Does the paucity of elastic fibres contribute to the process of keloidogenesis?

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Key words: Elastic fibres; wound healing fibroblasts; deformation forces; histomorphometric analysis.Running title: Comparative histomorphometric analysis of elastic fibres in lesional and non-lesional skin of patients with keloids

List of abbreviations

AI	Area of inflammation	FFWA	Fine fibrous wavy areas
ATP	Adenosine tri-phosphate	Fig	Figure
CT	Connective tissue	FFT	Fine fibrous tubular
DRWA	Dense regular wavy areas	HCB	Hyalinising collagen bundles
DRWW	Dense regular wavy areas with	K/PD	Keloid/papillary dermis border
wide spaces		LAS	Leica Application Suite
ECM	Extracellular matrix	SEM	Standard error of mean
EVG	Elastic-Van Gieson	SPSS	Statistical Package for the
FFA	Fine fibrous areas	Social Sciences	
FFCA	Fine fibrous cellular areas	TGF- β	Transforming growth factor
		beta	

SUMMARY

Introduction:Keloids are a prototype of excessive wound healing. Keloid fibroblasts generate traction force, which deforms and realigns the collagen network during migration. Cells move bidirectionally along aligned fibres resulting in nonuniform cell distribution. This and the anisotropic collagen properties displayed in keloids, may be governed by traction force. Excessive traction force (>elastic limit), causes permanent deformations. As recovery from deformational forces is attained mainly by elastic recoil, we hypothesised that in keloids the elastic limit is decreased by reduced numbers of dermal elastic fibres, leading to permanent plastic deformation of dermal tissue by traction force.

Objective: To quantitate and compare the elastic fibre content of keloids and non-lesional skin

Methods: Sections of keloids and non-lesional skin from 32 patients were stained with elastic Van Gieson. The elastic fibre content was histomorphometrically quantified and the mean (± SEM) percentage area of elastic fibres in lesional and non-lesional skin was compared.

Results: Elastic fibres at the border of keloids were increased whereas internally they were minimal or absent. Statistical analysis (Wilcoxon signed ranks test) showed significant differences ($p < 0.05$) in elastic fibre content between non-lesional dermis and keloids.

Conclusions: The lack of elastic fibres in keloids decreases the elastic limit, leading to effects of excessive deformational force. These include compression and stiffening of tissue, increased mitogenesis and cell contractility, modified DNA and protein synthesis and increased collagen biosynthesis. The manifestation of these effects in keloids, supports the hypothesis that decreased elasticity in keloids promotes permanent dermal deformation by traction forces.

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Introduction

As keloids are a prototype of excessive wound healing, the lesional fibroblastic cells constantly exert traction forces to facilitate migration and extracellular matrix remodelling. During this process the collagen network may be realigned to provide contact guidance for 3D migration of cells along the axis of fibre alignment, resulting in bi-directional cell migration and non-uniform cell distribution. This and the anisotropic collagen properties displayed in the various keloid regions identified2 occurred, probably, to offer greater resistance to prolonged traction forces generated by migrating fibroblasts. Cell adhesion and migration are prominent in the angiogenesis and remodeling stages of wound healing [1, 2, 3]. During excessive wound healing, as in keloids, in addition to the constant production and progressive accumulation of scar tissue, traction force is continuously generated by migrating fibroblasts. Excessive, prolonged traction forces deform the extracellular matrix up to their elastic limit; beyond this limit the friction force that the tissue can withstand is exceeded [4] and the collagenous matrix is unable to resist cellular traction force, resulting in movement of the matrix [7]. Cell movement only occurs if the collagenous matrix can resist the traction force exerted by cells [5]. Stress applied beyond the elastic limit, results in plastic changes that occur at a rate greater than the tissue can tolerate causing injury and permanent deformation; this is called plasticity stress [4]. Beyond the plasticity level, tissue cannot tolerate more stress and it ruptures4. Could the disfiguring dermal deformations in keloids be a manifestation of gradually accumulated effects of stress from many lesser loads exerted by continuous traction force generated by wound healing fibroblastic cells, especially during granulation tissue formation, angiogenic and tissue remodeling stages? Supportive evidence for this includes: 1) formation of keloids months to years post injury, after apparent successful healing and 2) use of pressure therapy in the effective treatment of keloids [6]. Pressure therapy compensates for the elastic insufficiency of the affected dermal tissue and reduces scar tissue formation by inducing localised hypoxia and subsequent fibroblastic degeneration [6]. However, pre-existing hypoxia was confirmed in keloids where the accumulation of hypoxia-inducible factor-1α protein was found[7]. The effectiveness of pressure therapy treatment of keloids may, therefore, be by exacerbation of the existing hypoxic state. This corroborates an association between stress (force applied), strain (deformation when stress is applied), elastic insufficiency, hypoxia and keloid formation; thus reinforcing the necessity to investigate the role of traction forces in the pathogenesis of keloids. As elastic recoil is the principal function responsible for recovery of tissue deformed by traction forces, we hypothesise that, in keloids, connective tissue elasticity is decreased, reducing the elastic limit and resulting in augmented tissue deformation; progressive accumulation of deformed dermal tissue leads to keloid formation. To assess this, we (1) histologically assessed elastic fibre distribution in keloids, (2) histomorphometrically quantified and compared the mean (± SEM) percentage area of elastic fibres in lesional and non-lesional skin of patients with ear lobe keloids, (3) histomorphometrically quantified and compared the mean (± SEM) percentage area of collagen fibres in lesional and non-lesional skin of patients with ear lobe keloids and (4) compared the collagen:elastic fibre ratios of keloids, non-lesional skin and normal skin. This study is the first to show the distribution of elastic fibres in keloids and implicate abnormal elastic fibre distribution in the aetiopathogenesis of keloid formation.

