Adipose-Derived Stem Cells Inhibit the Contractile Myofibroblast in Dupuytren's Disease

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Background: In an attempt to provide minimally invasive treatment for Dupuytren's disease, percutaneous disruption of the affected tissue followed by lipografting is being tested. Contractile myofibroblasts drive this fibroproliferative disorder, whereas stem cells have recently been implicated in preventing fibrosis. Therefore, the authors tested the role of stem cells in modulating myofibroblast activity in Dupuytren's disease.
Methods: The authors compared the effect of co-culturing Dupuytren's myofibroblasts with either adipose-derived or bone-marrow–derived stem cells on isometric force contraction and associated levels of α-smooth muscle actin mRNA and protein expression. The authors also tested the effect of these stem cells on Dupuytren's myofibroblast proliferation and assessed whether this was mediated by cell-to-cell contact or by a paracrine mechanism.
Results: Addition of adipose-derived stem cells to Dupuytren's myofibroblasts reduced the contraction of the latter, with a corresponding reduction of α-smooth muscle actin protein expression, probably through a dilution effect. In contrast, bone marrow–derived stem cells increased myofibroblast contractility. In addition, adipose-derived stem cells inhibit myofibroblast proliferation and mediate these effects by soluble factors, influenced by cell-to-cell contact–dependent signaling.
Conclusion: Adipose-derived stem cells inhibit the contractile myofibroblast in Dupuytren's disease, and these findings lend support to the potential benefit of lipografting in conjunction with aponeurotomy as a novel strategy for the treatment of Dupuytren's disease. (*Plast. Reconstr. Surg.* 132: 1139, 2013.)

Dupuytren's disease is a common fibroproliferative disorder, characterized by development of contractile cellular nodules that progress to form fibrotic cords, leading to digital contracture.[1,2] Contractures are most commonly treated by surgical excision or using less invasive alternatives such as division of the cord by percutaneous needle fasciotomy or, more recently, by enzymatic digestion with collagenase injections.[3] These less invasive treatment alternatives promote earlier postoperative recovery, although recurrence rates following these procedures remain high.[4–6]

In an attempt to overcome high recurrence rates after minimally invasive needle fasciotomy, a novel approach is being tested using a more extensive percutaneous needle aponeurotomy technique. With this technique, numerous superficial nicks are performed along the affected tissue to disintegrate the fibrous cord. Subsequent release of the skin from the subcutaneous layer followed

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Received for publication February 27, 2013; accepted May 15, 2013.
Presented at the First Research Council Meeting of the European Association of Plastic Surgeon, in Munich, Germany, May 23 through 24, 2012; and the Winter Scientific Meeting of the British Association of Plastic, Reconstructive, and Aesthetic Surgeons, in London, United Kingdom, December 4 through 7, 2012.

DOI: 10.1097/PRS.0b013e3182a3bf2b

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

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by injection with autologous lipoaspirates restores the subcutaneous fat layer.[7]

There is now increasing evidence for the role of mesenchymal stem cells as a potential treatment strategy to alleviate fibrosis. Mesenchymal stem cells are multipotent cells that can be easily obtained from a variety of sources, and have been shown to play a role in decreasing fibrosis in models of lung, liver, kidney, and heart, and also in an animal model of related Peyronie disease.[8–11] Mesenchymal stem cells exhibit potent immunomodulatory and antiinflammatory effects, either by direct cell-to-cell contact or by indirect secretion of various soluble factors.[12] Their use has also been examined in antitumor therapy, where they show antiproliferative effects.[13–16]

In Dupuytren's disease, the cell responsible for both contraction and extracellular matrix deposition is the myofibroblast[17] and is characterized by the expression of highly contractile α-smooth muscle actin. Research into treatment strategies should be targeted at modulating these myofibroblasts and inhibiting fibroproliferation. Therefore, we set out to examine the effect of lipoaspirates on myofibroblast activity, as these lipoaspirates are known to be a rich source of stem cells with regenerative potential.[18–20] Specifically, we tested the potential therapeutic benefit of adipose-derived mesenchymal stem cells to inhibit fibroproliferation in Dupuytren's disease, and whether this effect was specific to adipose-derived mesenchymal stem cells.

PATIENTS AND METHODS

Tissue Samples and Cells

Tissue samples were obtained following informed consent (MEC-2010–294). Dupuytren's nodular tissue was obtained from patients with Dupuytren's disease undergoing surgery, and normal skin was obtained from adult human skin discarded after reconstructive surgery. Human adipose-derived mesenchymal stem cells (Lonza, Berkshire, United Kingdom) were positive for CD13, CD29, CD44, CD73, CD90, CD105, and CD166 surface markers; and negative for CD14, CD31, and CD45 surface markers. Bone marrow–derived mesenchymal stem cells (Lonza) were positive for CD29, CD44, CD105, and CD166 surface markers; and negative for CD14, CD34, and CD45 surface markers. Cells positive for these markers of adipose-derived mesenchymal stem cells and bone marrow–derived mesenchymal stem cells have been reported to differentiate down various lineages, including chondrogenic, osteogenic, adipogenic, myogenic, neural, and endothelial.

