The systemic influence of chronic smoking on skin structure and mechanical function

Abigail K Langton1,2*, Evridiki Tsoureli-Nikita3, Holly Merrick1, Xuegen Zhao4, Christina Antoniou3, Alexander Stratigos3, Riaz Akhtar5, Brian Derby4, Michael J Sherratt6, Rachel EB Watson1,2 and Christopher EM Griffiths1,2†

1 Centre for Dermatology Research, The University of Manchester & Salford Royal NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
2 NIHR Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
3 First Department of Dermatology, Andreas Syggros Hospital of Cutaneous & Venereal Diseases, Athens University Medical School, Athens, Greece
4 School of Materials, The University of Manchester, Manchester, UK
5 Department of Mechanical, Materials and Aerospace Engineering, School of Engineering, University of Liverpool, Liverpool, UK
6 Division of Cell Matrix Biology & Regenerative Medicine, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK

*Correspondence to: AK Langton, NIHR Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, 1.726A Stopford Building, Oxford Road, Manchester M13 9PT, UK. E-mail: abigail.langton@manchester.ac.uk
†Joint senior authors.

Abstract

One of the major functions of human skin is to provide protection from the environment. Although we cannot entirely avoid, for example, sun exposure, it is likely that exposure to other environmental factors could affect cutaneous function. A number of studies have identified smoking as one such factor that leads to both facial wrinkle formation and a decline in skin function. In addition to the direct physical effects of tobacco smoke on skin, its inhalation has additional profound systemic effects for the smoker. The adverse effects on the respiratory and cardiovascular systems from smoking are well known. Central to the pathological changes associated with smoking is the elastic fibre, a key component of the extracellular matrices of lungs. In this study we examined the systemic effect of chronic smoking (>40 cigarettes/day; >5 years) on the histology of the cutaneous elastic fibre system, the nanostructure and mechanics of one of its key components, the fibrillin-rich microfibril, and the micromechanical stiffness of the dermis and epidermis. We show that photoprotected skin of chronic smokers exhibits significant remodelling of the elastic fibre network (both elastin and fibrillin-rich microfibrils) as compared to the skin of age- and sex-matched non-smokers. This remodelling is not associated with increased gelatinase activity (as identified by in situ zymography). Histological remodelling is accompanied by significant ultrastructural changes to extracted fibrillin-rich microfibrils. Finally, using scanning acoustic microscopy, we demonstrated that chronic smoking significantly increases the stiffness of both the dermis and the epidermis. Taken together, these data suggest an unappreciated systemic effect of chronic inhalation of tobacco smoke on the cutaneous elastic fibre network. Such changes may in part underlie the skin wrinkling and loss of skin elasticity associated with smoking.

© 2020 The Authors. The Journal of Pathology published by John Wiley & Sons Ltd on behalf of Pathological Society of Great Britain and Ireland.

Keywords: elastic fibres; elastin; fibrillin-rich microfibrils; skin; tobacco smoke exposure

Received 15 April 2020; Revised 15 May 2020; Accepted 20 May 2020

No conflicts of interest were declared. Although supported in part by a programme grant from industry, funders had no editorial control over this manuscript.

Introduction

One of the major functions of human skin is to provide protection from the environment, a major component of which is sun exposure. Chronic ultraviolet radiation (UVR) in sunlight is known to result in profound changes to both the collagenous and elastic dermal matrices, resulting in a specific clinical phenotype termed photoageing [1]. Although we cannot entirely avoid sun exposure, exposure to other environmental factors, including those where personal choice plays a role, may influence cutaneous function. A number of studies have identified smoking as one such factor [2–5]; these epidemiological studies identify smoking as an independent risk factor for the occurrence of facial wrinkles.

© 2020 The Authors. The Journal of Pathology published by John Wiley & Sons Ltd on behalf of Pathological Society of Great Britain and Ireland. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
Clinically, facial wrinkles of smokers differ from those of non-smokers; they are narrower but deeper, with sharply contoured periorbital and perioral lines [6]. The physical movement of pursing the lips and squinting (due to smoke irritation of the eyes when inhaling) is likely to enhance wrinkle formation at these anatomical sites. However, as well as direct physical effects, inhalation of tobacco smoke has systemic effects on the smoker. The adverse effects on the respiratory and cardiovascular systems from smoking are well known [7]; each of these systems requires tissue to expand and recoil many millions of times over an individual’s lifespan, mediated by the elastic fibre [8], a key component of extracellular matrices in dynamic tissues such as lungs [9], blood vessels [10], and skin [11].

Elastic fibres are a compound biomaterial, having a central core of amorphous, hydrophobic cross-linked elastin surrounded by a mantle of fibrillin-rich microfibrils (FRMs) [12]. These microfibrils are thought to provide a range of biological functions, which include: directing elastogenesis [13]; structural reinforcement of the elastic fibre and force transduction [14,15]; cell signalling via arginine-glycine-aspartic acid (RGD) sequences [16]; and allowing transforming growth factor beta (TGFβ) sequestration [17,18] (for a review on elastogenesis see [19]). Extracted FRMs have a characteristic ultrastructural appearance in electron and atomic force microscopy (AFM), with an average bead-to-bead distance (or periodicity) of 56 nm [20,21]. By using a molecular combing technique it is also possible to characterise the ability of isolated microfibrils to withstand applied surface tension forces [14,21].

