Supporting Information

On-chip Selective Capture of Cancer Cells and Ultrasensitive Fluorescence Detection of Survivin mRNA in Single Living Cell

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1 Table S1. DNA Sequence Used in This Work

TableS1 DNA sequences	
DNA	Sequences (5′ to 3′)
antisense oligonucleotide for cell viability and apoptosis detection (S1)	CCCAGCCTTCCAGCTCCTTG
antisense oligonucleotide labeled with FITC (F-S1)	FITC -CCCAGCCTTCCAGCTCCTTG
chemical synthesized target survivin strand (S2)	CAAGGAGCTGGAAGGCTGGG
random oligonucleotide labeled with FITC (F-S3)	FITC -TCTCCCCAGGACGCTCTCCT

2 Characterization of NGO-PEG

In order to avoid NGO aggregated in solutions rich in salts or proteins such as cell medium and serum, we conjugated PEG-NH_2 to the carboxylic acid groups on NGO by the previously reported method[1]. The chemical changes occurring upon treatment of NGO with PEG-NH_2 can be observed by FT-IR spectroscopy as both NGO and NGO-PEG display characteristic IR spectra (Figure S1 A). The most characteristic features in the FT-IR spectrum of NGO are the adsorption bands corresponding to the existence of -OH (~3430 cm^{-1}), C=O (1739 cm^{-1}), C=C (1600 cm^{-1}) and C-O (1054 cm^{-1})[2] functional groups. The FT-IR spectrum of NGO-PEG samples involved strong -CH_2- (2887 cm^{-1}) vibrations due to PEG-NH_2 chain, and amide-carbonyl (-NH-CO-) stretching vibration (1647 cm^{-1}), suggesting that PEG-NH_2 molecules have been successfully grafted onto NGO sheets.

Atomic force microscopy (AFM) observed NGO sheets were mostly single layered (topographic height ~1.0 nm). Owing to sonication steps after PEG molecules grafted onto NGO sheets, the complex can be divided into ultra-small size, the sizes of NGO-PEG sheets were mostly lower than 60 nm according to atomic force microscopy (AFM) characterization and the average thickness of NGO-PEG sheets were measured to be 1.2 nm(Figure S1 B and C). Dynamic light scattering (DLS) measurements also showed the size distribution of the NGO-PEG (Figure S1 D), was mostly within a narrow range from 20 nm to 60 nm. This will increase the transfer efficiency of NGO.

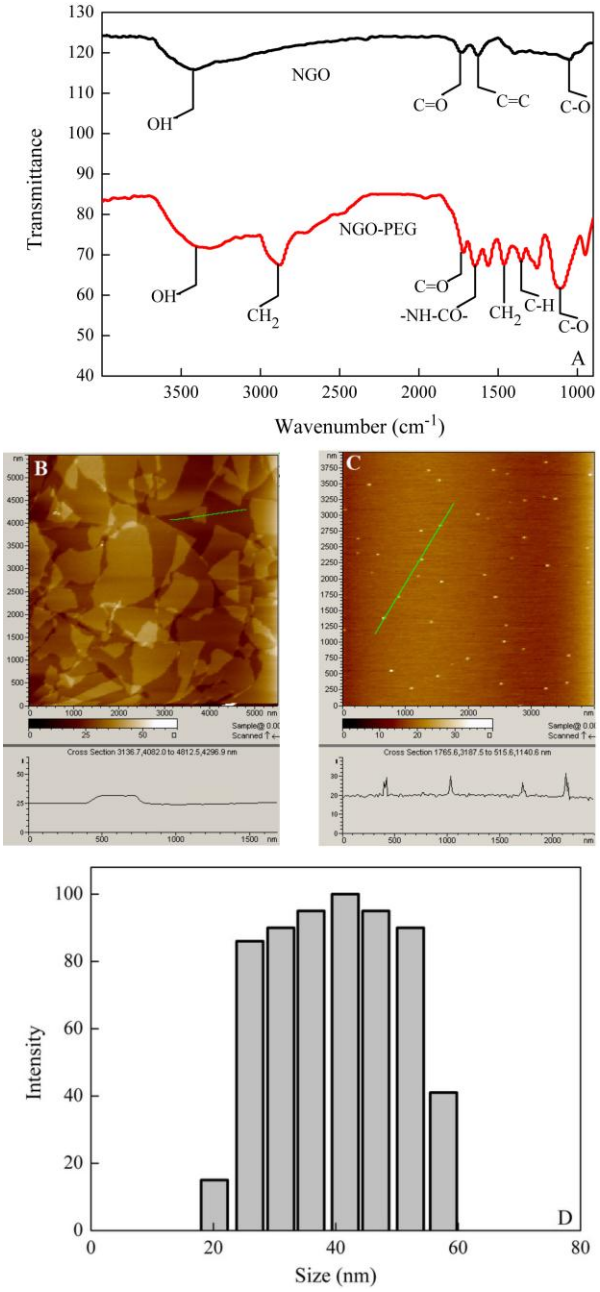

Figure S1 Infrared (IR) spectra of NGO and NGO-PEG(A), AFM characterization of NGO and NGO-PEG (B and C), DLS characterization of NGO-PEG (D).

