

Skin-Derived Fibroblasts for the Treatment of Refractory Achilles Tendinosis: Preliminary Short-Term Results

Haron Obaid, MBChB, FRCS, FRCR, FRCPC, Andrew Clarke, MBBS, FRANZCR, Peter Rosenfeld, MBBS, FRCS(Orth), Christopher Leach, BSc, MCSP, and David Connell, MBBS, FRANZCR

Investigation performed at the Royal National Orthopaedic Hospital, Stanmore, Middlesex, United Kingdom

Background: Chronic Achilles tendinosis is a common musculoskeletal disorder often refractory to conservative management. Our study aimed to assess the safety and efficacy of the use of autologous skin-derived collagen-producing cells in the treatment of refractory Achilles tendinosis.

Methods: We conducted a randomized, double-blind study on forty Achilles tendons in thirty-two patients (eight with bilateral involvement) who had a clinical and radiographic diagnosis of Achilles tendinosis. The patients ranged from twenty-two to sixty-seven years old and had a mean age of 45.2 years. The patients with unilateral involvement were randomized into the treatment group (twelve patients) and control group (twelve patients). The eight patients with bilateral involvement were individually randomized into treatment and control groups, with eight Achilles tendons in each group. Achilles tendons in the treatment group were injected under ultrasound guidance with laboratory-expanded, skin-derived fibroblasts suspended in autologous plasma. The control group received ultrasound-guided injection of a local anesthetic and physiotherapy. The Victorian Institute of Sport Assessment (VISA) questionnaire and visual analog scale (VAS) scores were used as the main outcome measures for both groups.

Results: Significant differences in the mean VISA and VAS scores were detected between the treatment and the control groups for the patients with unilateral involvement at six months ($p < 0.001$ for both). With use of the Mann-Whitney U Test, significant differences in the VISA score were observed at the second visit and at the three-month and six-month visits ($p = 0.02$, $p = 0.007$, and $p < 0.001$ respectively). The VAS scores also showed significant differences at the second visit and at the six-month evaluation ($p = 0.014$ and $p < 0.001$, respectively). The eight patients with bilateral involvement were analyzed separately; with the number of patients studied, no significant differences in the VISA or VAS scores were observed between the treatment group and the control group.

Conclusions: These preliminary short-term results demonstrate that the injection of skin-derived fibroblasts for the treatment of Achilles tendinosis is safe. However, larger studies with a longer duration of follow-up are required to determine the long-term effectiveness before wider clinical application is considered.

Level of Evidence: Therapeutic Level II. See Instructions for Authors for a complete description of levels of evidence.

Disclosure: One or more of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of an aspect of this work. None of the authors, or their institution(s), have had any financial relationship, in the thirty-six months prior to submission of this work, with any entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. Also, no author has had any other relationships, or has engaged in any other activities, that could be perceived to influence or have the potential to influence what is written in this work. The complete **Disclosures of Potential Conflicts of Interest** submitted by authors are always provided with the online version of the article.

JBJS | *Express*

This article was chosen to appear electronically on December 28, 2011, before publication of the final, definitive version.

Achilles tendinosis is a common musculoskeletal disorder that predominantly affects physically active individuals and is characterized by pain and swelling. The lifetime risk of Achilles tendinosis in men who are former elite distance runners is 52%¹, and the disorder represents 5% to 18% of the total number of injuries sustained by runners². Individuals with Achilles tendinosis constitute 4% of all patients who are seen in the sports clinics³⁻⁵. The exact pathophysiology of Achilles tendinosis is poorly understood, and a variety of extrinsic and intrinsic causal factors have been implicated. Intrinsic factors are predominantly biomechanical in origin, resulting from overuse injury, gastrocnemius-soleus dysfunction, lateral instability of the ankle, and excessive motion of the hind foot in the frontal plane (so-called whipping action) due to lateral heel strike with compensatory pronation^{6,7}. Additionally, posterior impingement due to a Haglund deformity (osseous bump), forefoot varus, and pes cavus are recognizable causes of Achilles tendinosis⁸. Extrinsic factors occur as a consequence of poor training technique, changing training pattern, previous injuries, poorly fitted footwear, and suboptimal training conditions^{9,10}.

Achilles tendinosis is commonly treated with eccentric physiotherapy exercises¹¹. A recent systematic review by Magnusson et al.¹² suggested that eccentric exercises are most effective in the treatment of midportion Achilles tendinopathy. A corrective orthotic device can help to improve the biomechanics of the foot and ankle and alleviate the pain^{13,14}, and such devices have been shown to be effective in up to 75% of runners¹⁵. Although no inflammatory process is present in Achilles tendinosis, nonsteroidal anti-inflammatory drugs and corticosteroid injections have been used with no scientific evidence to support their effectiveness¹⁶. Investigators have experimented with several other treatment modalities, such as laser therapy¹⁷, radiofrequency coblation¹⁸, and extracorporeal shock wave therapy¹⁹, in the treatment of Achilles tendinosis. However, the long-term effectiveness of these modalities remains unclear. Failure to respond to six months of conservative treatment can lead to surgical intervention with use of a variety of techniques²⁰⁻²³. The success rate of surgical intervention has reached 85% in some series²⁴. Nevertheless, a long-term study by Paavola et al.²⁵ showed that the outcome after nonoperative treatment of chronic Achilles tendinosis was more favorable.

There has been recent emphasis in the literature on treating refractory Achilles tendinosis with minimally invasive procedures such as intratendinous injection of dextrose, injection of sclerosing agents (e.g., polidocanol)²⁶, dry needling with autologous whole blood injection, and injection of platelet-rich plasma. Gene therapy has also been used by manipulating cellular function and protein synthesis through the delivery of genetic material into the cells with use of viral vectors or liposomes^{27,28}. Recent studies, however, have shown that cell therapy has a greater potential for tendon regeneration by repopulating tendon fibers with tenocyte-like cells^{29,30}. Our study aimed to assess the safety and efficacy of the use of autologous skin-derived collagen-producing cells in the treatment of refractory Achilles tendinosis.