Cell Culture

Dupuytren's cells, myofibroblasts, were isolated from α-smooth muscle actin–rich nodules,[21–23] and human dermal fibroblasts were isolated from full-thickness skin samples. Briefly, tissue samples were dissected into small pieces and digested in Dulbecco's Modified Eagle Medium (Lonza) containing 1% penicillin/streptomycin (PAA Laboratories, Somerset, United Kingdom) and Gibco 5% fetal bovine serum (Invitrogen, Paisley, United Kingdom.) with type I collagenase (Worthington Biochemical Corp., Lakewood, N.J.) and frequent gentle agitation for up to 2 hours at 37°C. Cells were then collected following centrifugation and cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in 5% carbon dioxide in a humidified incubator. Cells up to a maximum of passage 3 were used for experiments to prevent passage-dependent phenotypic alterations.

Culture Force Monitor

Measurement of the isometric contractile forces generated by cells within three-dimensional collagen matrices was performed as described previously.[21,24] Briefly, 2×10^6 cells were seeded onto 2.5 ml of type I collagen gel (First Link, Birmingham, United Kingdom), and three-dimensional matrices tethered between two flotation bars, one attached to a fixed point and the other attached to a force transducer. [**See Figure, Supplemental Digital Content 1**, which shows the culture force monitor, ***http://links.lww.com/PRS/A883***. Rectangular collagen gels were seeded with cells (*above*) and floated in culture medium (*below*), tethered between two flotation bars, one attached to a force transducer (*1*) and the other attached to a fixed point (*2*).] Fibroblast-populated collagen matrix–generated tensional forces were measured continuously and data logged every minute (dynes, 1×10^{-5} N) and a contraction profile was generated over 24 hours. Cell-populated matrices were cultured in Dulbecco's Modified Eagle Medium with 2% fetal bovine serum[25,26] and 1% penicillin/streptomycin at 37°C in 5% carbon dioxide in a humidified incubator. Matrices were seeded with co-cultures of different cell proportions (100 percent myofibroblasts; 75 percent myofibroblasts plus 25 percent adipose-derived mesenchymal stem cells, bone marrow–derived mesenchymal stem cells, or human dermal fibroblasts; 25% myofibroblasts plus 75% adipose-derived mesenchymal stem cells, bone marrow–derived mesenchymal stem cells, or human dermal fibroblasts; and 100% adipose-derived mesenchymal stem

cells, bone marrow–derived mesenchymal stem cells, or human dermal fibroblasts). The rate of contraction (dynes per hour) was calculated by measuring the average gradient of the curve between 8 and 24 hours. Experiments shown are from three individual patients, each performed in triplicate.

Quantitative Reverse-Transcription Polymerase Chain Reaction

Cells were cultured in monolayer and in adherent collagen matrices, floating collagen matrices, or tethered collagen matrices in the culture force monitor. Co-cultures were set up with varying cell proportions over 24 hours. Total RNA was extracted from each sample using the QIAamp RNeasy Mini Kit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions. Isolated RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop, Cambridge, United Kingdom). For real-time reverse-transcription polymerase chain reaction, inventoried TaqMan gene expression assays were used for α-smooth muscle actin (Hs00426835-g1) and type I collagen (Hs00164004-m1) (Applied Biosystems, Paisley, United Kingdom) with Reverse Transcriptase qPCR Mastermix No ROX (Eurogentec, Southampton, United Kingdom). Samples were run on the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems). Expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (Hs02758991-g1) (Applied Biosystems) and compared to the level of gene expression in myofibroblasts , which were assigned the value of 1 using ΔΔCt analysis performed with SDS software (Applied Biosystems).

Western Blots

Cells were cultured in monolayer, adherent collagen matrices, floating collagen matrices, or tethered collagen matrices cultured in the culture force monitor with co-cultures of different cell proportions for 24 hours (as stated above). Cell lysates were prepared using lysis buffer [25 mM hydroxyethyl piperazineethanesulfonic acid (pH 7.0), 150 mM sodium chloride, and 1% Nonidet P-40], containing protease inhibitor cocktail (Roche Biochemicals, Lewis, United Kingdom), and then electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gels, followed by electrotransfer of proteins onto polyvinylidene difluoride transfer membranes (Perkin Elmer, Cambridge, United Kingdom). Membranes were blocked in 5% bovine serum albumin/tris-buffered saline plus 0.05% Tween and incubated overnight at 4°C with primary antibodies against α-smooth muscle actin (Sigma, Dorset, United Kingdom) and vimentin (Abcam, Cambridge, United Kingdom). Horseradish peroxidase–conjugated anti-mouse immunoglobulin G (Amersham Biosciences, Amersham, United Kingdom) was used as a secondary antibody. Bound antibody was detected using the enhanced chemiluminescence kit (Amersham Biosciences) and visualized using Hyperfilm MP (Amersham Biosciences).

