of change in migration for each treatment relative to the unstimulated control and were interpreted as trivial (0–0.19), small (0.2–0.49), medium (0.5–0.79) or large (>0.8) as described (Cohen, 1992).

Results

Selection of factors for use in this study

Previous work comparing adult, fetal and ESC-tenocytes cultured in 3D demonstrated that Gene Ontology terms, including cell migration and cell mobility, were over-enriched when comparing genes that were differentially expressed between adult and fetal tenocytes (Paterson et al., 2020a). A range of chemokines, growth factors and growth factor binding proteins were associated with these terms (Supplementary Fig. 1). Eleven factors were selected based on the differential expression of the factor or its receptors, its upregulation following tendon injury, having a known role in migration and/or availability of equine proteins or similarity between the human and equine protein (Table 1).

Factors upregulated during tendon injury have differential effects on cell proliferation and migration of adult, fetal and ESC-tenocytes cultured in 2D

Of the 11 factors tested, TGFβ3, SDF1, IL8 and FGF9 had no significant effects on proliferation of any cell type at any of the doses tested (Fig. 1). FGF2, PDGF-BB and IGF1 all significantly increased the proliferation of adult and fetal tenocytes using at least one of the doses, but they had no significant effect on the proliferation of ESC-tenocytes (Fig. 1). The inflammatory cytokines IL-1β, TNFα and IFNγ resulted in a significant increase in proliferation in only fetal tenocytes, with no effect on adult and ESC-tenocytes (Fig. 1). In contrast, IL10 significantly increased the proliferation of ESC-tenocytes at all doses but had no effect

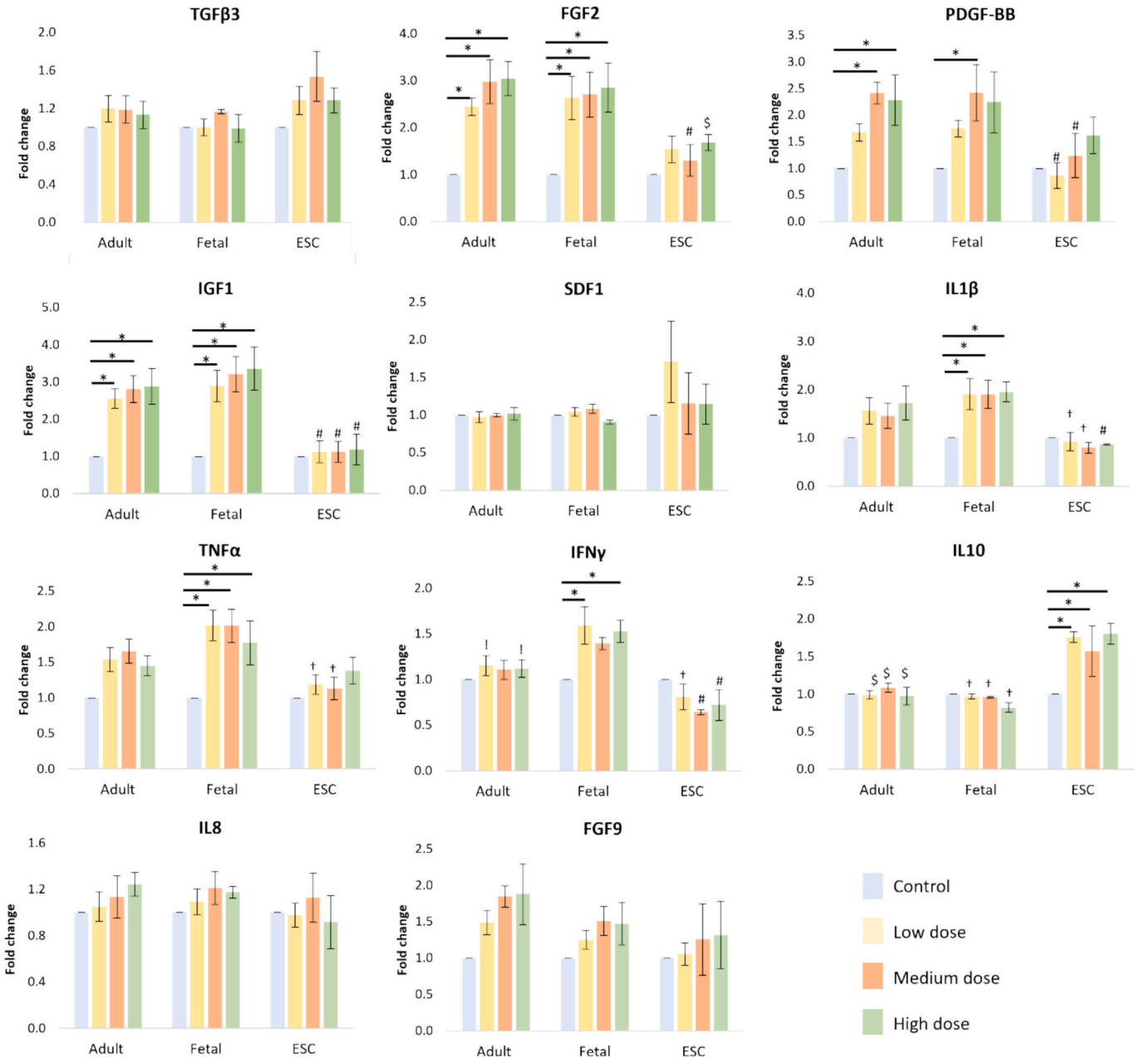

Fig. 1. Fold change in proliferation rates of adult, fetal and ESC-tenocytes following 72 h exposure to varying concentrations of different growth factors, cytokines, and chemokines. All cell types were stimulated with increasing concentrations of the growth factors the specific concentrations of which are given in Table 1. Within cell type differences ($p < 0.05$) versus control are denoted as *. Between cell type differences ($p < 0.05$) are denoted as # (adult and fetal vs. ESC-tenocytes), $ (adult vs. ESC-tenocytes), † (fetal vs. ESC-tenocytes), and! (fetal vs. adult tenocytes), respectively. All values are mean ± S.E.M of three biological replicates per cell type.

on adult or fetal tenocytes (Fig. 1).

