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Microrelief of Hirudinomorpha Hemocytes under Osmotic Stress.

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ABSTRACT

The purpose of this work is to study the effect of hypo- and hyperosmotic stress on morphometric parameters of hemolymph formed elements of Hirudinomorpha species. Application of atomic force microscopy allowed estimating such hemocyte parameters as cellulated surface microrelief, linear dimensions of the cells, including the height. Reduction of osmotic pressure is reflected in the cell microrelief that contains a large number of protrusions and furrows. In hypotonic environment, the surface of hemocytes looks heterogeneous on the scans. The distance between the elevations and their maximum height increases. The density of invaginations and their curvature in hypertonic conditions does not credibly differ from these parameters in normal conditions. The changes in the surface topography of hemocytes are described in contact interactions with solid substrate, and when exposed to environments other than physiologically normal. Roughness coefficient of all cell types in this environment increases, however this is not always associated with an increase in the number of micro elevations – deepening of depressions and increase in the height of microrelief elements also plays a significant role.

Keywords: hemocytes, surface microrelief, hypo- and hyperosmotic stress.

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INTRODUCTION

Relief or cellular surface topography is highly mobile characteristics: it varies depending on the functional state of the cell [2, 6, 8]. Surface roughness is a set of irregularities forming microrelief [7]. Quantitative evaluation of membrane surface roughness is of practical importance, since it allows identifying the effect of surface homogeneity and heterogeneity on the processes of capture of foreign objects and resistance to hypo- and hyperosmotic stress [9, 10].

MATERIALS AND METHODS

Hirudo medicinalis (Linnaeus, 1758), *Haemopis sanguisuga* (Linnaeus, 1758), *Erpobdella octoculata* (Linnaeus, 1758) hemocytes were used as subjects of research, previously classified into 4 types by morphofunctional features [1]. The resulting hemolymph was divided into three parts, each of them was placed in a separate Petri dish. 10 mcl of NaCl solution of a certain concentration (hypotonic solution – 0,4% NaCl, isotonic solution – 0,8% NaCl, hypertonic solution – 1.2%) was added to each part of hemolymph. Incubation was conducted for 1 minute. A drop of hemolymph was placed on the glass and smear preparations were made. The research was conducted using Integra Vita NT-MDT scanning probe microscope in atomic force spectroscopy mode applying the load at 25 areas of the cellular surface. The obtained AFM images were processed using Image Analysis 3.5 software [3]. We have analyzed the following amplitude average surface roughness parameters in accordance with the international standards: average square roughness Sq (nm); height of the highest peak Sp (nm); depth of the deepest trough Sv (nm); asymmetry Ssk – characterizes the skewness of profile distribution when one decline is steep, and the other is flat; excess Sku characterizes the length of profile distribution; Sz is a parameter characterizing the thickness of the perturbed surface layer not completely filled with the material where a relief changes. Also, the values of one of the functional parameters characterizing the relief in the local area and the degree of surface smoothness – density of peaks Sds ($1/\mu m^2$) were defined. This parameter shows a number of peaks per unit area [4, 5, 9].

RESULTS AND DISCUSSION

Changing under the influence of environmental factors, cellular surface microrelief reflects the features of their functional status. Using atomic force microscope images allowed estimating the behavior of *H. medicinalis* hemocyte surface microrelief after incubation in solutions of different concentrations.

The reduction in osmotic pressure leads to a significant volume increase of all cell types. On scanning images, hemocytes have a spherical shape and smooth membrane without folds. Micro elevations of the surface dominate, and large elevations and depressions were not detected.

In a hypertonic environment, surface of the blood cells of medicinal leech varies considerably. Protrusion of cytoplasmic granules, and fibrils of the cytoskeleton through plasma membrane were detected; micro elevations are absent. Amoebocytes flatten on the substrate. All cells have a tendency to form vacuoles.

Analysis of surface roughness shows smoothing of micro elevations and micro depressions on the cell membrane in a series of isotonic conditions – hypotonic conditions – hypertonic conditions.

In hypertonic conditions, the values of root-mean-square and average surface roughness reduce several times, but the distance between the elevations decreases. In hypotonic environment, heights of the peaks and depths of the depressions increase; in case of the increase in osmotic pressure, relief smoothing is observed. Changes in the symmetry of the distribution of various relief structures when changing the osmotic pressure were not observed. The entire cellular surface is uniformly transformed under the effect of uncharacteristic salinity. Under the influence of hypotonic conditions, SA microrelief has a smoother structure, low peaks and troughs are located remotely from each other (Table 1).