Cell Proliferation

Cell proliferation was measured using a 5-ethynyl-2′-deoxyuridine microplate assay (Invitrogen), which incorporates into DNA during active DNA synthesis. Co-cultures of different cell proportions were cultured in monolayer or adherent collagen matrices and incubated with 5-ethynyl-2′-deoxyuridine (10 mM) for 12 hours. 5-Ethynyl-2′-deoxyuridine incorporation was assessed with a fluorescent microplate reader (Gemini XPS; Molecular Devices, Sunnyvale, Calif.) by measuring the excitation/emission at a wavelength of 560 to 585 nm. To assess DNA content for proliferation studies, co-cultures of different cell proportions were cultured in monolayer or seeded in adherent collagen matrices up to 5 days and stained with Hoechst 33258 dye (Sigma-Aldrich) at days 1, 3, and 5. Briefly, the cells were lysed by freeze-thawing in distilled water and stained with the fluorochrome in TNE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid, 2 M sodium chloride, pH 7.4). The samples were analyzed with a fluorescent microplate reader by measuring the excitation/emission at a wavelength of 350 to 460 nm. DNA from calf thymus (Sigma-Aldrich) was used as a standard.

Conditioned Medium

Adipose-derived mesenchymal stem cells or myofibroblasts were incubated with 2% Dulbecco's Modified Eagle Medium at 37°C in 5% carbon dioxide for 24 hours, after which supernatants were harvested and used as conditioned medium from noncontact culture experiments. For experiments examining conditioned medium of direct contact cultures, co-cultures of 50 percent adipose-derived mesenchymal stem cells plus 50 percent myofibroblasts were incubated with 2% Dulbecco's Modified Eagle Medium at 37°C in 5% carbon dioxide for 24 hours, after which supernatants were harvested. Transwell chambers (8-mm membrane insert) (Sigma-Aldrich) were

used to collect conditioned medium from indirect contact cultures: co-cultures of 50 percent adipose-derived mesenchymal stem cells (upper chamber) and 50 percent myofibroblasts (lower chamber) were incubated with 2% Dulbecco's Modified Eagle Medium at 37°C in 5% carbon dioxide for 24 hours and supernatants were harvested. Conditioned medium was filtered through a 0.22-mm filter (Sigma-Aldrich) and stored at −80°C for later use in proliferation experiments.

Statistical Analysis

A paired *t* test was used to compare proliferation of myofibroblasts in control medium and adipose-derived mesenchymal stem cell–conditioned medium from noncontact cultures, conditioned medium from direct contact cultures, or conditioned medium from indirect contact cultures. One-way analysis of variance using the Bonferroni multiple comparison test was used to compare all other conditions. All statistical analyses were performed using GraphPad Software version 5.0c (GraphPad Software, Inc., La Jolla, Calif.). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of the Mechanical Environment on the Myofibroblast Phenotype

We compared the effect of two-dimensional monolayer, three-dimensional adherent matrices, and floating collagen matrices on type I collagen and α-smooth muscle actin mRNA and protein expression in Dupuytren's myofibroblasts, adipose-derived mesenchymal stem cells, bone marrow–derived mesenchymal stem cells, and human dermal fibroblasts. Bone marrow–derived mesenchymal stem cells expressed significantly higher levels of type I collagen mRNA, α-smooth muscle actin mRNA, and protein under all environments investigated ($p < 0.05$) compared with myofibroblasts, adipose-derived mesenchymal stem cells, and human dermal fibroblasts. [**See Figure, Supplemental Digital Content 2**, which shows cells studied in monolayer, adherent three-dimensional collagen matrices, and free-floating three-dimensional collagen matrices, ***http://links.lww.com/PRS/A884***. (*Left*) In monolayer (two-dimensional), levels of α-smooth muscle actin and collagen mRNA and α-smooth muscle actin protein expression were significantly higher in bone marrow–derived mesenchymal stem cells compared with the other cell types. Human dermal fibroblasts demonstrated a decrease in α-smooth muscle actin protein expression, although there was no significant difference in α-smooth muscle actin and collagen mRNA expression. Adipose-derived mesenchymal stem cells and myofibroblasts demonstrated no differences in α-smooth muscle actin or collagen gene or α-smooth muscle actin protein expression. (*Center*) Adherent three-dimensional collagen matrices led to a significant increase of α-smooth muscle actin and type I collagen mRNA and α-smooth muscle actin protein expression in only bone marrow–derived mesenchymal stem cells. There was a decrease in α-smooth muscle actin protein expression in adipose-derived mesenchymal stem cells and human dermal fibroblasts compared with myofibroblasts. (*Right*) Free-floating three-dimensional matrices demonstrated significantly increased α-smooth muscle actin and type I collagen mRNA and α-smooth muscle actin protein expression in bone marrow–derived mesenchymal stem cells compared with the other cell types. Adipose-derived mesenchymal stem cells and human dermal fibroblasts demonstrated a significant decrease in α-smooth muscle actin protein expression (although not type I collagen) and a concomitant decrease in α-smooth muscle actin protein expression compared with myofibroblasts. Gene expression was assessed by quantitative reverse-transcription polymerase chain reaction, and data are presented as fold change compared with glyceraldehyde 3-phosphate dehydrogenase, and normalized to expression in myofibroblasts. Protein expression was assessed by Western blotting, using vimentin as a loading control. Gels shown are representative of gels from three patients. Data are shown as the mean ± SEM from three patients (each assay was performed in triplicate) (*p < 0.05, **p < 0.01). *MF*, myofibroblasts; *ADMSC*, adipose-derived mesenchymal stem cells; *BDMSC*, bone marrow–derived mesenchymal stem cells; *αSMA*, α-smooth muscle actin; *HDF*, human dermal fibroblasts.]

