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Stem Cells and Basic Fibroblast Growth Factor Failed to Improve Tendon Healing

An in Vivo Study Using Lentiviral Gene Transfer in a Rat Model

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Background: The aim of this controlled study was to investigate the influence of mesenchymal stem cells (MSCs) and lentiviral (LV) expression of basic fibroblast growth factor (bFGF) on tendon remodeling in an in vivo rat model of an Achilles tendon defect.

Methods: In eighty-four male Lewis rats, complete 2.4-mm tendon defects were created and were either left untreated (the phosphate-buffered saline solution [PBS] group) or were treated with mesenchymal stem cells expressing enhanced green fluorescent protein (the MSC-LV-eGFP group) or with mesenchymal stem cells expressing basic fibroblast growth factor lentivirally (the MSC-LV-bFGF group). After fourteen and twenty-eight days, the tendons were harvested and analyzed biomechanically and immunohistologically.

Results: After fourteen days, both mesenchymal stem cell groups were slightly superior in biomechanical testing. However, only the PBS control group showed a significant increase in biomechanical results over time (fourteen versus twenty-eight days; $p = 0.012$). Biomechanical results were better after twenty-eight days for the control group than for both MSC groups. However, the difference was significant only with regard to the stiffness results in the comparison of the PBS control and the eGFP stem cell group ($p = 0.024$). Histologically, the MSC groups had no better results than the control group after fourteen and twenty-eight days. In immunohistology, only labeling for type-I procollagen was strongly increased in both MSC groups in comparison with the PBS control group ($p = 0.0009$ for the MSC-LV-bFGF group and $p = 0.0041$ for the MSC-LV-eGFP group at fourteen days, and $p = 0.004$ and $p = 0.132$, respectively, at twenty-eight days). There were no significant differences in the immunohistological results between the stem cell groups.

Conclusions: The biomechanical and immunohistological results showed that mesenchymal stem cells in both groups had only partially positive effects on tendon remodeling in the initial stages; however, in later stages, stem cells had potentially negative effects on biomechanical results. The additional expression of bFGF in stem cells had negligible effects on tendon remodeling.

Clinical Relevance: Preliminary studies using stem cells are partially promising; however, there are no relevant clinical data showing that stem cells are of significant benefit. The present study should lead to a more critical evaluation and thoughtful use of stem cells in humans until more clinical data are available.

*T.M. Kraus, MD, and F.B. Imhoff, MD, contributed equally to the writing of this article.

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The number of Achilles tendon injuries and ruptures has increased in recent decades¹⁻³. The reasons for Achilles tendon ruptures, as summarized by Kannus and Józsa⁴ and Okamoto et al.⁵, may be repetitive microtrauma in sports⁶, hypoxic and mucoid degeneration⁴, degenerative changes from decreased perfusion, tendolipomatosis or calcifying tendinopathy⁴, or they may occur after local infiltration of corticosteroids⁷.

Efforts have been made to accelerate primary tendon healing as well as to increase the quality of the repaired tissue. Methods, including the local injection of growth factors⁸ or platelet concentrate⁹ or the systemic administration of vitamins¹⁰, have been reported to be a successful treatment in animal studies. Promising results have also been reported in studies that applied mesenchymal stem cells (MSCs) in order to accelerate healing of the Achilles tendon after injury in a rabbit model¹¹ or after rupture in a rat model⁵. The effects and the improvement of tendon healing or tendon and/or bone healing with the application of mesenchymal stem cells or bone-marrow-derived cells have been well investigated^{5,12}. Mesenchymal stem cells may contribute to healing not only by direct differentiation but also by the release of growth factors such as insulin-like growth factor (IGF)-1, transforming growth factor (TGF)- β , vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF)^{13,14}. Therefore, not only the differentiation potential but also the constant release of growth factors distinguishes mesenchymal stem cells from tenocytes or fibroblasts.

Of all growth factors, bFGF showed an increase of collagen type-I and collagen type-III production¹⁵, which might strengthen the biomechanical and histological properties of regenerative tendons.

For these reasons, the aim of this study was to assess the healing potential of a rat Achilles tendon defect after treatment with lentiviral bFGF-transduced mesenchymal stem cells. To our knowledge, the effect of the combination of mesenchymal stem cells together with lentiviral bFGF transduction as a constant bFGF-producing "factory" on tendon healing in an in vivo animal model has not been analyzed.

The hypothesis of the study is that stem cells expressing the growth factor bFGF would increase healing of rat Achilles tendons more than stem cells alone.

Materials and Methods

Study Design

In eighty-four male Lewis rats, complete tendon defects were created and either were left untreated (the phosphate-buffered saline solution [PBS] group) or were treated with mesenchymal stem cells lentivirally expressing enhanced green fluorescent protein (eGFP; the MSC-LV-eGFP group) or with stem cells expressing bFGF lentivirally (the MSC-LV-bFGF group). After fourteen days and twenty-eight days, the tendons were harvested and analyzed biomechanically and immunohistologically. In each group, eight tendons underwent biomechanical testing and six underwent immunohistological analysis.

Stem Cells

Isolation, Cultivation, and Lentiviral Transduction of Rat Mesenchymal Stem Cells (rMSCs) from Bone Marrow

Mesenchymal stem cells were isolated from the tibia and femur of male Lewis rats as described by Lennon et al.¹⁶ and were cultured as described by

Neuhuber et al.¹⁷ for lentiviral transduction, differentiation assays, and cell transplantation.

