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ORIGINAL RESEARCH

MUSCULAR-APONEUROTIC SYSTEM OF THE SKIN OF THE FACE, AS A UNIQUE STRUCTURE OF THE FACIAL SKULL (CRANIUM FACIALIS) OF HIGHER ANIMALS

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ABSTRACT

Aim: The purpose of this research was to study the organization of collagen in animal skin in order to find similar SMAS structures.

Materials and Methods: The studies were conducted on skin samples from sheep and pigs (from the facial area), as well as trout. The obtained samples were studied under ultraviolet radiation. The Qscan Plus (AIOBIO, South Korea) device with a wavelength of 405 nm was used as a source of ultraviolet radiation. Skin sections were made at right and oblique angles in order to establish the spatial configuration of collagen fibers. The density (concentration) of fibers per unit area was calculated qualitatively by micro- and macroscopic methods with photometry. The results were evaluated in the Microsoft Office software package.

Results: In all the studied facial skin samples, the presence of collagen luminescent white in ultraviolet light was detected. The collagen was located lamellarly, parallel to the skin surface, without the formation of a three-dimensional mesh structure characteristic of the SMAS of human facial skin. **Conclusions:** The data confirm the presence of collagen in the facial skin of the studied animals. However, unlike human facial skin, the characteristic three-dimensional collagen organization of the SMAS has not been detected. Further research is needed to determine the evolutionary origin of the SMAS and identify the factors that determine its formation.

Conclusion: No structures similar to SMAS were found in the animals used in the experiment. This is most likely due to the function performed by SMAS – the transmission of facial contractions to the skin. Probably, the structural organization of collagen on the human face is unique. Further research is required to accurately determine the evolutionary development of SMAS.

Key words: SMAS; facial skin; collagen; ultraviolet radiation.

1. INTRODUCTION

Collagen is a fibrillar protein that is the main component of the extracellular matrix and provides structural support and tissue function. The diversity of types, properties, and wide distribution of this protein make it valuable for both scientific research and various industrial applications (cosmetology, medicine, food industry).¹ Differences in the biomechanical characteristics of collagen obtained from the skin of different animals (fish, pigs, sheep) make it important to study its origin and organization. Also, the structure and quantity of collagen are important diagnostic indicators.

Fibrillar collagen is the most abundant collagen in the body. Collagens types I, II, and III account for at least 80–90% of all collagen in the body.² The fundamental subunit of a collagen fibril is called tropocollagen. It is a protein molecule 1.5 nm in diameter and 300 nm in length, consisting of three polypeptide α -chains organized into a triple helix.³ The triple helix of type I collagen molecules (the most common type) consists of polypeptide chains, each of which contains a repeating sequence GXY, where G is glycine, and X and Y usually correspond to proline or hydroxyproline. The diameter of collagen fibrils ranges from 50 to 200 nm. When arranged together, fibrils form fibrous bundles with a diameter of 500 to 3000 nm.^{4,5} The three-dimensional structure of the collagen network varies significantly depending on its location in the body and its biological purpose. For example, collagen fibers in the arterial wall are densely packed and oriented along the length of the vessel.⁶

It is clear from the literature that collagen as a structural unit is present in many animals, especially in marine fish^{7, 8}, making them a natural source of additional nutrition for individuals with deficiencies. Non-invasive methods based on the use of ultraviolet radiation with a wavelength of 405 nm allow for the effective diagnosis of oral diseases and assessment of tissue quality due to their high sensitivity. Moreover, in the facial skin of humans and monkeys, collagen is

organized into a unique three-dimensional structure – SMAS. The evolutionary origin of SMAS remains a subject of research.

It is evident from the literature that collagen as a structural unit is present in many animals, especially in marine fish^{7, 8}, making them a natural source of additional nutrition for individuals with deficiencies. The aim of study is to compare the organization of collagen in the facial skin of different animal species (fish, pigs, sheep) and humans to find structural analogs of SMAS and to clarify its evolutionary history. Our preliminary data confirm the presence of collagen in facial skin samples from all animals studied, but the characteristic three-dimensional organization inherent to SMAS has not been identified.

