










Is “Initial Size of the Graft the Real Culprit behind Primary Contraction of Full-Thickness Skin Graft”?—A Cross-Sectional Study

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Arch Plast Surg 2023;50:106–115.

Abstract

Background Primary contraction of full-thickness graft has been traditionally quoted to be 40%. There are lacunae in literature to elaborate on the factors influencing it ever since.

Methods About 75 subjects who underwent full-thickness grafting procedures to resurface small defects were included in the study. The initial and final graft dimensions after primary contraction were traced on X-ray templates and the percentage of contraction was evaluated using the graphical method. This was further correlated with age, collagen, elastic matrix metalloproteinases-1 (MMP-1) and -2 content along with dermal thickness of the skin specimen sent from the graft.

Results The primary contraction of the graft had a very significant correlation only with the initial size of graft harvested with a linear regression of 33.3% and a Spearman’s correlation of 0.587 significant at a *p*-value of 0.001.

Conclusion This study though preliminary tries to highlight an important factor that primary contraction of grafts is a physical phenomenon independent of its contents like collagen, elastin, or MMP-1 and -2 or age and dependent on its initial size of harvest instead.

Keywords

- ▶ skin grafts
- ▶ elastin fibers
- ▶ collagen
- ▶ dermal thickness

Introduction

A full-thickness skin graft is used by reconstructive surgeons for resurfacing small defects. The main advantage of these grafts over split-thickness skin grafts is that the full-thickness grafts have a better color match and are hence cosmetically appealing. Due to lesser secondary graft contraction, the full-thickness grafts yield better functional results too.

The main disadvantage of full-thickness skin grafts, however, is the increased primary contraction (▶ **Supplementary Figs. S1, S2, S3**, available in the online version only) when compared with split skin graft which results in smaller effective graft harvests. Though traditionally quoted as 40% primary contraction rate for these grafts, further search in the literature revealed very sparse information regarding

received
November 12, 2021
accepted after revision
March 31, 2022

DOI <https://doi.org/10.1055/s-0042-1756297>.
eISSN 2234-6171.

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the same. The factors like age of the patient, dermal thickness, elasticity of skin (determined by the total elastin content), MMP-1 (matrix metalloproteinases-1) and -2 have not been considered in any of the studies so far. So the article aims to explore these and any other factors if present to understand their role in the primary contraction of full-thickness skin grafts.

Materials and Methods

Seventy-five subjects planned for small defect resurfacing with full-thickness skin grafts were selected on random for the study after ruling out any congenital skin conditions. Institute ethical clearance was sought (IM/RC108/2016/33) for the study and after taking written informed consent from the individual patients, full-thickness skin grafts were harvested for their groin region of sufficient size to resurface their skin defects resulting from correcting their deformities like syndactyly, finger contractures, etc. An X-ray template was designed initially based on the resulting skin defect to be resurfaced. This was placed in the groin region and the outline was traced. Infiltration with 2% lidocaine with adrenaline was done. The graft was harvested by incising on the marked region. De-fattening of the graft was done except for the tip of the graft on one end which was chosen for histopathology. The graft was kept wrapped in saline-soaked gauze. After 15 minutes the graft was then spread over another X-ray template, its outline traced, and the template cut accordingly. The pre- and post-harvest X-ray templates were collected and named according to their hospital number. Such pairs were traced on graph paper and their areas were assessed. Thus, the graft contraction was assessed using the formula:

Primary contraction = $\frac{\text{initial area} - \text{final area}}{\text{initial area}} \times 100$ (→ Fig. 1A, B).

The tip of the graft which was not de-fattened was excised and sent for histopathology to assess the dermal thickness, elastin, and collagen content. The elastin fibers and collagen content were evaluated on Verhoff von Gieson's stain. The elastin fibers were stained black whereas collagen was stained pink (→ Fig. 2). A photomicrograph of a constant area (0.0625 mm^2) was obtained for all the cases. The scale was set for a known measurement on the ImageJ software. The photographs were evaluated on the software by splitting the color channels and adjusting the threshold for color. The area-based analysis was used to quantitate the region of interest.¹

Immunohistochemistry (IHC) for MMP-1 and MMP-2 (Isotype: IgG1, Source: mouse from Chongqing Biospes) was simultaneously performed on the sections. IHC was done on 0.1% poly-L-lysine-coated slides by manual method. The slides were stained by DAB (3,3'-diaminobenzidine) and counterstained by hematoxylin. (→ Fig. 3A-D)

Positive controls on human placenta sections were put along with the batches of MMP-1 IHC, whereas breast carcinoma acted as a positive control for MMP-2.

