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APPLICATION OF FLOW CYTOMETRY TO ANALYZE EFFECT OF ALUMINUM-SILICON SORBENT ON BLOOD ERYTHROCYTES

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MOTIVATION AND AIM

- One effective method of detoxification is the method of hemoperfusion. The accumulated experience of using sorbents in medicine encourages the development of new, more advanced and safe materials and methods of their use [1-3]. A promising and modern method for such an assessment is the scanning flow cytometry, the use of which has been widely presented in published materials in recent years [4,5].
- Study's purpose is to use the scanning flow cytometry method to investigate the effect of the sorbent, with a gamma aluminum oxide and polydimethylsiloxane (PDMS) base, on the red blood cells of donor blood.

MATERIALS AND METHODS

- Aluminum silicon sorbent Al_2O_3@PDMS was studied. The sorbent was obtained by immobilization of polydimethylsiloxane (PDMS) on the surface of a porous aluminum oxide at room temperature. The porous structure of the sorbent sample was evaluated from experimental nitrogen sorption isotherms [6]. The adsorption activity was evaluated in relation to the sorption of blue methylene dye and vitamin B_{12} on a spectrophotometer (ApelPD-303UV, Japan). The patient's blood was taken from the vein before surgery and from the sterile wound drainage system HandyVac, hemoperfusion was performed with the latter according to the scheme in Fig. 1.. The morphological (Fig.2) and functional characteristics of red blood cells were determined by scanning flow cytometry (SFC) [4].

RESULTS

- **Table 1** shows that the porous structure of the sorbent is almost identical to the structure of the original carrier. The sorbent has a slightly higher adsorption activity in relation to the methylene blue dye compared to the original aluminum oxide. **Table 2** shows that the morphological parameters of blood before and after surgery are different. The sorbent does not negatively affect the morphological parameters of red blood cells. **Table 3** shows that the number of active band 3 proteins prior to the operation and the average maximum membrane extensibility were higher than after the operation.

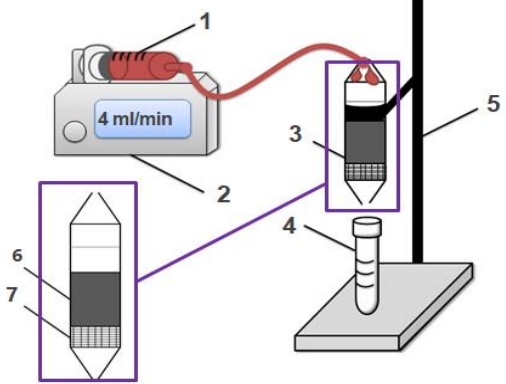

Fig. 1. Blood perfusion setup chart: 1 - syringe with blood, 2 - infusomat (to ensure uniform perfusion), 3 - glass column with a sorbent, 4 -vakuteyner with EDTA (anticoagulant), 5 - tripod for fixing the column, 6 - hemosorbent, 7 – glass filter

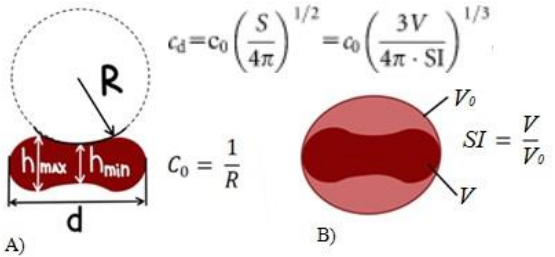

Fig. 2. A) d - diameter of the disk; hmin, hmax – minimum & maximum thickness; V – volume; S - surface area; SI - sphericity index; C_o - spontaneous curvature of the membrane; Cd - dimensionless spontaneous curvature of the membrane.
B) V_o - Spherical red blood cell with volume V_o; V - non-spherical red blood cell with volume V

Table 1. Some physical and chemical properties of the sorbent with a particle size of 0.2-0.8mm. Key: S – specific surface area, m^2/g; V - pore volume, cm^3/g; p – bulk weight, g/cm^3; MB - sorption of methylene blue, mg/g; B_{12} - sorption of vitamin B_{12}, %; γ-$A1_20_3$@PDMS is the aluminum-silicon sorbent.

Sample	S, m^2/g	V, cm^3/g	p, g/cm^3	MB, mg/g	B_{12}, %
γ-$A1_20_3$@PDMS	180±0,22	0,35±0.003	0,79±0,006	10,4±0.08	0,6±0.01
γ-$A1_20$	201,00±0,20	0,35±0.001	0,79±0,005	6,9±0,08	0,6±0.01

RESULTS (continue)

Table 2. Erythrocytes morphological parameters evaluated by blood analysis data before and after sorbent contact (M± SE).

