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Biological and medical application of SR from the storage rings of VEPP-3 and "Siberia-2". The origin of specific changes of small-angle X-ray diffraction pattern of hair and their correlation with the elemental content

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Abstract

X-ray diffraction and fluorescent investigations of human hair were carried out using SR of VEPP-3. The small-angle techniques were used for ecological monitoring and medical diagnostics of pathological states of human organisms with the usage of non-invasive methods for express analysis of biological objects, including human and animal tissues: nails, wool and hair samples.

Possibility using of hair fibre diffraction to test for pathological conditions is the subject of this paper. X-ray diffraction data allowed us to suppose the two-component structural model of the hair tissue. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

For the last 28 years, three Pushchino's institutes: Institute of Theoretical and Experimental Biophysics of RAS, Institute of Cell Biology of RAS, Institute of Mathematical Problems in Biology of RAS in collaboration with Budker Institute of Nuclear Physics SB RAS and Institute of Physiology of Azerbaijan AS have been working with SR, using it in the field of biology and medicine.

The recent correspondence from James et al. "Using hair to screen for breast cancer" [1] shows

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the presence of a diffuse ring in keratin fibres that corresponds to a molecular spacing of 4.44 nm. This spacing is thought to be related to small amounts of lipids in the sample, attributed to a membrane "bonding" fibrils together (James' words). The basis for the relationship of lipids with susceptibility to breast cancer remains unclear. The observation of "lipid" diffraction of keratin samples is not a new phenomenon and was reported by Fraser et al. [2]. At least five teams were involved in the attempt to replicate her results. But none of the studies managed to find the correlation between the diagnostic features mentioned in the "Nature" article and clinical knowledge of the state of patients [3–7]. Our group aimed to investigate the origin of the specific changes of X-ray diffraction pattern of hair. We studied hair from breast-cancer patients and from healthy subjects and made the following conclusion: the possibility of using of synchrotron fibre diffraction of hair to screen for pathologic conditions such as breast cancer proposed by Veronica James cannot be verified [8].

2. Materials and methods

X-ray diffraction and fluorescent investigations of human hair were carried out using SR of VEPP-3 and the small-angle techniques [9]. A stretching frame construction for hair clamping was used (Fig. 1).

We studied more than 500 hair samples and carried out more than 1000 different experiments. We collected hair from donors aging from 1 to 92 years with hair lengths ranging from 3 to 1000 mm. Scalp-hair samples from donors of different regions with anthropologic "pressure" and hairs of patients from specialized hospitals were also analysed. The samples of hair were obtained from the following sources: Blokhin Scientific Center of Oncology, the Department of Breast Tumor, Moscow; The 12th Municipal Hospital, Department of Gastroenterology, Novosibirsk; the Institute of Gynecology and Obstetrics, Novosibirsk; the Phthisiology Clinic, Novosibirsk; The public boarding school for tundra nenets, Samburg, Tyumen region; Chelyabinsk Branch Office 4 of Biophysics Institute. The hair elemental content (K, Ca, Cu, Zn, Fe, Mn, Cr, Se, Br, Co, Mo, Ni, V, Sr, Rb, Y, Sc, Ti, Ga, Zr, Nb, I, Sn, Sb, Ba, La, Ce, Nd, As, Pr and Pb) was determined.

3. Results and discussion

A hair X-ray pattern is a fibre diffraction pattern: the meridional reflections are due to supercoiling of the α-helical bundle packing in the keratin intermediate filaments (IF); the equatorial reflections, elongated parallel to the equator, give a diamond-shaped appearance, such a shape may arise from helical twisting and correspond to the cylindrical Fourier-transform of an α-helical

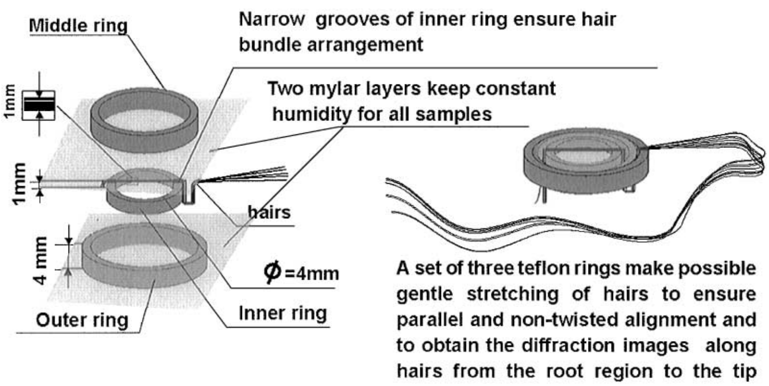

Fig. 1. The stretching frame construction for hair clamping.

