Biomaterials 67 (2015) 323–334

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Persistent luminescence nanoprobe for biosensing and lifetime imaging of cell apoptosis via time-resolved fluorescence resonance energy transfer

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ARTICLE INFO

Article history:
Received 4 April 2015
Received in revised form
17 July 2015
Accepted 20 July 2015
Available online 21 July 2015

Keywords:
Biosensor
Fluorescent probe
Caspase
Image analysis
Persistent luminescence nanoparticles

ABSTRACT

Time-resolved fluorescence technique can reduce the short-lived background luminescence and auto-fluorescence interference from cells and tissues by exerting the delay time between pulsed excitation light and signal acquisition. Here, we prepared persistent luminescence nanoparticles (PLNPs) to design a universal time-resolved fluorescence resonance energy transfer (TR-FRET) platform for biosensing, lifetime imaging of cell apoptosis and *in situ* lifetime quantification of intracellular caspase-3. Three kinds of PLNPs-based nanoprobes are assembled by covalently binding dye-labeled peptides or DNA to carboxyl-functionalized PLNPs for the efficient detection of caspase-3, microRNA and protein. The peptides-functionalized nanoprobe is also employed for fluorescence lifetime imaging to monitor cell apoptosis, which shows a dependence of cellular fluorescence lifetime on caspase-3 activity and thus leads to an *in situ* quantification method. This work provides a proof-of-concept for PLNPs-based TR-FRET analysis and demonstrates its potential in exploring dynamical information of life process.

1. Introduction

Time-resolved fluorescence (TRF), which can reduce the short-lived background luminescence and auto-fluorescence interference from cells and tissues by exerting the delay time between pulsed excitation light and signal acquisition, provides a background-free approach in life science [1–3]. In particular, coupling with the advantage of fluorescence resonance energy transfer (FRET), the time-resolved fluorescence resonance energy transfer (TR-FRET) assay offers high sensitivity in comparison with conventional FRET [4]. TR-FRET assays have been extensively applied in detection and imaging of biomolecules by employing organic fluorophores [5], fluorescent proteins [6,7], polymer materials [8], and nanoparticles as luminescent probes [9–17]. However, most of the reported TRF probes suffer from the vulnerability to environmental media, and the interference of multi-emission peaks, thus limit their further applications.

Persistent luminescence nanoparticles (PLNPs, also called long-lasting afterglow nanoparticles) can store the excitation energy and then slowly release it by a photonic emission [18–20]. The persistent luminescence can last several hours after removal of excitation resource, providing many conveniences in optical assay and imaging [21–23]. Scherman's group pioneered a series of applications of near-infrared PLNPs in real-time optical imaging of small animals without the need of any external illumination source [24–27]. Recently, several PLNPs probes have been developed for biosensing based on their inhibition of FRET or electron transfer to the quencher [28–31]. However, the attempt to employ PLNPs for TR-FRET analysis and lifetime imaging has not been made yet. Obviously, the energy transfer from the afterglow of PLNPs to short-lived organic dyes can apparently lengthen the fluorescence lifetime of acceptors, which greatly meets the TRF requirement. Thus PLNPs as a class of long-lifetime probes are very powerful for TR-FRET biosensing and time-resolved lifetime imaging of cellular biomolecules without background interference.

Here, we synthesized PLNPs probes of highly efficient persistent luminescence to design a universal TR-FRET platform for biosensing by setting appropriate delay time and gate time (Scheme 1a). The luminescence of fluorescein isothiocyanate (FITC) is effectively suppressed due to its shorter lifetime than delay time, which represents an interval between pulsed excitation light and signal acquisition. The long-lived luminescence of PLNPs is

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http://dx.doi.org/10.1016/j.biomaterials.2015.07.037
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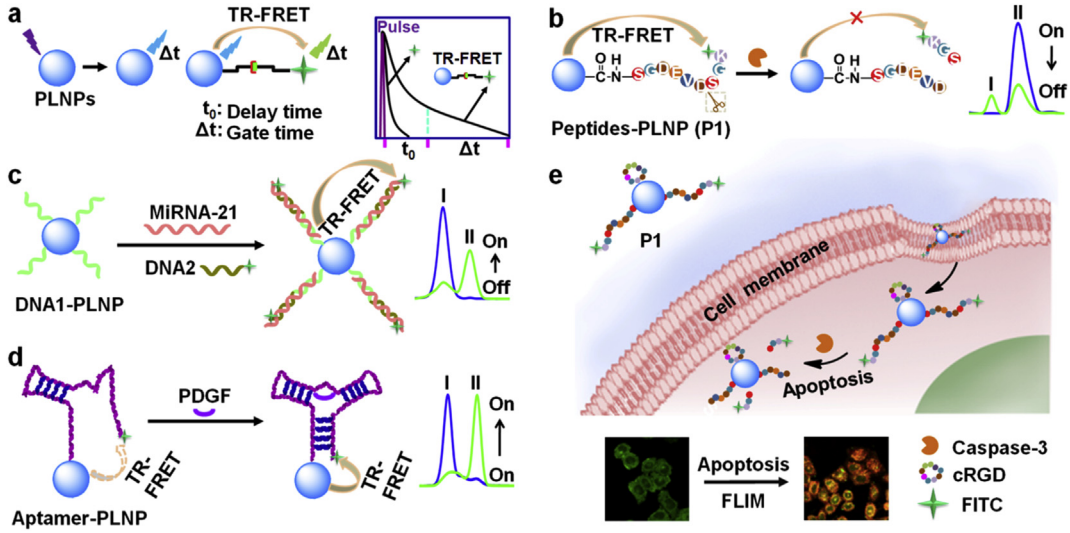

