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DIRECTIONAL BIOMECHANICAL PROPERTIES OF PORCINE SKIN TISSUE

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Skin is a multilayered composite material and composed principally of the proteins collagen, elastic fibers and fibroblasts. The direction-dependent material properties of skin tissue is important for physiological functions like skin expansion. The current study has developed methods to characterize the directional biomechanical properties of porcine skin tissues as studies have shown that pigs represent a useful animal model due to similarities between porcine and human skin. It is observed that skin tissue has a nonlinear anisotropy biomechanical behavior, where the parameters of material modulus is 378 ± 160 kPa in the preferred-fiber direction and 65.96 ± 40.49 kPa in the cross-fiber direction when stretching above 30% strain equibiaxially. The result from the study provides methods of characterizing biaxial mechanical properties of skin tissue, as the collagen fiber direction appears to be one of the primary determinants of tissue anisotropy.

Keywords: Mechanical property; skin tissue; collagen fiber orientation.

1. Introduction

Skins are complex in their structure and composition, and the properties of the skin vary from species to species.[1–4] It is comprised of several different types of proteins (e.g., collagens, elastin and proteoglycans) aligned in different directions and have molecular chains that have a directional orientation.[5,6] A previous study reported various mechanical properties before and after skin stretching, though failed to identify preferred-fiber and cross-fiber directions before mechanical characterizations.[7]

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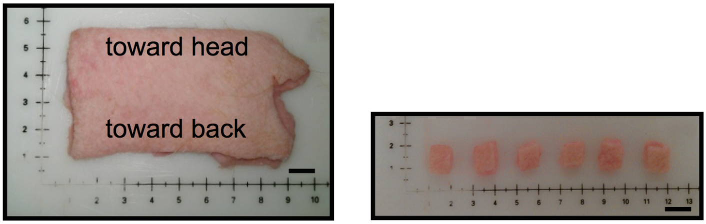
Mechanical testing under uniaxial tensile loading allows lateral contraction of the specimen during extension, which can further facilitate axial elongation.[7] Biaxial testing does not permit this process (or, more specifically, does so to a programmable degree), and hence skin loaded biaxially will facilitate accurate delineation of mechanical properties. Fibers composed of types I, III and V collagens are found in the tissue[8] and studies have shown that the spaces between collagen bundles change, reflecting a variation in collagen fiber directions.[9,10] Moreover, shearing tests on skin tissues were conducted to address its importance complementary to existing uniaxial and biaxial studies.[11]

In regenerative medicine, a significant effort is focused on increasing the area and volume of skin tissue using mechanical strain methods. The main intent is to plastically deform this biological material so that its surface area increases and over time regain its full thickness due to cell proliferation in a bioreactor. The driving force is the high fatality of severely burnt patients with a large area of thermally or chemically burnt skin tissue.[7,12,13] To treat patients with severe burns, small skin grafts can be harvested from healthy places on the body, quickly expanded to much larger areas *in-vitro*, compared to the original area and then be transplanted back to a burnt area of the body. Thus, skin expansion has become a valuable method in plastic and reconstructive surgery.[14,15] However, current prototype skin expansion bioreactors have been reported to cause tissue tearing due to uneven loading, gripping methods, stress concentration and other factors intrinsic to the tissue properties.[5] Tissue tearing is the key obstacle faced by *in-vitro* bioreactors, hindering many potential medical applications, such as reconstructing birth defects, burn injuries or breast tissue after mastectomy. Therefore, the current study aims to provide a method to better obtain fundamental quantitative data delineating direction-dependent mechanical properties of porcine skin tissue. It is expected that the identification of directional mechanical properties will optimize functional surface area enlargement of skin tissue, and hence better serve burnt patients in healthcare communities.

2. Materials and Method

2.1. *Sample preparation*

In the current study, effects of variability due to the sample thickness, age and species have been minimized via a well-controlled specimen source. Briefly, porcine skins from the belly area of six 6-month-old pigs were obtained from the Swine Education Unit at North Carolina State University immediately after euthanization for reasons unrelated to the current project, and tissues were prepared with a dermatome. The samples from our Swine Education Unit are selected in terms of the age and weight of pigs within the same species. Tissue samples were returned to the laboratory within 60 min after being euthanized. Subcutaneous fat tissue was removed and samples were then stretched out and held taut while being cut (Fig. 1(a)).



