Review

# Lipotransfer for radiation-induced skin fibrosis

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**Background:** **Radiation-induced fibrosis (RIF) is a late complication of radiotherapy that results in progressive functional and cosmetic impairment. Autologous fat has emerged as an option for soft tissue reconstruction. There are also sporadic reports suggesting regression of fibrosis following regional lipotransfer. This systematic review aimed to identify cellular mechanisms driving RIF, and the potential role of lipotransfer in attenuating these processes.**

**Methods:** **PubMed, OVID and Google Scholar databases were searched to identify all original articles regarding lipotransfer for RIF. All articles describing irradiated fibroblast or myofibroblast behaviour were included. Data elucidating the mechanisms of RIF, role of lipotransfer in RIF and methods to quantify fibrosis were extracted.**

**Results:** **Ninety-eight studies met the inclusion criteria. A single, definitive model of RIF is yet to be established, but four cellular mechanisms were identified through *in vitro* studies. Twenty-one studies identified connective tissue growth factor and transforming growth factor β1 cytokines as drivers of fibrotic cascades. Hypoxia was demonstrated to propagate fibrogenesis in three studies. Oxidative stress from the release of reactive oxygen species and free radicals was also linked to RIF in 11 studies. Purified autologous fat grafts contain cellular and non-cellular properties that potentially interact with these processes. Six methods for quantifying fibrotic changes were evaluated including durometry, ultrasound shear wave elastography, thermography, dark field imaging, and laser Doppler and laser speckle flowmetry.**

**Conclusion:** **Understanding how lipotransfer causes regression of RIF remains unclear; there are a number of new hypotheses for future research.**

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## Introduction

Radiation-induced fibrosis (RIF) is a common late complication of radiotherapy that results in progressive functional and cosmetic impairment. Half of patients with cancer receive regional radiotherapy as part of their management, in addition to surgery and chemotherapy[1]. RIF can affect activities of daily living secondary to tissue scarring, induration and contracture, diminishing quality of life[2]. As cancer survival improves, an increased number of patients will suffer from the debilitating sequelae of RIF[3]. New insights into the genetic susceptibility to RIF are emerging[4,5]. Corrective surgical procedures are limited by the friability and poor healing of irradiated tissue[6].

Purified autologous fat has been used as a filler to contour deformities for several years. Coleman and colleagues devised the method used for most reconstructive purposes today[7–9] (*Fig. 1*). A number of papers have described regression of RIF following autologous lipotransfer[10–13]. Rigotti and colleagues[10] demonstrated improved symptom scores after lipotransfer, in addition to visible improvement in fibrosis. Transmission electron microscopy of subcutaneous tissue biopsies showed well vascularized and hydrated tissue, mature adipocytes and total regression of fibrosis at 1 year after lipotransfer. These findings were attributed to adipose-derived multipotent stromal cells, which were isolated from lipoaspirates[10]. Other authors[11,12] noted similar results.

The underlying mechanism for improvements in both function and quality of fibrotic skin after lipotransfer remains unknown. Characterizing the cellular influence of lipotransfer would allow the isolation of key factors that could be targeted.

This review explores current understanding of cellular mechanisms underlying RIF, discusses the possible role