2D migration assays demonstrated that in the presence of high or low serum, there was no significant difference in the rate of cell migration between adult, fetal and ESC-tenocytes (i.e. cell type x FBS, $p = 0.884$), but all cells migrated significantly faster in the presence of high serum (i.e. FBS $p < 0.01$) (Supplementary Fig. 2). The effect of the 11 factors at each dose was then assessed in the presence of 2 % serum (Fig. 2). TGFβ3, FGF2, PDGF-BB, IGF1, SDF1, TNFα and FGF9 all had a significant ($p < 0.05$) three-way interaction across time, cell type and concentration (Table 2). Whereas IL-1β, IFNγ, IL8 and IL10 did not have a significant three-way interaction. TGFβ3 decreased migration in fetal and ESC-tenocytes but had no effect on adult tenocytes. FGF2, PDGFBB, IGF1 and SDF1 increased migration in fetal and adult tenocytes but not in ESC-tenocytes. TNFα increased migration in adult tenocytes, but decreased migration in fetal and ESC-tenocytes and FGF9 decreased migration in adult versus ESC-tenocytes (Fig. 2).

Based on the 2D proliferation and migration results using single cell type cultures (monocultures), we then shortlisted the factors to TGFβ3, IL10, FGF2, PDGFBB and IGF1 for further experiments at the lowest effective dose. TGFβ3 has no effect on proliferation by any cell type, but significantly inhibited fetal and ESC-tenocyte migration. In contrast, IL10 had no significant effect on migration but a cell type specific effect on proliferation (increasing proliferation of ESC-tenocytes). FGF2, PDGFBB and IGF1 all increased the proliferation and migration of adult and fetal tenocytes but had no effects on ESC-tenocytes.

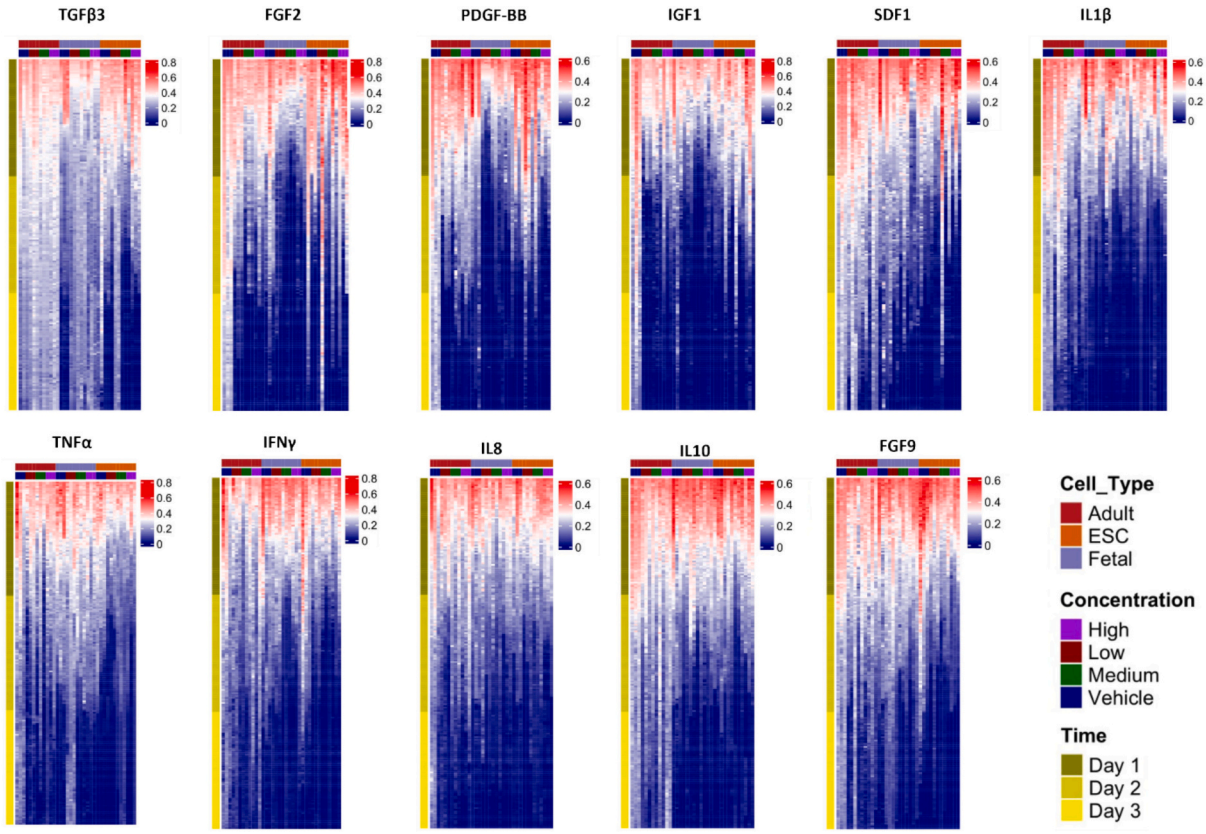

Fig. 2. Heatmaps depicting the change in migration over time (vertical annotation) for each cell type (top horizontal annotation) following stimulation with varying concentrations of each protein (bottom horizontal annotation). Samples are not clustered or scaled and data represents raw values (i.e., 1 = 100 % open wound area).

Table 2
Main effects and interactions following each three-way ANOVA. Significant three-way interactions are in bold. No post-hoc tests are shown.

Protein	Time	Cell type	Concentration	Time x cell type	Time x concentration	Cell type x concentration	Time x cell type x concentration
TGFß3	<0.001	<0.001	0.196	<0.001	<0.001	0.036	**<0.001**
FGF2	<0.001	0.002	0.004	<0.001	0.005	0.311	**<0.001**
PDGFBB	<0.001	<0.001	<0.001	<0.001	0.526	<0.001	**<0.001**
IGF1	<0.001	0.002	<0.001	<0.001	0.210	<0.001	**0.015**
SDF1	<0.001	<0.001	0.024	<0.001	<0.001	0.143	**0.003**
IL1ß	<0.001	<0.001	0.017	<0.001	0.128	0.009	0.09
TNFα	<0.001	<0.001	0.011	0.002	0.004	0.007	**<0.001**
IFNγ	<0.001	0.018	<0.001	<0.001	0.005	0.657	0.110
IL8	<0.001	0.001	0.018	<0.001	0.297	0.544	0.165
IL10	<0.001	0.001	0.017	<0.001	0.128	0.009	0.090
FGF9	<0.001	0.002	<0.001	<0.001	0.133	0.149	**0.048**

Co-culture assays confirm the differential effects of the factors on 2D wound closure

The effect of the short-listed five factors on 2D wound closure was next determined when combinations of mitotically active cells were co-cultured (Fig. 3). As in the monoculture conditions, high serum (10 % or 15 %) resulted in faster wound closure than low (2 %) serum for all combinations. The combination of "ESC plus fetal tenocytes" resulted in the fastest wound closure, completely closing the wound within one day of culture in high serum (Fig. 3). FGF2, IGF1 and PDGFBB all had a large effect in increasing wound closure by "fetal plus adult tenocytes" at day 1. In "ESC plus adult tenocytes" IL10 had a large effect in increasing wound closure at day 1 and day 2. IGF1 and TGFβ3 also had large effects in increasing wound closure by this combination of cells at day 2. In "ESC plus fetal tenocytes" FGF2 and IL10 had a large effect in increasing wound closure at day 1, and PDGFBB had a large effect at day 2 (Fig. 3). Cohen's d effect sizes are provided in Supplementary table 2.