3 Characterization of the assembly process of NGO-PEG/F-S1.

The assembly process of NGO-PEG/F-S1 was characterized by Zeta potential analysis. The NGO exhibited a negative ζ potential of -44.5 mV, as this material contains hydroxyl and carboxylic acid groups. After PEG molecules grafted onto NGO sheets, the NGO-PEG displayed a ζ potential of -5.32 mV (Figure S2), the decrease is just attributed to a large number of carboxylic acid groups on the NGO sheets combined to PEG bis (amine) during the PEGylation process. The

obtained NGO-PEG/F-S1 nanocomplex showed a ζ potential of -20.6 mV might be due to the adsorption of F-S1 contained more negative phosphate groups onto the surface of the NGO-PEG which is facilitated by non-covalent π-π stacking interactions involving both purine and pyrimidine bases of the DNA.[3]

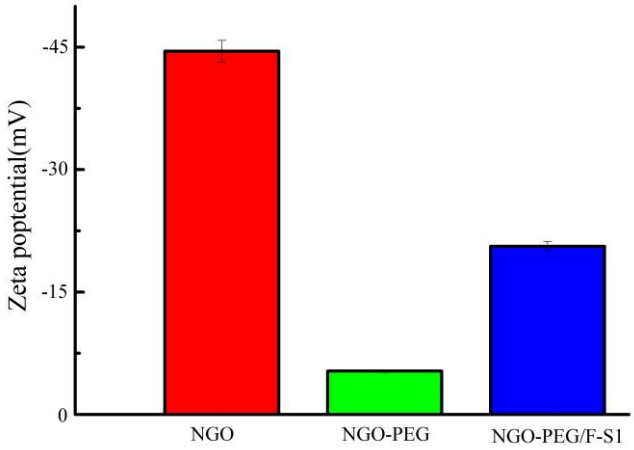

Figure S2 Zeta potentials of NGO, NGO-PEG, and NGO-PEG/F-S1.

4 Cell concentration effects.

As the design can be specific to their target cells on anti-PSCA modified microchannel device, further experiments have been done to analyse the influence of cell concentration on cell capture efficiency. Cell concentrations varied from 100 000 to 1 000 000 cell/mL, and the cell densities increased almost linearly with the increase of the cell concentrations (Figure S3A). Further, the percentage of cells captured showed no obvious change within this concentration range (Figure S3B). This result suggests that the device has a large dynamic range to capture cells for analysis and that the capture efficiency in the device is independent of cell concentration within this range.

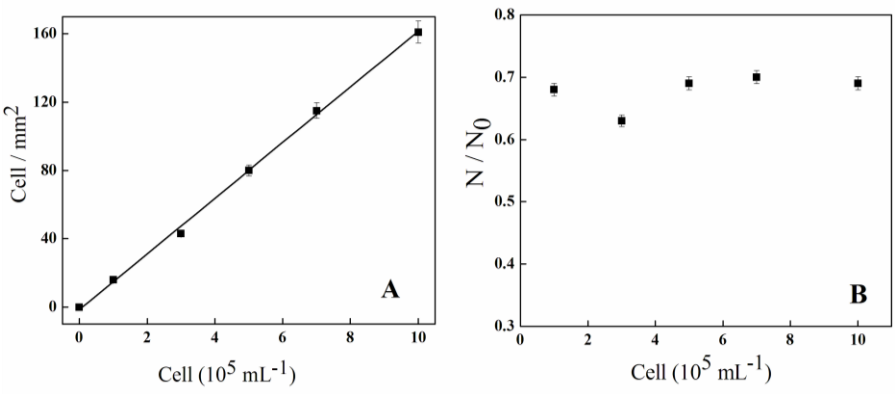

Figure S3. Effect of Cell concentration on capture intensities (A) and capture percentage (B).

5 Cells viability assay

Some works had revealed that NGO as a sensing platform was not intrinsically toxic and can be applied to living cells. To further investigate biocompatibility and toxicity of NGO-PEG and NGO-PEG/S1, we compared the toxicities (MTT tests) of NGO-PEG alone, or NGO-PEG/S1 composites (Figure S4) towards PC-3 cells. The results revealed that NGO-PEG exhibited negligible effects on the growth in PC-3 cells, and the advantages of NGO-PEG shown here make it a robust candidate for use in cellular detection.