Patients and Methods

The Ethical Committee of the Nelson R Mandela School of Medicine approved this study and all patients gave informed consent. The ethical policy is in compliance with the rules for human experimentation stated in the 1975 Declaration of Helsinki. Wedge biopsies were taken from lesional and non-lesional skin of thirty patients with keloids and no other underlying medical condition. Patient details are recorded in Table 1. The specimens were placed in 10% formalin saline (4% formaldehyde) immediately after procurement and were processed for paraffin wax embedment using conventional methods. Four and five micron sections of each specimen were stained with haematoxylin and eosin and elastic Van Gieson (EVG), respectively. Semiquantitative assessment of elastic fibre distribution in all sections of keloids and non-lesional skin biopsies was performed using the Olympus BH-2 microscope. From the 30 patients, a group of ten closely-matched patients with a common site of keloid formation and cause of initial injury (Table 1, blue rows) was selected for statistical analysis of the elastic fibre content in the keloid and adjacent normal skin. Close equivalence of patients minimized preexisting differences between the groups and maximized probability that differences found were associated with keloid formation. Patients chosen for the study were aged between 15 and 21 years, of a common race (African) and gender (females) who showed common cause of injury (ear-piercing) and site of keloid formation (ear lobes). The non-lesional skin biopsies were taken from the apparently normal area adjacent to the keloid.

Table 1: Patient details

Patient number	Race	Sex	Age	Diagnosis, location and size	Period after injury	Nature of initial injury and number of biopsies
1	C	M	31.5	Keloid. Presternal. 3.5 x 13 cm^2	4 years	Post thoracotomy. Bx, 2
2	A	F	19	Keloid, preauricalar, right. 7x11cm^2	2 years	Trauma. Bx,2
3	C		11.5	Keloid Suprapubic area. 2x4 cm^2	1year	Scar from surgery for undescended testis. Bx,2
4	A	M	21	Keloid. Left ear lobe. 2x2x2.5 cm^3	2 years	Ear piercing. Bx,1
5	A	F	15	Keloid. Right ear lobe	7 years	Ear piercing. Size NA. Bx,1
6	A	F	18	Keloids. Left and right ear lobes. 33x24x16 cm^3	Few months	Ear piercing. Bx,2
7	A	F	16	Keloids. Left and right ears lobes. Recurred after 1 year.R = 90x85x31 cm^3. L<R	1 year	Ear piercing. Bx,2
8	A	F	26	Keloids. Left and right ear lobes. 19x15X10 cm^3	2 months	Ear piercing. Bx,2
9	A	F	16	Keloid Left and right ear lobes 14x9x7 cm^3	1-2 years	Ear piercing. Bx,2
10	A	F	27	Multiple keloids. Recurred. Neck, back, presternal, chest. >18x3.5x1.1 cm^3	20 years	Plastic burns. Bx,8
11	A	F	29	Keloid. Suprapubic area. 2.1x3.5x1.1 cm^3	3 years	Ceasarian section scar. Bx,1
12	A	F	33	Keloids. Left and right ears lobes. Recurrent. 4.2x3.5x1.7 cm^3	2 years	Ear piercing. Bx,2
13	A	F	18	Keloids. Left breast, neck.	12 years	Neck, wire injury. Breast, abscess injury. Bx,2
14	A	F	21	Keloids. Left and right ear lobes. Recurrent	NA	Ear- piercing. Size NA. Bx,2
15	A	F	23	Keloid. Left ear lobe	NA	Ear-piercing. Size NA. Bx,1
16	A	F	18	Keloids. Bilateral ear lobes	NA	Ear piercing. Bx,2
17	A	F	26	Keloids. Left ear lobe, shoulder. Recurrent.	NA	Ear piercing. Shoulder, NA. Bx,2
18	A	F	22	Keloid. Left ear lobe. Recurrent	NA	Ear piercing. Bx,1

19	A	M	24	Keloids. Left and right ear lobes	NA	Ear piercing. Bx,2
20	A	M	49	Keloid. Left ear	NA	Stab wound. Bx,1
21	A	F	17	Keloid. Right ear	NA	Ear piercing. Bx,1
22	A	F	27	K. Left and right ears lobes. Recurrent for 3rd time	4 years	Ear piercing. Bx,2
23	A	M	24	Keloid. Left ear lobe.	2 years	Accident. Bx.1
24	A	F	18	Keloid. Left ear lobe	NA	Ear piercing. Bx,1
25	A	F	26	Keloid. Left ear lobe	5 years	Ear piercing. Bx,1
26	A	M	45	Keloid. Back of head. Occipital region. Prone to skin rash	2 years	Spontaneous. Bx,2
27	A	M	26	Keloid. Left ear lobe, posterior	2-3 years	Ear piercing. Bx,1
28	A	F	18	Keloids. Bilateral ear lobes	3 years	Ear piercing. Bx,2
29	A	M	28	Neck (below right ear), submandibular area	NA	NA. Bx,2
30	A	M	60	Keloid. Occipital area	NA	NA. Bx,2

C – Caucasian
A – African
M – Male
F – Female
Bx - Biopsy
RHS - Right hand side
NA - Not available

Morphometric and image analysis

The various regions composing the keloid were identified according to the classification of Bux and Madaree [8] and digital images of areas containing elastic fibres were captured using the EC 3 camera (Leica, Heerbrugg, Switzerland). Image analysis was performed using the Leica Application Suite (LAS), version 3.0. (Wetzlar, Germany). Prior to photography, the microscope was adjusted for optimal viewing by aligning for Kohler illumination and the system was calibrated using a 1000-µm stage micrometer. To exclude bias and repetition, the areas photographed were adjoining, non-overlapping fields of view showing the presence of elastic fibres; in non-lesional skin and each of the elastic fibre containing keloid regions. Acquired images were exported into Leica Qwin Pro (Wetzlar, Germany) for histomorphometrical evaluation using LAS version 3 image processing and analysis system and Leica Qwin Pro version 3.0, software (Wetzlar, Germany). The analysis included:

Non-lesional skin

1. Percentage area of elastic fibres in 100 fields of view (10 specimens in 10 fields of view at 40X)
2. Percentage area of collagen fibres in 100 fields of view (10 specimens in 10 fields of view at 40X)

Lesional skin

1. Percentage area of elastic fibres in 400 fields of view (4 areas X10 specimens in 10 fields of view at 40X)
2. Percentage area of collagen fibres in 400 fields of view (4 areas X10 specimens in 10 fields of view at 40X)

Also the mean % elastic and collagen fibre content of keloids and non-lesional were compared with that of normaldermis[9,10.]