Materials and Methods

We conducted a prospective, randomized, blinded, controlled trial on thirty-two patients with Achilles tendinosis, including twenty-four with unilateral involvement and eight with bilateral involvement. Inclusion criteria were based on the clinical and sonographic evidence of Achilles tendinosis; symptoms of more than six months; and failure of conservative treatment including rest, analgesia, immobilization in a boot cast, acupuncture, and physiotherapy. Patients were initially assessed by a foot and ankle specialist for clinical signs and symptoms of Achilles tendinosis such as pain, swelling, and focal tenderness. The diagnosis of Achilles tendinosis was further confirmed with an ultrasound scan, which demonstrated noninsertional fusiform thickening of the Achilles tendon with or without areas of neovascularization or interstitial tears. The study was confined to midportion Achilles tendinopathy; patients with insertional tendinosis were not included. Patients with previous tendinous injections, previous surgery, and a bleeding tendency were excluded from the study.

There were twenty male and twelve female patients, and the mean duration of symptoms was 17.2 months (range, seven to thirty-five months). The patients ranged in age from twenty-two to sixty-seven years, and the mean age was 45.2 years. The twenty-four patients with unilateral involvement were randomized into a treatment group (twelve patients) and a control group (twelve patients) (Fig. 1). The eight patients with bilateral involvement (sixteen Achilles tendons) were also equally randomized into treatment and control groups, with eight limbs in each group (Fig. 2). The patients were randomized with use of a sequence of random numbers from a computer-generated sequence. Blinding was carried out at all evaluations. Patients were followed for six months.

To comply with the regulatory guidelines, all patients had undergone serological tests to exclude syphilis, hepatitis B and C, and human immunodeficiency virus.

All patients had undergone physiotherapy assessment and treatment and had been given a standardized program of increased eccentric loading and stretching exercises for six months that had been designed by Fahlström et al.³¹. Subsequently, an ultrasound assessment was repeated, and all of the patients were reassessed with use of visual analog scale (VAS) and Victorian Institute of Sport Assessment (VISA) scoring systems (described below), which were labeled as baseline. At this point, patients who had shown substantial improvement were excluded from the study. However, the patients who failed to show any sign of improvement were randomized into two groups. Informed written consent was obtained from all of the patients prior to any intervention.

With use of a 4-mm-diameter punch-biopsy needle, a skin sample was obtained from the lateral aspect of the thigh on all patients. To save costs, only samples from the cell therapy group were processed, cultured, and expanded in number to produce a collagen-producing fibroblast population. The skin samples were sent to the GMP (Good Manufacturing Practices) laboratory in cell transport medium (Dulbecco's Modified Eagle Medium [DMEM/F-12; Invitrogen, Carlsbad, California] and gentamicin) with 4° to 10°C refrigeration. A computer tracking system was used to generate bar codes for all samples to avoid any mix-up.

Skin samples were washed in a phosphate-buffered saline solution and underwent mechanical disintegration. Additionally, HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid)-containing medium with type-1 collagenase and fetal calf serum were added to digest the connective tissue. At fourteen to twenty-four hours after sampling, phosphate-buffered saline solution was added to the samples and the connective tissue cells were isolated by centrifugation. A fibroblast medium was added to each pellet in a 50-mL tube. The medium containing the connective tissue cells was placed into a tissue culture flask and kept in an incubator (5% CO₂ at 37°C). Fetal bovine serum was used as a medium additive for the propagation of the connective tissue cells. In the laboratory, tenocyte-like cells were successfully grown in the culture media (Figs. 3-A and 3-B). These skin-derived fibroblasts showed exponential growth in cell medium culture, and a mean of 17.3 million cells (range, 10 to 28 million cells) was achieved over a four-week period in each preparation. The criteria for successful growth were cell viability (a mean [and standard deviation] of 90% ± 10% were living cells), Cluster of Differentiation 90 (CD90) expression for fibroblast marker (a mean of 80% ± 20% were positive), CD56 expression for muscle marker (a mean of 90% ± 10% were negative), CD34