A third-generation packaging system was used to produce vesicular stomatitis virus-G protein (VSV.G)-pseudotyped, self-inactivating lentiviral vectors expressing eGFP or bFGF as described¹⁸. Passage-2 mesenchymal stem cells were infected with lentiviral supernatants in the presence of 8 μ g/mL of polybrene (Sigma-Aldrich, Germany) overnight.

Characterization of Transduced rMSC

Transduction efficiency was determined by flow cytometry (FACSVantage; Becton Dickinson, Germany) of eGFP-transduced cells¹⁸. Expression of bFGF was analyzed by reverse transcription-polymerase chain reaction (RT-PCR). RNA was isolated from 10⁶ transduced mesenchymal stem cells using TRIzol reagent (Invitrogen, Germany) according to the manufacturer's instructions. Lysates were snap-frozen immediately and stored at -80°C until time of assay. Enzyme-linked immunosorbent assay (ELISA) was carried out according to the manufacturer's instructions with 100 μ L of lysate per sample.

Differentiation Assays

Differentiation assays for adipogenic, osteogenic, and chondrogenic differentiation were essentially carried out as described by Neuhuber et al.¹⁷. Adipogenesis was visualized by oil-red staining and microscopy, and subsequent semiquantification was by optical density 490-nm measurement of isopropanol extracted dye. Chondrogenesis was monitored by pellet assay. Pellets were digested with papain, and sulfated glycosaminoglycans (normalized to DNA content) were quantified as described by Toh et al.¹⁹.

Animals

Fourteen-week-old male Lewis rats (LEW/Crl inbred) were obtained from Charles River (Sulzfeld, Germany) with a mean body weight (and standard deviation) at surgery of 412 ± 20.37 g. Acclimatization lasted seven days; animals were kept at room temperature (mean, $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$). Two to four animals were housed together in a standard open-top Makrolon type-IV cage (Techniplast, Italy) with autoclaved sawdust bedding and pulp as nesting material. The rats had ad libitum access to water and food (Altromin, Germany). Lighting was controlled by a twelve-hour on-off cycle. Procedures involving animal care and treatment were conducted in conformity with national and international laws (Directive 86/609/EEC [European Communities Council]; German animal welfare law; FELASA [Federation of European Laboratory Animal Science Associations] guidelines). The protocol was approved by the district government of Upper Bavaria.

Surgical Procedures

Anesthesia was induced by an intramuscular injection of a combination of medetomidine (0.15 mg/kg), midazolam (2 mg/kg), and fentanyl (0.005 mg/kg). The surgical procedures were performed under sterile conditions after shaving and disinfection (Cutasept F; isopropanol; Bode, Germany) of the left hindlimb.

An approximately 15-mm skin incision was performed on the medial side over the Achilles tendon (see Appendix). The Achilles tendon was freed from surrounding tissue. Then, an arthroscopic punch (2.4 mm) was inserted to fully surround the Achilles tendon at 2 mm proximal to the calcaneal bone. One milliliter of cell suspension (PBS; 10⁶ cells/mL) was injected with use of a syringe in a fan-shaped manner distally and proximally to the arthroscopic punch (see Appendix). The tendon was then cut and was not sutured.

The skin was closed in an intracutaneous fashion with 4-0 absorbable Monocryl suture (poliglecaprone; Johnson & Johnson, Germany) added with OpSite spray-on dressing (Smith & Nephew, Germany). No immobilization device was applied; weight-bearing was not restricted.

Sample Collection

The rats were killed with an overdose of pentobarbital (80 mg/kg) administered intravenously. Tendons were prepared and harvested as a whole with triceps surae muscle on the proximal site and calcaneal bone distally. For biomechanical

TABLE 1 Absolute Counts of the Testing for Ultimate Load and Stiffness

Group	Ultimate Load* (N)		Stiffness* (N/mm)	
	2 Weeks	4 Weeks	2 Weeks	4 Weeks
bFGF	47.5 ± 7.5	47.6 ± 12.8	13.7 ± 4.4	18.8 ± 5.0
eGFP	45.4 ± 8.9	53.1 ± 10.8	10.8 ± 2.8	16.3 ± 7.9
PBS	42.4 ± 8.5	53.1 ± 15.6	12.8 ± 4.3	23.9 ± 7.2

*The values are given as the mean and the standard deviation. On the contralateral side, the ultimate load was a mean of 73.7 ± 11.1 N and the stiffness was a mean of 50.2 ± 13.5 N.

assessment, the contralateral tendons were also harvested. Tendons designated for biomechanical analysis were immediately frozen at -18°C. For histological analysis, tendons underwent a more precise preparation removing muscle and bone; fixation was done with 70% methanol (specimens were stored at +8°C).

Statistical Analysis

The study has 80% power with use of a two-sided test of 5% to detect a 7.5-N/mm difference in stiffness at fourteen days or twenty-eight days. The power analysis was performed with R software (version 2.10.1; R Foundation for Statistical Computing, Vienna, Austria), and the numbers were chosen after a review of the relevant literature. The biomechanical properties of the tendons were analyzed across treatments and repair times with use of analysis of variance with the level of alpha set at 0.05. Pairwise comparisons were performed with use of two-tailed paired t tests. Statistical analysis was performed with use of SPSS software (version 12.0; SPSS, Chicago, Illinois).

