A New World of Anti-Aging Skincare Targeting the Face-Wrapping "Tensional Network": "Ring-Collagen"

-Paradigm Shift of Skin Analysis: From Visualizing Tangible to Intangible Targets-

Ezure, Tomonobu[1]; Amano, Satoshi[1]; Matsuzaki, Kyoichi[2]; Ohno, Nobuhiko[3,4]

[1] MIRAI Technology Institute, Shiseido Co., Ltd., Kanagawa, Japan;
[2] Plastic and Reconstructive Surgery, International University of Health and Welfare, Chiba, Japan;
[3] Department of Anatomy, Jichi Medical University, Tochigi, Japan;
[4] National Institute for Physiological Sciences, Aichi, Japan

Corresponding author; Tomonobu Ezure, 1-2-12, Takashima, Nishi-Ku, Yokohama 220-0011, Japan, +81-45-222-1600, tomonobu.ezure@shiseido.com

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ABSTRACT

Background: Facial skin tightly "wraps" internal structures to retain facial morphology. However, the physical properties of the facial wrapping system are unknown, since intangible physical dynamics is inaccessible to current technology. Thus, we aimed to establish technology to visualize skin's physical dynamics, apply it to clarify the wrapping force, and develop an impactful anti-aging solution.

Methods: Skin deformation/recovery was observed by X-ray micro-CT. Gene expression analysis (micro-array/RNA-seq/real-time PCR) and immunohistochemistry were conducted in facial skin and cell/organ-culture systems. Skin physical/physiological properties and our skincare solution were evaluated in volunteers.

Results: Skin 4D-physical dynamics was digitally reconstructed by artificial-intelligence-based skin movement tracking. Moreover, our high-speed autostereoscopy enables operating this digital skin in real-space, allowing free/intuitive analysis of complex physical dynamics (designated "skin-mechanics reality").

Skin-mechanics reality revealed ring-shaped high-tension areas in facial dermis. These consist of ring-shaped collagen, designated "ring-collagen", encircling a proteoglycan (PG) layer around fine hairs. Ring-collagen creates center-directed tension due to its characteristic fiber direction, and generates a tensional network that tightly "wraps" the face to maintain facial morphology. Ring-collagen deteriorates with aging, resulting in aged-appearance.

Surprisingly, application of young skin's physical environment to organ-cultured skin ("environmental transplantation") regenerated ring-collagen via Wnt16 secretion (from hair follicles), which increases the PG layer. In volunteers, this treatment improved skin-wrapping force and aged-appearance.

Conclusion: We realized 4D-visualization of intangible physical properties in real-space: "skin-mechanics reality". This enabled us to discover "ring-collagen", which produces facial wrapping force, fundamental machinery maintaining facial morphology. By targeting ring-collagen, we developed "environmental transplantation" as novel anti-aging skincare.

INTRODUCTION

Facial morphology changes drastically with aging in various ways, such as loss of facial contour, descent of eyelids, and appearance of deep ditches (nasolabial folds, marionette lines, etc.) [1]. These are critical matters of concern to many people, but there are few impactful solutions for this. Previously, we have shown that these changes are due to sagging (ptosis of skin), and thus sagging is a critical target for anti-aging skincare [2].

In young face, the skin tightly fits onto the face, namely, it "wraps" the underlying structures. However, with aging, the skin loses this wrapping force, and then gravity causes the loose skin to sag down, no longer fitting tightly to the face. Although we have established the role of gravity in this later sagging process [2], little is known about the fundamental cause of sagging, namely how skin wraps the face and how the skin's wrapping force is lost with aging.

The main reason for this is that the wrapping force is inaccessible to current skin analysis, since visualizing the physical dynamics of the skin is not possible. We have progressed skin visualization technology from conventional 2D to 3D, and finally 4D, as we reported in previous IFSCC congresses [3]. Nevertheless, existing methods of skin analysis can only target tangible structures, and cannot visualize the dynamics of the skin's physical properties. This requires a fundamental breakthrough in imaging technology.

The dermal layer of skin contains various extracellular matrix components. The major component is Type I collagen, in the form of collagen fibers [4]. These fibers contribute to skin stiffness, and the deterioration of collagen fibers with aging has been extensively studied, both quantitatively and qualitatively. However, the actual status of collagen fibers in the body is largely unknown, especially in facial skin, which is not readily available for research.

Besides collagen, the dermal layer contains proteoglycans (PG), which are highly glycosylated proteins, such as versican, periostin and so on [5]. Since PGs can accommodate many water molecules, they are considered to contribute to the viscosity and volume of the skin.

Skin also contains multiple internal organs, such as sebaceous glands, sweat glands, etc. [6]. Furthermore, although human beings have lost body hair with evolution, fine hair still exists on the face and body [7]. These hairs are often removed for aesthetic reasons, though their contribution to the physical properties and youthful appearance of skin have yet to be studied.

Signaling molecules secreted from cells tightly control the amount and condition of extracellular matrix components in the skin. Among them, Wnts are involved in many biological processes, including cell fate determination, cell migration, and organogenesis, but the role of Wnt16 is largely unknown, especially in skin [8].

In this context, we aimed to develop an impactful solution for facial rejuvenation, and in this study, we addressed this topic through the following steps:

1) developing novel technology to visualize skin physical dynamics,

2) using this technology to investigate the skin-wrapping system of the face and its contribution to facial aging,

3) applying the findings to obtain rational solutions for facial rejuvenation.

METHODS

Skin specimens

137 specimens of surplus facial skin (subjects' age: 0-103 years) from plastic surgery were used for this study. 30 abdominal female skin specimens (subjects' age: 22-73 years) were purchased from Biopredic International (Rennes, France). All studies were approved by the ethics committees of all participating institutions.

Human testing and protocol

30 female volunteers (age: 30-40s) participated in the noninvasive skin analysis. The efficacy of novel skincare was examined in 6 female volunteers (40s). Sagging was evaluated using our photograph-based grading criteria [9]. Skin physical properties were measured with a Cutometer 580® [10].

X-ray micro CT

Skin specimens were cut into cubes of about 4 mm^3 and observed with an X-ray micro CT device (Xradia; Zeiss, Oberkochen, Germany) at low voltage (50 kV, 80 μA) [11]. Skin specimens retained their physical properties during study, as confirmed by recovery of their original state after deformation. Skin structures in each micro CT image were automatically identified by an artificial intelligence (AI) deep-learning system, Dragonfly (Object Research Systems, Montréal, Canada), and spatially reconstructed in real space with our newly developed high-speed autostereoscopy program for viewing on an autostereoscopy display (SONY, Tokyo, Japan) [12].

Organ and cell culture

Human skin specimens and dermal fibroblasts (Lonza; Basel, Switzerland) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS under humidified 5% CO_2/air at 37°C. Human outer root sheath (ORS) cells established from human skin specimens were cultured in keratinocyte serum-free medium (K-SFM; Gibco, NY, USA). Wnt16 was purchased from R&D Systems (MN, USA).

Gene expression analysis

Microarray analysis and RNA sequencing (RNA-seq) were conducted with an Agilent (CA, USA) or an Illumina (CA, USA) system, respectively. Real-time PCR was conducted by using a Light-cycler (Roche, IN, USA) as reported [13] with the following primer sets: Wnt16 forward; CTACGGAGCCCAAGGAAACT, reverse; GGTTTCCTCTTGCACAGCTC, versican forward; ACGGTGTCACTGACTGTGGA, reverse; TCCAAACAAGCCTTCTGAGC and periostin forward; GCCCTGGTTATATGAGAATGGA. reverse; GATGCCCAGAGTGCCATAAA.

Histological observation

Immunohistochemical observation was conducted by the Amex procedure [14] with anti-versican, anti-periostin (Abcam; Cambridge, UK) and anti-Wnt16 antibodies (LS-Bio; WA, USA), followed by detection with the EnVision system (Agilent; CA, USA).

Statistical analysis

Statistical analysis employed Pearson's correlation coefficient or Spearman's correlation coefficient by rank test. Differences between groups were evaluated by means of Student's t test or the Wilcoxon rank test. P<0.05 was considered significant.

RESULTS

1. Visualization of intangible 4D skin physical properties in real space: "skin-mechanics reality"

1.1. Visualization of skin physical dynamics on computer

Skin specimens were deformed, and we observed the deformation and recovery process to the original state by means of X-ray micro CT (Fig. 1a,b). Internal skin structures were identified by an AI deep-learning system, and reconstructed to obtain 3D images (Fig. 1c). We then set meshes with more than twenty thousand observation points (per mesh), and followed the movements of several million observation points during deformation/recovery (Fig. 1d,e). The direction and extent of the movement at each point was indicated with a cone, and the 4D physical properties were digitally reconstructed on computer from millions of these cones (Fig. 1f).

1.2. Spatial reconstruction of 4D physical properties of skin

In order to see the 4D physical properties of skin in real space, we developed a program for high-speed autostereoscopy [12], which makes it possible to view 4D images in real space (not on computer) without using any special equipment (it does not require special glasses or headset) (Fig. 1g,h). The whole stereoscopic image appears to move naturally in the observer's field of vision, as if it were being handled, based on tracking the position of the observer's eyes. Using this system, we achieved the first visualization of the spatiotemporal (4D) mechanical properties of skin in real space. We designated this technology as "skin-mechanics reality" (Fig. 1i).