bundle [10]. There is a slight trace of arcing across the equator which corresponds to a molecular spacing of 4.5 nm. Spacing of this reflection is not variable, but the angular size of this arc (texture) and arc width can increase so that the diffuse arc will be transformed into the full diffuse ring with higher orders. The X-ray patterns with a slight arc were called the "*no ring*" ones and those with rings were called the "*ring*" ones.

A set of diffraction patterns including "*no ring*" and "*ring*" ones can be obtained from the hairs of an individual donor by scanning along the sample point by point from the root region to the tip at 5–100 mm interval, making approximately $n = 5 - 15$ images per sample depending on the length of the hair (hair age) (Fig. 2). It was shown that there were donors whose hairs did not give the "ring" X-ray pattern from the root region to the tip.

Small-angle X-ray scattering (SAXS) shows the central region of diffuse scattering: the equatorial streak and central diffuse disk (Fig. 3a–d). There is an evident correlation between intensities of the streak and disk depending on the intensity of the diffuse ring of 4.5 nm. The transformation from the equatorial streak (a) to the circular symmetrical *disk* (d) takes place *via* intermediate forms of intensity distribution, where the 6-fold rotational symmetry (b, c) is supposed.

The X-ray fluorescent element analysis study has shown that there are changes in element content along the hair from the root region to the tip for donors with the "*ring*" type of hair. There is a definite correlation between the contents of Ca–Br in donor's hair and their X-ray fibrillar diffraction patterns (Fig. 4).

There is a class of donors whose hair always gives X-ray patterns without *ring* throughout the hair length. Such diffraction patterns were called "*ring free*" ones. In this case the law of conservation of integral intensity was realized. The X-ray fluorescent element analysis study has shown that:

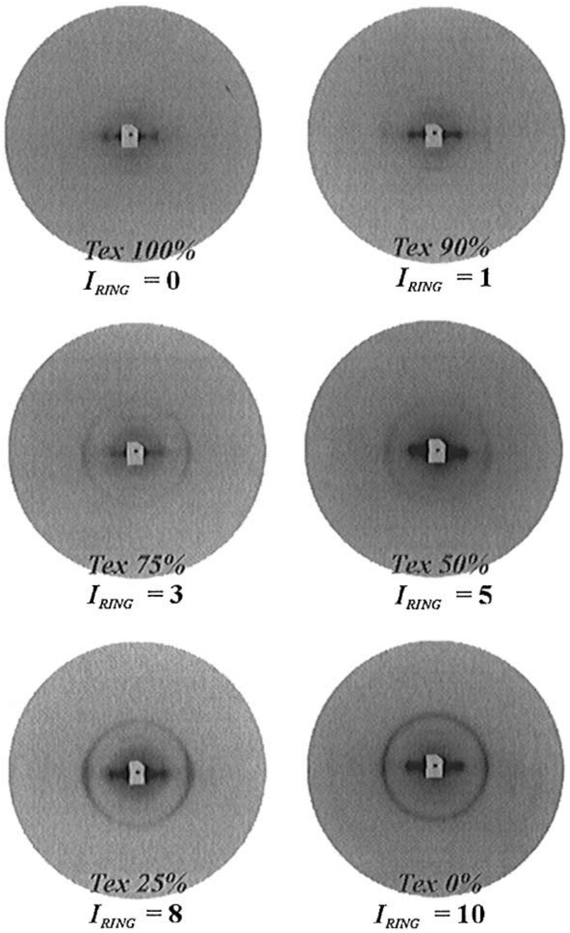

Fig. 2. A set of diffraction patterns from "*no ring*" to "*ring*".