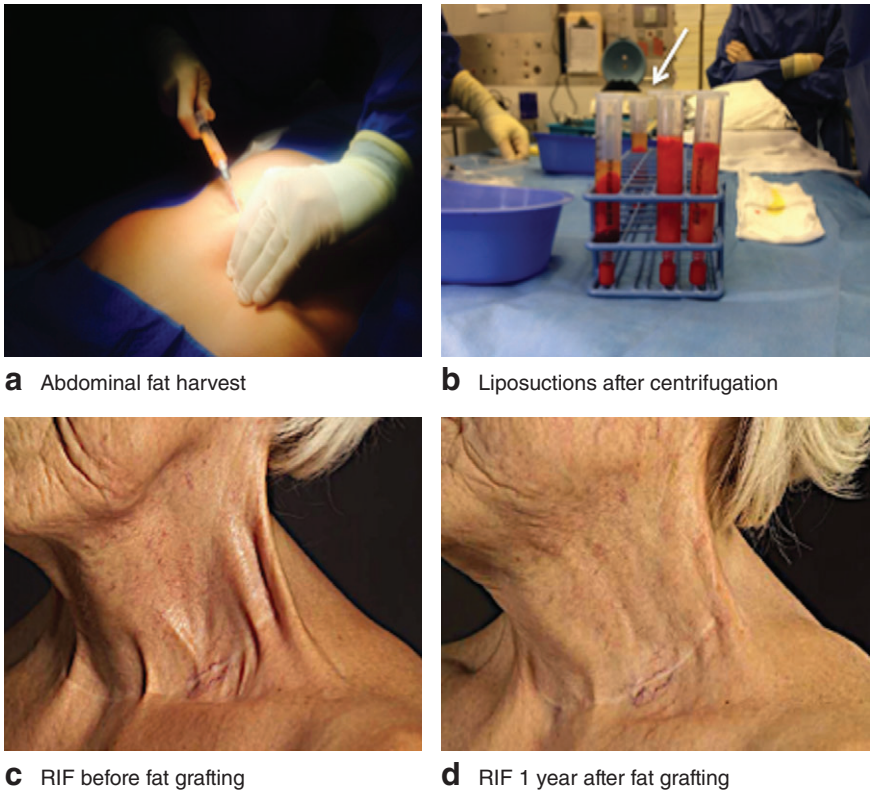


**Fig. 1** Coleman's technique. **a** Abdominal liposuction using a 3-mm Coleman cannula and Luer Lock syringe. **b** Products of centrifugation; centrifugation of the lipoaspirate yields three layers, with the purified fat forming the central layer. **c** Example of radiation-induced fibrosis (RIF) of the neck following modified neck dissection and adjuvant radiotherapy. **d** One year after three sessions of fat grafting, resulting in softer skin and restoration of the subcutaneous plane

of lipotransfer in attenuating these processes, and proposes areas for further investigation. In addition, available methods for quantifying fibrosis are reviewed in order to promote the use of objective data collection in research.

## Methods

A literature search was performed using PubMed, OVID and Google Scholar. Words used for the search included 'radiation fibrosis', 'fat grafts', 'cellular mechanisms', 'irradiated fibroblasts', 'breast', 'head and neck'. Reference lists of articles identified were examined to identify further relevant studies. Studies written in languages other than English were excluded but relevant data were used if the abstract was in English. Other exclusion criteria were: not relevant to lipotransfer and radiation, and review articles. Papers included were those published before 10 March 2015. Papers describing irradiated fibroblast or myofibroblast behaviour were included. Studies describing the behaviour of myofibroblasts in other fibrotic diseases were also considered. Finally, studies describing instruments for the quantification of tissue changes in radiation fibrosis were analysed. Initial exclusions were based on the article title; final exclusion was based on full-text review.

## Results of literature search

A total of 374 abstracts were identified from the database search (*Fig. 2*). The title and abstract of articles were screened for relevance. After excluding 276 studies, 98 papers published in English were ultimately reviewed, of which 28 were excluded. Four themes were identified in the studies, which explored current understanding of cellular mechanisms underlying RIF.

## Cellular mechanisms of radiation-induced fibrosis

Fibrosis is a complex pathological process involving multiple cellular and non-cellular factors, which mediate an aberrant healing process following ionizing injury. A single, definitive model is yet to be established, but key

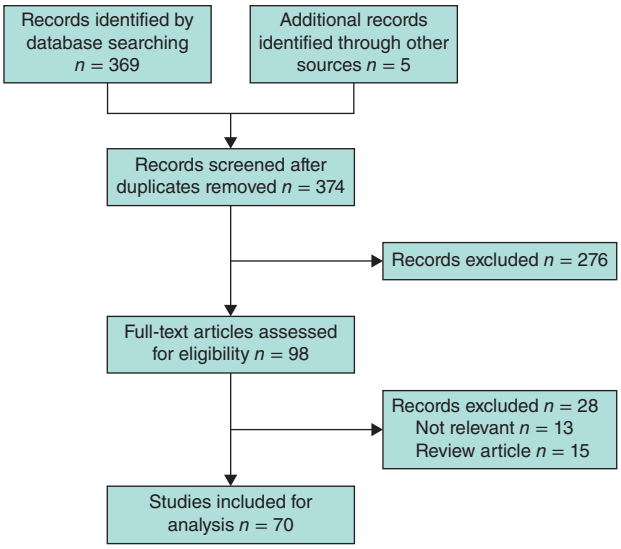


**Fig. 2** PRISMA diagram showing selection of studies for analysis

events in the pathogenesis of RIF are well described in the literature. Other fibrotic disease processes have unique and intricate features, but these likely converge on common pathways with RIF. Exploring this nexus of molecular and cellular interactions is essential to generate hypotheses for the role of lipotransfer in cutaneous disease regression (*Fig. 3*).

Morphologically, RIF involves progressive interstitial and microvascular deposition of extracellular matrix (ECM) proteins[14]. Cytokine-mediated activation and proliferation of myofibroblasts plays a crucial role in fibrogenesis. Key cytokines include transforming growth factor (TGF) β1 and connective tissue growth factor (CTGF).