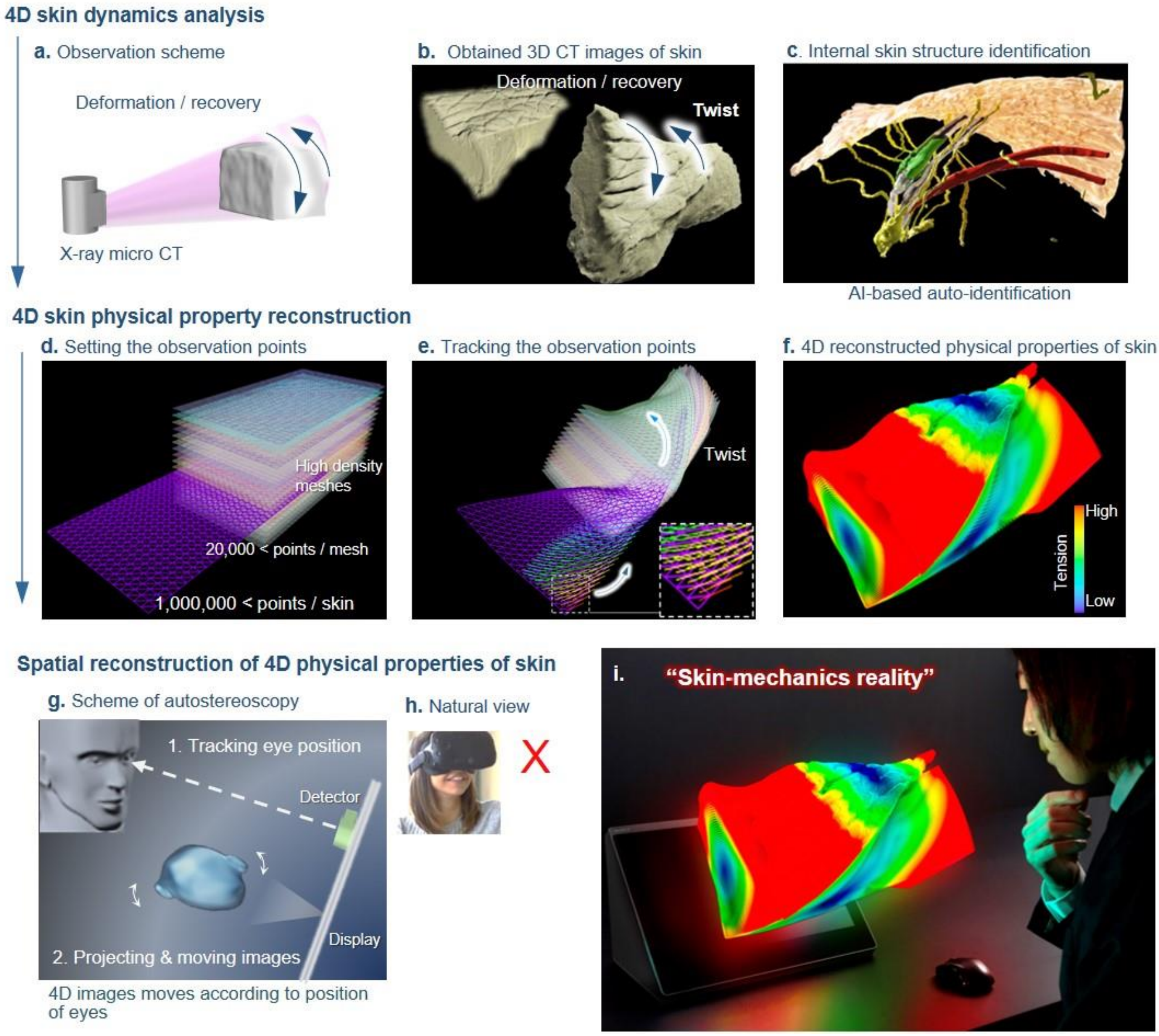

Figure 1. Visualization of intangible 4D skin physical properties in real space: "skin-mechanics reality"
a) Scheme of X-ray micro CT observation. Typical 3D CT images of b) whole skin and c) internal skin structures. To analyze the 4D dynamics of internal skin, d) high-density meshes were set in the skin, generating millions of observation points/skin. e) The dynamics of each point was monitored during deformation/recovery, and the direction and magnitude of changes in position were indicated by cones (see enlarged square). f) 4D skin physical properties reconstructed on computer. The color map shows the magnitude of the tension, as a representative example, determined as the difference between the initial and subsequent states of each mesh point. g, h) Schematic illustration of high-speed autostereoscopy. i) This illustrates the spatially reconstructed 4D skin mechanics image in real space, designated as "skin mechanics reality".

1.3. “Skin-mechanics reality” provides unprecedented information about the physical properties of skin

Skin-mechanics reality is composed of millions of physical 4D data (Fig. 2a,b) with ultra-high resolution (100 nm order). This technology can show real-time images of intangible physical information, such as distortion, tension, and stress, and is quite different from current 4D technology, which just shows the 4D movements of tangible structures. Further, this technology provides information anywhere in the skin, even where there is no clear structure (blood vessels, sweat glands and so on), using cones (to show in detail the information at each of the observation points; Fig. 2b) and/or arrows (to show summary information over larger areas; Fig. 2c,d). Since this skin is digitally reconstructed from millions of data points, the resulting object can be freely manipulated digitally, enabling dissecting, sorting and measuring (Fig. 2e,f). Further, real-space analysis drastically advances our understanding of the complex 4D physical dynamics, compared with current computer display (2D screen) observation. Therefore, this technology provides a paradigm shift of skin analysis, as compared with the current tangible target analysis using conventional on-computer analysis.

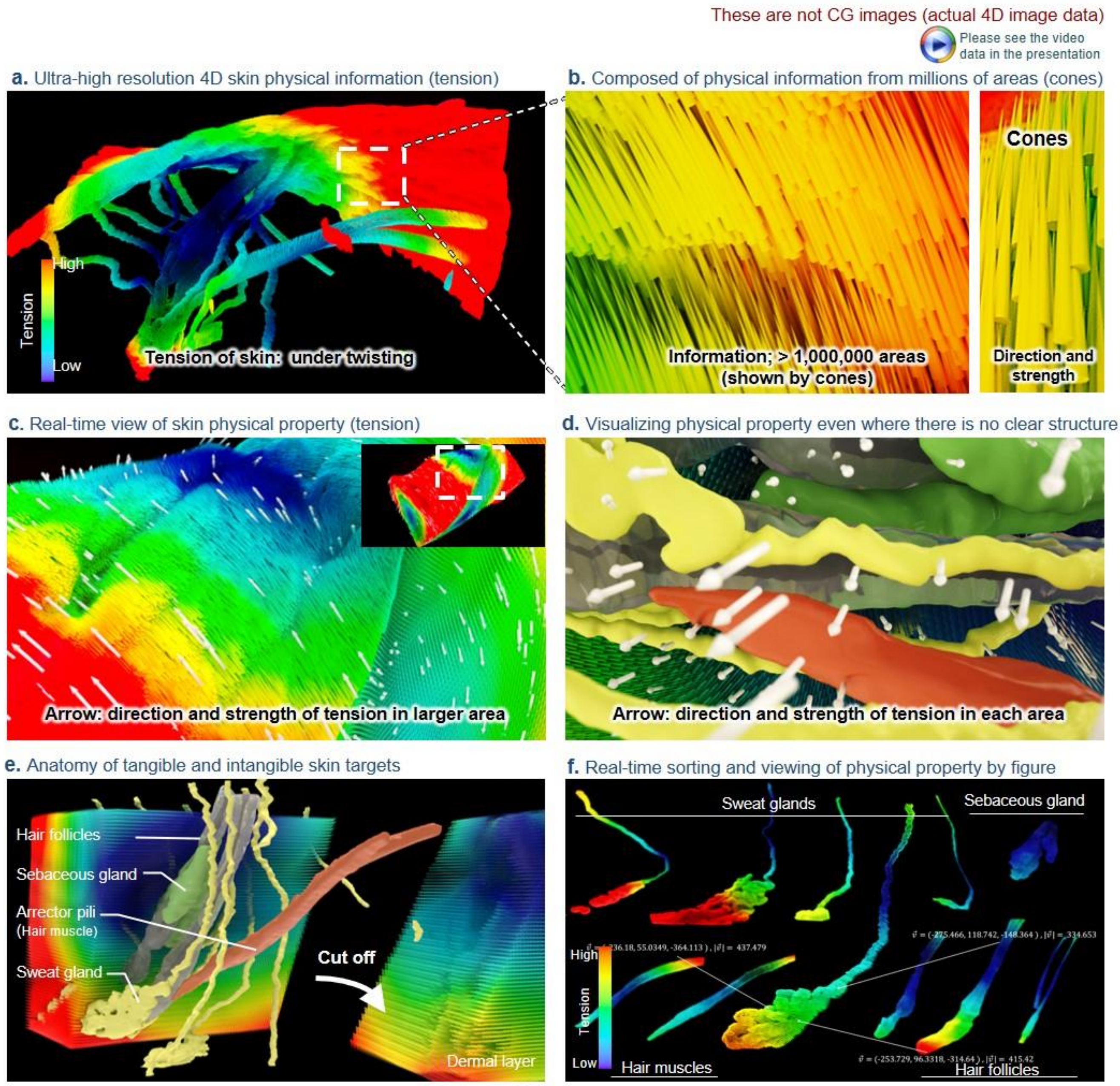

Figure 2. “Skin-mechanics reality” provides a paradigm shift in skin analysis as compared with current tangible target observation on computer

a) 4D skin physical properties in real space with ultra-high resolution (100 nm order). b) This digital skin is composed of millions of cones which carry physical and area information. c) Arrows show the direction and strength (indicated by the length of the arrow) of skin tension, in larger areas. d) Physical properties can be visualized anywhere in the skin, even where there is no clear structure. e) Dissection of digital skin. f) Real-time sorting and measurement of any target.

