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Original paper

Determination of the mechanical properties of the skin of pig foetuses with respect to its structure

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Skin is an important barrier protecting the organism against external environmental factors. Determination of its mechanical characteristics as regards its structure has significant scientific and application value. In this work, uniaxial tensile tests were conducted to determine the basic mechanical parameters of skin with respect to its structure. The subject of the study were skin samples taken from domestic pig foetuses. They were excised from different parts of body, in the direction parallel to the long axis of the body.

Regardless of the sampling site, the tests revealed no significant differences in the values of the maximum tensile strength (2.08 ± 0.25 MPa) and the conventional Young's modulus (5.87 ± 1.52 MPa). The mechanical and structural tests confirmed that regardless of the sampling region the skin of domestic pig foetuses may constitute a human skin substitute model.

Key words: *skin, uniaxial tensile test, mechanical properties, histology, animal specimens*

1. Introduction

The skin, being a multilayer material, is adapted to a number of functions important for the body, ensuring its proper operation. We can distinguish three main layers in the histological structure of the skin: epidermis, dermis, and hypodermis [18], [22]. In the case of individual regions of the body, the integumentary system (epidermis and dermis) undergoes modifications during its development to adapt to the performance of specific functions, depending on the local specific load conditions. In terms of mechanical properties, the most important role is played by the dermis layer [20], [21]. The physicochemical properties of individual structural elements of skin are affected by a number of biological factors: an individual's genotype, diet, and physiological status, including the level of hormones, vitamins, micro- and macronutrients [6], [26].

So far, mechanical properties have been studied on animal models (including mice, rats, sheep, and domestic pigs) because this ensures high homogeneity of the material. This stems from a uniform way of feeding, similar impact of external factors, and the same age of the animals forming the research group [2], [8], [22], [23], [24], [29]. The aim of this study was to determine the basic mechanical properties of the skin of domestic pig foetuses, sampled in a direction parallel to the long axis of the body from different regions of the body (nuchal region, dorsal region, lateral abdominal region, cranial abdominal region, and thoracic limb region), with respect to its structure. The analysis of an up-to-date knowledge suggests that mature porcine skin shows the greatest similarity to human skin both in terms of structure and mechanical

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properties [11], [15], [19], [22]. There is a particularly close similarity between the thickness of the individual layers of the skin of pig foetuses used in the studies and the corresponding integuments in humans [3], [4], [12], [17], [22]. There are as yet no study results confirming that the skin of domestic pig foetuses resembles human skin also in terms of the mechanical properties. At the same time, the description of the basic structural and mechanical properties of skin would enable the use of the skin from pig foetuses as a human skin substitute.

2. Material and methods

The research material was the skin of 5 domestic pig foetuses with an average weight of 800 ± 100 g, 30 ± 3 cm long, obtained thanks to our cooperation with the Department of Animal Physiology and Biostructure, Wrocław University of Environmental and Life Sciences. Skin samples were excised from different regions of the body (figure 1): nuchal region (NR), dorsal region (DR), lateral abdominal region (LAR), cranial abdominal region (CAR), and thoracic limb region (TLR). The samples, cut out with a special punch, had the same geometric dimensions: 50 mm in length and 5 mm in width. Until the time of examination (no longer than 5 hours) they were stored at a room temperature in 0.9% normal saline. According to the analysis of an up-to-date knowledge, short-term storage of tissues in normal saline does not adversely affect the appearance or the behaviour of the samples during examination [7].

