International Orthopaedics
https://doi.org/10.1007/s00264-020-04693-9

ORIGINAL PAPER

Autologous microfragmented adipose tissue reduces inflammatory and catabolic markers in supraspinatus tendon cells derived from patients affected by rotator cuff tears

Marco Viganò[1] · Gaia Lugano[1] · Carlotta Perucca Orfei[1] · Alessandra Menon[2,3] · Enrico Ragni[1] · Alessandra Colombini[1] · Paola De Luca[1] · Pietro Randelli[2,3] · Laura de Girolamo[1]

Received: 23 January 2020 / Accepted: 29 June 2020

Abstract

Purpose Rotator cuff tears are common musculoskeletal disorders, and surgical repair is characterized by a high rate of re-tear. Regenerative medicine strategies, in particular mesenchymal stem cell–based therapies, have been proposed to enhance tendon healing and reduce the re-tear rate. Autologous microfragmented adipose tissue (μFAT) allows for the clinical application of cell therapies and showed the ability to improve tenocyte proliferation and viability in previous in vitro assessments. The hypothesis of this study is that μFAT paracrine action would reduce the catabolic and inflammatory marker expression in tendon cells (TCs) derived from injured supraspinatus tendon (SST).

Methods TCs derived from injured SST were co-cultured with autologous μFAT in transwell for 48 h. Metabolic activity, DNA content, the content of soluble mediators in the media, and the gene expression of tendon-specific, inflammatory, and catabolic markers were analyzed.

Results μFAT-treated TCs showed a reduced expression of PTGS2 and MMP-3 with respect to untreated controls. Increased IL-1Ra, VEGF, and IL-6 content were observed in the media of μFAT-treated samples, in comparison with untreated TCs.

Conclusion μFAT exerted an anti-inflammatory action on supraspinatus tendon cells in vitro through paracrine action, resulting in the reduction of catabolic and inflammatory marker expression. These observations potentially support the use of μFAT as adjuvant therapy in the treatment of rotator cuff disease.

Keywords Tendon · Supraspinatus · Microfragmented adipose tissue · Rotator cuff · Mesenchymal stromal cells · Paracrine action

Introduction

Rotator cuff surgical repair represents an effective approach for the treatment of rotator cuff tears (RCT) in patients unresponsive to conservative therapies [1]. RCT represent a major cause of shoulder pain and disability and the prevalence is growing due to population aging [2, 3]. Nevertheless, it is characterized by a high rate of tear re-occurrence [4–6] mainly given by the failure of healing process in the supraspinatus tendon [7–11]. In order to improve tendon healing and reduce the incidence of re-tears after repair, different strategies have recently been proposed, many of which in the realm of regenerative medicine [12]. These approaches are aimed at exploiting the potential of platelets and mesenchymal stem cells to improve the regenerative potential of tissues through their production of growth factors and cytokines [13]. Several studies have demonstrated the ability of mesenchymal stem/stromal cells (MSCs) in the treatment of tendon injuries in both preclinical and clinical settings [14]. In particular, cultured adipose-derived stem/stromal cells (ASCs) injected in the injured supraspinatus tendon of fifty-five dogs allowed for functional improvements correlated by imaging

Pietro Randelli and Laura de Girolamo contributed equally to this work.

✉ Carlotta Perucca Orfei
carlotta.perucca@grupposandonato.it

[1] Orthopedics Biotechnology Lab, IRCCS Istituto Ortopedico Galeazzi, via Riccardo Galeazzi 4, 20161 Milan, Italy

[2] Laboratory of Applied Biomechanics, Department of Biomedical Sciences for Health, Università degli Studi di Milano, Via Mangiagalli 31, 20133 Milan, Italy

[3] 1° Clinica Ortopedica, ASST Centro Specialistico Ortopedico Traumatologico Gaetano Pini-CTO, Piazza Cardinal Ferrari 1, 20122 Milan, Italy

Published online: 08 July 2020

assessment [15]. In a recent report, nine patients with rotator cuff tendinopathy showed improvements after intra-tendinous administration of cultured autologous ASCs [16]. Adipose tissue, together with bone marrow, is considered one of the most convenient sources of MSCs [17], but the use of cultured cells in the clinical practice is limited by regulatory restrictions concerning the advanced therapy medicinal products (ATMPs) and the high costs of good manufacturing practice (GMP) cell expansion [18, 19]. For this reason, different one-step techniques have been developed for obtaining autologous cell concentrates from adipose tissue intra-operatively without need for cell culturing. Among the proposed products, microfragmented adipose tissue (μFAT) have been extensively characterized in vitro [20–22] and found to enhance human tendon cell (hTC) viability, proliferation, and growth factor production [23], providing the basis for the present study.

Herein, the potential of autologous μFAT in promoting tendon healing have been investigated in vitro on tendon cells derived from matched injured supraspinatus tendon (TCs). The working hypothesis was that the paracrine action of autologous μFAT would reduce the expression of catabolic and inflammatory markers in TCs derived from pathological tissue, favoring the establishment of a regeneration-prone microenvironment, thus providing evidence of the putative mechanism of action of μFAT in the context of rotator cuff disorders.