2. "Ring-collagen" produces a tensional network to form wrapping force of the face, and loss of it promotes facial aging

2.1. Facial skin contains ring-shaped high-tension areas in the deep dermal layer

Skin specimens contract immediately after excision. Thus, we reconstructed skin tension by stretching specimens to their pre-dissected size using a micro-manipulator, and observed the stretching/recovery process to reconstruct skin-mechanics reality (Fig. 3a). This technology revealed that in the static state of skin (in the face; tensioned), the skin tension is not uniform (Fig. 3b), but sites of high tension exist deep in the dermal layer (Fig. 3c,d). These sites are composed of ring-shaped high-tension areas (Fig. 3e,f). Directional analysis of tension of these ring-shaped areas showed that center-directed tension exists in these structures (Fig. 3g,h). Thus, facial skin contains ring-shaped areas with center-directed high tension, and the sum of these areas provides the skin tension over the whole face (Fig. 3i).

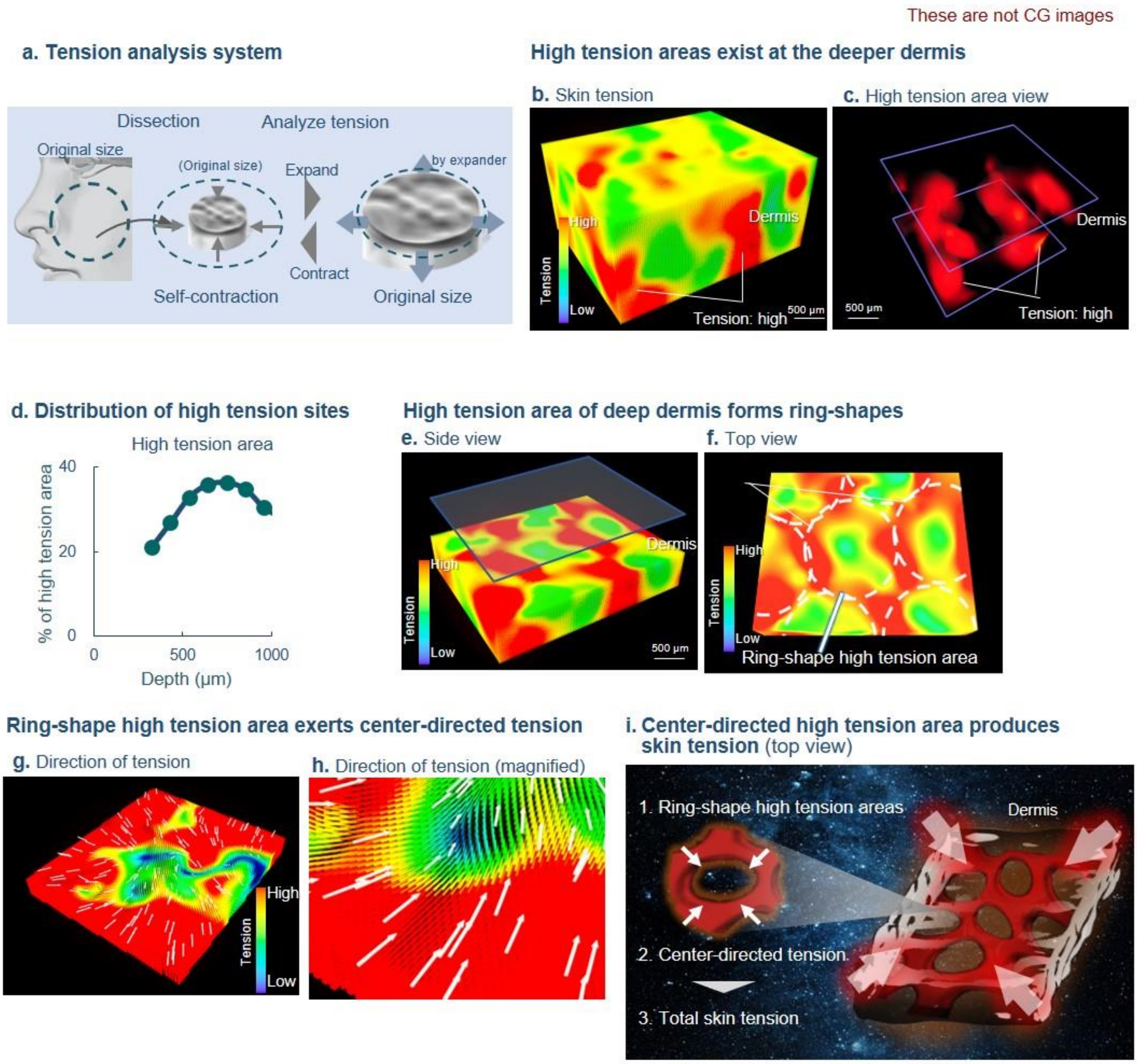

Figure 3. Facial skin tension is generated as the sum of the ring-shaped high-tension areas in the deep dermal layer

a) Schematic illustration of facial skin tension analysis. b) Reconstructed actual skin tension of the face (female, age 19). Tension is indicated by a color map of whole skin. c) Only high-tension areas are indicated. d) Distribution of high-tension areas. e) Side view and f) top view of horizontal section at the deep dermal layer (1,000 μm depth). Ring-shaped high-tension areas exist densely. g) The tension in these ring-shaped areas is directed towards the center, as shown in h) the magnified image (female, age 20). i) Ring-shaped center-directed high-tension areas in the deep dermal layer create the overall skin tension in facial skin.

2.2. Ring-shaped collagen structures, called "ring-collagen", surrounding proteoglycans produce high tension

To clarify the nature of the ring-shaped high-tension areas in the dermal layer, we investigated the relationship between tension and structure with our skin-mechanics reality system (Fig. 4a). We found that there are few structures in the high-tension areas (Fig. 4b). In fact, the high-tension areas consist of collagen in the form of rings (Fig. 4d,e). In contrast, low-tension areas contain single follicular units consisting of several fine hairs, with no clear skin structure (Fig. 4c,f). Immunohistochemical observation showed that these areas consist of proteoglycans, such as versican and periostin (Fig. 4g,h). This proteoglycan layer (PG layer) makes little contribution to the skin tension (Fig. 4i). Thus, taken together, our results indicate that facial skin contains ring-shaped collagen around fine hairs with PG layers; each ring-collagen structure produces center-directed tension, the sum of which creates the facial skin tension. We designated this facial skin tension-producing machinery as "ring-collagen" (Fig. 4j).