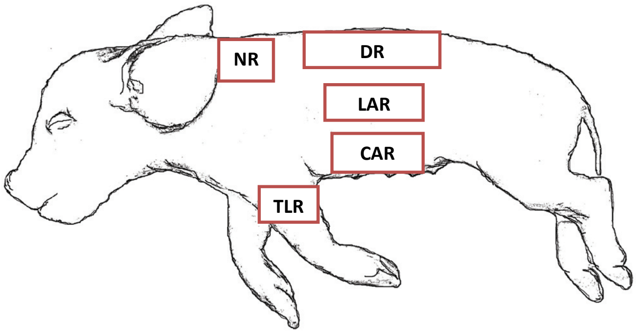

Fig. 1. The regions of sampling of the research material: NR – nuchal region, DR – dorsal region, LAR – lateral abdominal region, CAR – cranial abdominal region, and TLR – thoracic limb region

The methodology of examining the skin of domestic pig foetuses involved mechanical and structural tests. The mechanical tests were conducted on the day of sample collection. The tests involved uniaxial stretching of the samples at a constant rate of 5 mm/min [13], [20], [24], [25] until they broke. For this purpose, the samples were mounted on an MTS Synergie 100 testing machine by means of special flat clamps. The geometric dimensions of the samples were measured without contact during their stretching using an MTS ME 46 MG videoextensometer system. Before the proper test, each sample was subjected to three loading–unloading cycles [9], [5].

The samples underwent histological tests before (control) and after the mechanical tests. The skin samples were fixed in 4% buffered formalin solution, dehydrated in alcohol series, and embedded in paraffin. Paraffin blocks were cut with the use of a Microm HM 315 rotating microtome into 5 µm thick sections, which were then stained with Delafield's hematoxylin and eosin according to van Gieson's method. Histological images of the skin were examined under a Nikon 80i Eclipse light microscope in transmitted and polarized light. Next, using Nikon's Nis-Elements AR software, a morphometric analysis was performed on collagen fibres because those structural elements mainly affect the mechanical properties of skin. The length and thickness of individual collagen fibres of each specimen were measured in ten independent fields of view. The area occupied by all collagen fibres was also calculated.

All the results obtained were statistically analysed using OriginPro 8 software and were presented as the mean values with standard deviation ($X \pm$ SD).

3. Results

The non-linear stress–strain curves obtained (figure 2) were used to determine basic mechanical parameters of skin, i.e.: conventional Young's modulus (E) and the maximum value of stress ($\sigma_{\max}$). The cross-section area of the sample was determined as follows:

$$A = S \times G\,, \tag{1}$$

where: A (mm^2) – the cross-section surface area of the sample, S (mm) – sample width, G (mm) – sample thickness.

The conventional Young's modulus (E) was obtained from the slope of the linear section of the stress–strain curve. The maximum stress ($\sigma_{\max}$) was obtained as the peak of a stress–strain curve (figure 3).

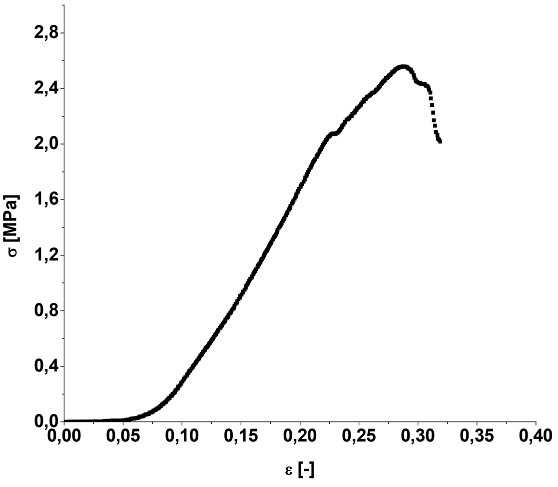

Fig. 2. Stress–strain curve representing a skin sample subjected to uniaxial stretching, collected from the lateral abdominal region (LAR)