A major function of elastic fibres is to provide the host organ with the ability to extend and recoil many times over the lifetime of the individual [22]. Hence, alterations to either the distribution of elastic fibres or to their capacity for recoil will significantly affect the tissue in which they reside. Previous studies have identified remodelling of the elastic fibre system in the pathophysiology of emphysema [23], chronic obstructive pulmonary disease [24,25], and aortic aneurysm [26]. Similarly, in human skin, the histological distribution of dermal elastic fibres has been studied [27–29]. These histological morphometric studies have identified that the dermal elastic fibres of smokers were fragmented and appeared to be increased significantly in number, and that the observed alterations were confined to the reticular and deeper dermis.

We were able to confirm the altered distribution of elastic fibre components in photoprotected skin from smokers. This was done by quantitative immunohistochemistry for both elastin and FRMs, and the use of in situ zymography (ISZ) to assess whether the remodelling is driven via the induction and increased activity of elastic fibre-degrading matrix metalloproteinases (MMPs). We further characterised the biological impact of changes to the micro-architecture and molecular ultrastructure of the cutaneous elastic fibre network using two independent, although complementary methods. The methods were: scanning acoustic microscopy (SAM), which facilitates investigation of mechanical properties of tissues using ultra-high frequency sound vibrations (100 MHz–1 GHz) where the reflected acoustic wave speed is related to tissue stiffness and density [30]; and molecular combing, which facilitates quantification of extensibility from extracted, tissue-derived microfibrillar assemblies, observed by AFM [14].

Materials and methods

Skin biopsy procurement

Studies conformed to The Declaration of Helsinki Principle. The study was approved by the ethics committee of Attikon General University Hospital, and informed consent was obtained from all participants. We recruited 36 volunteers to this study; of these, 18 had never smoked (9 M, 9 F; age range, 25–55 years), whereas the remaining 18 (9 M, 9 F; age range, 25–63 years) had an accumulated exposure to cigarette smoke of at least 10 pack-years (1 pack-year is defined as 20 manufactured cigarettes [one pack] smoked per day for 1 year). Except for their smoking status, all volunteers were in good general health as assessed by a clinician (ETN). All volunteers provided a single 6 mm punch biopsy from their photoprotected buttock following 1% lignocaine local anaesthesia. Samples were bisected, one-half embedded in optimal cutting temperature compound and the remainder snap frozen in liquid nitrogen. Samples were stored at −80 °C prior to analyses. Biopsies were cryosectioned at 7 μm in a single run, using the same blade and the same cryostat settings (Bright OTF cryostat; Cambridge, UK).

Immunohistochemistry

To investigate the distribution of elastic fibre components in skin of smokers and non-smokers, we performed immunohistochemistry for elastin and FRM as described previously [31,32]. In brief, frozen sections (7 μm) were fixed in 4% paraformaldehyde and hydrated in Tris-buffered saline (TBS; 100 mM Tris, 150 mM NaCl; pH 7.4). Sections were pre-treated with 0.5% Triton X-100 and endogenous peroxidase activity abolished by incubation with 0.6% hydrogen peroxide in methanol. Non-specific binding was blocked by incubation with 3% bovine serum albumin plus 3% normal horse serum. Primary antibodies were applied overnight at 4 °C. These were either mouse anti-elastin (clone BA4; Sigma-Aldrich; Watford, UK) diluted 1:1000 or mouse anti-human FRM (clone 11C1.3; ThermoFisher Scientific, Altrincham, UK) diluted 1:100. Negative controls were performed by omission of primary antibody. Sections were washed in TBS prior to incubation with the appropriate biotinylated antibody for 30 min. Antibody staining was visualized using a well-characterised immunoperoxidase reaction (VECTASTAIN Elite ABC system; Vector Laboratories, Peterborough, UK) utilising Vector SG as the chromogen. Sections
were counterstained with nuclear fast red (Vector Laboratories), dehydrated, and permanently mounted. Following randomisation and ‘blinding’, bright field images were captured using a BX53 microscope (Olympus Industrial; Southend-on-Sea, UK) and image analysis was performed using ImageJ software [33].

**In situ zymography**

To investigate whether smoking initiated tissue remodelling via induction of MMPs we assessed in situ gelatinase activity (MMP-2 and -9) on a sub-sample of volunteer tissue (non-smokers, \( n = 10 \), 5 M, 5 F; age range 26–55 years; smokers, \( n = 10 \), 5 M, 5 F; age range 32–63 years). Unfixed frozen sections (7 μm) were incubated with DQ-gelatin following the manufacturer’s instructions (ThermoFisher Scientific). As a control, cryosections were also pre-incubated with the MMP inhibitor 1-10-phenanthroline monohydrate (10 mM) for 1 h before continuing with the in situ zymography protocol [34]. Following incubation, gelatinase activity was visualised by fluorescence microscopy and intensity analysed using ImageJ software.

**Scanning acoustic microscopy**

To assess whether smoking induced changes in the mechanical properties of skin, we performed scanning acoustic microscopy on a sub-sample of volunteer tissue (non-smokers, \( n = 4 \), 3 M, 1 F; age range 26–45 years; smokers, \( n = 4 \), 3 M, 1 F; age range 36–45 years). Frozen sections (5 μm) were imaged using a SAM 2000 instrument (KSI Gmbh, Heborn, Germany). Images were acquired at a frequency of 770 MHz. Experiments were performed at room temperature using distilled water as the coupling solution. For quantitative analysis, three images were recorded for each subject and subsequently analysed as previously described using the \( V(f) \) method [30].

