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Conference Paper

Design of the Model of Ratiometric Polymer Nanobiothermometer Based on Quantum Dots

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Abstract

To solve many modern biological and biotechnological tasks it is necessary to realize strictly control and regulation of temperature of the cells and their organelles. This tasks include control of various exo- and endothermic reactions, monitoring of tissues' and individual cell's temperature in in vitro researches and in vivo procedures such as the hyperthermia procedure that used for cancer treatment. The today known methods of measuring and controlling of temperature at the cellular level can not provide the necessary level of locality and accuracy due to too big size and heightened sensitivity to external factors. The real alternative of existing today methods is nanoscale temperature biosensor operating on a ratiometric principle and based on the composite structure from polymers and colloidal quantum dots. In this paper we present a working model and plan of investigation of ratiometric nanoscale polymer nanobiothermometer based on quantum dots.

Keywords: thermosensors, quantum dots, local temperature, polymers, temperature measurement

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1. Introduction

An extremely popular trend in many natural sciences is the desire to miniaturize and subminiaturize the devices used. In the early 50's there were electronic biosensors [1], in the early 80's - biochips [2], and today the main object of research is nanoscale biosensors.

The emergence of a wide range of commercially available sensitive polymers [3], as well as high-quality (quantum yield more than 60%) colloidal quantum dots (QDs) [4, 5] has opened the possibility of developing nanobiosensors sensitive to wide range of physical properties of investigated objects and characterized by high level of feedback.

In contrast to organic dyes, QDs are characterized by narrow and symmetrical peak of fluorescence and, most importantly, by wide excitation band [5], which allows the creation of devices containing simultaneously several different types of QDs excited by the same radiation source without its additional tuning. Furthermore, QDs are characterized by extremely high photostability [5], which allows the use of high-power radiation sources (lasers) without fear of sudden "burnout" of the nanobiosensor during the study. Thus, the emergence of accessible and qualitative colloidal QDs opened the way to creation of multicoded ratiometric nanoscale biostructures.

One of the most demanded sensors for today is a nanoscale temperature sensor that can perform local measurements at the cellular level. Numerous biological and chemical reactions are characterized by a certain temperature change. The exact value and direct monitoring of this temperature can be a significant factor for researchers seeking to increase or decrease the effectiveness of any process, same as for the people studying the general properties of any cells, their response to certain types of stimuli. Nanoscale in vitro sensor of local temperature can be used for control various exo- and endothermic reactions, for research in the field of optogenetics, and it can serve as a prototype for in vivo temperature sensor for conducting hyperthermia procedure for cancer patients.

In this paper, we present methods of preparation and investigation of the working model of nanoscale biosensor of local temperature acting by ratiometric principle and based on the sensitive polymers and colloidal QDs. Ratiometric systems include systems whose data are deciphered on the basis of the ratio of several indicators strictly dependent on each other and regulated by a factor to which the device is directly sensitive. In the presented models as such indicators we used CdSe/ZnS QDs emitting at the wavelength of red and green light. The temperature of the model itself is their regulation factor. The obtained model of nanobiothermosensor is the polymer/QDs composite with polystyrene core on which two layers of QDs isolated from each other and a thermally sensitive "smart" polymer polyvinylcaprolactam functioning in the region from 21 °C to 41 °C are deposited.

The temperature measurement by the nanodevice's model of such structure is based on the strictly dependent from temperature compression by the "smart" polymer of all the entire composite structure. The degree of compression determines the change in the distance between the two different types of QDs and, accordingly, the level of resonance energy transfer between them. The level of resonance transfer, in turn, determines the ratio of the emission peaks of the QDs relative to each other, which is a parameter for measure the temperature.

To investigate the situation around the development of nanoscale temperature biosensors, an analysis of articles published during the last five years has been made. A total of twelve articles were analyzed [6-17], of which ten are devoted to the use of different techniques for creating the stable polymer microsphere/colloidal QDs hybrid structures [6-15] and two articles are devoted specifically to sensors of temperature [16, 17]. It is need to point out, that one of them [16] contains information about the new temperature sensitive material only, but does not contain any real prototypes of how to use it in quality of temperature sensor. Moreover, the most popular for today method for measuring the temperature of cells in vitro [17] is able to estimate only in the micrometer range and does not realize direct measurement. So, we can say that a nanoscale biosensor of temperature or a model capable of performing local measurements at the cellular level has not yet been constructed.

What about our research group, it is necessary to mention the two publications [18, 19], preceding the development of the model of the nanoscale biosensor of temperature presented in this article. In the paper of 2013 [18], a nanothermometer based on the use of only one type of QDs (not ratiometric one) was developed; and in the paper of 2016 [19] the prototype of the model of the nanoscale sensor of temperature is presented.

