INORGANIC BIOMIMETICS FOR CLINICAL HEMATOLOGY

This article presents an alternative method of synthesis of erythrocyte models based on laser-induced self-assembly of inorganic biomimetics. Obtained artificial red blood cells are shown to be appropriate objects for simulating cellular pathology in clinical hematology. The results of using inorganic erythrocytes for modeling some hematological abnormalities are given.

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Introduction

At present, the fundamental problem of hematology is the development of biologically compatible non-heme protein-free blood substitutes with the same functional properties as red blood cells. Vesicular synthesis of artificial red blood cells by means of hemoglobin encapsulating is not appropriate because the presence of vesicular structures in blood causes immune response of the organism (Ming and Chang, 2007). Hemoglobin is fixed by blood-plasma proteome and is transformed into haptoglobin, later utilized in kidneys, bone marrow and spleen, which leads to hemoglobinuria and thrombosis.

There were several attempts to create alternative blood substitutes based on perfluorocarbon emulsions in order to avoid the intoxication by hemoglobin-containing blood substitutes (Geyer, 1973; Naito and Yokoyama, 1975; Riess, 1991; Shumakov et al., 1993). However, the oxygen capacity of perfluorocarbon (liquid F-carbons ≈ 40 vol.%, per ftoran ≈ 7 vol.%) does not correspond to the oxygen capacity of native blood and plasma (blood ≈ 20 vol.%, plasma ≈ 2.4 vol.%). Besides, the practical application of perftorans is accompanied by a number of medical problems: infusion of perfluorocarbon Fluosol-DA can cause anaphylactic reaction (Watanabe et al., 1988).

Also perfluorocarbons are not stable enough due to their lipophilicity (Kabalnov et al., 1990; 1992) and, therefore, tend to interact with plasma membrane lipids and receptors. Thus, the clinical introduction of perfluorocarbon-based blood substitutes is rather difficult nowadays.

However, there are original projects of creation of new nanomechanical devices (Freitas, 1998; 2001) that mimic the functions of blood cells: both erythrocytes and lymphocytes. Respirocytes - artificial analogs of red blood cells, according to (Freitas, 1998), should be carbon nanomachines with a diameter of 1 micron, containing mesoscopic tanks - reservoirs of O2, CO2, water and glucose for respiration and energy supply. Mechanical equivalents of phagocytes (lymphocytes of the immune system), according to (Freitas, 2001), were described as nanomedical microscopic devices, imitating the functions of T-killers. In addition, nanostructured analogs of platelets - clottocytes were proposed to prevent hemorrhage (Freitas, 2000). Finally, vasculoid, mechanical analogue of the circulatory system, based on the listed nanomechanisms, is expected to be implemented by the method of probe assembly only in 2030 (Freitas and Phoenix, 2002).

Still it's almost obvious that a construction of a completely functional analogue of hematological systems in this way is almost impossible because of the systemic character of hematopoietic and respiratory processes. Therefore, the only possible method to create adequate functional analogues of biological cells (vasculocytes of any type) is self-assembly
(Trevors, 2002). For example, a synthesis of functional analogues of cells, based on colloidal micelles, is proposed in (Bedau et al., 2000; Mayer and Rasmussen, 2000; Rasmussen et al., 2004). It should be noted, that such cell models may not correspond to their living prototypes in chemical composition. A similar object based on metal-oxide capsules with selective permeability and capacity for gas exchange is described in (Muller and Roy, 2005). But it is impossible to form a microstructure of such biomorphic objects obtained via vesicular synthesis, which also inevitably leads to impossibility of a full simulation of cytological systems.

Meanwhile, laser forceps are appropriate for managing the assembly of supramolecular associates, such as colloidal micelles and membrane-vesicular structures (Sasaki et., 1991). Known are similar methods of manipulating of biological cells and their ultra-structure (Weber and Greulich, 1992; Moroz et al., 1996). These laser technologies provide for work with both organic and inorganic dispersed systems (Ozkan et al., 2006). That allows producing laser-optical assembly of vasculocytes, using hematopoietic prototypes. If respirocytes, microfagocytes and clottocytes, according to (Freitas, R., 1998; 200; 2001), represent a kind of nanorobotic devices, it can be assumed that laser-optical synthesis of vasculocytes can be considered as a biologically inspired self-assembly of robotic systems with a high ultrastructural organization (Pfeifer et al., 2007; Ummat et al., 2005).

The work (Granick, 1965) presents a model of cytological system that mimics hematological and respiratory functions using redox processes on magnetite with impurities adsorbed on it. It is well known that some iron compounds, such as magnetite and emulsion of iron trichloride, being semiconductors, possess photosensitivity. In this connection, they can be used as precursors for laser-optical self-assembly.