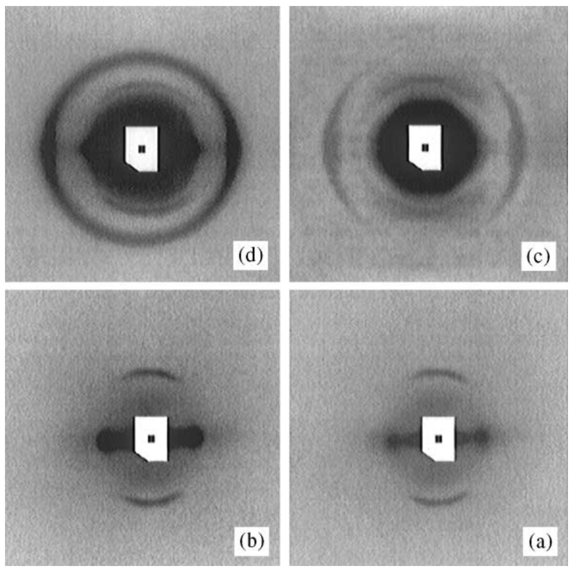

Fig. 3. (a–d) Four different patterns of human hair.

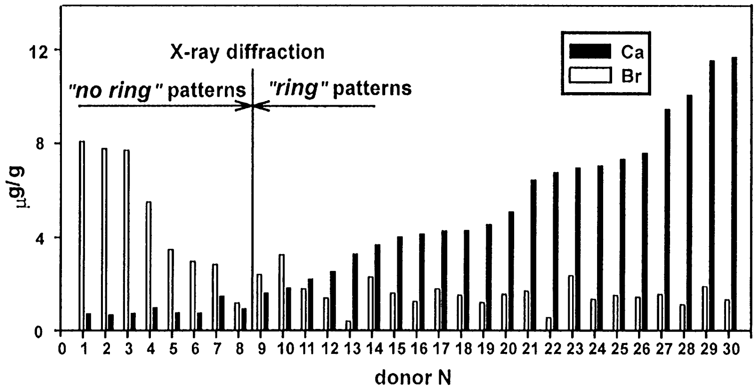

Fig. 4. Content of Ca (× 450) and Br in the hairs of 30 donors from Novosibirsk and Pushchino.

there are no changes in element contents along the hair for the "*ring free*" type donors; but there are some changes in element contents along the hair from the root region to the tip for donors with the "*ring*" type hair.

The correlation between the character of X-ray patterns and element content of Ca, Sr and Br was shown: for the "*no ring*" type—low Ca and Sr concentration and high Br concentration; for the "*ring*" type—high Ca and Sr concentration and low Br concentration.

We managed to show that prolonged soaking of hairs in 1 M $CaCl_2$ at pH 10–11 could transform a hair sample initially giving a typical "*ring free*" X-ray pattern in such a way that it was able to produce the "*ring*" X-ray pattern (Fig. 5).

The sensitivity of X-ray diffraction patterns to various reagents and heavy metals is presented in Table 1.

It was shown that the origin of the diffuse ring of 4.5 nm is not "lipid" but most likely glycoprotein in nature (proteoglycan). It is interesting to note that the "lipid ring" [2] is a very sharp reflection of hair in its natural state. But the patterns of hair soaked in solutions containing various heavy atoms underwent a transformation from a sharp ring to a diffuse halo and a change of small-angle intensity distribution from the equatorial streak to the central diffuse disk (Fig. 3). Obviously, the presence of a diffuse diffraction ring attributed possibly to "oil" was reported [1] to be

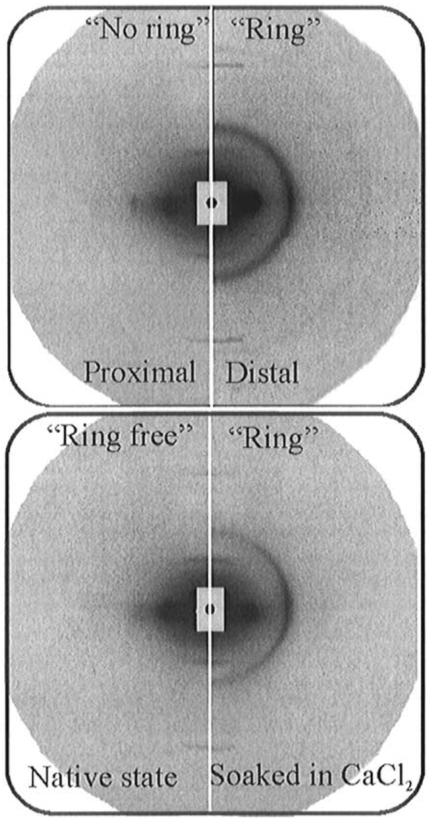

Fig. 5. (top) Two different X-ray patterns were obtained from *proximal* and *distal* fragments of the hair of an individual donor. (bottom) Two different X-ray patterns were obtained from *the same segment* of the hair in the native state and after soaking in $CaCl_2$ solution.