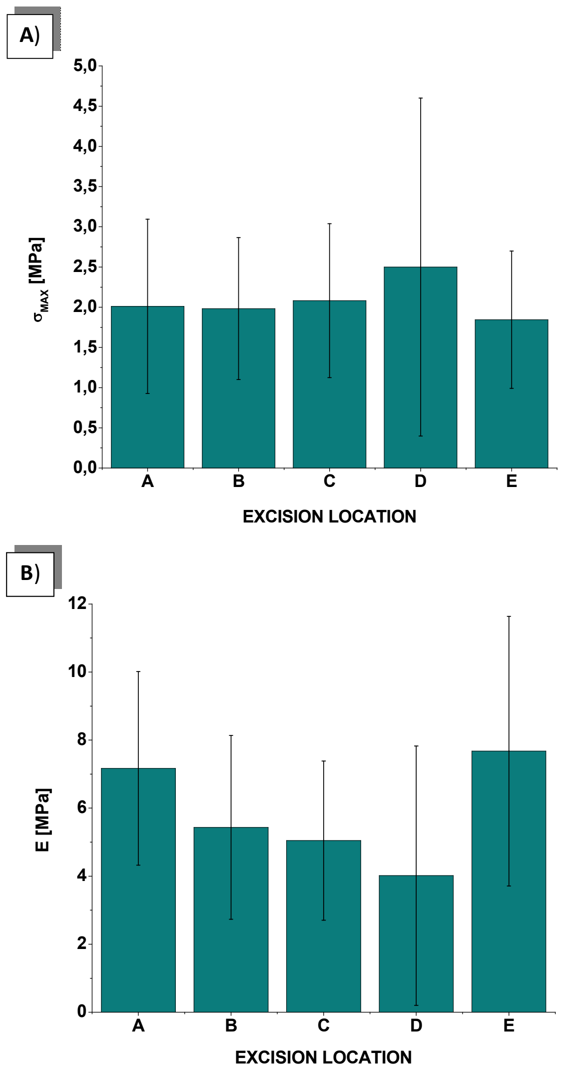

Fig. 3. A total of average values of the determined mechanical parameters: A) the value of the maximum tensile strength ($\sigma_{\max}$), B) the value of the conventional Young's modulus (E), depending on the body region from which the specimen was collected

Skin from different regions of the body shows similar values of the maximum tensile strength, which amounted to 2.07 ± 1.22 MPa at a force not exceeding 12.19 ± 5.54 N. The highest value of 2.50 ± 2.10 MPa was obtained for the samples collected from the cranial abdominal region (CAR), and the lowest value of 1.85 ± 0.85 MPa was revealed by the samples collected from the thoracic limb region (TLR) (figure 3a). The conventional Young's modulus (*E*) demonstrated different values, depending on the sampling site. At a stress value not exceeding 2.5 MPa the samples collected from the cranial abdominal region (CAR) demonstrated the lowest values of the conventional Young's modulus, i.e., 4.02 ± 3.81 MPa, compared to the skin samples collected from the thoracic limb region (TLR) that showed 7.68 ± 3.96 MPa and from the nuchal region (NR) that showed 7.17 ± 2.84 MPa. This testifies to lower skin stiffness at a given body region (figure 3b).

Measurement of skin thickness and analysis of the histological images obtained confirmed the observations made in mechanical tests. Changes in skin thickness were observed mainly in the cranial abdominal region (CAR), where, as a result of stretching, the values dropped by 37%. Skin thickness values also decreased by 12% in the lateral abdominal region (LAR) and by 16% in the dorsal region (DR) (figure 4). These changes were connected with retraction of the epithelium, and also, in the case of dorsal region (DR), with a high concentration of collagen fibres in the dermis layer. In the case of the skin from the nuchal region (NR), there was a slight increase in the skin thickness due to the presence of muscle tissue, which, after stretching, caused a strong shrinkage of the skin. This

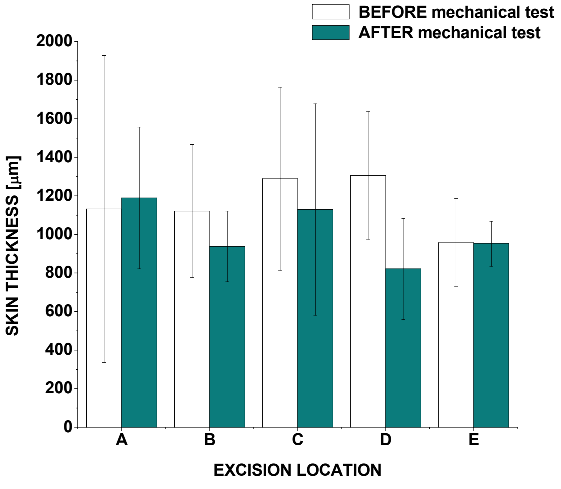

Fig. 4. A total of mean values of skin thickness (the epidermis and dermis layers) for the specimens collected before and after the mechanical test, depending on the body region from which the specimens were collected

showed that the skin from the nuchal region (NR) was not an appropriate region to examine changes in the mechanical and structural properties of skin.