**Materials and methods**

Artificial analogues of red blood cells were synthesized via laser-induced self-assembly according to the technique developed. As an example, consider the implementation of the method based on a pulsed tunable Ti: sapphire laser CF131MA with a large tuning range (189-950 nm), which allows to vary the parameters of the radiator, depending on the spectral characteristics of a cellular matrix and the colloidal substrate. As the pump source it is possible to use lasers LF114 or LF117, depending on the amount of energy per pulse. Figure 1 shows the flow-chart of the described method (all the intermediate regulators and detectors are omitted for the purpose of simplicity). The emitting system (G) beam is projected onto the hematological prototype (blood smear M), located on a quartz glass coverslip, and then it gets to the underlying light-sensitive iron-containing colloidal substrate (S), located in contact with the supported quartz glass coverslip on a translucent or transparent glass.

![Figure 1. Installation scheme](image)

Photo-induced processes of self-organization in the colloidal substrate lead to generation of dissipative structures, isomorphic to the cellular structure of a hematological prototype M.

The essence of this method is that in the course of interaction with a biological cellular structure beam flux is modulated by the adequacy of the structure: combinational beam scattering in cell ultrastructure takes place and also a change of its polarization in optically
active media of the cells, a change in its intensity depending on the absorption of the medium, etc. In output the beam parameters at each point are in compliance with the copied cellular structure.

If we use nanodispersed colloidal semiconductors as a substrate (precursor), sensitized to this spectral range, passing through the M radiation interacts with the precursor (S) and activates in it the processes of laser-induced self-organization at the expense of the generation-recombination phenomena in the semiconductor, which depends on the parameters of the radiation (Sholl, 1987). When placing an inorganic colloid precursor nearby an imitated hematological structure along the beam, one can obtain self-organizing dissipative structures isomorphic to the structure of radiant flux in output of the cellular prototype, hence copying its structure to the limits, determined by the wavelength. Thus, it should be taken into account that beam spectrum in output of the cellular prototype should correlate with absorption spectrum of an inorganic colloidal precursor.

Vasculocytes, as functional models of hemocytes, should simulate reversible gas transfer and other typical blood properties - hemocoagulation, immunohematological functions, etc. Due to participation of the iron atom in a hemoglobin molecule it is advisable to use Fe-containing precursors for hematology modeling. In this work photosensitized emulsions of iron trichloride and desorbed suspensions of magnetite were used as a precursor.

The using of a number of different inorganic colloidal substance as precursors and other types of biological tissues as cellular prototypes in the above method is also rather possible.

Results

As a result of laser-induced self-organization heterogeneous dissipative structures - inorganic copies of blood cells were obtained in a light-sensitive active medium. Obviously the structures obtained differ from the initial precursor in chemical composition.

According to X-ray diffraction (Figures 2, 3), their composition includes the following compounds - the products of chemical transformation of ferric chloride under the laser beam (Figure 2):

- Fe₈(O, OH)₁₆Cl₁.₅ (peaks 1, 2, 5, 10);
- Fe(OH)₃ (peaks 11, 14, 19);
- FeOCl (peaks 8, 10, 13, 15);
- Fe₂O₃ (peaks 4, 9, 16);
- Minor intermediate compounds.

In contrast to the product of self-organization, the initial precursor is the nature of the noise schedule that indicates its low structural organization (Figure 3). Thus, the formed chemical composition of the cellular structures depends on the spectral characteristics of radiant flux in the output of the cellular prototype.

Figure 4a presents micrographs of inorganic analogs of erythrocytes, synthesized on the basis of nanodispersed magnetite, and Figure 4b - the ones self-organized in hydrolysed iron trichloride. Both structures are completely isomorphic to donor erythrocytes, used as biological prototype.

In addition, the obtained structures were also found to possess a number of morphofunctional properties, which correlate with the ones of their biological prototypes due to dependence of their chemical composition on spectrochemical parameters of the original erythrocytes.

In this case it is possible to simulate a number of cytochemically caused membranopathies for the purposes of clinical hematology using the obtained models of erythrocytes. Thus
the present article shows the results of using inorganic erythrocytes in simulating of such hematological abnormalities as microspherocytosis, elliptocytosis, stomatoctyosis, acanthocytosis and some haemoglobinopathies.