The analysis for the histological testing was performed with the Fisher exact test and the Kruskal-Wallis test also with use of SPSS software (version 12.0; SPSS).

Biomechanical Testing

Each thawed Achilles tendon was moistened with phosphate-buffered saline solution to anticipate drying out. The proximal end (musculotendinous junction) was press-fixed in a cryoclamp, while the distal end of the Achilles tendon was placed in a mounting grid with osseous calcaneal fixation (see Appendix). Ten milliliters of liquid nitrogen caused freezing of the clamp and the musculotendinous junction for rigid fixation without affecting the free tendon. The angle between the calcaneus and Achilles tendon corresponded to 30° of dorsiflexion of the foot.

The tendons were then mounted onto a mechanical testing machine (Zwicki 1120; Zwick, Ulm, Germany) (see Appendix). The construct was initially set to a basic axial stress of 2 N for straightening and adjustment. Each tendon was then axially pulled at a constant speed of 0.16 mm/s until maximum load to failure. Simultaneously, contralateral tendons were tested in the same way for percentage statistical analysis, considering the diverse constitution of rats and avoiding inexact cross-sectional area measurements. Stiffness and maximum load to failure were calculated with SPSS software (version 12.0; SPSS).

Histological Analysis

After methanol fixation, specimens were decalcified using 5% EDTA solution for one week and then spent twenty-four hours in a 5% sucrose and phosphate-buffered saline solution prior to cryosectioning of 12-µm-thick sections.

Hematoxylin and eosin staining was then performed. Tendon fiber structure, fiber arrangement, rounding of the nuclei, regional variations in cellularity, increased vascularity, and collagen staining were assessed with use of a semiquantitative 4-point scoring system, ranging from 0 (normal appearance) to 3 (markedly abnormal appearance), as described by Longo et al.²⁰. The final score resulted from the sum of the individual scores given to each indicator.

Immunohistological Analysis

Detailed immunohistological analyses were conducted to detect changes in the composition and structure of the regenerated tendons following an established protocol described by Tischer et al.²¹. For immunohistological staining, primary monoclonal antibodies for type-I and type-III collagen (Sigma, Munich, Germany) in dilutions of 1:2000 (type-I collagen) and 1:4000 (type-III collagen) were used. Furthermore, the following antibodies from the Department of Biological Sciences of the University of Iowa were applied: the antibody M38 for type-I procollagen, the antibody AMF-17b for vimentin, the antibody HFN-7.1 for fibronectin, the antibody 2EA for laminin, and the antibody 12C5 for versican; all diluted 1:5.

For further evaluation, the tendons were divided into three areas: proximal, middle, and distal third. Each area was graded, depending on the staining reaction as 0 (no staining), 1 (partial staining), or 2 (strong staining).

Histological and immunohistological analyses were performed by two independent and blinded investigators.

Source of Funding

This study was supported by the Society for Joint Surgery and Arthroscopy (AGA). There is no commercial interest related to this study.

Results

Characterization of rMSCs

Due to the absence of one specific marker, mesenchymal stem cells are commonly characterized by the presence or absence of sets of surface markers^{22,23}. Thus, rat bone-marrow-derived mesenchymal stem cells (rMSCs) were verified by fluorescence-activated cell sorter (FACS) for the presence of a set of typical surface markers such as the ecto-5'-nucleotidase CD73 (mean and standard deviation, 38% ± 2%), the integrine 1α subunit CD49a (mean, 88% ± 2%), and the surface protein Thy-1 CD90 (mean, 99% ± 1%). Expected cells stained negative for the leukocyte marker CD45 (mean, 0.09% ± 0.02%). The mesenchymal stem cell characteristics were verified by differentiation assays and showed adipogenic, chondrogenic, and osteogenic differentiation potential in vitro (Fig. 1-A).

To determine transduction efficiency of lentiviral vectors, mesenchymal stem cells were transduced with an eGFP-expressing lentiviral vector and were highly positive for eGFP as analyzed by FACS (mean, 96.3% ± 2.5%). Nontransduced cells and bFGF-transduced cells showed background fluorescence of <0.5%. Transduction efficiency was assumed to be comparable between eGFP and bFGF-expressing lentiviral vectors. Expression of bFGF was verified by RT-PCR on their mRNA level (data not shown), and bFGF protein was quantified in cell lysates by ELISA. The adipogenic as well as the osteogenic differentiation (Ca²⁺ mg/mg protein) (Fig. 1-B) of eGFP-transduced cells was reduced compared with bFGF-transduced cells and nontransduced controls. Basic FGF transduction resulted in a sixtyfold increase of bFGF proteins in comparison with nontransduced and eGFP-transduced cells (mean, 3.045 ± 0.020 pg bFGF/10⁶ cells in comparison with 0.051 ± 0.019 pg bFGF/10⁶ cells [controls] and 0.058 ± 0.024 pg bFGF/10⁶ cells [eGFP-transduced cells]) (Fig. 1-C).

Animals

All eighty-four rats were able to use their left hindlimb after five to seven days and showed a normal gait pattern after twelve to fourteen days. There were no infections or other complications.