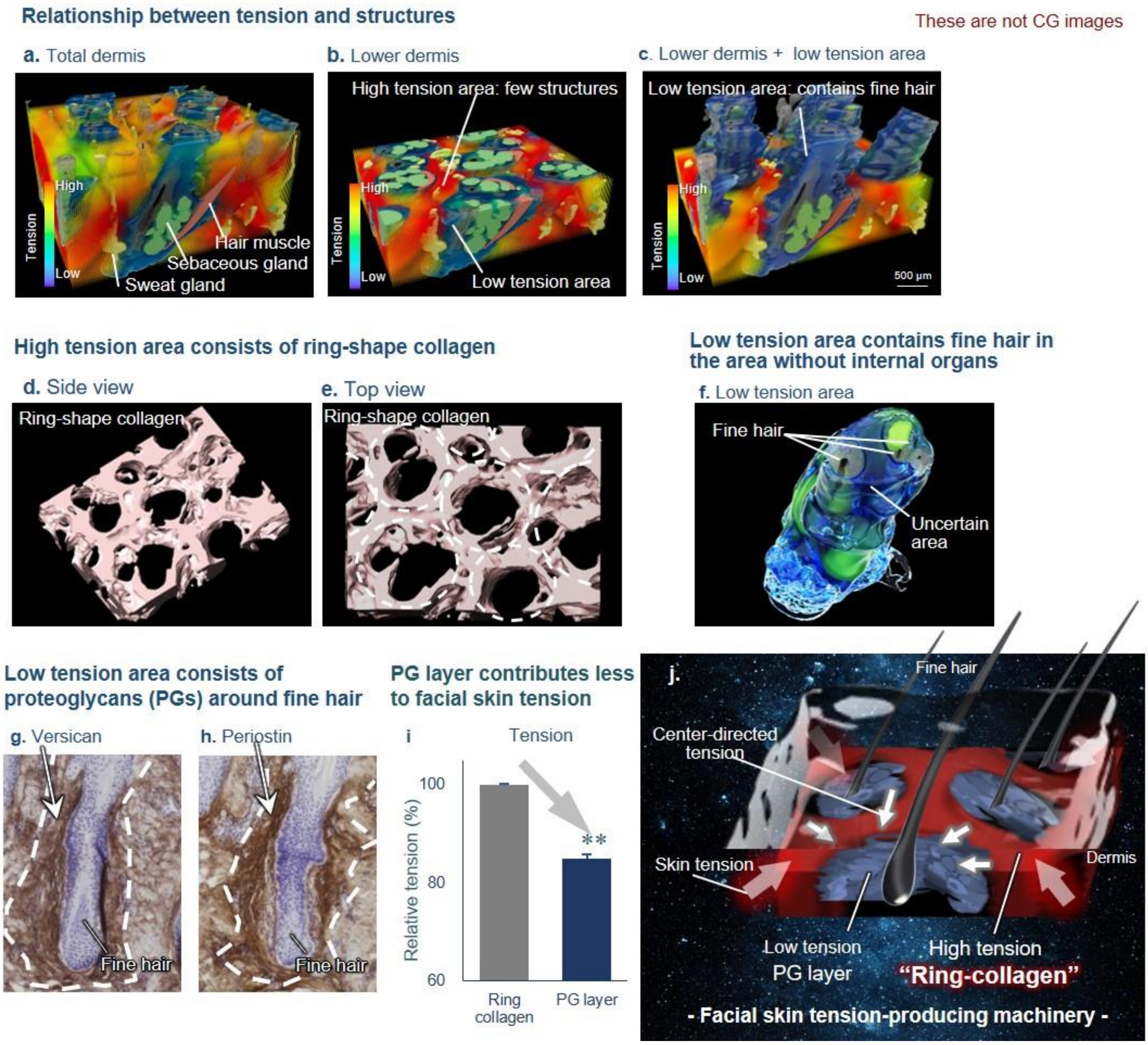

Figure 4. Ring-shaped collagen structures, called "ring-collagen", surrounding proteoglycans produce high tension

a) Relationship between reconstructed skin tension and the skin's internal organs (female, age; 19). b) Horizontal section of lower dermis. Few internal skin structures exist in the high-tension areas. c) Horizontal section of lower dermis with low-tension areas, composed of fine hairs and an area without internal organs (uncertain area). Components of a high-tension area in d) top and e) side view. Ring-shaped high-tension areas are consistent with the ring-shaped collagen. f) Components of a low-tension area, containing a follicular unit with fine hairs and an area with no internal organs. Immunohistochemical observation of low-tension area of young facial skin (age <40 years). The proteoglycans g) versican and h) periostin are present in the low-tension area around the follicular unit. i) Comparison of tension in the PG layer (low-tension area) and the ring-shaped collagen area (high-tension area) in young facial skin (age <40, n = 3) (**: $P<0.01$, Student's t test, means ± SEM). j) Schematic illustration of facial skin tension-producing machinery. Ring-shaped collagen, designated as ring-collagen, surrounds the PG layer and fine hairs (low tension), and this unique structure creates center-directed tension. The sum of the tension of these structures produces the overall skin tension.

2.3. Ring-collagen produces a tensional network, which creates “wrapping force” to maintain facial morphology

We found that the content of collagen in facial skin is low (<50 %) compared to body skin (>90 %), due to the presence of dense fine hairs (Fig. 5a-c). Then, we investigated how facial skin compensates for this disadvantage in order to maintain facial morphology (Fig. 5d). We found that when body skin tension was reconstructed, collagen fibers stretched and crossed each other (Fig. 5e,f), whereas when facial skin tension was reconstructed, collagen fibers in ring-collagen structures stretched and pulled each other in a circular manner (Fig. 5g,h). As shown in Fig. 5l.1-2, this suggests that stretched, circularly aligned ring-collagen fibers in facial skin produce a contraction force that generates tension, the sum of which is directed to the center of the ring. Moreover, when facial skin is deformed (Fig. 5i), the sum of this center-directed tension of all the rings (Fig. 5j,k) produces a large force that resists deformation. Thus, the ring-collagen structures form a “tensional network” to produce skin tension (Fig. 5l.3,4). Since the skin is fixed by ligaments and muscles [15], the interaction between the tensional network and ligaments, etc., generates a “wrapping force”, which causes the skin to wrap around the facial features, thereby serving to retain the facial morphology. This wrapping force enables facial skin to conform to the facial morphology despite the low content of collagen.

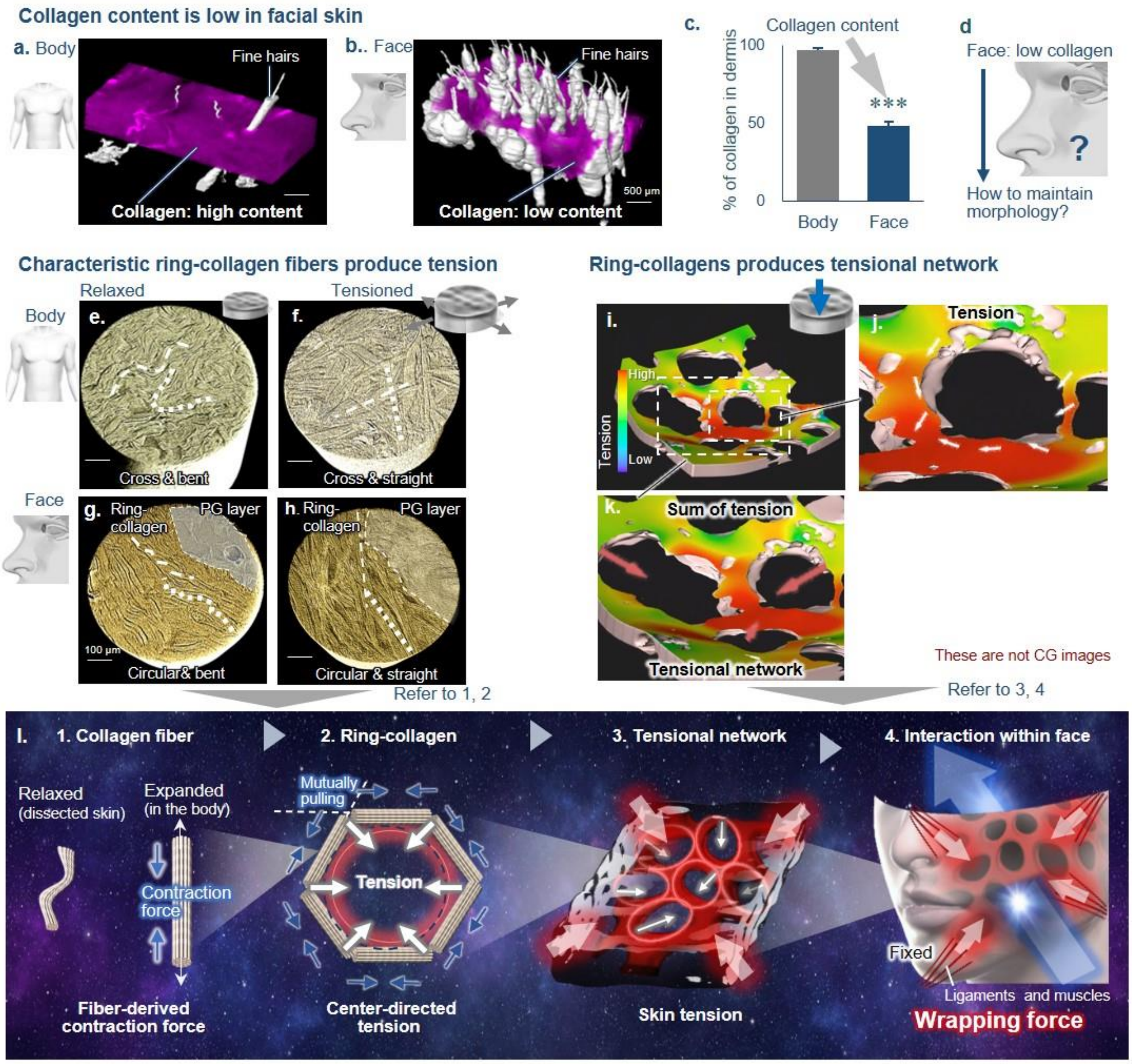

Figure 5. Ring-collagen system in facial skin produces wrapping force to maintain facial morphology
Collagen content of a) body and b) facial skin in the deep dermal layer of young skin (age<40 years). c) Collagen content in horizontal section of young dermis (1,000 µm depth) (N = 5, ***: P<0.001, Student’s t test, means ± SEM). d) How does facial skin maintain morphology despite its low collagen content? CT images of collagen fibers of e) body and g) facial skin in relaxed state (dissected), and f) body and h) facial skin after re-tensioning. i) Strength of tension in deformed (blue arrow direction) deep facial dermis. j) Direction of tension (white arrow) of ring-collagen. k) The sum of the tension of several ring-collagens (red arrow); tensional network. l) Schematic illustration of the way in which collagen fibers in ring-collagen generate a tensional network that interacts with skin structures to form large “wrapping force” that maintains facial morphology (see the text for details).

