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Human Fetal Progenitor Tenocytes for Regenerative Medicine

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Tendon injuries are very frequent and affect a wide and heterogeneous population. Unfortunately, the healing process is long with outcomes that are not often satisfactory due to fibrotic tissue appearance, which leads to scar and adhesion development. Tissue engineering and cell therapies emerge as interesting alternatives to classical treatments. In this study, we evaluated human fetal progenitor tenocytes (hFPTs) as a potential cell source for treatment of tendon afflictions, as fetal cells are known to promote healing in a scarless regenerative process. hFPTs presented a rapid and stable growth up to passage 9, allowing to create a large cell bank for off-the-shelf availability. hFPTs showed a strong tenogenic phenotype with an excellent stability, even when placed in conditions normally inducing cells to differentiate. The karyotype also indicated a good stability up to passage 12, which is far beyond that necessary for clinical application (passage 6). When placed in coculture, hFPTs had the capacity to stimulate human adult tenocytes (hATs), which are responsible for the deposition of a new extracellular matrix during tendon healing. Finally, it was possible to distribute cells in porous or gel scaffolds with an excellent survival, thus permitting a large variety of applications (from simple injections to grafts acting as filling material). All of these results are encouraging in the development of an off-the-shelf cell source capable of stimulating tendon regeneration for the treatment of tendon injuries.

Key words: Cell banking; Fetal cell therapy; Tendon healing; Tendon injuries; Tenocytes

INTRODUCTION

Tendons have the role to transmit forces from muscle to bone and protect the surrounding tissues by working as buffers against tension and compression. Unfortunately, tendon injuries are very common and affect wide and heterogeneous populations, which leads to potential loss of activity due to decreased mobility and chronic pain. Tendon injuries can be divided into two main categories depending on their acute or chronic nature; acute afflictions usually include traumatic partial or total tears or avulsions, while chronic afflictions are due to an overuse degenerative process presently referred to as tendinopathies (49). Although there is a wide range of tendinopathies, the majority are due to tendinosis (35). In this case, the histopathological observations of overused tendons generally present a disorganized extracellular matrix with separation of collagen fibers, decrease in fiber diameter and in density, but increase in type III collagen and in glycosaminoglycans. Simultaneously, hypercellularity and neovascularization are detected, but inflammation and granulation tissues are not common and are normally only seen in tendon ruptures. Subcategories of tendinosis showing lipoid degeneration or calcifications between collagen fibers can also be found (34,46,49). However, the separation between acute and degenerative tendon injuries is not always clear as spontaneous ruptures appear almost always from degenerative states, and thus tendinopathies can be considered as a predisposition to tendon ruptures (28,33). In many countries, with the democratization of sport practice, the number of overuse injuries has augmented during the last decades, and frequent sport-related injuries to tendons include the Achilles, the patellar, the tractus iliotibialis, the biceps femoris (Hamstring syndrome), the rotator cuff, the common wrist extensors (tennis elbow), and the common wrist flexors (golfer's elbow) (21,39). From a large joint clinical database, it has been shown that for 100.4 million injuries of any kind reported in 2006/2007 in the US, about 61.2 million are musculoskeletal, and among them, 44% are due to sprains and strains of soft tissue including

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ligaments or tendons (54). Taking results globally in the US, surgical interventions to repair tendons were estimated at 51,000 per year for rotator cuff tendons, 44,000 for Achilles tendon, and 42,000 for patellar tendon (11). Acute injuries to the hand tendon system also represent numerous cases (53). Globally, a variety of tendons are implicated with frequent impact on work or leisure.

The conventional treatments depend on the gravity of the injury, but range from conservative treatments with physiotherapy or infiltration and extend to surgical interventions such as tenotomy, tenodesis, suture, transfer, graft, or even prosthetic implants. Controlled mobilization is recommended in parallel because it has been shown that mechanical stimulation leads to fewer adhesions and increased strength (27). Regardless, tendon healing is always long, and this process can be divided in three overlapping steps. At first the inflammatory phase starts immediately following injury with the synthesis of chemoattractive substances by erythrocytes and inflammatory cells to attract and stimulate tenocytes. After a few days, the second step consists in the production of matrix mostly composed of type III collagen and under the control of tenocytes and macrophages. The last step takes place after 1 or 2 months; extracellular matrix proteins are still produced, but with an increase in type I collagen deposition and the reorganization and optimization of the fiber alignment. With time, the cell density and activity diminishes, and the overall procedure can take as long as 1 year or more. Unfortunately, even if long, the healing process is not perfect, and the natural structure is never attained again. Instead, scar tissue and adhesions remain and can lead to decreased mobility and rerupture (27,49,55).

Along with diminished healing outcomes, another issue is the potential lack of material for tissue replacement. For example, in the case of hand tendon injuries, a graft is frequently needed. The harvest of the palmaris longus or plantaris tendon is the prime choice as other graft sites potentially lead to disability. Nevertheless, these two sources are vestigial muscles, and thus are sometimes absent in individuals (26,56) and even if present are often not adequate (22,26).

To counter the poor healing outcomes and the potential lack of material, new strategies must be explored and tested. The field of regenerative therapy has encountered much interest lately. Off-the-shelf methods of cellular therapies able to stimulate tendon regeneration while preventing scar tissue maintenance would be of particular interest. It has been well documented in animals and humans that the early to midgestation fetuses (<24 weeks gestation for human) heal wounds through a mechanism that differs from adult tissue and is more rapid and leads to a regenerated tissue without scar (1), which is intrinsic to the fetal tissue itself and not to the fetal environment (10,14,20). It was shown in fetal animal models that bone (30), articular cartilage (37), and tendon (8) have the same ability as the skin to heal without scar. A recent study highlighted the change in expression of multiple genes during tendon development, which could explain the different type of healing (48). In the same manner, the use of cells harvested from fetal tissue has proven to be positive in wound healing. Two clinical trials including pediatric patients suffering from burns or wounds obtained excellent functional and esthetical results with the use of biological bandages composed of fetal skin progenitor cells. The damaged sites stimulated by fetal fibroblasts healed so well that the autografts initially intended were finally not necessary (15,23). In another study on adults suffering from refractory venous ulcers, the use of fetal skin progenitor cells also improved the healing (45). Along with their ability to stimulate healing, fetal cells present other interesting characteristics. They are already tissue specific and thus have innate good phenotypic stability. Moreover, with their high proliferation capacity, it is notably possible to create large cell stocks (cell banks) showing a high level of consistency and safety with respect to stringent requirements for clinical practice. Indeed, as the cell bank can be based on only one donor tissue, it is possible to trace all levels of the process from tissue to cells to final preparations including extensive screening for infectious diseases (2,44).

Therefore, fetal cells with their off-the-shelf availability and their capacity to stimulate healing in a scarless manner are a very attractive source for tissue regeneration. In this study, we evaluate the characteristics of human fetal progenitor tenocytes (hFPTs) that have undertaken clinical processing as these cells could be a potential safe and stable source of therapeutic cells available for tissue engineering and for treatment of tendon injuries.

MATERIALS AND METHODS

Tissue Culture and Cell Banking

Human Fetal Progenitor Tenocyte Harvesting and Cell Banking. Human fetal progenitor tenocytes were isolated from the Achilles tendon of a male 14 week gestation organ donation according to a protocol approved by an ethics committee [University Hospital of Lausanne (CHUV), Ethics Committee Protocol No. 62/07: 14 week gestation organ donation, registered under the Federal Transplantation Program and its Biobank complying with the laws and regulations]. The procedure was already explained in detail for other tissues (3,44). Briefly, the tendon biopsy of approximately 1 mm^3 was dissected by a fetal pathologist and placed in phosphate-buffered saline (PBS: NaCl 6.8 g/L, Na_2HPO_4 1.5 g/L, KH_2PO_4 0.4 g/L, code No. 100 0 324; Bichsel, Interlaken, Switzerland). Within several hours, the biopsy was transported to the certified human tissue laboratory for cell culture for

clinical use. The biopsy was washed three times for 15 min in PBS with 1% penicillin–streptomycin (Pen-Strep: code No. 15140-122; Gibco, Life Technologies Ltd., Paisley, UK). The tissue was dissected into smaller fragments, placed into culture dishes of 10 cm diameter (TPP, Trasadingen, Switzerland), and cultured in clinical growth medium composed of Dulbecco's modified Eagle medium (DMEM, code No. 41966-029; Gibco) containing 25 mM dextrose, 1 mM sodium pyruvate, supplemented to 5.97 mM L-glutamine (l-glut: code No. 25030-024; Gibco) and with 10% clinical-grade fetal bovine serum (FBS, code No. 10101145; Invitrogen, Life Technologies Ltd), without antibiotic supplementation. The medium was changed every 3 to 4 days and first cell growth emitting from tissue was seen already at 24 h. These cells (passage 0) were grown to 50% confluence, detached with trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA: 0.05% trypsin–0.02% EDTA, code No. 25300-054; Gibco), distributed into 75-cm^2 flasks (TPP) at passage 1, and they were used to develop a parental cell bank 11 days later when they reached full confluence. A total of 50 cryovials, each with 10 million cells in 1 ml cryosolution [50% DMEM, 40% FBS, 10% dimethyl sulfoxide (DMSO: code No. D2438; Sigma-Aldrich, St. Louis, MO, USA)], were frozen at a cooling rate of 1°C/min within a Nalgene cryo freezing container placed at −80°C. After 24 h, cells were transferred to the vapor phase of liquid nitrogen (−165°C) for long-term storage. The developed process has provided a unique cell population with the associated cell deposits for patent application (WO 2013/008174 A1: European publication No. 2732030: European Collection of Cell Cultures, ECACC deposit reference 12070302).

