BIOMATERIALS

BIOSENSOR FOR LACTATE DEHYDROGENASE ACTIVITY DETERMINATION

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Lactate dehydrogenase (LDH: EC 1.1.1.27) is the most clinically important dehydrogenase of those occurring in human serum. The application of serum LDH is relevant in the diagnosis of myocardial infarction (late detection), hemolytic anemia and prognostic serum biomarker and independent predictive factor of median survival in patients with oncology.

Lactate dehydrogenase catalyzes the reversible reaction: L-lactate + NAD^+ ↔ pyruvate + NADH.

New electrochemical assay for LDH detection in the drop (50 μL) based on of screen-printed carbon modified electrode for NADH determination is proposed. Portable electrochemical sensors can be a good alternative to the other methods for LDH determination due to low cost, simplicity of operation and providing rapid screening for early cancer diagnostics.

This study included the development of high-performance nanocomposite sensor for the NADH determination and optimization of conditions, method and procedure for LDH determining using the obtained chip. Carbon screen-printed electrode (SPE) (DropSens), poly (allylamine hydrochloride) (PAH), NAD^+ and NADH, L-Lactic Dehydrogenase (Sigma-Aldrich), L-lactic acid (AppliChem) was used in this investigation. Activity of LDH samples was controlled by spectrophotometry. The amperometry, cyclic voltammetry (CV) (DropSens 400), chronoamperometry and chronocoulometry were applied for investigation of the sensor electrochemical properties.

As was found the modification of carbon electrode surface by nanocomposite film of DND with PAH resulted in the shift of the optimal value of applied potential close to 0.45 V, and improved the sensitivity of NADH determination three times.

Optimal conditions for the LDH assay were estimated as follow: 0.1 M phosphate buffer solution, pH 7.5, with NAD^+ (10 mM) and L-lactate (80 mM), sample pre-incubation (5 min) at 37 °C. Principal possibility of LDH activity detection in the linear range 1-25 U/L with sensitivity 2.98 μA/U/L was shown by CV method.

APPLICATION OF METAL ENHANCEMENT OF DYE FLUORESCENCE FOR SENSING OF AMYLOID FIBRILS

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Neurodegenerative diseases and amyloidoses are connected with the formation and accumulation of beta-pleated protein aggregates (amyloid fibrils) in different tissues. Fluorescent probes are among the convenient tools for the detection and study of biological macromolecules, particularly proteins and their aggregates. In the case of low concentration of biomolecules, detection sensitivity provided by the probes could be increased by metal enhancement of the dye fluorescence.

Previously we proposed trimethine cyanine dyes as amyloid-sensitive probes for fibrillar aggregates detection. Such amyloid-specific dyes are considered to bind with a fibril via placing into the row formed by beta-pleats of polypeptide chains.

Here we study the efficiency of metal enhancement of the fluorescence intensity for the cyanine dyes complexed with fibrils. For this, the dye and dye-fibril complex were deposited on the polyvinylpyrrolidone (PVP)-covered silver-island films (SIF) on glass. We explored the trimethine cyanine dye D-51 with N-sulfoalkyl substituent giving high fluorescent response in aqueous solution upon binding both to insulin and lysozyme fibrils (up to 70 times).

In order to estimate the level of metal enhancement of the dye fluorescence intensity, we compared intensities of the dye deposited on PVP-covered glass and PVP-covered SIF. The shape of the fluorescence emission spectra of the dye on PVP and SIF-PVP remains the same as in aqueous solution with maximum near 576 nm that corresponds to dye monomers (so dye molecules does not aggregate on surface). Fluorescence intensity of the free dye deposited on Ag-covered surface was demonstrated to be 5.2 times higher as compared to the glass surface without SIF. As for the dye complexed with insulin fibril, the 6-fold metal enhancement of fluorescence was shown, while in the case of fibrillar lysozyme this enhancement was estimated to be little bit lower (about 3.5 times).

Thus deposition of the free dye and its complexes with fibrils on PVP-covered SIF results in the metal surface enhancement of D-51 dye fluorescence intensity and could be used to enhance the signal at low concentrations range. Besides, the origin of the fibrils could affect the intensity of this enhancement.