2.4. Ring-collagen decreases with aging, leading to aged appearance

How does this facial morphology-retaining system relate to facial aging? Skin-mechanics reality showed that ring-collagen is drastically decreased in aged facial skin compared to young skin due to loss of the PG layer (the core of the ring shapes) (Fig. 6a-g, j) , Indeed, high tension areas composed of ring-collagen are drastically decreased in aged skin (Fig. 6h,i). Thus, we established a non-invasive evaluation system for ring-collagen. We found that the diameter of fine hairs is significantly positively related to ring-collagen diameter (or PG layer diameter) (Fig. 6k-n). Thus, we established a 6-grade photograph-based evaluation system of fine hair status based on a combination of size and density to predict ring-collagen status (Fig. 6o). Human testing revealed that loss of ring-collagen decreases skin elasticity, and worsens aged appearance (sagging) (Fig. 6p). Therefore, loss of ring-collagen with aging results in decreased wrapping force, leading to aged facial appearance (Fig. 6q).

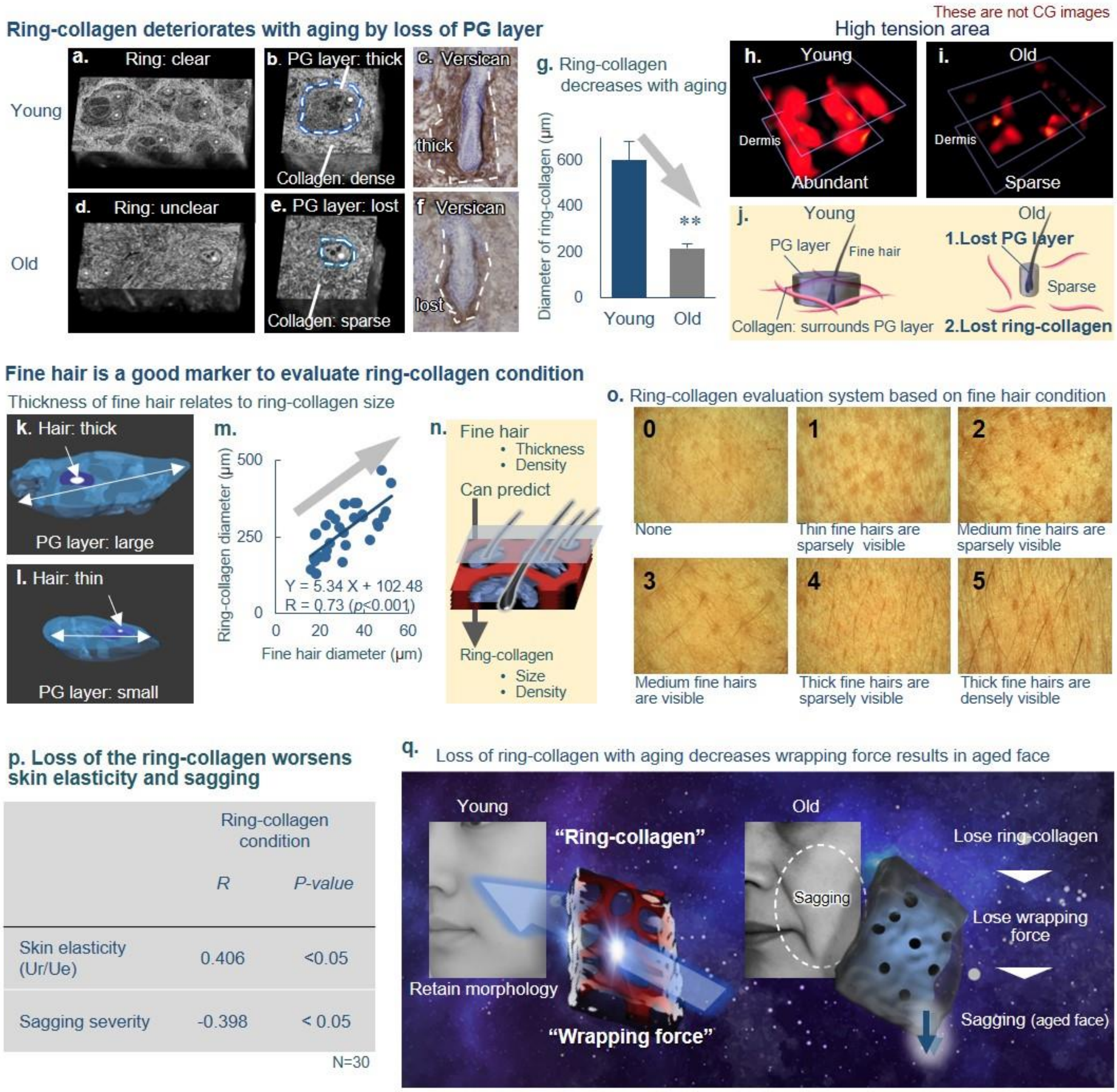

Figure 6. Loss of ring-collagen with aging decreases the wrapping force, leading to aged appearance

Collagen condition in a,b) young (age<40 years) and d,e) old (>60 years) facial dermal layer (1,000 µm depth), observed by CT. The dotted lines show the PG layer (the core of ring-collagen). Immunohistochemistry of PG (versican) in c) young and f) old skin showed loss of PG with aging (periostin gave similar results: data not shown). g) The size of ring-collagen (diameter of the PG layer) decreased with aging (N = 5, **: P<0.01, Student's t test, means ± SEM). High-tension areas of facial skin: h) young (age 19 years; shown in Fig. 3c) and i) aged (age 86 years). j) Loss of the PG layer with aging leads to deterioration of ring-collagen structure. CT images of the PG layer of k) young and l) old facial skin. m) The diameter of fine hairs is positively related to ring-collagen diameter (N = 31, Pearson's correlation coefficient). n) Ring-collagen condition can be predicted from the status of fine hairs. o) Newly established 6-grade evaluation system of ring-collagen condition based on fine hair status. p) Ring-collagen condition is significantly positively related to skin elasticity and negatively related to sagging severity (Spearman's correlation coefficient; 30 female cheeks). q) Ring-collagen decreases with aging, which decreases the wrapping force, leading to aged appearance.

3. "Transplantation" of the physical condition of young skin remodels ring-collagen and rejuvenates the face

3.1 Ring-collagen senses skin tension via fine hairs, and high tension regenerates ring-collagen

Our results suggest that ring-collagen is a critical target for facial rejuvenation, so we sought a way to improve it. First, we searched for an inducer of ring-collagen. We microdissected fibroblasts in the PG layer (the core of ring-collagen) and fine hair follicular cells (outer root sheath; ORS), and then we compared gene expression in the two systems (Fig. 7a,b). We found that Wnt16 induces PGs in fibroblasts (Fig. 7c-e). Further, Wnt16 is strongly expressed in ORS, but is drastically decreased in aged skin (Fig. 7f-h). Thus, Wnt16 can act as an inducer of ring-collagen. As shown in Fig. 7i, the internal/external physical burden of aged skin decreases due to the loss of internal volume (fat, muscle and bone) and decreased facial expression [9, 16]. Thus, we imposed the physical burden of young skin, high tension, on skin in an organ culture model (designated "environmental transplantation"), and found that this tensional burden increased the size of ring-collagen concomitantly with induction of Wnt16 (the putative inducer of ring-collagen) and increased PG production (Fig. 7j-o). These results suggest that ring-collagen is a skin tension organizer (sensor/regulator). Namely, it senses the tensional burden of skin via fine hairs, and ORS secrete Wnt16 in response to imposed tension, inducing PGs production in fibroblasts in the PG layer to expand the core of ring-collagen, thereby reconstructing ring-collagen (Fig. 7p).