Table 1
The sensitivity of X-ray diffraction patterns to various reagents and heavy metals[a]

Reagents		Change of intensities of different reflections				
		Ring of 4.5 nm	Diffuse small-angle scattering		Meridional reflection	Equatorial reflection
			Disk	Streak		
Ethanol		0	0	0	0	0
Chloroform		0	+	+	−	+
100% Acetone		−	0	0	−	−
Hydrogen peroxide 30% H_2O_2		0	0	0	0	0
8 M Urea		0	0	−	− −	−
β-mercaptoethanole		−	+	+	− −	−
0.6 M $AgNO_3$	"Ring"	+ +	+ +	+ +	+ +	+ +
	"No ring"	0	0	+ +	−	+ +
1.2 M $AgNO_3$	"Ring"	+ +	+ +	+ + +	+ +	+ + +
	"Ring free"	0	0	+ +	−	+ +
0.6 M $AgNO_3$ after chloroform	"Ring"	+ + +	+ +	+ + +	+ + +	+ + +
	"Ring free"	+	+	+ +	+	+ +
1.2 M $AgNO_3$ after chloroform	"Ring"	+ + +	+ + +	+ + +	+ + +	+ + +
	"Ring free"	+ +	+ +	+ + +	+ +	+ + +
Paint shampoo	"Ring free"	+	+	+	+	+
Tinting "Ursul" (Au, Ag, Cu)	"Ring free"	+			+	+
Permed liquid	"Ring free"	0	0	0	0	0
1 N HCl	"Ring"	0	0	0	0	0
NaOH pH 10–11	"Ring free"	0	0	0	0	0
1 M $CaCl_2$ pH 5	"Ring"	0			0	0
	"No ring"	+ + +			+	+
	"Ring free"	0			0	0
1 M $CaCl_2$ pH 11	"Ring free"	+ + +			+	+
5 mM EDTA	"Ring"	0	0	0	0	0

[a] "0"—no change; "+ +"—strong increase; " − "—weak decrease; "+"—weak increase; "+ + +"—very strong increase; "− −"—strong decrease.

an integral component absolutely of all samples of human scalp hair and that is why it is impossible to explain the "no ring" X-ray pattern.

The X-ray diffraction data allowed us to superimpose the two-component structural model of hair tissue, which consists of the flexible component of the extracellular matrix (ECM) in series with the inflexible component of the keratin intermediate filaments (IF) (Fig. 6).

We have interpreted the weak diffuse arc at a spacing of 4.5 nm as arising from the interference between assemblies of flexible ECM units consisting of glycoproteins, which can be either fibrillar and ribbon-like or random-coil in morphology and have low electron density.

During the last 15 years, we have been studying the structure of glycoproteins by X-ray small-angle diffraction [11,12]. X-ray patterns have been obtained from different components of the gastrointestinal tract (Fig. 7). They consist of a large number of clear diffraction rings at spacing between about 10 and 0.4 nm. Some reflections were common for all components: 4.65, 2.33, 1.56 and 1.24 nm. All the patterns are interpreted as arising from the order of glycoprotein molecules ordered in the liquid-crystalline structure.

The soaking of the "*ring free*" type hair in solutions of heavy metal salts after preliminary washing in chloroform results in a modified X-ray pattern, and the appearance of the significant