Negative controls were also run with each batch of IHC.

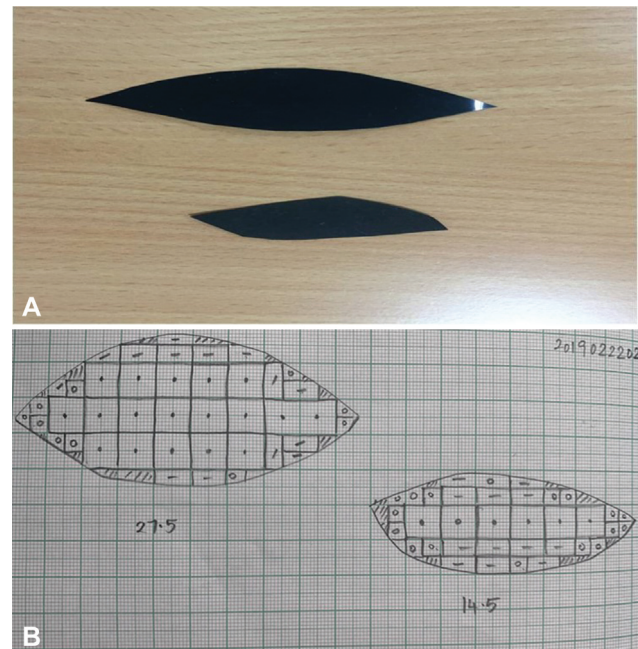


Fig. 1 (A) X-ray template of pre- and post-contracted graft tracings. (B) Pre- and post-contracted graft tracings of X-ray templates over graph paper.

MMP-1 was evaluated in the cytoplasm whereas MMP-2 expression was noted in the cytoplasm and extracellular matrix.

The expression of the antibodies was quantified on ImageJ software by evaluating the area of cells stained in the hotspot. The slides were scanned at $\times 400$ magnification and a hotspot of IHC expression was selected. A photomicrograph of a constant area (0.0625 mm^2) was obtained for all the cases. The scale was set on the software for a known measurement. The photographs were processed for the percentage area expression of DAB on ImageJ software using the color deconvolution plugin.² Each case was evaluated twice by the first author only and the average value was noted as percentage area and absolute value in mm^2 .

The documented values were subjected to statistical analysis. Percentage contraction of the graft was correlated

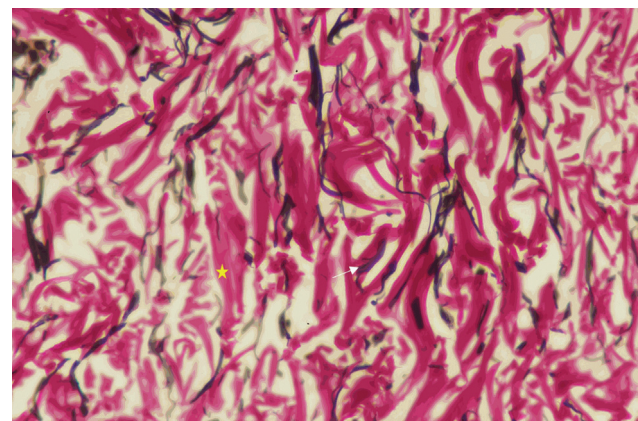


Fig. 2 Collagen and elastin staining with VVG stains and viewed using ImageJ software.

- Collagen: ★
- Elastin: →

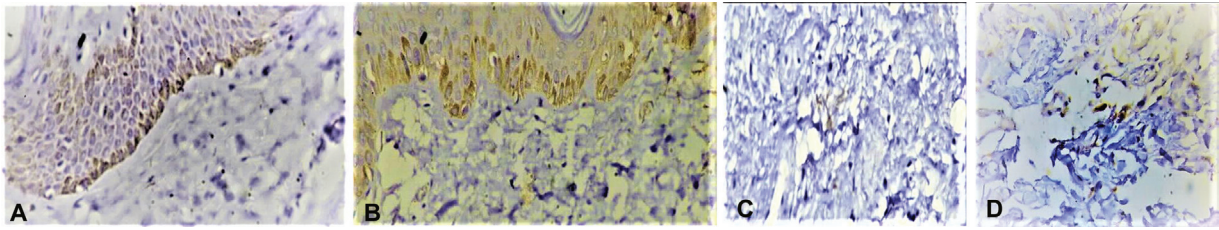


Fig. 3 Immunohistochemistry. (A) MMP-1 staining of epidermis. (B) MMP-2 staining of epidermis. (C) MMP-1 staining of the dermis. (D) MMP-2 staining of the dermis. MMP-1, matrix metalloproteinase-1.