The factors have differential effects on cell viability, collagen contraction and wound closure in a 3D wound model

The five shortlisted factors were next used in a 3D wound model. The model was developed by generating a central hole in the collagen construct which underwent closure and repair over the 14-day culture period (Fig. 4A). The effect of the five shortlisted factors on cell viability, wound closure and overall gel contraction was then determined. Initial experiments were performed using 2 % serum. However, at this low serum level, gel contraction and wound closure by fetal and ESC-tenocytes was significantly inhibited (Supplementary Fig. 3). Optimisation revealed that 7.5 % serum was the minimum required to produce normal contraction and complete wound closure by all cell types and was therefore taken forward. Under these conditions, PDGFBB significantly increased cell viability of adult tenocytes and FGF2 significantly increased cell viability of fetal tenocytes. TGFβ3, IL10 and IGF1 had no effects on viability of any cell type (Fig. 4B). None of the factors had

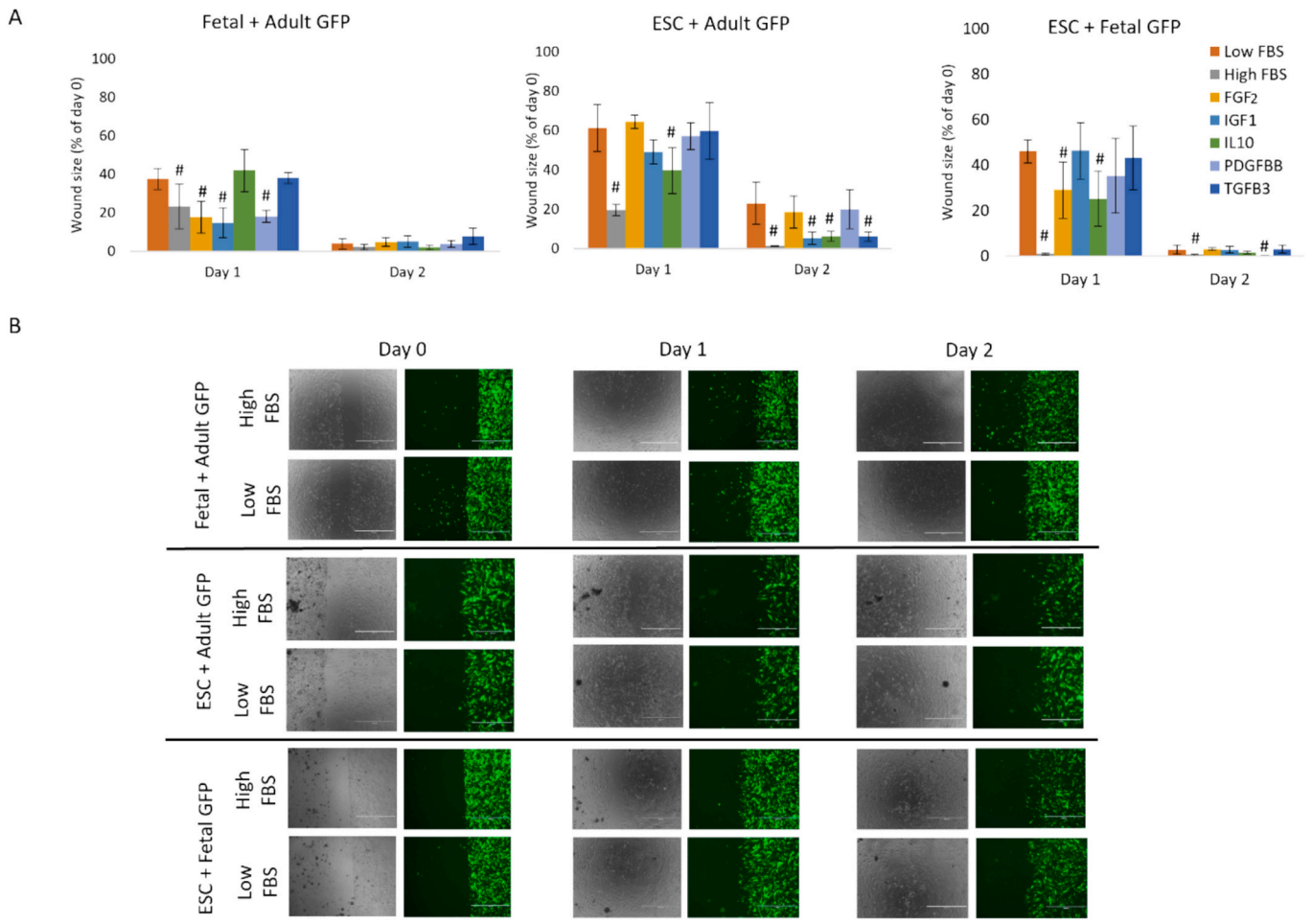

Fig. 3. Wound closure by combinations of fetal plus adult tenocytes, ESC plus adult tencoytes and ESC plus fetal tenocytes, in response to culture with five different factors. A) Wound size is shown as a percentage of day 0 starting size. Error bars represent the S.E.M of three biological replicates. # indicates a large Cohen's d effect size (Supplementary table 2). B) Representative images are shown for each combination of cells in both low (2 %) serum and high (10 % or 15 %) serum (B). Scale bar = 1000 μM.

significant effects on wound closure or overall gel contraction in ESC-tenocytes (Fig. 4C). In adult tenocytes, TGFβ3 significantly increased wound closure and gel contraction whereas FGF2 inhibited wound closure and gel contraction. In fetal tenocytes, TGFβ3 also significantly increased wound closure and gel contraction, and although FGF2 showed a trend to inhibit wound closure and gel contraction, this was only significant for gel contraction at days 13 and 14 of culture. IL10 also significantly increased wound closure of fetal tenocytes at days 9 and 10 (wound height) and days 13 and 14 (wound width) but did not effect overall gel contraction (Fig. 4C).