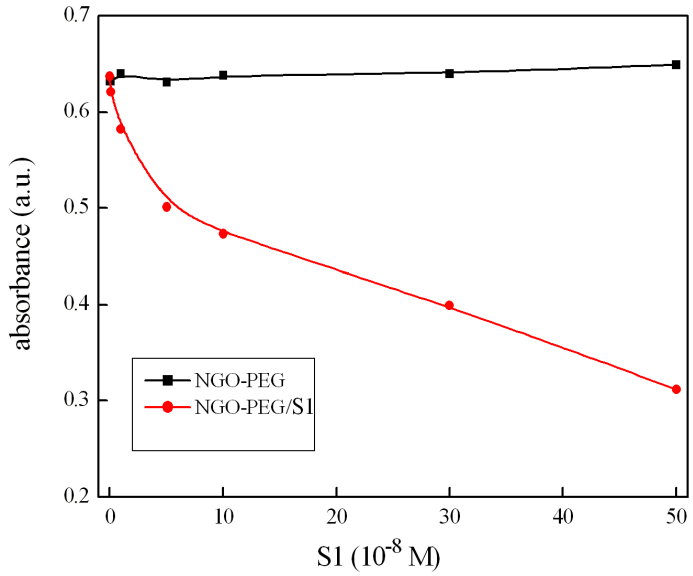

Figure S4 Cytotoxicity detection of NGO-PEG and NGO-PEG /S1 nanocomplex.

6 Cells apoptosis assay

In order to analysis the level of apoptosis induced by NGO-PEG/S1, PC-3 cells treated with a well-known apoptosis inducer paclitaxel as a positive control experiment (Figure S5). Target cells treated with paclitaxel for different times were further analyzed by flow cytometry analysis. As shown in Figure S5, the percentage of early apoptotic cells increased from 3.84% at 3 h (Figure S6A) to 28.3% at 24 h (Figure S5D), so the percentage of early stage apoptotic cells increased with the increasing incubation time of paclitaxel. When cells were incubated with paclitaxel for 12h, the percentage of early stage apoptotic cells was up to 11.2%, which reached the similar apoptosis level of target cells induced by NGO-PEG/S1 composites. The results reveal the NGO-PEG/S1 composites hold great promise in early diagnosis for cancers.

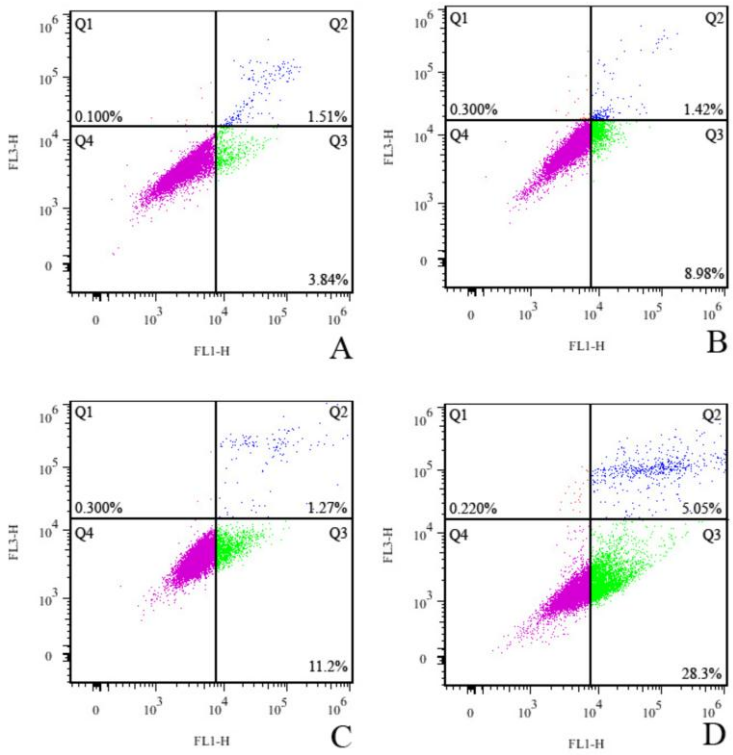

Figure S5 Flow cytometric analysis of apoptosis for cells induced by paclitaxel with different incubation time: (A) 3 h, (B) 6 h, (C) 12 h, and (D) 24 h. FL1-H represents AnnexinV-FITC signal and FL3-H represents propidium iodide (PI) signal. Region Q4 includes live cells (AnnexinV-FITC－/ PI－), region Q3 represents early apoptotic cells (AnnexinV-FITC＋/ PI－), region Q2 contains late apoptotic cells (AnnexinV-FITC＋/ PI＋), while quadrant Q1 shows necrosis cells (AnnexinV-FITC－/ PI＋).

References

(1) Z. Liu; J. T. Robinson; X. M. Sun; H. J. Dai, *J. Am. Chem. Soc.* 2008, **130**, 10876-10877.

(2) X. M. Sun; Z. Liu; K. Welsher; J. T. Robinson; A. Goodwin; S. Zaric; H. J. Dai, *Nano Res.* 2008, **1**, 203-212.

(3) A. J. Patil; J. L. Vickery; T. B. Scott; S. Mann, *Adv. Mater.* 2009, **21**, 3159-3164.