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Technology for Forecasting and Early Detection of Disease Based on Analysis of Human Saliva by Means of Implantable Biosensor

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This article is devoted to the development of a device implanted in human oral cavity for continuous monitoring of the patient's salivary fluid in order to detect occurring cardiac diseases. Miniature sensor draws in saliva at certain intervals and analyzes it for the presence of biological markers that indicate, for example, approaching myocardial infarction. If cordial markers are identified there is an automatic notification of the patient and/or medical personnel. The paper presents the basic requirements to be met by the sensor that will help to improve the quality and speed of diagnosis of various cardiovascular diseases.

Key words: Biological markers (biomarkers), biosensor, cardiomarker, myocardial infarction, saliva, implant, cardiovascular system.

In recent decades, salivary fluid has been proposed as an alternative to screening diagnostics. An important advantage of using saliva compared to blood as a diagnostic environment is easy and non-invasive sampling of this material, which helps to eliminate the discomfort and pain associated with blood sampling, as well as to avoid problems of privacy associated with the intake of urine. In addition, in comparison with blood, the saliva consists of fewer proteins, thereby reducing the risk of errors due to non-specific effects and hydrostatic interaction. Blood protein concentration can vary by several orders of magnitude, the half-life of proteins ranges from several seconds to several months or more. The composition of saliva is not so complex and variable, so saliva more accurately reflects the current state of the organism in a specific period of time. [1-3]

The vast quantity of information contained in saliva as in a biological fluid makes it a promising alternative to blood. Analysis applicability of saliva caused in the first place by no need of invasion, and therefore painful sensations that accompany the intake of blood. Secondly, any invasion potentially increases the risk of infectious complications. Therefore, saliva became the subject of great attention of scientists all over the world.

The saliva has been proposed as a non-invasive alternative to screening-diagnosis, however, the use of saliva had been hampered by the low sensitivity of the methods and low

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concentrations of salivary biomarkers. The development of high technology has resulted in learning how to detect many important biological substances in saliva: electrolytes[4], drugs[5-6], proteins (cytokines, hormones, enzymes)[7-9], antibodies[10-11], bacteria[12-13], RNA[14-17]. Due to high technologies saliva was used as an indicator of various diseases, to identify infectious agents, therapeutic drug monitoring, hormones, and to obtain genetic information. Nowadays, experience on correlating levels of biomarkers of blood and saliva has been gathered, which will allow in many cases to refrain from blood sampling. Technologically advanced and reliable methods of diagnosis using saliva specimens can be executed repeatedly without any damage to the health of the patient.

It is expected that modern analytical technologies will greatly expand the potential diagnostic value of saliva. Early identification in saliva biomarkers of cardiovascular disease, especially predictors of acute myocardial infarction (AMI), creates conditions for a qualitative leap in the diagnosis of cardiovascular events.

Thus, the use of biochemical indicators of saliva for a comprehensive assessment of the condition of the human body opens new possibilities for diagnosis and predicting the course of various socially significant diseases.

MATERIAL AND METHODS

Diseases of the circulatory system are the main cause of death and disablement. In the structure of total mortality in the Russian Federation they are accounted for 56%, mainly strokes and heart attacks[18], 30-40% of victims die at the pre-hospital stage. Sudden cardiovascular death could have been prevented, if timely professional assistance was available. However, this can be realized only under the condition of constant monitoring condition of the patients relating to the risk group.

An implanted into the oral cavity sensor (biosensor), focused on the dynamic analysis of protein in the saliva, may be the solution. This technical device will monitor the patient's condition and send information to the appropriate health care organization. It will help to prevent myocardial infarction in cases of severe attacks of coronary heart disease, and also will significantly improve the results of treatment of cardiovascular patients through timely taken remedial measures.

This work is focused on identification of biomarkers of myocardial infarction, because of its high medical and social significance[18-20].

As part of the work there was analysis of world literature, patent development, interdisciplinary consultations with leading experts (technical, medical) carried out, the aim of which was: to explore and describe changes in the chemical composition of saliva upon the occurrence of socially significant diseases, to develop proposals for the detection of such changes and their detection using an implantable biosensor systems.

Water and dissolved organic and inorganic substances compose saliva. The solid components are dissolved in the liquid phase and differ from person to person, in addition, their composition can vary during the day. Organic substances of saliva include enzymes (á-amylase, maltase, kallickrein, lysozyme, mucin, vitamins, urea, uric acid, amino acids, cholesterol, agglutinin and agglutinogen (appropriate to the blood group), the components of the blood coagulation, glucose (diabetics), etc. Amylase and maltase enzymes break down carbohydrates to oligo - and monosaccharides. Inorganic substances of saliva are the salts of various acids, trace elements, cations and anions, mainly, different ions: K^+, Na^+, Cl^-, Mg^{2+}, HCO_3^-, Ca^{2+}, HPO_3^{2-} è NH_3[21]

Among proteins the most important are those which are produced by the organs of internal secretion (á-amylase, histamine, cystatin, lactoferrin, lysozyme, mucines, proline-rich proteins (PRP) or which are derived from plasma (albumin, secretory immunoglobulin A (sIg A) and transferrin[22].

