Accuracy and reliability of a hand-held in vivo skin indentation device to assess skin elasticity

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Keywords: Skin, Elasticity, Indentation, Stiffness, Anti-aging, skin firmness, skin aging
ABSTRACT

**Background:** Because most functions of skin depend on its mechanical properties an understanding of tissue mechanics is important to diverse areas including medicine and the cosmetic industry. The aim of this study was to evaluate the performance of a hand-held indentation device for fast and reliable determination of skin stiffness.

**Methods:** Device accuracy to indentation depths of 0.3 and 0.6 mm, was evaluated first on plastic foam materials with mechanical properties verified by an industrial standard material testing apparatus. Subsequently, the device’s sensitivity to detect changes in skin stiffness was evaluated for age-related changes by comparing anterior forearm skin stiffness among 53 healthy women (18-79 years). Sensitivity to detecting skin stiffness changes was also evaluated in facial skin of 49 women who utilized an anti-aging cream over a period of six weeks.

**Results:** High correlation was detected between stiffness of plastic foam phantoms measured with the indentation device for different intender lengths and Young’s modulus determined with industrial standard mechanical testing device (0.6 mm indenter: p < 0.01; 1.3 mm indenter: p = 0.06). Age-related decrease of 38% in the elasticity of the skin was detected in healthy volunteers (p < 0.05). Finally, significant increase in skin stiffness was detected after 6 weeks’ application of anti-aging cream. The coefficient of variation for 0.6 and 1.3 mm indenters were 7.4% and 8.5%, respectively.

**Conclusions:** The presented indentation technique was shown to be accurate against the industrial standard material testing device. Detection of skin changes related to aging and usage of anti-aging cream could be detected with the indentation technique. The new device was found to be feasible for monitoring skin stiffness in cosmetics and clinical conditions.
INTRODUCTION

Because most functions of skin depend on its mechanical properties an understanding of tissue mechanics is important to diverse areas including medicine and the cosmetic industry (1,2). In clinical environments, the integrity of soft tissues is often evaluated with manual palpation. Since this procedure is highly subjective, various novel quantitative techniques have been introduced for evaluation of mechanical function of skin (3). In the cosmetic industry, the measurement techniques are commonly based on applications of indentation, suction, torsion or tension measurements (3–8) with a large number of different quantitative parameters reported. However, the physical basis, and thus, the relation of the determined parameters to skin physiology is not always clear (3). To best interpret detected changes in skin mechanics it is important that physiological and anatomical characteristics affecting the measurements be known. Because skin has a layered structure determination of tissue components affecting measured mechanical properties is difficult but essential for the proper design of quantitative tools used to assess skin mechanical properties. These layers that consist of a porous solid matrix and fluid result in complex, anisotropic, heterogeneous and viscoelastic properties (6,9) although its mechanical properties are mainly determined by collagen and elastin fiber networks along with ground substance of the dermis (6,10). Collagen fiber network structure determines anisotropic and non-linear skin properties (9,11) and permits high strains with low forces as well as high tensile strength with high forces (12,13). Elastin is responsible for skin’s mechanical integrity at low loads (13). The ground substance, composition of tissue water and collagen determine skin’s viscoelastic properties (9,13). In its relaxed state skin is in tension so that determining its mechanical behavior in vivo is complex (14).
Various indentation-based devices have been used to determine skin stiffness (5,15). The basic technique uses skin compression with an indenter with a measurement of the force resisting the strain. Accordingly, an indentation principle seems a feasible method to assess skin stiffness (16). In recent studies, a finite-element modeling was used to optimize the indentation geometry and to determine the tissue components affecting the indentation measurement (16). Mechanical properties and volume of the skin were found to be dominant in determining the indentation response of forearm skin when a short indenter was used (indenter length = 0.5 mm and diameter = 2 mm) (16). Since the effect of adipose tissue and muscle on indentation response was small, it was concluded that a narrow, short and round ended indenter combined with a short measurement time might be feasible for robust clinical determination of the mechanical integrity of skin (16). Since the performance characteristics of this technique are still unknown, the aim of the present study was to introduce a new indentation-based device, compare the technique with an industrial material tester and evaluate the fast indentation technique for the determination of skin stiffness changes with age in healthy female volunteers and also after application of anti-aging cream.
METHODS

Measurement Device

Skin Stiffness was determined using a commercially available hand-held device applying an indentation principle (Elastimeter, Defin Technologies, Kuopio, Finland). Two identical devices with cylindrical indenter lengths 0.6 or 1.3 mm were used with each having a diameter of 2.5 mm. Each device has two force sensors, one sensor is connected to the indenter and the other to a reference 23 mm diameter base plate as shown in Figure 1. The force required to indent the skin to depths of 0.6 mm and 1.3 mm is determined by characteristic indenter-to-reference plate force sensor curves.