Materials and methods

μFAT harvesting and TC isolation

Supraspinatus tendon biopsies and lipoaspirate adipose tissue were collected from ten patients (five females and five males, mean age 54.0 ± 11.7 years old) during arthroscopic rotator cuff repair with μFAT infiltration. The collection of spare tissues was approved by the Institutional Review Board (no. 148/INT/2015, January 13, 2016), and an informed consent was obtained from all patients. The microfragmented adipose tissue was prepared from lipoaspirate adipose tissue collected before surgery using Lipogems60™ device (Lipogems International S.p.A., Milano, Italy), following manufacturer's instructions [24]. An aliquot was procured before injection for the in vitro experiments. The fragmented tissue was centrifuged at 376×*g* for five minutes at room temperature in order to separate the aqueous phase. The tissue (at this stage including adipocytes, stromal cells, extracellular matrix, and endothelial cells [24]) was frozen at − 80 °C after dilution in the same volume of freezing mix (90% FBS, 10% dimethyl sulfoxide, Sigma Aldrich, St. Louis, MO, USA) to preserve cell viability, while the aqueous phase was stored at − 20 °C. This solution may contain molecules and other elements (such as microvesicles) that may play a role in μFAT action. An aliquot (1 ml) of μFAT was digested by 0.075% w/v collagenase type I (Worthington, Lakewood, NJ, USA) for 45 min at 37 °C before and after the freezing/thawing procedure. Cell count and viability were evaluated by Nucleocounter NC-3000 using cell viability staining (Chemometech, Allerod, Denmark) [25]. The mean cell count was of $1.1 \pm 0.7 \times 10^6$ cells/ml. Cell viability of freshly harvested μFAT was 51.0 ± 9.9%, and it was reduced to 31.4 ± 9.9% post-thaw. Human tendon cells (TCs) were isolated from torn supraspinatus tendon (SSP) biopsies. The tissue was digested in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) with 0.3% w/v collagenase type I (Worthington, Lakewood, NJ, USA) overnight at 37 °C, as previously reported [26]. The isolated TCs were cultured in complete medium (high glucose-DMEM, 10% v/v fetal bovine serum (FBS; Euroclone, Pero, Italy) 100 U/ml penicillin, 100 μg/ml streptomycin, 0.29 mg/ml l-glutamine (Gibco, Waltham, MA, USA), supplemented with 5 ng/ml fibroblast growth factor (bFGF; Peprotech, London UK). During culture, medium was replaced every 3 days. Cells were cultured up to passage three when all the experiments were performed.

TC-μFAT co-cultures

After counting by hemocytometer, TCs were seeded in 12-multiwell plate at the density of 50.000 cells/well and co-cultured with 250 μl of μFAT (containing 125 μl of tissue thawed before usage in complete medium and 125 μl of aqueous phase previously stored at − 20 °C), using transwell inserts with pores of 0.4 μm diameter (Corning, Corning, NY, USA). This allowed to maintain TCs separated from μFAT. TCs cultured in absence of μFAT were considered as untreated controls. After 48 hours of incubation, cell viability was measured and then culture medium and TCs were harvested for the following experiments. Culture medium was stored at − 20 °C, while cell pellets were stored at − 80 °C.

Cell viability assay

Cell viability was tested by Alamar Blue assay in all the samples. Briefly, after 48 h of co-cultures, the culture medium was replaced by a 10% v/v solution of Alamar Blue (ThermoFisher Scientific, Waltham, MA, USA) in DMEM. TCs were incubated for 2 h at 37 °C, and then the fluorescence was measured by a spectrophotometer (ex. 540–em. 590, Victor X3, Perkin Elmer, Waltham, MA, USA).

RNA and DNA isolation and quantification

Total RNA and DNA were extracted from all samples using TRI reagent (Sigma Aldrich). Briefly, 300 μl of TRI reagent was added to all the cell pellets in order to obtain cell lysate. Then, 100 μl of 1-bromo-3-chloropropane (Sigma Aldrich) was added to the each lysate. Samples were centrifuged for

ten minutes at 12,000×*g*, and the interphase was collected for DNA extraction by addition of 100% ethanol and centrifugation at 2000×*g* for five minutes. The precipitated DNA pellet was washed for 30 minutes in a 0.1 M trisodium citrate solution added with 10% v/v ethanol, and then centrifuged five minutes at 2000×*g*. After one wash in 75% ethanol DNA was solubilized using 50 μl 8 mM NaOH and stored at − 20 °C. DNA quantification was performed at 260 nm absorbance by Nanodrop spectrophotometer using software version 3.7.1 (Nanodrop nd-1000, ThermoFisher Scientific) [27]. The RNA was precipitated from the remaining aqueous phase by incubation with isopropanol. RNA pellet was washed twice by 75% ethanol, re-suspended in 20 μl RNAse-free water, and then stored at − 80 °C.

Gene expression analysis

The cDNA was obtained from total RNA by reverse transcription with iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA,USA) as per manufacturer instructions. Quantitative real-time PCR was performed using 10 ng of cDNA in a reaction mix comprising TaqMan® Universal PCR MasterMix and the following Assays-on-Demand Gene expression probes (Life Technologies Waltham, MA, USA): MMP1 (metalloproteinase-1, Hs00899658_m1), MMP3 (metalloproteinase-3, Hs00968305_m1), PTGS2 (cyclooxygenase-2, Hs00153133_m1), SCX (scleraxis, Hs03054634_g1), COL1A1 (collagen type I alpha 1 chain, Hs01076777_m1), and COL3A1 (Collagen type III alpha 1 chain, Hs00943809_m1). The experiments were performed using the Applied Biosystems StepOnePlus® (Life Technologies), with the following protocol: 50 °C for two minutes, 95 °C for ten minutes, 40 cycles at 95 °C for 15 seconds, and 60 °C for one minute. The mean expression of two housekeeping genes, ACTB (β-actin, Hs99999903_m1) and YWHAZ (Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta, Hs03044281_g1), was used for normalization [28]. Two replicates were analyzed for each sample, and data were presented according to ΔCt method [29].