**Figure 2. X-ray diagram of products**

Initial angle=10, final angle=60, step=0.02, exposure=0.3, rate=4, max. number of pulses=563

![X-ray diagram of products](image)

It is well known that a change of osmotic resistance of erythrocytes (Fox, 1964) is characteristic of microspherocytosis, because spherocytic erythrocytes become osmotically less resistant. This hemolytic anemia is considered as a molecular defect of the erythrocyte membrane structure and can be reliably diagnosed by the acid erythrogram method at pH 3.0, when microspherocytosis acid erythrogram shows a sharp elongation of the hemolysis.

In inorganic hematological systems acidic pH also leads to microspherocytosis and violation of the osmotic resistance, followed by bursting of erythrocyte membranes. Figure 4a presents microspherocytic inorganic cells and Figure 5(b, c) demonstrates broken cell membranes under the pH-shift. Over time, the lysed membranes, according to the acoustic microscopy, disintegrate, and the sediment (Figure 5d) analogous to so-called "shades of red blood cells", is obtained under similar conditions.
Another membranopathy simulated is elliptocytosis when a content of oval-shaped red blood cells rises from 10% to 25-75% and more. It should be noted that the presence of elliptical erythrocytes in the donated blood is also possible in hematological modeling, although a number of inorganic elliptical erythrocytes were objectively recorded in our experiments. The latter also broke cell membranes and an increase in autohemolysis was accompanied by the increase in the osmotic resistance. Photomicrographs of inorganic cells, imitating the following hematological abnormalities, are pictured in Figure 6(a, b).

The hemolytic anemias described are attributed to membranopathies, so their dependence on the physicochemical parameters of the membranes is obvious. However, as the morphometric characteristics of inorganic cells are homeomorphic to the ones of normal blood cells (morphometric characteristic of elliptocytosis is an affine transformation of the morphometric characteristic of normal erythrocytes, while stomatocytosis in this transformation takes place only in its central zone), the nature of initiation is not clear.
In order to prove the possibility of the occurrence of hemolytic anemia in inorganic copies of healthy red blood cells under the influence of physicochemical factors of the medium, hereditary blood diseases were modeled by the method described.

Acanthocytosis or hereditary abetalipoproteinemia is a rare disease, described for 30 cases. Therefore, simulating acanthocytosis on the iron-containing inorganic cell models, we can be almost sure that the effect was caused by physical and chemical conditions of the medium, but not by the form of donor blood cells. Considered pathology in clinical hematology is associated with the defects in the structure of membrane lipids. So it is possible to simulate the morphologic-structural features of acanthocytosis by changing the membrane Eh and initiating the Fe-deficiency, using the copies of red blood cells from a healthy donor. Figure 6c shows photomicrographs of inorganic echynocytes - the products of secondary effects on the initial inorganic structures, similar to those given in Figure 4.

**Figure 6. Photomicrographs of inorganic erythrocytes, imitating various membranopathies**

![Photomicrographs of inorganic erythrocytes](image)

a. Model of ellyptocytosis.  
b. Model of stomatocytosis.  
c. Model of echynocytes

**Figure 7a. Profilogram of inorganic erythrocyte**

![Profilogram of inorganic erythrocyte](image)

**Figure 7b. Profilogram of inorganic proerythroblast**

![Profilogram of inorganic proerythroblast](image)

The similar echynocyte shapes are observed when homozygous β-(Hb A2)-thalassemia. But, since echynocytosis is also characteristic of iron-deficiency anemia, it is possible to interpret the experiments with the degradation of the inorganic erythrocyte membranes as the induced Fe-deficiency. With the method proposed, it is possible to influence erythropoiesis of the copies of immature erythroblasts by changing the parameters of the
medium (pH, dH, Eh, electrical conductivity and ion concentrations, etc). Figure 7 presents profilograms of inorganic erythropoietic cells, taken in the proximal region. It is obvious that, unlike the erythrocyte, proerythroblast has a ripple profilogram in the central region, due to the presence of heterodispersed polymorphic granules.

The presence of polymorphic granules in organic proerythroblasts indicates the activity of acid phosphatase and a low degree of cell proliferation. Thus, the above principle in cytology can be applied for simulating the processes of metabolic and functional cell specialization.

**Conclusion**

Inorganic morphofunctional models of the blood cells were synthesized. The biomimetic structures obtained were found to generate oxygen, in particular, its active forms. The mechanisms of redox processes in inorganic erythrocyte models are rather different from the ones in their biological prototypes. Inorganic copies of the blood cells can be used in clinical hematology for the diagnosis of hemolytic anemia and other pathologies. Also it is possible to create a new type of inorganic blood substitutes based on them. Using the techniques of laser-optical assembly, it is possible to obtain morphofunctional copies of blood cells of every type, in order to create a system of interacting biomimetical structures - vasculoid (Freitas and Phoenix, 2002).

**References**