The principle of the indentation technique of this device is presented in previous papers (5,15,18). Briefly, it consists of two coaxial 1500 g load cells (Honeywell micro switch force sensor FSG-15N1A, Columbus, Ohio, USA). The indentation measurements are conducted by manually pressing the indenter onto skin surface. To make standardized measurements, the device monitors the speed and force applied on the skin and rejects the measurements done e.g. too slowly/quickly or with too low/high force. After five sequential measurements, the device calculates an average on the display of the device in units of (N/m). The time to make five successful measurements is about 5-10 sec.

Reference Materials for Assessing Indenter Accuracy

For later use to determine indenter accuracy, the mechanical properties five different plastic foams of varying stiffness were determined. These materials were cut into cylinders with a diameter of 19.0 ± 0.1 mm [mean ± SD] and a height of 10.0 ± 0.8 mm. The reference material
properties were determined by measurements done using a custom designed linear servo motorized material testing device (Figure 2A) consisting of a precision motion controller (Newport PM500-C, Irvine, CA, USA), an actuator (displacement resolution of 1 µm, Newport, PM1A11939) and a 1 kg load cell (AL311AR, Honeywell, Columbus, OH, USA).

The test procedure was a one-step unconfined compression test. First, the surface of reference material was adjusted to be parallel to the compressing plate using a goniometer (Edmund Optics inc., Barrington, NJ, USA). Then, the compressing plate was driven into contact with the surface of reference material and the thickness of the sample was recorded from the actuator. To assure good initial contact between the reference material and compressing plate, the reference material was compressed to 2% pre-strain (i.e. 2% compression relative to sample thickness). After one-hour relaxation the reference material was compressed to a 10% strain in one second (i.e. 10% compression from remaining thickness of the sample). The corresponding load was recorded via the load cell to achieve force – deformation data from which a stress – strain curve for the reference material was created (Figures 2B and 2C). Elastic modulus $E$ for each foam material was determined as a slope of the linear part of the stress-strain curve (Figure 2C). Each reference material was obtained from NMC Cellfoam PLC (Laitila, Finland) with the following designations: $E30$, $L30PS$, $PU+E25$, $S60$, Eurocell 130 and $Superseal-W$.

**Indenter Measurements on Test Materials**

Each reference material type was used as a target material for the ElastiMeter indentation device in which forces associated with two different indenter lengths (0.6 and 1.3 mm) were determined. The same six reference materials for which properties were now
known were used but a different shape was used. For the indenter tests, square planes were used with surface areas as supplied by the company of 260-650 cm² and thicknesses of 9.0-29.8 mm. For each material lying on a hard table surface, 20 repeated indentation measurements were performed. Each indentation was conducted at different locations of the material to ensure that measurements were being done on fully relaxed materials. From the measured indenter force, a stiffness value in a unit of Newtons per meter (N/m) was calculated by dividing the measured indenter force by material deformation (length of the indenter). This stiffness unit, when applied to skin, is also referred to as Instant Skin Elasticity (ISE). The term ISE is used to emphasize that the indentation measurements are done nearly instantaneously (within 0.5 sec). Thus, for the indenter lengths used the present measurements describe elastic, not viscoelastic properties of the skin. The stiffness values measured with the ElastiMeter device were compared to the elastic modulus of the test materials as determined with material testing device via a correlation analysis.

Subjects for Skin Stiffness Measurements

A total of 102 healthy volunteers were recruited for this study. All were from the Degree Programme in Beauty and Cosmetics, Laurea University of Applied Sciences, Vantaa, Finland. Recruiting was performed by the students under the supervision and guidance of their teachers. The selected participants gave their written consent for the measurements of the study.
**Skin Stiffness Measurements**

To evaluate various aspects of the indentation device the study was divided into three parts.