ELISA

The production of IL-1Ra, IL-6, TNFα (Peprotech, London, UK), and VEGF (R&D Systems, Minneapolis, MN, USA) in the culture media of differently treated TCs after 48 hours was analyzed by ELISA, following manufacturer's instruction. The detection ranges were 23–1500 pg/ml for IL-1Ra, 24–1500 pg/ml for IL-6, 31.3–2000 pg/ml for VEGF, and 31–2000 pg/ml for TNFα.

Statistical analysis

All the analyses were performed using GraphPad Prism v5.0 (Graphpad Software, La Jolla, CA, USA). Normal distribution of data was assessed by Shapiro–Wilk test. Paired *t* test was applied to measure differences among treatments when data presented a normal distribution; otherwise, the Wilcoxon matched paired test was applied. A level of $p < 0.05$ was considered statistically significant.

Results

μFAT did not influence TC viability and proliferation

Neither viability nor DNA content of TCs was influenced by the presence of autologous μFAT. Indeed, similar values were obtained in treated and non-treated TCs after 48 h of co-culture in transwell with μFAT (Fig. 1A, B). μFAT demonstrated a mean cell content of $2.2 \pm 1.5 \times 10^6$ cells/ml. No correlations between the effects provoked on TCs and cell count were observed.

μFAT inhibits the expression of SCX, COL1A1, COL3A1, MMP-3, and COX-2

A reduction of SCX expression was observed in TCs co-cultured with μFAT for 48 hours, even if not in a statistically significant manner (− 45%, $p = 0.064$; Fig. 2A). Similarly, the paracrine action of μFAT resulted in the reduction of both collagen type I and type III expression (− 54%, $p = 0.53$ and − 65%, $p < 0.05$, respectively; Fig. 2B, C), leading to a slight increase in the collagen type I/type III expression ratio (not significant, n.s.). For what concern the expression of catabolic enzymes, μFAT was able to reduce the expression of MMP-3 in TCs by 73% ($p < 0.05$) with respect to untreated controls (Fig. 2E), but not that of MMP-1 (Fig. 2D). At the same time, μFAT inhibited the expression of cyclooxygenase-2 (PTGS2, − 78%, $p < 0.05$) (Fig. 2F).

μFAT enhanced the production of cytokines and growth factors

While TNFα resulted undetectable in all samples (data not shown), the other molecules tested were modulated in the supernatant of TCs cultured in presence of μFAT for 48 hours. Increases were observed in IL-1Ra (+ 123%, $p < 0.05$; Fig. 3A), IL-6 (+ 267%, $p < 0.01$; Fig. 3B), and VEGF content (+ 59%, $p < 0.01$; Fig. 3C) with respect to the untreated controls. Conversely, the quantity of IL-1β was reduced in the culture media of TCs in co-culture with μFAT (− 23%, $p < 0.05$; Fig. 3D).

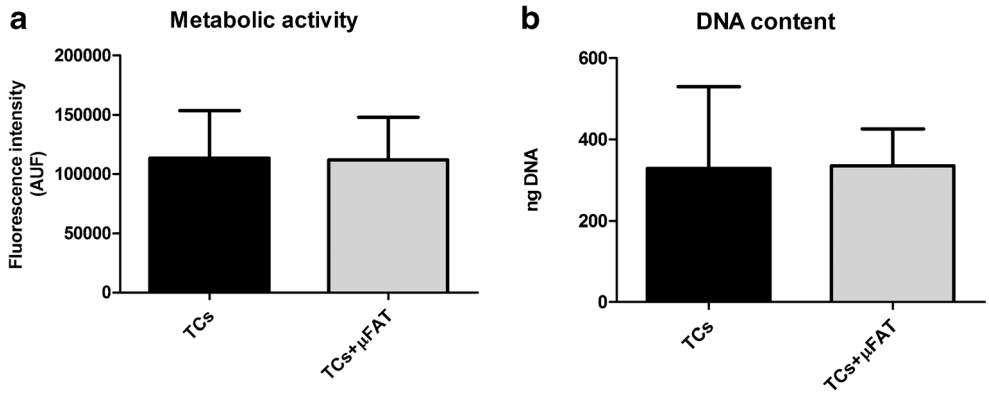

Fig. 1 TC metabolic activity and DNA content. Metabolic activity (**A**) measured by Alamar assay and DNA content (**B**) of TCs co-cultured with autologous µFAT (TCs + µFAT) for 48 hours and untreated controls (TCs) ($n = 10$)

Discussion

This study showed that µFAT effectively increased the content of soluble anti-inflammatory and trophic mediators in the cell supernatant of the TCs co-cultured for 48 hours in an in vitro setting of paracrine communication. As a result, inflammatory, catabolic, and fibrotic marker expression in tendon cells derived from injured supraspinatus tendon was found to be reduced. Supraspinatus tendon undergoes continuous shear stress and load due to its anatomical positioning, and given this high functional demand, it is often subject to injury and degeneration requiring surgical intervention [30, 31]. In addition, the frequency of re-tears after these procedures is high and often due to failure of tendon healing, which is often limited by the meager cellularity and vascularization of this particular tissue [7–11]. The tendon degenerative processes are known to involve inflammation, especially in rotator cuff disease [32], where mediators such as IL-1β and NLRP3 inflammasome contribute to the progression of the pathology [33], stimulating the expression of inflammatory and catabolic elements, such as cyclooxigenase-2 and metalloproteases [34–36]. In this context, the application of regenerative medicine treatments is intended to counteract the inflammatory process and stimulate the viability of tendon cells, in order to favor restoration of tissue

