Biological sciences

SKIN ACOUSTIC SCANNING

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In recent years the *acoustic method* is widely used for diagnostics and observation of the skin treatment (soft tissues) which is based on the measurement of the propagation speed of an audible frequency surface waves in tissues. This method is widely used in various branches of medicine. This work represents investigation results of different tissue systems (rats' skin, different scars on human skin, rabbits' eyes sclera) mechanical properties, obtained by noninvasive acoustic express-method, which is based on the measurementof the propagation speed of audible frequency surface waves. The morphological method was applied to these systems in parallel with the acoustic method. The correspondence between mechanical properties of tissue systems and structural organization of the examined tissues was found. It was shown the possibility of partial replacement of the diagnostic biopsies for acoustic imaging.

Keywords: acoustic method, speed of propagation of audible frequency surface waves, morphological method, connection between mechanical properties and tissue structure

Nowadays the following various noninvasive visualization technologies for skin studies [9], are being used: optical video monitoring, optical tonometry, 3D-modelling, tevametry, (vapometry), corneometry, superficial pH-metry, reviscometry, ballistometry, dermatoscopy; optical coherent skin tomography; confocal laser imaging in vivo microscopy; ultrasound skin imaging (syn.- dermatosonography).

In the diagnostics and assessment of treatment effectiveness, the above methods are quite informative, but expensive. They require the particular training of specialists to work with the corresponding stationary devices.

In recent years the *acoustic method* [10, 5] is widely used for diagnostics and observation of the skin treatment (soft tissues) which is based on the measurement of the propagation speed of an audible frequency surface waves in tissues. This method is widely used in various branches of medicine [6,7,8].

Purpose of the work: to show the possibilities of the acoustic method use for differential objective diagnostics of the tissues condition by comparison of the obtained results with the morphological studies of these tissues.

Materials and methods of research

Acoustic method

Study of tissue mechanical properties was performed by using two instruments, peculiar for their practical characteristics of wave disturbance generation: acoustic tissue analyzer (ATA) [10] and acoustic medical diagnostic instrument (AMDI) [5], which determine the propagation speed of surface wave V.

The following parameters were calculated from the measured values of the velocity:

• $\Delta V = V_s - V_n$ - speed difference in pathological and normal tissues (or speed difference, measured during different periods of treatment);

• $Z = V_s/V_n$ – ratio of speeds in pathological and normal tissues;

• V_y , V_x – speeds in the mutually perpendicular directions which are used in mechanical anisotropy examination;

• Acoustic anisotropy coefficient $K = V_y/V_x - 1$. Morphological method

Tissue samples were analyzed as per standard morphological method: preserved in 4% neutral formaldehyde solution with acetate buffer (pH = 7,2-7,4). After 24 hours rinsing the tissue samples are dehydrated in ascending concentration alcohols and poured in paraffin as per regular procedure. The prepared sections are coloured with haematoxylin and eosin to verify the general morphological picture and by Van Gieson method as per standard histological method. The coloured preparations are examined with the help of the microscope Nu (Karl Zeiss, Germany) under diffuse light.

Test object

To show the acoustic method possibilities, this project represents previously made investigations on different biological systems: rats' skin after hydrocarbon gel subcutaneous introduction, human skin with scar changes; rabbits' eyes sclera after collagenous plastic procedure.

Results of research and their discussion

1) Polyacrylamide hydrocarbon gel "Argiform"

Polyacrylamide gels are used for soft tissues endoprosthesis replacement.

The project [1] represents the possibility of acoustic in vivo method use for objective evaluation of rats' skin properties after hydrocarbon gels introduction. The gel is subcutaneously injected in a particular place on the thigh. Acoustic measurements are made in two directions: the first one (V) corresponds to the natural vertical orientation of the animal's leg; the second one (Xaxis) – is perpendicular to the first one.

The gel is injected subcutaneously with a syringe, the needle of which is directed along the V axis. The wave rate was measured in the central spot over the gel introduction zone.

Speed change dynamics ΔV_y and ΔV_x is represented in Table 1.