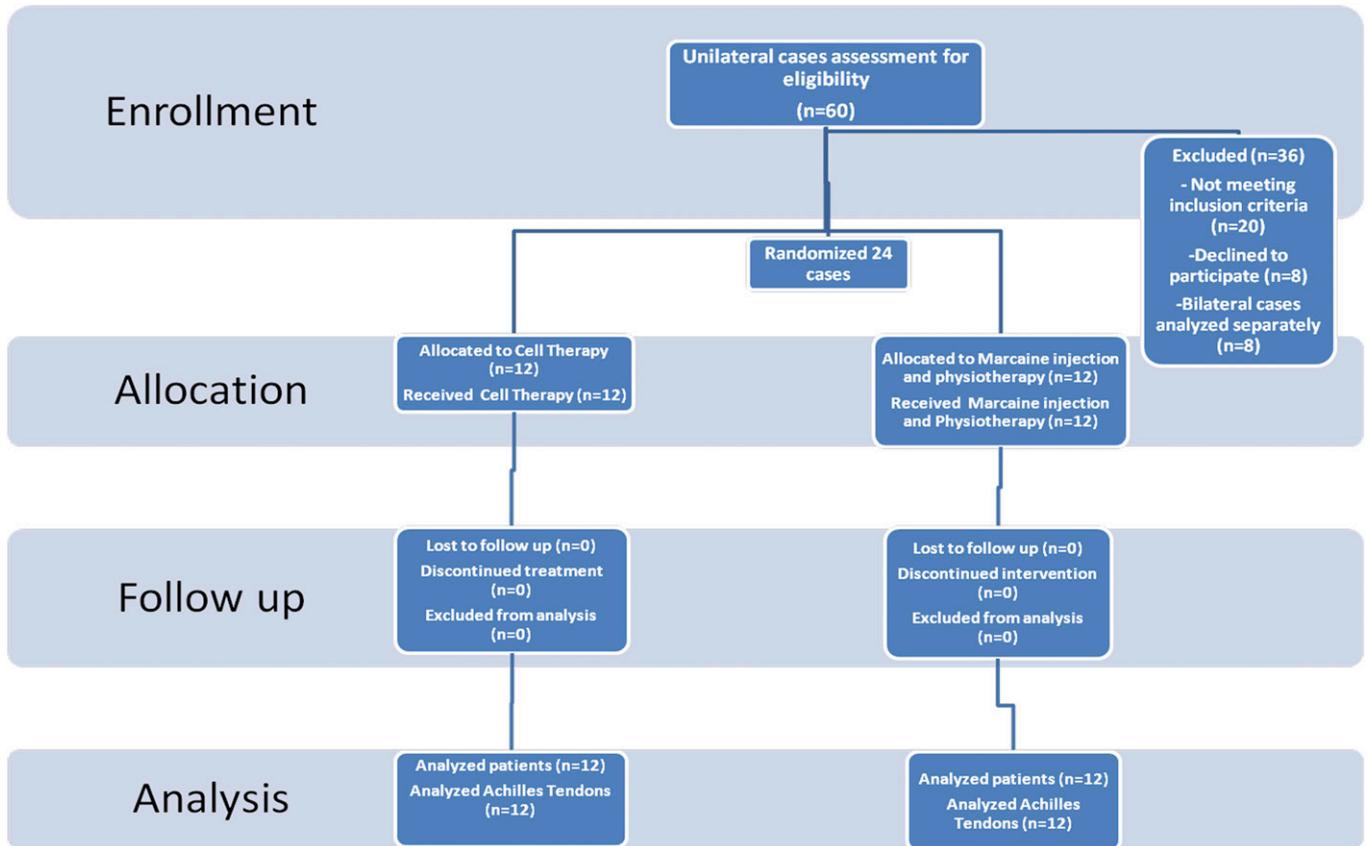


Fig. 1
Flow diagram for the patients with unilateral involvement, demonstrating the enrollment, allocation, follow-up, and analysis stages of the trial.

expression for bone stem-cell marker (a mean of $90\% \pm 10\%$ were negative), and a cell count of approximately 10 million. The expanded cells showed tenocyte-like behavior in an ex vivo stretch model (stretch-oriented collagen type-I and II

production). The cultured skin fibroblasts demonstrated histological appearances, such as elongated nuclei and spindle-shaped cytoplasm, similar to those of tenocytes. Cells were characterized by flow cytometry^{32,33} and the releasing criteria for

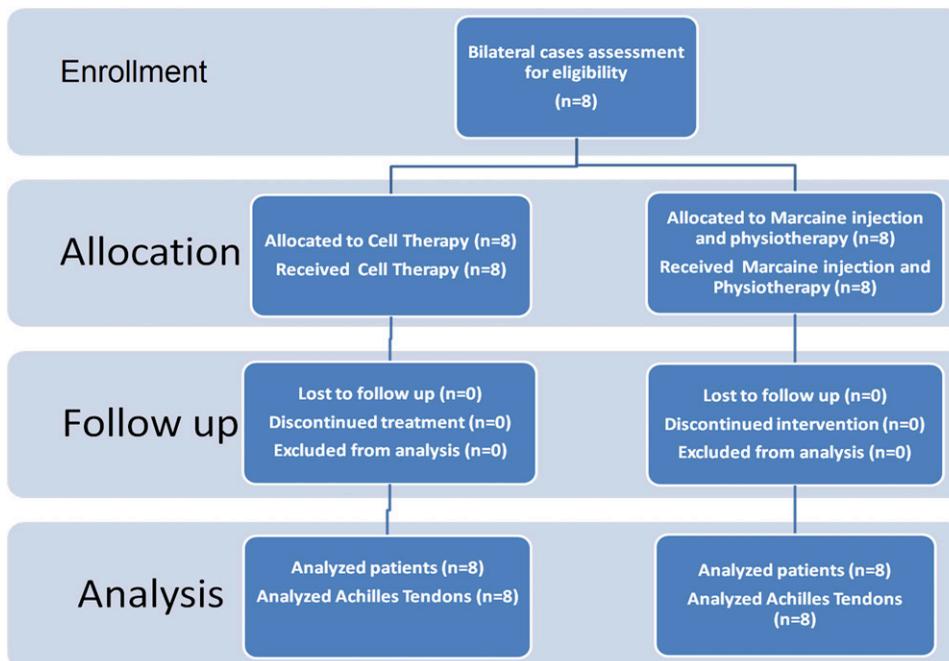


Fig. 2
Flow diagram for the patients with bilateral involvement, demonstrating the enrollment, allocation, follow-up, and analysis stages of the trial.

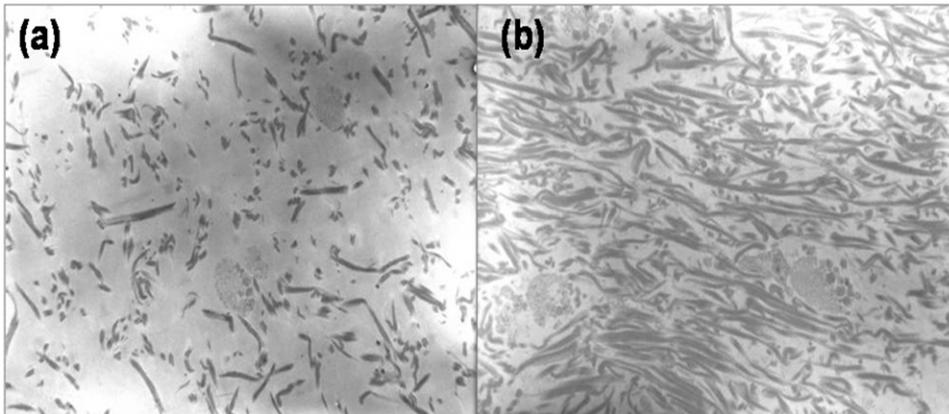


Fig. 3
Skin-derived fibroblasts on day 0 (**Fig. 3-A**) and eleven days after culture (**Fig. 3-B**) (magnification, $\times 200$). Note the expansion in cell number in Figure 3-B.

tenocyte-like cells, including positive CD90 marker and mean viability by 7-aminoactinomycin D staining of $90\% \pm 10\%$.