Adipose-derived mesenchymal stem cells and human dermal fibroblasts expressed lower levels of α-smooth muscle actin mRNA and protein when cultured in floating matrices ($p < 0.05$) under minimal tension, compared with myofibroblasts and bone marrow–derived mesenchymal stem cells. (**See Figure, Supplemental Digital Content 2**, ***http://links.lww.com/PRS/A884***.) The stiffness of both three-dimensional adherent matrices and monolayer cultures exerts effects on cell differentiation on the cell types used, and cells exhibited a similar contractile myofibroblast phenotype under these conditions. Therefore, all our experiments were conducted under these conditions.

Adipose-Derived Stem Cells Reduce the Dupuytren's Myofibroblast Phenotype, whereas Bone Marrow–Derived Stem Cells Increase the Myofibroblast Phenotype

To test the effect of adipose-derived mesenchymal stem cells on Dupuytren's myofibroblasts, we co-cultured different percentages of adipose-derived mesenchymal stem cells with myofibroblasts and examined their effect on the myofibroblast phenotype compared with control co-cultures of bone marrow–derived mesenchymal stem cells or human dermal fibroblasts, in the culture force monitor. Co-cultures with adipose-derived mesenchymal stem cells resulted in a dose-dependent decrease in contractility of myofibroblasts ($p < 0.05$) associated with a corresponding reduction of α-smooth muscle actin protein expression. However, there was no effect on α-smooth muscle actin mRNA expression (Fig. 1). Control human dermal fibroblasts demonstrated a similar decreasing effect on myofibroblast contractility and α-smooth muscle actin protein expression ($p < 0.01$), again without affecting α-smooth muscle actin mRNA expression (Fig. 1). In contrast, co-cultures with bone marrow–derived mesenchymal stem cells led to a dose-dependent increase in contractility and α-smooth muscle actin protein expression, without affecting α-smooth muscle actin mRNA expression (Fig. 1).

Stem Cells Inhibit Proliferation of Dupuytren's Myofibroblasts

Co-cultures in monolayer of adipose-derived mesenchymal stem cells with myofibroblasts led to