**Part 1:** In part 1 the capability of device to detect age-related changes on anterior forearm skin stiffness was determined in a group of 46 seated women (age 18-79 years). Using the 1.3 mm indenter, the indentation force at the right anterior forearm was measured in triplicate at an arm site eight cm distal to antecubital crease. Skin stiffness was calculated as the measured indentation force/indenter length, with the average of the triplicate values used to characterize the arm site stiffness. Subsequently, subjects were divided into six age groups (<20, 20-29, 30-39, 40-49, 50-59, >60 years, consisting of 5, 12, 7, 7, 10 and 5 subjects, respectively) and mean and standard deviation were determined for each group.

**Part 2:** The sensitivity of the indentation device to detect changes in facial skin stiffness associated with anti-aging cream use was determined in 49 other females (age 40-69 years). The cream product and its manufacturer are protected by a confidentiality agreement. Pre-treatment baseline stiffness using the 1.3 mm indenter was determined on left cheek skin at a location about 6 cm laterally from mid-point of the nasolabial line. During the seated measurements, the subjects were asked to keep their face relaxed. Thereafter all subjects started using the anti-aging cream daily for six weeks. The indentation measurements were repeated at 2, 4 and 6 weeks. Subsequently, subjects were divided into three age groups (40-49, 50-59 and 60-69 years) consisting of 23, 17 and 9 subjects, respectively.
Part 3: Indentation device short-term reproducibility was evaluated by one operator. For this part, eight healthy male and female volunteers (age 30-65 years) were recruited. Skin stiffness was determined bilaterally on anterior forearm sites using indenter lengths of 0.6 mm and 1.3 mm). At each site, measurements were repeated 10 times within a time interval of about 90 seconds. The reproducibility was determined as a coefficient of variation (CV).

Data-analysis

Data-analysis was done with Matlab (version 7.12.0, Mathworks Inc., Natick, MA, USA). Statistical analyses were done with SPSS (version 19, IBM, USA). Assessments of correlations between elastic moduli and stiffness determined using the plastic reference materials was done using Pearson correlations. Statistical significance in stiffness values determined among different age groups was determined with a Kruskal-Wallis one-way ANOVA. Pair-wise analysis was conducted using Dunn-Bonferroni method. Furthermore, the subjects younger and older than 30 years where pooled and the statistical significance in ISE values between these two age groups was determined using Mann-Whitney U test. Statistical significance between different time points in the anti-aging cream experiment was determined using a Freedman test. Pair-wise analysis was conducted using Dunn-Bonferroni method. The level of significance was set to 0.05. The reproducibility of the indentation measurement was determined as a coefficient of variation (Glüer 1999). The confidence intervals were calculated using non-parametric bootstrap sampling method (Gulhar et al. 2013).
RESULTS

Test Material Elastic Modulus vs. ElastiMeter Determined Stiffness (ISE value)

The elastic modulus of the plastic foam materials determined from the slope of the stress-strain curves ranged from 60 kPa for the PU+E25 material to 173 kPa for the Superceal W material (Table 1). The corresponding stiffness (ISE) values determined using the indenter depended on the indenter length and varied as shown in Table 1. Further the correlation between the ISE value and the measured elastic modulus also depended on indenter length with the maximum correlation ($r = 0.98$) observed at an indenter length of 1.3 mm.

Age Dependence of Anterior Forearm Skin Stiffness

Indentation forces, measured with the 1.3 mm indenter at forearm, ranged from 15 mN to 118 Mm with corresponding ISE values ranging from 12 N/m to 91 N/m over the entire age range. A significant decrease in ISE was observed with increasing age until the 40-49 years was reached (Figure 3). Above this age, there were slight variations in the magnitude of ISE with the appearance of a decline in ISE after age 50-59. However, no age group above 40-49 differed significantly from the ISE values of the 40-49 age-group. However, when only two age groupings were considered, with group-young being subjects < 30 years of age (n=17) and group-older being subjects ≥ 30 years (n = 29) a highly significant difference in ISE values was found. For this analysis, ISE values for group-older were significantly less than for group-young (50.4 ± 14.4 N/m vs. 66.2 ± 10.7 N/m, p<0.001)
**Facial Skin Stiffness Changes**

ISE tended to increase during the 6-week usage of anti-aging cream on facial skin as represented in **Figure 4**, but the increase was statistically greater than baseline only at the 6-week evaluation. In addition, the increase in ISE (16.8%) from the baseline to the 6-week time point was significant only in the youngest age group \((p < 0.01)\). In the youngest age group (40-49 years) the ISE values measured at 6-weeks were significantly greater than at 2 and 4 weeks \((p < 0.01 \text{ and } p < 0.05, \text{ respectively})\).