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MEMBRANE VESICLES PRODUCTION BY KOMBUCHA CULTURE UNDER STRESS CONDITIONS

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Extracellular Membrane Vesicles (EMV) play role in intercellular communication, transportation and protection of microorganisms. Microbial EMVs may be used as scaffolds for smart drug delivery or as vaccines against infectious diseases. It is still unknown whether the size of natural vesicles might be an essential factor that determines how easily they can diffuse in a tissue. The purpose of this study was to know how stress factors affect the size of membrane vesicles in kombucha, known as a probiotic polymicrobial culture.

A wide range of EMV with sizes of 30-1200 nm has been isolated from kombucha culture. Total preparation of extracellular vesicles, including outer membrane vesicles and post-Golgi vesicles showed round-shaped size distributions according transmission electron microscopy (TEM) records. Size estimation of total vesicleome has been done by DLS approach (Distribution Light Scattering, Malvern Instrumental Ltd); values of found peaks at 180 and 220 nm were consistent with TEM measurements. In kombucha culture grown with anorthosite rock, the range of size distribution of EMVs was more narrow (55-1055 nm) than in the control variant (without anorthosite) (35-1080 nm) with the peak of 160 nm. Derived count rate in both variants showed that the total putative number of membrane vesicles produced by kombucha community members in the presence of anorthosite may exceed control (without anorthosite) 20 times. The 16-fold increased amount of released vesicles has been detected after a low-dose high-speed electron irradiation of the culture. It should be emphasized that the average size of membrane vesicles produced by the stressed microbial cells (both mineralized and irradiated) was reduced. The same tendency was observed in kombucha culture specimens exposed outboard of International Space Station in a 25 month space-flight experiment, the returned samples produced smaller average size vesicles (91 nm) in more narrow range of size distribution (68-105 nm). It may be suggested that physical properties of the EMVs may affect the way they mediate intercellular communication.

IN SITU SURFACE FUNCTIONALIZATION OF IRON OXIDE MAGNETIC NANOPARTICLES (IONPS) WITH NATURAL AMINO ACIDS: A POTENTIAL CARRIER FOR BIOMEDICAL APPLICATIONS

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In this work we reported the synthesis of various natural amino acids (AAs) coated iron oxide magnetic nanoparticles (IONPs) under one-pot reaction in an aqueous medium.

Several AAs, which were made up of hydrophilic and hydrophobic groups were selected to study their effects on size, morphology and toxicity of IONPs. Functionalized IONPs were characterized by X-ray diffraction (XRD), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) techniques. Cellular toxicity of IONPs was also investigated on HFF2 and HEK-293 cell lines.

Natural AAs coated IONPs show the possibility of using this nanoparticles in the development of *in vitro* and *in vivo* biomedical fields due to do not possess a toxic effect, good ζ-potential and related small and narrow size distribution.

The results show that so prepared IONPs are biocompatible.

ISOLATION AND EXPANSION OF HUMAN PERIPHERAL BLOOD-DERIVED ENDOTHELIAL PROGENITOR CELLS FOR CLINICAL APPLICATION

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Dysfunctional endothelial cells (vasculature) play a key role in the pathogenesis of a range of human diseases. The endothelium alterations are directly involved in peripheral vascular diseases, stroke, heart diseases, diabetes, chronic kidney failure, tumor growth, venous thrombosis, endothelial dysfunction implicated in tissue injury caused by ischemia-reperfusion, etc. The aim of our study was to assess the expansion rate of endothelial progenitor cells isolated from peripheral blood (PB-EPCs) with the use of two different isolation protocols; to use cultured autologous PB-EPCs for 3D bone tissue engineered equivalent manufacturing for critical size bone defects' restoration.

Peripheral blood specimens (per 20 ml, heparinized, from 20 donors) were obtained by venipuncture. Specimens were seeded in T75 flasks directly (group 1, n = 10) or via PB-MNCs obtaining and seeding with use of Histopaque-1077 (group 2, n = 10). PB-EPCs were cultured in EGM-2MV medium containing growth factors (Lonza) in multigas incubator at 37 °C, saturated humidity, 5% CO_2 and 5% O_2. Colony-forming capacity, phenotype, karyotype and capillary-like structures formation in Matrigel (Corning) have been determined for expanded EPCs cultures.