with other factors like age, dermal thickness, collagen, elastin content along with MMP-1 and -2 using Spearman's correlation coefficient and regression analysis was derived. Any other factor if found significant was also correlated accordingly and the results were interpreted appropriately.

Results

Out of the initially recruited 75 patients, samples of two patients were discarded due to irregular processing. Seventy three patients were included in this study. The age groups ranged from 9 months to 60 years with an average age of 20 years. The male to female ratio was 45:28. The predominant primary pathology for which full-thickness skin graft was considered was post-burn contracture of fingers followed by syndactyly and post-traumatic contracture. All the full-thickness grafts were harvested from the groin region. The size of the grafts ranged from 2 to 36 cm² (average = 13.14 cm²). Though the average percentage of primary contraction was 41%, the rate of contraction varied from one individual to another. (► **Table 1**).

On correlating the primary contraction rate with parameters like age, dermal thickness, MMP-1 and -2 of epidermis and dermis, and elastin content, the following were the observations. No significant correlation was found between primary contraction and factors like age, dermal thickness, collagen, elastin, MMP-1 dermis, and 2 epidermis and dermis. But surprisingly there was a very significant correlation between the initial size of the graft and the graft contraction with a *p*-value of 0.000. In addition there was also a correlation between MMP-1 epidermis with a *p*-value of 0.033 (<0.05). On further analysis, linear regression of initial factor was found to be 33.3%. This tells us that for every unit increase in the area of the graft harvested, there will be an increase in the graft contraction rate by 1.257% (► **Table 2**, ► **Figs. 4** and **5**).

The graft contraction rate did not reveal any significant correlation with gender differences using the independent *t*-test. Further correlations of subsets like the correlation between dermal thickness and collagen, elastin or MMP-1 and -2 as well as that between the initial size of the graft between collagen elastin and MMP-1 and -2 did not yield any significant result.

Discussion

Literature reveals several articles published on the secondary contraction of grafts on account of the clinical relevance.³ But we rarely find any study regarding the primary contraction

of grafts. The primary contraction of grafts has been traditionally attributed to the change in physical dimensions of graft owing to the dermal components including collagen, elastin, and glycosaminoglycan.

The primary contraction was demonstrated initially by Dupuytren's⁴ and Langer.⁵ In the original study conducted by Davis and Kitlowski,⁶ the primary contraction of full-thickness skin graft was quoted as 43.6% on an average. The authors did not find any correlation between the different ages and the rate of contraction. In addition, they noticed that this rate of contraction decreased to 24.86 and 11.26% as the thickness of the graft decreased from mid thickness split skin graft to thick Ollier-Thiersch graft. By this, they concluded that the rate of contraction varied positively with the thickness of the dermis. Though Davis emphasized that an elastic substance is responsible for the contractility of skin and it increases with the increase in depth, it was Pinkus⁷ who considered the network of elastic fibers as a cutaneous skeleton that counteracts the normal shrinking capacity of the fibrillary matrix and changes of the skin during a stretch, pressure and, rotation. These studies also indicated that the primary contraction of grafts was independent of the direction of harvest and that it was uniform in all directions.

Some studies reviewed in literature have suggested ways of reducing the secondary contraction of full-thickness using a dermal template such as Integra⁸ but no such studies have been performed to determine factors to reduce primary contraction of such grafts.

The importance of primary contraction of the graft is understood by the observation of skin retraction following trivial trauma. Despite no effective loss of skin, there is still a defect observed if the wound is unattended. This mechanism, though indirectly understood, has found its clinical applications such as top closure system in the management of wounds.⁹ Here the authors have tried to realize that if an equal or slightly more amount of tension is applied to skin margins in the opposite direction of retraction, then the defect of the wound created can be decreased and this maneuver subsequently can promote primary closure of such defects. They have further extrapolated and used this technique to close actual defects of skin produced due to excision of tumors or trauma.