Human Adult Tenocyte (hAT) Harvesting and Cell Banking. Adult tenocytes can be harvested from pieces of tendon resected during an operation. As Achilles tendon is generally not resected, it was decided to work with another anatomical source more readily available. Healthy abductor pollicis longus tendon of a 69-year-old male donor was obtained in the CHUV in accordance with the institutional policy on tissue donation and under the Department Biobank regulations (CHUV, Regulations for Biobank for Musculoskeletal Medicine Department No. 3-12-2012). Tissue was placed in PBS and directly transferred to the laboratory. The tissue fragment was rinsed three times for 15 min in PBS with 5% Pen-Strep. It was then dissected into smaller fragments and transferred into dishes of 6 cm diameter with growth medium (further called standard growth medium) composed of DMEM containing 25 mM dextrose, 1 mM sodium pyruvate, 5.97 mM l-glut, and 10% laboratory-grade FBS (code No. F7524; Sigma-Aldrich), without antibiotic supplementation. The cells could be seen to migrate from the tissue after several days (5–7), and the medium was changed every 3 to 4 days. At confluency, cells were detached with trypsin–EDTA and grown in 75-cm^2 flasks up to passage 2 to develop a standard cell stock. Cells were trypsinized and distributed into 24 vials, each with 1 million cells in 1 ml cryosolution (50% DMEM, 40% FBS, 10% DMSO) and frozen in the same manner as for hFPTs (lower cell concentration to obtain sufficient number of vials). A parental adult cell bank composed of 24 million cells in passage 2 was therefore created.

Cell Growth and Morphology: 2D and 3D Conditions

Both human fetal progenitor and adult tenocytes were grown under the same conditions for 2D culture. Cells were expanded into tissue culture polystyrene flasks of 75 cm^2 with filter screw cap (TPP) placed in cell culture incubators at 37°C in a humidified atmosphere containing 5% CO_2. The standard growth medium was composed of DMEM with 25 mM dextrose, 1 mM sodium pyruvate, 5.97 mM L-glutamine, and 10% laboratory-grade FBS, free of antibiotic supplementation. It was changed every 3 to 4 days, and cells were trypsinized when reaching confluence. Seeding densities of 3,000 and 6,000 cells/cm^2 were tested, but as higher seeding densities did not accelerate the overall growth, cells were then seeded at the low density of 3,000 cells/cm^2. Cell growth of hFPTs in 2D standard culture conditions was analyzed between passage 4 and 15. The morphology was controlled and recorded every 3 to 4 days by imaging (CKX41 and E-330; Olympus, Tokyo, Japan). After 13–15 days of growth, cells of at least three flasks were trypsinized, pooled together, and counted. The series were repeated multiple times, and each passage was evaluated at least four times. Mean population doublings (PD) and doubling time (DT) were determined for each passage using the formulas:

$$\mathrm{PD} = \frac{\ln\left(\dfrac{\text{final cell number}}{\text{initial cell number}}\right)}{\ln(2)} \tag{1}$$

$$\mathrm{DT} = \frac{\mathrm{Time[d]}}{\mathrm{PD}} \tag{2}$$

The linearity of the lengthening in doubling time for late passages was evaluated statistically by determining the regression line with the root mean square error model and calculating the coefficient of determination (R^2) in excel software (Microsoft, Redmond, WA, USA).

hFPTs were also seeded in six wells at passages 4 and 9, and the same formulas were used to determine the shortest doubling time during the most rapid growth period occurring between day 1 or 2 and day 7 of culture (each time point in triplicate and experiments repeated three times).

For some experiments, the cells were also cultured as microtissue pellets with a 3D configuration. They were produced by centrifuging 500,000 cells for 10 min at 250×*g* and then placing individual tubes in cell culture incubators at 37°C in a humidified atmosphere containing 5% CO_2 with the cap unscrewed half a rotation to allow gas circulation. Standard growth medium was changed every 3 to 4 days. Some pellets were also produced with a supplementation of 284 μM L-ascorbic acid (code No. A4544; Sigma-Aldrich) to evaluate the impact on protein deposition.

Cell Conservation, Viability, and Recovery

The cells were preserved in a standard cryosolution (50% DMEM, 40% FBS, and 10% DMSO) for long-term conservation. The percentage of living cells at thawing was based on cell counting with a hemocytometer and the trypan blue exclusion technique (code No. T-8154; Sigma-Aldrich). The cells were then plated in 75-cm^2 tissue culture flasks and counted when cells reached confluency (after 13 to 15 days in culture with a seeding density of 3,000 cells/cm^2). The results were compared to cells seeded at the same passage from trypsinization instead of thawing.

Tendon Marker Profiling

hFPTs and hATs were both evaluated in 2D culture to compare their morphology and the expression of characteristic markers. The cells were plated at 3,000 cells/cm^2 in 48-well plates and incubated under standard culture conditions for 72 h. Immunohistochemical stainings were then realized as follows. Permeabilization of membranes was made during treatment for 10 min in 0.1% Triton X-100 (code No. A1388; Applichem, Darmstadt, Germany). The slides were washed three times in PBS and blocked for 30 min in bovine serum albumin solution 1% (BSA: code No. A7906; Sigma-Aldrich), before incubation with primary antibody at 4°C overnight. Primary antibodies were diluted in BSA 1% and targeted type I collagen (1:1,000; code No. ab292; Abcam, Cambridge, UK), scleraxis (1:100; code No. ab58655; Abcam), and tenomodulin (N-14) (1:100; code No. sc-49325; Santa Cruz Biotechnology, Heidelberg, Germany). After three washes in PBS, the slides were incubated with their complementary secondary antibody for 40 min at room temperature, namely Alexa Fluor® 488 donkey anti-goat IgG (1:800 in PBS; code No. A-11055; Molecular Probes, Life Technologies Ltd, Paisley, UK) or Alexa Fluor® 568 donkey anti-rabbit IgG (1:1,000 in PBS; code No. A-10042; Molecular Probes). The nuclei were then counterstained with 4,6-diamidino-2-phenylindole (DAPI; code No. 10236276001; Roche, Rotkreuz, Switzerland), and images were taken with an inverted microscope equipped for fluorescence (Olympus IX81) and a digital camera (Andor iXon). Control stainings were performed with the secondary antibodies only (primary antibodies replaced by 1% BSA) and were used as negative to adjust display of fluorescent signals within ImageJ software (NIH, Bethesda, MD, USA).

hFPTs and hATs were also grown in 3D pellet culture to evaluate their ability to produce tendon matrix. After 14 days, pellets were fixed in 4% (w/v) neutralized formalin solution (code No. 3933; J.T. Baker, Deventer, The Netherlands) for 24 h at 4°C, washed three times with PBS, and subsequently dehydrated and embedded in paraffin (code No. 111609; Merck Millipore, Darmstadt, Germany). The sections of 3 μm were then rehydrated and washed with PBS prior to staining. Type I collagen, tenomodulin, and scleraxis immunohistochemical stainings were realized with the same method as described above for monolayer cells, but with an additional step consisting of antigen retrieval for 10 min in heated Tris (hydroxymethyl) aminomethane (Tris: 10 mM; code No. T-6066; Sigma-Aldrich)–EDTA (1 mM; code No. 03680; Sigma-Aldrich) at pH 9.0, before blocking the section. Instead of addition of DAPI, the slides with 3D pellet culture were then mounted with Vectashield mounting medium with DAPI (code No. H-1200; Vector Laboratories, Burlingame, CA, USA). Control staining and treatment of images were done as explained above for 2D monolayer cells.