Scheme 1. Schematic illustration for (a) PLNPs-based TR-FRET principle, (b–d) TR-FRET detection strategies of caspase-3 protease, miRNA-21 and PDGF protein by using caspase-specific peptide & cRGD-, DNA1-and aptamer-functionalized PLNPs probes, respectively, and (e) lifetime imaging of intracellular caspase-3 activity using **P1** during cell apoptosis by FLIM. I and II represent TRF signals of PLNPs donor at 468 nm and FITC acceptor at 520 nm, respectively. Blue and green lines represent the TRF curves before and after the response of the corresponding target, respectively.

captured in the gate time designating the time for signal acquisition. Once TR-FRET occurs from PLNPs to FITC, the signal of FITC is acquired in the gate time. Using caspase-3 (related to cell apoptosis) [32–34], microRNA-21 (miRNA-21) and platelet-derived growth factor (PDGF) as the model targets, three recognition nanoprobes are prepared by covalently assembling cyclic arginine–glycine–aspartic acid peptide (cRGD) and FITC-labeled substrate peptide, DNA or aptamer on PLNPs for "on–off", "off–on" and "on–on" TR-FRET detection of enzyme activity, nucleic acids and proteins, respectively. The "on–off" method results from the enzymatic cleavage reaction to release the FITC from the peptide and thus inhibits the FRET from PLNPs to FITC (Scheme 1b). The "off–on" switch is generated by the sandwich hybridization among DNA1, miRNA-21 and DNA2 to achieve the FRET from PLNPs to FITC (Scheme 1c). After the recognition of the FITC-labeled aptamer to target protein, their structure change brings the FITC closer to PLNPs and thus produces an "on–on" TR-FRET strategy (Scheme 1d).

Indeed, many elegant fluorescent probes including graphene oxide–peptide conjugate [35], aggregation light-up tetraphenylethene fluorogen [36], and a genetically encoded fluorescent protein [37], have been developed for detection of caspase activity in live cells [38,39]. However, these proposed methods were usually susceptible to the short-lived auto-fluorescence interference from cells and tissues. Considering that fluorescence lifetime of the probe depends on the recognition to the target, the "on–off" TR-FRET strategy can be further used for lifetime imaging of caspase-3 activity in live cells via fluorescence lifetime imaging microscopy (FLIM) (Scheme 1e), which is an imaging technique based on the differences in the exponential decay rate of the fluorescence from a fluorophore, rather than its intensity [40]. More importantly, this work presents the dependence of cellular lifetime of PLNPs on caspase-3 activity, which produces a novel protocol for *in situ* quantification of caspase-3 activity in single cell based on fluorescence lifetime, and thus provides a robust approach for dynamic evaluation of caspase-dependent cell apoptosis.

2. Materials and methods

2.1. Materials and reagents

Human caspase-3 (active) recombinant protein and cell lysis buffer were purchased from millipore (Billerica, MA, USA). Platelet-derived growth factor (PDGF)-BB was purchased from R&D System (Minneapolis, MN, USA) and was dissolved in 4 mM HCl containing 0.1% BSA. Caspase-3 inhibitor (Ac-DEVD-CHO), caspase-3 fluorogenic assay kit, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), HeLa cells and Annexin V-FITC/propidium iodide (PI) cell apoptosis kit were purchased from Nanjing Keygen Biotechnology Co. Ltd. (Nanjing, China). DNA hybridization buffer (pH 7.4) contained 10 mM Tris–HCl, 1 mM EDTA, 50 mM NaCl and 10 mM $MgCl_2$. Caspase assay buffer contained 40 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM EDTA, 10% sucrose, 0.1% CHAPS and 10 mM DTT. Phosphate buffer saline (PBS, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4 and 1.41 mM KH_2PO_4. Human caspase-3 (active) recombinant protein was reconstituted to 1.0 unit μL^{-1} with PBS containing 15% glycerol. All other reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water ($\geq$18 MΩ, Milli-Q, Millipore).

Caspase-3 specific peptide SGDEVDSGK-FITC, control peptide SGDEVGSGK-FITC, cRGD and DNA oligonucleotides were synthesized and purified by Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China). RNA oligonucleotides were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The DNA and RNA oligonucleotides included:

DNA1, 5′-NH_2 TCA ACA TCA GT-3′; (note: DNA1 was functionalized with FITC at 3′ for FRET parameter measurements.)

DNA2, 5′-NH_2 CTG ATA AGC TA (FITC)-3′;

PDGF-BB aptamer, 5′-NH_2 CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG (FITC)-3′;

PDGF-BB control DNA, 5′-NH_2 CAG CGT ACG GCA CGT ACC GAT TCA CCA TGA AGC TG (FITC)-3′;

miRNA-21, UAGCUUAUCAGACUGAUGUUGA;

single-base mismatched miRNA-21, UAGCUUAUCAGACUGAU