**Fibrillin-rich microfibril isolation**

A sub-sample of volunteers were used for examination of FRM characteristics (non-smokers, \( n = 3 \), age range 26–31 years; smokers, \( n = 3 \), age range 25–30 years). These assemblies were extracted from skin biopsies using bacterial collagenase type IA (0.5 mg/ml suspended in 0.4 M NaCl, 0.05 M Tris–HCl, 0.01 M CaCl\(_2\) at pH 7.4, and supplemented with protease inhibitors: 2 mM phenylmethanesulphonyl fluoride and 5 mM N-ethylmaleimide). Skin biopsies were incubated in 2 ml collagenase buffer with agitation for 4 h at room temperature followed by further digestion overnight at 4°C. The digested tissue was centrifuged to remove any cell debris, and the supernatant was fractionated by gel filtration using a Sepharose CL-2B column (ÄKTA prime plus system; GE Healthcare, Little Chalfont, UK) that was equilibrated in high salt buffer (0.4 M NaCl, 0.05 M Tris–HCl at pH 7.4). FRMs were eluted from the column in the excluded volume (\( V_{ex} \)) peak.

Atomic force microscopy, molecular combing, and data processing

FRM ultrastructure was characterized by AFM. Using the Multimode 8 AFM (Bruker AFM Probes, Camarillo, CA, USA) fitted with ScanAsyst-Air cantilevers, randomly selected 10 × 10 μm locations were scanned at a rate of 1.97 Hz. The morphologic metrics assessed were the number of beads per FRM and inter-bead periodicity. Periodicity was determined by measuring the distance between individual beads. For each experimental group, microfibril periodicity (\( n = 3000 \)) and bead number per microfibril (\( n = 150 \) microfibrils) were determined from AFM images using ImageJ, WSxM scanning probe microscopy software, and by routines written in Microsoft Visual Basic 6.0 (MJS). Samples were also subject to molecular combing, whereby surface tension forces are employed to cause viscous drag on any partially adsorbed FRM (\( n = 2000 \)). Inter-bead periodicity is a widely used, reliable, and quantitative marker for analysis of FRM ultrastructure [35–37].

**Statistics**

Statistical analysis was performed using GraphPad Prism 8.1.2 (GraphPad Software, Inc. San Diego, CA, USA). Results were considered significant if \( p < 0.05 \) (95% confidence level).

**Results**

Photoprotected skin of chronic smokers exhibits significant remodelling of the elastic fibre network

Immunohistochemical analyses of the major dermal elastic fibre components elastin and FRMs were performed on photoprotected buttck skin biopsies from smokers and non-smokers. In the skin of non-smokers, elastin and FRMs were seen to be arranged in distinctive candelabra-like arrays, connecting oxytalan fibres of the dermal-epidermal junction (DEJ) to elaunin fibres of the superficial papillary dermis. In contrast, immunohistochemical staining for elastin and FRMs in the skin of smokers revealed significant loss of elastic fibre architecture (Figure 1A). This remodelling of the elastic fibre network was accompanied by a significant increased deposition of both elastin (mean ± standard deviation [SD]; non-smokers = 6.4 ± 1.9%; smokers = 8.7 ± 2.2%; \( p < 0.01 \); Figure 1B, D) and FRMs (non-smokers = 19.3 ± 6.3%; smokers = 23.4 ± 6.0%; \( p < 0.05 \); Figure 1C, E) in the photoprotected skin of smokers, as compared to age- and sex-matched non-smoker controls.

Altered elastic fibre function in smokers is not mediated via changes in gelatinase activity

To assess whether changes in elastic fibre deposition were mediated via the expression and activity of the gelatinases MMP-2 and -9, enzymes known to remodel elastic fibres, we performed immunohistochemistry to detect pro- and active enzyme isoforms, and ISZ to detect activity. We
observed no alterations in the distribution by immunohistochemistry of MMP-2 or -9 (data not shown). However, as antibodies identify both the pro- and active forms of these enzymes, we also performed ISZ utilising a gelatin substrate to assess enzyme activity (Figure 2). We found these enzymes localised primarily to the epidermis (Figure 2A, B) and there is no significant difference in their activity (integrated fluorescence intensity; mean ± SD arbitrary units; non-smokers: 50.7 ± 11.1; smokers: 46.4 ± 11.2; \( p = 0.402 \); Figure 2C). As a control, pre-incubation with the MMP inhibitor 1-10-phenanthroline monohydrate blocks gelatinase activity (Figure 2D). Further analysis revealed that, irrespective of smoking status, the epidermal basal keratinocytes were devoid of gelatinase expression (Figure 2E). Within the dermis, gelatinase activity was low and there was no significant difference in activity between smokers and non-smokers (mean ± SD arbitrary units; non-smokers: 4.7 ± 2.5; smokers: 5.5 ± 2.5; \( p = 0.50 \); Figure 2C).

Smokers’ skin exhibits increased tissue stiffness

We assessed if alterations to the architecture and abundance of the elastic fibres resulted in changes to the biomechanical properties of the skin of smokers. Tissue stiffness was assessed using SAM, where increasing acoustic wave speed (\( \lambda \)) indicates increasing stiffness. Epidermal and dermal stiffness was determined by performing SAM on cryosections of skin biopsies mounted on glass microscope slides; skin was imaged from the external section edge, through the epidermis and into the dermis (Figure 3A). Using this method we observed that the skin of smokers had a significantly higher acoustic wave speed than non-smokers in both the epidermis (non-smokers: 1579.1 ± 9.4 ms\(^{-1} \); smokers: 1614.7 ± 15.0 ms\(^{-1} \); \( p < 0.01 \)) and the dermis (non-smokers: 1565.4 ± 10.7 ms\(^{-1} \); smokers: 1611.2 ± 11.3 ms\(^{-1} \); \( p < 0.001 \); Figure 3B).