Cell Population Homogeneity and Stability: Flow Cytometry and Karyotyping

Population homogeneity and stability were evaluated at passages 3, 6, and 9 by flow cytometry. Cells were tagged with ethidium monoazide (EMA: 1 μg/ml, code No. E1374; Molecular Probes) to exclude the cells with damaged membranes. Primary monoclonal mouse antibodies targeting fibroblast/epithelial marker D7-FIB (1:50 in PBS; code No. MCA1399GT, clone D7-FIB; Abd Serotec, Dusseldorf, Germany), macrophage/neutrophil marker CD14 (1:10; code No. MCA2185T, clone MEM-18; Abd Serotec), hematopoietic and endothelial cell marker CD34 (1:50; code No. MCA547GT, clone QBEND/10; Abd Serotec), cell adhesion marker CD90 (1:10; code No. MCA90, clone F15-42-1; Abd Serotec), and the coreceptor of the transforming growth factor-β (TGF-β) superfamily CD105 (1:50; code No. MCA1557, clone SN6; Abd Serotec) were used. A secondary antibody, Alexa Fluor® 488 goat anti-mouse IgG (1:1,000 in PBS; code No. A-11001; Molecular Probes), was used in combination. Conjugated monoclonal antibodies were also used to tag human leukocyte antigens (HLA), which are implicated in transplant rejection. HLA-DR, DP, DQ antibody was coupled with fluorescein isothyocyanate (FITC) and HLA-A, B, C antibody with phycoerythrin (PE) (1:1; code No. 555558, clone Tu39 and 555553, clone G46-2.6; BD Pharmingen, San Diego, CA, USA). The nonimmunogenic HLA-G protein was also tested with a primary monoclonal

mouse antibody (1:10; code No. ab52455, clone 4H84; Abcam) and with the same secondary antibody. Negative controls were made with nonspecific antibodies of the same isotypes at equal concentrations (Abd Serotec, code No. MCA928EL, code No. IC002P and BD Pharmingen, code No. 555573; R&D Systems, Minneapolis, MN, USA). Data were obtained with a CyAN™ADP analyzer (Beckman Coulter, Indianapolis, IN, USA) and processed with FlowJo software (Ashland, OR, USA).

A conventional karyotype was realized in order to observe potential numerical or structural abnormalities and to identify low-degree mosaic conditions of the hFPTs. For these analyses, dividing cells were exposed for 1.5 h to colchicine (code No. 27650; Sigma-Aldrich) at a final concentration of 0.16 µg/ml to block cell division in metaphase. The cells were then exposed to a hypotonic shock with 37.5 mM KCl (code No. 60121; Sigma-Aldrich) for 20 min, fixed with 25% acetic acid (code No. 320099; Sigma-Aldrich) and 75% methanol (code No. 179337; Sigma-Aldrich), and spread onto a glass microscope slide. The metaphases were exposed for 10–20 s to trypsin (1% in EDTA), washed with 150 mM NaCl, and dried at room temperature. The slides were then stained with Giemsa solution (2% in PBS; code No. 109204; Merck Millipore) for 8 min for visualization of the chromosomal G-band pattern. Karyotyping of hFPTs was accomplished with the observation of 60 metaphases at passages 3, 6, and 12. Forty supplementary metaphases were observed for chromosome 19 at passage 3.

Phenotypic Stability With Osteogenic- or Adipogenic-Inducing Condition

hFPTs were plated at 3,000 cells per cm^2 in 12-well plates, alongside with human adipose-derived stem cells (ASCs) and human bone marrow-derived mesenchymal stem cells (BM-MSCs), both obtained in the CHUV in accordance with the institutional policy on tissue donation and under the Department Biobank regulations (CHUV, Ethics protocol No. 124/10: "Development of cell banks from bone marrow for the development of treatments and bioengineering" and Regulations for Biobank for Musculoskeletal Medicine Department of the CHUV No. 3-12-2012). The cells were grown for 4 days with standard growth medium. An osteogenic induction was then accomplished over 21 days using a medium composed of α-MEM (code No. 22571-020; Gibco), 10% FBS, 5.97 mM L-glut, 284 µM L-ascorbic acid, 5 mM β-glycerophosphate (code No. G9422; Sigma-Aldrich), and 100 nM dexamethasone (code No. D4902; Sigma-Aldrich), which was changed every 3 to 4 days. Cells were then rinsed with deionized water and fixed in 4% formalin solution for 10 min at room temperature before von Kossa staining (code Nos. 85228, S-4019, and S-1648; Sigma-Aldrich) or Alizarin red (pH 9) staining (code No. A5533; Sigma-Aldrich) to observe the production of mineralized matrix, with the first showing phosphate deposition and the second calcium deposition (43). An adipogenic induction was realized over 21 days using a medium composed of DMEM, 5.97 mM L-glut, 1% insulin–transferrin–selenite (ITS, final concentration: 10 mg/L bovine insulin, 5.5 mg/L human transferrin, and 5 µg/L sodium selenite; code No. I3146; Sigma-Aldrich), 1 µM dexamethasone, 100 µM indomethacin (code No. I7378; Sigma-Aldrich), and 100 µM 3-isobutyl-1-methylxanthine (IBMX, code No. I5879; Sigma-Aldrich). Cells were then rinsed with deionized water, fixed in 4% formalin solution for 10 min at room temperature, and stained with Oil red O staining kit (code No. 010303; Diapath, Martinengo, Italy), according to the protocol of the manufacturer, to highlight the presence of neutral lipids.

Biocompatibility in Porous Scaffold and Gel Matrix

For evaluation of cell survival in porous scaffolds, hFPTs were trypsinized at passage 4, and solutions containing 250,000 cells in 500 µl standard growth medium were inoculated onto solid 2.25 cm × 3 cm commercial horse collagen membranes (code No. B2240100999999; TissuFleece E, Baxter, Nuremberg, Germany) and placed in culture dishes of 10 cm diameter. After 1 h of incubation at 37°C and 5% CO_2 to allow cells to attach to the membranes, standard growth medium was added in the dishes, which were then replaced in the incubator. At several time points up to day 14, the membranes were rinsed with PBS and 250 µl of LIVE/DEAD solution (code No. L-3224; Molecular Probes) added over the entire membranes. The membranes were incubated for 30 min at room temperature, before images were taken with an IX81 Olympus fluorescent microscope and Andor iXon digital camera and treated within ImageJ.

For evaluation of cell survival in hydrogel, hFPTs were trypsinized at passage 4, rinsed with PBS, and resuspended in PBS at 1 million cells/ml (PBS was used because growth medium cannot be removed from gel). Forty microliters was distributed into wells of a 96-well plate, mixed with 60 µl of a commercial 0.8% hyaluronic acid (HA) gel (Sinovial, IBSA, Lodi, Italy) with a displacing pipette to homogenize the suspension, and the plate was placed at 4°C overnight. After 24 and 72 h, 100 µl of LIVE/DEAD solution was added to the wells and mixed with the displacing pipette. Images were taken after 30 min of incubation at room temperature and treated within ImageJ.

Growth-Stimulating Ability of hFPTs Toward hATs

A coculture assay was realized using hATs in a 12-well plate and γ-irradiated hFPTs in Transwell inserts placed just above the hATs (code No. 3460-COR; Costar, Corning Incorporated, Lowell, MA, USA) (Fig. 1). The 0.4-µm polyester membrane at the bottom of Transwell allowed liquid diffusion while preventing any cell exchange. On

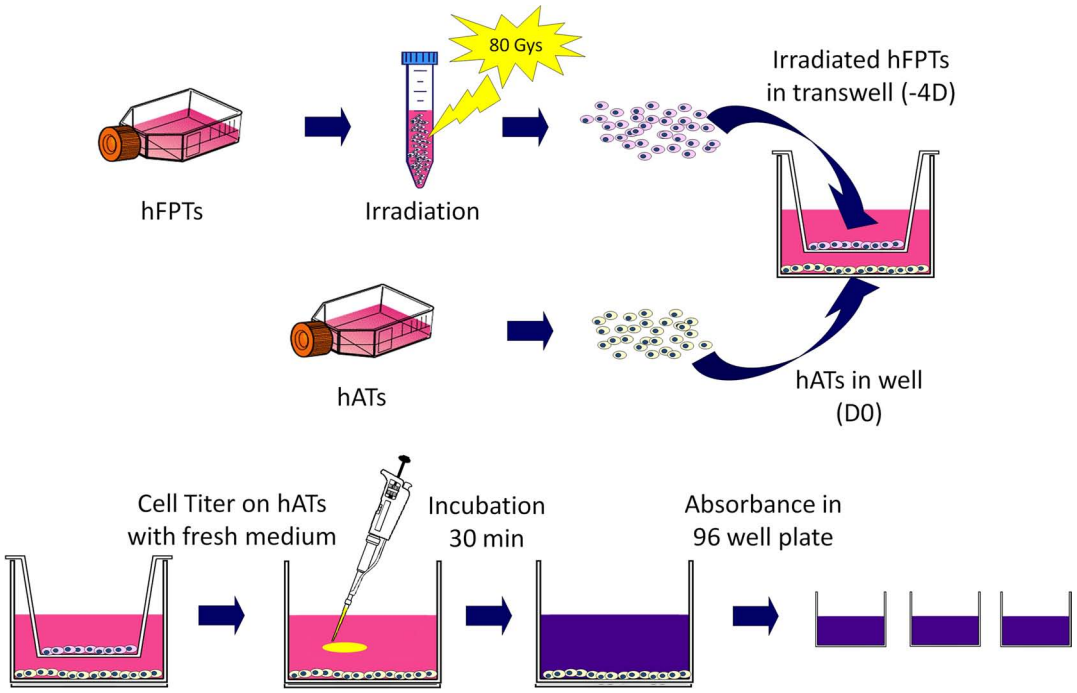

Figure 1. Coculture method for stimulation assay. Coculture method employed to assess metabolic activity of human adult tenocytes (hATs) when stimulated by human fetal progenitor tenocytes (hFPTs). hFPTs irradiated (to block division) and placed in Transwell inserts 4 days (−4D) before hATs to already condition the medium. hATs seeded in wells on D0 for beginning of growth. Cell titer assay done on hATs with fresh medium, and supernatant transferred into 96-well plate after reaction for absorbance reading at 492 nm.

