ZINC IONS CARRIED BY LIPOSOMAL TRANSPORTER

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ABSTRACT

Microfluidic techniques have been developed intensively in recent years. Electrochemical detectors are preferred in microfluidic systems, whereas liposomes can be used for amplification of the electrochemical signals. The aim of this study was to design a nanodevice for targeted anchoring of liposome as transport device. In this study liposome with encapsulated Zn(II) was prepared. Further, gold nanoparticles were anchored onto the liposome surface allowing binding of SH-modified molecules (DNA). For targeted capturing of the transport device, DNA loops were used. DNA loops were represented by paramagnetic microparticles with oligodT chain, on which a connecting DNA was bound. Capturing of transport device was subsequently done by hybridization to the loop. For detection of Zn(II) encapsulated in liposome a microfluidic system was used. The study succeeded in demonstrating that liposome is suitable for the transport of Zn(II) and nucleic acids. Such modified liposome may be used as a transporter for targeted binding using DNA anchor system.

MATERIALS AND METHODS

Chemicals

Cholesterol, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt, chloroform, Zn(NO3)2•6H2O, sodium citrate, HAuCl4•3H2O and water were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise. Hydrogenated phosphatidylcholine from soybean was a gift from Lipoid GMBH (Ludwigshafen,Germany). Magnetic particles (MPs) oligodT were purchased from Invitrogen (Oslo, Norway).

Preparation of gold nanoparticles (AuNPs), liposome film and liposome filled with Zn(II)

Gold nanoparticles were prepared by citrate method at room temperature according to Kimmling et al. and Polte et al. (Kimling, et al. 2006; Polte, et al. 2010) .Liposome was prepared according to published method (Kunjachan, et al. 2012) with modification. Solutions containing 0.25, 0.5, 1 and 2 mg of Zn(II) were added to lipid film (20 mg). Samples were homogenized with Ultra – Turrax T8 (IKA Werke GMBH, Staufen, Germany) for 10 min. The homogenized mixtures were then heated and shaken for 15 min at 60 °C in at Thermomixer Comfort (Eppendorf, Germany). The samples were then washed several times with MiliQ water on Amicon 3k (Merck Millipore, Merck KgaA, Darmstadt, Germany).

Modification of liposome surface by gold nanoparticles and thiolated oligodeoxynucleotides Modification of liposomes by gold was done according to Bhuvana et al. [38]. The sequence of ODN-SH was 5´ CTGGAATGCAGA (SH) 3´ (Sigma Aldrich, St.Louis, MO, USA). This probe was complementary to target oligodeoxynucleotide (ODN) probe (5´ TCTGCATTCCAG AAAAA, Sigma Aldrich, St.Louis, MO, USA), which was anchored to MPs.

INTRODUCTION

Microfluidic techniques and devices have rapidly developed in recent years due to lower reagent consumption, and faster and more sensitive analysis (Kitazoe, et al. 2012; Kwakye, et al. 2006). Numerous diagnostic applications of microfluidic techniques associated with genes, proteins and cells have been described in accordance to the advantages related with miniaturization, automation, sensitivity and specificity. Electrochemical analysis is one of the most sensitive methods for determination of inorganic and also organic substances. To enhance electrochemical signal, nanoparticles and magnetic microparticles can be used (Li, et al. 2012; Nam, et al. 2003). Application of liposomes, member of nanoparticle based materials, is advantageous for this purpose. In this case, electroactive reagent is controlled, released from liposome and determined. Numerous sensitive voltammetric methods is based on the presence of electroactive compound in the cavity of the liposome.

Isolation of nanogold modified liposomes using magnetic microparticles and its detection

For isolation of nanogold modified liposomes was used MPs. A first 5´ TCTGCATTCCAG AAAAA was hybridized to MPs. The next step was second hybridization with 10 µl of lipoZn-AuNPs-ODN-SH. Electrode system was designed and fabricated as a disposable planar three-electrode sensor in LabSensNano laboratories of Brno University of Technology, Czech Republic. Working electrode was screen-printed using special carbon paste for electrodes of electrochemical sensors (DuPont BQ221) from DuPont Company (DuPont, Wilmington, USA) and reference electrode was screen-printed using special polymer Ag/AgCl paste (DuPont 5874, Ag:AgCl ratio 65:35). Other experimental details of the electrode fabrication can be found in Chudobova et al. (Chudobova, et al. 2013). Printed three-electrode system was coupled with the flow cell according to the following scheme (Fig.1 A). The sample was pumped using peristaltic pump (Amersham Biosciences, Uppsala, Sweden). Change of oxidative signal was recorded with potentiostat PGSTAT 101 (Methrom, Herisau, Switzerland) and the results were evaluated by the Software NOVA 1.8. All measurement was carried out by method of difference pulse voltammetry (DPV).