Myofibroblasts have heterogeneous origins, but the most abundant and well described progenitor cell is the dermal fibroblast[15]. Quiescent fibroblasts produce few proteins and interact minimally with neighbouring cells or the native ECM. However, on exposure to fibrogenic cytokines, they differentiate into myofibroblasts, expressing contractile proteins that promote cell–cell and cell–matrix anchoring[15]; this process is known as activation. Cell–matrix adhesion protein morphology ranges from dot-like focal complexes to elaborate supermature focal adhesions composed of diverse contractile fibres[16]. Expression of α-smooth muscle actin (α-SMA), a component of anchorage molecules, is specific to myofibroblasts, and increasing concentrations of this protein are associated with promotion of focal adhesion formation[16]. Mechanotransductive forces transmitted through the cytoskeleton via these adhesion proteins likely play an important role in establishing the myofibroblast phenotype[16–18].

## Transforming growth factor β1 in radiation-induced fibrosis

Several signalling pathways have been linked to myofibroblast progenitor activation in the literature, but TGF-β1-driven cascades appear to be chief. TGF-β1 is a protein bound in a latent form to the native ECM, and is released acutely by radiation-mediated oxidative cleavage of binding motifs[19]. Functional TGF-β1 binds to specific receptors on progenitor cell membranes that signal the production of Smad signalling proteins, which are intracellular transducers of TGF-β-dependent gene expression[20] (*Fig. 4*). In terms of fibrosis, the integral role of Smad3 intermediary signals was demonstrated *in vitro* by blocking its expression within dermal fibroblasts, inhibiting the TGF-β1 signalling pathway[21]. This was reflected in an *in vivo* study by Lakos and colleagues[22], who reported attenuation of skin fibrosis in a Smad3-null murine model of scleroderma. An alternative route for TGF-β1-based progenitor activation has also been described: the p38 mitogen-activating protein kinase signalling route, which is well characterized with dermal fibroblasts[23]. This is an intracellular signalling molecule that transduces TGF-β1-mediated collagen expression. A pathological role for this molecular pathway is yet to be linked to RIF.

Further targets of TGF-β1 have been identified in other fibrotic cutaneous diseases. Notably, activation of dermal fibroblast RhoA GTPase by this cytokine is a key process in the pathogenesis of scleroderma[24]. This molecule plays a role in regulating the organization of actin and myosin within the cytoskeleton, via downstream effectors termed Rho-associated kinases (Rocks)[25]. Akhmetshina and co-workers[24] demonstrated that fibroblasts differentiate into myofibroblasts in response to Rock upregulation. Correspondingly, an *in vitro* analysis of scleroderma myofibroblasts revealed a reduction in ECM protein gene expression on inhibition of the Rock pathway[24] (*Fig. S1*, supporting information). Similar findings were reported with intestinal smooth muscle cells isolated from patients afflicted by radiation fibrosis in the intestinal mucosa, suggesting that Rock plays a role in the pathogenesis of RIF[26]. However, it is yet to be characterized in irradiated skin, and remains an area for further research[26].

CTGF, a fundamental fibrogenic protein, is another downstream target of TGF-β1 that is featured in scleroderma and radiation enteritis[27,28]. In these disease processes, it promotes fibroblast activation via a Smad3-independent mechanism, linked instead to the TGF-β1–Rho–Rock pathway[28]. There is a paucity of evidence demonstrating this with irradiated dermal fibroblasts, although CTGF has been shown to act synergistically with TGF-β1 to induce skin fibrosis in a murine