All subjects had 10 mL of venous blood aspirated from the antecubital fossa. The blood was injected into a tube containing citrate, after which it was centrifuged (2000 rev/min for fifteen minutes in an LC6 centrifuge; Sarstedt, Montreal, Quebec, Canada) to obtain the plasma supernatant. The control group subsequently had their sample discarded. All patients were asked to lie prone on the examination table with their feet dangling over the edge of the

bed. Hypoechoic areas and intratendinous tears were identified on ultrasound prior to injection. All patients then received a local anesthetic with a bolus injection of 5 mL of bupivacaine (0.25% Marcaine; AstraZeneca, London, United Kingdom) on the ventral surface of the Achilles tendon in its midsection with use of a 21-gauge needle. In the treatment group, the needle was repositioned into the tendon substance so that an additional injection could be made. A combination of cells with autologous platelet-rich plasma (labeled the implant stage) was injected into the site of tendinous and fibril discontinuity of the

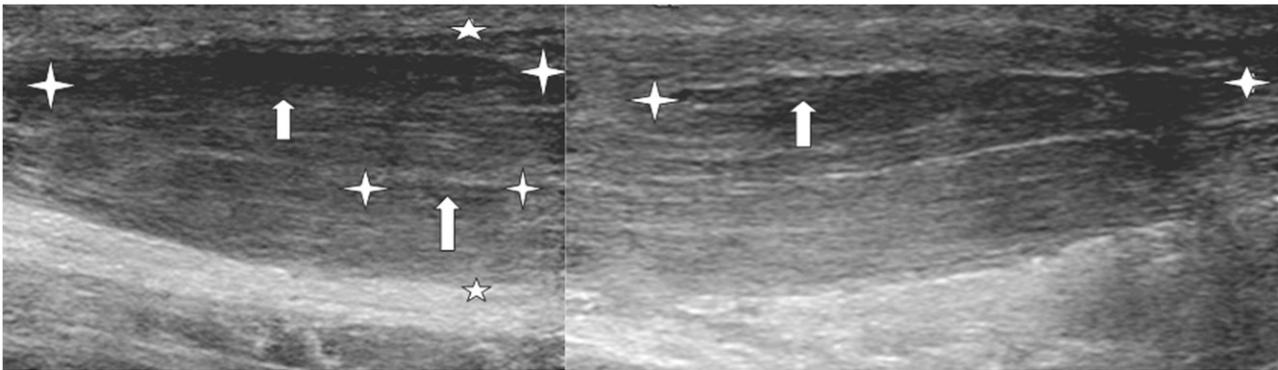


Fig. 4-A

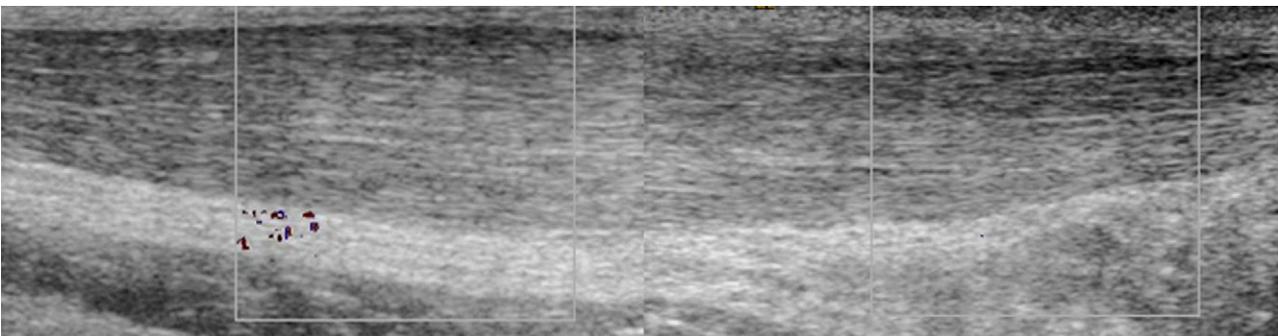


Fig. 4-B

Figs. 4-A and 4-B A sixty-three-year-old man with a three-year history of chronic midportion Achilles tendinopathy who had failed conservative treatment, including an eccentric loading program, casting, and injection therapy. **Fig. 4-A** The preliminary ultrasound shows severe tendinosis manifested by marked fusiform thickening, hypoechoic changes, and longitudinal intrasubstance tears. The four-point stars denote the beginning and the end of the tear, and the arrows point to the tear midsection. Tendon thickness was measured at maximum tendon thickening (five-point stars). **Fig. 4-B** An ultrasound examination performed six months after cell therapy, showing a reduction in tendon thickness, increased echotexture, and resolution of the intrasubstance tears. There was almost complete replacement of the initial hypoechoic abnormality with normal-appearing fibrillar tendon material. Power Doppler interrogation (gray boxes) showed reduced vascularity.

Achilles tendons of the cell group with use of a double-barreled syringe technique containing equal amounts (2 mL) of cell suspension in one chamber and autologous centrifuged plasma in the other.

Following the procedure, patients were asked to remain in the prone position for five minutes without plantar flexing the foot. Exercise and heavy lifting were not allowed for the ensuing forty-eight hours. The patients were examined by the physiotherapist on the day of treatment and were given a leaflet with instructions to begin a standardized eccentric-loading physiotherapy program after forty-eight hours; they returned for follow-up evaluations at one, three, and six months.

At the first visit (labeled the screening stage), all patients were assessed for pain and functional disability parameters with use of validated outcome measures. Subjective and objective assessments of the Achilles tendon were achieved by gauging functional status with use of the VISA questionnaire³⁴, a validated index of Achilles tendinopathy that includes questions regarding symptoms, functional tests, and ability to play sports. All patients were asked to complete the VISA questionnaire before undergoing an ultrasound scan or injection at the screening, baseline, and implant stages and at the six-week, three-month, and six-month follow-up evaluations. Six questions in the questionnaire regarding the level of health were scored independently with use of a VAS, ranging from 0 to 10 (with 0 being the worst and 10 being the best level of health). The VISA score ranged from a maximum of 100 for a physically fit and asymptomatic person to a minimum of 0 for a symptomatic individual who was not physically fit.