The experiments conducted by Davis and Kitlowski suggested that the thickness of the skin graft correlates with primary contraction. Though this experiment indirectly tried to suggest that the quantity of collagen and elastin is responsible for the primary contraction, no effective

Table 1 Table stating data to correlate age and primary contraction

	Shapiro-Wilk		
	Statistic	df	p-Value
Age in months	0.888	73	0.0001
Initial size	0.899	73	0.0001
Final size	0.971	73	0.088
Percentage of + 1:28 contraction	0.964	73	0.036
Collagen area	0.912	73	0.0001
Collagen percentage	0.898	73	0.0001
Elastin area	0.791	73	0.0001
% Area elastin	0.788	73	0.0001
Dermis thickness	0.868	73	0.0001
Mmp 1 epi	0.675	73	0.0001
Mmp1 epi %	0.691	73	0.0001
Mmp1 der	0.669	73	0.0001
Mmp1 der%	0.696	73	0.0001
Mmp2 epi	0.632	73	0.0001
Mmp2 epi %	0.637	73	0.0001
MMP-2 der	0.757	73	0.0001
MMP2 der %	0.771	73	0.0001
Nonparametric correlations			
Spearman's rho		Percentage of + 1:28 contraction	
Age in months	Correlation coefficient	0.281*	
	p-Value	0.016	
	N	73	
Initial size	Correlation coefficient	0.587**	
	p-Value	0.000	
	N	73	
Final size	Correlation coefficient	0.140	
	p-Value	0.237	
	N	73	
Collagen area	Correlation coefficient	0.020	
	p-Value	0.867	
	N	73	
Collagen percentage	Correlation coefficient	0.058	
	p-Value	0.626	
	N	73	
Elastin area	Correlation coefficient	-0.032	

(Continued)

Table 1 (Continued)

	Shapiro-Wilk		
	Statistic	df	p-Value
	p-Value	0.791	
	N	73	
% Area elastin	Correlation coefficient	-0.024	
	p-Value	0.843	
	N	73	
Dermis thickness	Correlation coefficient	0.056	
	p-Value	0.637	
	N	73	
Mmp 1 epi	Correlation coefficient	0.250*	
	p-Value	0.033	
	N	73	
Mmp1 epi %	Correlation coefficient	0.271*	
	p-Value	0.020	
	N	73	
Mmp1 der	Correlation coefficient	0.054	
	p-Value	0.650	
	N	73	
Mmp1 der%	Correlation coefficient	0.016	
	p-Value	0.895	
	N	73	
Mmp2 epi	Correlation coefficient	0.166	
	p-Value	0.160	
	N	73	
Mmp2 epi %	Correlation coefficient	0.147	
	p-Value	0.214	
	N	73	
MMP-2 der	Correlation coefficient	0.132	
	p-Value	0.266	
	N	73	
Mmp2 der %	Correlation coefficient	0.155	
	p-Value	0.190	
	N	73	
Nonparametric correlations			
Spearman's rho			Dermis thickness
Age in months			0.260*

(Continued)

Table 1 (Continued)

	Shapiro-Wilk		
	Statistic	df	p-Value
	p-Value		0.446
	N		73
MMp2 epi %	Correlation coefficient		0.155
	p-Value		0.189
	N		73
MMP-2 der	Correlation coefficient		-0.165
	p-Value		0.164
	N		73
MMp2 der %	Correlation coefficient		-0.160
	p-Value		0.176
	N		73
Nonparametric correlations			
Spearman's rho			Initial size
Dermis thickness	Correlation coefficient		0.156
	p-Value		0.188
	N		73
Age in months	Correlation coefficient		0.517**
	p-Value		0.000
	N		73
Final size	Correlation coefficient		0.838**
	p-Value		0.000
	N		73
Collagen area	Correlation coefficient		0.090
	p-Value		0.449
	N		73
Collagen percentage	Correlation coefficient		0.064
	p-Value		0.589
	N		73
Elastin area	Correlation coefficient		-0.018
	p-Value		0.883
	N		73
% Area elastin	Correlation coefficient		-0.010
	p-Value		0.935
	N		73
MMP 1 epi	Correlation coefficient		0.280*

Table 1 (Continued)

