

## Non-invasive *In vivo* Techniques to Differentiate Photodamage and Ageing in Human Skin

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**It is important to differentiate skin changes due to the intrinsic ageing process from those due to chronic photodamage in the development of therapies to assist the latter condition, and in this study we have used instrumental techniques to differentiate between changes in a range of properties of skin due to ageing and those due to photodamage, especially with regard to elasticity.**

We measured three sites of differing sun exposure in a group of younger and in a group of older subjects. A pulsed A-scan ultrasound system was used to measure skin thickness, and a uniaxial extensometer was employed to assess elastic properties. Skin surface roughness measurements were made using silicone rubber impressions and a stylus profilometer.

We demonstrated significant differences in skin roughness between young and old subjects at every site and differences between sun-exposed and sun-protected sites only in the older group. Parameters of the elastic properties of skin differed between the groups, and also between sites of most different sun exposure.

The uniaxial extensometer can demonstrate a loss of the skin's elasticity predominantly by photodamage, and the roughness of the skin surface can be shown to increase mostly by chronological ageing but to decrease modestly by photodamage. This demonstrates that differences between the two processes can be quantified, and indeed they should be.

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Photodamaged skin is identified by a sallow yellow discoloration, the presence of wrinkles, changes in pigmentation, the development of small tumours in the skin, dryness, and a loss of elasticity (1, 2). These characteristics are usually most pronounced in the elderly, where the accumulation of sun damage is greatest. Indeed, skin from habitually sun-exposed sites on the elderly shows great differences when compared to covered sites. It has been difficult to differentiate between the changes in skin due to chronological ageing and those due to photodamage (3).

Non-invasive objective and quantitative methods have much to offer in the study of the physical characteristics of the ageing process (4, 5). For instance, the elasticity of skin and the thickness of skin have both been demonstrated to decrease with age (6, 7).

It has become important to differentiate skin changes due to the intrinsic ageing process from those due to chronic photodamage because of the development of therapies to assist the latter condition, and in this study we have used instrumental techniques to differentiate between changes in a range of properties of skin due to ageing and those due to photo-

damage, especially with regard to elasticity. A pulsed A-scan ultrasound system has been used to measure skin thickness, and a uniaxial extensometer has been employed to assess elastic properties. Skin surface roughness measurements were made using silicone rubber impressions and a stylus profilometer.

### MATERIAL AND METHODS

Measurements were performed on 22 normal, healthy, white male volunteers, aged 22 to 79 years, who had given their written, witnessed informed consent, with Boston skin types I, II, or III. Subjects were divided into a young group (age 20–28,  $n=6$ ) and an older group (age 54–79,  $n=16$ ). Three defined sites were examined: 1) the extensor aspect of the forearm, 10 cm proximal to the wrist, 2) the flexor aspect of the forearm, 10 cm proximal to the wrist, and 3) the lateral aspect of the thigh, 5 cm distal to the trochanteric protuberance. These sites were chosen to represent moderately exposed, moderately protected and non exposed skin, respectively. Each site was first shaved of any hair with a safety razor. For each measurement, the volunteer was in the supine position with both arms and legs in full extension. For skin surface replica measurements of the lateral aspect of the thigh, the volunteer was lying on his right side, with both legs fully extended, so that the skin site was in a horizontal plane. Ambient temperature was 20–25 degrees Centigrade, and relative humidity was 30–40%. Ethical approval for this study was granted by the Local Research Ethics Committee of the South Glamorgan Area Health Authority and the University of Wales College of Medicine.

#### *Ultrasound*

An A-scan ultrasound system (Dermotronics Ltd., Cardiff) was used to measure skin thickness at each of the three sites, where three readings were taken successively and averaged (7). A calibrated oscilloscope was used to display the A-scan, and a velocity of ultrasound in skin of 1,580m/s was used to transform the measured time between echoes from the skin to a thickness value.

#### *Measurement of skin surface roughness*

At each study site, a silicone dental impression material (Silflo) was applied to the skin to take a resting, natural negative impression of the skin surface, which was mounted on a glass slide. Later, each of the Silflo impressions was analysed with a Hommeltester stylus profilometer (Hommelwerke GMBH, Germany) to assess the roughness of the skin surface, in the direction perpendicular to the long axis of the limb. The parameters used to describe roughness are those defined by the German DIN 4768 standard. Recorded roughness values were the mean of three measurements of each silicone impression.