PB-EPCs cultures were obtained only from 15 donors per 20 (6 in group 1; 9 in group 2). Cultured PB-EPCs possess characteristic morphology and CD31+CD34+CD73+CD105+CD309+D90-CD45-HLA-DR- phenotype. When seeded in Matrigel, all cultures formed capillary-like structures. Colony-forming capacity was higher in Histopaque-1077-processed than in directly seeded blood specimens: 7.8 ± 3.5 (n = 9, group 2) vs 0.6 ± 0.4 (n = 6, group 1) colonies per T75. In group 2 over 24 days 22.6 ± 1.0×10^6 PB-EPCs were obtained vs 7.0 ± 2.0×10^6 over 46 days for group 1. The obtained autologous PB-EPCs cultures have been used for pre-vascularization of 3D bone tissue engineered equivalents which have been applied in patients with combat-related limb trauma aimed to restore the critical size bone defects.

Cell isolation method has a significant effect on the efficiency of PB-EPCs growth rate and expansion. For further broad clinical use of PB-EPCs, the development of humanized xeno-free media is necessitating.

ENDOMETRIAL MULTIPOTENT MESENCHYMAL STROMAL CELLS FOR REGENERATIVE MEDICINE APPLICATION: ISOLATION, CULTURING, MORPHOLOGICAL AND FUNCTIONAL PROPERTIES STUDY

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The endometrium is a unique structure that is able to complete self-renewal over the month cycle, and undergoes these changes over 400 times during women's reproductive life. A significant regenerative potential is due to the presence of stem cells in the endometrium, such as mesenchymal, epithelial and endothelial progenitor cells. Endometrium is a promising object for MSCs isolation for their further use in regenerative medicine and ART. The aim of the study was to isolate, expand and characterize the endometrial MSCs from minimal endometrial biopsy for further endometrium hypoplasia treatment.

The endometrium biopsy (n = 10) was obtained at diagnostic hysteroscopy in the first phase of the menstrual cycle from women with endometrial hypoplasia and previously unsuccessful ART cycles. In all cases, a voluntary written informed consent was obtained from the patients. Endometrial fragments were dissociated by enzymatic treatment for 1 h in 0.05% collagenase IA and 0.05% pronase. The cells were cultivated in DMEM:F12 supplemented with 10% FBS, 2 mM L-glutamine and 1 ng/ml FGF-2 in a multi-gas incubator at 5% CO_2 and 5% O_2. The following assays were done: clonogenic potential (CFU test), phenotype by flow cytometry, karyotype (GTG-banding), directed multilineage differentiation potential and growth factors/cytokines production by Bio-Plex Pro Human 27-plex assay (BioRad).

Primary population of endometrial cells was heterogeneous and contained cells with fibroblast-like and epithelial-like morphology. We used the 3rd passage cells for characterization when the majority of cell population had fibroblast-like morphology. The cells possessed CD49f + CD73 + CD90 + CD105 + CD140a + CD140b + CD146 + CD166 + CD31-CD34-CD45-CD106-CD184-CD227-CD326-HLA-DR-Lgr5-phenotype. They were capable of direct osteogenic, adipogenic and chondrogenic differentiation. The cells showed 35.7±6.2% colony forming efficiency and a tendency to 3D spheroid formation in colonies. The GTG-banding assay confirmed the stability of eMSC karyotype during long-term culturing (up to P10). After 48 h incubation period in serum-free medium eMSC secreted following proteins: cytokines – IL-1ra (74.6 ± 9.5 pg/ml), IL-6 (29.8 ± 8.3 pg/ml), IL-8 (138.5 ± 33.3 pg/ml), IL-10 (9.6 ± 5.5 pg/ml) and IFNγ (55.9 ± 3.8 pg/ml); growth factors – VEGF (92.2±19.8 pg/ml), GM-CSF (133.2 ± 5.1 pg/ml) and FGF-2 (17.8±4.3 pg/ml); chemokines – IP-10 (39.9 ± 3.3 pg/ml) and MCP-1 (41.1 ± 6.7 pg/ml).

Thus, obtained endometrial MSCs meet minimal ISCT criteria for MSCs, such as adherence to plastic in standard culture conditions, expression of typical phenotype markers and ability for the directed differentiation *in vitro*. They also produce a range of cytokines, chemokines and growth factors, which make them a perspective object for the use in the regenerative medicine application, e.g. endometrium hypoplasia and Asherman's syndrome treatment.