Atomic force microscope scans of *E. octoculata* amoebocyte clearly show the raised central region of the nucleus and perinuclear space and the cytoplasm area, which is almost even with the substrate. The membrane covering the nucleus forms the elevations caused by protrusion of cytoskeleton fibrils arranged randomly. The presence of longer fibrils radially directed from the nucleus was found in the peripheral part of the cell.

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Table 1: Parameters of surface microrelief heterogeneity of *H. medicinalis* hemocytes

Parameters	BA	AA	NA
Isotonic environment			
Sq, nm	91,89±7,54	67,25±5,16	125,43±34,56
Sa, nm	69,82±9,65	51,82±2,84	105,53±10,71
Sp, nm	676,51±43,87	653,67±4,67	776,97±63,92
Sv, nm	237,64±23,19	279,95±9,58	212,93±31,28
Sds, 1/um·um	39,65±6,51	45,13±6,83	10,97±2,13
Ssc, nm	37,02±3,22	25,87±4,92	16,48±3,41
Hypotonic environment			
Sq, nm	57,19±8,93*	65,18±7,22	0,11±0,01*
Sa, nm	45,92±4,48*	53,08±7,48	0,09±0,01*
Sp, nm	713,56±12,04	859,18±73,11*	1,18±0,01*
Sv, nm	400,83±21,28*	487,74±90,08*	0,65±0,01*
Sds, 1/um·um	79,11±6,39*	79,48±10,61*	1,53±0,05*
Ssc, nm	38,99±2,97	26,70±3,34	3,72±0,15*
Hypertonic environment			
Sq, nm	29,48±4,68*	20,02±2,57*	62,31±3,93*
Sa, nm	23,14±2,71*	16,22±1,42*	50,71±3,92*
Sp, nm	279,56±4,09*	196,26±5,45*	356,33±7,79*
Sv, nm	104,53±11,95*	60,81±6,81*	45,59±6,27*
Sds, 1/um·um	20,47±2,31*	36,82±2,25	14,86±0,56*
Ssc, nm	8,89±2,11*	14,39±3,68*	10,88±2,10*

Note: BA – BA – big amoebocytes; AA – average amoebocytes; NA – not amoebocytes; * – credibility of differences between the values of the parameters in isotonic conditions and in conditions of changed osmotic pressure ($p<0,05$); credibility of differences was assessed by Student t-test.

In hypertonic conditions, amoebocyte cells have uniform morphological structure. Folds and furrows form on the surface of hemocytes.

Analysis of surface topography of *E. octoculata* coelomocytes showed a slight increase in the values of root-mean-square and average roughness in hypotonic environment and a decrease in hypertonic conditions.

Table 2: Parameters of surface microrelief heterogeneity of *E. octoculata* hemocytes

Parameters	BA	AA	SA	NA
Isotonic environment				
Sq, nm	0,13±0,04	49,74±4,66	63,56±1,97	40,09±3,01
Sa, nm	0,09±0,03	37,81±1,41	51,89±8,06	31,25±0,76
Sp, nm	1,46±0,02	126,81±0,08	515,34±19,66	417,98±13,66
Sv, nm	0,67±0,06	236,35±3,43	151,55±6,51	209,95±11,43
Sds, 1/um·um	2,41±0,81	1,08±0,19	4,74±1,14	34,19±5,61
Ssc, nm	11,34±0,05	5,21±0,009	8,24±0,02	11,43±1,98
Hypotonic environment				
Sq, nm	43,33±4,65*	51,11±5,72	81,97±3,84*	68,77±2,44*
Sa, nm	36,42±8,31*	35,93±9,76	62,55±5,65	55,02±1,58*
Sp, nm	485,59±60,42*	468,33±5,37*	545,74±4,84*	268,16±29,09*
Sv, nm	209,95±30,22*	154,02±4,51*	57,63±4,91*	287,34±8,04*
Sds, 1/um·um	2,87±0,19	2,29±0,31*	1,33±0,03*	1,48±0,42*
Ssc, nm	12,17±1,78	5,19±0,01	6,26±0,03*	9,27±0,05
Hypertonic environment				
Sq, nm	48,80±9,02*	43,44±2,12	0,12±0,01*	46,47±0,44*
Sa, nm	41,30±9,36*	35,09±8,72	0,09±0,03*	36,14±0,31*
Sp, nm	395,06±41,67*	392,47±20,53*	1,59±0,16*	627,10±2,51*
Sv, nm	125,72±11,24*	258,32±6,52*	0,82±0,24*	286,55±6,65*
Sds, 1/um·um	2,11±0,31	2,62±0,66*	1,12±0,13*	2,29±0,38*
Ssc, nm	17,19±2,13*	6,21±0,04*	7,28±0,43*	5,22±0,02*

Note: BA – big amoebocytes; AA – average amoebocytes; SA – small amoebocytes; NA – not amoebocytes; * – credibility of differences between the values of the parameters in isotonic conditions and in conditions of changed osmotic pressure ($p<0,05$); credibility of differences was assessed by Student t-test.