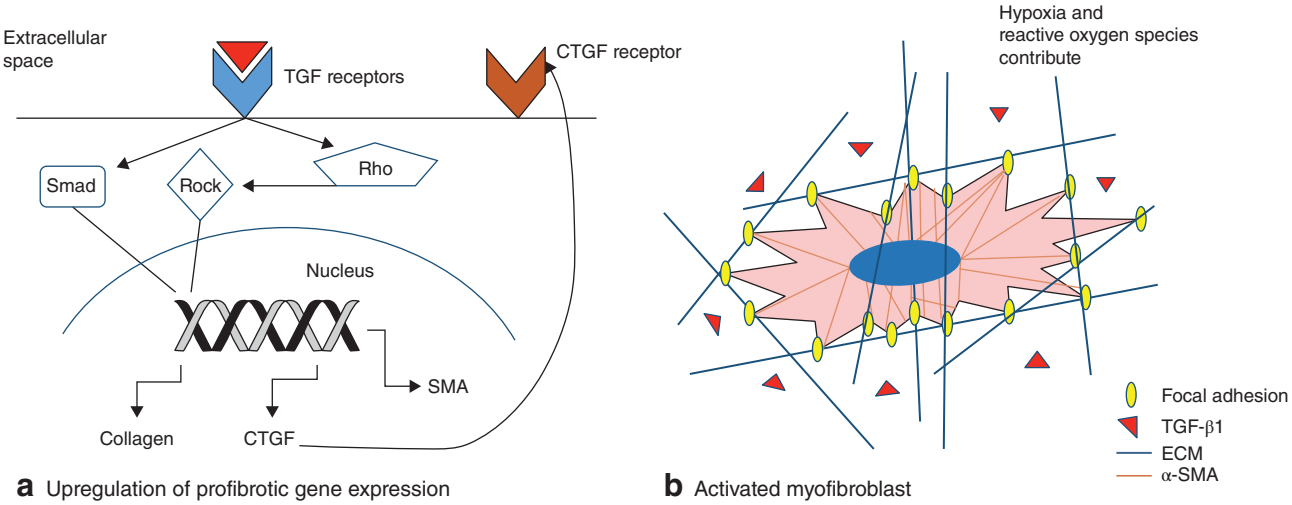


**Fig. 3** Overview of fibroblast to myofibroblast differentiation: **a** common products and pathways in transforming growth factor (TGF) β-mediated profibrotic gene expression; **b** schematic of an activated myofibroblast showing interaction between contractile α-smooth muscle actin (SMA), focal adhesions and extracellular matrix (ECM). CTGF, connective tissue growth factor; Rock, Rho-associated kinase

model[29]. CTGF is particularly significant as it can stimulate its own expression via an autocrine positive feedback loop[28]. Consequently, in the chronic setting, where TGF-β1 levels may be diminished, CTGF levels can remain raised, autonomously maintaining the pathological fibrotic process[28].

## Tumour necrosis factor α and platelet-derived growth factor in radiation-induced fibrosis

Preclinical models have generated some evidence for the potential roles of platelet-derived growth factor (PDGF) and tumour necrosis factor (TNF) α in RIF. Abdollahi and co-workers[30] found that PDGF expression was increased following irradiation of lung tissue in murine specimens, and therapeutic targeting of PDGF resulted in attenuation of RIF. PDGF has been identified as a major cytokine mediating the pathogenesis of scleroderma[31]. Notably, TGF-β1 upregulates PDGF activity in scleroderma fibroblasts, but not in healthy fibroblasts[32]. The specific role that PDGF plays in RIF requires further investigation.

Nawroth and co-workers[33] also used a murine model, and showed that intraperitoneal administration of TNF-α-targeting nanoparticles prevented RIF. Recent data using a three-dimensional model of Dupuytren's disease have revealed that TNF-α can induce a myofibroblast phenotype and upregulate profibrotic gene expression in affected palmar dermal fibroblasts, via the Wnt/β-catenin signalling pathway[34]. Non-palmar fibroblasts from patients did not replicate this phenomenon, and the mechanism for this is undetermined. Wnt/β-catenin pathways are also activated in irradiated fibroblasts[35]. These studies may represent an as yet undescribed molecular network in the pathogenesis of RIF.

## Hypoxia and oxidative stress in radiation-induced fibrosis

Vasculitis is a common feature of radiation injury and also participates in propagation of fibrogenesis[36]. Luminal narrowing occurs owing to intravascular and perivascular matrix deposition, resulting in hypoxia and ischaemia[37]. Chronic hypoxia has been shown to induce ECM protein synthesis *in vitro* using scleroderma-sourced fibroblasts, promoted by a hypoxia-inducible factor 1α pathway. However, gene upregulation also occurred independently, suggesting a pleiotropic effect[38].

Oxidative tissue stress results from the release of reactive oxygen species (ROS) and free radicals following irradiation, due mainly to ionization of water molecules[39,40]. Alterations in DNA methylation mediated by these products of ionizing radiation result in chronic epigenetic changes, compounded by direct damage to DNA base pairs[41]. The relationship of these processes to RIF pathogenesis remains to be fully elucidated, but numerous reports of reduction of RIF with antioxidant therapy are available in the literature[42–48].

Individually targeting hypoxia and oxidative stress has yielded suboptimal results and questionable therapeutic benefit[44]. However, combination therapy with