Extracted fibrillin-rich microfibrils from the skin of smokers are shorter and less able to resist strain than those from the skin of non-smokers

To assess whether there were any ultrastructural changes to the FRMs, assemblies from experimental groups were isolated by size exclusion chromatography following bacterial collagenase digestion, as described previously [36,37]. Using AFM, images of FRMs from each of

Figure 1. Smokers’ skin contains significantly more dermal elastic fibres than the skin of non-smokers. Photoprotected skin of smokers contains more elastin-positive fibres and fibrillin-rich microfibrils than photoprotected skin of non-smokers. Immunoperoxidase staining was performed on fresh frozen skin sections using monoclonal antibodies to elastin (BA4) and fibrillin-rich microfibrils (11C1.3). (A) Discrete elastic fibre-positive assemblies are seen in photoprotected skin of non-smokers, whereas the skin of smokers contains fragmented elastin fibres and fibrillin-rich microfibrils. Scale bar, 50 μm. Plots reveal a significant increase in both (B, D) elastin and (C, E) fibrillin-rich microfibrils in smokers as compared to non-smokers. *\( p < 0.05 \); **\( p < 0.01 \); scale bar = 50 μm.
the experimental groups were captured and the number of beads per microfibrillar assembly enumerated.

Microfibrillar assemblies extracted from the skin of smokers were significantly shorter than those extracted from age- and sex-matched non-smokers (mean ± SD; non-smokers: 26.4 ± 21.0; smokers: 21.6 ± 15.8; p < 0.05; Figure 4A–C). Smoking also influenced the inter-bead periodicity of the FRM; characteristically, FRMs have a bead-to-bead periodicity of approximately 56 nm [38]. The mean periodicity for FRMs extracted from non-smokers was in agreement with this (mean ± SD: 55.5 ± 13.4 nm); however, for smokers, periodicity was significantly increased (60.2 ± 14.9 nm; p < 0.001; Figure 4D).

To assess whether these observed changes affect the biomechanics of the FRMs, extracted FRMs were subjected to molecular combing to assess whether they were more or less able to resist tensile forces. Following molecular combing, FRM periodicity was significantly increased in the smoking group (mean ± SD; 65.1 ± 19.6 nm) compared with the non-smoking group (57.1 ± 17.6 nm; p < 0.001, Mann–Whitney U-test; Figure 4E, F). Furthermore, significantly more periodicity measurements (99/2000) were extended beyond 100 nm in the FRMs extracted from smokers as compared with non-smokers (30/2000; χ² test, p < 0.001).

Discussion

Smoking is regarded as an independent risk factor for skin ageing [2–5], the clinical presentation of which includes the premature appearance of coarse and fine wrinkles [39]. Here, we confirm the findings of previous studies that identify a significant remodelling of the elastic fibre network occurs in the photoprotected skin of smokers [27–29] and provide further evidence for alterations in both the macromolecular organisation and tensile strength of FRMs, fundamental components of elastic fibres in diverse tissue systems.

Investigators have sought to assess whether smoking has a systemic effect on elastic fibres, with opposing results; Allen and co-workers [40], and more recently
Knuutinen and colleagues [41], failed to identify significant changes in the distribution of elastic fibres in the photoprotected skin of smokers using histological methodologies. However, studies employing the immunohistochemical identification of elastin have found significant increases in both the number and total area attributable to elastin-positive fibres in the reticular and deeper dermis of the photoprotected skin of smokers [27,29]. In addition to confirming these findings for elastin, we also used immunohistochemical methods to identify a similar increase in FRM abundance and loss of FRM architecture in the photoprotected skin of smokers. One potential mechanism by which elastic fibres may undergo remodelling is through the local activity of MMP-2 and -9 [42]; therefore, we performed ISZ, which has the advantage of localizing active enzyme activity to the tissue under investigation. In agreement with other studies, we showed that the activity of MMP-2 and -9 was largely restricted to the epidermis [43,44] and that regardless of smoking status, the level of MMP activity was invariant. Our study suggests that MMP-2 and -9 may not be the main mechanistic driver for the observed alterations in elastic fibres; however, we cannot rule out that small changes to enzyme activity over many years could result in the observed alteration to the cutaneous architecture, abundance, and distribution of papillary dermal elastic fibres. The role of MMPs in the pathogenesis of wrinkle formation in the skin of smokers may be more important for the collagenous rather than the elastic fibre network. It has been shown previously that smoking decreases the synthesis of type I and III collagens in skin in vivo [41] and alters the balance of dermal extracellular matrix (ECM) turnover [41,45]. However, there are conflicting opinions on precisely which MMPs are responsible for the remodelling of collagen, with both MMP-1 [45] and MMP-8 [41] suggested as likely candidates. Increased activation of circulating neutrophils [46] and neutrophil elastase [47] in response to cigarette smoking has also been noted and may provide a further mechanism by which elastin and FRMs are degraded [48,49].