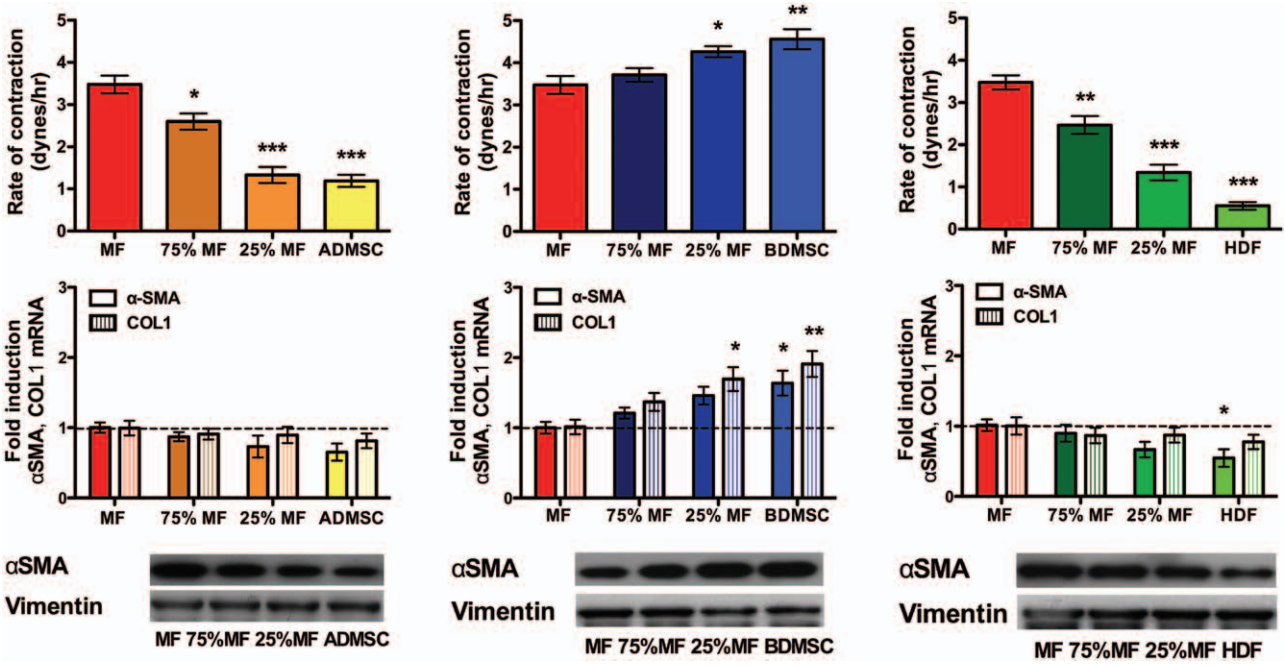

Fig. 1. Co-cultures with adipose-derived mesenchymal stem cells led to inhibition of the contractile myofibroblasts in Dupuytren's disease, whereas bone marrow–derived mesenchymal stem cells promoted myofibroblast contractility. (*Left*) Co-cultures of Dupuytren's myofibroblasts and adipose-derived mesenchymal stem cells led to a dose-dependent decrease in contractility when cultured in the culture force monitor. There was a concomitant decrease in α-smooth muscle actin protein expression, although α-smooth muscle actin and type I collagen mRNA expression demonstrated no significant change. (*Center*) Co-cultures with myofibroblasts and bone marrow–derived mesenchymal stem cells led to a dose-dependent increase in contractility in the culture force monitor. A corresponding increase in α-smooth muscle actin and type I collagen mRNA expression and an increase in α-smooth muscle actin protein expression were observed. (*Right*) Co-cultures with myofibroblasts and human dermal fibroblasts led to a dose-dependent decrease in isometric contraction in the culture force monitor. α-Smooth muscle actin and type I collagen mRNA expression demonstrated a corresponding decrease, as did α-smooth muscle actin protein expression. Gene expression was assessed by quantitative reverse-transcription polymerase chain reaction, and data are presented as fold change compared with glyceraldehyde 3-phosphate dehydrogenase, and normalized to expression in myofibroblasts. Protein expression was assessed by Western blotting, using vimentin as a loading control. Gels shown are representative of gels from three patients. Data are shown as the mean ± SEM from three patients (each assay was performed in triplicate) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). *MF*, myofibroblasts; *α-SMA*, α-smooth muscle actin; *ADMSC*, adipose-derived mesenchymal stem cells; *BDMSC*, bone marrow–derived mesenchymal stem cells; *HDF*, human dermal fibroblasts.

a reduction in cell numbers at day 5 and a decrease in DNA synthesis after 12 hours compared with myofibroblasts or adipose-derived mesenchymal stem cells in culture alone ($p < 0.05$). Similarly, co-cultures of bone marrow–derived mesenchymal stem cells with myofibroblasts demonstrated a decrease in cell number ($p < 0.05$) and a corresponding decrease in DNA synthesis, whereas co-cultures of myofibroblasts with control human dermal fibroblasts demonstrated no difference in cell proliferation. Testing proliferation of co-cultures in adherent collagen matrices yielded similar results compared with monolayer (Fig. 2).

Conditioned Medium from Adipose-Derived Stem Cells and Myofibroblast Co-cultures Leads to Reduced Dupuytren's Myofibroblast Proliferation

First, we tested the effect of conditioned medium of adipose-derived mesenchymal stem cells or myofibroblasts to examine the effect of key soluble factors secreted by these cells when cultured alone, having no contact with each other. Conditioned medium taken from adipose-derived mesenchymal stem cells led to decreased myofibroblast cell number at day 5 (83 percent; $p < 0.05$), whereas conditioned medium taken from myofibroblast cultures had no effect (104 percent; $p = 0.93$) (Fig. 3). Next, we tested whether myofibroblasts influenced the production of soluble inhibitory factors by adipose-derived mesenchymal stem cells in an indirect fashion, in a situation when there would be no direct cell-to-cell contact between these cells. We cultured adipose-derived mesenchymal stem cells and myofibroblasts in a Transwell chamber, separating these cells by a 0.8-μm membrane, allowing communication only by means of culture media. Conditioned medium taken from indirect contact co-cultures also led to decreased myofibroblast cell number at day 5 (73 percent; $p < 0.001$) (Fig. 3). Subsequently, we tested whether myofibroblasts influenced the production of soluble inhibitory factors by adipose-derived mesenchymal stem cells in a direct fashion, in a situation when adipose-derived mesenchymal stem cells would be in direct contact