2. Materials and Methods

2.1. Materials

Semiconductor CdSe/ZnS nanocrystals with core/shell structure stabilized with tri-n-octylphosphine oxide (TOPO) (emission wavelength 545 and 615 nm), kindly provided by M.V. Artemyev, Institute of Physical and Chemical Problems of the Belarusian State University; bovine serum albumin (Serva); sodium chloride, sodium hydroxide, "p. a." (Reanal, Hungary); methanol, chloroform, ethanol, "puriss. spec."; mercaptoacetic acid (Acros Organics); polydiallyldimethylammonium chloride (PDADMAC) (Floerger, France); alternating copolymer of maleic anhydride and 1-octadecene (PMAO) (Sigma); polystyrene (PS) microspheres with a diameter of 370 nm are kindly provided by Generalova A.N. (Laboratory of Polymers for Biology, IBCh RAS); and poly-N-vinylcaprolactam (PVC) 40% ethanol solution (Acros Organics); aqueous ammonia brand "puriss. spec." ("Sigma Tech"); tetraethoxysilane (Aldrich); hexamethylene diamine (Acros Organics); PBS (pH 7.4) (Sigma).

To prepare all the solutions used in the work, MilliQ's highest purification water obtained from the Millipore plant was used.

2.2. The method of functionalization of QDs with mercaptoacetic acid

The QDs (CdSe/ZnS with λ_{fl} = 610 nm), 3 mg, were purified from an excess of TOPO by triple dispergation/centrifugation in chloroform (800 µl) and precipitation in methanol (1000 µl). After that by careful stirring were added chloroform and 200 µl of mercaptoacetic acid, followed by triple centrifugation and dispersion in methanol. The resulting precipitate was dispersed in the mixture of 20 µl of 0.1 M NaOH and 100 µl of water and transfered into the new tube.

The obtained modified QDs must be stored at a temperature 4-C6°C. The obtained modified QDs will remain their colloidal stability for at least 14 days.

2.3. The method of functionalization of QDs with poly(maleic anhydride-alt-1-octadecene) (PMAO)

The QDs (CdSe/ZnS with λ_{fl} = 545 nm), 3 mg, were purified from an excess of TOPO by triple dispergation/centrifugation in chloroform (500 µl) and precipitation in methanol (500 µl). After that by careful stirring was added the solution of PMAO in chloroform. Then the sample was incubated for 2 hours at room temperature, followed by evaporation. To the resulting precipitate was added 0,02 ml hexamethylenediamine in chloroform and incubation was repeated. The solvent was again evaporated and dispersed in PBS (pH 7.4), followed by centrifugation (14,000 rpm, cycle 30 min).

The obtained modified QDs must be stored at a temperature 4-C6°C. The obtained modified QDs will remain their colloidal stability for at least 6 months.

2.4. The method of creation of the models of ratiometric nanoscale sensor of local temperature

During the work, the model of nanoscale sensor of local temperature consisting of the core, thermosensitive polymer, 2 types of QDs and insulating layer between them has been developed.

We used the polystyrene microspheres with diameter ∼ 370 nm as the core, QDs modified in accordance with the above procedures red and green QDs with λ_{fl} = 615 nm and λ_{fl} = 545 nm, respectively. polyvinylcaprolactam functioning in the region from C21°C to 41°C was used as the thermosensitive polymer.

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To apply the models obtained in this work as ratiometric instruments, it is necessary that the total number of attached QDs to each individual microsphere and the uniformity of their distribution in each deposited layer be approximately the same. It has been experimentally established that for the realization of this task it is necessary to work with an excessive number of QDs, i.e. with an amount significantly higher than the number of vacancies on the polymer microsphere. In the course of the work, it was found that the optimal concentration of QDs for a sample with a latex concentration 20 mg/ml is 0.0059 μM.

2.5. The methods of investigation of obtained samples

The obtained thermosensor samples were investigated by optical microspectroscopy. The optical images of the control sample and the fluorescence intensity distribution among the individual microspheres in the sample were examined by collected experimental setup. To excite the fluorescence of the samples we operated at a wavelength 488 nm.

Thermosensitive properties, namely the fluorescence dependence on the temperature in the obtained model of ratiometric nanobiosensor of local temperature were performed by modified experimental setup using the 100X lens replacement for the LMPLFLN 20X (Olympus) lens focusing laser radiation per fiber end with core diameter 200 μm. The opposite end of the optical fiber, serving both for excitation and for collection of fluorescence, was dropped directly into the sample with a volume 500 μl. The scheme of the modified setup is shown in Figure 1. The temperature control of the sample was carried out by thermocouple. As a heater we used Reacti-Therm Heating Module (PIERCE). The measurement was carried out by careful stirring of the sample. The accumulation time of the fluorescent signal is 0.3 sec.

For the experiments the elements of the data processing system of the unique scientific setup “System for probe-optical 3D correlative microscopy” IBCh RAS (http://ckp-rf.ru/usu/486825/), was used. The equipment provided by the IBCH core facility (CKP IBCH, supported by Russian Ministry of Education and Science, grant RFMEFI62117X0018).