**Forearm Skin ISE Value Reproducibility**

The coefficient of variation of ISE values determined in the eight healthy volunteers was (mean and 95% confidence interval): 7.4 (4.5-8.6) %, and 8.5 (6.1-8.7) % for indenter lengths of 0.6 and 1.3 mm, respectively. ISE values obtained using indenter lengths of 0.6 mm and 1.3 mm were highly correlated \((r = 0.94, p < 0.001)\).

**DISCUSSION**

In the present study, the feasibility of a novel hand held indentation device for determination of skin stiffness was evaluated. The stiffness values determined for phantoms with different mechanical properties correlated well with laboratory measurements done with material testing device. Furthermore, changes in skin stiffness related to aging and the usage of anti-aging cream could be sensitively demonstrated with the indentation device. Finally, the reproducibility of the technique was found to be adequate for cosmetic industry and clinical use.
In literature, several techniques for in vivo determination of mechanical properties of the skin are reported. To determine meaningful parameters related to skin mechanics the contributions of different skin layers on measurement technique need to be known. Due to the complex layered composite structure of the skin the determination of the contributions of the different tissues layers to the measured mechanical response is challenging. In previous studies the finite element modelling was used to optimize the indentation geometry and to determine the contributions of major tissue components to indentation response of skin (16). Skin was found to be mainly responsible for the indentation response when a short indenter (0.5 mm) was used. Furthermore, the effect of skin thickness or adipose tissues on stiffness values was found to decrease as the length of indenter was decreased from 2 mm to 0.5 mm (16). Use of a short indenter is theoretically optimal for the determination of skin stiffness. However, application of very short indenter of 0.2 or 0.3 mm (1, 27) might make the measurement prone to errors and thus jeopardize the reproducibility of the technique. Therefore, longer indenter such as 0.6 mm or 1.3 mm might be optimal compromise for accurate and reproducible determination of skin stiffness. In the present study, the reproducibility determined with 0.6 mm was comparable to that determined with 1.3 mm indenter.

High correlations were detected between the ISE values and Young’s elastic moduli determined with 0.6 and 1.3 mm indenters for phantom materials. Furthermore, measurements in human skin yielded significant correlations between intender lengths. The stiffness values determined in present study with 1.3 mm indenter for the forearm and facial skin were in the same range as those reported previously for a 0.3 mm indenter (28). Mayrovitz et al (28) also noticed that in facial skin the 0.3 mm indenter data correlated well with the 1.3
mm indenter results. However, the 1.3 mm indenter might be too long to assess elasticity of a thin skin but for the determination of subcutaneous firmness a longer indenter of 1.3 mm is feasible. Therefore, we consider the 0.6 mm indenter adequate for the assessment of elasticity in human skin.

The skin stiffness of healthy female volunteers was found to decrease with aging. This finding is in line with several previous studies conducted with indentation principle (16), Cutometer (16,19) and various other techniques (20–22). The age-related decrease of skin stiffness is caused by the degeneration of the skin elastin and collagen network and a decrease of skin thickness (23). The dimensions of indenters applied in the present study were designed to minimize the effect of skin thickness and the mechanical properties of adipose tissues on the measured ISE values (16). In the recent study the effect of skin water content on indentation stiffness was reported to be small (27). Thus, the observed decrease of skin stiffness with age is most probably caused by the degeneration of the skin collagen and elastin. Interestingly, the decreasing trend in skin stiffness was momentarily reversed in the age group of 50-59 years. As all subjects in the present study were women, this finding might be due to hormonal changes related to menopause and hormonal replacement therapy. In previous studies menopause have been related to decrease in skin collagen, glycosaminoglycan and water content (24–26). These changes lead to decrease in skin elasticity and strength (26). However, the hormone replacement therapy has been reported to prevent the degeneration of the skin composition and restore some of the skin mechanical function (27). Thus, hormone replacement therapy may explain the transient increase in skin elasticity in patients in the age group of 50-59 years. Although the ISE values of the oldest age group (>60 years) were markedly reduced compared
with age groups <20 and 20-29 years, the decrease was not statistically significant due to a limited number of volunteers in the oldest age group.