(a) (b)

Fig. 1. A representative sample preparation. (a) Tissue samples were returned to the laboratory within 60 min after being euthanized. Subcutaneous fat tissue was removed and samples were then stretched out and held taut while being cut. (b) Six tissue samples ($\sim 7\,\text{mm} \times 7\,\text{mm}$) from two different skins were prepared for each testing angle. Scale bar = 1 cm.

Six tissue samples ($\sim 7\,\text{mm} \times 7\,\text{mm}$) from two different skins were prepared for each testing angle (Fig. 1(b)). A micrometer was used to measure the thickness of the samples (ca. 1.08 mm) and the samples were stored in Hank's Balance Salt Solution (HBSS) for 2 h for the relaxation.

2.2. *Mechanical characterization of skin tissue*

Though biaxial testing of skin has been studied by Lanir and Fung,[16–18] the current work focuses on establishing a method to characterize direction-dependent mechanical properties. A biaxial tissue tester (BioTester 5000, CellScale, Waterloo, Canada), equipped with two load cells ($500\,\text{mN} \pm 1\,\text{mN}$) and actuators for each axis of loading, was used for measuring the force and displacement of the skin tissues[19] (Fig. 2(a)). The measured values were used to further obtain stress-strain curves and to calculate the parameters of material's modulus.[20–22] Synchronized time lapse video for real-time monitoring and post-process analysis was provided by the charged-couple device (CCD) camera, which acquires images with a pixel resolution of 1280×960 at an acquisition rate of 15 Hz, with a lens focal length of 75 mm.

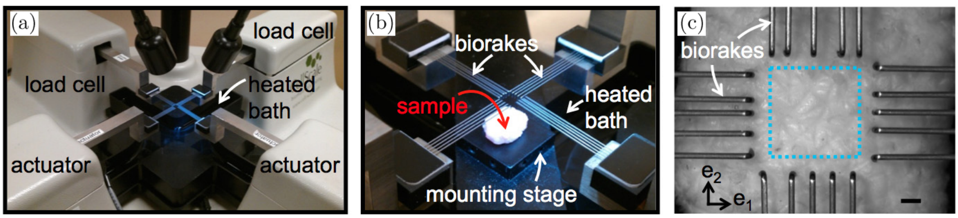


Fig. 2. The Biotester is capable of applying physiological plausible biaxial loading and stretching states to tissue samples. (a) The biotester is equipped with two load cells and two actuators for each axis. (b) The mounting stage enables samples being pierced through by the tungsten biorakes. (c) The biorakes provide evenly distributed loading/stretching states and this unique feature assures the control of loading conditions. Scale bar = 1 mm.

A temperature controlled saline bath with data logging capability provided a physiological environment for testing soft tissue specimens.

Specimen mounting is considered as one of the major challenges of biaxial testing.[19] For example, artifacts such as suturing procedures usually cause discrepancy in results due to inconsistent boundary conditions.[19,23] In contrast, biorakes provide fast and accurate sample mounting: each biorake consists of five tungsten tines used to anchor one edge of the specimen[19–22,24] (Fig. 2(b)). Four rakes provide uniform attachment across the edges of the samples and evenly distribute the load spanning 4 mm in length on each side of the sample (Fig. 2(c)). This unique feature not only assures the control of boundary conditions, but also significantly reduces the variability between sample sizes. In other words, if the sample size prepared is larger than $16\,\mathrm{mm}^2$, the active loading area remains $4\,\mathrm{mm} \times 4\,\mathrm{mm}$. After the sample was mounted, the sample was lowered into the HBSS bath, which was heated to 37°C to simulate an *in-vivo* physiological environment.

Prior to testing, a preload (average = 0.005 N) was applied to flatten the initially slack tissues and the flatten sample is chosen as our reference configuration. The tissue samples were tested up to 35% strain in both axes with a 15 s stretch cycle and a 15 s recovery cycle (i.e., strain-rate = 2.33%), with no hold time (Fig. 3(a)).