To further assess whether smoking compromises elastic fibre function, FRM assemblies were extracted from skin biopsies, thus allowing analysis of macromolecular structure. Perhaps unsurprisingly, given the histological description of fragmented fibres in smokers’ skin, we found a significant reduction in the length of FRM assemblies and a significantly increased inter-bead periodicity, as compared to the skin of non-smokers. Extracted FRMs were also put under extension stress via molecular combing. Previous studies have identified the ability of FRMs to reversibly extend up to a periodicity of ~100 nm, after which they are unable to reform [14]. In FRM populations extracted from the skin of non-smokers the proportion of beads with periodicity ≥100 nm is between 1 and 2%; however, when we subjected the FRMs extracted from the skin of smokers to this mechanical force, this proportion significantly increased to ~5%. Hence, FRMs from the skin of smokers appear to be less able to withstand mechanical forces.

Loss of elastic fibre architecture and function occur not only as the result of tobacco smoke exposure but also in the rare genetic condition cutis laxa. Individuals with cutis laxa have mutations in key structural components of the elastic fibres (including elastin and fibrillin), causing both excess inelastic skin and pulmonary disease [50–52]. Perhaps indicative of shared biology across systems, it has also been identified that the distribution of elastic fibres in skin correlates well with that in lung [53]. It is therefore tempting to postulate that weakened elastin and FRMs may also affect the physiological behaviour of the lung and potentially the cardiovascular system in individuals who smoke. Collectively, there is mounting evidence that affirms the biological plausibility underlying the association of pulmonary disease phenotypes with alterations in skin biology in tobacco-exposed individuals. Chronic obstructive pulmonary disease (COPD) is a condition where cigarette smoking is an important risk factor. However, only a subgroup of smokers develops COPD and it is unclear why these individuals are more susceptible to the detrimental effects of cigarette smoking [54]. In individuals with smoking-related COPD, there is an association between facial wrinkling and airflow obstruction, suggesting that lung and skin share a common susceptibility to the deleterious effects of tobacco smoke exposure [55]. Individuals with smoking-related COPD have increased
elastosis in their photoprotected skin, and the degradation of dermal elastin is associated with increased emphysema severity and carotid pulse wave velocity, indicating that elastin breakdown is a systemic condition [25]. More recently, it has been shown that biomechanical loss of skin elasticity is associated with pulmonary emphysema, biomarkers of inflammation, and MMP activity in the skin of smokers with COPD [56]. Functional measurement of elasticity using non-invasive methods is an accessible and objective determinant of the biomechanical properties of skin ECM that has been validated in several studies [57–59], although its utility as an indirect measure of elastin degradation in COPD has only recently emerged [56].

Elastic fibres make up only a small proportion of the dermal ECM; therefore, to assess the effects of smoking on skin as a whole, we performed SAM. The main advantages of this method, as compared to nanoindentation, for example, is that micromechanical data can be obtained at a higher spatial resolution (−1 μm with a 1 GHz lens). Using this method, we identified that the skin of smokers is significantly stiffer than that of non-smokers. It has previously been demonstrated that the skin of smokers has increased activity of lysyl oxidase enzymes [60,61]. Lysyl oxidases primarily drive the catalysis of cross-links in both fibrillar collagens and elastin in the dermis [62]; however, they are also present within the epidermis of both human and mouse skin [61,63–65], where it has been suggested that they play a role in maintaining epidermal homeostasis and normal keratinocyte differentiation [66,67]. Although the function of these enzymes in the epidermis remains to be fully elucidated, we suggest that epidermal stiffness could, at least in part, be a consequence of increased lysyl oxidase activity. Furthermore, a recent study employing skin autofluorescence measurements revealed an independent association between the accumulation of advanced glycation end products (AGEs) in the skin and parameters of lung function in subjects with COPD [68]. Thus, the increase in overall skin stiffness described herein may be attributable to an increased level of protein cross-linking within the skin and may be further indicative of an association between changes in the skin and other smoking-related conditions such as idiopathic pulmonary fibrosis [69] and arterial stiffening [70].

This study characterises the effect of cigarette smoking on the dermal elastic fibre system, yet the mechanism via which it asserts these effects remains elusive. However, it is clear that dermal homeostasis is disrupted in the photoprotected skin of smokers due to the systemic effects of tobacco smoke. Tobacco smoke is a complex mixture of many thousands of chemical components known to have toxicological, carcinogenic and mutagenic properties [71,72]. Mainstream (smoker-inhaled) smoke is divided into a particulate solid phase (tar) and the gas phase (toxic gases, volatile organic compounds) containing both stable and unstable free radicals and reactive oxygen species (ROS) that have the potential for biological oxidative damage [72,73]. Cigarette smoking enables these toxic free radicals to be distributed systemically via the bloodstream. ROS in the gas-phase promotes the destruction of endogenous antioxidants and impairs the vital role of cellular antioxidant defences [74]. Furthermore, several studies show that antioxidant vitamins are lower in smokers resulting in systemic oxidative stress [75,76]. Thus, the highly vascularised dermis may be particularly sensitive to oxidative stress, leading to a cascade of downstream consequences that ultimately result in elastin and FRM degradation and remodelling [77].

Premature facial ageing, altered skin texture, and skin wrinkling may help to influence a smoker’s decision to quit and are important deterrent factors for the uptake of tobacco products [78]. It has been shown in several studies that smoking cessation has a rejuvenating effect on the skin both in reducing perceived age [79] and improving skin colour [80]. Increased public health awareness of the positive effects of stopping smoking and maintaining abstinence on both facial appearance and respiratory health [81] should be encouraged.