The wide range of biochemical saliva, the reactivity of salivary glands in pathological and functional disorders, unusually high intensity of circulation with the possibility of large amount of secret, as well as the classical doctrine of the mechanism of homeostasis of the internal environment of organism in stress conditions led to the formation of new views on the function of the salivary glands[23-24]. Changes in the composition of saliva under different conditions of a person not only play a role in the diagnostic criteria, but are prognostic markers[25].

The relation between biomarkers of saliva and acute forms of ischemic heart disease is reflected in many scientific works[23, 26-29]. Therefore, given the importance of cardiovascular disease and, mainly, myocardial infarction, technique presented in this article is aimed at prevention and early detection of MI that will allow to take timely and appropriate medical decisions, which ultimately will give the opportunity to reduce premature mortality.

Diagnosis and prediction of cardiovascular events are demonstrated in the following eligible patent documents, which are the closest analogues of the developed device. John T. Mcdevitt and others offered a number of methods for the determination of cardiovascular biomarkers (index – cardiobioindex, CBI; each biome; combination of biomarkers) for risk assessment, diagnosis or prognosis of cardiovascular diseases. Technical diagnostic device works on the principle of lab-on-chip[30]. The same authors three years later patented a new method of rapid diagnosis of AMI in humans, giving preference to saliva[31].

Joachim Struck and John GF Cleland[32] propose an invention that allows one to diagnose and predict the course of chronic heart failure by determining the level of procalcitonin in biological fluids (blood, serum, plasma, cerebrospinal fluid, saliva, sputum, urine).

Among Russian technical solutions a device for the detection and prediction of heart disease based on the analysis of saliva was found. Special feature of the salivary sensor is that it is combined with a radio frequency identification (RFI) chip, i.e. data from the sensor is transmitted to the reader via radio-frequency electromagnetic field. If necessary, the sensor has access to saliva through a small tube. In case of myocardial infarction danger the signal is triggered in the bracelet. The authors suggest installing this device in tooth-implant or dentures of various modifications[33]

The main disadvantages of this technology is the limited battery life (up to 1 month), as well as of a cartridge. The cartridge inside the implant must be regularly recharged with new reagents for the subsequent analyses, which obliges the patient to visit the dentist on a monthly basis. Limited capacity for medicines draws attention. Furthermore, in this solution there is no possibility of remote monitoring.

To eliminate the disadvantages of the art, the paper describes the developed miniature device for implantation in the oral cavity, which is capable of determining for long time the concentration of protein markers of acute myocardial infarction (main: high-sensitivity troponin, a binding fatty acid protein, myoglobin, troponin T) by the method of immunochemiluminescence analysis of saliva and to inform the patient and medical-preventive institution of vascular catastrophe and/or its predictors.

In the course of work it was required to develop the following scientific and technological basis:

· systemsof the lab-on-chip class, including tanks for storage of reagent used in the chemiluminescence reaction, the system of micro pumps for collection of saliva samples and transferring the reagent from the tank to a storage tank for the reaction (if necessary), the silicon photomultiplier to register luminescence;

· lab-on-chip electronics, ensuring the functioning of the silicon photomultiplier (bias voltage, load), as well as the count of pulses with a duration of not less than 25 ns at a rate not exceeding 10 million pulses per second;

· power supply capable of supporting the functioning of the lab-on-chip for a long time.

The principle of operation of the developed device is the following. The collection of saliva sample is carried out through micro pumps, frequency of sampling varies and can be programmed at any time (from 1 time per 10 minutes to 1 time per month and less). Saliva enters the lab-on-chip, equipped with fluorescent labels. When interacting with the protein-markers these markers begin to emit the illumination of different wavelengths, the intensity of which is registered. The received information is recorded on request in the reader, which is located on the patient's body (for example, in the form of a necklace or a bracelet) and contains the microprocessor. When exceeding the threshold concentration of myocardial infarction predictors, bracelet generates audio and kinesthetic alarms. Using GPS and GPRS/ Wi-Fi information comes in the health care organization and primary care physician.

The biosensor will have minimal dimensions (less than 0.9 cm in diameter), so it can be embedded in a tooth, a denture or false teeth. The device, consisting of a microchip and metal rings, can operate as an antenna. In some cases, power supply or other microchips that allow to increase the computing power of the device can also be embedded.

Microchip sensor will store the result of analysis of saliva (at least the last analysis). The results will be stored in memory of at least 256 bits in size. Each bit will be encoded as at least one biomarker of cardiovascular disease. This creates the opportunity to make at least 26 reactions, if necessary, their number can be increased, which will ensure the timeliness and accuracy of diagnosis.