day minus 4 (−4D), hFPTs were irradiated with a dose of 80 Gy (cesium 137) to inactivate their cellular division capacity in order to maintain a stable amount of cells in Transwells throughout the experiment (parallel test without growth for four timepoints up to day 25). They were seeded in triplicates to test four conditions corresponding to 0, 10,000, 50,000, and 100,000 irradiated hFPTs per Transwell with a total of 2 ml of standard growth medium between the well and Transwell. On day 0 (D0), the wells were all seeded at a density of 3,000 cells/cm^2 with adult cells. The 4-day delay between the two seeding sessions was done to already have a conditioned medium on day 0. The activity of hATs was measured every 3 to 4 days up to day 17, and at each time point the Transwells were placed apart in incubator with 1 ml of conditioned medium, which was preserved. The medium remaining in wells was discarded, 500 µl standard growth medium and 20 µl Cell Titer (code No. G3580; Promega, Madison, WI, USA) were added in each well (and in three supplementary empty wells for blanks), and the metabolic reaction took place during 30 min at 37°C. After 30 min, the reaction was stopped by rapidly transferring the supernatants to an empty 12-well plate. The supernatants were then transferred into a 96-well plate, and 10 min after the end of reaction, the absorbance was read at 492 nm (Tecan Infinite F50, Männerdorf, Switzerland), the blank absorbance was subtracted, and the mean corrected absorbance was calculated for the three wells of each condition. The percentage increase in activity was also calculated for each condition in comparison to hATs without stimulation. The hATs were rinsed once with PBS, 1 ml of standard growth medium was added to the wells, and the corresponding Transwells with 1 ml conditioned medium were placed back in the plate and into the incubator until the next time point. The whole experiment was performed three times successively, and the results are presented as mean of the three experiments with error bars indicating standard deviation. For each experiment and each condition, the average increase throughout the five timepoints was calculated, and the global increase per condition was then determined for the three experiments.

RESULTS

Cell Harvesting, Cell Culture, and Cell Banking

From one single fetal tendon biopsy, it was possible to isolate hFPTs and to expand them to obtain a clinical cell bank consisting of 50 vials of 10 million cells each at the end of passage 1. Starting from the adult pollicis longus tendon biopsy, it was possible to create a cell bank of 24 vials of 1 million cells each at the end of passage 2. Concerning the morphology, hFPTs maintained very similar spindle-shaped morphology from low passages up to passage 12 with highly aligned configuration when

the density increased. In higher passages (passage 15), the cells were slightly larger, and there was weaker cellular alignment when the density increased (Fig. 2A). The mean doubling time of hFPTs during passage showed two different phases. From passage 3 to passage 9, the growth was very steady, and the mean doubling time was always more rapid than 4 days (mean = 3.75 days, SD 0.16 days) with a mean of 3.72 population doubling per passage (SD 0.18 PD). The minimal doubling time was recorded between day 1 or 2 and day 7 and was also very similar between passage 4 (mean = 1.2 days SD 0.2 days, $n = 3$) and passage 9 (mean = 1.3 days, SD 0.1 days, $n = 3$). From passage 10 to passage 15, the proliferation rate slightly decreased linearly ($R^2 = 0.94$). For each passage, one population doubling took an additional 8 h than in the previous passages to be achieved, which represents

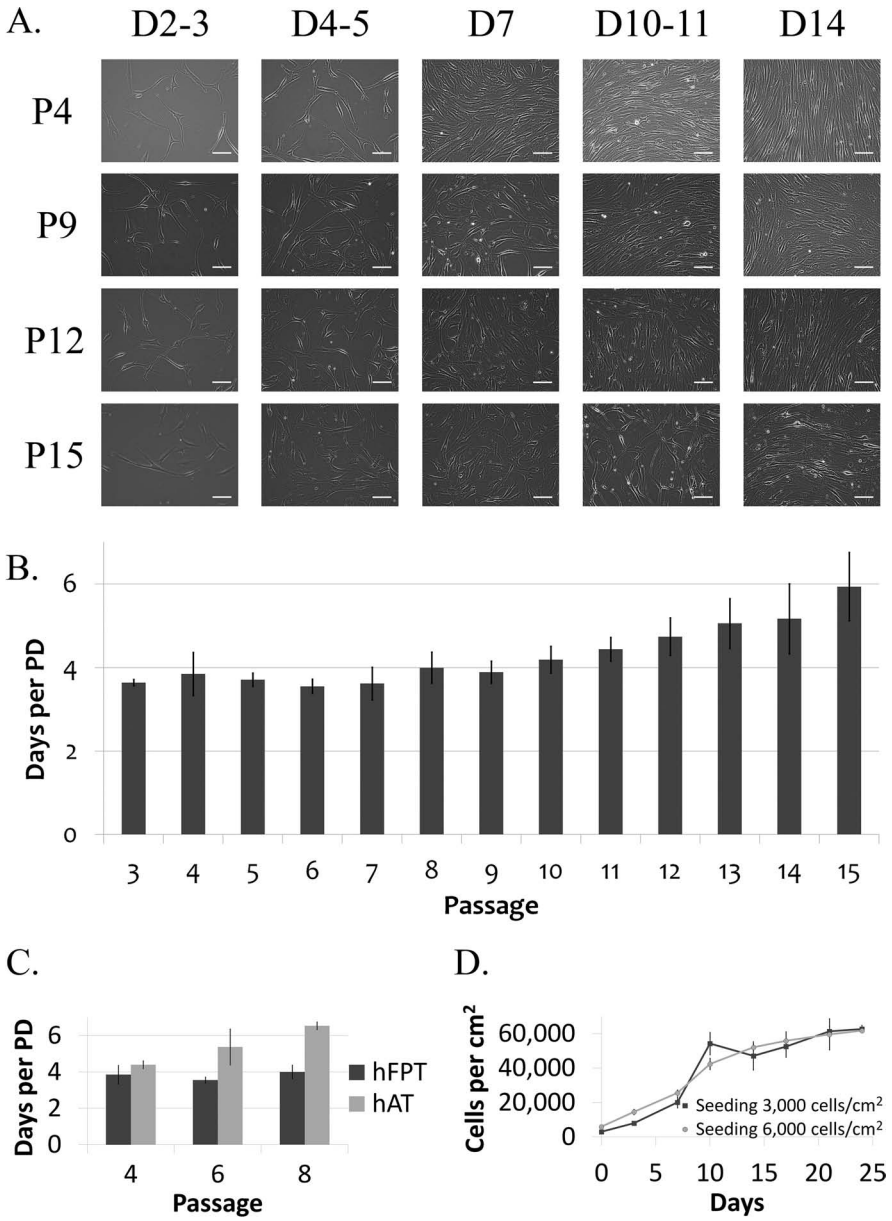

Figure 2. hFPT growth in monolayer culture. Cellular morphology of human fetal progenitor tenocytes (hFPTs) during cellular growth from low seeding density to confluency during early (P4–9), mid (P12), and late passages (P15) (A). Doubling time of hFPTs accomplished from early passages to late end-of-passage culture. Each passage tested four times with average number of days to allow cell doubling presented with standard deviation (B). Doubling time of hFPTs compared to the one of adult tenocytes (hATs) at similar passages (C). Influence on cell growth depending on low to intermediate seeding densities represented by actual cell counts from 0 to 24 days of culture. Experimentation done in triplicate with average and standard deviation indicated (D). Scale bars: 100 μm.

around 8.5% increase in doubling time (Fig. 2B). In comparison, hATs have already a longer doubling time at passage 4 with a mean of 4.40 days per doubling (SD 0.21 days, $n=4$), which then slows down to 5.38 days (SD 0.99 days, $n=3$) at passage 6 and 6.54 days (SD 0.21 days, $n=2$) at passage 8 (Fig. 2C). It was shown that low seeding densities (3,000 cells/cm^2) could be implemented with hFPTs with results very similar to higher seeding densities (6,000 cells/cm^2) (Fig. 2D). Low seeding densities allowed for a rapid early expansion and development of a very large clinical cell bank.