In conclusion, we identified a significant remodelling of the cutaneous elastic fibre system in smokers. This remodelling, which appears to be independent of MMP digestion of elastic fibres, is apparent at both the histological and ultrastructural levels and results in skin, which is significantly stiffer in smokers than in non-smokers. These changes may not only affect the appearance of skin – via the formation of features associated with premature ageing – but may also influence the function of other elastic fibre-rich tissues, such as those found in the respiratory and cardiovascular systems. Characterisation of the linked pathology between degeneration and remodelling of the ECM in skin and other organs as a consequence of tobacco smoke exposure warrants further investigation.

Acknowledgements

We are grateful for the technical assistance of Dr Nigel Hodson from The University of Manchester BioAFM Facility. CEMG is a National Institute for Health Research (NIHR) Emeritus Senior Investigator.

Author contributions statement

AKL, MJS, RA, BD, REBW, and CEMG designed the research. ET-N, AC, and AS provided skin biopsies. AKL, HM, and XZ performed the experiments and acquired data. AKL, REBW, and CEMG wrote the manuscript.

Funding

This study was funded in part by the MRC Lifelong Health and Wellbeing programme (MRC Grant No. G1001398; awarded to MJS, REBW and BD) and by a programme grant from Walgreens Boots Alliance.
Nottingham, UK (awarded to CEMG, REBW, and MJS). CEMG and REBW are supported in part by the National Institute for Health Research Manchester Biomedical Research Centre.

References

1. Kligman AM. Early destructive effect of sunlight on human skin. JAMA 1969; 210: 2377–2380.

2. Kadunce DP, Burr R, Gress R, et al. Cigarette smoking: risk factor for premature facial wrinkling. Ann Intern Med 1991; 114: 840–844.

3. Rexbye H, Petersen I, Johanssen M, et al. Influence of environmental factors on facial ageing. Age Ageing 2006; 35: 110–115.

4. Martires KJ, Fu P, Polster AM, et al. Factors that affect skin aging: a cohort-based survey on twins. Arch Dermatol 2009; 145: 1375–1379.

5. Green AC, Hughes MC, McBride P, et al. Factors associated with premature skin aging (photoaging) before the age of 55: a population-based study. Dermatology 2011; 222: 74–80.

6. Daniell HW. Smoker’s wrinkles. A study in the epidemiology of “crow’s feet”. Ann Intern Med 1971; 75: 873–880.

7. Kenfield SA, Wei EK, Rosner BA, et al. Burden of smoking on cause-specific mortality: application to the nurses’ health study. Tob Control 2010; 19: 248–254.

8. Sherratt MJ. Tissue elasticity and the ageing elastic fibre. Age (Dordr) 2009; 31: 305–325.

9. Miller J. The arrangement of the elastic fibres in the bronchi and lung. J Anat Physiol 1906; 40: 162–170.

10. Ayer JPHG, Philpott DE. Aortic elastic tissue; isolation with use of formic acid and discussion of some of its properties. AMA Arch Pathol 1958; 65: 519–544.

11. Dick JC. Observations on the elastic tissue of the skin with a note on the reticular layer at the junction of the dermis and epidermis. J Anat 1947; 81: 201–211.

12. Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. Birth Defects Res C Embryo Today 2007; 81: 229–240.

13. Trask TM, Trask BC, Ritty TM, et al. Interaction of tropoelastin with the amino-terminal domains of fibrillin-1 and fibrillin-2 suggests a role for the fibrillins in elastic fiber assembly. J Biol Chem 2000; 275: 24400–24406.

14. Sherratt MJ, Baldock C, Haston JL, et al. Fibrillin microfibrils are stiff reinforcing fibres in compliant tissues. J Mol Biol 2003; 332: 183–193.

15. Ashworth JL, Kiely CM, McLeod D. Fibrillin and the eye. Br J Ophthalmol 2000; 84: 1312–1317.

16. Bax DV, Mahalingam Y, Cain S, et al. Cell adhesion to fibrillin-1: identification of an Arg-Gly-asp-dependent synergy region and a heparin-binding site that regulates focal adhesion formation. J Cell Sci 2007; 120: 1383–1392.

17. Olivieri J, Smaldone S, Ramirez F. Fibrillin assemblies: extracellular determinants of tissue formation and fibrosis. Fibrogen Tissue Rep 2010; 3: 24.

18. Massan-Wu T, Chiu M, Choudhury R, et al. Assembly of fibrillin microfibrils governs extracellular deposition of latent TGF beta. J Cell Sci 2010; 123: 3006–3018.

19. Mittheux SM, Weiss AS. Elastin. Adv Protein Chem 2005; 70: 437–461.

20. Kiely CM, Cummings C, Whitaker SP, et al. Isolation and ultrastructural analysis of microfibrillar structures from foetal bovine elastic tissues. Relative abundance and supramolecular architecture of type VI collagen assemblies and fibrillin. J Cell Sci 1991; 99: 797–807.

21. Sherratt MJ. Tissue elasticity and the ageing elastic fibre. Age (Dordr) 2009; 1–21.

22. Gooe J, Lillie M, Carrington E, et al. Elastic proteins: biological roles and mechanical properties. Philos Trans R Soc Lond B Biol Sci 2002; 357: 121–132.

23. Janoff A, Sloan B, Weinbaum G, et al. Experimental emphysema induced with purified human neutrophil elastase: tissue localization of the instilled protease. Am Rev Respir Dis 1977; 115: 461–478.