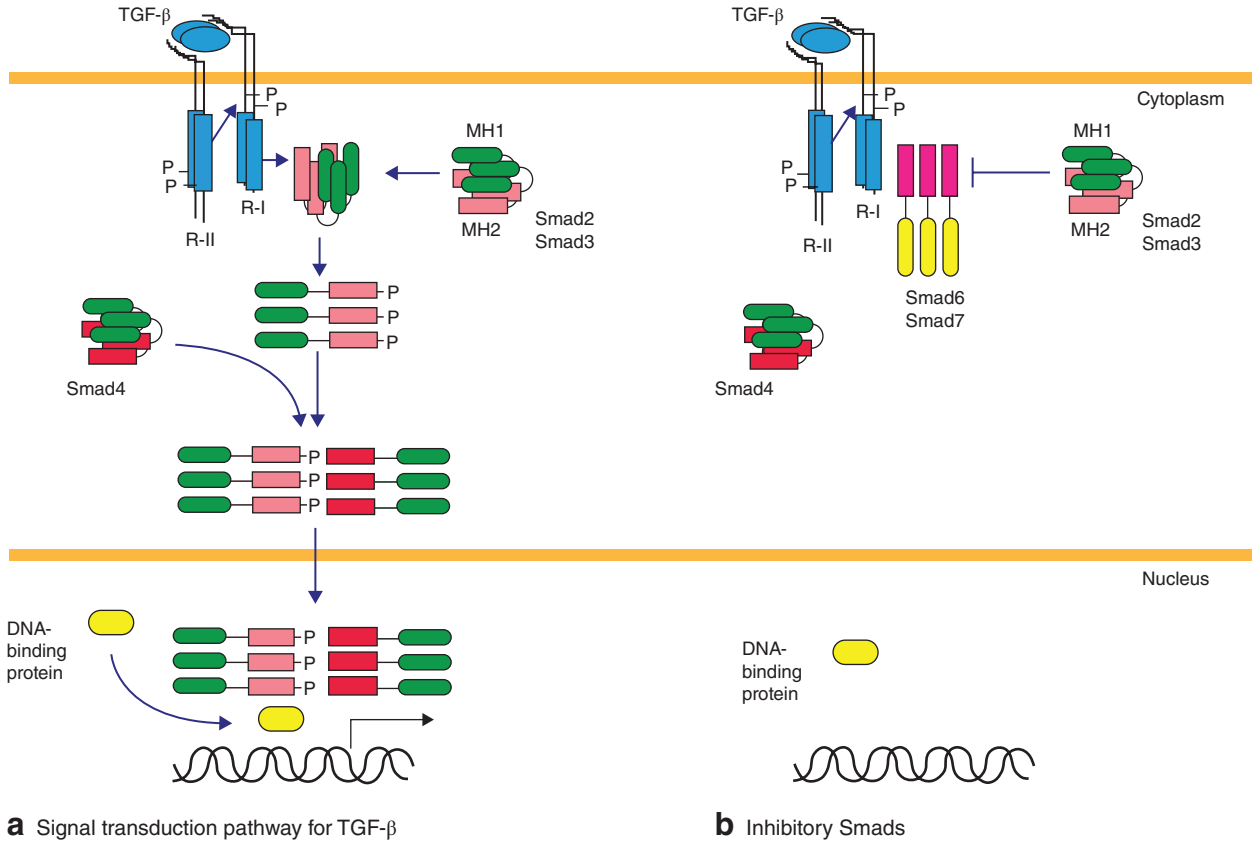


**Fig. 4** Transforming growth factor (TGF)/Smad signalling. **a** A hypothetical signal transduction pathway for TGF-β. TGF-β binding leads to the assembly of a receptor complex in which the type II receptor (R-II) phosphorylates and activates the type I receptor (R-I). Pathway-restricted Smads (Smad2 and Smad3), which may be anchored in the cytoplasm, are phosphorylated, which leads to heteromerization with Smad4, a common mediator Smad. The Smad complex is then translocated to the nucleus, where it binds to DNA directly or in complex with other components and affects transcription of specific genes. **b** Inhibitory Smads (Smad6 and Smad7) bind to the receptors, and prevent the phosphorylation and signalling activity of pathway-restricted Smads. MH1 and MH2 represent domains for binding DNA and forming complexes with other Smads. Reproduced with permission from Heldin and colleagues[20]

pentoxifylline and tocopherol (vitamin E) has significantly reversed RIF in animal studies, as well as in one double-blind placebo-controlled clinical trial[45,46]. Pentoxifylline is a methylxanthine derivative that increases blood flow and has antifibroblast properties, whereas tocopherol is an antioxidant that combats ROS activity[47,48]. *In vitro* studies using irradiated dermal fibroblasts showed a reduction in ROS generation and DNA damage with this treatment[49].

## Quantifying fibrosis

Clinical scoring systems are commonly used to study RIF. They provide data based on clinical examination, which is cost-effective and easy to perform. Grading of fibrosis usually uses ordinal Likert scales to generate semiquantitative scores. Davis and colleagues[50] evaluated the interobserver reliability of commonly used systems using Landis and Koch's κ statistic, and reported marked variability in scoring between experienced clinicians, highlighting a disadvantage in using these subjective techniques to determine the response of RIF to therapeutic interventions[50,51]. Quantifiable measures of fibrosis could circumvent these issues, but they are rarely used.

Some methods for quantifying fibrotic regions, such as MRI[52] or CT[53], are expensive and time-consuming, precluding their use for research. Other instruments may not be validated in patients, and safety concerns disqualify their application.