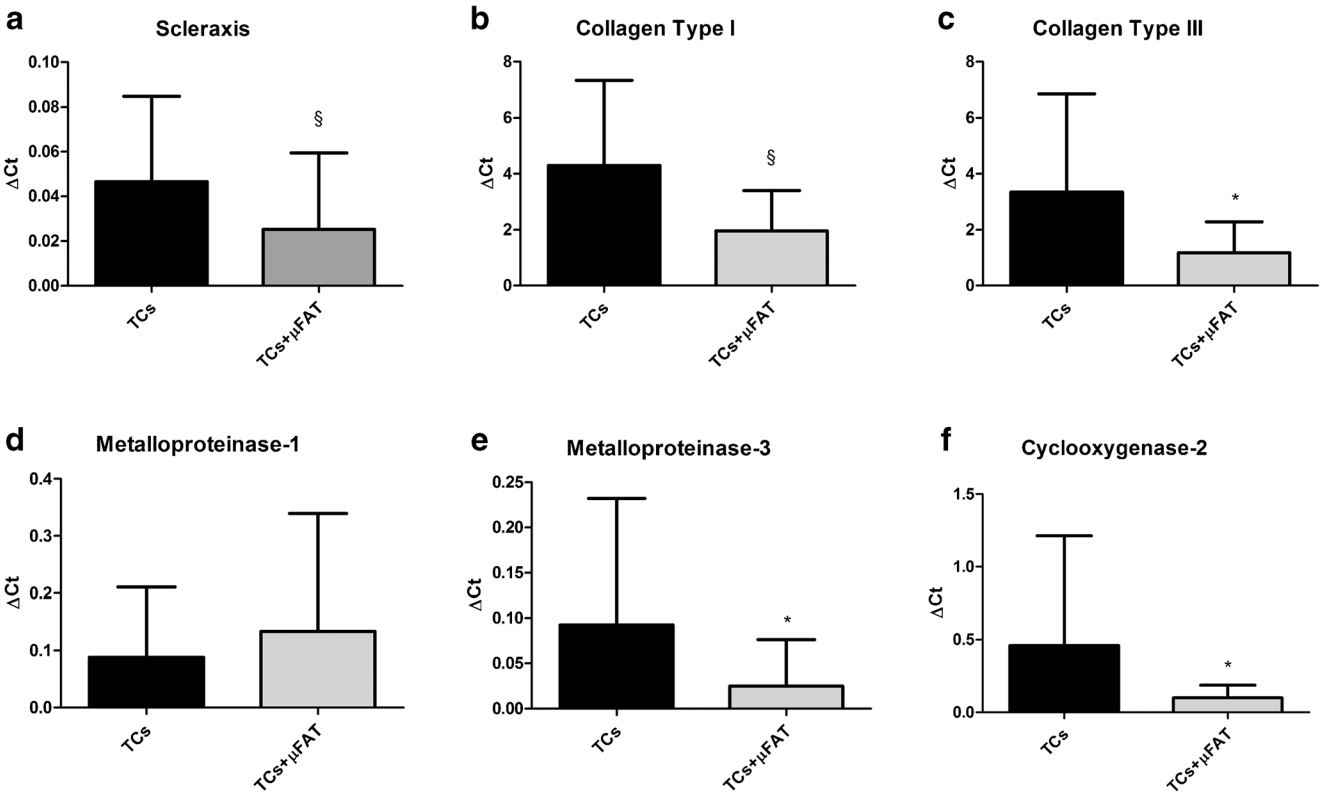

Fig. 2 TC gene expression. Gene expression of SCX (**A**), COL1A1 (**B**), COL3A1 (**C**), MMP-1 (**D**), MMP-3 (**E**), and PTGS2 (**F**) in TCs cultured in the presence or absence of µFAT for 48 hours. *$p < 0.05$; §$p < 0.1$ (tendency). Data are expressed as ΔCt mean ± SD with respect to ACTB/YWAHZ expression ($n = 10$)