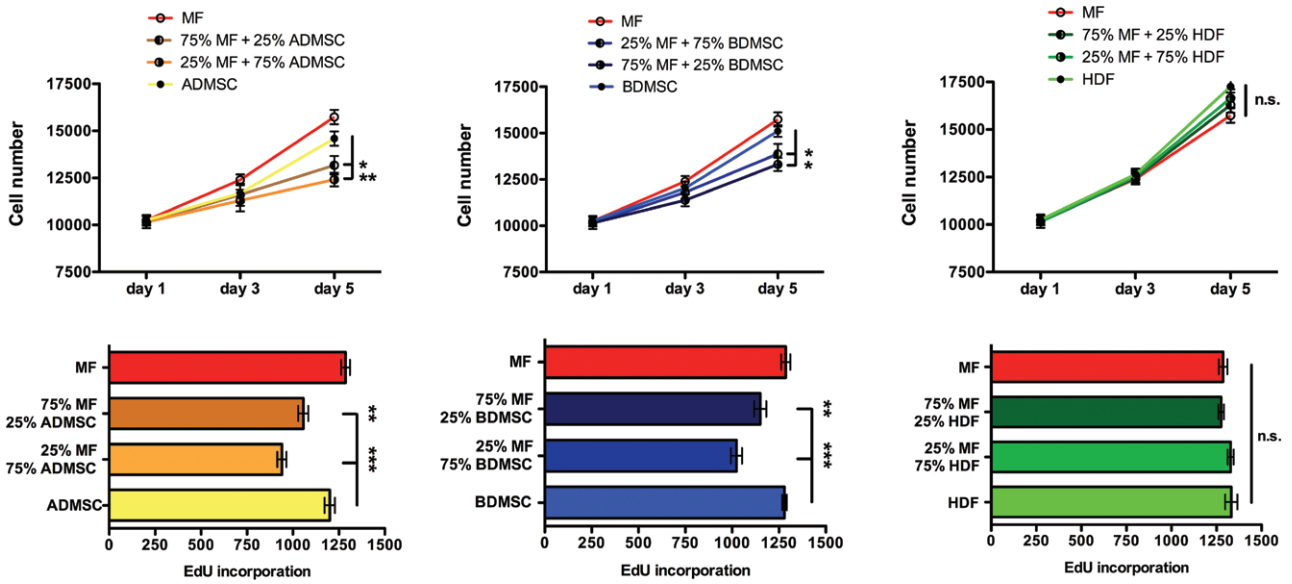

Fig. 2. Stem cells inhibit proliferation of Dupuytren's myofibroblasts. (*Left*) Co-cultures of Dupuytren's myofibroblasts with adipose-derived mesenchymal stem cells were cultured up to 5 days and stained with Hoechst dye to quantify DNA content. Co-cultures of myofibroblasts with adipose-derived mesenchymal stem cells demonstrated a significant decrease in cell number compared with either myofibroblasts or adipose-derived mesenchymal stem cells. Myofibroblasts co-cultured with adipose-derived mesenchymal stem cells demonstrated a corresponding significant decrease in DNA synthesis compared with respective cell types alone, evaluated by 5-ethynyl-2′-deoxyuridine incorporation. (*Center*) Similarly, co-cultures of Dupuytren's myofibroblasts with bone marrow–derived mesenchymal stem cells demonstrated a significant decrease in cell number and DNA synthesis compared with respective cell types alone. (*Right*) Co-cultures of Dupuytren's myofibroblasts with human dermal fibroblasts demonstrated no difference in cell number or DNA synthesis compared with respective cell types alone. Data are shown as the mean ± SEM from three patients (each assay was performed in triplicate) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). *MF*, myofibroblasts; *ADMSC*, adipose-derived mesenchymal stem cells; *BDMSC* , human bone marrow–derived mesenchymal stem cells; *HDF*, human dermal fibroblasts; *EdU*, 5-ethynyl-2′-deoxyuridine.

with myofibroblasts. The production of soluble inhibitory factors by direct cell-to-cell cultures led to decreased myofibroblast cell number at day 5 (69 percent; $p < 0.001$) and a significant decrease compared with conditioned medium from adipose-derived mesenchymal stem cells of noncontact cultures ($p < 0.05$) (Fig. 4).

DISCUSSION

This study shows that adipose-derived mesenchymal stem cells release soluble factors through cell-to-cell contact–dependent signaling to inhibit Dupuytren's myofibroblast proliferation. Furthermore, using the culture force monitor as a functional outcome, we showed that adipose-derived mesenchymal stem cells reduce myofibroblast contractility, with a corresponding reduction of α-smooth muscle actin protein expression, probably through a dilution effect. Myofibroblasts are the primary cells that contribute to the pathologic processes leading to fibrosis and digital contractures in Dupuytren's disease.[27] Therefore, our findings represent a potential translational strategy using adipose-derived mesenchymal stem cells in the treatment of Dupuytren's disease. We also demonstrate that mesenchymal stem cells isolated from different tissues have similar characteristics, but they can exhibit differential effects on myofibroblast contractility, and thus the source of mesenchymal stem cells should be considered when planning their use in the clinical setting.[28,29] There is no animal model for Dupuytren's disease, and in vivo conditions are most reliably examined by studying myofibroblasts in three-dimensional collagen lattices under isometric tension.[24] Myofibroblasts maintain their phenotype only under stress, and loss of tension is associated with disassembly of α-smooth muscle actin stress fibers.[30] The substrate stiffness of both three-dimensional adherent matrices and monolayer cultures exerts effects on cell differentiation on the cell types used, and in our system, cells exhibited a similar contractile myofibroblast phenotype under these conditions. Therefore, all of our experiments were performed under these conditions to ensure that the culture conditions would similarly influence cell-to-cell and cell-to-matrix adhesion formation, and also cell differentiation.[31,32]