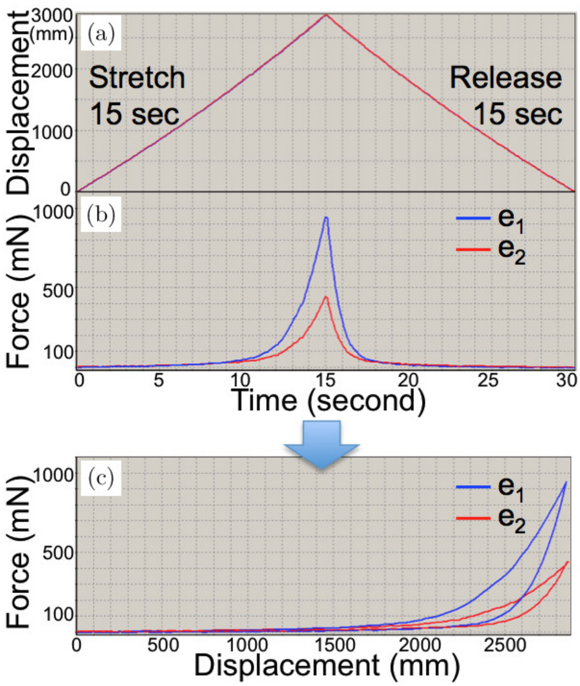


Fig. 3. (a) The tissue samples are tested up to 35% strain in both axes with a 15 s stretch cycle and a 15 s recovery cycle, with no hold time. (b) An integrated analysis module for Biotester outputs measured force data in both directions. (c) The module also generates force versus displacement relationships for both directions in real time during the equibiaxial testing.[21]

After the sample was mounted, one test was executed to pre-stretch the tissue to release the residual stresses inside tissue samples, i.e., pre-conditioning. The result of the pre-conditioning was not included in the material property measurements. Each sample was then tested five times to obtain the stress–strain curves of the tissue as accurate as possible, and it is defined as one set of tests spanning about 7 min to finish one set of testing. The image tracking and analysis software (LabJoy, CellScale, Waterloo, Canada), an integrated analysis module for Biotester, was used to output corresponding force data and to generate force versus displacement relationships for both directions in real time during the equibiaxial testing[21] (Figs. 3(b) and 3(c)).

2.3. *Collagen fiber directions identification*

When tissue samples are prepared, it is difficult to visualize preferred and cross-preferred collagen fiber directions as sometimes the Langer lines[25–28] are not clear in the samples. For an isotropic material, the moduli measured in any direction will be the same, and the differences between moduli (measured from different direction) will be minimum, i.e., zero. It leads to the hypothesis of the current study that the minimized coupling effects between preferred and cross-fiber directions are generally observed when their mechanical properties exhibit most differences between them, i.e., anisotropy. Therefore, to identify preferred and cross-fiber directions, the samples are rotated counterclockwise at approximately 25°–30° after each set of tests. Due to the symmetry, three testing angles are used: 0°, 30° and 60°. In Fig. 4, 0° samples "a" were prepared as described previously and as shown in Fig. 2(c), in which sample edges were aligned with stretching directions, e_1 and e_2 directions, respectively. Samples "b" were the ones after 30° rotation from the sample edges and samples "c" were the ones with a total of 60° rotation from the sample edges (Fig. 4). The goal is to develop a method that could be used to identify the preferred- and cross-fiber directions of the tissue and to determine the intrinsic direction-dependent mechanical property of skin samples (Fig. 4).

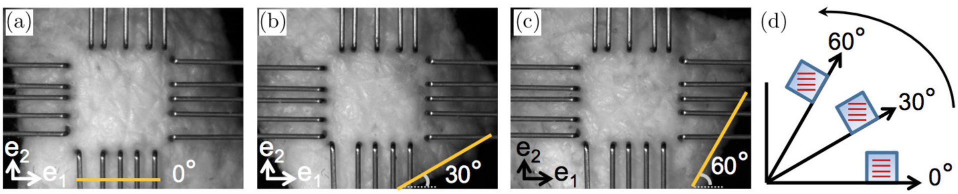


Fig. 4. (Color online) The samples are rotated counterclockwise at approximately 30° after each test to determine the direction-dependent mechanical property of skin samples. e_1 and e_2 directions are defined as stretching directions. Preferred-collagen fibers orientation is represented in color red, as shown in (d).