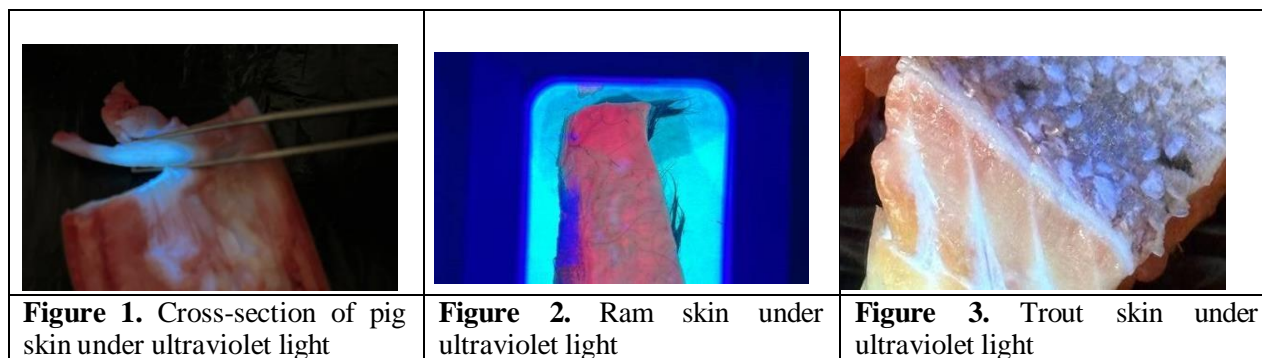
2. MATERIAL AND METHODS

The studies were conducted on biological material: ram (n=10), pig (n=10), trout (n=10). Freshly obtained material was used in the work, storage conditions were natural cold without preliminary embalming. The skin material of the ram and pig was collected from the facial area of the skull. The obtained samples were immediately studied under ultraviolet radiation. The Qscan Plus device (AIOBIO, South Korea) with a wavelength of 405 nm was used as a source of ultraviolet radiation. Skin sections were made at right and oblique angles in order to establish the spatial configuration of collagen fibers. The density (concentration) of fibers per unit area was calculated qualitatively by micro- and macroscopic methods with photometry.

The solubility of acid-soluble and pepsin-soluble collagens was studied. The results were evaluated in the Microsoft Office software package.

3. RESULTS

The skin samples of sheep, trout and pig were examined. The macrophotographic results obtained were photographed and presented in figures 1 – 3.



The presence of collagen was detected in all samples. Collagen is known to fluoresce white under ultraviolet light, which was detected. Arrows in the figures indicate areas where collagen accumulation was detected. In the studied samples, the highest collagen content was detected in trout (Table 1).

Table 1. Collagen content in the samples

Sample number and its characteristics	Sample 1 (n=10)	Sample 2 (n=10)	Sample 3 (n=10)
Density of collagen fibers in the superficial layers	low	low	high
Collagen fiber density	high	low	high
Skin flap thickness in mm	2,5±0,2	1,4±0,1	0,6±0,1

In all three samples, collagen was arranged linearly, laminae, without forming a three-dimensional structure. This fact was confirmed by performing several tissue sections in different places. The direction of the collagen fibers was parallel to the skin surface, and no mesh structure was observed.

The study examined the solubility of acid-soluble and pepsin-soluble collagens isolated from pig, sheep and trout skin, depending on the pH level. The results are presented below.:

1. High solubility in acidic environments (pH 1–4):

- Collagens from all three species (porcine, sheep, and trout) demonstrated high solubility in the pH range of 1–4.
- Maximum solubility was observed at pH 3.
- Acid-soluble collagen: $90.2 \pm 1.5\%$.
- Pepsin-soluble collagen: $93.4 \pm 2.1\%$. This indicates that collagens are largely soluble in strong acid.