Statistical analysis

All data were represented as means and standard errors (± SEM); these results are summarized in Table 2. Data obtained was entered in a computerized statistical analysis program, SPSS 15 for Windows, (SPSS, Chicago, IL) and comparative analysis of the mean percentage area of elastic and collagen fibres between non-lesional skin and each of the elastic fibre-containing keloid regions was performed using

Wilcoxon signed-rank non-parametric test. A p value of less than 0.05 was considered to be statistically significant.

Table 2: Specimen grouping details, mean and ± SEM of % area of elastic and collagen fibres in lesional and non-lesional skin of patients with keloids

Non-Lesional Skin (n = 10)							
Sp No	Region	No of Fields		Elastic Fibre % Area		Collagen Fibre % Area	
				Mean	±SEM	Mean	±SEM
1	Dermis	10		1.85	±0.078	71.89	±1.149
2	Dermis	10		10.64	±0.298	37.42	±0.919
3	Dermis	10		9.09	±0.196	43.05	±0.500
4	Dermis	10		4.2	±0.188	53.34	±0.718
5	Dermis	10		4.05	±0.191	59.2	±0.435
6	Dermis	10		3.52	±0.236	72.22	±0.687
7	Dermis	10		7.1	±0.314	57.99	±0.618
8	Dermis	10		9.09	±0.278	43.05	±0.375
9	Dermis	10		7.73	±0.227	55.81	±0.654
10	Dermis	10		8.92	±0.281	41.96	±0.593

Lesional Skin (n = 10)								
	Elastic Fibre % Area							
	FFT		DRWW		AI		K/PD	
Sp No	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
1	0.12	±0.012	0.39	±0.018	4.22	±0.1056	3.17	±0.10006
2	0.09	±0.007	0.09	±0.0114	5.02	±0.1013	4.52	±0.1483
3	0.46	±0.027	0.17	±0.0123	9.59	±0.1319	10.26	±0.1483
4	1.12	±0.137	0.42	±0.0149	2.4	±0.138	10.94	±0.2179
5	0.6	±0.052	0.26	±0.017	5.49	±0.1665	6.78	±0.1447
6	0.38	±0.027	0.22	±0.015	4.99	±0.1487	7.88	±0.0967
7	0.42	±0.028	0.18	±0.018	4.32	±0.1297	6.43	±0.148
8	0.45	±0.025	0.24	±0.014	7.01	±0.1137	7.98	±0.1191
9	0.19	±0,016	0.11	±0.019	4.80	±0.1307	5.55	±0.2095
10	0.67	±0.033	0.29	±0.014	5.21	±0.1378	8.97	±0.1294
	Collagen Fibre % Area							
	FFT		DRWW		AI		K/PD	
Sp No	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM
1	70.01	±0.507	63.97	±1.09	6.72	±0.1414	55.3	±1.393
2	65.91	±0.561	33.41	±1.05	5.21	±0.1273	41.35	±0.941
3	76.36	±1.518	59.73	±0.945	23.25	±0.3099	53.24	±1.446
4	58.52	±1.437	46.19	±1.33	5.69	±0.1383	28.82	±0.901
5	60.33	±1.194	66.13	±1.73	20.56	±0.2078	31.04	±0.7148
6	90.62	±1.811	39.22	±0.527	5.63	±0.1404	46.98	±1.2407
7	64.17	±1.945	56.87	±1.357	6.43	±0.1643	30.33	±0.7008
8	64.39	±1.3576	48.07	±0.844	6.32	±0.1758	45.32	±1.1521
9	75.87	±1.635	58.99	±1.199	5.71	±0.1268	40.23	±0.7891
10	64.84	±1.652	49.6	±1,034	6.22	±0.1505	50.54	±0.705

Sp No – Specimen Number FFT –Fine fibrous tubular
AI – Area of inflammation K/PD – Keloid/papillary dermis border
DRWW – Dense regular wavy connective tissue with wide spaces

Results

Histological semiquantitative assessment of the elastic fibre content of non- lesional dermis and keloids showed that non-lesional skin contained the largest amount of elastic fibres with 66% showing a great increase in elastic fibres and 28% a moderate increase; this was followed by the keloid/papillary dermis border where there was a great increase in 28% of the specimens and a moderate increase in 47% (Table 3). Areas of inflammation located at the keloid/papillary dermis border showed moderate (19%) to scattered (25%) elastic fibres in 44% of the keloid specimens. Within keloids there was a paucity of elastic fibres; fibrous tubular regions showed the highest content (22% moderate and 66% few elastic fibres) followed by dense regular wavy areas (9% moderate and 53% few elastic fibres). Moderate to few elastic fibres were present around blood vessels in 69% of keloids and in nodular areas in 57% of the keloid specimens. Only 9% of keloid specimens showed the presence of elastic fibres in hyalinising collagen bundle regions; the distribution was sparse. Areas of angiogenesis displayed moderate numbers of elastic fibres in 15% and few in 41% of the keloid specimens.