24. Deslee G, Woods JC, Moore CM, et al. Elastin expression in very severe human COPD. Eur Respir J 2009; 34: 324–331.

25. Maclay JD, McAllister DA, Rabinovich R, et al. Systemic elastin degeneration in chronic obstructive pulmonary disease. Thorax 2012; 67: 606–612.

26. Campa JS, Greenhalgh RM, Powell JT. Elastin degradation in abdominal aortic aneurysms. Atherosclerosis 1987; 65: 13–21.

27. Frances C, Boisnic S, Hartmann DJ, et al. Changes in the elastic tissue of the non-sun-exposed skin of cigarette smokers. Br J Dermatol 1991; 125: 43–47.

28. Boyd AS, Stasko T, King LE, et al. Cigarette smoking-associated elasticotic changes in the skin. J Am Acad Dermatol 1999; 41: 23–26.

29. Just M, Ribera M, Monsé E, et al. Effect of smoking on skin elastic fibres: morphometric and immunohistochemical analysis. Br J Dermatol 2007; 156: 85–91.

30. Akhtar R, Schwarzer N, Sherratt MJ, et al. Nanoindentation of histological specimens: mapping the elastic properties of soft tissues. J Mater Res 2009; 24: 638–646.

31. Watson REB, Craven NM, Kang SW, et al. A short-term screening protocol, using fibrillin-1 as a reporter molecule, for photoaging repair agents. J Invest Dermatol 2001; 116: 672–678.

32. Watson REB, Long SP, Bowden JJ, et al. Repair of photoaged dermal matrix by topical application of a cosmetic ‘antiaging’ product. Br J Dermatol 2008; 158: 472–477.

33. Abramoff MD, Magelhaes PJ, Ram SJ. Image processing with ImageJ. Biophotonics Int 2004; 11: 36–42.

34. Garcia-Alleza M, Prada C, Lattarulo C, et al. Matrix metalloproteinase inhibition reduces oxidative stress associated with cerebral amyloid angiopathy in vivo in transgenic mice. J Neurochem 2009; 109: 1636–1647.

35. Eckersley A, Mellody KT, Pilkington S, et al. Structural and compositional diversity of fibrillin microfibrils in human tissues. J Biol Chem 2018; 293: 5117–5133.

36. Hibbert SA, Watson REB, Gibbs NK, et al. A potential role for endogenous proteins as sacrificial molecules that mediate wound healing. J Pathol 2009; 217: 513–523.

37. Sherratt MJ, Baylce CP, Reilly SM, et al. Low-dose ultraviolet radiation selectively degrades chromophore-rich extracellular matrix components. J Pathol 2010; 222: 32–40.

38. Kiely CM, Sherratt MJ, Marson A, et al. Fibrillin microfibrils. Adv Protein Chem 2005; 70: 405–428.

39. Tsoureli-Nikita E, Watson REB, Griffiths CEM. Photageing: the darker side of the sun. Photochem Photobiol Sci 2006; 5: 160–164.

40. Allen HB, Johnson BL, Diamond SM. Smoker’s wrinkles? JAMA 1973; 215: 1067–1069.

41. Knutinen A, Kokkonen N, Risteli J, et al. Smoking affects collagen synthesis and extracellular matrix turnover in human skin. Br J Dermatol 2002; 146: 588–594.

42. Ashworth JL, Murphy G, Rock MJ, et al. Fibrillin degradation by matrix metalloproteinases: implications for connective tissue remodeling. Biochem J 1999; 340: 171–181.

43. Inomata S, Matsuura Y, Amano S, et al. Possible involvement of Gelatinases in basement membrane damage and wrinkle formation in chronically ultraviolet B-exposed hairless mouse. J Invest Dermatol 2003; 120: 128–134.

44. Quan T, Qin Z, Xia W, et al. Matrix-degrading metalloproteinases in photoaging. J Invest Dermatol Symp Proc 2009; 14: 20–24.