									Т	able 1
Time after injection			30 min		1 day		14 days		30 days	
Wave changes rate along X and Y axes $(\Delta V, m/c)$			ΔV_y	$\Delta V_{\rm X}$	ΔV_y	$\Delta V_{\rm X}$	ΔV_y	$\Delta V_{\rm X}$	ΔV_y	$\Delta V_{\rm X}$
			17	3	17	23	30	27	35	28
Anisotropy	Before	Af	ter 30 1	nin	Af	ter 14 c	lays	Af	T ter 30 c	T able 2 lays
Absent	20%	10%		0%		6%				
"positive" $(V_y > V_x)$	60%	27%		82%		89%				
"negative" ($\vec{V_y} < \vec{V_x}$)	20%	63%		18%			5%			

 ΔV parameter was changing in different periods after the gel introduction. The parameter stabilization is observed within 14–30 days. By this time the speed values are finally stabilized.

Gels introduction causes the redistribution of the mechanical tension in the skin. Table 2 represents the anisotropy development before and along different periods after the gel introduction.

While the subcutaneous gel introduction one can observe the "positive" anisotropy predominance which is basically the reflection of tensile stress towards the natural orientation of the animal's leg (Y axis).

The acoustic characteristics were correlated with the morphological results of the biopsy samples taken directly from those areas where the speed was measured. Fig. 1 represents the results obtained after 30 days after hydrocarbon gel introduction (increase \times 32).

According to the obtained histological data by 30 days after hydrocarbon gel introduction the maturation processes and connective tissue remodeling are completed. Till this very moment the capsule formation period is stabilized and density of its borders is increased. Orientation of the capsule under formation corresponds to the acoustic skin anisotropy at the place of hydrocarbon gel introduction – the capsule is oriented towards the most prominent acoustic anisotropy evidence.

As the biomechanical properties of the connective tissues structure are determined by the architecture of the dense collagenous fibers bundles, this is the fact which determines the speed increase. Another thing influencing the speed increase is the density of the capsule filled with the gel. Speed values stabilization in the area over the capsule (by 30 days) witnesses the completion of the principal structural changes in this area.

The same results are obtained for another 3 hydrocarbon gels [1].

In such a way it is shown that the dynamics of the tissue acoustic characteristics changes corre-

sponds to the dynamics of its structural organization formation in the area of the gel introduction

2) Combined scars

Non-invasive determination of a skin scar injuries type is topical. It is caused by the necessity of some adequate treatment selection to prevent the functional and cosmetic defects appearance. The acoustic method is applied for differential diagnostics of the scar types by Pligin [3]. A scar change is followed by the surface wave V value increase in comparison with the speed in the intact and visually healthy skin V_n . As per standard clinical parameters all scars under investigation were divided into 3 groups: uncomplicated (normotrophic) scars; hypertrophic and keloid scars. Fig. 2 represents empiric densities of propagation speed of the surface wave for the scars of different type.

Based on these graphics one can perform previous evaluation of a scar belonging to a certain type.

Histological analyses of hypertrophic and keloid scars has shown the following. Epidermis thickness in a keloid scars is more comparatively to the hypertrophic one; Stratum Lucidum is more prominent in the keloid scar; collagen thickness is more in the keloid scar; collagen fibers orderliness is available in the hypertrophic scar, in the keloids one the collagen fibers orderliness is almost not expressed; fibroblasts infiltration is more expressed in the keloids.

Comparison of the histological data with the acoustic parameters enables to approach to the understanding of a tissue structural organization influence on its acoustic characteristics.

Based on the acoustic data, to make the diagnostics of a tissue with scar changes objective, there was chosen a quantitative parameter $Z = V_c/V_c$, table 3.

3) Post burn scars

The children's post burn scars were investigated by Soboleva [4]. Scar type determination was performed by the acoustic method as per Z parameter and clinical features which were observed.



Fig. 1. a) Subcutaneous hydrocarbon gel location, around it one can observe a thin capsule (¬); b) encapsulatedhydrocarbongel (g), capsule (k), derma (d), epidermis (e)



Fig. 2. Empiric densities of propagation speed of the surface wave for the scars of different types



Fig. 3. Normotrophic scar, ageof 1 year 6 months, corresponds to the clinical features of this scar type:
1) over a large area the epidermis is clearly structured; 2) papillary and reticular dermis are distinguished; 3) thin collagenous fiber bundles with separated fib

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For the morphological investigation there was taken a part of the scar in which the scanning was performed. The morphological investigations results are shown on Fig. 3, 4, 5. (Colouring withhaematoxylin and eosin.Increase ×100).

Table 3 represents the acoustic and morphological features of three scars types.