Ultrasound assessment was performed at the time of recruitment (the screening stage); six weeks following physiotherapy, at the time of skin biopsy (baseline); at the time that the cells were implanted; and at six weeks, three months, and six months after the cells were implanted (Figs. 4-A and 4-B). Patients were scanned in the prone position with the feet hanging over the edge of the table. The Achilles tendons were scanned in both longitudinal and

transverse dimensions on gray-scale and color Doppler imaging with minimal pressure applied³⁵ and were assessed for thickness, echotexture, interstitial tears, and neovascularity³⁶⁻³⁸.

The trial was registered with the National Research Ethics Service (National Patient Safety Agency) in the United Kingdom, with a registration number 08/H0724/27.

Statistical Analysis

In this prospective, randomized, controlled, blinded trial, cell treatment was compared with physiotherapy treatment (the control). Five repeated measurements, including the baseline primary outcome measure with the VISA score and the secondary outcome measure with the VAS score, were carried out. Both VISA and VAS scores were tested for normal distribution, which was not fulfilled across all repeated measurements; therefore, nonparametric significance testing with use of the Mann-Whitney U test for between-group comparison was applied. In total, five between-group comparisons were performed. To correct for multiple comparisons, the Bonferroni procedure was applied and p values of <0.001 were considered to indicate significant differences.

Source of Funding

The study was funded by the Austrian biotech company Innovacell.

Results

Twenty-four patients with unilateral involvement were randomized into two groups, with twelve patients in the cell therapy group and twelve patients in the control group.

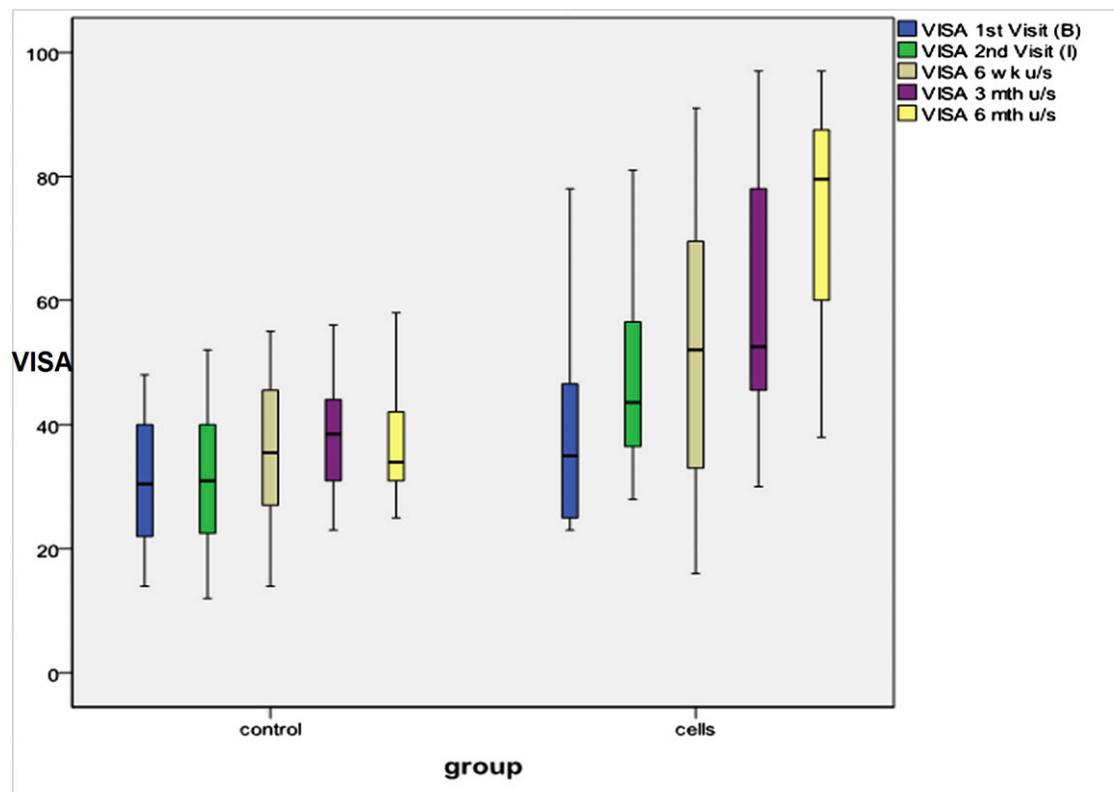


Fig. 5
The VISA scores (y axis) before and after treatment for the cell therapy group and the control group (intergroup) and between baseline and six months after treatment (within-group). The whiskers represent the range of data, the bars represent the upper and lower quartiles, and the midline represents the median. The VISA score ranges from 0 to 100. B = baseline, I = implant, and u/s = ultrasound.