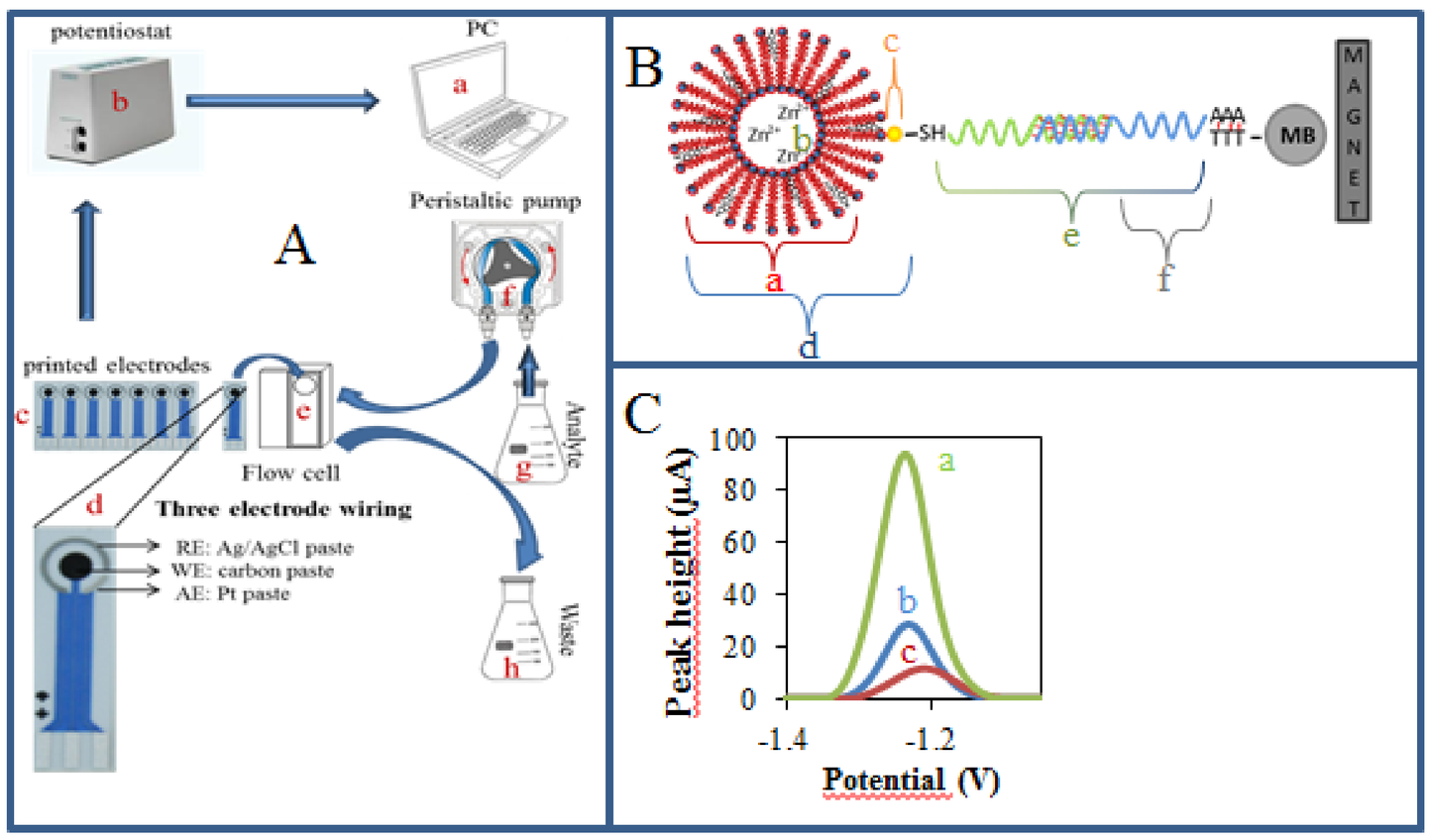

Fig.1
(A) Scheme of microfluidic devide. a) PC, b) potentiostat, c) screen-printed electrode, d) three electrode wiring, e) flow cell, f) peristaltic pump, g) analyte, h) waste.
(B) LipoZn-AuNPs-SH-ODN-ODN-MB-complex a) lipoZn, b) Zn2+, c) gold nanoparticles, d) AuNPs-lipoZn, e) oligonucleotides bond to gold nanoparticles using SH groups, f) The created lipoZn-AuNPs-SH-ODN transporter was isolated using magnetisable particles (MB) with immobilized complementary oligonucleotides.
(C) The decreasing trend in the zinc reduction signal depending on the decreasing concentration of oligonucleotide bound to lipoZn. a) 200 µM Zn(II), b) 60 µM Zn(II), c) 10 µM Zn(II).

CONCLUSION

In this study, nanodevice for targeted delivery was suggested. As the transporter zinc containing liposome, which was modified with gold nanoparticles, which served to establish the oligonucleotide binding, was used. This nanodevice was anchored to a magnetisable particle with a complementary oligonucleotide. Functionality of the nanodevices was proven through electrochemically determination of Zn(II) enclosed in liposomes. Designed nanotransporter may also be used for targeted and controlled transport to specific tissues (tumour) and to prevent the multiplication of viruses (Miyako, et al. 2012; Swaminathan and Ehrhardt 2012; van Kouwenhove, et al. 2011). Besides this, magnetic nanoparticle clusters, which can, under the influence of an external magnet, target both the tumour and its microenvironment, may be also enclosed (Mikhaylov, et al. 2011).

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