The culture force monitor permits real-time force quantification in three-dimensional collagen lattices under isometric tension. Using this measure of contractility and associated α-smooth muscle actin gene and protein expression, we found that myofibroblasts co-cultured with adipose-derived mesenchymal stem cells and human dermal fibroblasts led to a dose-dependent decrease in the contractile myofibroblast phenotype. This dose-dependent decrease is most likely attributable to serial dilution of myofibroblasts and, in turn, reduced overall force contraction.[33] In direct contrast to our findings with adipose-derived mesenchymal stem cells, we found a dose-dependent increase in the myofibroblast phenotype when bone marrow–derived mesenchymal stem cells were co-cultured with myofibroblasts. Sarraf et al.[34] showed a comparable contractility of bone marrow–derived mesenchymal stem cells, although these data were from mouse-derived cells examined in the culture force monitor. Other authors have also shown lower contractility of bone marrow–derived mesenchymal stem cells,[35] although these studies used cells up to passage 10, which is recognized to have a negative influence on cell contractility.[33] It is worth noting that our co-culture model experiments were performed over only 24 hours in the culture force monitor and therefore are not truly representative of the processes involved in vivo, as progression of Dupuytren's disease may evolve over several months or years. To further characterize the interaction between mesenchymal stem cells and myofibroblasts, we tested whether stem cells inhibited myofibroblast proliferation. Mesenchymal stem cells have been shown to inhibit proliferation in various tumor and nontumor cells, including hematopoietic cancers and in cardiac and renal fibrosis.[11,13,36,37] In line with this, we found a decrease in proliferation of Dupuytren's myofibroblasts with both adipose-derived mesenchymal stem cells and bone marrow–derived mesenchymal stem cells when the cells were cultured in direct contact.

Several lines of evidence suggest that mesenchymal stem cells may in part mediate their inhibitory effects on proliferation by paracrine mechanisms. We examined whether proliferation of myofibroblasts was inhibited in response to soluble factors produced by adipose-derived mesenchymal stem cells, conditioned medium from adipose-derived mesenchymal stem cells co-cultured with myofibroblasts separated by either a Transwell insert to prevent cell-to-cell contact, or by allowing direct adipose-derived mesenchymal stem cells and myofibroblast cell-to-cell contact. We found that, under all conditions, Dupuytren's myofibroblast proliferation was inhibited and that, using conditioned medium from adipose-derived mesenchymal stem cells, similar inhibitory results were seen as have been reported in the literature using conditioned medium from bone marrow–derived mesenchymal stem cells and cultured with cardiac fibroblasts.[11] However, the greatest inhibition of proliferation