TGFβ3 and FGF2 have different effects on adult, fetal and ESC-tenocyte gene expression and cell distribution in the 3D wound model

As FGF2 and TGFβ3 had the greatest effect on wound closure in 3D, we also analysed IL6 secretion, gene expression and cell distribution after 7 days of culture (and in 7.5 % serum). No IL6 was detected in the media of any cell type under any condition (data not shown). We examined the expression of tendon-associated genes (*SCX* and *COMP*), along with a selection of MMPs (*MMP1*, *3*, *9*) and *TIMPs* (*TIMP1*, *2*, *3*). These genes all showed differences in expression between adult, fetal and ESC-tenocytes cultured under control conditions in 3D (Supplementary Fig. 4 and (Paterson et al., 2020a)). FGF2 had no significant effects on gene expression in any cell type but had a trend to increase *MMP9* in ESC-tenocytes (Fig. 5C). TGFβ3 resulted in a significant increase in *MMP9*, *COMP* and *SCX* in adult cells (Fig. 5A). In fetal cells, significant increases in the expression of *MMP9* and *TIMP1* were observed along with a small but significant decrease in *SCX* (Fig. 5B). However, the scale of the changes in expression induced by TGFβ3 is smaller in fetal than adult cells. In contrast, in ESC-tenocytes no significant changes were induced by TGFβ3, although there was a trend for *MMP9* to increase (Fig. 5C).

Cell distribution in fixed 3D collagen constructs was determined through H&E staining after 7 days of culture (as the wounds had closed under many conditions at later time points). As we have reported previously (Barsby et al., 2014), adult and fetal cells tend to distribute throughout the collagen gel, whereas ESC-tenocytes are primarily located at the edges of the gels (outer gel edge and wound edge where present) (Fig. 6A). TGFβ3 and FGF2 had no effect on the distribution of adult tenocytes (Fig. 6A and B). In fetal tenocytes, TGFβ3 had no effects on cell distribution, but FGF2 significantly increased the number of cells located at the wound edge (Fig. 6A and C). In ESC-tenocytes, cells were distributed at the wound edge in all conditions in high numbers, which prevented cell numbers from being quantified (Fig. 6A).

Discussion

In this study we tested the effects of eleven factors on cell proliferation, migration and gene expression in adult, fetal and ESC-tenocytes. These factors have all been found to be upregulated in adult tendon injuries previously (see Table 1). IL8 (Ribitsch et al., 2021; Herdrich et al., 2010) and TGFβ3 (Herdrich et al., 2010) are also upregulated in

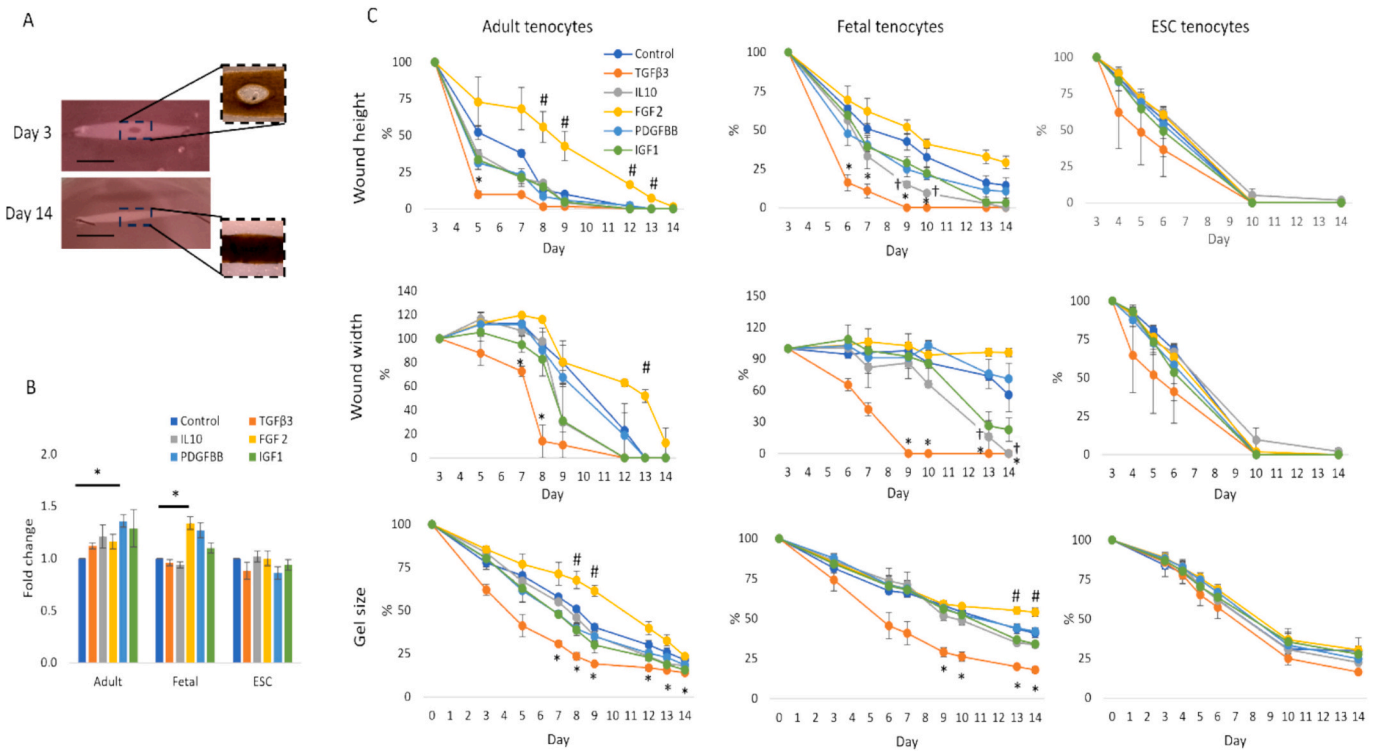

Fig. 4. The effect of the factors on tenocytes cultured in a 3D wound model. A) Representative images of the central wound which closes over a 14-day culture period. Scale bars = 5 mm. B) Cell viability shown as a fold change compared to the vehicle control. C) Wound closure and gel contraction over time are shown for each cell type. Top panel shows the wound height, middle panel shows the wound width and the bottom panel shows the overall gel size (diameter). In each case, wound measurement and gel size is shown as a percentage of the starting size (day 3 for wounds, day 0 for overall gel size). Error bars represent the S.E.M of three biological replicates. * $p < 0.05$ TGFβ3 versus control (wound plus vehicle control). # $p < 0.05$ FGF2 versus control. † $p < 0.05$ IL10 versus control.