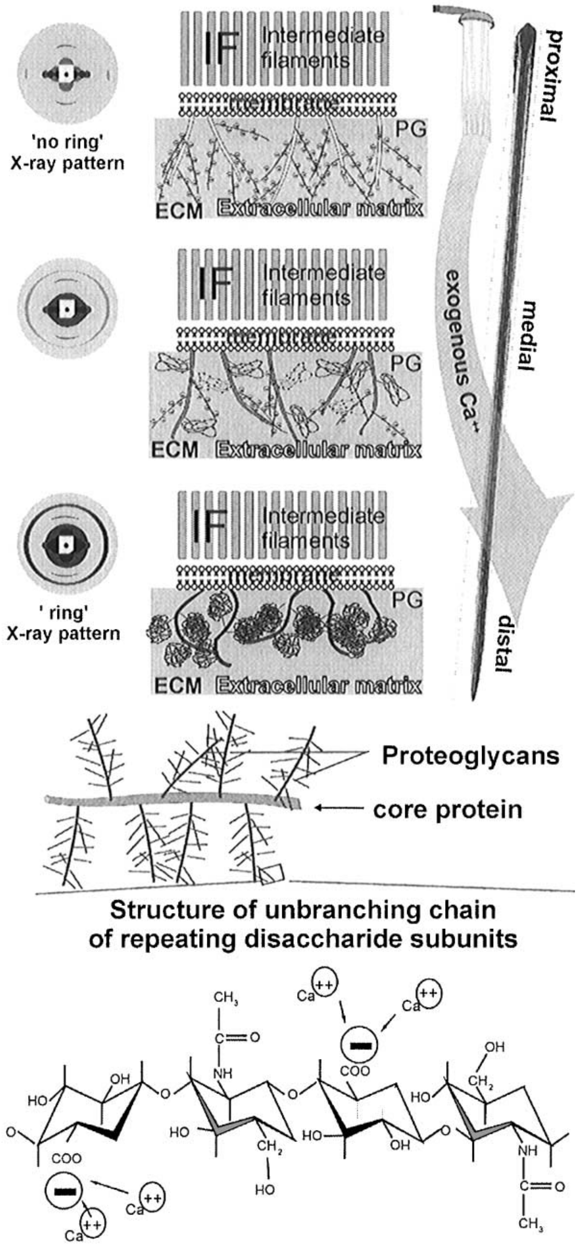

Fig. 6. (top) Two component structural model of the hair tissue. The diagram of ECM structure transformations and corresponding X-ray patterns from proximal, medial and distal segments of the hair. (bottom) The model of molecular organization of proteoglycans (PG). The positive charged metals transform the configuration of PG chains due to electrostatic interaction with multiple anion groups of polysaccharide chains.

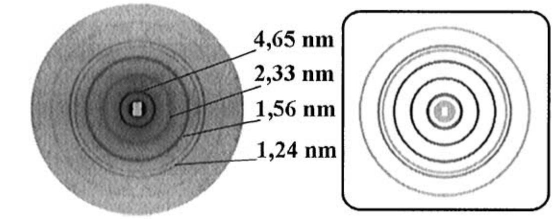

Fig. 7. The X-ray diffraction pattern from concentrated solution of isolated glycoprotein and its diagram.

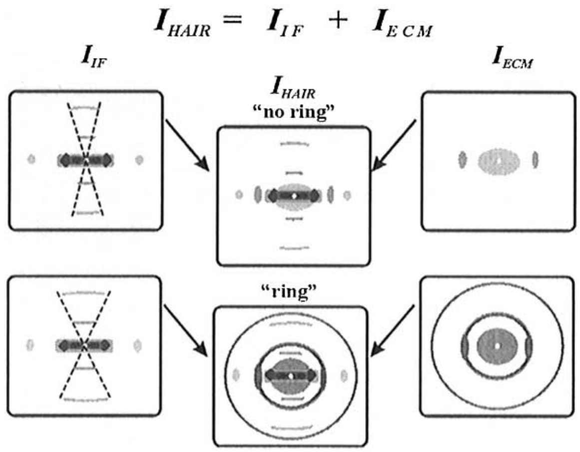

Fig. 8. (a, b) The X-ray pattern of the two-component structural model.

intensity ring is evident (the "*ring*" type of pattern). It is necessary to note that the derivatives of heavy metals are not isomorphous: the positively charged metals transform the configuration of glycoproteins from a fibrillar structure in to a random-coil one. In this case, the equatorial diffuse arc will be transformed into a full ring with its higher orders. The strong higher orders of an Ag-derivative arise from the increasing electron density due to electrostatic interactions of positively charged ions with multiple anion groups of polysaccharide chains.