	Shapiro-Wilk		
	Statistic	df	p-Value
	Correlation coefficient		
	p-Value		0.026
	N		73
Initial size	Correlation coefficient		0.156
	p-Value		0.188
	N		73
Final size	Correlation coefficient		0.131
	p-Value		0.268
	N		73
Collagen area	Correlation coefficient		0.266*
	p-Value		0.023
	N		73
Collagen percentage	Correlation coefficient		0.196
	p-Value		0.097
	N		73
Elastin area	Correlation coefficient		-0.309**
	p-Value		0.008
	N		73
% Area elastin	Correlation coefficient		-0.302**
	p-Value		0.009
	N		73
MMp 1 epi	Correlation coefficient		0.181
	p-Value		0.126
	N		73
MMp1 epi %	Correlation coefficient		0.222
	p-Value		0.059
	N		73
MMp1 der	Correlation coefficient		-0.008
	p-Value		0.950
	N		73
Mmp1 der%	Correlation coefficient		0.089
	p-Value		0.453
	N		73
MMp2 epi	Correlation coefficient		0.091

Table 1 (Continued)

	Shapiro-Wilk		
	Statistic	df	p-Value
	p-Value		0.016
	N		73
MMp1 epi %	Correlation coefficient		0.339**
	p-Value		0.003
	N		73
MMp1 der	Correlation coefficient		0.013
	p-Value		0.912
	N		73
Mmp1 der%	Correlation coefficient		0.030
	p-Value		0.800
	N		73
MMp2 epi	Correlation coefficient		0.293*
	p-Value		0.012
	N		73
MMp2 epi %	Correlation coefficient		0.327**
	p-Value		0.005
	N		73
MMP-2 der	Correlation coefficient		0.076
	p-Value		0.524
	N		73
MMp2 der %	Correlation coefficient		0.098
	p-Value		0.409
	N		73
Percentage of +1:28 contraction	Correlation coefficient		0.587**
	p-Value		0.000
	N		73

*correlation is significant at the 0.05 level (2-tailed).

**correlation is significant at the 0.01 level (2-tailed).

evidence could be retrieved from the existing literature. Our study similarly could not find any significant correlation between the primary contraction and collagen, elastin content, or MMP-1 and -2.

The network of elastic fibers of the dermis was determined as the factor responsible for shrinkage of grafts by Ragnell.¹⁰

Dermal thickness is normally complemented by the blood cells which add volume to the ground substance and thus add to the thickness, viscoelasticity, and resilience of skin other than collagen and elastin. But after harvesting a skin graft

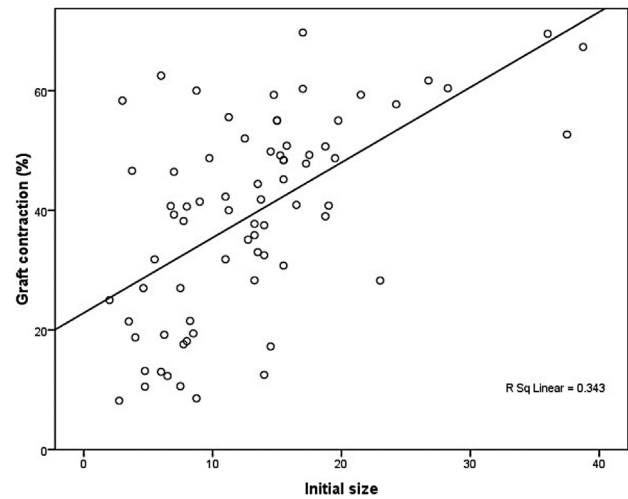


Fig. 4 Correlation between graft contraction and other parameters.

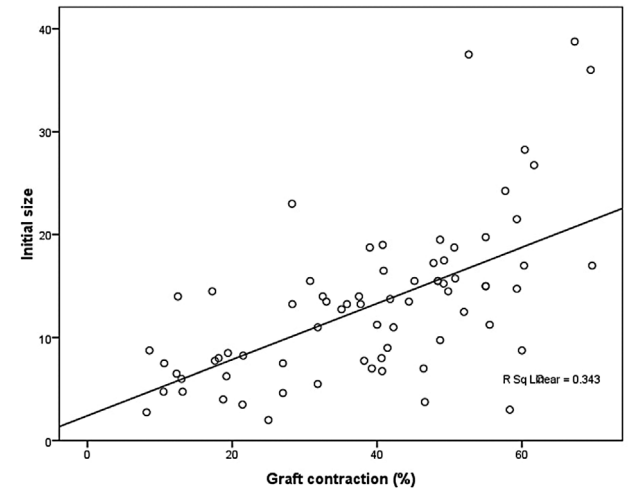


Fig. 5 Linear regression of graft contraction with an initial size of the graft.

due to the lack of circulation, the thickness of the dermis calculated will never reflect the true thickness in vivo due to the loss of this fluid component of the dermis. Hence the lack of correlation between the dermal thickness and graft contraction in our study probably has less significance as the assessment was done in vitro and hence the thickness may be used only to quantify the collagen and elastin content of that part of the skin graft.