With regard to the morphology of elastic fibres, at the papillary dermis/keloid border they were long and fairly thick, short and stubby, or thin and wispy; the long elastic fibres generally were oriented parallel to the long axis of the collagen fibre bundles (Fig 1a). Elastic fibres were increased in and around inflamed vessels of the sub papillary plexus at the papillary dermis/keloid border; elastic fibres were also present in the inflammatory infiltrate (Fig. 1b). It was remarkable that increased numbers of elastic fibres were located on the keloid side of the sub papillary plexus in 50% of the specimens, while on the papillary dermis side minimal amounts were found (Fig. 1b). At the papillary dermis/keloid border, some keloid zones, viz., fibrous tubular regions and dense regular wavy connective tissue areas, contained abundant elastic fibres (Fig. 1c, d) whereas matched areas within the keloid were devoid of elastic fibres or contained very few frail fibres (Fig. 1e, f). In non-lesional skin, the elastic fibres were irregularly arranged, less well-defined, clumped and paler staining when compared with those at the papillary dermis /keloid border (Fig. 2a); also the elastic fibres reoriented from parallel to perpendicular to the axis of collagen fibres and compacted to form thickened bands (Fig. 2b). This often occurred in close proximity to areas of tissue rupture or areas where collagen and elastic fibres appeared disheveled and degenerate (Fig. 2b). Within keloids there was a paucity of elastic fibres and this was restricted to certain keloid zones which included the fibrous tubular region and the dense regular wavy connective tissue areas (Fig. 1e, f); other zones such as fine fibrous cellular, and hyalinising collagen bundle areas were devoid of elastic fibres or showed occasional spots or blurred specs of positivity for elastin (Fig. 2c, d). Six % of the specimens displayed blurred patches of elastin staining within few hyalinizing collagen bundles (Fig. 2e). A consistent and conspicuous finding was the absence of elastic fibres in very cellular regions of the keloid, viz., fine fibrous cellular and wavy fine fibrous cellular areas (Fig. 2f).

Statistical analysis of ten ear lobe specimens using the Wilcoxon signed ranks test, showed statistically significant differences in the elastic fibre content between non-lesional dermis and: 1) fine fibrous tubular areas and 2) dense regular wavy areas in keloids (Table 4 and Fig. 4).

Table 4. Comparative statistical analysis of % elastic fibres in area of inflammation, fine fibrous tubular area, keloid/papillary dermis border area and dense regular wavy area with apparently normal area of keloids

	Elastic fibres in normal area of keloids versus Elastic fibres in keloids in area of Inflammation	Elastic fibres in normal area of keloids versus Elastic fibres in keloids in fine fibrous tubular	Elastic fibres in normal area of keloids versus Elastic fibres in keloids/papillary dermis border	Elastic fibres in normal area of keloids versus Elastic fibres in keloids in dense regular wavy wide
Z	-1.580(a)	-2.803(a)	-.764(b)	-2.803(a)
Asymp. Sig. (2-tailed)	.114	.005	.445	.005

a Based on negative ranks.
b Based on positive ranks.
c Wilcoxon Signed Ranks Test

Differences in collagen fibre content between non-lesional dermis and fine fibrous tubular areas in keloids as well as between non-lesional dermis and areas of inflammation were statistically significant (Table 5 and Fig. 5).

Table 5. Comparative statistical analysis of % collagen fibres in area of inflammation, fine fibrous tubular area, keloid/papillary dermis border area and dense regular wavy area with apparently normal area of keloids

	Collagen fibres in normal area of keloids - Collagen fibres in keloids in area of inflammation	Collagen fibres in normal area of keloids - Collagen fibres in keloids in fine fibrous tubular	Collagen fibres in normal area of keloids - Collagen fibres in keloid/papillary dermis border	Collagen fibres in normal area of keloids - Collagen fibres in keloids in dense regular wavy wide
Z	-2.803(a)	-2.599(b)	-1.784(a)	-.051(a)
Asymp. Sig. (2-tailed)	.005	.009	.074	.959

a Based on negative ranks.
b Based on positive ranks.
c Wilcoxon Signed Ranks Test

The elastic:collagen fibre ratios were calculated from the statistical data on % collagen and % elastic fibres in keloids and non-lesional dermis (Table 6). The elastic:collagen fibre ratios were as follows: 1) 1:1.73 in the areas of inflammation, 2) 1:154 in fine fibrous tubular areas, 3) 1:5.8 in the keloid/papillary dermis border area, 4) 1:220 in dense regular wavy areas and 5) 1:8.1 in the dermis of non-lesional skin. In normal dermis the elastic fibre content is 2-5% 9 and collagen 71.9% 10, giving a proportional ratio range of 1:14 to 1:36.

Table 6: Comparative analysis of mean % elastic and collagen fibre content of keloids, non-lesional and normal dermis

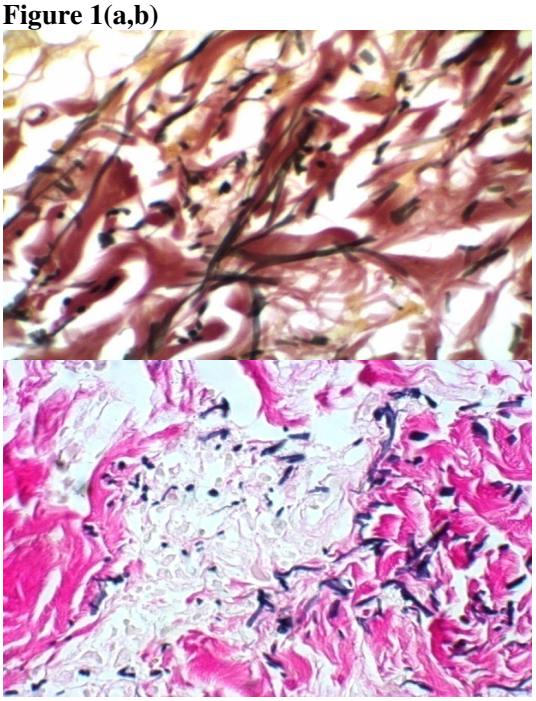
	Normal dermis	**Non-lesional dermis (100 fields)**	**Keloid/pap-illary dermis border (100 fields)**	**Areas of inflammation (100 fields)**	**Fibrous tubular areas (100 fields)**	**Dense regular wavy areas (100 fields)**
% elastic fibre content	2-5 (Gibson et al., 1965)	6.519	7.25	5.31	0.45	0.24
% collagen fibre content	71.9 (Neuman and Logan, 1950)	53.6	42.31	9.17	69.10	52.21
Proportional elastic fibre: collagen fibre ratio	1:14 to 1:36	1:8.2	1:5.8	1:1.73	1:154	1:220
Rating when compared with normal dermis % elastic fibre content		↑ ++	↑ ++	↑ +	↓ +++	↓ +++
Rating when compared with normal dermis %		↓ +	↓ ++	↓ +++	↓	↓ +