3. Results and Discussion

The fluorescence dependence on the temperature in the obtained model of ratiometric nanobiosensor of local temperature was investigated by the procedure described in

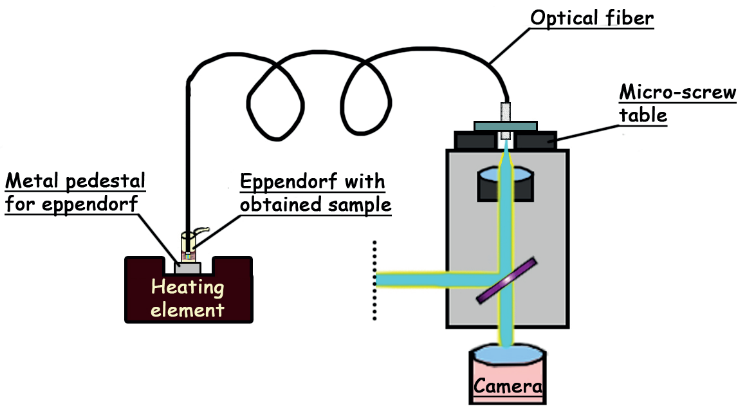

Figure 1: The optical scheme of the modified experimental setup for investigation of fluorescence dependence on temperature in obtained model.

Step 2.5 (Fig. 1) at three temperatures levels: 21, 41 and C51°C. The measurements at C21°C were carried out twice to evaluate the reproducibility of the results. The selected temperature values are the boundary values and are intended to demonstrate the principle possibility of the correct functioning of the nanoscale sensor of local temperature. In accordance with the concept of ratiometric sensors, the temperature dependence was estimated from the ratio of the fluorescence intensity of the green and red QDs, i.e. by the value of the parameter ($I_{GreenQDs}/I_{RedQDs}$). Ideally, the produced models of nanoscale sensors of local temperature should be characterized by the same value of this parameter at the temperature C21°C both: at its primary measurement and at a measurement carried out after the model were brought to the upper boundary temperature (C41°C) and extra heated (C51°C). The spectrum showing the fluorescence dependences of the obtained model on the temperature are shown in Figure 2. The total measurement error is no more than 10%, which is determined by the value of the signal-to-noise ratio.

In our model, the contribution of the reirradiation effect between QDs of the same type, following the general compression of the entire design of the model, is much smaller than the contribution made by resonance energy transfer (FRET) between QDs isolated from each other. This is an evident from the correspondence of the fluorescence peaks of the green and the red QDs to the donor and acceptor positions in the FRET pair [20].

4. Summary

The multi-stage method for creation of the nanoscale biosensor model of local temperature, working according to the ratiometric principle has been developed. The results

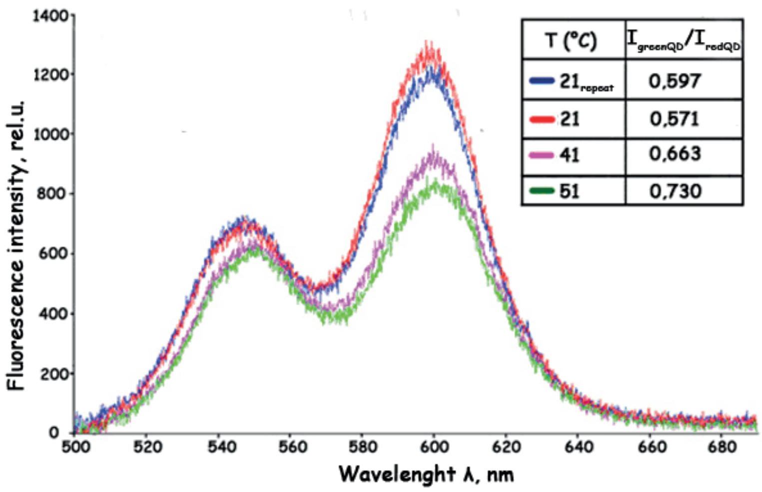

Figure 2: The fluorescence dependence of the obtained model on the temperature. In the table on the right, the values of the parameter ($I_{GreenQDs}/I_{RedQDs}$) for each measured temperature are indicated. Symbol 21_{repeat} - re-measurement of the parameter ($I_{GreenQDs}/I_{RedQDs}$) at a temperature of C21°C after the model have been heated to (C41°C) and over (C51°C) the boundary temperature of the thermosensitive polymer.

obtained in this work demonstrate the fundamental possibility of constructing multi-coded ratiometric nanosystems based on colloidal QDs. The possibility of developing such devices not only opens the door to the world of nanomedicine, but also includes all innovative possibilities for the development of biosensors, that became realizable by the availability of high-quality colloidal QDs and a wide range of sensitive ("smart") biopolymers.

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