Cell Conservation, Viability, and Recovery

hFPTs conditioned in freezing medium and frozen at −80°C in passage 3 for 4 to 10 months presented a survival rate of 97.6% (SD 0.6%, $n=5$) after thawing. When seeded in 75-cm^2 flasks at passage 4 for 14 days, they had a mean doubling time of 3.73 days (SD 0.13 days, $n=3$), which is very close to what is found for the same passage from enzymatic detachment with 3.62 days (SD 0.42 days, $n=2$). hFPTs frozen in the vapor phase of liquid nitrogen for 30 to 36 months presented a survival rate of 93.8% (SD 2.9%, $n=3$) and a mean doubling time of 3.43 days (SD 0.21 days, $n=3$).

Expression of Surface Markers in 2D and 3D Culture Conditions

When cultured in 2D monolayer culture, fetal progenitor and adult tenocytes presented a very similar spindle-shaped morphology, and both expressed the three different markers. The normally extracellular type I collagen was present in the cytoplasm, but not in the nucleus. The type II transmembrane protein tenomodulin was expressed over the entire surface. The transcription factor scleraxis was detectable as a light staining both within the nucleus and in close proximity (Fig. 3A).

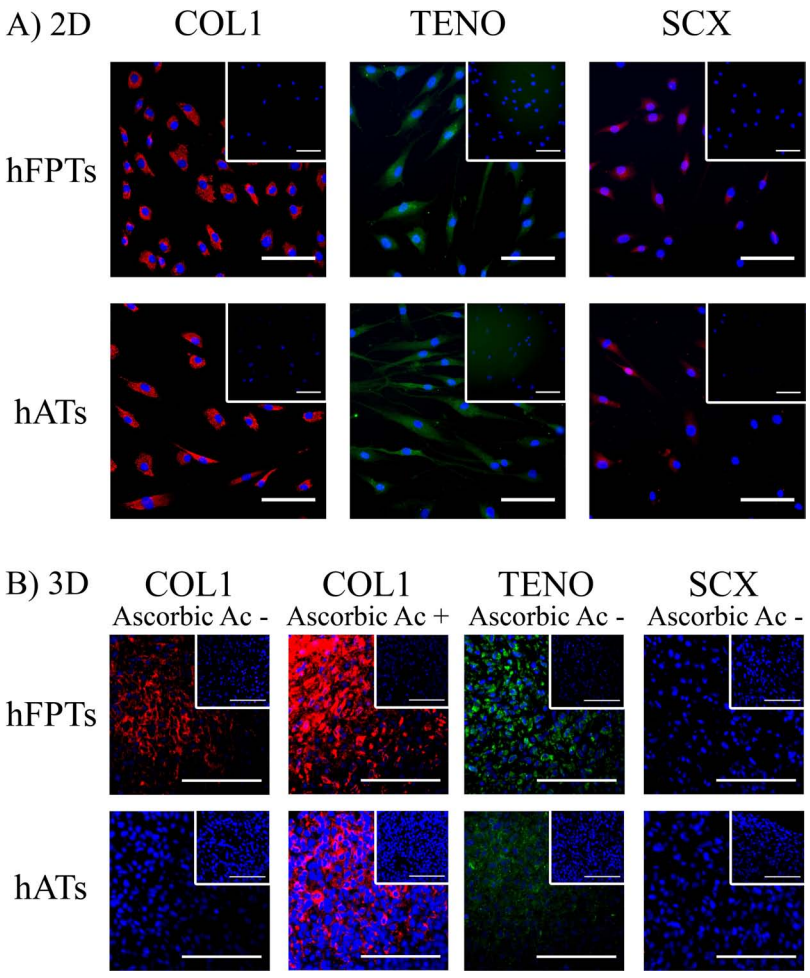

Figure 3. hFPT immunohistochemical staining in 2D and 3D (compared to hAT). Immunohistochemical staining of human fetal progenitor tenocytes (hFPTs) and adult tenocytes (hATs) for collagen I (COL1), tenomodulin (TENO), and scleraxis (SCX) in 2D monolayer culture (A) and 3D pellet culture with (+) or without (−) ascorbic acid supplementation (B). Negative controls shown in upper right corner of images. Scale bars: 100 μm.

Human progenitor and adult tenocytes had both the ability to form 3D pellets, and in such a configuration they were able to deposit extracellular matrix during the creation of microtissues. The matrix-to-cell ratio was slightly higher in hFPT pellets with the nucleus appearing more distant with more matrix in between. Interestingly, type I collagen was only detectable in the extracellular matrix surrounding fetal progenitor cells, but not around adult cells. The supplementation of the medium with ascorbic acid allowed to detect type I collagen also with hATs, but still in lower quantity compared to hFPTs. Tenomodulin (extracellular domain) was positive both in progenitor and adult tenocytes. Scleraxis is not an extracellular matrix protein, but was also tested in 3D to evaluate its expression. Although visible in monolayer culture, we were not able to detect the presence of this transcription factor in 3D culture for either fetal progenitor or adult tenocytes (Fig. 3B). The addition of ascorbic acid did not change the expression of tenomodulin or scleraxis (data not shown).

Cell Population Homogeneity and Stability: Flow Cytometry and Karyotyping

The cells showed a homogenous population distribution at all tested passages, and there was no shift in the expression of the observed surface markers. hFPTs stained positive for the fibroblast marker D7-fib, for the cell adhesion marker CD90, and for the coreceptor of the TGF-β superfamily CD105. There was no discernible contaminating subpopulation enrichment, as seen by the negative macrophage/neutrophil marker CD14 and hematopoietic/endothelial cell marker CD34. As expected, the proteins of MHC class II (HLA- DR, DP, DQ) were not present, while the ones from MHC class I (HLA-A, B, C) were mildly positive. The particular MHC class I protein HLA-G was not present (Fig. 4A).

hFPTs presented a male karyotype as seen by the presence of both X and Y chromosomes, and the quality was very good at all passages tested with a resolution of 500 G-bands. At passage 3, on the 60 metaphases tested, 57 were normal, and there was one nonclonal numerical abnormality observed during experimentation. Two metaphases presented structural abnormalities with a possible deletion of terminal short arm of chromosome 19. Therefore an additional 40 supplementary metaphases were tested for chromosome 19 without observation of other deletion. At passage 6, there was no clonal abnormality. Fifty-six metaphases were considered normal, one with numerical abnormality and three with structural abnormalities. At passage 12, the 60 metaphases presented all a normal karyotype. For each passage, the karyotype was always 46, XY. No mosaicism was detected at any passages, and with 60 metaphases tested for each passage, the risk of mosaicism remains smaller than 5% with a 0.95 confidence level as demonstrated by Hook (24) (Fig. 4B).

Phenotypic Stability With Osteogenic- or Adipogenic-Inducing Conditions

After 3 weeks of osteogenic induction, hFPTs remained stable, and there was no mineral deposition as shown with the Alizarin red and the von Kossa stainings, which both remained negative. Both of the adult stem cells derived from adipose tissue and bone marrow (ASCs and BM-MSCs) cultured under the same conditions deposited phosphate and calcium matrix as expected. A similar observation was made for adipogenic induction with hFPTs being negative for the Oil red O staining, while ASCs and BM-MSCs presented lipid accumulation (Fig. 5).

Biocompatibility in Porous Scaffold and Gel Matrix

Twenty-four hours after seeding, hFPTs demonstrated a good biocompatibility with commercial horse collagen as indicated using the LIVE/DEAD assay. The cells were homogeneously dispersed, they could attach to the scaffold, and more than 95% were metabolically active (green). Several time points were evaluated, and this viability was still found after 14 days. Once cells were successfully attached to the matrix (1 h), it was possible to rinse the membrane multiple times with PBS with no cell detachment seen. In contrast, cells seeded in PBS were mostly alive, but remained round and could not attach without standard growth medium. hFPTs could be homogeneously dispersed in a 0.8% HA gel by using a displacement pipette. They did not need growth medium and could be directly mixed in PBS or NaCl before being dispersed uniformly throughout a viscous gel matrix. More than 95% of the cells were alive and metabolically active when conserved for 24 h at 4°C. After 72 h, there was an increase in dead cells, and the overall viability approached 70% (Fig. 6).

Stimulating Ability of hFPTs Towards hATs

In vitro cell culture models for tendon regeneration can provide general results on dose response. Such models may lead to crucial preclinical information for critical assessment of potential responses of injury in vivo. The results within the coculture assay showed a dose-dependent stimulation of hFPTs on adult tenocytes. A quantity of 10,000 cells per Transwell seemed insufficient to stimulate adult tenocytes (0% increase, SD 2.3%, $n=3$). But with higher amounts of cells, between day 3 and 17, there was a mean increase of 10% (SD 1.3%, $n=3$) of the metabolic activity of adult tenocytes with 50,000 hFPTs per Transwell and of 25% (SD 3.8%, $n=3$) with 100,000 hFPTs per Transwell, compared to an absence of hFPTs (Fig. 7). The increased global activity means that the number of adult cells was increased or that the metabolic activity per cell was increased, or that both were simultaneous.