Thus, principles of the surface wave spread rate changes in different scars types, determined in the works [3, 4] correlate both with clinical features, and morphological features of these scars.

4) Sclera

The work [2] represents studies of maturity degree of rabbits' eyes sclera influence on its

acoustic and morphological parameters. The experimental animals underwent sclera collagenous plastics (introduction of collagen hemostatic sponge into the Tenon's space). The investigation was performed in 4 areas of posterior sclera (upper-external, upper-internal, lower-external, lower-internal). Measurements were made in two mutually perpendicular directions.

The acoustic investigations have shown that sclera tissue with low maturity level (1 month after surgery) has lower values of the surface wave spread rate than that with higher maturity level (4 months after surgery). Results of the acoustic imaging are represented in Table 4.



Fig. 4. Hypertrophic scar, age1 year 6 months, corresponds to the clinical features of this scar type: 1) epidermis is thickened with dyskeratosis signs and absence of its normal layers; 2) papillary and reticular dermis are slightly distinguished; 3) collagenous fibers are of middle density



Fig. 5. Keloid scar, age 3 years, corresponds to the clinical features of this scar type: 1) epidermis cellar layer is absent; 2) collagenous fibers bundles of different directions and separated; 3) small cells quantity

Table 3

Table 4

Normotro	phic	scar	Z=	109-	-116	%
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Morphological features: epidermis moderate atrophic and dystrophic changes; lateral and parallel location of collagenous fibers and bundles;

Predominance of fibrocytes over fibroblasts;

Capillaries pausity;

Moderate vasculitis;

Significant amount of elastic fibers.

Hypertrophic scar Z = 130–140 %

Morphological features: epidermis dystrophic changes; orderly arranged powerful collagenous bundles; Fibroblasts predominance with signs of dystrophic and parabiotic changes;

Numerous capillaries with productive vasculitis signs;

Big amount of elastic fibers which have linear organization and oriented as per collagenous fibers bundles. Keloid scar Z = 198-228 %

Morphological features: big amount of functionally active fibroblasts with presence of the giant cellar forms; Functionally active capillaries reduction;

Collagenous fibers myxomatosis;

Absence of elastic fibers; small capillary amount

Topographic zones of	Superficial waves rate V, m/c					
posterior sclera	After 1 month after surgery		After 4 months after surgery			
	Imaging directions		Imaging directions			
	У axis	X axis	y axis	X axis		
upper-outer	$54,7 \pm 0,7$	$57,5 \pm 0,5$	65,1 ± 0,8	63,1 ± 0,9		
upper-inner	$53,6 \pm 0,8$	53,6±0,8	$62,4 \pm 0,8$	59,6 ± 1,2		
lower-outer	$56,2 \pm 1,0$	$55,1 \pm 1,7$	$62,3 \pm 0,8$	$65,9 \pm 1,1$		
lower-inner	$52,4 \pm 1,1$	$54,4 \pm 1,4$	$64,3 \pm 0,9$	$64,3 \pm 0,9$		
P < 0,05				•		

	Table 5
Morphological features after 1 month (low maturity level)	Morphological features after 4 months (increased maturity level)
Low maturity level of the differentiate sclera fibroblasts which is expressed in fibroblasts proliferation, tissue granulation growth as well as structural randomness of the sclera collagenous fibrils is peculiar for smaller surface wave rate values.	Increase of newly formed sclera cellar maturity level is expressed in the granulation tissue maturity and its refor- mation into a fibrocicatrical one; Fibrous tissue is peculiar for its significant thickness; collagenous fibers of connective tissue body have ar- ranged path, significant thickness, high collagenous blast activity is preserved.

The obtained results witness that sclera maturity level causes an increase of the surface wave speed both in transversal and lateral directions.

Table 5 represents morphological data comparison after 1 month after surgery (at low maturity level of the newly formed sclera connective tissue) and after 4 months after surgery (after its maturity level increase).

Thus, according to the surface wave speed increase one can conclude the sclera maturity level.

The work [2] also represents that the same dependence of the surface wave propagation

speed on the maturity level takes place in a cornea too.

Conclusion

Based on the results represented in this work one can conclude that the acoustic features of different tissues which are determined by the non-invasive acoustic method, properly reflect the tissue structural peculiarities. Simplicity and high informative capacity of the non-invasive acoustic method in some cases allows using the acoustic scanning instead of diagnostic biopsy.

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