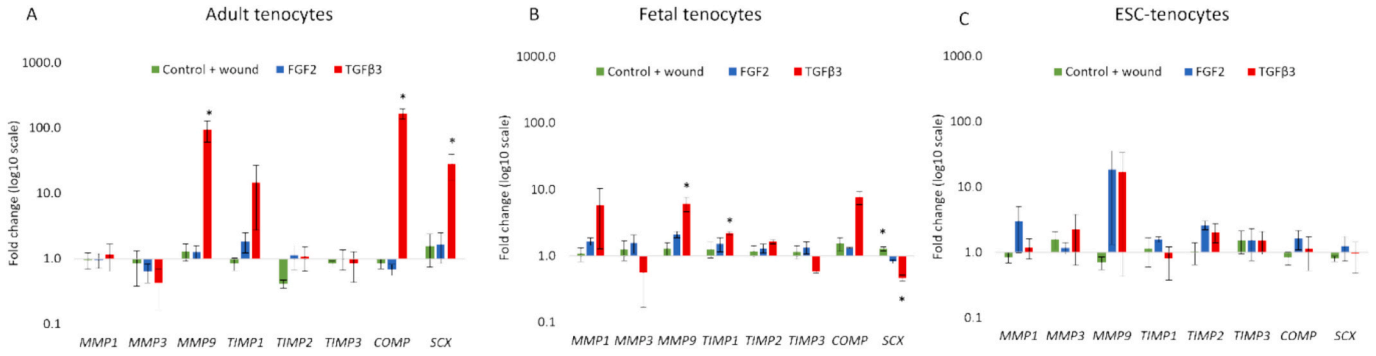

Fig. 5. Gene expression in 3D cultures of A) adult tenocytes, B) fetal tenocytes and C) ESC-tenocytes in response to a wound alone (green bars), a wound and FGF2 (blue bars) or a wound and TGFβ3 (red bars). Expression is shown as a fold change compared to the control (no wound, no growth factors) on a log10 scale. Error bars represent the S.E.M of three biological replicates. *$p < 0.05$ compared to the no wound control.

fetal tendon injuries, but the expression profiles of the other factors in fetal tendon injuries are not known.

Testing these factors in 2D migration and proliferation assays revealed that IL8 did not affect the proliferation or migration of any cell type. FGF2, PDGFBB, IGF1 and TNFα had cell type dependent effects on both proliferation and migration. IL1β, IFNγ and IL10 had cell type dependent effects only on proliferation, while SDF1, TGFβ3 and FGF9 had cell type dependent effects only on migration. We have previously demonstrated that the inflammatory cytokines IL1β, TNFα and IFNγ have detrimental effects on adult tenocytes but not ESC-tenocytes (Beaumont et al., 2023; Smith et al., 2023; McClellan et al., 2019a) and further work to investigate their effects at the different stages of tendon healing is warranted (Ellis et al., 2022). In contrast, FG2F2, PDGFBB, IGF1, IL10, SDF1, TGFβ3 and FGF9 have been shown to have beneficial effects on tendon healing (Lu et al., 2022; Chen et al., 2022; Miescher et al., 2023; Lin et al., 2023; Ricchetti et al., 2008; Sun et al., 2018). In this study we initially took forward FGF2, PDGFBB, IGF1, TGFβ3 and IL10.

Both FGF2 and PDGFBB have previously been shown to promote fibroblast migration (Song et al., 2016; Li et al., 2004) and IGF1 promotes the migration of bone marrow derived stem cells (Lin et al., 2020). In this study, they all increased the migration and proliferation of adult and fetal tenocytes, but not ESC-tenocytes. In 2D wound co-cultures using proliferating cells, FGF2 and PDGFBB had a large effect on increasing wound closure when fetal tenocytes were present, whereas IGF1 increased wound closure in all cultures where adult tenocytes were present.

We have previously shown that TGFβ3 inhibits the migration of fetal,

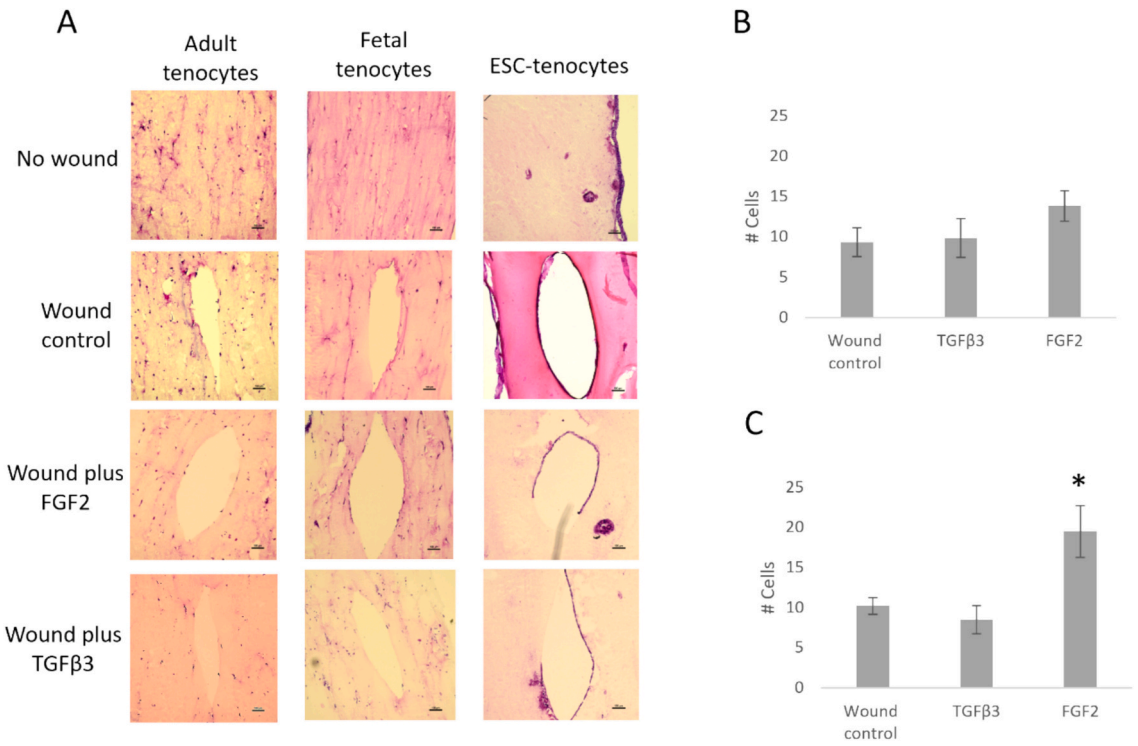

Fig. 6. Cell distribution in fixed 3D collagen gels after 7 days of culture. A) H&E staining of gel sections. Images are representative of three biological replicates. Scale bar = 100 μm. B) Quantification of adult tenocytes at the wound edge. C) Quantification of fetal tenocytes at the wound edge. Error bars represent the S.E.M of three biological replicates. *$p < 0.05$ compared to the wound control.

but not adult tenocytes (Paterson et al., 2020b), using a transwell system. Here we confirmed this result and further demonstrated that the migration of ESC-tenocytes is also inhibited by TGFβ3, despite them expressing a significantly lower level of TGFβR1 and R2 (Paterson et al., 2020b). In 2D wound co-cultures using proliferating cells, TGFβ3 had a positive effect only on day 2 of wound closure when ESC and adult tenocytes were co-cultured. This could reflect the trend for TGFβ3 to increase the proliferation of ESC-tenocytes.