#### *Measurement of extensibility*

A hand-held uniaxial extensometer (Dermotronics Ltd., Cardiff) was used at each of the three sites in a longitudinal position. Plastic rectangular feet (12.5 mm × 6.25 mm), initially at a separation of 10 mm, were glued with cyanoacrylate adhesive (Powabond) to the skin surface. The skin between these feet was stretched by 30% at a rate of 3% per second, via motor-driven arms, and the forces on the arms were measured with strain gauges. The resultant force time curve

shows an initial peak extension force required to attain the 30% extension, which while maintaining the extension then drops with time to some final steady value. This stretching behaviour of skin is likely to vary with the thickness of the skin. In order to take account of this, the measured force curve is described by three parameters which are divided by skin thickness: 1)  $F_v$  (Newtons/mm), the time-varying component of the stretch force, 2)  $F_e$  (Newtons/mm), the time-independent (steady) component of the extension force, and 3)  $\tau$  (seconds/mm), the time constant of the time-dependent curve approximated as an exponential decay curve, which is defined as the time required for  $F_v$  to fall to  $F_v/e$ , where  $e = 2.718$  (8).

#### Clinical assessment

Each of the three study sites on the volunteers was examined by an experienced dermatologist, and evaluated for photodamage. Each site was given a score of 0 (none), 1 (mild), 2 (moderate), or 3 (severe).

#### Histological assessment

4-mm punch biopsies of the skin were taken after local anaesthesia with 2% lignocaine from the extensor aspect of the forearm and flexor aspect of the forearm of each volunteer. After routine histological processing, sections were stained with haematoxylin and eosin and with Halmi's modified trichrome stain for elastic tissue. Each section was evaluated blindly by one of the authors (RM) for the extent of elastosis. After viewing both the H- and E-stained and the Halmi's-stained sections, each biopsy site was assessed using a 10-cm visual analogue score (VAS) for elastosis, ranging from 0 (none) to 10 cm (extreme solar elastosis).

## RESULTS

#### Ultrasound

The mean skin thickness was less for the older group at all sites, although the difference reached statistical significance only at the flexor aspect of the forearm site (younger group mean skin thickness 1.69 SD 0.13 mm; older group mean skin thickness 1.40 SD 0.06 mm,  $p = 0.035$ , Mann-Whitney U-test). No significant correlation was seen when comparing skin thickness to the histological VAS assessment of elastosis, a subjective semi-quantitative indication of photodamage.

#### Skin surface roughness

$R_a$  and  $R_z$ , which are the resting skin surface profile parameters of roughness, showed significantly rougher skin in the older group compared to the younger group at each site (Table I).

Within each volunteer, differences in roughness parameters between pairs of sites were taken, and the mean of these

Table I. Roughness parameters  $R_a$  and  $R_z$  of unstretched skin

Values quoted are mean  $\pm$  standard deviation. Site 1: Extensor aspect of forearm; Site 2: Flexor aspect of forearm; Site 3: Lateral thigh.

| Group  | $R_a$ ( $\mu\text{m}$ ) |                    | $R_z$ ( $\mu\text{m}$ ) |                    |
|--------|-------------------------|--------------------|-------------------------|--------------------|
|        | Older                   | Younger            | Older                   | Younger            |
| Site 1 | 13.05 $\pm$ 0.61        | ***9.78 $\pm$ 0.63 | 60.15 $\pm$ 2.55        | *48.92 $\pm$ 2.24  |
| Site 2 | 14.71 $\pm$ 1.00        | **10.00 $\pm$ 0.65 | 66.25 $\pm$ 3.61        | **49.12 $\pm$ 3.06 |
| Site 3 | 15.56 $\pm$ 1.25        | **10.25 $\pm$ 0.98 | 70.74 $\pm$ 5.14        | **49.93 $\pm$ 4.22 |

Comparison between older and younger groups by Mann-Whitney U-test: \* $p \leq 0.04$ ; \*\* $p \leq 0.02$ ; \*\*\* $p \leq 0.01$ .

differences computed for each group. A significant difference in roughness was found between the extensor aspect of the arm (most sun-exposed) and the thigh (least sun-exposed), but only in the older group and not in the younger group (Table II). No significant differences were seen between any two sites in the younger group at all.

