Supporting Information

Peptide-Conjugated PEGylated PAMAM as a High Affinitive Nanocarrier towards HER2-overexpressing Cancer Cells

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Methods
The peptide was synthesized by solid phase method in our lab (Fig. S1). The purity was validated by a single peak in HPLC and matrix assisted laser desorption ionization time-of-light mass spectrometry (MALDI-TOF) was used for determining the mass of peptide H6 (1121.1 m/z) (Fig. S2).

![Peptide H6 configuration](image)

Fig. S1: Peptide H6 configuration (M.W. 1119.57).

The synthesized peptides were analyzed and purified by using a waters HPLC system (2489 UV/vis Detector) on a C18 5μm column (250 mm × 4.6 mm). Purification of peptides was characterized by MALDI-TOF MS (Bruker Daltonics) equipped with a nitrogen laser with reflectron and positive-ion modes. The laser power energy was adjusted between 0% and 100% to provide laser pulse energy between 0 and 100 μJ per pulse (Fig. S2).

![Molecular mass and purification performance](image)

Fig. S2: Molecular mass of final synthesized peptide was confirmed with MALDI-TOF-MS (left) and the purification performed by HPLC (right).

1H-NMR spectroscopy is shown in Fig. S3 with 3 profile peaks (up: PAMAM G4, middle: peptide H6 and bottom: synthesized nanocarrier) was done to evaluate the success of synthesis and the ratio of PEG and peptide on the surface of PAMAM. PAMAM full generation has multiple peaks between 2.22 and 2.67 ppm which come from the methylene protons of its branching units (the top and the bottom). The typical methylene protons of PEG at 3.51 ppm was used for calculation of PEG ratio (bottom). The typical peaks of benzene groups at 6.62 and 6.91 ppm was used for calculation of peptide ratio (middle and bottom). The disappearance of the characteristic peak of the Mal group in PEG at 6.7 ppm in the NMR spectrum of PMAM–PEG–H6 indicated that the Mal group had successfully reacted with the amino group of peptide H6 (bottom).
Zeta potential measurement by DLS showed the charge values of PAMAM (Fig. S4 A) and PAMAM–PEG–H6 (Fig. 2 C) were 3.60 mV and 2.98 mV respectively, indicating the slightly positive charge of PAMAM and no significant change in the charge values of PAMAM due to attachment with peptide H6.
Fig. S4: The surface zeta potential of PAMAM (A) and the size distribution histogram of PAMAM–PEG–Peptide H6 (B). The hydrodynamic size of the nanocarriers was around 7 nm.

PAMAM–PEG–Peptide H6 was immobilized in three replicates on the SIP–COOH surface gold coated chip by the amino group of the peptide at the concentration of 100μM (ESI Fig.5). To complete the binding of peptide on the surface, the chip was incubated in 4 °C for 24 hours in a humid plate. After the immobilization, the SPRi chip was blocked using 1 M ethanolamine. The curves of association and dissociation rates were recorded by real-time binding data on the chip image and analyzed using a PlexArray HT system. SPRi analysis procedure was followed by running of PBST buffer and five concentration of the protein (5.56×10⁻⁹M, 2.28×10⁻⁸M, 1.14×10⁻⁸M, 5.70×10⁻⁹M and 2.85×10⁻⁹M).

Fig. S5: Schematic view of SPRi in combination with Peptide H6 microarray to detect HER2 protein.

Encapsulation of DOX into the interior of dendrimers was carried out by using an equilibrium method. 2 mg of DOX hydrochloride was dissolved in 2 mL PBS and mixed with 20 mg of PAMAM–PEG–H6. Then the solution was equally divided to two parts and each one diluted to reach 5 mL separately. One part was made up with DMEM medium for MTT assay and the other part with PBS for SPRi assay and stirred vigorously in dark for 24 hours. The unincorporated DOX was removed by a centrifuge at 10,000 rpm by 3 minutes and the precipitates related to the DOX-encapsulated polymers were collected and redissolved in related solutions with the same volumes. The encapsulation percentage of DOX loaded in nanocarriers was characterized using spectrofluorophotometer (Shimadzu RF-5301PC) at 460 nm that showed the same intensities for both matrixes. Native DOX with determined concentration was utilized as control (Fig. S6).
Cumulative DOX release from the nanocarriers as a function of time at pH 5.5 and 7.4 showed a relatively fast rate in pH 5.5 compared with pH 7.4 in 24 hours. More than 95% of DOX were released from the nanocarriers at pH 5.5. This phenomenon might be attributed to the inherent acidic property of PAMAM and repulsion with the acidic solvent (ESI Fig. 7).

Fig. S7: Time-dependent release diagram of DOX from PAMAM–PEG–H6/DOX in PBS buffer (pH 7.4) and acetate buffer (pH 5.5).