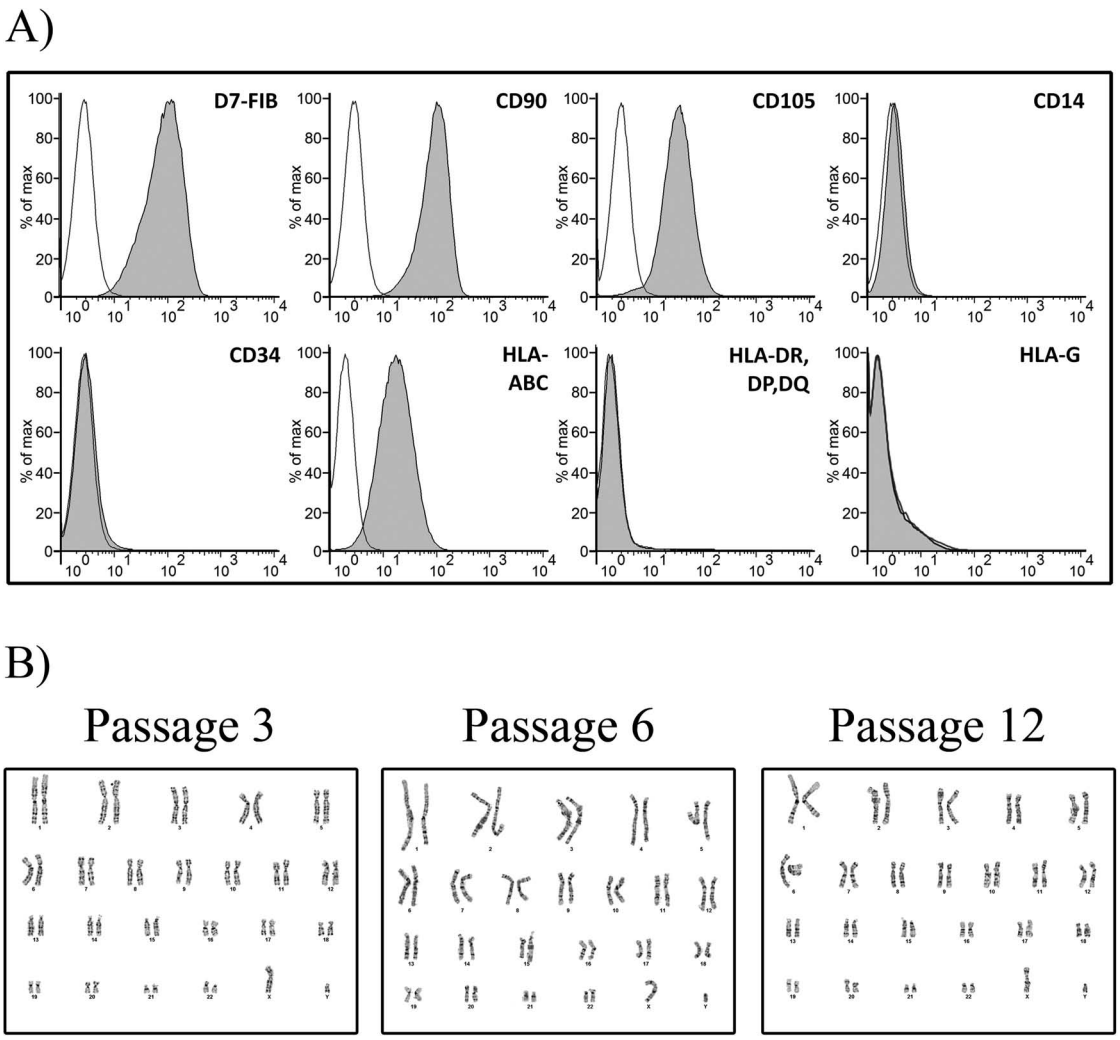

Figure 4. hFPT population homogeneity and genetic stability. Cell surface proteins tested by flow cytometry for human fetal progenitor tenocytes (hFPTs) at passage 6 (A). Karyotyping of hFPTs at low and high passages with low cell culture seeding protocols (B).

DISCUSSION

In case of tendon injury, the major negative outcomes are the presence of scar tissue and adherence between tendon and its surrounding structures. Fetal tendon heals in a different manner, without fibrotic tissue or adhesion. The differences seen between fetal regenerative healing and adult reparative healing relies probably on different cytokine profiles. The idea to use isolated cytokines is appealing; however, tissue healing is a complex process with multiple interactions between the different cytokines, and there is most likely a defined balance to be found to ameliorate tendon healing (8). Nevertheless, fetal cells, which naturally present such a tuned balance of cytokines and which moreover are supposed to be themselves the effectors of scarless healing (32), could be optimized for a unique cell source for therapeutic use.

To be compatible with a potential clinical use in the future, it is crucial to be in accordance with the laws and regulations. Tissues and cells for clinical use are regulated nationally in most countries worldwide. In the present case, the tissue was obtained as an organ donation, which is registered with Swissmedic (Swiss Agency for Therapeutic Products) in a Federal Transplantation Program and with respect to the Law for Transplantation RS810.21 of Switzerland and its related directives on research with human subjects. Moreover, it was possible to integrate current good manufacturing practice (GMP) for cell processing for the creation of the parental cell bank, which is of primary importance. Banking cells allows the creation of an off-the-shelf stock, which presents a major advantage to have cells available easily and rapidly for their use. In parallel to the legal aspects, the

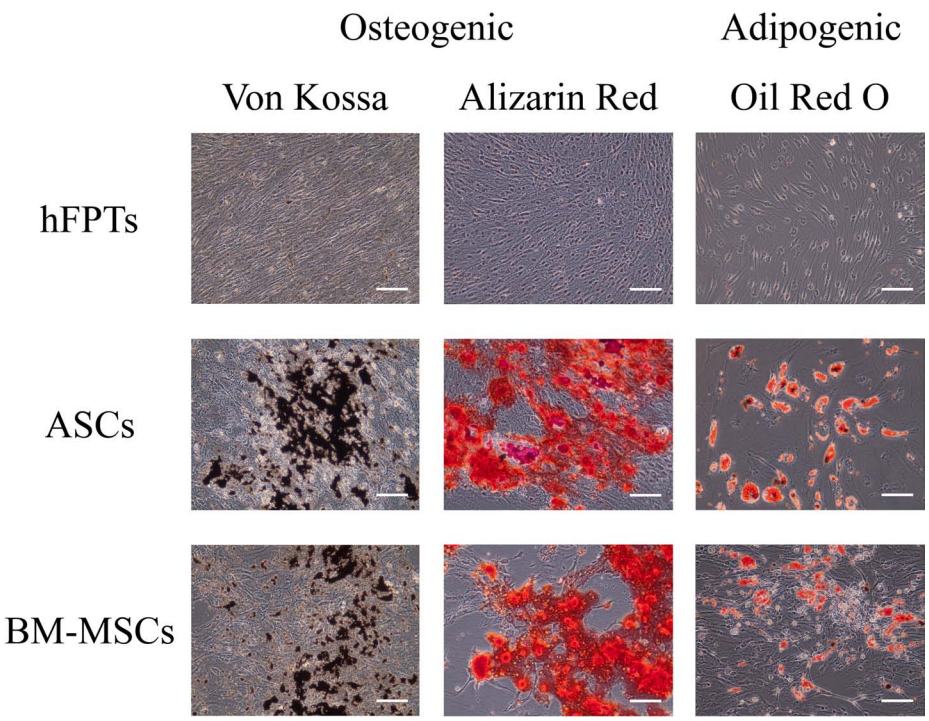

Figure 5. hFPT phenotypic stability with inducing conditions (compared to ASCs and BM-MSCs). Osteogenic and adipogenic differentiations of fetal progenitor tenocytes (hFPTs), adipose-derived stem cells (ASCs), and bone marrow-derived mesenchymal stem cells (BM-MSCs) over 21 days of induction. Osteogenic induction yields calcified nodules or lipid accumulation for BM-MSCs and ASCs, which are in brown-black with von Kossa staining (phosphate deposition) and in red with Alizarin red staining (calcium deposition). Adipogenic induction yields lipid accumulation for BM-MSCs and ASCs, which are in red with Oil red O staining. No matrix deposition or lipid accumulation seen for hFPTs. Scale bars: 100 μm.