IL10 has previously been shown to promote the proliferation and migration of tendon-derived stem cells through JAK/STAT signalling (Deng et al., 2018). In our study, IL10 also significantly increased the proliferation of ESC-tenocytes, but had no significant effects on cell migration. In 2D wound co-cultures using proliferating cells, IL10 had a large effect in increasing the rate of wound closure when ESC-tenocytes were present. This is likely due to its positive effects on ESC-tenocyte proliferation. Interestingly ESC-tenocytes express lower levels of the IL10 receptors than fetal and adult tenocytes ((Paterson et al., 2020a) and Supplementary Fig. 6). Therefore, the differential response does not appear to be due to differences in receptor availability.

We next adapted our 3D culture system to include a central "wound". This recapitulates the core lesion generally seen in horse superficial digital flexor tendon injuries (O'Sullivan, 2007), but in our model, the lesion is totally empty, rather than consisting of damaged extracellular matrix. Wound closure was directly related to overall gel contraction, and we demonstrated that of the five factors tested, only TGFβ3 and FGF2 had significant effects on wound closure and gel contraction. TFGβ3 has been shown to have a positive effect on tendon healing (Lin et al., 2023). We previously demonstrated that TGFβ3 promoted tendon differentiation of ESCs (Barsby and Guest, 2013) and increased the degree of 3D gel contraction by ESCs but not adult tenocytes (Barsby et al., 2014). In contrast, in this study we demonstrated that TGFβ3 significantly increased the degree of wound closure and gel contraction by both adult and fetal tenocytes, but not by ESC-tenocytes. The discrepancy in the results likely reflects the different concentrations of both serum (7.5 % in this study versus 10 % for adult cells and 15 % for ESCs in the previous study) and TGFβ3 (5 ng/mL in this study versus 20 ng/mL in the previous study). The concentrations tested here are based on previous in vitro studies, but it is not yet clear how well they correlate to levels found in vivo (Ellis et al., 2022). This may be critical, if different doses have differential effects on cellular behaviour. It has previously been shown that ESC-tenocytes cultured in 3D express significantly lower levels of the TGFβ3 receptors (Paterson et al., 2020a), which may explain the lack of response of ESC-tenocytes to the low dose of TGFβ3 used in 3D in this study. We saw no changes in cell distribution of any cell type within the collagen gels in response to TGFβ3, but gene expression changes in *MMPs*, *TIMPs*, *COMP* and *SCX* were detected in adult and fetal tenocytes, but not ESC-tenocytes. However, differences in the scale and direction of effect were found, with larger changes observed in adult tenocytes. It is not clear if the observed changes in gene expression indicate improved tendon regeneration or not. An increased rate in collagen contraction may reflect a "scarring" response. It would be beneficial to determine the mechanical properties of the resulting constructs (McClellan et al., 2019a; Atkinson et al., 2020), the expression of genes associated with scarring e.g. *COL3A1,* and collagen fibre size and alignment to get a more complete understanding of the quality of the resulting collagen matrix.

In contrast to TGFβ3, FGF2 inhibited 3D wound closure by adult and fetal tenocytes but had no effects on ESC-tenocytes. ESC-tenocytes cultured in 3D have a significantly lower level of FGFR1 than both fetal and adult tenocytes and a trend for lower levels of FGFR3 and FGFR4 ((Paterson et al., 2020a) and Supplementary Fig. 5). This may explain why the ESC-tenocytes are less responsive to FGF2. However, a larger inhibition of contraction was observed in the adult compared to the fetal cells, which cannot be explained by differences in receptor expression. FGF2 also affected the distribution of fetal tenocytes by promoting the cells to migrate to the edges of the construct and wound. This was not observed using adult tenocytes, but interestingly, the ESC-tenocytes appear to preferentially localise at the periphery of the constructs

under all conditions. FGF2 had no significant effects on candidate gene expression in any cell type and so the mechanisms behind its differential effects are currently unclear. Global gene expression analysis is warranted in future work.

FGF2 has been shown to have positive effects on some tendon repair parameters such as cell proliferation, collagen production and stimulation of angiogenesis. However, its effects on mechanical properties have been variable (reviewed in (Lu et al., 2022)). Our data demonstrating that FGF2 inhibits collagen gel contraction suggests that it could have a negative effect on tendon healing. Interestingly, we have previously demonstrated that IL1β and TNFα impair collagen gel contraction through activation of the NFκB signalling pathway (Beaumont et al., 2023; Smith et al., 2023; McClellan et al., 2019a), and NFκB has also been demonstrated to be activated by FGF2 (Tang et al., 2003). Further work to determine if NFκB activation is responsible for the inhibition of 3D wound healing and gel contraction is required.

This study had a number of limitations. For instance, the models did not incorporate vascular cells or immune cells which play a critical role in tendon healing (Crosio and Huang, 2022). In our 3D wound model, we did not detect any IL6 protein secretion in the presence or absence of TGFβ3 and FGF2 (although *IL6* gene expression was not measured). We have previously demonstrated that equine tenocytes cultured in 3D do not produce detectable levels of IL6 under control conditions, but high levels are produced following IL-1β stimulation (Beaumont et al., 2023). Therefore, while the current 3D model was used to study wound closure, in order to refine it to better represent the injured tendon, additional inflammatory factors would be required. The models also did not examine the effect of combinations of factors which would be present in vivo, and they were very short term (maximum 14 days duration). Hence, they cannot not fully capture the complexity of the in vivo situation. In our 2D and 3D models, we used different serum concentrations (2 % in 2D and 7.5 % in 3D) as the 3D collagen gels containing fetal and ESC-tenocytes did not contract well at very low serum levels. This could have affected comparisons between the results. The stiffness of the matrix and species of collagen used is also different in 2D (collagen coated tissue culture plates) and 3D (collagen gels). This could also produce different effects on cell migration/distribution in the different in vitro models (Yi et al., 2022), and affect the translatability of the results to the in vivo situation. In our 2D models, the ESCs were exposed to 20 ng/mL TGFβ3 for 14 days to differentiate them into tenocytes (Barsby and Guest, 2013; McClellan et al., 2019a), prior to use in the proliferation and migration assays. However, how this affects their subsequent response to TGFβ3 applied during these assays is unknown. In contrast, as we have shown that 3D culture alone can drive tenocyte differentiation of ESCs (Barsby et al., 2014), our 3D models started with undifferentiated ESCs. It is therefore possible that in 3D the factors we added affected the differentiation of the ESCs and gene and protein analysis would be required to confirm this.