The effect of anti-aging cream on facial skin stiffness could be detected during 6-month usage of the product. Interestingly, the effect was statistically significant only in the age group of 40-49 years. This might indicate that age-related changes might be repaired with anti-aging cream. However, the repair capacity of the skin seems to decrease with age. Previously, the effect of an applicant to mechanical properties of the skin have been evaluated in situ for rat skin (12). In that study the applicant was found to moisturize and soften the skin surface leading to decrease in skin elastic modulus as compared to untreated skin.

To conclude, the Elastimeter was found to be feasible for the determination of skin stiffness. The age and anti-aging cream related changes in the skin stiffness could be detected with the device. Furthermore, the accuracy and reproducibility of the technique was found to be good for cosmetic industry and clinical use. As the device is battery operated and light weighted the clinical usability of the technique is high.

Acknowledgement

The authors wish to thank students of Laurea University of Applied Sciences (Helsinki, Finland) for assistance making skin measurements.

Conflict of interest statement

Authors have no conflicts of interests
REFERENCES


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### TABLE 1

<table>
<thead>
<tr>
<th>Plastic foam</th>
<th>Newport material testing device (kPa)</th>
<th>0.6 mm indenter (N/m)</th>
<th>1.3 mm indenter (N/m)</th>
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</thead>
<tbody>
<tr>
<td>PU+E25</td>
<td>57.6</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>E30</td>
<td>48.2</td>
<td>75</td>
<td>64</td>
</tr>
<tr>
<td>L30 PS</td>
<td>131.8</td>
<td>88</td>
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</tr>
<tr>
<td>S60</td>
<td>124.0</td>
<td>90</td>
<td>88</td>
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<tr>
<td>Superceal W</td>
<td>274.1</td>
<td>140</td>
<td>144</td>
</tr>
<tr>
<td>Eurocell 130</td>
<td>537.8</td>
<td>374</td>
<td>344</td>
</tr>
</tbody>
</table>

Correlation between ISE and Elastic modulus

\[ r = 0.97, p < 0.01 \quad r = 0.98, p < 0.01 \]

**Table 1.** Elastic modulus (kPa) and ISE (N/m) values determined for the plastic foam materials (n = 6). The greatest correlation was observed for an indenter length of 1.3 mm.
Figure 1. Indenter Device

(A) Hand-held ElastiMeter. (B) Probe of the ElastiMeter with an indenter localized in the center of the reference base plate. Essential feature of the device is a mechanical isolation of indenter from reference base plate.
Figure 2. Test setup and procedure for determining elastic modulus

A) A custom designed linear servo motorized material testing device was used to characterize the mechanical properties of reference materials. The material testing device consisted of actuator, load cell, compression plate and goniometers. B) A representative measured compression palate force as a function of reference material deformation. C) A representative stress-strain curve determined for a phantom material. The elastic modulus (E) was determined in the linear part of stress-strain curve (shown in red).
Figure 3. Skin Stiffness vs. Aging

Mean and standard deviation of the ISE with different age groups. Decreasing trend was detected in the values of ISE as a function of age up-until age 40-49 years. The ISE values were increased temporarily in the age group of 50-59 years. *P<0.05 Kruskal-Wallis test.
Figure 4. Skin Stiffness vs. Time Post-Age-Cream

Mean and SD of ISE after measurement of facial skin at baseline and 2, 4, and 6 weeks after the start of anti-aging cream usage. As compared to baseline the usage of anti-aging cream was found to increase the skin stiffness 16.8%, 14.9% and 8.8% during six weeks in age groups of 40-49, 50-59, 60-69 years, respectively. The effect was statistically significant in the 40-49 year age group. **P<0.01, *P<0.05, Freedman test.