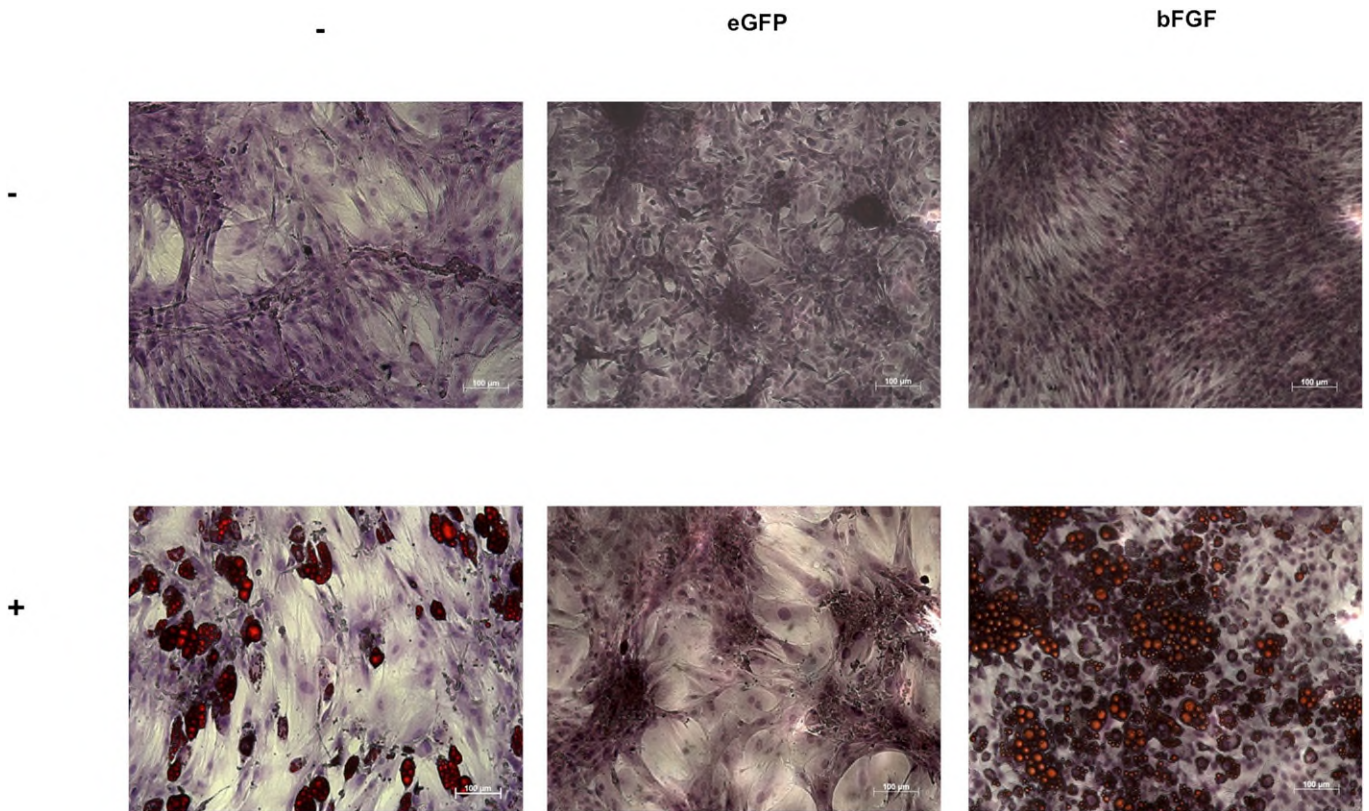


Fig. 1-A

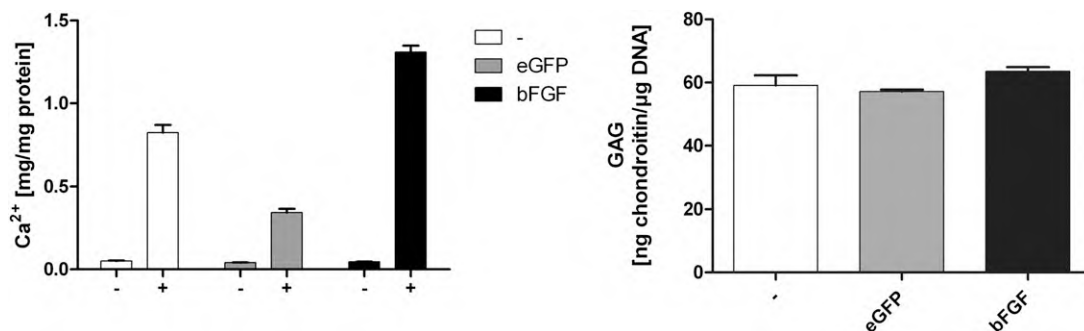


Fig. 1-B

Figs. 1-A, 1-B, and 1-C Differentiation potential of control mesenchymal stem cells (-) and lentivirally transduced mesenchymal stem cells (eGFP and bFGF). **Fig. 1-A** Adipogenic differentiation: Control and transduced mesenchymal stem cells were subjected to three cycles of adipogenic induction (+) or were left noninduced (-). Oil-red staining of mesenchymal stem cells demonstrates retention of adipogenic differentiation of lentivirally transduced cells. **Fig. 1-B** Osteogenic differentiation (Ca^{2+}) (left): Control and transduced mesenchymal stem cells were induced for osteogenesis (+) or were left noninduced (-) (fourteen days of incubation). Quantification of calcium deposits demonstrates retention of osteogenic differentiation of lentivirally transduced cells. Chondrogenic differentiation (right): The deposition of sulphated glycosaminoglycans (GAG) in pellets was quantified and normalized to DNA content after twenty-one days. Control and lentivirally transduced mesenchymal stem cells show the same chondrogenic differentiation potential. The values are given as the mean and the standard deviation.