Studies on intrinsic aging show rarefied collagen and elastin fibers. They also reveal decreased production of collagen type 1 fibers, degeneration of oligosaccharide in addition to elastin, fibrillin, and collagen that influences the ability of the skin to retain bound water. Although based on these studies, we expect that the dermal thickness would decrease with age and there would be more degeneration or elastosis with aging, and hence would lead to decrease in primary contraction, our study on the contrary showed no correlation of age with primary contraction. In our study since we are comparing the original and final size of the same

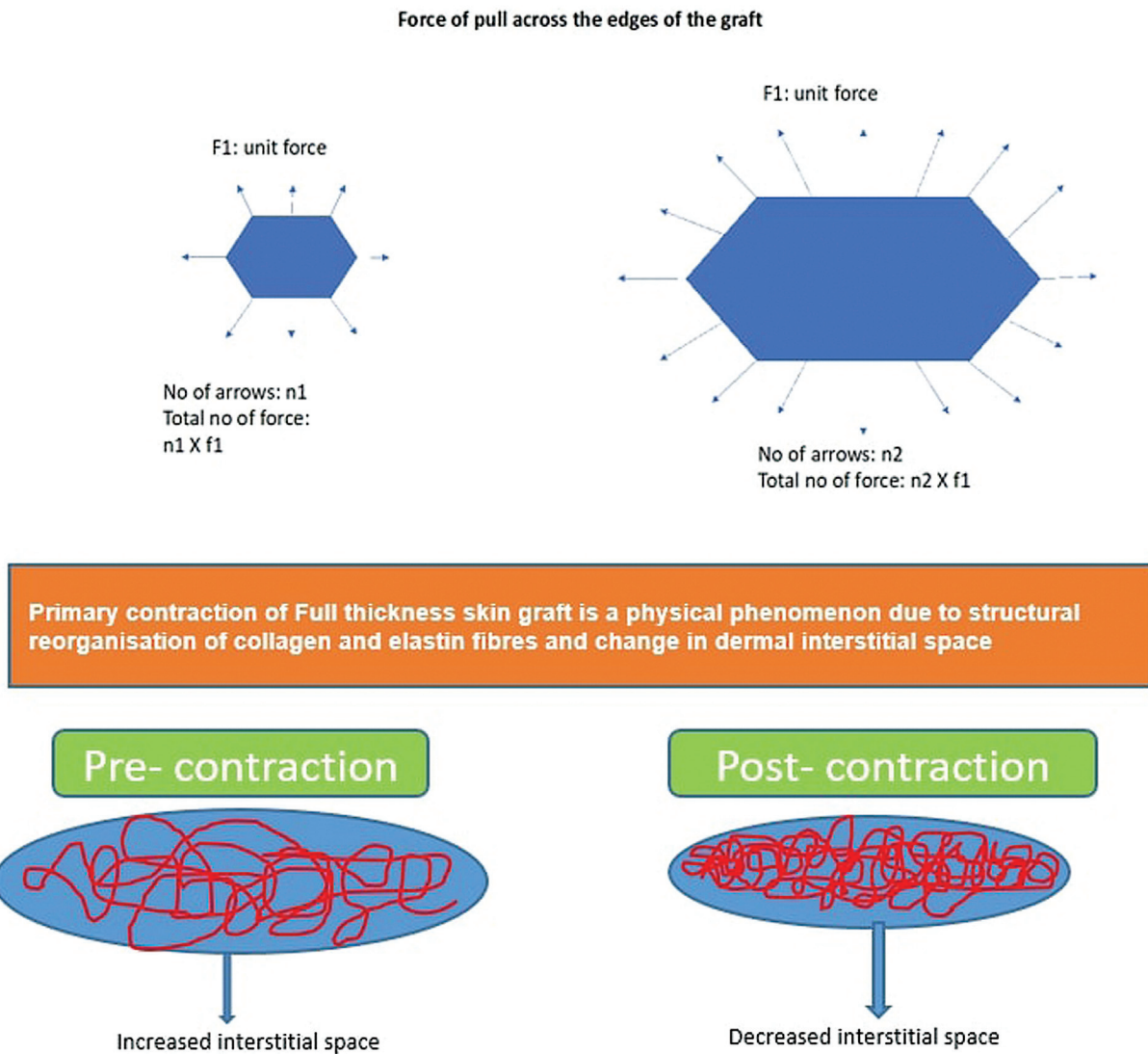


Fig. 6 Graphical abstract—schematic diagram to show the difference of pull by unit force on different sizes of graft and the resultant elastic recoil.

piece of skin harvested from a particular sample, the control factor is the same with regards to the percentage of collagen, elastin, etc. Hence the lack of correlation between age and contraction rate can be explained.

The only factor in our study that showed a significant correlation between the primary contractions was the initial size of graft harvest. Considering the absence of a relation between dermal content and primary contraction, the only possible explanation for the following result may be attributed to the physical properties of the skin.

The creep mechanism is one important property of skin. Creep and skin viscoelasticity property has been attributed to the cross-linking of collagen peptide chains which provide for the resiliency of skin when subjected to stress and shear deformation. Though the literature dealing with this phenomenon does not talk about basic resting stress that exists between cross-linking polymers of collagen when no deformation occurs, we can understand that the skin of a normal

person at rest will have a basic force that prevents the skin from wrinkling or contracting (even though not stretched out).¹¹

Consider a piece of skin is being attached on all sides by the cross-linking peptides of dermal collagen. The moment a skin graft is harvested, the existing elastin and collagen inside the harvested part of the skin crinkle due to lack of attachment as well as due to the new space between the peptides which was previously occupied by the interstitial fluid¹² component in vivo.