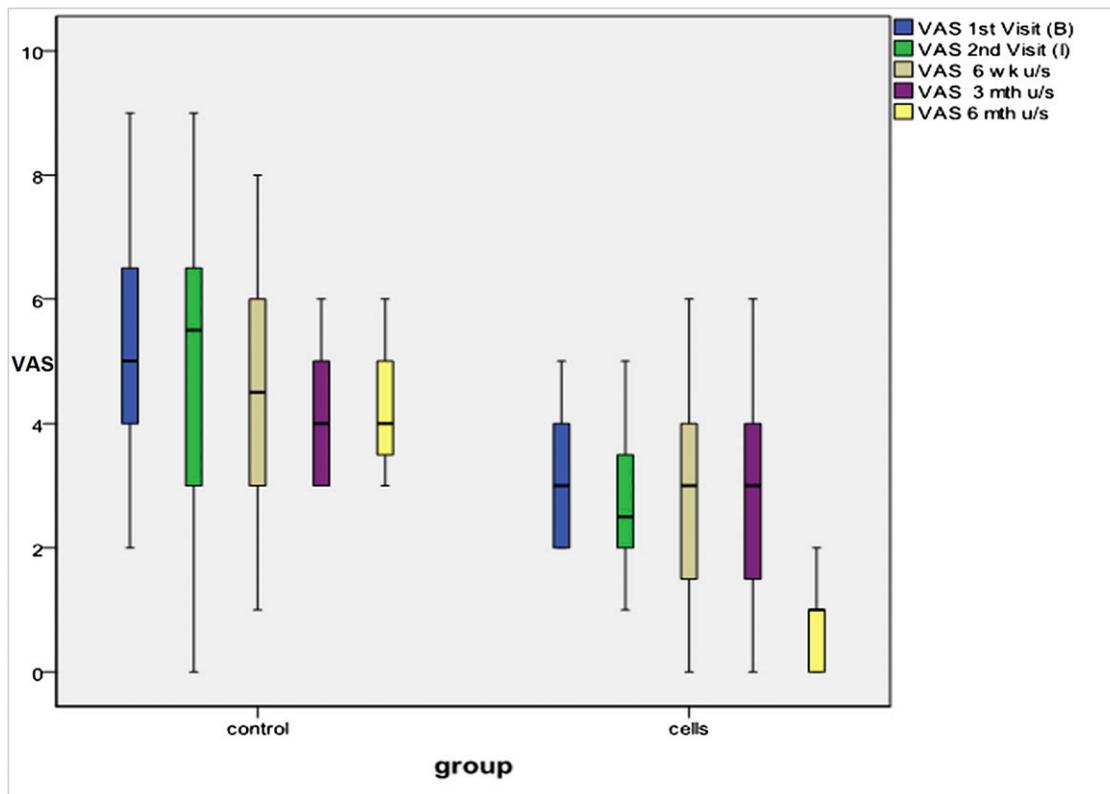


Fig. 6
The VAS scores (y axis) before and after treatment for the cell therapy and control groups (intergroup comparison) and between baseline and six months after treatment (within-group). The whiskers represent the range of data, bars represent the upper and lower quartiles, and the midline represents the median. The VAS score ranges from 0 to 10, with 0 indicating no pain and 10, maximum pain. B = baseline, I = implant, and u/s = ultrasound.

With use of the Mann-Whitney U Test, significant differences in the VISA scores were observed between groups at the second visit ($p = 0.02$), at three months ($p = 0.007$), and at six months ($p < 0.001$). The median VISA values increased from 35.00 (before treatment) to 79.50 (six months after treatment) in the treatment group, while the values marginally increased from 30.50 (before treatment) to 34.00 (six months after treatment) in the control group. The between-group VAS scores were significantly different at the second visit and at six months ($p = 0.014$ and $p < 0.001$, respectively). The median VAS values decreased in the treatment group from 3.00 (before treatment) to 1.00 (six months after treatment), whereas the median values slightly decreased in the control group from 5.00 (before treatment) to 4.00 (after treatment). For the patients with unilateral involvement, the distribution of VISA and VAS scores before treatment and twenty-six weeks after treatment in the cell therapy and control (physiotherapy) groups is illustrated in Figures 5 and 6. No adverse effects were observed in the cell group.

The eight patients with bilateral involvement were analyzed separately. The eight patients (sixteen Achilles tendons) were divided equally into treatment and control groups, with eight Achilles tendons in each group. With the number of patients studied, there were no significant differences in VISA and VAS scores between treated and control groups, with the exception of the VAS score at six months ($p = 0.038$).

Discussion

Although the literature has recently reflected a great interest in platelet-rich plasma as a treatment modality in chronic tendinopathy, a recent study by de Vos et al.³⁹ found no advantage after platelet-rich plasma injection in the Achilles tendon compared with placebo. However, this treatment continues to be advocated in sports medicine circles.

Over the past few years, the application of mesenchymal stem-cell therapy in promoting Achilles tendon-healing with use of animal models has shown promising early results^{40,41}. One of the major disadvantages of therapy with mesenchymal stem cells, however, is their potential to differentiate into undesirable tissue types such as bone, cartilage, or muscle tissues. Tendon-derived progenitor cells have also been utilized for the treatment of tendon disorders^{42,43}. Nonetheless, compromising the donor site for this purpose remains a great obstacle in any future development of this technique.

Both animal and human studies have produced encouraging early results in the utility of skin-derived fibroblasts in promoting tendon-healing^{44,45}. Additionally, skin cells are widely available and relatively easy to harvest. In a stretch model, skin fibroblasts can express the extracellular matrix glycoprotein tenascin and develop gap junctions, enabling collagen fibers to align in accordance with tension lines⁴⁶. Moreover, skin fibroblasts produce type-I and III collagen fibers along the tenocytes when

used with plasma gel and subjected to mechanical stimulation-stretch by careful training of the tendon involved. In our study, plasma scaffold was used to apply linear periodic stretch to these cells, which led to increased type-I and III collagen expression.

In a pilot study of twelve patients, Connell et al.⁴⁷ demonstrated that the use of skin-derived fibroblasts in the treatment of refractory lateral epicondylitis is both safe and effective. Our study is a further extension to these findings. Although no fully objective examination data supporting the improvement were reported, our preliminary short-term results showed improvement in the pain (VAS) and function (VISA) scores in the cell therapy group of patients with unilateral involvement. However, no significant differences were observed in the VISA or the VAS scores of the patients with bilateral involvement. This could be attributed to the small sample size of the patients with bilateral involvement and to the fact that patients with bilateral Achilles tendinopathy might have found it difficult to score each side independently.

It is presumed that the combination of cell preparation and plasma together forms a gel-like structure, which can be used to fill up interstitial tears in the tendon tissue. Additionally, the gel helps to retain injected cells within the tendon tissue. A second effect of the plasma gel is the transfer of the mechanical force through to the cell surfaces. This transfer of the mechanical force appears to stimulate the cells to produce and secrete collagen fibers that help to repair the damaged tendon. We believe that by placing collagen-producing cells into the site of a tendon injury and/or tears, these cells will lay down the so-called building blocks for repair, i.e., type-III collagen. Furthermore, the addition of plasma also utilizes any potential positive effects of growth factors present in the plasma.