collagen fibre content						
Rating when compared with non-lesional dermis % elastic fibre content	↓ +		↑ +	↓ +	↓ +++	↓ +++
Rating when compared with non-lesional dermis % collagen fibre content	↑ ++		↓ ++	↓ +++	↑ ++	↓
Key: + mild, ++ moderate, +++ greatly ↑ Increase						
↓ Decrease						

When comparing the mean % elastic and collagen fibre content of normal dermis with that of lesional and non-lesional skin of patients with ear lobe keloids (Table 6), the following results were noteworthy:

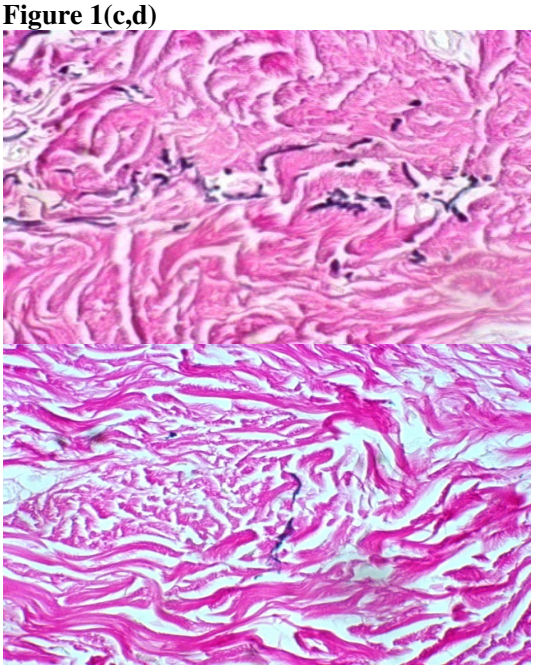
1. The mean % elastic fibre content of non-lesional dermis and the keloid/papillary dermis border were moderately increased
2. The mean % elastic fibre content within keloids were greatly decreased
3. The mean % collagen content of non-lesional dermis and all keloid areas were decreased
4. All proportional elastic fibre:collagen fibre ratios were outside the range of normal skin, being low in the keloid border regions and very high within keloid

Figure 1(a,b)



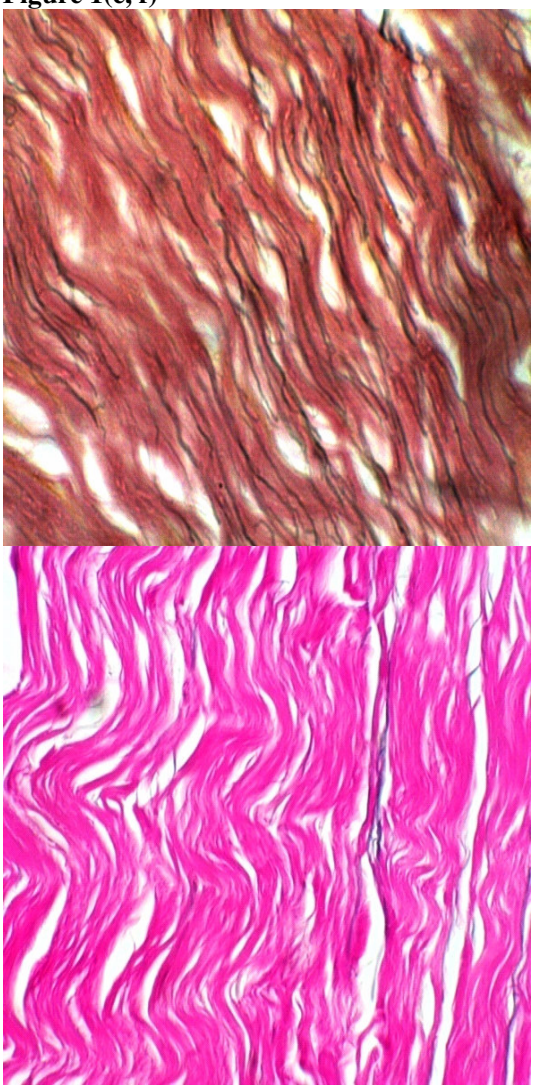
a. Scale bar = 20µm; Stain: EVG
b. Scale bar = 100µm; Stain: EVG