In conclusion, we have demonstrated that factors that are upregulated in the injured tendon have differential effects on the proliferation, migration, gene expression and wound healing by adult, fetal and ESC-tenocytes in 2D and 3D models. TGFβ3 and FGF2 appear to have a greater effect in adult tenocytes than fetal tenocytes and little effect on ESC-tenocytes. While TGFβ3 promotes 3D wound closure by adult and fetal cells, FGF2 inhibits wound closure, particularly by adult cells. A deeper understanding of the mechanisms behind their responses is required to aid our understanding of scarless fetal regeneration and inform the development of cell-based therapies.

CRediT authorship contribution statement

Ross E. Beaumont: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Emily J. Smith:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Clara David:** Writing – review & editing, Validation, Investigation, Formal analysis. **Yasmin Z. Paterson:** Writing – review & editing, Methodology, Conceptualization. **Elena Faull:** Writing – review & editing, Validation, Investigation. **Deborah J. Guest:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cdev.2025.204003.

Data availability

Data will be made available on request.

References

Adelmann-Grill, B.C., et al., 1987. Inhibition of fibroblast chemotaxis by recombinant human interferon γ and interferon α. J. Cell. Physiol. 130 (2), 270–275.
Atkinson, F., et al., 2020. Cyclical strain improves artificial equine tendon constructs in vitro. J. Tissue Eng. Regen. Med. 14 (5), 690–700.
Avella, C.S., et al., 2009. Ultrasonographic assessment of the superficial digital flexor tendons of National Hunt racehorses in training over two racing seasons. Equine Vet. J. 41 (5), 449–454.
Barsby, T., Guest, D., 2013. Transforming growth factor beta3 promotes tendon differentiation of equine embryo-derived stem cells. Tissue Eng. Part A 19 (19–20), 2156–2165.
Barsby, T., Bavin, E.P., Guest, D.J., 2014. Three-dimensional culture and transforming growth factor Beta3 synergistically promote Tenogenic differentiation of equine embryo-derived stem cells. Tissue Eng. Part A 20, 2604–2613.
Beaumont, R.E., et al., 2023. Exogenous interleukin-1 beta stimulation regulates equine tenocyte function and gene expression in three-dimensional culture which can be rescued by pharmacological inhibition of interleukin 1 receptor, but not nuclear factor kappa B, signaling. Mol. Cell. Biochem. 479 (5), 1059–1078.
Beredjiklian, P.K., et al., 2003. Regenerative versus reparative healing in tendon: a study of biomechanical and histological properties in fetal sheep. J. Biomed. Eng. 31, 1143–1152.
Chen, M.S., et al., 2018. IL-1β-induced matrix Metalloprotease-1 promotes mesenchymal stem cell migration via PAR1 and G-protein-coupled signaling pathway. Stem Cells Int. 2018, 3524759.
Chen, Y., et al., 2022. A promising candidate in tendon healing events-PDGF-BB. Biomolecules 12 (10).
Cohen, J., 1992. Statistical power analysis. Curr. Dir. Psychol. Sci. 1 (3), 98–101.
Crosio, G., Huang, A.H., 2022. Innate and adaptive immune system cells implicated in tendon healing and disease. Eur. Cell. Mater. 43, 39–52.
Dahlgren, L., Mohammed, H.O., Nixon, A.J., 2005. Temporal expression of growth factors and matrix molecules in healing tendon lesions. J. Orthop. Res. 23, 84–92.
DeFoor, M.T., et al., 2024. Treatment of tendon injuries in the service member population across the spectrum of pathology: from exosomes to bioinductive scaffolds. Bioengineering (Basel) 11 (2).
Deng, G., et al., 2018. Interleukin-10 promotes proliferation and migration, and inhibits tendon differentiation via the JAK/Stat3 pathway in tendon-derived stem cells in vitro. Mol. Med. Rep. 18 (6), 5044–5052.
Dunlevy, J.R., Couchman, J.R., 1995. Interleukin-8 induces motile behavior and loss of focal adhesions in primary fibroblasts. J. Cell Sci. 108 (Pt 1), 311–321.
Dyson, S.J., 2004. Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992-2000). Equine Vet. J. 36 (5), 415–419.
Ellis, I.M., Schnabel, L.V., Berglund, A.K., 2022. Defining the profile: characterizing cytokines in tendon injury to improve clinical therapy. J. Immunol. Regen. Med. 16, 100059.
Favata, M., et al., 2006. Regenerative properties of fetal sheep tendon are not adversely affected by transplantation into an adult environment. J. Orthop. Res. 24, 2124–2132.
Gebäck, T., et al., 2009. TScratch: a novel and simple software tool for automated analysis of monolayer wound healing assays. Biotechniques 46 (4), 265–274.
Guest, D.J., Allen, W.R., 2007. Expression of cell surface antigens and embryonic stem cell pluripotency genes in equine blastocysts. Stem Cells Dev. 16, 789–795.
Guest, D.J., Smith, M.R.W., Allen, W.R., 2010. Equine embryonic stem-like cells and mesenchymal stromal cells have different survival rates and migration patterns following their injection into damaged superficial digital flexor tendons. Equine Vet. J. 42 (7), 636–642.
Gurau, T.V., et al., 2023. Epidemiology of injuries in professional and amateur football men (part II). J. Clin. Med. 12 (19).