2. Decreased solubility in a neutral environment (pH 4–6):

o The solubility of both collagen types decreased sharply in the pH range from 4 to 6.

o The minimum solubility was observed at pH 6:

- Acid-soluble collagen: $35.2 \pm 3.1\%$
- Pepsin-soluble collagen: $38.4 \pm 3.3\%$

DISCUSSION

It is known that collagen is present in the skin, connective tissue and many other structures of various animals. Evolutionarily, it is not entirely clear in which animals collagen first appeared. It is known that proteins of the collagen family are found in sponges (Porifera) - animals phylogenetically distant from humans⁹

Animals are a classic source of collagen in industry, which is used for cosmetic procedures, wound treatment, stopping bleeding, and as a food additive.¹⁰

¹¹⁻¹² Collagen is most often obtained from the skin of fish and pigs, sheep are also used for this purpose, and the structure and properties of collagen vary depending on the source of its production.

Thus, pig collagen has better mechanical properties than collagen based on fish skin.¹¹

Collagen studies using ultraviolet radiation are gaining popularity due to their low invasiveness and high visibility of the results. Radiation with a wavelength of 405 nm is proposed by researchers for the early diagnosis of oncological diseases of the oral cavity. In this case, areas lacking collagen appear dark under the radiation, and areas with a normal collagen content

appear lighter.¹⁴ Also, the phenomenon of collagen autofluorescence can be used to assess the biological properties of tissue transplants.¹⁵

Despite the fact that the evolutionary predecessors of humans have collagen in the skin of the facial area, its structural organization differs from that of humans. It has been found that collagen on human facial skin is part of the SMAS - the Superficial Muscle-Aponeurotic System. This structure was first described in 1976 by researchers Mitz and Peyronie.¹⁶ Since then, several types of SMAS have been identified, differing in structure and location on the face. Using 3D reconstruction technologies, a 3D model of SMAS was constructed based on histological preparations. In the course of such a study, it was discovered that SMAS is not a chaotically arranged collagen fibers, but a three-dimensional network that forms interconnected spaces.

The fibrous septa of SMAS form communicating compartments filled with adipose tissue.^{17, 18}

The functions of SMAS, as well as the classification and number of its types, are the subject of discussion in the scientific community. It is assumed that SMAS transmits facial contractions to the skin of the face and induces facial expression.¹⁷ This function is consistent with the fact that SMAS is found only in humans and their closest evolutionary "relatives" - monkeys, since it is in these organisms that facial mimic activity is most pronounced among animals.¹⁹

Today, it is obvious that the extracted types of collagen correspond to the protein composition of type I collagen, consisting of $\alpha 1$, $\alpha 2$, β and γ chains²⁰ and can be used both locally and in the form of injections for inflamed tissues for the purpose of reconstruction.²¹ Our data are consistent with the data of previous researchers. Indeed, collagen is present in the facial skin of animals, and the amount of this protein was different in different animals. However, collagen does not form a 3D structure characteristic of the human face. The study did not find any structures that even remotely resembled the SMAS. It is likely that the SMAS is an evolutionarily young, unique structure that is unique to the human face. Further research is needed to determine who first developed the SMAS and how it has changed over the course of evolution.

CONCLUSION

No structures similar to SMAS were found in the animals used in the experiment. Collagen of non-monkey animals on the face does not have the corresponding spatial organization, it is located lamellarly. Most likely, this is due to the function performed by SMAS - the transmission of facial contractions to the skin. The structural organization of collagen on the human face is unique. Further research is required to accurately determine the evolutionary development of SMAS. The data obtained allow us to optimize the procedure for using collagen of animal origin as an additional source of nutrition for humans, as well as a structural intraoperative substrate

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Competing interest

The authors declare that there are no competing interest.

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