Using multiple regression analysis a significant negative correlation between age and both the  $R_a$  ( $r = -0.1$ ,  $p = 0.02$ ) and the  $R_z$  ( $r = -0.4$ ,  $p = 0.03$ ) parameters was found, but not between either parameter and the VAS assessment of elastosis on histological specimens.

#### Extensometry

In comparing extensometry parameters from each site,  $F_v$ ,  $F_e$ , and  $\tau$  are each significantly different between age groups for at least one of the three tested sites.

- (1)  $F_v$  was significantly higher in the older group at two sites.
- (2)  $F_e$  was significantly higher in the older group at the thigh.
- (3)  $\tau$  was the most consistent parameter, being significantly higher at all three sites in the older group.

On the extensor aspect of the arm,  $\tau$  was significantly positively correlated to the histological VAS score for elastosis ( $r = 0.51$ ,  $p = 0.015$ ), and on the flexor aspect of the arm,  $F_e$  was significantly positively correlated to the histological VAS score for elastosis ( $r = 0.45$ ,  $p = 0.047$ ) (Table III). Both correlations were tested using Spearman's correlation test. No extensometer parameters had any correlation with age.

#### Clinical score

On the extensor aspect of the arm, clinical photodamage scores of 0 (none), 1 (mild), and 2 (moderate) were given. No volunteer received a score of 3 (severe). When comparing these scores to the histological VAS assessment of elastosis, significant differences are seen between scores 0 and 1 ( $p < 0.0001$ ) and between scores 0 and 2 ( $p < 0.0001$ ), but not between scores 1 and 2.

No significant differences were seen on the flexor aspect of the arm when comparing the clinical scores to the histological VAS.

## DISCUSSION

Differentiating between the processes of chronological ageing and photodamage in skin is a difficult task, because most people with extensive clinical signs of sun damage are elderly and have experienced both ageing and the accumulation of considerable degrees of photodamage. It has been suggested that most of the cosmetic problems in the skin of the elderly are due to sun damage, rather than age (3, 9), and we have attempted to find non-invasive methods to aid in the differentiation of these two processes in a quantitative manner. It must be stressed that we have not measured gross or fine wrinkling or other clinical signs and have not studied facial skin. However, we believe that the information we have generated and the conclusions drawn can be extrapolated to any site, including the face.

Our study has identified two non-invasive methods that have been shown to differentiate the two processes. The roughness of the resting skin profile can be quantified and is

Table II. Differences in roughness parameters between sites of differing solar exposure within each volunteer

Comparison of the mean of the individual differences of the older and the younger groups. Sites as in Table I. Values quoted are mean difference  $\pm$  standard deviation.

| Group         | Ra ( $\mu\text{m}$ ) |                    | Rz ( $\mu\text{m}$ ) |                     |
|---------------|----------------------|--------------------|----------------------|---------------------|
|               | Older                | Younger            | Older                | Younger             |
| Site 1-Site 3 | -2.51 $\pm$ 3.8**    | -0.47 $\pm$ 3.4 ns | -10.59 $\pm$ 17.7*   | -1.02 $\pm$ 11.7 ns |
| Site 1-Site 2 | -1.66 $\pm$ 3.5 ns   | -0.22 $\pm$ 2.7 ns | -6.09 $\pm$ 12.6 ns  | -0.2 $\pm$ 10.8 ns  |
| Site 2-Site 3 | -0.86 $\pm$ 4.6 ns   | -0.25 $\pm$ 1.4 ns | -4.50 $\pm$ 18.2 ns  | -0.82 $\pm$ 5.8 ns  |

mostly affected by chronological ageing, and the elasticity of the skin can be quantified by a uniaxial extensometer and is mostly affected by solar damage.

Multiple regression analysis showed that roughness parameters were significantly dependent on age but not related to the histological assessment of photodamage. Also Table I shows that both Ra and Rz, indicators of roughness, are significantly higher in the older group than in the younger group, for each site tested. However, in comparing the two age groups it is not possible to separate the effects of age from those of photodamage, since these two factors are highly co-dependent.