2.4. *Statistical analysis*

Data is presented as the mean ± standard deviation. The number of experimental samples is represented as n. Student's t tests are used to test differences in population means. Differences with $p < 0.05$ are considered significant.

3. Results and Discussion

An integrated analysis module (Lab joy, CellScale) for Biotester directly outputs measured force and displacement data in both directions. The force data is directly output from the load cells. Stresses are calculated based on the force divided by the size of the sample × the recorded thickness of the sample. The displacements are obtained from the relative positions of the rigid biorakes (from the actuators), and it is different from what have been presented in Fung's and others' work,[16–18,29,30] where few dots on the samples or a rectangular grid consisting of few points were generally used to calculate the displacements or strains during the biaxial testing. After averaging over measured stress versus strain for tissue samples ($n = 6$) for each testing angle under equibiaxial testing, the results are shown in Fig. 5. The plot shows the correlation between the strain and the resulting stress in the preferred-fiber and cross-fiber directions for skin tissue samples. In Fig. 5, clear nonlinear and anisotropy material behaviors (solid versus dashed lines) of the skin tissue are

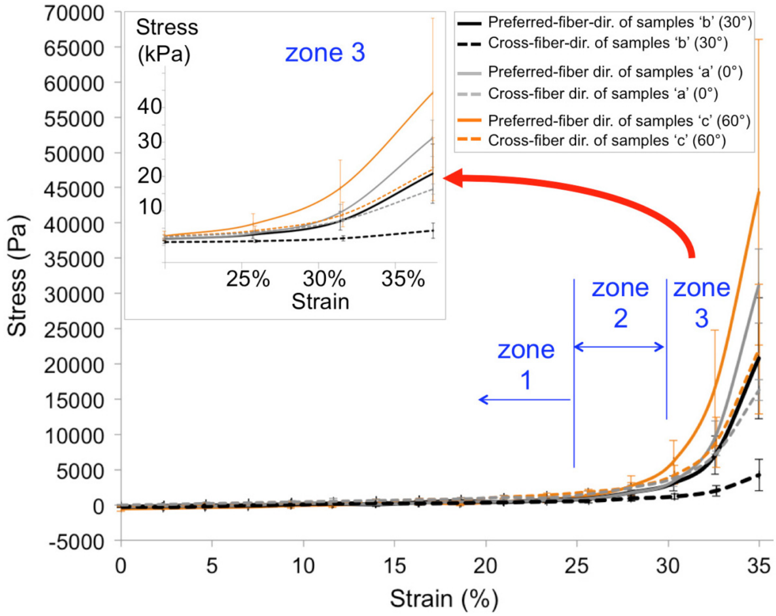


Fig. 5. Stress–strain curves for skin samples at different rotation angles ($n = 6$ for each testing angle). A nonlinear mechanical property is observed. Three zones are observed: zone 1 (0%–25% strain), zone 2 (25%–30% strain) and zone 3 (30%–35% strain). Inset: Stress–strain curves in zone 3.

Table 1. Piecewise parameters of materials modulus of samples "b" at 30° testing angle.

Modulus (kPa)	0%–25% of strain (Zone 1)	25%–30% of strain (Zone 2)	30%–35% of strain (Zone 3)
Preferred-fiber direction (p)	4.66 ± 1.01	46.70 ± 17.64	378.01 ± 160.13
Cross-fiber direction (c)	3.05 ± 0.65	13.27 ± 0.93	65.96 ± 40.49

observed in all samples at different testing angles. In addition, three zones are observed: zone 1 is between 0% and 25% strain stretching, zone 2 is between 25% and 30% strain stretching and zone 3 is between 30% and 35% strain stretching. Under the equal-biaxial stretching, it is observed that high standard deviations exist due to the higher mean stress values measured in the high strain (e.g., 30%) regime.