One limitation of this study is the small number of patients included in the trial. The pretreatment factors such as sensitivity to physiotherapy might have affected the subsequent differences in the intergroup functional scores of the patients with unilateral involvement. The patients with bilateral involvement had to be analyzed separately, and the results demonstrated no significant difference between the treatment and control groups. The patients with bilateral involvement could not have been considered independent because of the likelihood of systemic effect. The exact numbers of cells or injections that are actually needed to achieve the optimum regeneration of the tendon remain uncertain. We were not able to perform histopathological correlation, as no surgical specimens were available. Further studies are recommended to test the effectiveness of this

procedure in patients with insertional tendinosis. Furthermore, the long-term efficacy and safety of this novel technique needs to be determined through larger series with a longer duration of follow-up. We remain uncertain as to whether needling of the tendon during the procedure could have contributed to the healing response. Moreover, because plasma was used as a carrier medium to allow cells to be embedded in a set gel, the possible effect of growth factors on tendon-healing could not be excluded. Further studies are needed to clarify this potential synergistic effect.

Many challenges in the field of cell therapy exist and should be recognized. These include transportation of the cells, storage requirements, and costly cell preparation. In addition, cells need to be manufactured in a dedicated facility that must meet stringent regulatory guidelines. Regardless, cell therapy remains an exciting potential treatment for Achilles and other tendon disorders.

In conclusion, the use of skin-derived fibroblasts in the treatment of Achilles tendinopathy is safe. Although this novel technique may have a role in ameliorating a patient's symptoms and physical performance, larger studies with longer-term follow-up periods are needed to achieve maximum healing potential and determine the long-term effectiveness. ■

Haron Obaid, MBChB, FRCS, FRCR, FRCPC
Royal University Hospital, 103 Hospital Drive,
Saskatoon, SK S7N 0WB, Canada.
E-mail address: haron@doctors.org.uk

Andrew Clarke, MBBS, FRANZCR
Royal National Orthopaedic Hospital,
45 Bolsover Street, London W1W 5AQ, United Kingdom

David Connell, MBBS, FRANZCR
Royal National Orthopaedic Hospital,
Stanmore, Middlesex, HA7 4LP, United Kingdom

Peter Rosenfeld, MBBS, FRCS(Orth)
St Mary's Hospital, Praed Street,
London W2 1NY, United Kingdom

Christopher Leach, BSc, MCSP
Ember Physiotherapy, 18 Buckingham Avenue,
West Molesey, Surrey, KT8 1TG, United Kingdom

References

- Kujala UM, Sarna S, Kaprio J. Cumulative incidence of Achilles tendon rupture and tendinopathy in male former elite athletes. *Clin J Sport Med.* 2005;15:133-5.
- McCroly JL, Martin DF, Lowery RB, Cannon DW, Curl WW, Read HM Jr, Hunter DM, Craven T, Messier SP. Etiologic factors associated with Achilles tendinitis in runners. *Med Sci Sports Exerc.* 1999;31:1374-81.
- Clement DB, Taunton JE, Smart GW. Achilles tendinitis and peritendinitis: etiology and treatment. *Am J Sports Med.* 1984;12:179-84.
- Devereaux MD, Lachmann SM. Athletes attending a sports injury clinic—a review. *Br J Sports Med.* 1983;17:137-42.
- Soma CA, Mandelbaum BR. Achilles tendon disorders. *Clin Sports Med.* 1994;13:811-23.
- James SL, Bates BT, Osternig LR. Injuries to runners. *Am J Sports Med.* 1978;6:40-50.
- Maganaris CN, Narici MV, Almekinders LC, Maffulli N. Biomechanics and pathophysiology of overuse tendon injuries: ideas on insertional tendinopathy. *Sports Med.* 2004;34:1005-17.
- Kvist M. Achilles tendon injuries in athletes. *Sports Med.* 1994;18:173-201.
- Kvist M. Achilles tendon injuries in athletes. *Ann Chir Gynaecol.* 1991;80:188-201.
- Subotnick SI, Sisney P. Treatment of Achilles tendinopathy in the athlete. *J Am Podiatr Med Assoc.* 1986;76:552-7.
- Silbernagel KG, Thomeé R, Thomeé P, Karlsson J. Eccentric overload training for patients with chronic Achilles tendon pain—a randomised controlled study with reliability testing of the evaluation methods. *Scand J Med Sci Sports.* 2001;11:197-206.