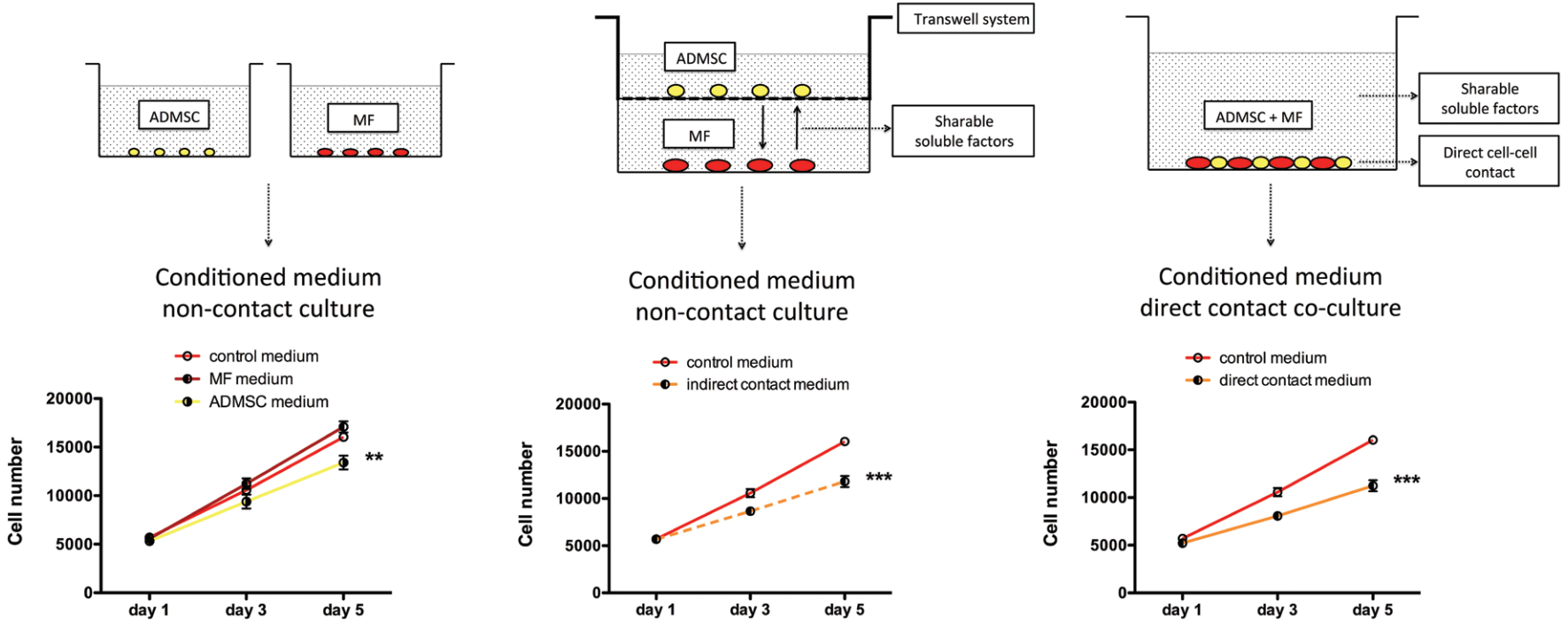

Fig. 3. Conditioned medium of adipose-derived mesenchymal stem cells and adipose-derived mesenchymal stem cells and myofibroblast co-cultures inhibited Dupuytren's myofibroblast proliferation. (*Left*) Myofibroblasts were cultured in monolayer up to 5 days treated with conditioned medium from noncontact cultures of either adipose-derived mesenchymal stem cells or myofibroblasts, and stained with Hoechst dye. Conditioned medium from adipose-derived mesenchymal stem cells led to a decreased myofibroblasts cell number, whereas conditioned medium from myofibroblasts had no effect on cell number. (*Center*) Conditioned medium from indirect contact co-cultures in a Transwell chamber with 50 percent adipose-derived mesenchymal stem cells and 50 percent myofibroblasts led to significantly decreased myofibroblast cell number. (*Right*) Similarly, conditioned medium from direct contact co-cultures of 50 percent adipose-derived mesenchymal stem cells and 50 percent myofibroblasts resulted in a significantly decreased myofibroblasts cell number. Data are shown as the mean ± SEM from three patients (each assay was performed in triplicate)($^{**}p < 0.01$, $^{***}p < 0.001$). *MF*, myofibroblasts; *ADMSC*, adipose-derived mesenchymal stem cells.

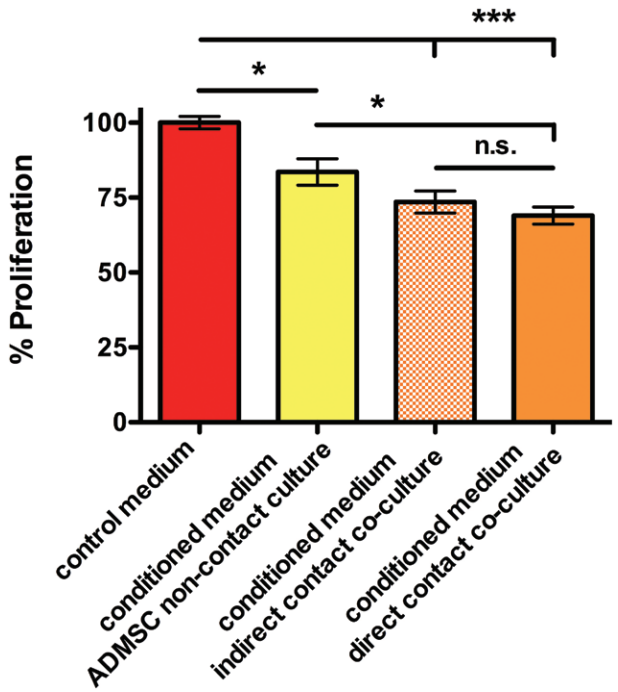

Fig. 4. Adipose-derived stem cells inhibit myofibroblast proliferation by soluble factors, influenced by cell-cell contact-dependent signaling. Conditioned medium from direct contact co-cultures in a Transwell chamber with 50 percent adipose-derived mesenchymal stem cells and 50 percent myofibroblasts led to a significantly decreased myofibroblast cell number compared with conditioned medium from noncontact cultures of adipose-derived mesenchymal stem cells. Data are shown as the mean ± SEM from three patients (each assay was performed in triplicate) (*$p < 0.05$, ***$p < 0.001$). *ADMSC*, adipose-derived mesenchymal stem cells.

was seen when direct cell-to-cell contact was permitted with co-cultures of adipose-derived mesenchymal stem cells and Dupuytren's myofibroblasts. This greatest inhibition of proliferation with direct cell-to-cell contact is also reported in the literature using bone marrow–derived mesenchymal stem cells cultured with T-cell leukemia, colon adenocarcinoma, and small-cell lung cancer cell lines.[3]

In Dupuytren's disease, localized inflammation has been shown to contribute to development and progression of the disease, and cells derived from Dupuytren's tissue produce an appreciable amount of these proinflammatory cytokines.[38–40] In a condition related to Dupuytren's disease, injections of adipose-derived mesenchymal stem cells in an animal model of Peyronie disease led to the prevention of fibrosis at the early inflammatory stage, with down-regulation of collagen III protein expression.[8] Similarly, mesenchymal stem cells have been shown to down-regulate inflammatory cytokines and reduce α-smooth muscle actin protein expression in an animal model of renal fibrosis.[36] Therefore, the beneficial antiinflammatory and antifibrotic effects suggest a potential role for adipose-derived stem cells in the prevention of progression of fibrosis in Dupuytren's disease. It is likely that following extensive percutaneous needle aponeurotomy, disruption of the affected tissue will lead to stress relaxation and subsequent loss of tension. Loss of tension is associated with disassembly of α-smooth muscle actin stress fibers and in turn may result in apoptosis.[30] Although aggregates of myofibroblasts remain, the injection of lipoaspirates containing adipose-derived mesenchymal stem cells results in a reduction of density of contractile myofibroblasts. Furthermore, the adipose-derived mesenchymal stem cells reduce the recruitment of additional myofibroblasts by inhibiting their proliferation.

These findings are consistent with and are the first to support the hypothesis that adipose-derived stem cells represent a potential translational strategy in the treatment of Dupuytren's disease, whereby adipose-derived stem cells down-regulate contractile myofibroblasts. This lends support to the potential benefit of lipografting in conjunction with percutaneous needle aponeurotomy in the treatment of Dupuytren's disease.

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