Identification of cardiac biomarkers in the saliva will occur through chemical reactions, defined by immunochemiluminescent analysis. The reaction of each biomarker definition will take place in a separate cell sensor: 1 is coded as a positive reaction to individual cardiac marker, 0 as negative reaction.

The RFI reader periodically requests the microchip and analyzes whether the results of the analysis are the symptoms of approaching MI. If the result is positive, the bracelet/necklace informs the person about the risk of onset of myocardial infarction. The decision about the signal is taken by a microchip embedded in the RFI card reader. In addition, in the case of identification of high risk of MI, an instruction will appear on the led screen, which can be used either by the patient, or the one who assists him, if the patient is unconscious. The reader will have a remote control function, with which one can query the sensor saliva to carry out an immediate analysis of suspected MI. And will be built in GPS system, which allows to provide a system for detecting the location of the patient, to obtain information about his condition and take appropriate timely actions.

To carry out continuous monitoring and processing of complex indices of levels of biomarkers new software, based on the resulting medical research decision rules, needs to be created. Wireless transmission of data about the change of physiological parameters of the cardiovascular system will be carried out through a radio channel of the microprocessor associated with the implanted biosensor. To do this, a set of interfaces for transmitting digital data to a computer of a medical institution and/or physician must be created. Such network communication system as Wi-Fi and/or GPRS can be used.

The biosensor will be installed in a dental implant is an artificial tooth, bridge or false teeth that are installed in the oral cavity, which uses technology that is used today in prosthetic dentistry to support restorations. A typical implant consists of a titanium screw with a roughened or smooth surface. The convenience of this implant is also justified by the fact that in dentistry, there are no absolute contraindications for the use of dental implants.

RESULTS

Thus, given the current state of the project development the following approaches are proposed to be implement, providing the most effective diagnosis and the possibility of its mass application:

About controlled cardiomarkers:

· leaving attempts to choose a single "ideal" biological indicator;

· confirmation of choice of biological markers and their combinations by proteomic data (the term for the totality of proteins of an organism, produced by cells, tissues or organism in a certain period of time [34]) characteristic of the protein composition of saliva from different representative groups of patients;

· during the analysis implementation of complex personalized account of relevant connections and their dynamics, reflecting the individual characteristics of the patient;

· the combination in the panel of controlled cardiomarkers of direct destruction of cardiac tissue indicators and proteins associated with pathological processes, which are indicators of inflammation, vascular system, determining the risk and potential severity of the disease;

About means of selective detection of cardiomarkers:

· applying immune chromatography approaches for rapid analysis with minimal effort on the side of operator, when the contact of the analytical system (cassettes) with the analyzed probe directly initiates the course of all subsequent interactions and the formation of detectable complexes on the surface of the membrane carrier;

· using the original methodology of two-dimensional immune chromatography for simultaneous high-throughput analysis of large numbers (20-30) of compounds, based on the formation of the analytical zone in the form of an ordered array of tiny dots with immobilized immunoreagents of different specificity;

· due to the use of colorimetric and fluorimetric nanodispersed markers and their complexes with antibodies of optimal size and composition, the limit of detection of target compounds is 10-25-fold lower in comparison with traditional immune chromatography;

About means of test results registration:

· development of two types of detectors for the detection and quantitative evaluation of the composition of nanoparticle markers in the immune chromatography systems - optical and thermometric;

· complete hardware-software complex, taking into account the initial probability of the risks of cardiovascular disease: one of the detectors is simpler and optical detector equipment is cheaper for mass control and specialized high-sensitivity option of thermometric detector for risk groups;

· original methodology of the highly sensitive thermometric detection of the colloidal gold nanoparticles on the membrane carrier;

· using video-digital registration of distribution of the marker on the surface of the carrier, and not the total level of binding to minimize erroneous diagnosis caused by nonspecific interactions and incorrect identification of the binding zones;

About software tools for the processing and recording of results:

· ensuring the transfer of information on test results using mass telecommunication devices;

· maintaining and accounting quantitative analysis and source images of test systems;

· the objectification of decision-making on test results;

· formation of individual databases on the results of periodic tests;

· personification of the decision about the risks of development of cardiovascular system diseases to suit individual cardiomarker profiles.

CONCLUSION

The main expected result of the proposed project will be the creation and characterization of the experimental sample of a portable biosensor system for individual non-invasive monitoring of the cardiovascular system. The principal advantages of the proposed system are:

· easy sampling for cardiomarker control;

· automation of the analysis;

· objectification of the evaluation results;

· dynamics of body condition monitoring according to the periodic testing;

· databases for personalized disease risk assessment;

· low cost of the analytical system, providing the possibility of its mass use.

Therefore, the developed system will help to improve the quality and speed of diagnosis of various cardiovascular diseases and to prevent their progress, evaluate the effectiveness of treatment and timely adjust it, to reduce psychological stress of patients and improve adherence to treatment.

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