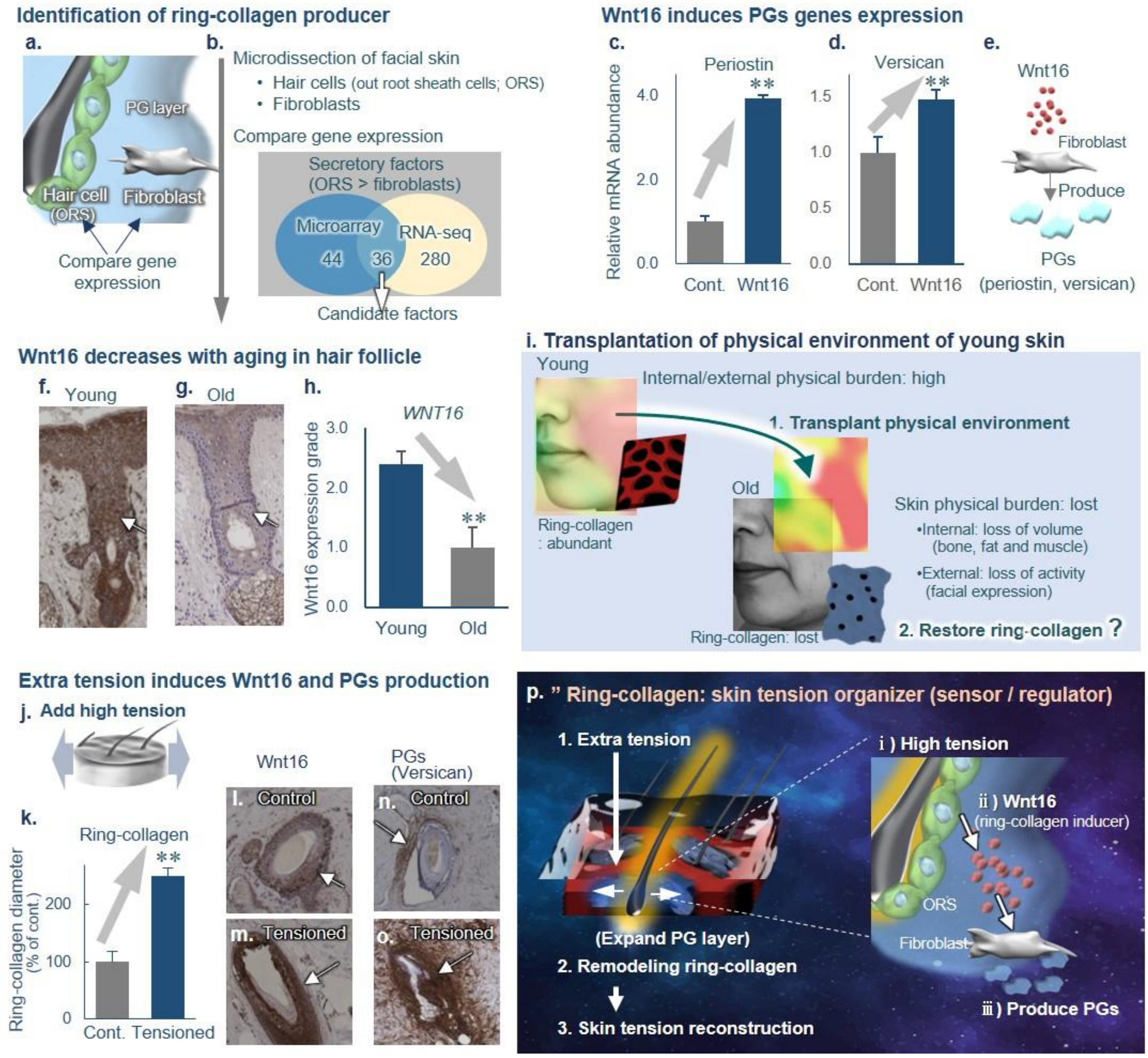

Figure 7. Ring-collagen as a tension organizer, and reconstruction of ring-collagen under high tension

a,b) Summary of identification of ring-collagen inducer by microdissection of young facial skin (age <40 years). Overexpressed secretory factors were selected as candidates. c-e) Wnt16 (1 μg/mL) induced gene expression of PGs in fibroblasts (N = 3, **: $P<0.01$, Student's t test, means ± SEM). Immunohistochemistry of f) young (age <40 years) and g) old (age >60 years) facial skin was conducted, h) and graded (o; none, 1; weakly detected, 2; moderately detected, 3; strongly detected) (young, N = 5; old, N = 6; **: $P<0.01$, Student's t test, means ± SEM). i) Aging decreases the physical burden on skin. j) Extra tensioning (30% expansion) of organ-cultured skin, cultured for 7 days (abdominal skin, ring-collagen sparsely detected, age<40 years). k) The size of ring-collagen was measured as the size of the PG layer (N = 3, **: $P<0.01$, Student's t test, means ± SEM). Immunohistochemistry of Wnt16 in l) control and m) extra-tensioned skin, and versican in n) control and o) tensioned skin. p) Ring-collagen as a skin tension organizer.

3.2. Ring-collagen senses the skin's environment, and "environmental transplantation" rejuvenates the face

Since the tensional burden of skin is important for facial appearance, we investigated the feasibility of physical skincare approaches. We found that other environmental stimulation, thermal stimulation, of follicular cells (ORS) induced expression of Wnt16 (ring-collagen inducer) (Fig. 8a), production of PGs (the core of ring-collagen) (Fig. 8c-i) and ring-collagen remodeling (Fig. 8j) in organ-cultured skin. These results suggest that ring-collagen senses and responds to a variety of skin physical conditions, physical forces and thermal conditions. We confirmed this by human testing, using a tensional approach as a representative modality. High tension for 4 weeks in middle-aged healthy volunteers significantly improved aged facial morphology (Fig. 8k-m) and skin elasticity (Ur/Ue), which is a parameter of skin wrapping force (Fig. 8n), with no increase of fine hairs. These results suggest that skin physically adapts to internal/external physical environments via ring-collagen, by remodeling itself (via the Wnt16-PG system) to regulate skin tension and control the wrapping force in order to maintain internal organs and the facial morphology (Fig. 8o).

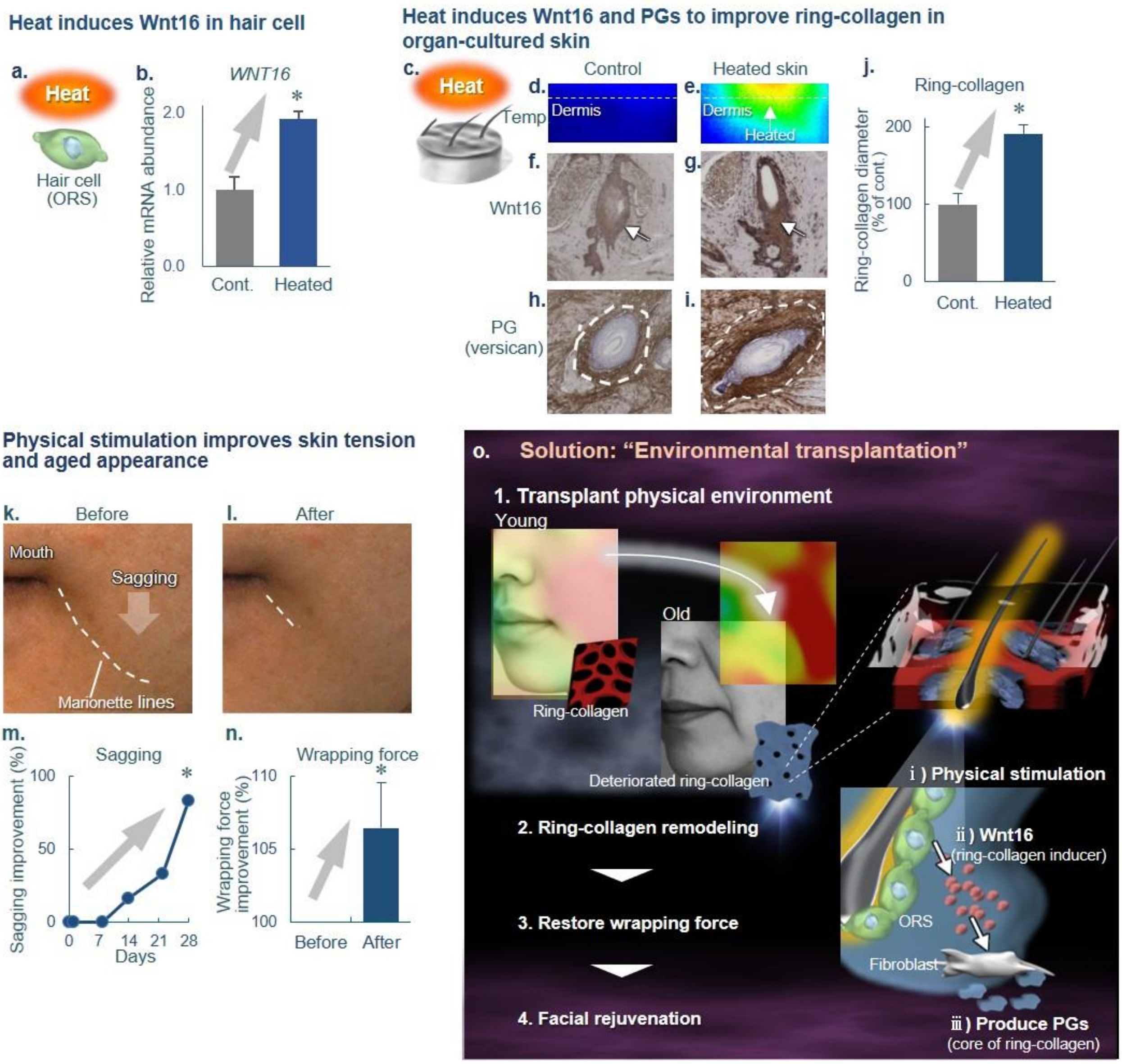

Figure 8. Skin physically adapts to the environment via ring-collagen, and environmental transplantation rejuvenates the face.
a) Thermal stimulation of hair follicular cells (ORS). ORS cells were thermally stimulated (42 degrees Celsius for 60 min). b) Wnt16 expression increased upon thermal stimulation ((N = 3, *: P<0.05, Student's t test, means ± SEM). c-e) Organ-cultured skin (abdominal, age<40 years) was thermally stimulated (42 degrees Celsius for 60 min). d, e) Thermography. Immunohistochemistry of Wnt16 in f) control and g) heated skin, and immunohistochemistry of versican (a representive of PGs) in h) control and i) heated skin. j) Ring-collagen size was determined as the size of the PG layer (immunohistochemistry of versican) (N = 3, **: P<0.01, Student's t test, means ± SEM). k,l) Human testing of the effect of a physical burden (high tension) showed an improvement in sagging of cheek skin (dotted lines show the marionette line, a marker of cheek sagging). m) Time course of improvement of sagging (*: P<0.05, Wilcoxon rank test, N = 6). n) Improvement of wrapping force. Ur/Ue was taken as a parameter of wrapping force (see Fig. 6n). o) Proposed solution for facial rejuvenation: "Environmental transplantation" of the physical condition of young skin.