The moduli of the preferred-fiber direction and the cross-fiber direction in zone 3 (30%–35% strain) for samples at 30° testing angle (sample "b") are 378.01 kPa and 65.96 kPa, respectively, and the difference between them is 378.01 kPa − 65.96 kPa = 312.02 kPa (Table 1). The moduli of the preferred-fiber direction and the cross-fiber direction in zone 3 (30%–35% strain) for samples at 60° testing angle (sample "c") are 592.47 kPa and 289.45 kPa, respectively, and the difference between them is 592.47 kPa − 289.45 kPa = 303.02 kPa (Fig. 5). It is observed that samples at 30° testing angles exhibit most differences in mechanical properties between preferred and cross-fiber directions, i.e., samples "b" exhibit slightly larger anisotropy than samples "c" (Fig. 5). It is due to the fact that most of the collagen fibers are straightened and aligned along the e_1 stretching direction at this testing angle (Fig. 6(b)). Therefore, a more compliant material property is measured in the cross-stretching direction, e_2 (Fig. 5). In contrast, collagen fibers in samples at other testing angles are not perfectly aligned along and perpendicular to both stretching

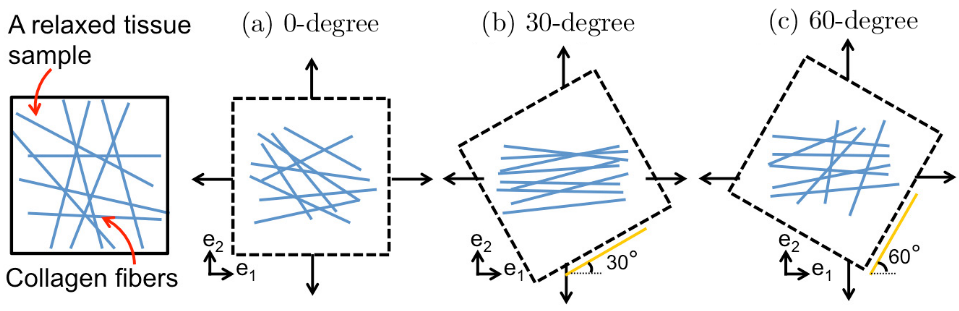


Fig. 6. Collagen fibers are randomly distributed in a relaxed tissue sample. From the stress–strain curves in Fig. 5, it is observed that samples at 30° testing angles have the most differences in mechanical properties between preferred and cross-fiber directions. It is implied that most of the collagen fibers are straightened and aligned along the e_1 stretching direction at this testing angle, as shown in (b). In contrast, collagen fibers in samples at other testing angles are not perfectly aligned along and perpendicular to both stretching directions e_1 and e_2, as shown in (a) and (c). Therefore, anisotropic behaviors of tissue samples are less obviously compared to the ones at the 30° testing angles, as shown in Fig. 5.

directions e_1 and e_2, respectively. Therefore, anisotropic behaviors of tissue samples are less obviously compared to the ones at the 30° testing angles.

Piecewise parameters of material modulus of samples "b" in three zones are listed in Table 1, and values are expressed as means ± standard deviations for skin tissue samples. Stress–strain curves in zone 1 provide moduli of elasticity of samples "b" and stress–strain curves in zones 2 and 3 provide tangent moduli of elasticity. The differences between the direction-dependent stress versus strain curves in samples "b" are mainly due to collagen fibers arrangements.[31–34] Most of the collagen fibers in samples "b" align along the e_1 direction, therefore stiffer mechanical properties in the preferred-fiber direction are observed (Figs. 5 and 6(b)).

In zone 1 (0%–25% strain), it is observed that the modulus in the preferred-fiber direction is higher than that in the cross-fiber direction ($E_p^{(1)}/E_c^{(1)} = 1.53$), suggesting that the skin sample has slightly anisotropic material properties in zone 1 (Table 1 and Fig. 5). This could be due to randomly distributed collagen fibers in skin tissue samples being not fully aligned along the preferred-collagen fiber direction before reaching 25% equibiaxial stretching, as shown in Fig. 5. In zone 2 (25%–30% strain), it is observed that the modulus in the preferred-fiber direction is more than 3 times higher than that in the cross-fiber direction ($E_p^{(2)}/E_c^{(2)} = 3.52$), as shown in Table 1 and Fig. 5. It is suggested that the skin sample has anisotropic material properties in zone 2 (Table 1 and Fig. 5). However, it is still not clear which intrinsic biological characteristic gives rise to the interesting microstructure features of collagen fibers that reflect back to the measured mechanical property in zone 2. In zone 3 (30%–35% strain), it is observed that the modulus in the preferred-fiber direction is more than five times higher than that in the cross-fiber direction for the skin tissue samples ($E_p^{(3)}/E_c^{(3)} = 5.73$) (Table 1 and Fig. 5). The increased ratio of this mechanical property in the skin tissue samples suggests that collagen fibers experience most realignment and straightening in zone 3.