Figure 1(c,d)



c. Scale bar = 100µm; Stain: EVG
d. Scale bar = 100µm; Stain: EVG

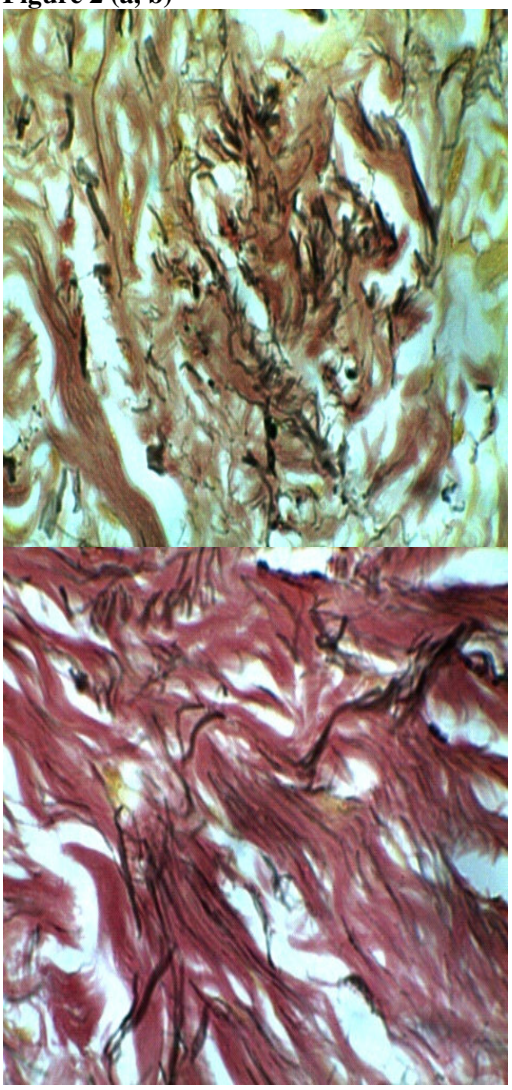
Figure 1(e, f)



e. Scale bar = 20µm; Stain: EVG
f. Scale bar = 100 µm; Stain: EVG

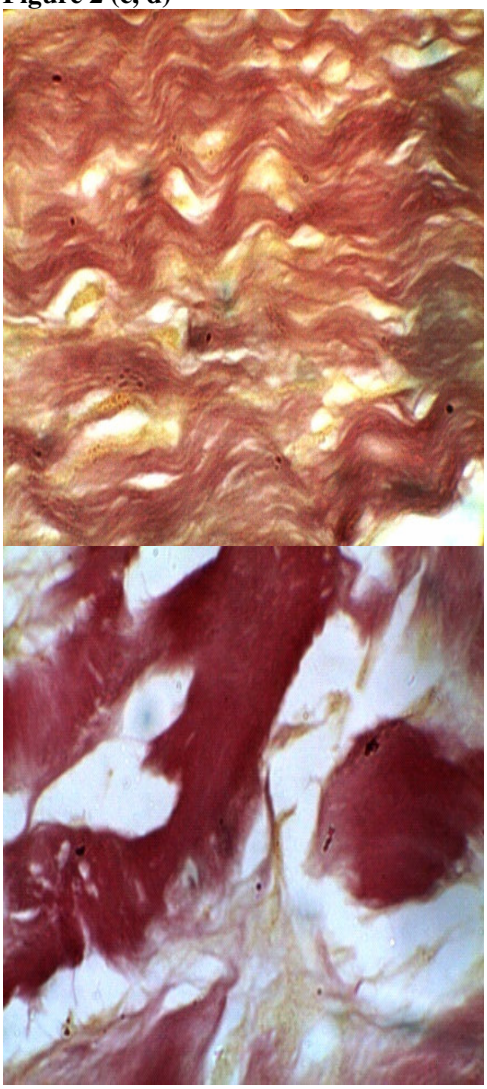
Figure 1. Photomicrograph of papillary dermis/keloid border showing: (a) long thick (green star), short stubby (blue arrows) and thin wispy (red arrow) elastic fibres. Note long elastic fibres oriented parallel to the long axis of collagen bundles (green arrows); (b) increase in elastic fibres adjacent to inflamed subpapillary plexus (yellow stars), few in area of inflammation (green stars) and increase in keloid side of the subpapillary plexus (violet line); (c,d,e,f) Fibrous tubular (c) and dense regular wavy (e) areas at the papillary dermis/keloid border showing abundant elastic fibres when compared with similar areas within keloids (d,f, respectively), with sparse, frail elastic fibres (green stars)**.**

Figure 2 (a, b)



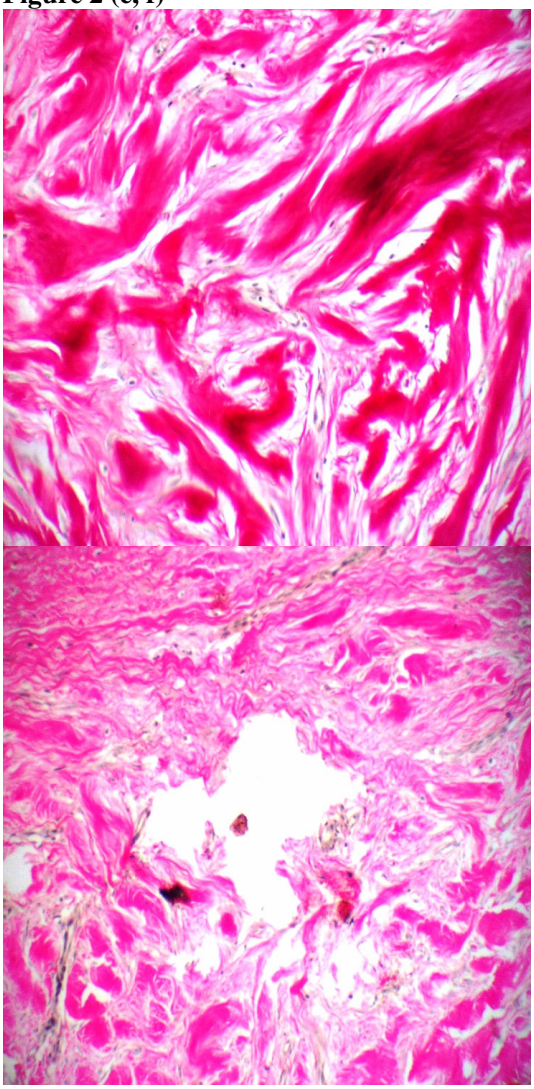
a. Scale bar = 100µm; Stain: EVG
b. Scale bar = 100µm; Stain: EVG