By comparing sites of differing sun exposure within each volunteer, we can isolate the effects of photodamage from age. In the younger group we found no significant differences in roughness between the three sites (Table II), presumably because *i*) there are no intrinsic differences in roughness between these sites in the younger group, and *ii*) insufficient photodamage has been suffered at the exposed sites to result in changes of magnitudes detectable by this method. However, in the older group, there was a significant difference in the parameters Ra and Rz between the least and the most sun-exposed sites: the thigh was significantly rougher than the extensor aspect of the arm. We also found that the thigh was rougher than the flexor aspect of the arm and the flexor aspect of the arm was rougher than the extensor aspect of the arm, although these differences failed to reach significance. The observation that the skin surface was less rough in more sun-exposed sites leads us to believe that these differences are caused by photodamage. In Table I, this decrease in roughness from the thigh to the flexor aspect of the arm to the extensor aspect of the arm is seen in both age groups but is more notable in the older group. We postulate that significant differences were only noted between the extensor aspect of the

arm and the thigh in the older age group because this is where the amount and the difference in sun exposure is greatest, and that a greater number of subjects would be needed to reliably detect the more subtle changes between other sites.

Using multiple regression analysis shows that both Ra and Rz are positively correlated with age and negatively correlated with photodamage. However, these parameters are affected much more by age than by sun damage, as is reflected in the levels of significance. Only age, and not the histological VAS score of elastosis, an indication of solar damage, significantly correlated with Ra and Rz. This data then leads us to conclude that age increases the roughness of the skin surface and photodamage decreases the roughness, although on the sites studied here, the effect of age is greater than the effect of photodamage.

Beside skin surface topography, its elastic properties – as determined with the uniaxial extensometer – were other qualities that we evaluated successfully in order to differentiate chronological ageing from solar damage.

In Table III, the extensometer parameters Fv, Fe, and Tau adjusted for skin thickness were all seen to be higher in the older group than the younger group on at least of one of the sites studied. Indeed, Tau was higher at all sites in the older group. This parameter relates to the viscoelastic and recovery properties of the skin, and a slower time indicates a less resilient material. Since the older group has experienced both ageing and photodamage, it cannot be ascertained from this analysis to which process these increases may be attributed.

A Spearman's rank correlation analysis was used to compare extensometry parameters with our VAS histology score for elastotic change. This showed that at the most sun-exposed site Tau was significantly correlated with photodamage ( $r=0.51$ ,  $p=0.015$ ), while on the inner arm site Fe, the elastic component of the extensometry parameters, correlated with

Table III. Elasticity parameters measured using uniaxial extensometry

All factors normalised with respect to skin thickness. Data are presented as mean (standard error)

Fe – Time independent stretch force Newton/mm, Fv – Time dependent (relaxation) stretch force Newton/mm,  $\tau$  – Time constant of relaxation curve Second/mm.

| Group  | Extensor arm site |                | Flexor arm site |                | Lateral thigh site |                |
|--------|-------------------|----------------|-----------------|----------------|--------------------|----------------|
|        | Old               | Young          | Old             | Young          | Old                | Young          |
| Fe     | 4.04 (0.53)       | 2.57 (0.54) ns | 2.86 (0.33)     | 1.92 (0.13) ns | 2.10 (0.24)        | 1.20 (0.31)*   |
| Fv     | 4.15 (0.42)       | 3.55 (0.61) ns | 3.44 (0.42)     | 2.12 (0.23)*   | 2.30 (0.21)        | 1.27 (0.28)*** |
| $\tau$ | 5.27 (0.43)       | 3.16 (0.54)*** | 4.96 (0.48)     | 3.36 (0.09)*** | 3.51 (0.31)        | 2.17 (0.38)**  |

Comparison between older and younger groups by Mann-Whitney U-test. ns: not significantly different.

Significantly different: \* $p \leq 0.05$ , \*\* $p \leq 0.03$ , \*\*\* $p \leq 0.02$ .

photodamage ( $r=0.45$ ,  $p=0.047$ ). Although these were the only significant results, they suggest that the extensometry parameters may be a measure of photodamage.

Multiple regression analysis revealed that the slopes of the Age versus Extensometry parameters were all at or around zero. This supports our conclusion that changes in the extensometry parameters were mainly caused by photodamage and were unrelated to age.

The extensometer parameter Fv did not correlate with photodamage or age, but it is unclear why this value was higher in the older age group at two sites.