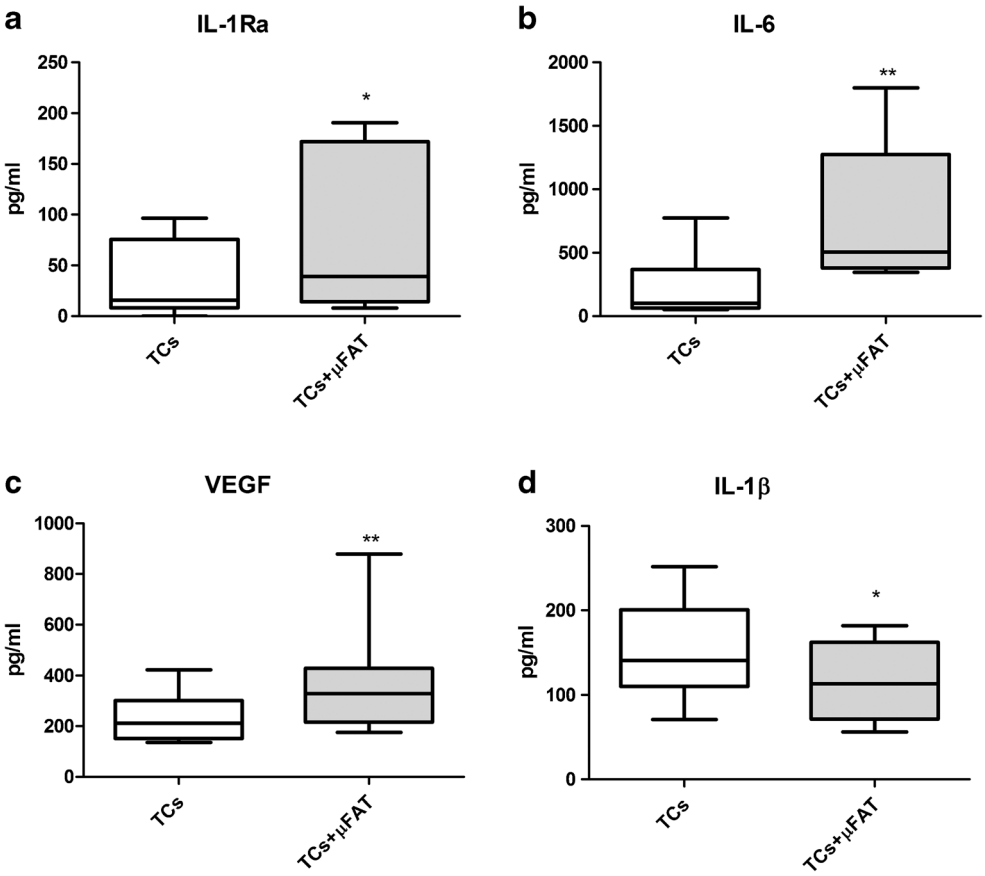

Fig. 3 Cytokines and VEGF content. IL-1Ra (**A**), IL-6 (**B**), VEGF (**C**), and IL-1β (**D**) content in the media of SSP-TCs cultured in the presence or absence of μFAT for 48 hours. Data are represented as median and 5–95% percentile ($n = 10$). *$p < 0.05$; **$p < 0.01$

homeostasis and healing, rather than increasing the cell number and/or proliferation.

The application of μFAT in our model significantly reduced the expression of cyclooxygenase-2 (COX-2), a key marker of inflammation. While in the early stage of tendon injury the inflammatory reaction, comprising COX-2 production, may contribute to tissue healing by eliciting cell proliferation and tissue repair [37–39], inflammation should progressively decrease in the following stages of the healing process, to allow for the restoration of tissue homeostasis. Conversely, the establishment of a chronic inflammatory condition appears to represent the crucial turning point towards pathology development and tissue degeneration [37]. The cellular model used in the present work originates from a pathological context where tissue homeostasis was lost and inflammatory mediators were produced chronically [40]. Indeed, the presence of the inflammatory mediator IL-1β was observed in the supernatants of TCs cultured in standard conditions, which was then reduced by the co-culture with μFAT, confirming the anti-inflammatory activity of this product. Thus, the reduction of PTGS2 gene expression observed in this model following μFAT treatment might be interpreted as a return of TCs to a physiological state.

On the contrary, TNFα was not present in our samples. This molecule was reported to be present in freshly harvested torn human supraspinatus tendons [41] and in cultures of tenocytes derived from the Achille's tendon [42]. Nevertheless, a previous report on cultured supraspinatus tendon cells was consistent with our observation, showing negligible expression of this molecule [43].

The reduction of these inflammatory mediators also demonstrated effects on the production of catabolic enzymes. MMP-1 and MMP-3 were described to be correlated with the tear size in rotator cuff lesions [44], even if to some extent they are also expressed in non-pathological conditions [45]. In our study, MMP-3 expression was significantly reduced in μFAT-treated samples with respect to untreated controls, while MMP-1 expression was unaltered. Involvement of these enzymes in rotator cuff tears has already been demonstrated at both protein and gene levels [46, 47] and similar results have been obtained by other authors observing co-cultures of tendon explants and ASCs [48], with a specific focus on MMP-3 [49]. High levels of MMP-3 have been associated to tendon re-tear after rotator cuff repair [50], and thus, the reduction of this catabolic enzyme expression would represent a strong rationale for the use of μFAT in conjunction with rotator cuff repair.

Despite these positive results regarding inflammatory and catabolic markers expression, after 48 hours of co-culture with μFAT, the TCs also reduced their tendon-specific markers'

transcription, such as SCX, COL1A1 and COL3A1. SCX is one of the main transcription factors involved in tenocytes differentiation, and it contributes to the expression of many tendon-specific molecules [51]. Our findings are consistent with the ASC-mediated reduction of tendon-specific marker expression previously described by other authors [49, 52]. Taken together, these observations suggest that the effect of μFAT in the context of tendon pathology is not able to stimulate tendon cell viability but rather to control inflammation and pathology progression. However, in our study, the reduction of collagen type I expression was balanced by the concomitant decrease of collagen type III transcription, thus maintaining the proportion of COL1A1/COL3A1 ratio, which is an important aspect for the prevention of fibrotic extracellular matrix deposition [53, 54].

Given the experimental setting used in this study, it is possible to state that all the effects induced by μFAT were exerted by its paracrine action. μFAT is known to release a wide number of soluble mediators, in particular those involved in the modulation of inflammation and chemotaxis, and comprising IL-1Ra, VEGF, and IL-6 [21]. In fact, in our study, higher levels of these molecules were observed in the media of TCs co-cultured with autologous μFAT. VEGF and IL-1Ra are strictly associated with the counteraction of inflammation and the promotion of tissue healing. IL-1Ra is a competitive inhibitor of IL-1β [55], and thus, it directly blocks the cascade of events elicited by this inflammatory molecule [56]. Our data showed that the increase of IL-1Ra effects is associated with a direct reduction of IL-1β release by TCs. At the same time, VEGF represents a pro-regenerative factor, not only stimulating angiogenesis but also fostering the restoration of proper tissue vascularization [57]. The production of VEGF is regulated by different factors including IL-6 [58] whose concentration appeared to be up-regulated by the presence of μFAT. While representing an actor in the inflammatory cascade [59, 60], IL-6 is also involved in a complex network of molecular signaling, which may be able to support TC proliferation and tendon healing [61–63], also reducing the catabolism in other cells such as inflamed synoviocytes in the in vitro setting [22].