A characteristic feature of the skin of the foetuses examined was the low degree of keratinisation of the epidermis, in which numerous observed invaginations constituted hair buds. In these sites the epidermis was often almost twice as thick, whereas keratinisation was lower than in the other regions (figure 5). After the stretching process there were no major destructive changes visible in the epithelium layer. The epithelium shape was usually determined by the degree of development of the connective tissue of dermis.

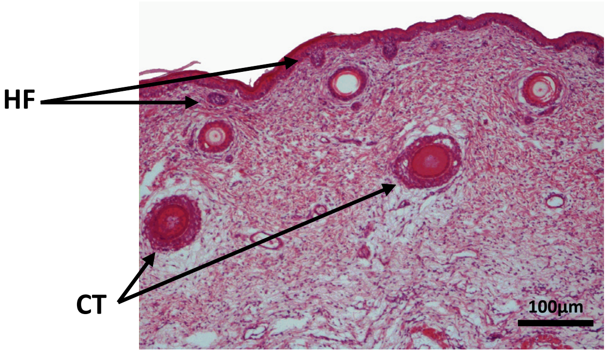

Fig. 5. Image of skin structure of a sample after the mechanical test. Skin fragments from the cranial abdominal region (CAR). The epithelium with a small degree of keratinisation with visible hair follicles (HF). Around the hair there is visible associated connective tissue (CT) that is not deformed. H&E staining (100× magnification)

The mechanical properties of skin are mostly determined by the dermis layer [20], [21]. Here, we can clearly distinguish two or even three layers, of which the layer lying under the epithelium is built of numerous thick collagen fibres forming trabeculae, whose spatial arrangement is specific to the particular site. As a result, we can observe in skin three zones of varying degrees of elasticity. The greatest degree of elasticity is demonstrated by the epithelium region and the adjoining connective tissue, as well as the region adjoining hypodermis. If some structural element (e.g., a hair) runs or is present in all those layers, it moves as a result of stretching at a rate characteristic of that layer.

In the dermis of foetuses, depending on the area, we can observe hairs in different stages of development together with glands and skin muscles and two distinct membranes containing numerous elastic fibres lying under the epithelium in the hypodermis region (figure 6). These membranes, together with skin muscles, were responsible for the phenomenon of skin retraction after stretching. This modified the appearance of the epithelium and the hypodermis, which in this case became wrinkled. As a result of stretching; the angle between hairs together with their tufts and the skin surface significantly decreased in accordance with the direction of the forces. This was related to the fact that a hair bulb lying deep in the dermis did not move as quickly as the site at which the hair tuft opened up to the surface. As a result, the hair underwent a characteristic "lie down".

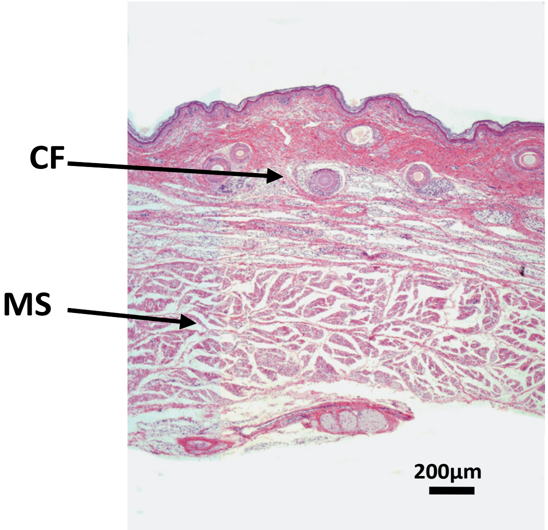

Fig. 6. Image of skin structure of a specimen collected before the mechanical test, a skin fragment from the cranial abdominal region (CAR). Clearly visible thin skin muscles (SM) and single hairs in the dermis layer. The degree of compaction of collagen fibres is the greatest under the epidermis (CF). H&E staining (40× magnification)

The hypodermis itself, unless collected together with muscles, was built of loose connective tissue with a small amount of fat cells and became fragmented under the influence of the acting forces so it did not constitute a uniform layer having a significant impact on the mechanical properties of skin.