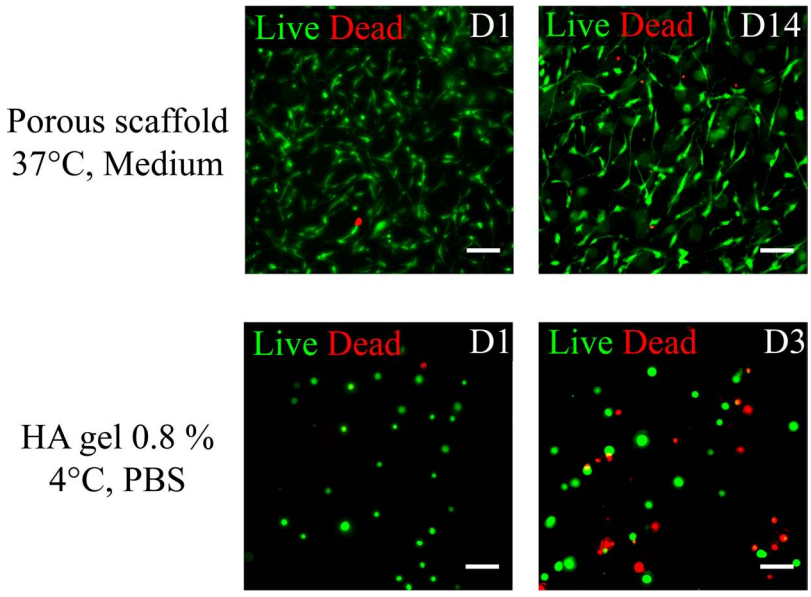

Figure 6. hFPT biocompatibility within delivery systems. Biocompatibility of human fetal progenitor tenocytes (hFPTs) with porous collagen scaffold and hyaluronic acid gel (0.8%) the day following seeding (D1) and again after 14 days (D14) for porous scaffold and 3 days (D3) for gel. Metabolically active live cells appear in green and damaged dead cells in red. Scale bars: 100 μm.

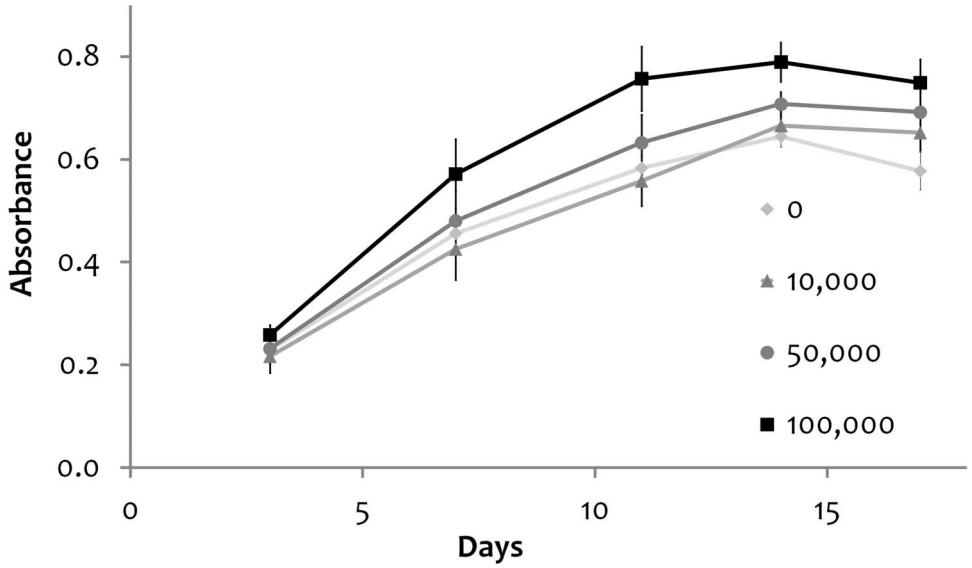

Figure 7. Stimulating ability of hFPTs. Metabolic activity of human adult tenocytes (hATs) stimulated by various amounts of irradiated human fetal progenitor tenocytes (hFPTs) through a coculture with Transwell inserts. Experimentation done three times and in triplicate with average and standard deviation indicated.

cells are expected to present features such as good proliferation capacity, good stability, and possibility of long-term storage.

The culture of hFPTs was rapid with a minimal doubling time of approximately 30 h in maximal growth period and a mean doubling time in passage 10 still more rapid than for hATs in passage 4. hFPTs only required very basic culture conditions, without need of specific growth factors, which permits inexpensive and easier growth conditions compared to other cell types such as MSCs, which necessitate demanding conditions for upscaling cells in an undifferentiated state or directing them in a specific phenotype. The overall rate of proliferation was very steady up to passage 9, even with low initial seeding densities. This long stability can probably be explained as hFPTs were obtained from a young tissue of 14 weeks gestation. Even if the cells had slightly slower proliferation at much higher passages, the morphology and karyotype accomplished at passage 12 did not show any abnormalities. A major advantage of progenitor cells is the possibility to expand them to create a large working cell bank and therefore an unlimited off-the-shelf reserve. An average of 3.72 population doublings per passage up to passage 9 means that at each passage, it is possible to increase the number of cells by a factor 13.18-fold ($=2^{3.72}$). If we imagine limiting all clinical work with the cells at passage 6 (two thirds of the observable signs of cell aging), this would lead to an increase by almost 400,000-fold (13.18^5) the initial number of cells banked at passage 1. Thus, we can estimate a total of 1.96×10^{14} cells at the end of passage 6 if the entire cell bank was expanded. Estimating approximately 1 million hFPTs per

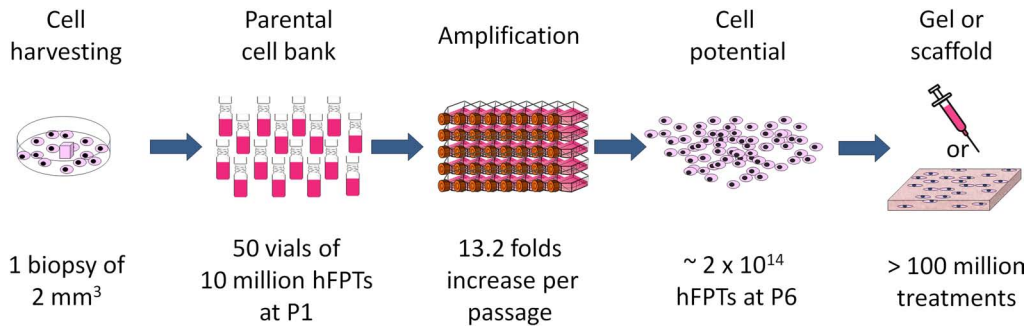

Figure 8. hFPT cell bank processing and capacity. Starting with one biopsy from fetal Achilles tendon, it was possible to create a cell bank of 50 vials with 10 million human fetal progenitor tenocytes (hFPTs) each. Based on growth capacity of hFPTs, more than 100 million treatments could be potentially created with cells at the end of passage 6.

treatment protocol, it would be possible to create more than 100 million treatments (Fig. 8). These estimations are hypothetical, but are based on clinical experience with our hospital for treatment of burns and wounds (between 270,000 and 540,000 cells were used per 9 cm × 12 cm scaffold for biologic bandages for wound healing), and it shows the potential of such a cell line when correctly banked for clinical use (15,23,45).

Our results show that it was possible to conserve the cells at −80°C for short-term conservation (with more than 95% survival over 4 to 10 months) or at −165°C in the vapor phase of liquid nitrogen for long-term storage (with 90–95% survival over 30 to 36 months). Moreover, there was no delay in the growth of frozen cells as the doubling times to reach confluency were very close to what was found with cells cultured at the same passage from enzymatic detachment. These results were obtained with standard cryosolution, and the period of conservation is probably much longer, as we have been able to conserve other progenitor cells at −165°C for more than 15 to 20 years with this manner (e.g., skin progenitor cells, unpublished data).