The following sections describe non-invasive, affordable, portable and safe techniques that can be implemented easily for research (and possibly clinical) purposes. They focus on quantifying the mechanical properties of fibrotic skin and alterations in the regional microcirculation. The

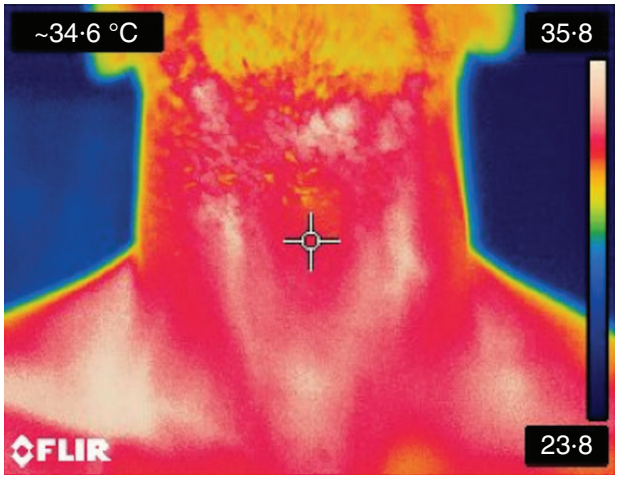


**Fig. 5** Thermography image of a healthy volunteer, taken with a FLIR® thermal camera. Targeted areas can be selected using the FLIR® software to generate/quantify regional temperatures. The detectable temperature range (right of image) can be adjusted to suit individual requirements

authors' centre deals mainly with RIF in the head and neck, but these modalities are applicable elsewhere in the body.

## Quantifying skin biomechanics

*Durometry*

A durometer is a hand-held probe that uses indentation force to measure skin hardness[54]. More sophisticated durometers are equipped with a transducer, connected to a computer, and use ultrasound to generate results[54]. The probe is applied to a desired area, where it indents the tissue. The degree of tissue displacement can be rendered as a function of the applied force. The resulting force–indentation curve is used to calculate Young's modulus of the tissue, a measure of the stiffness. Simpler devices use digital gauges to convert indentation force into standard units of measurement.

Durometry has been used to quantify tissue changes in scleroderma, fat grafting for contractures and burns[55–57]. Leung and colleagues[58] and Zheng *et al.*[59] separately studied durometry in neck fibrosis, and demonstrated a marked increase in tissue modulus compared with normal controls. Although the latter reported a 15·2 per cent rate of interobserver variability, this might improve with operator experience. Indeed, a study by Merkel and co-workers[55] calculated an interobserver correlation coefficient of up to 0·92 using durometry in patients with scleroderma.

A key limitation of this technique is that data are dependent on the pressure at which the probe is applied, and standardization can prove difficult. Furthermore, the probe must be placed perpendicular to the area of interest while underlying musculature is relaxed. Selecting consistent areas for data collection in patients who have had radiotherapy for cancer is difficult, as individuals typically undergo a variety of surgical resections and reconstructions leading to loss of anatomical landmarks. Poor mobility secondary to RIF can also pose problems.

*Suction elasticity meters*

Suction-based measurement of skin elasticity can provide an alternative to indentation. The Cutometer® (MPA 580; Courage and Khazaka, Cologne, Germany) is a commercially available device that uses a negative pressure probe to apply a vacuum to an area of interest. Skin is drawn into the probe and released after a specific time. Within the probe, a light-based measuring system uses the disruption of transmitted light to generate graphical representations of resistance to suction and elasticity. Data are expressed in millimetres of deformation.

This technique has been used to study biomechanical properties of skin in healthy subjects[60], burns[61] and patients with scleroderma[62]. Chin and colleagues[63] evaluated the Cutometer® in the skin of irradiated necks, and reported reduced skin elasticity in 251 patients. Killaars and co-workers[64] used it to study the biomechanical changes of skin affected by breast cancer-related lymphoedema, reporting reduced elasticity in affected regions.

The limitations described for suction-based techniques are similar to those for durometry.

*Ultrasound shear wave elastography*

Ultrasound shear wave elastography (USWE) uses ultrasound to induce multiple mechanical waves within target tissue, and uses the speed of wave propagation to quantify the regional Young's modulus. Qualitative information is also provided via the generation of a tissue stiffness map. USWE has been shown to be useful for assessing liver fibrosis[65], and predicting malignancy risk in thyroid nodules[66] or discrete breast lesions[67–69]. Sowa *et al.*[70] studied the use of USWE for identification and quantification of fat induration following autologous flap breast reconstruction, highlighting its value in assessing diffuse tissue modulus. In the context of RIF, Kaluzny and co-workers[71] used this technology to quantify fibrosis in the major salivary glands. They confirmed that there was a statistically significant difference in tissue elastic modulus between irradiated and healthy skin. Although USWE has not yet been used to quantify skin fibrosis, its clinical success in other areas makes it worth evaluating further.