The main limitation of the present study is the need to culture TCs for several passages before the experiments to obtain a suitable cell number for the completion of the analyses. Cell culturing outside of the pathophysiological environment may have altered the gene expression pattern of the cells, thus reducing the reliability of this pathological model. This also brought the need for freezing and thawing μFAT, reducing its cell viability, and thus possibly negatively influencing the results. Another limitation is represented by the short observation time applied in our study, which was primarily designed to evaluate the gene expression of specific markers as well as the production of soluble mediators. Therefore, it may lack suitable time points for the observation of other effects, such as those on proliferation and viability of TCs co-cultured with μFAT as observed by others [23, 64].

In conclusion, μFAT exerted an anti-inflammatory action on supraspinatus tendon cells in vitro through the release of biochemical mediators, resulting in the reduction of catabolic and inflammatory marker expression. These observations potentially support the use of μFAT in rotator cuff disease, as a conservative treatment aimed to symptoms relief or as an adjuvant therapy in conjunction with surgical repair, although further translational studies are needed.

Experimental data are available at https://osf.io/dfqs5/?view_only=09a66eb5367145ec81f96888f6cff908

Funding information This research was funded by the Italian Ministry of Health "Ricerca Corrente."

Compliance with ethical standards

Conflict of interest L.d.G.: paid consultant for Lipogems SpA. The other authors have no conflict of interest.

References

1. Schmidt CC, Jarrett CD, Brown BT (2015) Management of Rotator Cuff Tears. J Hand Surg Am 40:399–408. https://doi.org/10.1016/j.jhsa.2014.06.122
2. Svendsen SW, Frost P, Jensen LD (2012) Time trends in surgery for non-traumatic shoulder disorders and postoperative risk of permanent work disability: a nationwide cohort study. Scand J Rheumatol 41:59–65. https://doi.org/10.3109/03009742.2011.595375
3. Colvin AC, Egorova N, Harrison AK et al (2012) National trends in rotator cuff repair. J Bone Joint Surg Am 94:227–233. https://doi.org/10.2106/JBJS.J.00739
4. Chen M, Xu W, Dong Q et al (2013) Outcomes of single-row versus double-row arthroscopic rotator cuff repair: a systematic review and meta-analysis of current evidence. Arthroscopy 29:1437–1449. https://doi.org/10.1016/j.arthro.2013.03.076
5. Rhee YG, Cho NS, Yoo JH (2014) Clinical outcome and repair integrity after rotator cuff repair in patients older than 70 years versus patients younger than 70 years. Arthroscopy 30:546–554. https://doi.org/10.1016/j.arthro.2014.02.006
6. McElvany MD, McGoldrick E, Gee AO et al (2015) Rotator cuff repair: published evidence on factors associated with repair integrity and clinical outcome. Am J Sports Med 43:491–500. https://doi.org/10.1177/0363546514529644
7. Liu C-F, Aschbacher-Smith L, Barthelery NJ et al (2011) What we should know before using tissue engineering techniques to repair injured tendons: a developmental biology perspective. Tissue Eng Part B Rev 17:165–176. https://doi.org/10.1089/ten.TEB.2010.0662
8. Evans RB (2012) Managing the injured tendon: current concepts. J Hand Ther 25:173–189; quiz 190. https://doi.org/10.1016/j.jht.2011.10.004
9. Hoppe S, Alini M, Benneker LM et al (2013) Tenocytes of chronic rotator cuff tendon tears can be stimulated by platelet-released growth factors. J Shoulder Elb Surg 22:340–349. https://doi.org/10.1016/j.jse.2012.01.016