Macroscopic Assessment

Macroscopic assessment showed continuity was regained in all tendons. The defect zone in the Achilles tendon was easily detectable in all groups at fourteen days and was partially detectable at twenty-eight days after surgery (see Appendix).

Biomechanical Testing

Ultimate Load to Failure

The ultimate load to failure ratio (treated side to contralateral side) was significantly better in the MSC-LV-eGFP group ($p = 0.036$; 95% confidence interval [CI], 0.02 to 0.36) in comparison

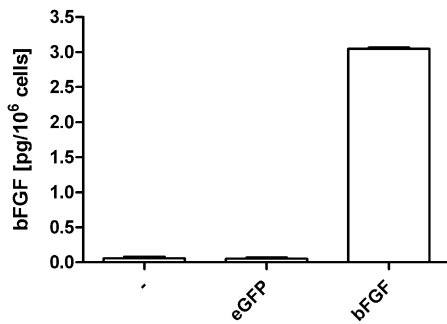


Fig. 1-C
Basic FGF expression of lentivirally transduced mesenchymal stem cells. Mesenchymal stem cells transduced with lentiviral vectors coding for eGFP or bFGF and nontransduced controls (-) were analyzed for bFGF expression by ELISA. The values are given as the mean and the standard deviation.

with the PBS control group after fourteen days (Fig. 2-A). In addition, the MSC-LV-bFGF group showed better results than the PBS control group; however, the difference was not significant ($p = 0.063$; 95% CI, -0.01 to 0.32). The additional expression of bFGF compared with eGFP had no significant effect after fourteen days ($p = 0.751$; 95% CI, -0.22 to 0.16) (Table I).

Over time, the ultimate load to failure ratio showed a significant improvement in the PBS group from day 14 to day 28 ($p = 0.012$; 95% CI, -0.58 to -0.10). A similar improvement was not observed in the mesenchymal stem cell groups ($p = 0.678$ [95% CI, -0.26 to 0.18] for bFGF and $p = 0.971$ [95% CI, -0.26 to 0.25] for eGFP). However, after twenty-eight days, there were no significant differences between any groups ($p = 0.285$ [95% CI, -0.46 to 0.16] for PBS versus eGFP; $p = 0.296$ [95%

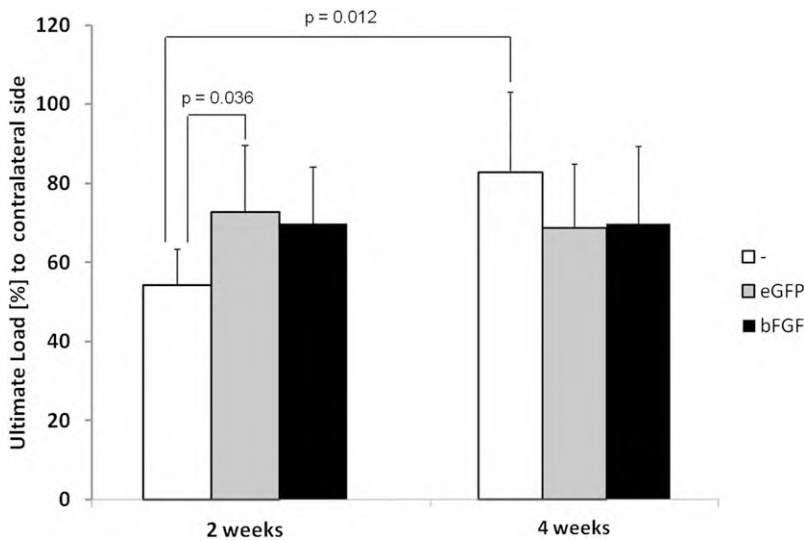


Fig. 2-A

Figs. 2-A and 2-B Ultimate load to failure and stiffness of the tendons. The values are given as the mean and the standard deviation. The dash indicates the PBS group.

Fig. 2-A Biomechanical load to failure after fourteen days and twenty-eight days. **Fig. 2-B** Biomechanical stiffness after fourteen days and twenty-eight days.