In the case of skin samples collected from the nuchal region (NR), the dorsal region (DR), and thoracic limb region (TLR), the analysis of the objects collected for morphometric calculations demonstrated certain similarities of the changes in the spatial arrangement of collagen fibres as a result of stretching.

Packing of collagen fibrils, i.e., compaction, concerned mainly small objects with the width of 1–2 µm, which, as a result of stretching, changed their arrangement to longitudinal, as proved by the increased number of objects measuring up to 10 µm in length. In the skin from the abdominal region, a change of the spatial orientation of fibres took place in objects less than 1 µm in length. The other objects showed no changes in either longitudinal or transverse dimensions. In the skin from the lateral abdominal region (LAR), there was an increase in the number of objects with a surface area of 20–40 μm^2. This change occurred most frequently with the change of the width of these objects.

Examination of the surface area or the volume of collagen fibres showed an increased density of the packing of fibres in all samples with the exception of the thoracic limb region (TLR), where, before stretching, a highly packed fibre layer formed a distinctive layout, the so-called "mattress", which was destroyed as a result of stretching (figure 7). This led to the coiling of collagen fibres and, consequently, to an increase in the number of spaces between the fibres observed in the morphological analysis. An increase in fibre density showed that, as a result of stretching, collagen fibres got closer to each other, which should contribute to increased tissue resistance to stretching between collagen fibres.

In the histological specimen, collagen fibres were seen as objects having their own length, width, and surface area. At the same time, knowing the specimen thickness, the volume of the examined object could be determined. However, it was a segment or a cross-section of an object stretched in three-dimensional space, so changes in the shaping of objects could reflect changes in the spatial arrangement of larger structures, of which they are components.

As can be seen by the present results, the behaviour of small objects in skin, which are the fragments of collagen fibres, confirms the existence of the phenomenon of compaction of collagen fibres and their rotation connected with movement. This caused the "tearing" of large fibres into a number of smaller objects. The fibres lost their original arrangement and their new layout corresponded to the directions of the forces in tissues. Such new, stabilised status under in vivo conditions may become fixated as a result of synthesis of new collagen fibres by connective tissue cells along the established patterns of fibre arrangement, giving new directional strength values to a particular skin fragment.

4. Discussion

Skin, as the largest organ linking the body with the external environment, shows differentiation of the mechanical and structural properties resulting from its heterogenic and anisotropic character [10], [27], [28].