Thus, we have discovered that facial skin contains a high density of “ring-collagen” structures, which produces a tensional network in skin due to the specific collagen fiber alignment (Fig. 9.1). This network produces a “wrapping force” that wraps the facial skin tightly onto underlying structures of the face to retain the facial morphology (Fig. 9.2, 3). Ring-collagen senses the internal/external physical environment of skin, and remodels itself via the Wnt16-PG system to adjust the skin tension to maintain the facial morphology in response to environmental changes (Fig. 9.4). Therefore, ring-collagen is a skin tension organizer (sensor and regulator). Aging decreases the skin’s physical burden and decreases Wnt16 (ring-collagen inducer), leading to the loss of PGs (Fig. 9.5). This in turn results in the deterioration of ring-collagen, thereby decreasing the wrapping force, which leads to sagging (aged facial appearance) (Fig. 9.6-8). We showed that environmental skincare, which we call environmental transplantation, imitating the physical burden of young skin, improves the state of ring-collagen, resulting in rejuvenation of facial appearance (Fig. 9.9). Thus, we believe ring-collagen is an emerging target of skincare.

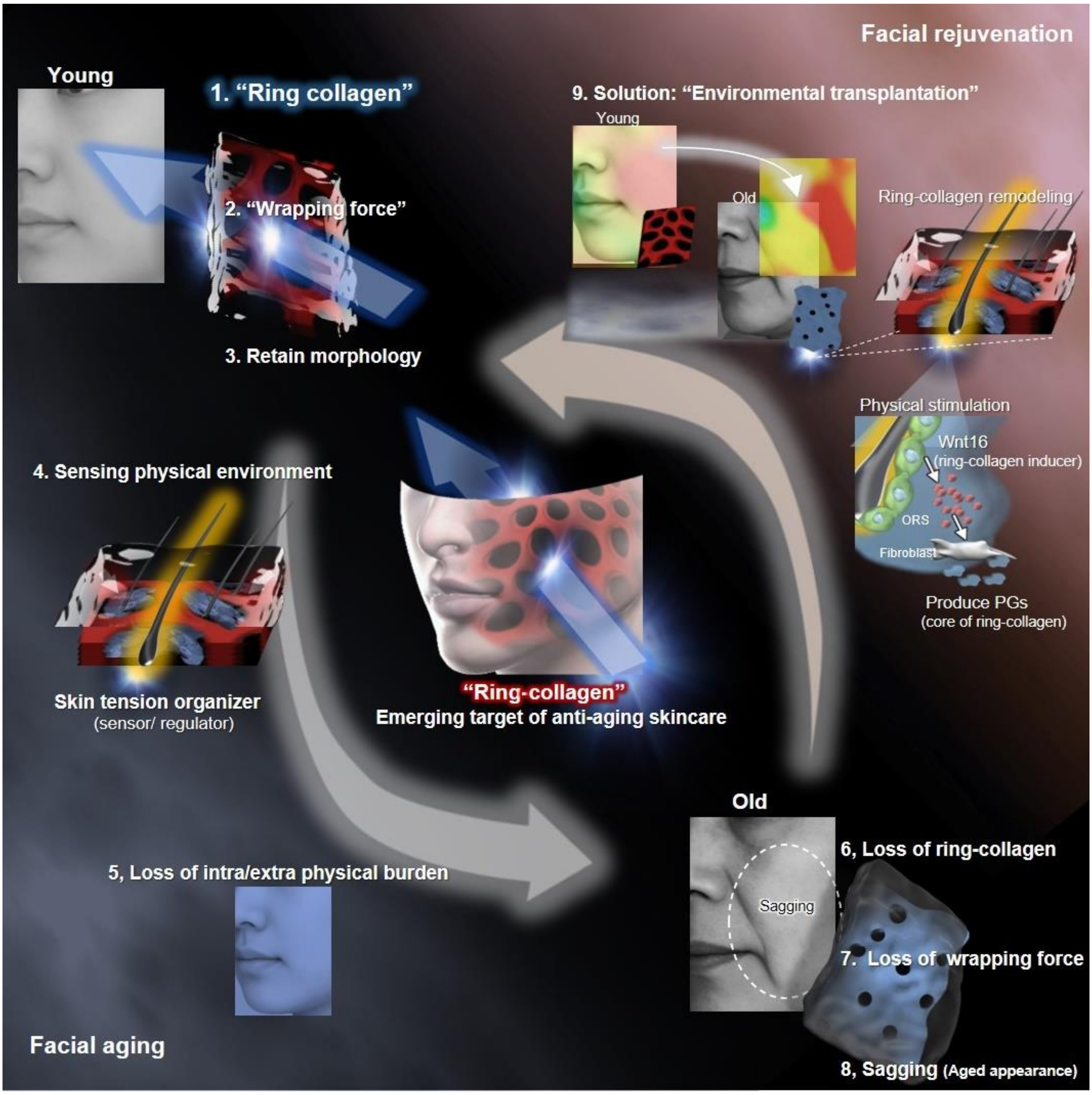

Figure 9. Ring-collagen is an emerging target for facial rejuvenation
1-4) Ring-collagen is an organizer of facial skin tension and wrapping force. 5-8) Loss of ring-collagen induces facial aging. 9) Intra/extra environmental skincare, “environmental transplantation”, is a promising approach for rejuvenation of facial appearance.

DISCUSSION

1. "Skin-mechanics reality" provides a paradigm shift for skin analysis

In this study, we aimed to clarify the fundamental mechanism of facial aging by identifying the physical machinery that enables facial skin to fit tightly onto underlying structures to maintain the facial morphology. For this purpose, we established a visualization system for intangible physical force, in real space. This technology was designated as "skin-mechanics reality", and represents a paradigm shift for skin analysis as follows.

1.1. "Skin-mechanics reality" revolutionizes skin analysis by moving from tangible structures to the intangible world (Fig. 10a)

Skin visualization technology has progressed from 2D to 3D, and then to 4D, but can still visualize only tangible targets, such as skin cells, internal organs and so on. In contrast, our skin-mechanics reality can visualize the 4D dynamics of intangible physical properties anywhere in the skin, even where there is no clear structure, for the first time. Indeed, we found variations of tension in the dermis in apparently uniform areas of extracellular matrix. This led to the unprecedented discovery of the ring-collagen system, and the concept of wrapping force.

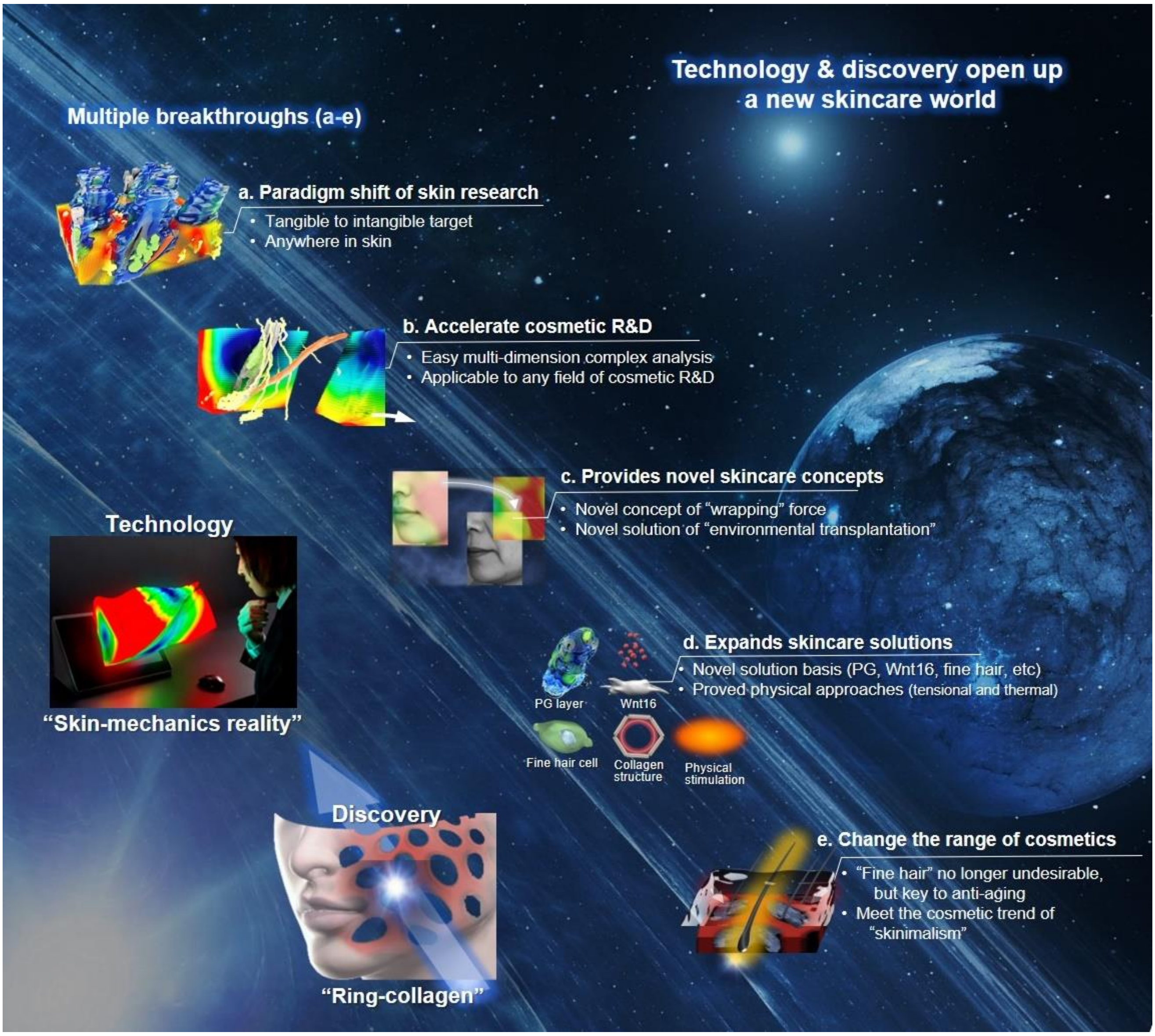

Figure 10. "Skin-mechanics reality" technology and discovery of "ring-collagen" provide multiple breakthroughs (a-e) that open up a new world of skincare.