45. Lahmann C, Bergemann J, Harrison G, et al. Matrix metalloproteinase-1 and skin ageing in smokers. Lancet 2001; 357: 935–936.
46. Hoonhorst SJ, Timens W, Koenderman L, et al. Increased activation of blood neutrophils after cigarette smoking in young individuals susceptible to COPD. Respir Res 2014; 15: 121.
47. Weitz JI, Crowley KA, Landman SL, et al. Increased neutrophil elastase activity in cigarette smokers. Ann Intern Med 1987; 107: 680–682.
48. Takeuchi H, Gomi T, Shishido M, et al. Neutrophil elastase contributes to extracellular matrix damage induced by chronic low-dose UV irradiation in a hairless mouse photoaging model. J Dermatol Sci 2010; 60: 151–158.
49. Kieltly CM, Woolley DE, Whittaker SP, et al. Catabolism of intact fibrillin microfibrils by neutrophil elastase, chymotrypsin and trypsin. FEBS Lett 1994; 351: 85–89.
50. Kozel BA, Su CT, Danback JR, et al. Biomechanical properties of the skin in cutis laxa. J Invest Dermatol 2014; 134: 2836–2838.
51. Urban Z, Davis EC. Cutis laxa: intersection of elastic fiber biogenesis, TGFbeta signaling, the secretory pathway and metabolism. Matrix Biol 2014; 33: 16–22.
52. Lebwohl MG, Schwartz E, Jacobs L, et al. Abnormalities of fibrillin in acquired cutis laxa. J Am Acad Dermatol 1994; 30: 950–954.
53. Just M, Monsé E, Ribera M, et al. Relationships between lung function, smoking and morphology of dermal elastic fibres. Exp Dermatol 2005; 14: 744–751.
54. Eisner MD, Anthonisen N, Coutlas D, et al. An official American Thoracic Society public policy statement: novel risk factors and the global burden of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010; 182: 693–718.
55. Patel BD, Loo WJ, Tasker AD, et al. Smoking related COPD and facial wrinkling: is there a common susceptibility? Thorax 2006; 61: 566–571.
56. O’Brien ME, Chandra D, Wilson RC, et al. Loss of skin elasticity is associated with pulmonary emphysema, biomarkers of inflammation, and matrix metalloproteinase activity in smokers. Respir Res 2019; 20: 128.
57. Langton AK, Graham HK, McConnell JC, et al. Organization of the dermal matrix impacts the biomechanical properties of skin. Br J Dermatol 2017; 177: 818–827.
58. Langton AK, Graham HK, Griffiths CEM, et al. Ageing significantly impacts the biomechanical function and structural composition of skin. Exp Dermatol 2019; 28: 981–984.
59. Langton AK, Alessi S, Hann M, et al. Aging in skin of color: disruption to elastic fiber organization is detrimental to skin’s biomechanical function. J Invest Dermatol 2019; 139: 779–788.
60. Langton AK, Griffiths CE, Sherratt MJ, et al. Cross-linking of structural proteins in ageing skin: an in situ assay for the detection of amine oxidase activity. Biogerontology 2012.
61. Langton AK, Tsourelli-Nikita E, Griffiths CEM, et al. Lysyl oxidase activity in human skin is increased by chronic ultraviolet radiation exposure and smoking. Br J Dermatol 2017; 176: 1376–1378.
62. Chenzo V, Andre V, Reymermier C, et al. LOXL as a target to increase the elastin content in adult skin: a dill extract induces the LOXL gene expression. Exp Dermatol 2006; 15: 574–581.
63. Hayashi K, Feng KS, Mercier F, et al. Comparative immunocytochemical localization of lysyl oxidase (LOX) and the lysyl oxidase-like (LOXL) proteins: changes in the expression of LOXL during development and growth of mouse tissues. J Mol Histol 2004; 35: 845–855.
64. Kobayashi H, Ishii M, Chanoki M, et al. Immunohistochemical localization of lysyl oxidase in normal human skin. Br J Dermatol 1994; 131: 325–330.
65. Noblesse E, Cenizo V, Bouez C, et al. Lysyl oxidase-like and lysyl oxidase are present in the dermis and epidermis of a skin equivalent and in human skin and are associated to elastic fibers. J Invest Dermatol 2004; 122: 621–630.
66. Le Provost GS, Debret R, Cenizo V, et al. Lysyl oxidase silencing impairs keratinocyte differentiation in a reconstructed-epidermis model. Exp Dermatol 2010; 19: 1080–1087.
67. Bouez C, Reynaud C, Noblesse E, et al. The lysyl oxidase LOX is absent in basal and squamous cell carcinomas and its knockdown induces an invading phenotype in a skin equivalent model. Clin Cancer Res 2006; 12: 1463–1469.
68. Hoonhorst SJ, Lo Tam Loi AT, Hartman JE, et al. Advanced glycation end products in the skin are enhanced in COPD. Metabolism 2014; 63: 1149–1156.
69. Antoniou KM, Walsh SL, Hansell DM, et al. Smoking-related emphysema is associated with idiopathic pulmonary fibrosis and rheumatoid lung. Respirolology 2013; 18: 1191–1196.
70. Doonan RJ, Hausvater A, Scallan C, et al. The effect of smoking on arterial stiffness. Hypertens Res 2010; 33: 398–410.
71. Talhout R, Schulz T, Florez E, et al. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health 2011; 8: 613–628.
72. Valavanidis A, Vlachogianni T, Fiotakis K. Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respiratory particles. Int J Environ Res Public Health 2009; 6: 445–462.
73. Huang MF, Lin WL, Ma YC. A study of reactive oxygen species in mainstream of cigarette. Indoor Air 2005; 15: 135–140.
74. Cross CE, Traber M, Eiserich J, et al. Micronutrient antioxidants and smoking. Br Med Bull 1999; 55: 691–704.
75. Panda K, Chattopadhayay R, Chattopadhayay DJ, et al. Vitamin C prevents cigarette smoke-induced oxidative damage in vivo. Free Radic Biol Med 2000; 29: 115–124.
76. Traber MG, van der Vliet A, Reznick AZ, et al. Tobacco-related diseases. Is there a role for antioxidant micronutrient supplementation? Clin Chest Med 2000; 21: 173–187.
77. Rinnerthaler M, Bischof J, Streubel MK, et al. Oxidative stress in aging human skin. Biogerontology 2012.
78. Demierre MF, Brooks D, Koh HK, et al. Public knowledge, awareness, and perceptions of the association between skin aging and smoking. J Am Acad Dermatol 1999; 41: 27–30.
79. Serri R, Romano MC, Sparavigna A. "Quitting smoking rejuvenates the skin": results of a pilot project on smoking cessation conducted in Italian. Skinmed 2010; 9: 23–29.
80. Cho YH, Jeong DW, Seo SH, et al. Changes in skin color after smoking cessation. J Investig Dermatol 2014; 151: 1379–1385.
81. Willemsse BW, Postma DS, Timens W, et al. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. Eur Respir J 2004; 23: 464–476.