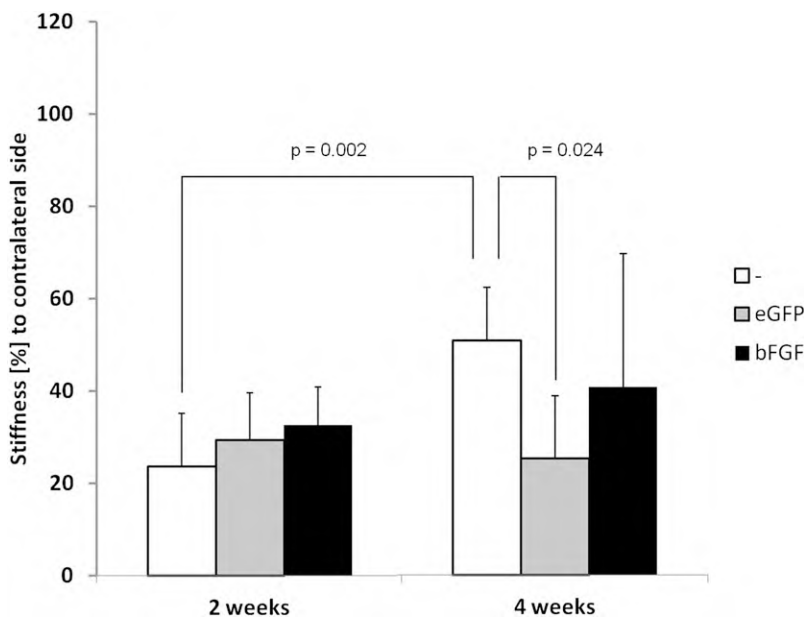


Fig. 2-B

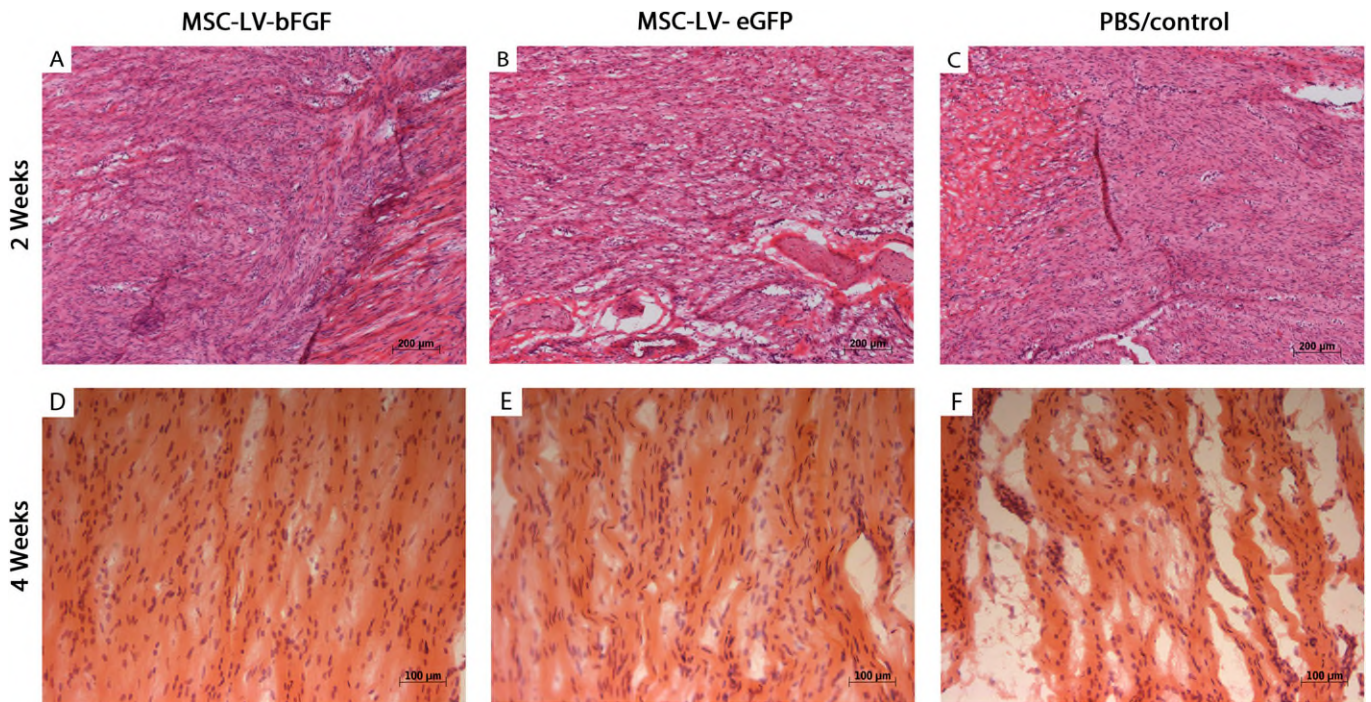


Fig. 3

Figs. 3-A through 3-F Hematoxylin and eosin staining of the tendons after healing periods of fourteen days and twenty-eight days. **Figs. 3-A, 3-B, and 3-C** After fourteen days, many collagen fibers are evident in the MSC-LV-bFGF group. In the PBS photomicrograph, granulation tissue is shown without real characteristics of tendon tissue, whereas the photomicrographs of the mesenchymal stem cells show initial fiber structure. **Figs. 3-D, 3-E, and 3-F** After twenty-eight days, there are more parallel and denser collagen fibers in the MSC-LV-bFGF group (**Fig. 3-D**) than in the MSC-LV-eGFP group (**Fig. 3-E**). The tissue in the PBS group seems fluffy and not as well organized, and there are fewer typically oval nuclei of tenocytes (**Fig. 3-F**).

CI, -0.41 to 0.14] for PBS versus bFGF; and $p = 0.929$ [95% CI, -0.27 to 0.29] for eGFP versus bFGF).