Figure 2 (c, d)



c. Scale bar = 20µm; Stain: EVG
d. Scale bar = 20µm; Stain: EVG

Figure 2 (e, f)



e. Scale bar = 100µm; Stain: EVG
f. Scale bar = 100µm; Stain: EVG

Figure 2. Photomicrograph of (a) Increased elastic fibres in non-lesional skin showing irregular (green stars), clumped (red stars) and paler staining (blue stars) forms; (b) Elastic fibres reoriented from a parallel (green stars) to perpendicular arrangement (blue arrows) to the length of collagen fibres and aggregate to form bands (green arrow) in the vicinity of ruptured tissue and disheveled , degenerate collagen and elastic fibres (yellow stars); (c, d) Fine fibrous cellular, and hyalinising collagen bundle areas showing occasional specs (green stars) and spots (yellow stars), respectively; (e) Blurred patches of elastin staining within HCB (blue stars) and (f) Ruptured tissue (green star) between WFFA (yellow star) and HCB area (blue stars).

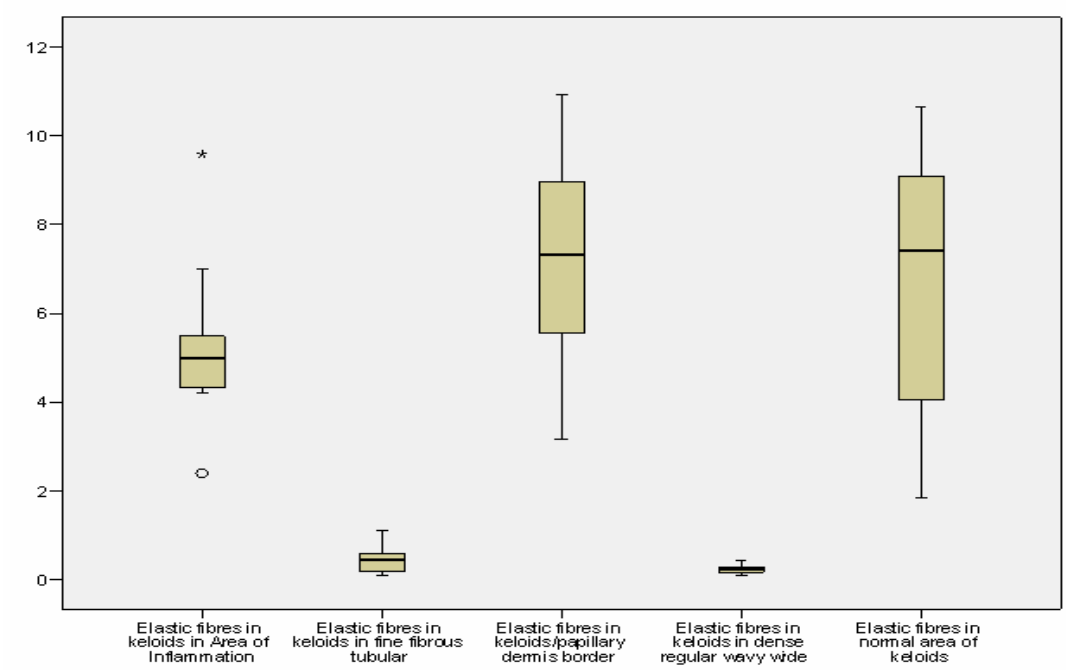


Figure 3. Elastic fibre content in area of inflammation, fine fibrous tubular area, keloid/papillary dermis border area, dense regular wavy area and apparently normal area of keloids.
Results are shown as mean percentage area of ten fields/specimen (n = 10). *p<0.05 compared with controls (normal area of keloids).

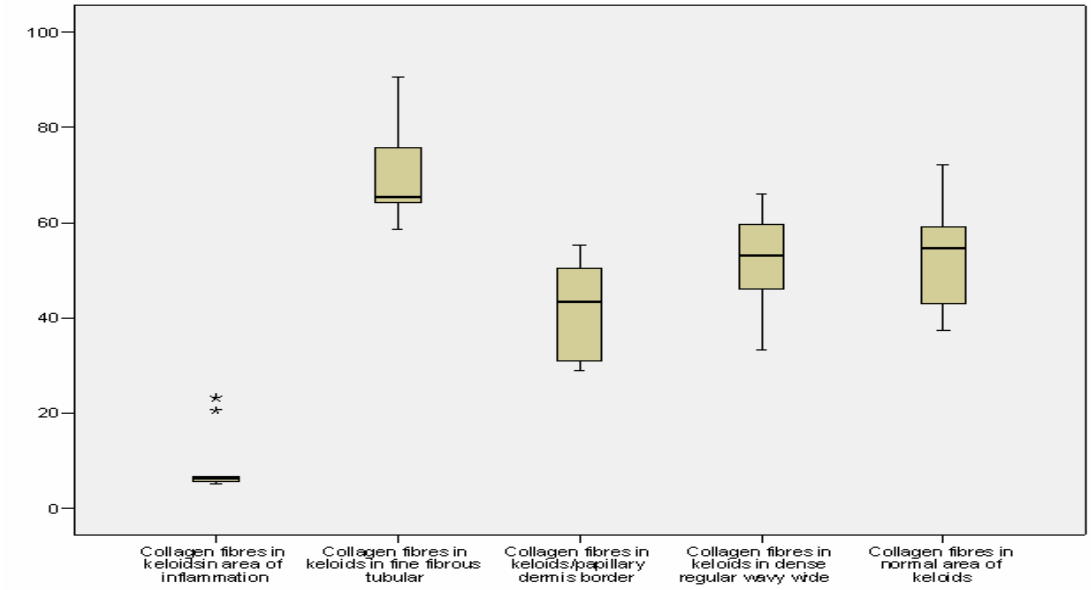


Figure 4. Collagen fibre content in area of inflammation, fine fibrous tubular area, keloid/papillary dermis border area, dense regular wavy area and apparently normal area of keloids. Results are shown as mean percentage area of ten fields/specimen (n = 10). **p<0.01 compared with controls (apparently normal area of keloids).