10. Weeks KD, Dines JS, Rodeo SA, Bedi A (2014) The basic science behind biologic augmentation of tendon-bone healing: a scientific review. Instr Course Lect 63:443–450
11. Abtahi AM, Granger EK, Tashjian RZ (2015) Factors affecting healing after arthroscopic rotator cuff repair. World J Orthop 6: 211–220. https://doi.org/10.5312/wjo.v6.i2.211
12. Randelli P, Randelli F, Ragone V et al (2014) Regenerative medicine in rotator cuff injuries. Biomed Res Int 2014. https://doi.org/10.1155/2014/129515
13. Murphy MB, Moncivais K, Caplan AI (2013) Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 45:e54. https://doi.org/10.1038/emm.2013.94
14. Abat F, Alfredson H, Cucchiarini M et al (2018) Current trends in tendinopathy: consensus of the ESSKA basic science committee. Part II: treatment options. J Exp Orthop 5. https://doi.org/10.1186/s40634-018-0145-5
15. Canapp SO, Canapp DA, Ibrahim V et al (2016) The use of adipose-derived progenitor cells and platelet-rich plasma combination for the treatment of supraspinatus tendinopathy in 55 dogs: a retrospective study. Front Vet Sci 3:61. https://doi.org/10.3389/fvets.2016.00061
16. Jo CH, Chai JW, Jeong EC et al (2018) Intratendinous injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of rotator cuff disease: a first-in-human trial. Stem Cells 36:1441–1450. https://doi.org/10.1002/stem.2855
17. Mussano F, Genova T, Corsalini M et al (2017) Cytokine, chemokine, and growth factor profile characterization of undifferentiated and osteoinduced human adipose-derived stem cells. Stem Cells Int 2017:6202783. https://doi.org/10.1155/2017/6202783
18. Salmikangas P, Schuessler-Lenz M, Ruiz S et al (2015) Marketing regulatory oversight of advanced therapy medicinal products (ATMPs) in Europe: the EMA/CAT perspective. Adv Exp Med Biol 871:103–130. https://doi.org/10.1007/978-3-319-18618-4_6
19. Guess AJ, Daneault B, Wang R et al (2017) Safety profile of good manufacturing practice manufactured interferon γ-primed mesenchymal stem/stromal cells for clinical trials. Stem Cells Transl Med 6:1868–1879. https://doi.org/10.1002/sctm.16-0485
20. Polancec D, Zenic L, Hudetz D et al (2019) Immunophenotyping of a stromal vascular fraction from microfragmented lipoaspirate used in osteoarthritis cartilage treatment and its lipoaspirate counterpart. Genes (Basel) 10. https://doi.org/10.3390/genes10060474
21. Nava S, Sordi V, Pascucci L et al (2019) Long-lasting anti-inflammatory activity of human microfragmented adipose tissue. Stem Cells Int 2019:5901479. https://doi.org/10.1155/2019/5901479
22. Paolella F, Manferdini C, Gabusi E et al (2019) Effect of microfragmented adipose tissue on osteoarthritic synovial macrophage factors. J Cell Physiol 234:5044–5055. https://doi.org/10.1002/jcp.27307
23. Randelli P, Menon A, Ragone V et al (2016) Lipogems product treatment increases the proliferation rate of human tendon stem cells without affecting their stemness and differentiation capability. Stem Cells Int 2016:4373410. https://doi.org/10.1155/2016/4373410
24. Bianchi F, Maioli M, Leonardi E et al (2013) A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell Transplant 22:2063–2077. https://doi.org/10.3727/096368912X657855
25. Shah D, Naciri M, Clee P, Al-Rubeai M (2006) NucleoCounter—an efficient technique for the determination of cell number and viability in animal cell culture processes. Cytotechnology 51:39–44. https://doi.org/10.1007/s10616-006-9012-9
26. Stanco D, Viganò M, Perucca Orfei C et al (2015) Multidifferentiation potential of human mesenchymal stem cells from adipose tissue and hamstring tendons for musculoskeletal cell-based therapy. Regen Med 10:729–743. https://doi.org/10.2217/rme.14.92
27. Desjardins P, Conklin D (2010) NanoDrop microvolume quantitation of nucleic acids. J Vis Exp. https://doi.org/10.3791/2565
28. Viganò M, Perucca Orfei C, de Girolamo L et al (2018) Housekeeping gene stability in human mesenchymal stem and tendon cells exposed to tenogenic factors. Tissue Eng Part C Methods 24:360–367. https://doi.org/10.1089/ten.TEC.2017.0518
29. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45
30. Bailey AJ, Robins SP, Balian G (1974) Biological significance of the intermolecular crosslinks of collagen. Nature 251:105–109
31. Nho SJ, Yadav H, Shindle MK, Macgillivray JD (2008) Rotator cuff degeneration: etiology and pathogenesis. Am J Sports Med 36: 987–993. https://doi.org/10.1177/0363546508317344
32. Abraham AC, Shah SA, Thomopoulos S (2017) Targeting inflammation in rotator cuff tendon degeneration and repair. Tech Shoulder Elb Surg 18:84–90. https://doi.org/10.1097/BTE.0000000000000124
33. Thankam FG, Roesch ZK, Dilisio MF et al (2018) Association of inflammatory responses and ECM disorganization with HMGB1 upregulation and NLRP3 inflammasome activation in the injured rotator cuff tendon. Sci Rep 8:8918. https://doi.org/10.1038/s41598-018-27250-2
34. Gotoh M, Hamada K, Yamakawa H et al (1997) Significance of granulation tissue in torn supraspinatus insertions: an immunohistochemical study with antibodies against interleukin-1 beta, cathepsin D, and matrix metalloprotease-1. J Orthop Res 15:33–39. https://doi.org/10.1002/jor.1100150106
35. Riley GP, Curry V, DeGroot J et al (2002) Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. Matrix Biol 21:185–195
36. Zhang J, Wang JH-C (2010) Production of PGE(2) increases in tendons subjected to repetitive mechanical loading and induces differentiation of tendon stem cells into non-tenocytes. J Orthop Res 28:198–203. https://doi.org/10.1002/jor.20962
37. Schneider M, Angele P, Järvinen TAH, Docheva D (2018) Rescue plan for Achilles: therapeutics steering the fate and functions of stem cells in tendon wound healing. Adv Drug Deliv Rev 129: 352–375. https://doi.org/10.1016/j.addr.2017.12.016
38. Hammerman M, Blomgran P, Ramstedt S, Aspenberg P (2015) COX-2 inhibition impairs mechanical stimulation of early tendon healing in rats by reducing the response to microdamage. J Appl Physiol 119:534–540. https://doi.org/10.1152/japplphysiol.00239.2015
39. Rundle CH, Chen S-T, Coen MJ et al (2014) Direct lentiviral-cyclooxygenase 2 application to the tendon-bone interface promotes osteointegration and enhances return of the pull-out tensile strength of the tendon graft in a rat model of biceps tenodesis. PLoS One 9:e98004. https://doi.org/10.1371/journal.pone.0098004
40. Bergqvist F, Carr AJ, Wheway K et al (2019) Divergent roles of prostacyclin and PGE2 in human tendinopathy. Arthritis Res Ther 21. https://doi.org/10.1186/s13075-019-1855-5
41. Millar NL, Wei AQ, Molloy TJ et al (2009) Cytokines and apoptosis in supraspinatus tendinopathy. J Bone Joint Surg (Br) 91:417–424. https://doi.org/10.1302/0301-620X.91B3.21652
42. Ackermann PW, Domeij-Arverud E, Leclerc P et al (2013) Anti-inflammatory cytokine profile in early human tendon repair. Knee Surg Sports Traumatol Arthrosc 21:1801–1806. https://doi.org/10.1007/s00167-012-2197-x
43. Millar NL, Akbar M, Campbell AL et al (2016) IL-17A mediates inflammatory and tissue remodelling events in early human tendinopathy. Sci Rep 6. https://doi.org/10.1038/srep27149
44. Yoshihara Y, Hamada K, Nakajima T et al (2001) Biochemical markers in the synovial fluid of glenohumeral joints from patients