The observation that the larger dimension of graft contracts more can also be explained similarly. Consider two different dimensions of skin graft being stretched by equal unit force at equal intervals at their edges. Since the circumference of the larger dimension of graft would be more than the smaller ones it is only natural to decipher that the net total force exerted on a larger graft would be more than that of a smaller graft. Once the graft is harvested and these

Table 2 Value of R^2 to predict the graft contraction rate

S. no	UHID/CR	Age	Gender	Site	Initial size	Final size	Percentage of contraction
1	515887	60	M	Groin	23	16.5	28.26
2	538447	60	M	Groin	36	22.5	62.5
3	511347	56	F	Groin	13.5	7.5	44.4
4	516776	24	F	Groin	19.5	10	48.7
5	503799	15	M	Groin	15.5	8	48.38
6	532205	14	F	Groin	2	1.5	25
7	150433	10	M	Groin	16.25	7.75	52.3
8	631236	35	M	Groin	15.5	8.5	45.16
9	625567	28	M	Groin	15	6.75	55
10	20170018853	30	F	Groin	17.25	9	47.8
11	526569	30	F	Groin	14	8.75	37.5
12	611465	4	M	Groin	6.75	3.5	40.7
13	20170068581	3	F	Groin	4.625	3.375	27
14	20180018239	21	M	Groin	17.5	8.25	9.25
15	20170010837	23	M	Groin	13.25	9.5	28.3
16	20180005232	14	M	Groin	5.5	3.75	31.8
17	20180010926	20	M	Groin	6	3.25	45.8
18	20180090891	13	F	Groin	13.5	7.75	5.75
19	20180083306	4	M	Groin	14	12.25	12.5
20	20180072901	20	F	Groin	14	5.25	62.5
21	20170007195	43	F	Groin	28.25	14	50.4
22	20180081469	15	M	Groin	17	6.75	60.3
23	201831875	55	M	Groin	19.75	17.5	11.39
24	20180211985	1	M	Groin	4	3.25	18.75
25	20180115248	19	M	Groin	21.5	8.75	59.3
26	20180175570	18	F	Groin	6.25	3	52
27	20180183188	36	M	Groin	9	5	44.44
28	201802079	8	M	Groin	8	4.75	40.62
29	20190005753	4	F	Groin	12.75	7	45.09
30	201809404	17	F	Groin	15.25	7.75	49.18
31	20190789554		M	Groin	19	11.25	40.78
32	603070	5	M	Groin	11	5.25	52.27
33	20190180433	17	M	Groin	38.75	15	61.29
34	20190173490	10	M	Groin	7.75	3.75	51.61
35	20190058900	53	M	Groin	11.25	6.75	40
36	20190197014	20	F	Groin	8.25	4	51.51
37	20190222021	2 mo	F	Groin	3.5	2.75	21.42
38	20170077379	9	F	Groin	3.75	2	46.6
39	20190169211	19	F	Groin	8.75	3.5	60
40	20190009722	4	F	Groin	3	1.25	58.33
41	20190052196	6	M	Groin	9.75	5	48.71
42	20190229909	9 mo	M	Groin	6.25	4	36
43	20190193953	59	M	Groin	13.75	10.75	21.81