The main disadvantage of USWE is the requirement for operator experience in the technique, precluding easy use with minimal training.


**Fig. 6** Laser speckle contrast flowmetry of a healthy volunteer. Flux values representing blood flow can be detected within defined areas of interest. A colour map or flux image can be generated for accompanying qualitative data

## Quantifying microcirculatory changes

### *Thermography*

Thermal imaging has been correlated with tissue perfusion in animal as well as human studies[72,73]. Schlager and colleagues[74] reported a statistically significant relationship between thermographic data and skin perfusion. Thermographic imaging detects electromagnetic radiation emitted from surfaces to generate a regional temperature map (*Fig. 5*).

The FLIR® thermal camera (http://www.flir.co.uk/home/) is a hand-held device that can be linked to computer software for image analysis. Preliminary, unpublished data collected in patients with RIF suggests that there are differences between fibrotic and healthy skin.

The main limitation of thermography is that the ambient temperature can compromise standardization of data collection. A temperature-controlled room is required for patients to acclimatize before images are taken.

### *Laser Doppler and laser speckle contrast flowmetry*

Laser Doppler flowmetry (LDF) and laser speckle contrast flowmetry (LSCF) are optical techniques that measure blood flow within the microcirculation non-invasively.

LDF technology has been used to quantify tissue perfusion since the 1980s[75]. Devices use coherent monochromatic light to illuminate target tissue, where the incident photons interact with red blood cells (RBCs) to create Doppler shifts. These are translated by computer software into a qualitative colour map, and a mean quantitative perfusion value within defined areas of interest. LDF is used clinically in burn assessment and healing[76,77]. Leutenegger and co-workers[78] demonstrated its value in real-time assessment of flow through the dermal microcirculation, as well as in skin flaps following burn reconstruction. Single-point LDF probes are limited by small measurement fields that do not permit convenient evaluation of the heterogeneous dermal microcirculation, compromising interobserver reproducibility owing to spatial differences in perfusion values[79]. Newer LDF technology permits video imaging with a wider field, minimizing this limitation.

LSCF involves a similar technology that also uses coherent light illumination of tissue. Light scattered by movement of RBCs forms an interference pattern with varying intensities in space, termed a speckle. Fluctuations in the rate and spatial orientation of blood flow cause blurring of the speckle pattern. These variable patterns are received by a photodetector, and the degree of blurring in different regions can be quantified to generate perfusion values (*Fig. 6*)[80]. Increased blood flow results in greater blurring of the speckle pattern. LCSF has a wide detection field, and data may be more reproducible than those from LDF[81].

Neither technique has yet enjoyed widespread use in the assessment of RIF anywhere in the body.

### *Sidestream dark field imaging*

Sidestream dark field imaging (SDFI) is a relatively new tool in microcirculatory research that permits live visualization of RBCs as they move through capillaries[82]. The device comprises a hand-held probe with concentrically arranged light-emitting diodes (LEDs) that emit photons at a wavelength of 530 nm, which are selectively absorbed by RBCs. A central light, isolated from the LEDs, provides tissue illumination via photon scattering.

Together, an image can be produced in which RBCs appear as mobile dark objects within grey blood vessels, on a lighter tissue background.

In humans, this technology has been used primarily in sites covered by mucosa (sublingual and buccal)[83,84]. Precursor devices have been used *in vivo* to assess skin flap viability in animal models[85]. It is yet to be used in fibrotic, indurated skin, and would need to be trialled before it could enjoy wider use in RIF research. It is likely that an increase in the depth of penetration of incident light will be required to monitor cutaneous microcirculatory changes. A possible use of SDFI could be to monitor perfusion following therapy for intraoral carcinoma.

Another limitation is that largely semiquantitative data have been reported in the literature up to now. De Backer and colleagues[86] developed scoring systems based on observer assessment of blood flow through vessels in captured videos. Perfusion is characterized as present, absent or intermittent in these scoring systems, and true RBC flow velocity is not provided. If software is developed that can reliably calculate RBC flow velocity through selected vessels, SDFI could represent a novel technique for quantification of microcirculatory changes in RIF before and after lipotransfer.

### Overview

The optimal method for monitoring RIF is still under debate, and part of an ongoing investigation. The authors' current method of monitoring the effects of RIF involves a variety of techniques, including a physical examination to assess the range of movement, durometry to evaluate skin mechanics evaluation, quality-of-life questionnaires and LSCF for analysis of the microcirculation.