The X-ray pattern of the two-component structural model can be considered as a result of superposition of two separate diffraction patterns of IF and ECM (Fig. 8). In this case, the scattering intensity of hair I_{HAIR} can be represented as the sum of the intensities of scattering of the

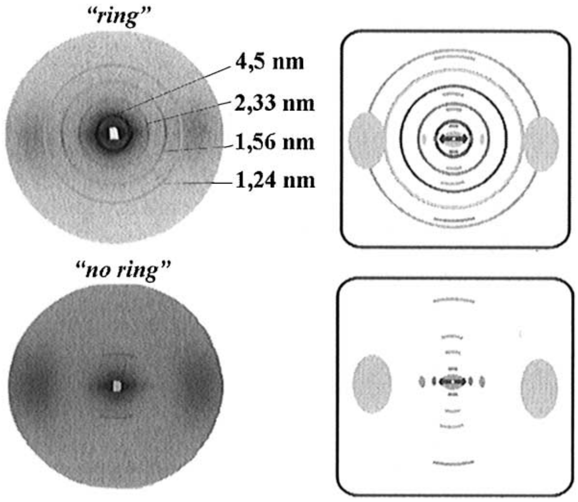

Fig. 9. Two types of hair X-ray diffraction patterns with their diagrams.

intermediate filament I_{IF} and extracellular matrix I_{ECM} : $I_{HAIR} = I_{IF} + I_{ECM}$.

Fig. 8 shows the diagrams of "*ring*" and "*no ring*" diffraction patterns of hair samples as a superposition of two diffraction patterns due to I_{IF} and I_{ECM}.

The model diagram of the ECM (Fig. 8b) completely coincides with the X-ray pattern of concentrated solutions of glycoproteins produced by epithelial cells of the gastrointestinal tract (Fig. 7).

Fig. 9 presents two types of hair X-ray diffraction patterns with their diagrams. It is evident that the "*ring*" X-ray pattern is a superposition of diffraction patterns of the "*no ring*" type and of glycoproteins.

Many factors cause variation in the mineral content of hair. The distribution of element contents along the hair and/or among a great number of samples has a bimodal character: there are two pools in distribution of element content. The first pool is due to the minor amount of intrinsic elements in the cell that have endogenous origin. The element content in the cell has specific invariant values and is regulated by selective membrane transport. The second pool—the maximal level in the element contents, is due to the specificity of the ECM. The element content in the ECM is variable, has exogenous origin and is determined by two factors: firstly, by the complex microenvironment of follicle whose secretions bathe the hair and, secondly, by the macroenvironment and is due to the ECM affinity and depends on the amount of ECM per cell. Pools of minerals are stored during the adult life.

The hair tissue is protected from the environment by the protective acidic sebum mantle; there are many different detergents which may strip the protective mantle from the skin and hairs and let the aggressive environment attack the exposed tissue. So, the protective mantle of the skin and hair plays an essential role in the absorption of metals by the ECM assembly. Disordering of the mantle results in the appearance of a diffuse ring at a spacing of 4.5 nm.

Conservation of the ordered structure in the tissue is ensured by means of two levels of protection. At the first level, cellular membranes protect the structure of cells as such. At the second level, the intercellular matrix structure is isolated from the environment by the protective acidic sebum mantle, which is a product of the sebaceous gland. There are a number of physical–chemical interventions capable of destroying one or more levels of protection. Cases of cellular membrane damage were omitted in the present discussion. In other cases, which are more interesting for us, when the intercellular matrix structure is disrupted. That results in degradation of the whole continuum of the tissue structural elements and in distortion of the orientation-polarized structure. The cells will lose their polarization and consequently the polarity of the structural orientation of the tissue ensemble will disappear. In this case, the cells become independent of each other and are capable of freeing themselves from the tissue "prison". This may be one of the reasons for the metastasizing processes.

Thus, all of the epithelial cells display extensive *keratin filament frameworks,* around which the cell shape and polarity are defined. The primary *cytoskeletal* components *in vivo* regulate and affect the interaction of epithelial cells, the *extracellular matrix* develops the structure orientation of the whole tissue, and simultaneously the *flexible extracellular matrix* is transformed into the *fibrillar* *matrix*. Our results propose the regulatory role of metal content in the matrix assembly.

So, the hair tissue should be considered as a continuous structural organ providing resistance to mechanical stresses externally applied to the tissue. Mutations that weaken this structural framework and any exogenous factors that change *the extracellular matrix* increase the risk of cell rupture and result in a variety of human disorders.

Acknowledgements

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