Discussion

Histomorphological and statistical analysis of the elastic fibre content in keloids showed that the highest concentrations were found at the keloid/papillary dermis border and non-lesional dermis. As the papillary dermis and non-lesional dermis adjacent to keloids are known to be normal with no evidence of scarring [11], it is anticipated that the elastic fibre content here is similar to that of normal unaffected skin where elastic fibres make up about 2-5% of the total volume of the dermis. In normal skin elastic fibres form an interconnecting network around collagen bundles to maintain normal skin tension by providing extensibility to accommodate deformation forces and elastic recoil to restore collagen to their original length after deformation9, provided that the strain among the collagen molecules is less than 3% when displacement of the collagen molecules is minimal [12]. Restoration of the collagen fibres to their original length also requires replacement of tissue fluid that was displaced during deformation; this permits associated collagen bonds to reorganize. In normal dermis fluid displaced into adjacent tissue compartments during deformation, is easily replenished by elastic recoil. In contrast, if elastic fibres are lacking, as in keloids, strain rapidly progress from the elastic to the plastic range, causing stiffening and permanent deformation of tissue and original tissue structure cannot be regained [4 9]. The higher than normal concentration of elastic fibres at the keloid/papillary dermis border and in non-lesional dermis may be a response to cope with traction forces exerted by keloid fibroblasts and resistance and shear forces wielded by firm inelastic keloids. The lower than normal concentration of collagen fibres quantified at the keloid/papillary dermis border and in non-lesional dermis was contrary to the proven finding that keloid fibroblasts overproduce collagen; this may be explained by gross tissue compression that occurs during permanent deformation. Lengthening and eventual rupture may also occur during permanent deformation. Connective tissue becomes progressively stiffer with lengthening and collagen fibres become aligned in the direction of the stretching; this is exhibited by keloids [13]. At a molecular level, in irreversibly deformed tissue, collagen cross-links are disrupted leading to ruptured collagen fibres, tissue failure and obstruction of the force-relaxation response [4] when stress cannot be absorbed and is transferred to adjacent areas. The compression of collagen fibres into thick bundles and elastic fibres into thick bands observed at the keloid border may be caused by stress transferred in this way. Areas of tissue failure with disheveled and degenerate collagen and elastic fibres may also be caused in this way.

The elastic:collagen fibre ratios of keloids and non-lesional skin are higher than that in normal skin; being 1.73 and 2.4 times higher in non-lesional dermis and the keloid/papillary dermis border respectively. This alters connective tissue biomechanics; the increase in collagen fibres may be a fibroblastic response to provide increased tensile strength to cope with stress transferred from the inflexible keloid. Non-lesional dermis and the keloid/papillary dermis border form the upper and lateral borders of the keloid and therefore, are the first areas around the keloid, to endure the consequences of transmitted compressive forces. Fibroblasts exposed to increased mechanical force showed stimulation of collagen and elastin synthesis [14]. The increased elastic fibre production at the upper and lateral keloid borders and the general increased collagen fibre production in keloids may thus be a consequence of cumulative forces: gravitational, displacement (exerted by rigid keloid tissue), traction (exerted by wound healing fibroblasts), shear, resistance and friction forces (wielded by regularly aligned and multi-directionally orientated dense collagen bundles). Shear, resistance and friction force may further be increased by movement of the matrix which occurs when keloid fibroblasts cannot resist traction forces [5]. In areas of inflammation the decreased elastic fibre:collagen fibre ratio was influenced by the high elastic fibre content. This was a consequence of the absence of elastase-producing neutrophils (infiltrate comprised lymphocytes and plasma cells [13]) and collagenase production mediated by inflammatory agents of wound healing fibroblasts [15].

In contrast with the large number of elastic fibres in the keloid/papillary dermis border and non-lesional dermis, the keloid proper exhibited a lack or absence of elastic fibres. Elastic fibres impart the properties of stretch and recoil to tissue, in their absence, the accumulative effect of deformation forces is compaction of fibres, a stiffer extracellular matrix, increased rigidity and alteration of shape and dimensions of the tissue. Increased rigidity alters matrix mechanics leading to activation of integrins, which promotes mitogenic signaling and cell contractility [16], further increasing matrix stiffness. This creates a positive feedback loop [17] that continually stimulates cell contractility, increasing stress, which, because of the lack of elasticity, progresses to plasticity stress and consequent permanent deformation. Evidence of stress reaching breaking point was found in ruptured tissue generally located below the papillary dermis/keloid border; this corresponded with the first part of the keloid with minimal elastic fibres, to encounter, additionally, the effects of atmospheric pressure (force of 1 kilogram per square centimeter). This and opposing upward

pressure rebound by the keloid, compress microvessels [2] leading to hypoxia and ischaemia.

The role of unrestrained stress in the pathogenesis of keloids is supported by innovative technical studies where custom-made silicon ear moulds were developed to apply homogeneous pressure to the ear [18]. There was a > 50% reduction in the size of keloids fitted with pressure devices made from Zimmer splints when assessed at one year 6. The success of pressure therapy was attributed to exacerbation of the previously existing hypoxic condition, reduction in number of myofibroblastic cells and reorientation of disorganized collagen to a parallel arrangement similar to that found in normal wound healing [19]. Pressure therapy also compensates for elastic insufficiency, as shown by the success of elastic bandages in the treatment of hypertrophic scars [22]. Zimmer splints abolish the need for elasticity by maintaining homogenous pressure.

This study is the first to confirm that elastic fibres are deficient in keloids. Reinstating elastic properties in keloids could gradually decrease the size of keloids, perhaps by conversion to the normal wound healing process. Treatment modalities for keloids should therefore include pressure therapy and research in the field of pressure therapy biomechanics, methodologies and devices should be continual.

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