- 12.** Magnussen RA, Dunn WR, Thomson AB. Nonoperative treatment of midportion Achilles tendinopathy: a systematic review. *Clin J Sport Med.* 2009;19:54-64.
- 13.** MacLellan GE, Vyvyan B. Management of pain beneath the heel and Achilles tendonitis with visco-elastic heel inserts. *Br J Sports Med.* 1981;15:117-21.
- 14.** Mohr RN. Achilles tendonitis. Rationale for use and application of orthotics. *Foot Ankle Clin.* 1997;2:439-56.
- 15.** Jørgensen U. Achillobodynia and loss of heel pad shock absorbency. *Am J Sports Med.* 1985;13:128-32.
- 16.** Paavola M, Kannus P, Järvinen TA, Järvinen TL, Józsa L, Järvinen M. Treatment of tendon disorders. Is there a role for corticosteroid injection? *Foot Ankle Clin.* 2002;7:501-13.
- 17.** Ozkan N, Altan L, Bingöl U, Akın S, Yurtkuran M. Investigation of the supplementary effect of GaAs laser therapy on the rehabilitation of human digital flexor tendons. *J Clin Laser Med Surg.* 2004;22:105-10.
- 18.** Tasto JP, Cummings J, Medlock V, Harwood F, Hardesty R, Amiel D. The tendon treatment center: new horizons in the treatment of tendinosis. *Arthroscopy.* 2003;19 Suppl 1:213-23.
- 19.** Chen YJ, Wang CJ, Yang KD, Kuo YR, Huang HC, Huang YT, Sun YC, Wang FS. Extracorporeal shock waves promote healing of collagenase-induced Achilles tendinitis and increase TGF-beta1 and IGF-I expression. *J Orthop Res.* 2004;22:854-61.
- 20.** Rosenzweig S, Azar FM. Open repair of acute Achilles tendon ruptures. *Foot Ankle Clin.* 2009;14:699-709.
- 21.** Doral MN, Bozkurt M, Turhan E, Ayvaz M, Atay OA, Uzümçügil A, Leblebicioğlu G, Kaya D, Aydoğ T. Percutaneous suturing of the ruptured Achilles tendon with endoscopic control. *Arch Orthop Trauma Surg.* 2009;129:1093-101.
- 22.** Chan KB, Lui TH, Chan LK. Endoscopic-assisted repair of acute Achilles tendon rupture with Krackow suture: an anatomic study. *Foot Ankle Surg.* 2009;15:183-6.
- 23.** Thermann H, Benetos IS, Panelli C, Gavriilidis I, Feil S. Endoscopic treatment of chronic mid-portion Achilles tendinopathy: novel technique with short-term results. *Knee Surg Sports Traumatol Arthrosc.* 2009;17:1264-9.
- 24.** Calder JD, Saxby TS. Surgical treatment of insertional Achilles tendinosis. *Foot Ankle Int.* 2003;24:119-21.
- 25.** Paavola M, Kannus P, Paakkala T, Pasanen M, Järvinen M. Long-term prognosis of patients with Achilles tendinopathy. An observational 8-year follow-up study. *Am J Sports Med.* 2000;28:634-42.
- 26.** Ohberg L, Alfredson H. Ultrasound guided sclerosis of neovessels in painful chronic Achilles tendinosis: pilot study of a new treatment. *Br J Sports Med.* 2002;36:173-7.
- 27.** Lou J, Kubota H, Hotokezaka S, Ludwig FJ, Manske PR. In vivo gene transfer and overexpression of focal adhesion kinase (pp125 FAK) mediated by recombinant adenovirus-induced tendon adhesion formation and epitenon cell change. *J Orthop Res.* 1997;15:911-8.
- 28.** Nakamura N, Shino K, Natsuume T, Horibe S, Matsumoto N, Kaneda Y, Ochi T. Early biological effect of in vivo gene transfer of platelet-derived growth factor (PDGF)-B into healing patellar ligament. *Gene Therapy.* 1998;5:1165-70.
- 29.** Juncosa-Melvin N, Boivin GP, Gooch C, Galloway MT, West JR, Dunn MG, Butler DL. The effect of autologous mesenchymal stem cells on the biomechanics and histology of gel-collagen sponge constructs used for rabbit patellar tendon repair. *Tissue Eng.* 2006;12:369-79.
- 30.** Kryger GS, Chong AK, Costa M, Pham H, Bates SJ, Chang J. A comparison of tenocytes and mesenchymal stem cells for use in flexor tendon tissue engineering. *J Hand Surg Am.* 2007;32:597-605.
- 31.** Fahlström M, Jonsson P, Lorentzon R, Alfredson H. Chronic Achilles tendon pain treated with eccentric calf-muscle training. *Knee Surg Sports Traumatol Arthrosc.* 2003;11:327-33.
- 32.** Jongkind JF, Verkerk A. Cell sorting and microchemistry of cultured human fibroblasts: applications in genetics and aging research. *Cytometry.* 1984;5:182-7.
- 33.** Jongkind JF, Verkerk A, Visser WJ, Van Dongen JM. Isolation of autofluorescent "aged" human fibroblasts by flow sorting. Morphology, enzyme activity and proliferative capacity. *Exp Cell Res.* 1982;138:409-17.
- 34.** Robinson JM, Cook JL, Purdam C, Visentini PJ, Ross J, Maffulli N, Taunton JE, Khan KM. Victorian Institute Of Sport Tendon Study Group. The VISA-A questionnaire: a valid and reliable index of the clinical severity of Achilles tendinopathy. *Br J Sports Med.* 2001;35:335-41.
- 35.** Dong Q, Fessell DP. Achilles tendon ultrasound technique. *AJR Am J Roentgenol.* 2009;193:W173.
- 36.** van Schie HT, de Vos RJ, de Jonge S, Bakker EM, Heijboer MP, Verhaar JA, Tol JL, Weinans H. Ultrasonographic tissue characterisation of human Achilles tendons: quantification of tendon structure through a novel non-invasive approach. *Br J Sports Med.* 2010;44:1153-9.
- 37.** Sengkerij PM, de Vos RJ, Weir A, van Weelde BJ, Tol JL. Interobserver reliability of neovascularization score using power Doppler ultrasonography in midportion Achilles tendinopathy. *Am J Sports Med.* 2009;37:1627-31.
- 38.** Mitchell AW, Lee JC, Healy JC. The use of ultrasound in the assessment and treatment of Achilles tendinosis. *J Bone Joint Surg Br.* 2009;91:1405-9.
- 39.** de Vos RJ, Weir A, van Schie HT, Bierma-Zeinstra SM, Verhaar JA, Weinans H, Tol JL. Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. *JAMA.* 2010;303:144-9.
- 40.** Chong AK, Ang AD, Goh JC, Hui JH, Lim AY, Lee EH, Lim BH. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit Achilles tendon model. *J Bone Joint Surg Am.* 2007;89:74-81.
- 41.** Young RG, Butler DL, Weber W, Caplan AI, Gordon SL, Fink DJ. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. *J Orthop Res.* 1998;16:406-13.
- 42.** Chen JM, Willers C, Xu J, Wang A, Zheng MH. Autologous tenocyte therapy using porcine-derived bioscaffolds for massive rotator cuff defect in rabbits. *Tissue Eng.* 2007;13:1479-91.
- 43.** Bi Y, Ehrchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, Li L, Leet AI, Seo BM, Zhang L, Shi S, Young MF. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat Med.* 2007;13:1219-27.
- 44.** Deng D, Liu W, Xu F, Wu XL, Wei X, Zhong B, Cui L, Cao YL. [In vitro tendon engineering using human dermal fibroblasts]. *Zhonghua Yi Xue Za Zhi.* 2008;88:914-8. Chinese.
- 45.** Liu W, Chen B, Deng D, Xu F, Cui L, Cao Y. Repair of tendon defect with dermal fibroblast engineered tendon in a porcine model. *Tissue Eng.* 2006;12:775-88.
- 46.** Chiquet M, Renedo AS, Huber F, Flück M. How do fibroblasts translate mechanical signals into changes in extracellular matrix production? *Matrix Biol.* 2003;22:73-80.
- 47.** Connell D, Dahir A, Alyas F, Curtis M. Treatment of lateral epicondylitis using skin-derived tenocyte-like cells. *Br J Sports Med.* 2009;43:293-8.