Erythrocyte parameters	Patient's blood analysis		Blood analysis (column without carrier)	Blood analysis γ- Al_2O_3@ PDMS
	Before surgery	After surgery		
d – diameter, μm	6,66 ± 0,02	6,04 ± 0,02	6,02 ± 0,02	5,940 ± 0,018
h – minimal thickness, μm	1,078 ± 0,011	1,45 ± 0,015	1,501 ± 0,017	1,501 ± 0,014
h – maximal thickness, μm	2,176 ± 0,008	2,632 ± 0,010	2,711 ± 0,011	2,675 ± 0,010
V – volume, μm^3	64,5 ± 0,5	62,6 ± 0,5	64,2 ± 0,5	61,6 ± 0,5
S – surface area, μm^2	101,4 ± 0,6	90,4 ± 0,5	90,7 ± 0,6	88,3 ± 0,5
SI –sphericity index	0,6734 ± 0,0018	0,7635 ± 0,0018	0,774 ± 0,002	0,7734 ± ,0017
C_0 – spontaneous curvature of the membrane, μm^{-1}	-0,060 ± 0,008	-0,393 ± 0,008	-0,413 ± 0,008	-0,422 ± 0,008
Cd – dimensionless spontaneous curvature of the membrane	0,05 ± 0,02	-0,85 ± 0,02	-0,92 ± 0,02	-0,93 ± 0,02
Hemoglobin concentration, g/dl	60,0 ± 0,3	49,3 ± 0,2	47,80 ± 0,2	48,19 ± 0,19
CHb – hemoglobin content, pg	34,3 ± 0,3	27,39 ± 0,17	27,14 ± 0,18	26,22 ± 0,15

- The average value of the number of active band 3 proteins and of the ratio of membrane stiffness to elasticity after contact with a glass porous filter increased compared to the blood sample after surgery. After contact with the sorbent, the average values of the number of band 3 proteins decreased and the average values of the ratio of membrane stiffness to elasticity decreased insignificantly.

Table 3. Changes in the functional parameters of red blood cells after contact with the sorbent. (M ± SE)

Sample	The number of active band 3 proteins in a single red blood cell, 10^6	Ultimate extensibility of the erythrocyte membrane, 10^{-2}
Blood before the operation	1,37 ± 0,07	12,0 ± 1,4
Blood after surgery	0,400 ± 0,005	1,67 ± 0,16
Blood after contact with an empty column	0,50 ± 0,04	5,3 ± 0,5
Blood after contact with the Al_2O_3@PDMS sorbent	0,280 ± 0,004	1,49 ± 0,13

CONCLUSIONS

This study using a modern method of scanning flow cytometry showed that the new porous aluminum-silicon sorbent based on highly durable and standardized aluminum oxide and polymethylsiloxane minimally changes the morphological parameters of red blood cells, the ratio of stiffness to plasticity of the membrane, and does not critically reduce the number of band 3 proteins in red blood cells responsible for anion exchange of cells. All changes are within the normal range for red blood cells. The obtained data allows us to confirm the safety of the new sorbent and the process of its production.

REFERENCES

[1]. H. W. Gil, S. J. Kim, J. O. Yang, E. Y. Lee and S. Y. Hong, "Clinical outcome of hemoperfusion inpoisoned patients", Blood Purif , vol. 30, pp. 84–88, Jul 2010.

[2]. A. S. Morozov, I. V. Bessonov, A. V. Nuzhdina and V. M. Pisarev, "Sorbents for Extracorporeal RemovalToxic Substances and Molecules with Adverse Biological Activity (Review)", General Reanimatology, vol. 12(6), pp. 82-107, 2016.

[3]. S. E. Khoroshilov and A. V. Nikulin, Detoxification in critical conditions: understanding the scientific problem in the XXI century (review), General Reanimatology, vol. 13 (5), pp. 85-108, 2017.

[4]. K. V. Gilev, E. S. Yastrebova, M. A. Strokotov, N. A. Yurkin, A. V. Karmadonova, V. V. Chernyshev, V. P. Lominorotov and V. P. Maltsev, "Advanced consumable-free morphological analysis of intact red blood cells by a compact scanning flow cytometer", Cytometry A, vol. 91, pp. 867-873, Sep 2017.

[5]. V. Maltsev, L. Rachkovskaya, V. Nimaev, A. Smagin, A. Pivkina, E. Yastrebova, A. Volodin, A. Vedyagin, A. Shurlygina, E. Rachkovsky and M. Korolev. "Carbon-Mineral Carriers for Active Pharmaceutical Substance" Proceedings. International Multi-Conference on Engineering, Computer and Information Sciences (SIBIRCON), Novosibirsk. 21-22 Oct. 2019, pp. 0305-0308

[6]. S. Greg and K. Sing, "A sorption, specific surface area, porosity", Moscow: Mir, 1984, 310 p.