with rotator cuff tear. J Orthop Res 19:573–579. https://doi.org/10.1016/S0736-0266(00)00063-2
45. Lo IKY, Marchuk LL, Hollinshead R et al (2004) Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. Am J Sports Med 32:1223–1229. https://doi.org/10.1177/0363546503262200
46. Assunção JH, Godoy-Santos AL, Dos Santos MCLG et al (2017) Matrix metalloproteases 1 and 3 promoter gene polymorphism is associated with rotator cuff tear. Clin Orthop Relat Res 475:1904–1910. https://doi.org/10.1007/s11999-017-5271-3
47. Del Buono A, Oliva F, Longo UG et al (2012) Metalloproteases and rotator cuff disease. J Shoulder Elb Surg 21:200–208. https://doi.org/10.1016/j.jse.2011.10.020
48. Costa-Almeida R, Berdecka D, Rodrigues MT et al (2018) Tendon explant cultures to study the communication between adipose stem cells and native tendon niche. J Cell Biochem 119:3653–3662. https://doi.org/10.1002/jcb.26573
49. Costa-Almeida R, Calejo I, Reis RL, Gomes ME (2018) Crosstalk between adipose stem cells and tendon cells reveals a temporal regulation of tenogenesis by matrix deposition and remodeling. J Cell Physiol 233:5383–5395. https://doi.org/10.1002/jcp.26363
50. Gotoh M, Mitsui Y, Shibata H et al (2013) Increased matrix metalloprotease-3 gene expression in ruptured rotator cuff tendons is associated with postoperative tendon retear. Knee Surg Sports Traumatol Arthrosc 21:1807–1812. https://doi.org/10.1007/s00167-012-2209-x
51. Shukunami C, Takimoto A, Oro M, Hiraki Y (2006) Scleraxis positively regulates the expression of tenomodulin, a differentiation marker of tenocytes. Dev Biol 298:234–247. https://doi.org/10.1016/j.ydbio.2006.06.036
52. Veronesi F, Della Bella E, Torricelli P et al (2015) Effect of adipose-derived mesenchymal stromal cells on tendon healing in aging and estrogen deficiency: an in vitro co-culture model. Cytotherapy 17:1536–1544. https://doi.org/10.1016/j.jcyt.2015.07.007
53. Thankam FG, Evan DK, Agrawal DK, Dilisio MF (2018) Collagen type III content of the long head of the biceps tendon as an indicator of glenohumeral arthritis. Mol Cell Biochem. https://doi.org/10.1007/s11010-018-3449-y
54. Pajala A, Melkko J, Leppilahti J et al (2009) Tenascin-C and type I and III collagen expression in total Achilles tendon rupture. An immunohistochemical study. Histol Histopathol 24:1207–1211. https://doi.org/10.14670/HH-24.1207
55. Dabrowski MP, Stankiewicz W, Płusa T et al (2001) Competition of IL-1 and IL-1ra determines lymphocyte response to delayed stimulation with PHA. Mediat Inflamm 10:101–107
56. Palomo J, Dietrich D, Martin P et al (2015) The interleukin (IL)-1 cytokine family – balance between agonists and antagonists in inflammatory diseases. Cytokine 76:25–37. https://doi.org/10.1016/j.cyto.2015.06.017
57. Luo J, Xiong Y, Han X, Lu Y (2011) VEGF non-angiogenic functions in adult organ homeostasis: therapeutic implications. J Mol Med; New York 89:635–645. https://doi.org/10.1007/s00109-011-0739-1
58. Cohen T, Nahari D, Cerem LW et al (1996) Interleukin 6 induces the expression of vascular endothelial growth factor. J Biol Chem 271:736–741
59. Legerlotz K, Jones ER, Screen HRC, Riley GP (2012) Increased expression of IL-6 family members in tendon pathology. Rheumatology (Oxford) 51:1161–1165. https://doi.org/10.1093/rheumatology/kes002
60. Liu Z, Simpson RJ, Cheers C (1995) Interaction of interleukin-6, tumour necrosis factor and interleukin-1 during Listeria infection. Immunology 85:562–567
61. Chen S, Deng G, Li K et al (2018) Interleukin-6 promotes proliferation but inhibits Tenogenic differentiation via the Janus kinase/signal transducers and activators of transcription 3 (JAK/STAT3) pathway in tendon-derived stem cells. Med Sci Monit 24:1567–1573. https://doi.org/10.12659/MSM.908802
62. Lin TW, Cardenas L, Glaser DL, Soslowsky LJ (2006) Tendon healing in interleukin-4 and interleukin-6 knockout mice. J Biomech 39:61–69. https://doi.org/10.1016/j.jbiomech.2004.11.009
63. John T, Lodka D, Kohl B et al (2010) Effect of pro-inflammatory and immunoregulatory cytokines on human tenocytes. J Orthop Res 28:1071–1077. https://doi.org/10.1002/jor.21079
64. Pauly S, Klatte-Schulz F, Stahnke K et al (2018) The effect of autologous platelet rich plasma on tenocytes of the human rotator cuff. BMC Musculoskelet Disord 19:422. https://doi.org/10.1186/s12891-018-2339-5