## Discussion

Adipose tissue is abundant in the human body and is harvested easily. Transplanted fat is biocompatible and integrates well with host tissue, thus exhibiting several qualities of an ideal filler[87]. Several pilot studies[10–13] have reported regression of cutaneous RIF following fat grafting, but the mechanisms underlying this phenomenon remain unknown.

An important next step in clinical research is to use objective methods of data collection to quantify alterations in tissue quality following lipotransfer. The efficacy of this treatment could thus be evaluated more reliably, and different treatment regimens compared. Different techniques of processing harvested adipose tissue may result in varying degrees of tissue rehabilitation when engrafted, and knowledge of the most effective method would improve clinical practice, and provide a better idea of components to investigate *in vitro*. Preliminary animal studies could also benefit from the use of the modalities outlined above. This would not only improve understanding of the effect of lipotransfer on irradiated tissue, but would act as an important bridge between laboratory-based and clinical research.

Several options are available to quantify skin fibrosis, but a standard assessment protocol is yet to be established. From a technical point of view, developing a reproducible method may be limited by the variable regional anatomy between patients after surgery. Each individual patient's tissue properties can be altered by coexisting pathology, such as lymphoedema. A range of techniques require evaluation in a large population of patients, in order to identify the most sensitive methods. It is likely that a combination of modalities will be required to quantify changes in cutaneous RIF. The studies evaluated here are somewhat limited by the lack of objective study design.

Learning more about how components of grafted fat affect the cellular mechanisms driving RIF is also essential to improve treatments. Current research has largely focused on adipose-derived multipotent stromal cells (ADMSCs), which have considerable plasticity and regenerative potential. These cells are found in the stromal vascular fraction of processed, centrifuged lipoaspirate, which can be used to enrich fat grafts with a higher concentration of ADMSCs[10,88]. ADMSCs are known to release a variety of cytokines, which elicit complex paracrine and autocrine effects, potentially interacting with the cellular processes described previously[89,90].

A favourable mechanism could be related to the release of proangiogenic factors, such as vascular endothelial growth factor and hepatocyte growth factor[89–91]. These could mitigate the chronic hypoxia associated with RIF by promoting tissue revascularization. An antioxidant effect protecting cells from ROS has also been attributed to ADMSCs[92].

Notably, the majority of the cytokines released by ADMSCs appear to be profibrotic factors: TGF-β1, CTGF and PDGF[93,94]. A homeostatic reaction that alters this cytokine profile within irradiated tissues could be an explanation for the paradoxical anti-RIF effect of these cells. No evidence yet exists for this. An alternative possibility is the existence of a cytokine-independent system.

Antifibrotic effects could result from a dilutional effect on abnormal myofibroblasts, restoring a cell population appropriate for normal healing. Verhoekx and colleagues[95] used ADMSCs on a population of Dupuytren's myofibroblasts in a three-dimensional collagen lattice model. They noted dose-dependent decreases in contractile force and α-SMA concentration at 24 h. When bone marrow-derived

multipotent stromal cells were co-cultured with myofibroblasts, the opposite effect occurred. ADMSC cultures exhibited no change in α-SMA mRNA expression, supporting a dilutional effect[95]. Cells were subsequently co-cultured within media in direct contact, or separated by an insert. In both cases, myofibroblast proliferation was inhibited, implying that a paracrine signalling process also plays a role[95]. Although this experiment provides new evidence for the benefit of fat grafting in Dupuytren's disease, not RIF, repeating this experiment with irradiated myofibroblasts could reproduce these findings. Research could then take a further step and characterize the specific antimyofibroblast molecules secreted by ADMSCs.

Following the development of a basic understanding of interactions between ADMSCs and pathological myofibroblasts, the putative role of other regional cells should be investigated, such as keratinocytes, which are known to modulate the fibroblast phenotype in other fibrotic diseases such as scleroderma[96].

Ultimately, the effects of engrafted fat may simply be due to its physical presence. By disrupting myofibroblast focal adhesion and reducing ECM stress, a graft may induce substantial apoptosis of pathological cells and promote normal dermal fibroblast survival, while restoring the integrity of subcutaneous tissue, thereby facilitating healing[97–99].

Understanding how autologous lipotransfer works remains challenging, owing to complexities of the underlying cellular mechanisms. Components of fat grafts likely influence irradiated myofibroblasts via cytokine-dependent and -independent processes, which may be elucidated by future *in vitro* research. Promoting the use of quantitative metrics to assess tissue fibrosis will permit objective analysis of treatment regimens, and help integrate laboratory-based and clinical research.

## Disclosure

The authors declare no conflict of interest.

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## Supporting information

Additional supporting information may be found in the online version of this article:

**Fig. S1** Prevention of differentiation of resting dermal fibroblasts into myofibroblasts in samples obtained from patients with systemic sclerosis, by inhibition of Rho-associated kinase signalling (Word document)