Herdrich, B.J., et al., 2010. Fetal tendon wound size modulates wound gene expression and subsequent wound phenotype. Wound Repair Regen. 18 (5), 543–549.
Hopkins, C., et al., 2016. Critical review on the socio-economic impact of tendinopathy. Asia Pac. J. Sports Med. Arthrosc. Rehabil. Technol. 4, 9–20.
Inness, C.M., Morgan, K.L., 2015. Polo pony injuries: player-owner reported risk, perception, mitigation and risk factors. Equine Vet. J. 47 (4), 422–427.
Joannes, A., et al., 2016. FGF9 and FGF18 in idiopathic pulmonary fibrosis promote survival and migration and inhibit myofibroblast differentiation of human lung fibroblasts in vitro. Am. J. Phys. Lung Cell. Mol. Phys. 310 (7), L615–L629.
Lam, K.H., et al., 2007. Descriptive analysis of retirement of thoroughbred racehorses due to tendon injuries at the Hong Kong Jockey Club (1992-2004). Equine Vet. J. 39 (2), 143–148.
Li, W., et al., 2004. Mechanism of human dermal fibroblast migration driven by type I collagen and platelet-derived growth factor-BB. Mol. Biol. Cell 15 (1), 294–309.
Li, X., et al., 2006. Horse embryonic stem cell-like cell lines from the proliferation of inner cell mass cells. Stem Cells Dev. 15, 523–531.
Lin, M., et al., 2020. IGF-1 enhances BMSC viability, migration, and anti-apoptosis in myocardial infarction via secreted frizzled-related protein 2 pathway. Stem Cell Res Ther 11 (1), 22.
Lin, M., et al., 2023. Growth factors in the treatment of Achilles tendon injury. Front. Bioeng. Biotechnol. 11.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-deltadeltaCT}$ method. Methods 25, 402–408.
Lu, J., et al., 2022. The functions and mechanisms of basic fibroblast growth factor in tendon repair. Front. Physiol. 13, 852795.
Mao, W.F., et al., 2020. Transcriptome profiling of digital flexor tendons after injury in a chicken model. Biosci. Rep. 40 (6).
McClellan, A., et al., 2019a. A novel mechanism for the protection of embryonic stem cell derived tenocytes from inflammatory cytokine interleukin 1 beta. Sci. Rep. 9 (1), 2755.
McClellan, A., et al., 2019b. Equine fetal, adult, and embryonic stem cell-derived tenocytes are all immune privileged but exhibit different immune suppressive properties in vitro. Stem Cells Dev. 28 (21), 1413–1423.
Miescher, I., et al., 2023. In vitro and in vivo effects of IGF-1 delivery strategies on tendon healing: a review. Int. J. Mol. Sci. 24 (3).
Misheff, M.M., 2010. Lameness in endurance horses. In: Ross MW and Dyson S J (eds) Diagnosis and Management of Lameness in the Horse, pp. 1137–1148.
Molloy, T., Wang, Y., Murrell, G.A.C., 2003. The roles of growth factors in tendon and ligament healing. Sports Med. 33 (5), 381–394.
Morita, W., et al., 2017. Cytokines in tendon disease: a systematic review. Bone Joint Res. 6 (12), 656–664.
Murray, R.C., et al., 2006. Association of sport and performance level with anatomical site of orthopaedic injury diagnosis. Equine Vet. J. Suppl 36, 411–416.
Murray, R.C., et al., 2010. Identification of risk factors for lameness in dressage horses. Vet. J. 184 (1), 27–36.
Nakamura, Y., et al., 2013. Enhanced wound healing by topical administration of mesenchymal stem cells transfected with stromal cell-derived factor-1. Biomaterials 34 (37), 9393–9400.
Nichols, A.E.C., Best, K.T., Loiselle, A.E., 2019. The cellular basis of fibrotic tendon healing: challenges and opportunities. Transl. Res. 209, 156–168.
O'Sullivan, C.B., 2007. Injuries of the flexor tendons: focus on the superficial digital flexor tendon. Clin. Techniq. Equine Pract. 6 (3), 189–197.
Paterson, Y.Z., et al., 2020a. Genome-wide transcriptome analysis reveals equine embryonic stem cell-derived tenocytes resemble fetal, not adult tenocytes. Stem Cell Res Ther 11 (1), 184.
Paterson, Y., et al., 2020b. The transcription factor scleraxis differentially regulates gene expression in tenocytes isolated at different developmental stages. Mech. Dev. 163, 103635.
Patterson-Kane, J.C., Becker, D.L., Rich, T., 2012. The pathogenesis of tendon microdamage in athletes: the horse as a natural model for basic cellular research. J. Comp. Pathol. 147 (2–3), 227–247.
Postlethwaite, A.E., Seyer, J.M., 1990. Stimulation of fibroblast chemotaxis by human recombinant tumor necrosis factor alpha (TNF-alpha) and a synthetic TNF-alpha 31-68 peptide. J. Exp. Med. 172 (6), 1749–1756.
Ribitsch, I., et al., 2021. Molecular mechanisms of fetal tendon regeneration versus adult fibrous repair. Int. J. Mol. Sci. 22 (11), 5619.
Ricchetti, E.T., et al., 2008. Effect of interleukin-10 overexpression on the properties of healing tendon in a murine patellar tendon model. J. Hand. Surg. [Am.] 33 (10), 1843–1852.
Shimode, K., et al., 2009. Local upregulation of stromal cell-derived factor-1 after ligament injuries enhances homing rate of bone marrow stromal cells in rats. Tissue Eng. Part A 15 (8), 2277–2284.
Singer, E.R., et al., 2008. Injuries in the event horse: training versus competition. Vet. J. 175, 76–81.
Smith, E.J., et al., 2023. Tumour necrosis factor alpha, interleukin 1 beta and interferon gamma have detrimental effects on equine tenocytes that cannot be rescued by IL-1RA or mesenchymal stromal cell-derived factors. Cell Tissue Res. 391 (3), 523–544.
Smith, E.J., et al., 2024. Equine embryonic stem cell-derived tenocytes are insensitive to a combination of inflammatory cytokines and have distinct molecular responses compared to primary tenocytes. Stem Cell Rev. Rep. 20 (4), 1040–1059.
Song, Y.H., et al., 2016. Distribution of fibroblast growth factors and their roles in skin fibroblast cell migration. Mol. Med. Rep. 14 (4), 3336–3342.
Steinmann, S., et al., 2020. Spectrum of tendon pathologies: triggers, trails and end-state. Int. J. Mol. Sci. 21 (3).
Suarez-Arnedo, A., et al., 2020. An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays. PLoS One 15 (7), e0232565.
Sun, J., et al., 2018. Controlled release of collagen-binding SDF-1α from the collagen scaffold promoted tendon regeneration in a rat Achilles tendon defect model. Biomaterials 162, 22–33.
Tang, J.B., et al., 2003. Tendon healing in vitro: promotion of collagen gene expression by bFGF with NF-kappaB gene activation. J. Hand. Surg. [Am.] 28 (2), 215–220.
Tang, Q.M., et al., 2014. Fetal and adult fibroblasts display intrinsic differences in tendon tissue engineering and regeneration. Sci. Rep. 4, 5515.
Wang, Y., Li, J., 2023. Current progress in growth factors and extracellular vesicles in tendon healing. Int. Wound J. 20 (9), 3871–3883.
Williams, R.B., Harkins, L.S., Wood, J.L.N., 2001. Racehorse injuries, clinical problems and fatalities recorded on British racecourses from flat racing and National Hunt racing during 1996, 1997 and 1998. Equine Vet. J. 33 (5), 478–486.
Yi, B., Xu, Q., Liu, W., 2022. An overview of substrate stiffness guided cellular response and its applications in tissue regeneration. Bioact. Mater. 15, 82–102.
Zhang, G., et al., 2022. Large animal models for the study of tendinopathy. Front. Cell Dev. Biol. 10, 1031638.