(Continued)

Table 2 (Continued)

S. no	UHID/CR	Age	Gender	Site	Initial size	Final size	Percentage of contraction
44	20190200948	17	M	Groin	37.5	17.75	52.66
45	20190030974	28	M	Groin	24.25	10.25	57.7
46	20190193555	14	F	Groin	4.75	1.75	63.15
47	20190230024	34	M	Groin	13.25	8.5	35.84
48	20190210098	40	F	Groin	15.75	7.75	50.79
49	20190216356	1	M	Groin	16.5	9.75	40.9
50	20190034054	19	M	Groin	13.25	8.25	37.73
51	20180155307	10	F	Groin	5	4	20
52	20180057563	1	M	Groin	9	8.75	2.7
53	20180160651	0.08	M	Groin	7	3.75	46.42
54	20190036884	3	M	Groin	2.75	2.25	18.18
55	20180002792	10	F	Groin	15.5	9.5	30.76
56	2018021687	25	M	Groin	12.5	6	52
57	20190006482	35	M	Groin	8.5	6	29.41
58	521489	10	M	Groin	14	6.5	53.5
59	53676	23	M	Groin	21	9.75	53.5
60	20190052893	1	F	Groin	7.5	7	6.6
61	20190044371	19	M	Groin	8	3.75	53.12
62	20190028384	18	F	Groin	26.75	10.25	61.68
63	20190110981	12	M	Groin	15.75	11	30.15
64	20190117537	5	F	Groin	11.25	5	55.55
65	20190058426	52	F	Groin	18.75	15	20
66	20180179536	20	F	Groin	6	2.25	62.5
67	20180095070	10	M	Groin	14.5	7	49.82
68	20190113519		F	Groin	15.5	8	48.38
69	2019010512	2.5	M	Groin	11	7.5	31.81
70	20190064794	67		Groin	14.5	12	17.24
71	20190129777	18	M	Groin	17	6	69.7
72	20190174529	6	F	Groin	14.75	6	59.3
73	20180160651	0.08	M	Groin	7	3.75	46.42

attachments are released, the amount of recoil would also be anticipated to be more in the larger graft than the smaller ones (► Fig. 6). This is also derived from the observation that a large fragment of skin has more stiffness than a smaller fragment.¹³ The force of recoil would be equal to K times x , where K is the size of the graft while x is the deformation. So as the K increases, the force also increases. However, further studies that measure the interstitial space before and after contraction would be necessary to prove this hypothesis. This is in consensus with the study by Braza and Fahrenkopf¹⁴ which states that the elastic recoil of elastin fibers is probably responsible for the primary contracture.

The present study is one of the first kind which correlates multiple parameters like age, gender, collagen, elastin content, MMP-1, and -2 with primary contraction of full-thickness skin

grafts. The study emphasizes the role of the initial size of skin graft in determining the percentage of contraction.

This study concludes that the primary contraction of full-thickness skin graft is related to the physical properties of the skin. Unlike the existing literature, the study reveals no correlation of primary contraction with other factors like age, dermal thickness, collagen, elastin, or MMP-1 and -2.

Authors' Contributions

All the authors have been involved in data collection, interpretation, and analysis of data. The first author has also been involved in the conceptualization of the study in addition.

Note

The paper was presented as an oral presentation at APSICON November 2018 National Conference. All schematic figures have been made by the first author.

Ethical Approval

This study was granted ethical approval under IM/RC108/2016/33.

Funding

The project has been funded by an intramural grant: IM/RC108/2016/33.

Conflict of Interest

None declared.

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