1.2. Real-space skin analysis takes skin analysis to the next generation of cosmetic R&D (Fig. 10b)

"Skin-mechanics reality" enables us to visualize skin physical properties in 4D, in addition to the 4D dynamics of skin structures. However, the resulting information is very complex and difficult to understand just by on-screen analysis. So, we developed high-speed autostereoscopy to allow detailed observation of 4D dynamics as a stereoscopic image in real-time and in real space. This is superior to virtual reality (VR), since it does not require special glasses or equipment, which would restrict research activities such as operating computer equipment or instruments, or discussions with colleagues. This technology should be widely useful not only for skin analysis but also in other areas, such as cosmetic research, product development and services, since currently data sets are becoming ever larger and more complicated to deal with on conventional 2D displays. Thus, this real-space technology moves cosmetics R&D to the next generation.

2. Ring-collagen opens up a new frontier in skincare

By means of this technology, we discovered "ring-collagen" in facial skin as the fundamental structure generating a tensional network, which in turn generates the wrapping force to tightly fit the skin onto the underlying structures, thereby maintaining facial morphology. Further, this machinery can sense the skin's physical environment, and respond by remodeling itself, namely environmental adaptation of skin physically. Deterioration of ring-collagen due to the change of physical condition with aging induces facial aging, but transplantation of the physical environment of young skin restores ring-collagen (via the Wnt16-PG system) and the wrapping force to rejuvenate facial morphology. These wide-ranging discoveries open up a new frontier of skincare as follows.

2.1. Ring-collagen provides novel concepts for skincare (Fig. 10c)

1) Wrapping force: Current skin care for sagging focuses on skin ptosis due to gravity, (i.e., in the vertical direction), although this occurs after skin fitting to the face becomes looser (sagittal direction). This study first focused on this fundamental cause of sagging, namely impaired fitting, and established "wrapping force" produced by ring-collagen, as a breakthrough concept for sagging. Indeed, based on this novel concept, we established a new solution for facial rejuvenation.

2) Environmental transplantation for rejuvenation: Our results suggest that ring-collagen can sense and respond to changes in intra/extra environmental conditions by modulating the tension. This is reasonable, since the physical properties of skin have been reported to change depending on the season [17, 18]. Further, we found that loading the physical condition of young skin on aged skin can reconstruct the wrapping force of aged skin and rejuvenate facial appearance. This novel concept of "environmental transplantation" provides a completely new approach to facial rejuvenation.

2.2. Ring-collagen expands skincare solutions (Fig. 10d)

This study provides a wide range of novel skincare targets. For example, the ring-collagen inducer Wnt16 could be targeted with drugs, and the new anti-aging skincare target of ORS cells might also be targeted with drugs delivered via fine hairs. Further, instead of the conventional approach of focusing on collagen amount and quality, we can proceed towards a collagen-targeting solution at the structure level. Moreover, we confirmed the efficiency of physical stimulations in this study, providing the opportunity for a variety of novel physical treatments, devices, services and supportive products to conduct them.

2.3. Ring-collagen fundamentally changes the range of skincare (Fig. 10e)

1) Fine hair is not an unsightly blemish: The high density of fine hairs in facial skin drastically decreases the collagen content, but the ring-collagen system induced by fine hair compensates for this by generating a wrapping force. Indeed, fine hair acts as a sensor and inducer for tensional remodeling through secreting Wnt16. Further, our solution targeting this fine hair system does not increase fine hairs itself, so that we can separate anti-aging effect from fine hair growth concerns. These discoveries drastically change the commonsense aesthetic view of fine hair; instead of an unsightly blemish, it is a key target of skincare.

2) New era of minimal skincare: Currently minimal skincare, "skinimalism", is attracting increasing interest, as more and more consumers demand cosmetics with simple ingredients, both because of safety concerns and to reduce the environmental burden. The trend towards skinimalism has been accelerating during and after the pandemic, so that meeting this big trend is an urgent issue for cosmetic R&D. Our novel skincare concept of a physical approach (environmental transplantation) provides a basis for services and devices to rejuvenate facial skin even for skincare minimalists, without depending on just ingredients. Thus, our physical concept can contribute to the advance of cosmetic R&D to meet this new requirement in the next era.

CONCLUSION

We established novel skin analysis technology, "skin-mechanics reality", which represents a paradigm shift in skin analysis, enabling visualization of the 4D dynamics of intangible properties (physical dynamics), rather than just tangible structures, in real space and in real time. This technology enabled us to identify “ring-collagen”, and to show that it produces a tensional network that tightly “wraps” the facial skin around underlying structures, and thereby serves to retain the facial morphology, acting as an anti-deformation system. We also clarified that ring-collagen senses and modulates the skin physical condition in response to environmental changes via the Wnt16-PG system. These discoveries provide a wide range of new targets and fundamentally change the concept of skincare (“wrapping force” and “environmental transplantation”). We believe our “skin-mechanics reality” technology and discovery of “ring-collagen” provide multiple breakthroughs that open up a new world of skincare.

Note: There is no conflict of interest.

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REFERENCES

1. Ezure T, Yagi E, Kunizawa N, Hirao T, Amano S (2011) Comparison of sagging at the cheek and lower eyelid between male and female faces. Skin Res Technol 17:510-515.
2. Ezure T, Amano S (2012) Involvement of upper cheek sagging in nasolabial fold formation. Skin Res Technol 18:259-264.
3. Ezure T, Amano S, Matsuzaki K, Ohno N (2020) New Horizon in Skincare Targeting the Facial-Morphology-Retaining Dermal “Dynamic Belt” 31th IFSCC congress
4. Mouw JK, Ou G, Weaver VM (2014) Extracellular matrix assembly: a multiscale deconstruction. Nat Rev Mol Cell Biol 15:771-785.
5. Lee DH, Oh JH, Chung JH (2016) Glycosaminoglycan and proteoglycan in skin aging. J Dermatol Sci 83:174-181.
6. Ezure T, Amano S, Matsuzaki K (2021) Aging-related shift of eccrine sweat glands toward the skin surface due to tangling and rotation of the secretory ducts revealed by digital 3D skin reconstruction. Skin Res Technol 27:569-575.
7. Grymowicz M, Rudnicka E, Podfigurna A, Napierala P, Smolarczyk R, Smolarczyk K, Meczekalski B (2020) Hormonal Effects on Hair Follicles. Int J Mol Sci 21:5342-5345.
8. Mendoza-Reinoso V, Beverdam A (2018) Epidermal YAP activity drives canonical WNT16/beta-catenin signaling to promote keratinocyte proliferation in vitro and in the murine skin. Stem Cell Res 29:15-23.
9. Ezure T, Hosoi J, Amano S, Tsuchiya T (2009) Sagging of the cheek is related to skin elasticity, fat mass and mimetic muscle function. Skin Res Technol 15:299-305.
10. Ryu HS, Joo YH, Kim SO, Park KC, Youn SW (2008) Influence of age and regional differences on skin elasticity as measured by the Cutometer. Skin Res Technol 14:354-358.
11. Kim IG, Park SA, Lee SH, Choi JS, Cho H, Lee SJ, Kwon YW, Kwon SK (2020) Transplantation of a 3D-printed tracheal graft combined with iPS cell-derived MSCs and chondrocytes. Sci Rep 10:4326-4340.
12. Chen F, Qiu C, Liu Z (2022) Investigation of Autostereoscopic Displays Based on Various Display Technologies. Nanomaterials (Basel) 12:1-16.
13. Ezure T, Amano S (2011)Negative regulation of dermal fibroblasts by enlarged adipocytes through release of free fatty acids. J Invest Dermatol 131:2004-2009.
14. Sato Y, Mukai K, Watanabe S, Goto M, Shimosato Y (1986) The AMeX method. A simplified technique of tissue processing and paraffin embedding with improved preservation of antigens for immunostaining. Am J Pathol 125:431-435.
15. Swift A, Liew S, Weinkle S, Garcia JK, Silberberg MB (2021) The Facial Aging Process From the "Inside Out". Aesthet Surg J 41:1107-1119.
16. Ramirez OM, Robertson KM (2001) Comprehensive approach to rejuvenation of the neck. Facial Plast Surg 17:129-140.
17. Uchegbulam I, Danby SG, Lewis R, Carre MJ, Maiti R (2022) Effect of seasonal change on the biomechanical and physical properties of the human skin. J Mech Behav Biomed Mater 127:105058.-105068.
18. Nam GW, Baek JH, Koh JS, Hwang JK (2015) The seasonal variation in skin hydration, sebum, scaliness, brightness and elasticity in Korean females. Skin Res Technol 21:1-8.