There is still no real specific tendon marker in the literature (51), so it is preferable to detect the presence of a pattern of several proteins, which are not specific, but nevertheless characteristic of tendon tissue. It was the case in this study and the tenocyte status was confirmed for hFPTs with the presence of type I collagen, tenomodulin, and scleraxis observable in monolayer culture, alongside with a morphology very similar compared to adult tenocytes. Concerning morphology, it is noticeable that fetal tenocytes present a more rounded morphology within tendon in vivo during development and at low ages (52). However, when placed in 2D culture, fetal progenitor tenocytes present a spindle-shaped morphology comparable to the one seen with older cells, even at low passages. Concerning the markers, type I collagen is an extracellular matrix component, and the strong cytoplasmic staining, which was observed, could be explained by the presence of type I procollagen. Type I procollagen shares the same amino acid sequence as type I collagen with the presence of propeptides at both terminal ends. It is synthesized in the cell before being converted to type I collagen by cleavage of propeptide during secretion in the extracellular matrix (6,25). Interestingly, when cultured as microtissue pellets, there were some differences between fetal progenitor and adult cells. The global deposition of matrix was visually increased with hFPTs compared to hATs, and there was a deposition of type I collagen in hFPT pellets, while it was not possible to detect this protein in 3D culture from hATs cultured under the same conditions. The addition of ascorbic acid (implicated in collagen synthesis) allowed the synthesis of type I collagen in both fetal and adult pellets, but still in lower quantities for hATs than for hFPTs. Fetal and adult tendons are known to be able to secrete different types of matrix, notably in the case of tendon healing. In adult healing, type I collagen peaks only after several weeks to replace the temporary fixative matrix (49), while in fetal healing the regenerated tendon appears rapidly indiscernible from unwounded tendons (8). Thus, it is possible that human progenitor tenocytes secrete preferably type I collagen, while adult cells are more turned toward other proteins. Further investigations would be necessary to compare the differences between the extracellular matrix compositions, which could be linked with the regenerative properties of fetal cells versus the reparative abilities of adult cells, and a recent study in sheep highlighted differences in morphological and molecular changes from fetal development to adult (48). Tenomodulin and scleraxis have both proven to have an implication in tendon development, as mice lacking either one have shown defects in tendon structure (18,36). In 3D culture, tenomodulin was secreted in both young and old cell lines. On the other hand, it was not possible to detect the presence of scleraxis in 3D culture in either fetal progenitor or in adult cells. Taylor et al. found a similar situation with a significantly reduced expression of scleraxis in 3D cell culture (51). They hypothesized that scleraxis is important in the early steps of tendon development, but that once the phenotype is acquired there is no further change in its expression. The similar expression of scleraxis between normal and diseased tendons supports these assumptions (51).

The surface markers of hFPTs seen with flow cytometry indicated a homogenous cell population. As expected, the overall profile was similar to fibroblastic adherent cells (positive for D7-fib and CD90). Potential contaminating subpopulations like hematopoietic or endothelial cells were not found (CD34 and CD14), which is important as it is known that the reduction of such cells (e.g., T lymphocytes) prevents significant graft-versus-host disease reactions (42). There was no shift in the expression of the surface markers between low and high passages, indicating a good stability. The presence of CD105 was really interesting as it increases the stability of the cells in the tenogenic phenotype. Indeed, it has recently been demonstrated that a lack of this protein in tenocytes leads to more chondrogeneration in healing tendon than when this protein is present (4).

hFPTs exhibited, as expected, a lack of MHC class II proteins (HLA-DR, DP, DQ), while MHC class I (HLA-A, B, C) was mildly present. This profile is very close to the one of MSCs (29). T lymphocytes are known to drive allograft rejection through HLA recognition, and the presence of MHC class I proteins on the surface of a cell should normally trigger an immune reaction from $CD8^+$ T cells. Nonetheless, it has largely been proven

that MSCs, even if they present MHC class I antigens, have immune-modulating effects and that they are able to be grafted with lack of immunological response. Indeed, they possess the ability to inhibit proliferation of T lymphocytes in vitro (29) and have also been well tolerated in vivo, notably in primates, even in the case of MHC mismatch (7,17). Neonatal foreskin cells found in the commercial product Apligraft also present a similar MHC profile and have also been well tolerated (50). The tolerance seen with these examples would indicate that the mechanism is not fully understood and that some allogenic cell types, although presenting HLA proteins, can be used for transplantation without immunosuppression. There is multiple evidence that fetal cells behave in the same manner with a capacity to modulate immune reaction. One of the most important observations supporting this is during gestation itself. It has been shown that even if paternal HLA-C are recognized during pregnancy, there is no harm to the fetus (38). The presence of HLA-G has been proposed as a potential modulator, as these proteins are elevated on fetal cells in contact with maternal tissue and have been shown to be able to inhibit different lymphocytes (12,41,47). hFPTs did not express these particular MHC class I proteins, but this is nonetheless not the only mechanism of immunomodulation in fetal tissue. Fetal fibroblasts and keratinocytes present an immunosuppressive activity and are able to inhibit lymphoproliferation, notably through their expression of indoleamine 2,3-dioxygenase (IDO) (59). Our past clinical trials have also illustrated an excellent tolerance for fetal cells. Biologic bandages containing fetal fibroblasts lead to improved outcomes in burn and wound healing, and even if there was no engraftment long term, multiple applications did not initiate immune reactions (15,23).

Altered cell differentiation is sometimes thought to play a role in the etiology of tendinopathies (16), as calcification and appearance of lipid droplets (normally not seen in tendons) are factors found in tendinopathies, which can precede spontaneous rupture of tendons (28). To use cell therapy for tendon treatment, it is then of primary importance to have a cell line with a stable phenotype, to avoid drift to osteogenic or adipogenic phenotypes. While ASCs and BM-MSCs showed differentiation into osteogenic or adipogenic phenotypes under inducing conditions, hFPTs demonstrated a very high stability in their phenotype without any sign of mineral or lipid accumulation. These results are particularly encouraging as it could prevent the differentiation of the cells and the appearance of calcification or lipid accumulation often seen in vivo with other cell types. MSCs, either from adipose tissue or from bone marrow, have been employed for tendon regeneration in various animal models (31), and even in a human clinical trial (19). In most of the cases, MSCs lead to improved outcomes, but they could not attain the quality found with fetal healing (8,58). Furthermore, there is still concern about the stability of MSCs to act as tenocytes when used in tendon repair. In a study conducted by Awad et al., the use of MSC–collagen composites improved the biomechanical parameters of the repaired tendon, but lead to the formation of bone in the repair site in 28% of the grafted tendons versus 0% in the natural repair control group (5). Bi et al. also warned that BM-MSCs expanded in vitro before transplantation into mice formed bone rather than tendon tissue and that the use of such cells could potentially lead to calcifications. In contrast, they showed that the use of the recently discovered tendon stem/progenitor cells (TSPCs) avoided this problem with a phenotype more tuned into tendon production. However, the harvesting of autologous TSPCs does not seem possible for clinical use as there is not an easily available source (9). Embryonic stem cells (ESCs) have also been tested in vivo, and Chen et al. have proposed a manner to first differentiate ESCs into MSCs before tendon treatment to avoid the risk of teratoma formation (13). More recently, induced pluripotent stem cells (iPSCs) improved the outcomes of tendon healing in a study (57). However, ESCs and iPSCs are also inducible cells and still present the same risk of dedifferentiation as MSCs. Differentiated cells such as human autologous tenocytes could present good stability, but as for TSPCs, there is a lack of an easily available source for harvesting, and this would also require a biopsy and lengthy culture period before clinical use would be possible. hFPTs present both advantages of a strong overall stability (phenotype and karyotype), along with an off-the-shelf availability whenever needed, which gives support for these cells as a potential source in clinical use.

Different clinically acceptable delivery systems would be critical to assure localized and targeted treatments. hFPTs had the ability to positively interact both with gel and porous matrix and therefore could provide interesting delivery modes that are routinely used in the clinic. On one side, injectable cell preparations capable of stimulating tendon regeneration could be particularly interesting to treat tendinopathies and simple acute injuries. The ability of hFPTs to survive in HA gel overnight (or even up to 3 days) at 4°C could provide an interesting logistic solution. It would be easy to prepare the formulation on the day before an intervention by removing cells from the frozen-stored bank, rinsing them with PBS or NaCl to eliminate undesired products, mixing them with gel and keeping the product at 4°C for transport. On the other hand, engineered tendon constructs would be useful for resistant degenerative or extended acute injuries, when filling bulk tissue defects with material is required (40). hFPTs can be uniformly and efficiently integrated into scaffold, and a rinsing step before delivery allows to

remove undesired products without detaching cells. The product can be delivered almost immediately or kept in culture for longer periods.

Tendon healing is known to be a long process in part driven by adult tenocytes. Our in vitro model of coculture has proven that hFPTs could stimulate adult tenocytes, which is interesting as an increase in their global activity could potentially accelerate the overall process. In addition, studies and literature indicate that wound stimulation provided by cell therapy with fetal progenitor cells could potentially provide a scarless healing with the absence of fibrotic tissue. An in vivo study to show safety and efficacy would be the next step to bring this cell therapy closer to the clinic.

CONCLUSION

hFPTs have been shown to be a unique stable cell source that can be produced under stringent current GMP manufacturing. They present a remarkable genetic and phenotypic stability and are able to conserve their tenogenic nature even in the presence of inductive conditions toward other phenotypes. The possibility to create rapidly a large cell bank to obtain off-the-shelf reserves is ideal for cell therapy and tissue engineering. Moreover, the cells show a good interaction both with porous and gel scaffolds for clinical preparations and delivery. As the cells are all derived from the same organ donation, it is also possible to include within the process extensive screening easily adaptable to GMP cell banking conditions. Finally, their ability to stimulate the activity of adult tenocytes is of importance as it could lead to a more rapid healing in vivo. The in vivo model chosen for further evaluation should also allow distinguishing whether rapid regenerative healing without scar could be attained all the while assuring safety and that no immune reaction would be elicited by these particular banked fetal progenitor tenocytes.

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