Stiffness

The stiffness ratio (treated side to contralateral side; Fig. 2-B) was not significantly different between any of the three groups after fourteen days ($p = 0.415$ [95% CI, -0.09 to 0.20] for PBS versus eGFP; $p = 0.186$ [95% CI, -0.04 to 0.22] for PBS versus bFGF; and $p = 0.522$ [95% CI, -0.08 to 0.15] for eGFP versus bFGF). As for the maximum load to failure, only the PBS group showed a significant increase in stiffness values from day 14 to day 28 ($p = 0.002$; 95% CI, -0.44 to -0.10). There were no significant differences from day 14 to day 28 with regard to stiffness observed for the mesenchymal stem cell groups ($p = 0.592$ [95% CI, -0.12 to 0.20] compared with $p = 0.523$ [95% CI, -0.35 to 0.19]) (Table I).

After twenty-eight days, the PBS control group showed significantly better results than the eGFP group ($p = 0.024$; 95% CI, -0.46 to -0.05). Also, the PBS group had higher values than the bFGF group after twenty-eight days, but the difference was not significant ($p = 0.431$; 95% CI, -0.41 to 0.21). Similarly, the bFGF group had higher values than the eGFP group after twenty-eight days but with no significant difference ($p = 0.259$; 95% CI, -0.20 to 0.50).

Histological Analysis

In general, hematoxylin and eosin staining after fourteen days showed mainly scar tissue, in particular at the intersections of the remaining parts of the original tendon (Fig. 3). The main differences between the tendons harvested after twenty-eight days compared with those harvested after fourteen days were their fiber structures and their more parallel fiber arrangements. Also, the rounding of the nuclei and the regional variations of cellularity clearly decreased, and the intensity of the collagen staining returned to normal appearance (Fig. 3).

There were no significant differences in the histological features of the control, bFGF, and eGFP groups (see Appendix).

Immunohistological Analysis

Immunohistological analysis revealed no significant difference between the staining patterns of type-I collagen, type-III collagen, fibronectin, laminin, versican, or vimentin in all three groups. However, collagen synthesis was especially activated in both the bFGF group and the eGFP group because the type-I procollagen staining was markedly more intense compared with the PBS group after fourteen days ($p = 0.0009$ for the bFGF group and $p = 0.0041$ for the eGFP group) and after twenty-eight days ($p = 0.004$ and $p = 0.132$, respectively) (Fig. 4 and see Appendix).

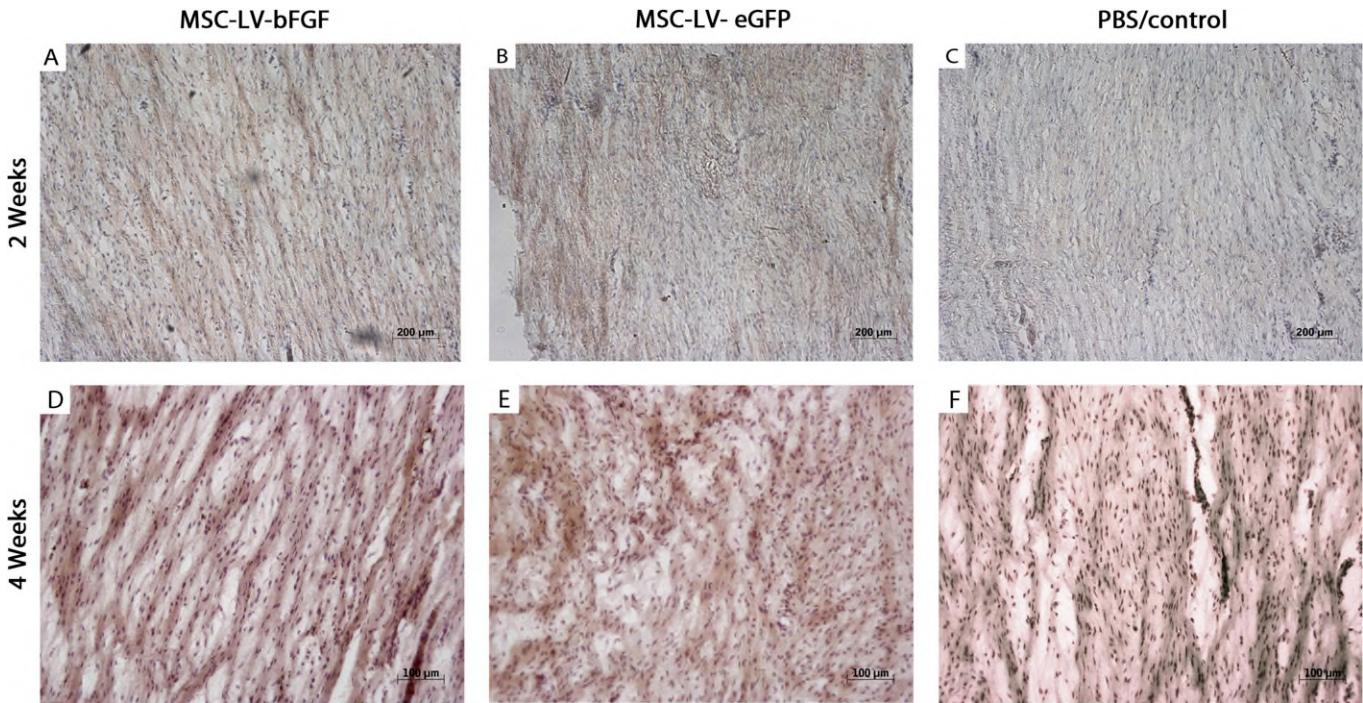


Fig. 4

Type-I procollagen staining after fourteen days (Figs. 4-A, 4-B, and 4-C) and twenty-eight days (Figs. 4-D, 4-E, and 4-F). The brown color indicates the type-I procollagen detection. More intense procollagen-I staining was seen in the stem cell groups (Figs. 4-A, 4-B, 4-D, and 4-E).