Big amoebocytes (BA) similarly respond to an increase and a decrease in environment salinity. In normal conditions, smooth surface has a complex structure with a maximum size of elevations and depressions under hypotension. Significant smoothing of NA microrelief is observed in hypertonic conditions. The remaining cell types are characterized by small increase in roughness in hypotonic conditions and a reduction in case of the increased environment salinity. Under the effect of varying osmotic pressure, there is a decrease in density of elevations on the surface of small amoebocyte (Table 2).

The surface topography of *H. sanguisuga* hemocytes in case of an increase in osmotic pressure is characterized by relief homogeneity on AFM scans. Cytoskeleton fibrils are noticeable; strands protrude on the surface of hemocytes through plasma membrane. The increase in osmotic pressure increases protrusion of cytoskeleton granules and fibrils. Cell surface is relatively flat.

In hypotonic conditions, hemocytes of this type flatten; radial strands of fibrils from the cell nucleus to cell membrane are visible. Reduction of osmotic pressure is reflected in the cell microrelief that contains a large number of protrusions and furrows. In hypotonic environment, the surface of hemocytes looks heterogeneous on the scans.

Table 3: Parameters of surface microrelief heterogeneity of *H. sanguisuga* hemocytes

Parameters	BA	AA	SA
Isotonic environment			
Sq, nm	41,39±4,32	44,66±9,41	33,09±2,41
Sa, nm	36,13±9,45	34,14±7,22	25,42±3,69
Sp, nm	512,30±42,7	494,76±61,11	433,91±10,46
Sv, nm	336,34±38,52	248,88±30,61	248,03±10,63
Sds, 1/um·um	4,64±0,43	10,03±2,31	26,69±2,97
Ssc, nm	3,94±0,09	9,31±1,17	7,81±0,45
Hypotonic environment			
Sq, nm	42,38±3,23	64,89±8,91*	61,57±4,06*
Sa, nm	32,71±2,04	44,76±3,56	49,49±5,51*
Sp, nm	456,95±4,29*	581,54±14,33*	643,81±10,23*
Sv, nm	229,41±7,53*	187,35±25,12*	347,71±7,48*
Sds, 1/um·um	22,09±4,10*	9,27±0,45	7,41±0,89*
Ssc, nm	10,37±1,49*	5,67±0,76*	8,63±0,57*
Hypertonic environment			
Sq, nm	64,82±7,21*	58,31±4,32	44,66±7,02*
Sa, nm	53,14±3,45*	45,84±7,11	34,14±4,21
Sp, nm	629,49±6,78*	658,11±22,95*	494,76±18,21*
Sv, nm	307,06±13,21	388,35±17,31*	248,88±32,12
Sds, 1/um·um	3,94±0,75	10,86±2,32	10,03±3,01*
Ssc, nm	4,69±1,31	12,57±2,31	9,31±2,31

Note: BA – big amoebocytes; AA – average amoebocytes; SA – small amoebocytes; * – credibility of differences between the values of the parameters in isotonic conditions and in conditions of changed osmotic pressure ($p<0,05$); credibility of differences was assessed by Student t-test.

The average roughness in hypotonic environment is not changing in big amoebocytes; an increase in a number of protrusions per unit area (Sds) and their mean curvature (Ssc) is observed. Other parameters do not undergo significant changes. In small amoebocytes and not amoebocytes, an increase of such parameters as Sq under increased salinity is observed; microrelief changes towards an increase in protrusions and reduction of the number of depressions per unit area. In hypertonic conditions, a slight increase in the main parameters of surface roughness was observed. The distance between the elevations and their maximum height increases. The density of invaginations and their curvature in hypertonic conditions does not credibly differ from these parameters in normal conditions (Table 3).

SUMMARY

Roughness coefficient of all cell types in this environment increases, however this is not always associated with an increase in the number of micro elevations – deepening of depressions and increase in the height of microrelief elements also plays a significant role.

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CONCLUSIONS

The changes in the surface topography of hemocytes are described in contact interactions with solid substrate, and when exposed to environments other than physiologically normal. An increase/decrease in the thickness of the disturbed layer in hypotonic and hypertonic environment respectively is characteristic for cells that perform phagocytic function, with maintenance or increase in the number of micro elevations per unit area. The prevalence of invaginations in hemocytes with abundant content of granules is observed when exposed to conditions with increased osmotic pressure.

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