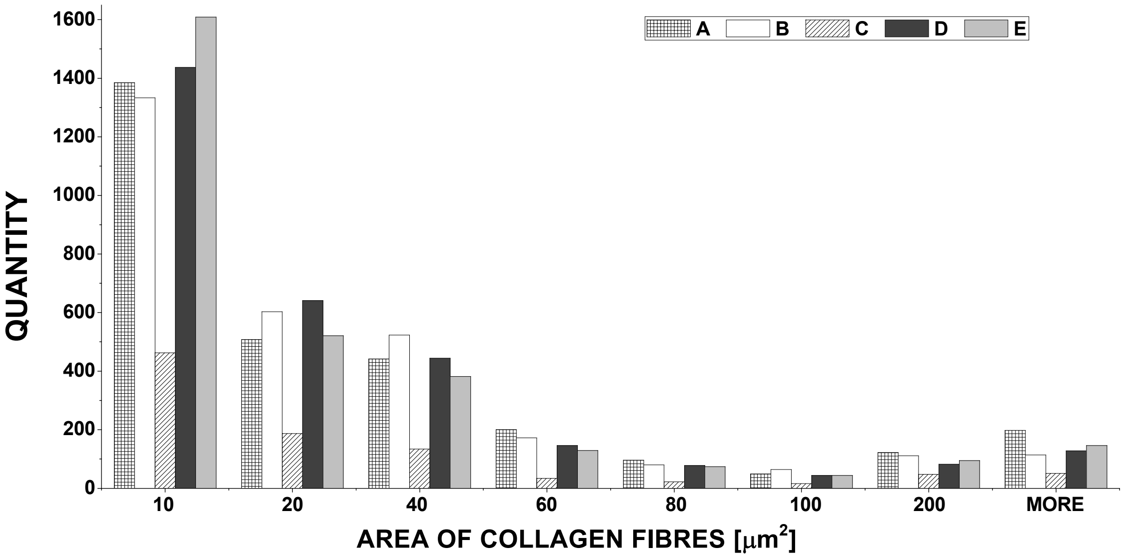

Fig 7. A histogram of the distribution of the surface area of collagen fibres for specimens collected before the mechanical test, depending on the body region from which the specimens were examined

The studies conducted showed no significant differences in the mechanical and structural properties of the skin of domestic pig foetuses collected from different regions in a direction parallel to the long axis of the body. The tensile strength values are comparable to the values obtained by ANKERSEN et al. who, on the basis of uniaxial tensile tests, showed a similarity of porcine skin to human skin [1]. At the same time, the obtained ranges of the values of mechanical parameters, i.e., the maximum tensile strength (2.08 ± 0.25 MPa), correspond to the strength values of human skin obtained by NÍ ANNAIDH et al. [16]. This confirms that, irrespective of the sampling area, the skin of domestic pig foetuses may be used as a human skin substitute in the studies carried out in vitro.

Physiological and mechanical properties of skin depend, to a large degree, on the specific arrangement of collagen fibres. The studies conducted by LIU and YEUNG [15] revealed the impact of the physical properties of the tissue connected mainly with the orientation of collagen fibres in skin on its mechanical parameters. In order to maintain an appropriate elasticity and adjust to the prevailing mechanical conditions, skin undergoes differentiation of the layout of collagen fibre bundles, which form shaped, mattress-like arrangements in response to the existing, varying mechanical conditions. In their natural state, the structural elements making up skin are arranged "loosely", which enables maintenance of tension within a certain range of skin elasticity. Under physiological conditions, the structural elements of skin are arranged in such a way that tension can be maintained within a certain rigid-elastic optimum.

When skin is violently stretched, collagen fibres almost immediately arrange themselves parallel to each other, which leads to increased local friction to prevent their further movement. Obviously, if the threshold strength is exceeded, the skin may tear and repair processes will be initiated [14]. The studies carried out by ZHANG et al. [30] on the porcine skin model showed that the formation of free, stretched grafts in a region other than the damaged area increases its surface area without affecting the mechanical properties of skin. The process of preventing tearing involves not only collagen fibres, but also the other elements, such as: elastic fibres, proteoglycans, intercellular matrix proteins and, above all, the cells of the fibroblastic line present in the skin, which together form a network of interconnections, thanks to which changes in tension cause numerous biological responses [14].

5. Conclusions

The type of changes in the collagen fibre arrangement under uniaxial stretching was specific to a given region. Also, the samples showed similar strength values of the individual skin fragments.

The microscopic examination of the structure of the skin of domestic pig foetuses before and after stretching, with respect to the mechanical results obtained, enabled us to conclude that, despite the differences in the arrangement of collagen fibres in the respective regions of skin examinations (nuchal region, dorsal region, lateral abdominal region, cranial abdominal region, and thoracic limb region), there were no significant differences affecting a change in the mechanical properties of skin. The most common parameter compensating for different arrangement of fibres was the skin thickness.

It was also concluded that changes between the respective regions of sample excision related mostly to the epidermis thickness and the presence of its products: hair and skin glands.

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