Previous work has shown the time constant for viscoelastic recovery to increase with age (10, 11), in contrast to our study which attributes this increase to photodamage. Since it has been shown that in photodamaged sites, the normal elastic tissue conformation is lost and replaced by actinic elastosis (9, 12), it is not unlikely that this might lead to a loss of the elastic properties of the skin, with a resulting increase in Tau. However, our study also found the elastic component Fe to increase with photodamage, whereas others have shown the elastic parameter to decrease with age (10, 11). In view of the histological loss of normal elastic tissue in sun damage, it would be expected that with photodamage, the elastic component would decrease, not increase as we have seen. This difference might be attributed to the fact that our values for Fe are normalised for skin thickness. Since skin thickness has been shown to be decreased in the aged (7), our normalised Fe values have been altered so that values are decreased much more in the young than in the elderly.

Nevertheless, we have demonstrated that the loss of a viscoelastic property of skin, as reflected in the extensometer parameter Tau, is more indicative of actinic damage than chronological age.

With ultrasound measurements of skin thickness, we were able to show significantly thinner skin in the older group at one site, but at the other two sites, which also showed a thinner skin in the older group, the difference was not statistically significant. This result is consistent with previous studies (7), but we were unable to differentiate ageing and actinic damage by skin thickness alone.

The clinical assessment of photodamage, when correlated to the histological assessment, could significantly differentiate between groups assessed as showing no sun damage and those showing some evidence of sun damage, but not between two different degrees of sun damage. This might be due to the fact that of the three sites we examined, the most sun-exposed would have been the extensor aspect of the forearm, which is not usually as photodamaged as other sites, such as the dorsum of the hands and the face. In effect, our study was only looking at a range of actinic damage from none to moderate, and

indeed no volunteer ever received a "severe" clinical score. Also, this difficulty in distinguishing between amounts of solar damage using a scale of 0, 1, 2, or 3 illustrates the disadvantages of using a short-interval scoring system.

## CONCLUSION

We have looked at several non-invasive methods to attempt to differentiate the changes in the skin caused by chronological ageing versus those caused by actinic damage. The uniaxial extensometer can demonstrate a loss of the skin's elasticity predominantly by photodamage, and the roughness of the skin surface can be shown to increase mostly by chronological ageing but to decrease modestly by photodamage. This demonstrates that differences between the two processes can be quantified, and indeed they should be. Certainly, there are differences between actinic damage and chronological ageing which we have demonstrated, and these findings mean that further investigations into both processes promise to be worthwhile.

## REFERENCES

- Warren R, Gartstein V, Kligman AM, Montagna W, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. *J Am Acad Dermatol* 1991; 25: 751-760.
- Lober CW, Fenske NA. Photoaging and the skin: differentiation and clinical response. *Geriatrics* 1990; 45(4): 36-42.
- Taylor CR, Stern RS, Leyden JJ, Gilchrist BA. Photoaging/photodamage and photoprotection. *J Am Acad Dermatol* 1990; 22(1): 1-15.
- Marks R. Methods for assessment of cutaneous ageing. *Int J Cos Sci* 1990; 12: 153-163.
- Berardesca E, Farinelli N, Rabbiosi G, Maibach HI. Skin bioengineering in the non-invasive assessment of cutaneous ageing. *Dermatologica* 1991; 182: 1-6.
- Escoffier C, de Rigal J, Rochefort A, Vasselet R, Leveque JL, Agache PG. Age-related mechanical properties of human skin: an in vivo study. *J Invest Dermatol* 1989; 93: 353-357.
- Tan CY, Statham B, Marks R, Payne PA. Skin thickness measurement by pulsed ultrasound: its reproducibility, validation and variability. *Br J Dermatol* 1982; 106: 657-667.
- Mourad MM, Edwards C, Marks R. Skin extensibility in the gravitational syndrome. *Bioeng Skin* 1988; 4: 199-215.
- Kligman AM. Early destructive effect of sunlight on human skin. *J Am Med Assoc* 1969; 210(13): 2377-2380.
- Daly CH, Odland GF. Age-related changes in the mechanical properties of human skin. *J Invest Dermatol* 1979; 73: 84-87.
- Finlay B. The torsional characteristics of human skin in vivo. *Biomed Eng* 1971; 6(12): 657-573.
- Braverman IM, Fonferko E. Studies in cutaneous ageing: I. The elastic fiber network. *J Invest Dermatol* 1982; 78: 434-443.