Comparing zones 1 and 2, a ten-fold increase in the modulus in the preferred-fiber direction is observed ($E_p^{(2)}/E_p^{(1)} = 10.02$). In contrast, only a four-fold increase in the modulus in the cross-fiber direction is observed ($E_c^{(2)}/E_c^{(1)} = 4.35$). The result confirms that this highly anisotropic material property in skin tissues is dominated by the orientation of the collagen fibers.[35–37] Moreover, comparing zones 2 and 3, the modulus of the skin tissue samples in the preferred-fiber direction is eight-fold higher than the one in zone 2 ($E_p^{(3)}/E_p^{(2)} = 8.09$), which is slightly lower than the one in zone 1 ($E_p^{(2)}/E_p^{(1)} = 10.02$). Interestingly, the modulus of the skin tissue samples in the cross-fiber direction is comparable to the one in zones 2 and 1 ($E_c^{(3)}/E_c^{(2)} = 4.97$ and $E_c^{(2)}/E_c^{(1)} = 4.35$). It is suggested that collagen fiber alignment in skin tissues is saturated when samples are stretched above 30% strain (Table 1 and Fig. 5). In the current *in-vitro* study, it was recognized in pilot experiments that skin samples began to tear when stretched above 35% strain, consistent with observations reported in other studies.[7] In this study, it is intended to conduct a parametric study to understand direction-dependent mechanical properties. Therefore, tissue samples are equibiaxially stretched only up to 35% of the strain.

One of the limitations in the current study is that only one cycle of pre-conditioning is used in the study where the test was executed to pre-stretch the tissue to release the residual stresses inside tissue samples. Hysteresis is observed during equibiaxial testing (Fig. 3(c)), therefore viscoelasticity play an important role in the mechanical behavior of skin tissue. Viscoelasticity of skin tissues have been characterized previously,[7,38] and it is observed that the stress relaxation of the tissue is highly dependent on the duration of the tissue stretching. Moreover, the results from the studies also revealed that the skin tissue slowly recovers back to the original mechanical properties. However, the studies failed to identify preferred-fiber and cross-fiber directions prior to these mechanical measurements.[7,38] Therefore, the current study aims to establish a measurement method to identify and quantify direction-dependent mechanical properties of skin tissue prior to viscoelasticity measurement. The follow up study focusing on directional-visco-elasticity of skin tissue prior to and after skin stretching is currently in preparation. While the focus of the current study was purely biomechanical, leveraging our laboratory's biaxial mechanical testing expertise, we intend in follow-up studies to conduct histological and biochemical evaluations, toward developing structure–function relationships for the skin. Moreover, surface-area gain for reconstructive surgery has been studied by varying tissue expander shapes[39] and tissue expansion speed and duration,[38] and has been characterized by histochemical,[40] histomorphological and ultrastructural analyses.[5] Therefore, the demonstrated method in the current study provides a great foundation on optimizing surface-area gain for the reconstructive surgery. That is, by stretching skin tissue with proper direction, force, speed and duration, it is expected to achieve a functional tissue expansion and helping patients who need a large surface area of skin grafts or reconstructions.

4. Conclusion

Biological tissues have a complex microstructure and their biomechanical behaviors are highly related to inhomogeneous collagen fiber architecture. The current study provides a method to quantify directional mechanical properties of skin tissues, which is very important prior to reporting mechanical properties of skin tissues. To the authors' knowledge, it is the first reported directional biomechanical properties of skin tissue at the large strain range (above 30%). The directional–mechanical properties of skin tissue is measured biaxially and identified via the anisotropic behavior of tissue samples. It is observed that skin tissue has a nonlinear anisotropy biomechanical behavior, where the parameters of material modulus are 378 ± 160 kPa in the preferred-fiber direction and 65.96 ± 40.49 kPa in the cross-fiber direction when stretching above 30% strain equibiaxially. The result from the study provides methods of characterizing biaxial mechanical properties of skin tissue, as the collagen fiber direction appears to be one of the primary determinants of tissue anisotropy.

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