Discussion

This analysis showed only partially positive effects on the repair tissue structure and composition by stem cells. However, biomechanical improvements are marginal in the early stage and not present in the later stage. The stem cell groups did not show significant improvement in histologic features. All immunohistological parameters after fourteen days and twenty-eight days were comparable among all three groups, besides type-I procollagen. There was a significant increase in type-I procollagen staining for both the eGFP and bFGF stem cell groups in comparison with the PBS group. However, these improved immunohistological results are not represented by the biomechanical data after twenty-eight days. The additional expression of bFGF was without a significant effect on tendon remodeling.

One of the most promising approaches to improve tendon healing is the application of mesenchymal stem cells^{5,11,12,24}. Among their proliferation and differentiation capacity into osteoblasts, chondrocytes, adipocytes, and fibroblasts¹³, the beneficial effect of mesenchymal stem cells in sites of injury may not necessarily involve their differentiation into the regenerating tissue type, but rather the local production of growth factors such as IGF-1, TGF- β , VEGF, PDGF, and bFGF^{14,25,26}.

An established way to express certain growth factors has been developed by gene therapy methods. In particular, a retroviral gene transduction is very efficient with regard to amounts of expressed protein and long-term production^{27,28}. In contrast, transient gene transfer methods such as plasmid-based systems are not efficient with regard to the results of tissue repair²⁹. Therefore,

we used lentiviral vectors (retroviral family) to achieve the highest growth-factor concentrations in the repair tissue for the biggest possible effect.

In a comparison of the histological data with those of other studies, we found that Chan et al.¹⁵ used bFGF as a one-time administered protein for patellar tendon healing in a rat model. Those authors showed a positive effect on histological and immunohistological repair characteristics only after seven days. Specifically, they found higher amounts of type-III collagen levels and higher cell proliferation rates¹⁵. Type-III collagen, however, is thinner and more extensible than type-I collagen and is, together with type-I procollagen, an important factor in the early phases of tendon healing.

As an innovative alternative to the above-mentioned study, in our study we used stable expressed bFGF by lentiviral transduction of mesenchymal stem cells. We thought that the short-term effect of bFGF described in the study of Chan et al. was due to the transient effect of a single protein application. Although we were able to show that bFGF transduction was very efficient, there was no clear long-term effect with regard to the biomechanical results. In addition, in comparing the mesenchymal stem cell groups, we found that differences regarding all analyses were marginal. One explanation could be that the needed bFGF levels for tendon repair are low, native bFGF production of stem cells is sufficient, and additional bFGF may be without further effects. In a study involving a rat Achilles tendon model, Zhang et al.⁸ described a substantial positive effect of VEGF with regard to biomechanical properties in the early period of healing (seven days and fourteen days). In contrast, there were

no significant influences in biomechanical characteristics after four weeks. These results are similar to our data.

Our animal model allowed full mobilization and weight-bearing after the creation of a tendon defect. Okamoto et al.⁵ showed, in a similar rat Achilles tendon model, that mesenchymal stem cells or bone marrow cells alone achieved higher biomechanical properties than an empty control group. However, additional growth factors were not analyzed. A possible explanation for the better outcome of their stem cell groups might be the transection rather than a defect and also the postoperative cast immobilization. However, our model may be more appropriate as the rat Achilles tendon has a higher regeneration potential than human tendon, and transection leads to rapid healing and hinders the analysis of certain factors on repair tissue. In contrast to the study by Okamoto et al., Bring et al.³⁰ showed, in a rat Achilles tendon defect model, that prolonged immobilization had a negative effect on tendon healing. An explanation for this finding was the upregulation of different genes in the mobilized group in comparison with the immobilized group. In our study, all tendons showed macroscopically a continuous repair tissue after fourteen days and twenty-eight days.

In conclusion, we established an Achilles tendon in vivo defect model based on stem cells and stable lentiviral growth factor expression. Our analysis showed only partially positive effects on the repair tissue structure and composition by stem cells. However, biomechanical improvements are marginal in the early stage and not present in the later stage.

Because preliminary in vitro and in vivo stem cell studies are partially promising, the use of stem cells is becoming popular. However, because of poor study designs and/or missing long-term results, the benefits and transferability to clinical use are often limited. In addition, missing or negative effects of stem cells in the orthopaedic field are rarely reported. Our study should lead to a more critical and objective use of stem cells in humans until more relevant clinical data are available.

Appendix

eA Tables showing scores for hematoxylin and eosin staining results after fourteen and twenty-eight days and for type-I procollagen staining after fourteen and twenty-eight

days as well as figures demonstrating the surgical procedure, biomechanical testing, and macroscopic assessment of a tendon after a twenty-eight-day healing period are available with the online version of this article as a data supplement at jbjs.org.

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