Scalable Nanomanufacturing of Cyclic Peptide-based Nanorobots for *In Vivo* Sensing

NSF Grant 1300167. PD: Dr. Khershed P. Cooper

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I. Research Area and Field of Impact

Nanomanufacturing. Impact to the fields of nanorobot and nanomedicine.

II. Project Objective

This grant provides funding for the development of a scalable nanomanufacturing platform to fabricate cyclic peptide-based nanorobots for biomedical applications, such as *in vivo* sensing, disease diagnosis, and targeted drug delivery. The nanomanufacturing platform will be used to assemble various types of cyclic peptide-based nanotubes conjugated with DNA-based aptamers. The core body of the nanorobots will be formed by self-assembling individual cyclic peptide subunits under controlled reaction conditions. After the core body has been formed, aptamers will be conjugated to the open ends of the nanotubes, and serve as a sensing and actuating components. Upon binding of a target biomarker to the aptamers, a conformational change takes place allowing the nanorobots to release their payload. In an effort to optimize the design, a library of cyclic peptides with varying diameters, controlled by the number of peptide subunits will be fabricated. To further demonstrate the modularity of the approach, aptamers for a variety of biomarkers related to specific diseases will be conjugated to the nanorobots and tested. To scale-up the fabrication process, phase equilibrium method, self-assembly in bulk solution, and layer-by-layer assembly method will be examined. After prototype fabrication, the nanomanufacturing process will be further optimized in terms of reliability, yield and manufacturing efficiency.

If successful, the results of this research will lead to manufacturing of cyclic peptide-based nanorobots for *in vivo* sensing, disease diagnosis and targeted drug delivery. The primary goal of this research is to determine the fundamental engineering principles related to scalable nanomanufacturing of self-assembled bio-molecules. The principles learned through this research will be applicable to various nanomanufacturing processes with self-assembly as a key step for bottom-up manufacturing. Upon development and optimization of the fabrication process, the medical community will benefit from the nanorobots.

III. Methods

A cyclic peptide nanotubes (CPNTs) used for antitumor drug delivery *in vitro* has been investigated in our group [1]. For such a purpose, a model cyclic peptide *cyclo*-(-L-Gln-D-Ala-L-Glu-D-Ala-L-Gln-D-Ala-L-Cys-D-Ala-) was synthesized using solid peptide synthesizer (ABI 433A, Applied Biosystems, USA). The suspension (~1 mM) of peptide subunit was dissolved by adding NaOH drop-wise to a pH of 12.0, and the effect of pH on the formation of CPNTs was examined by phase equilibrium method [2-3]. The particle sizes and zeta potentials of the resulting nanotubes at different pH ranging from 12.0 to 2.0 were detected at 25°C using a Malvern Zetasizer, NANO ZS (Malvern Instruments Limited, UK). The morphology of the CPNTs was

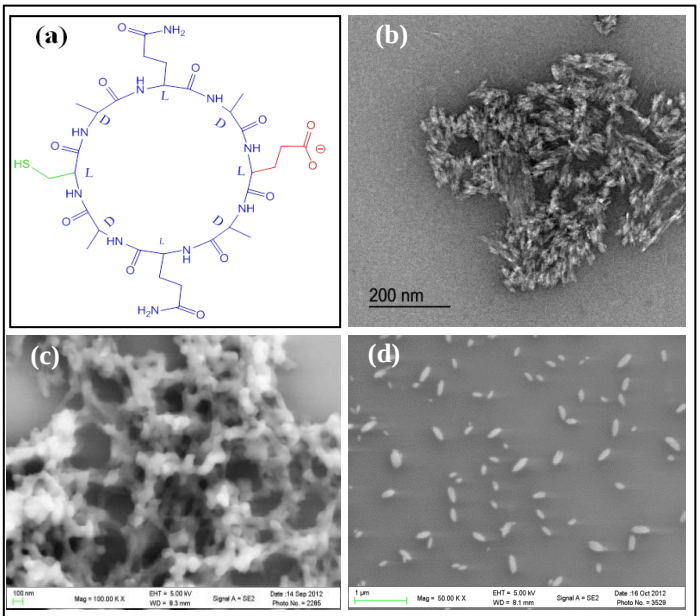

Fig. 1 Preparation and characterization of the cyclic peptide nanotube (CPNT) bundles, DOX-loaded CPNTs, and PEG-modified DOX-loaded CPNTs. (a) Structure of the cyclic octapeptide; (b) TEM images of the CPNT bundles prepared at pH 2.0; (c) The SEM images of DOX-loaded CPNT bundles in aggregates at pH=7.0; (d) The SEM images of PEG-modified DOX-loaded CPNT bundles.

analyzed using transmission electron microscopy (Zeiss LIBRA 200 FE, Germany) at 80 kV. For the purpose of drug delivery to tumor cells, doxorubicin (DOX) was used as a model antitumor drug. The DOX-loaded CPNT bundles (CPNT/DOX) and PEG-modified CPNT/DOX bundles were prepared [1], and the morphologies of both CPNT bundles were examined using SEM (LEO 1525, high resolution FE-SEM system, Germany).

Upon successful preparation of the drug-loaded CPNTs, the cytotoxicity (MTT assay [4-5]), cellular uptake (flow cytometry [6]), and intracellular distribution (confocal imaging [6]) of the of PEG-modified CPNT/DOX bundles on human breast cancer cell lines, MCF-7 and MCF-7/ADR were examined. To further elucidate the underlying mechanism of the elevated DOX uptake by the CPNTs, a P-gp function analysis were conducted in MCF-7/ADR cell line using a specific substrate of P-gp (Hoechst 33342) as an index of P-gp activity[7].

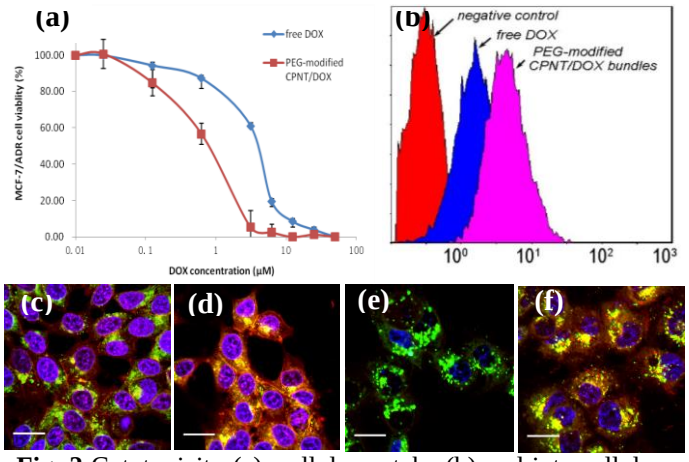

Fig. 2 Cytotoxicity (a), cellular uptake (b) and intracellular distribution (c-f) of PEG-modified DOX-loaded CPNT bundles. (a) cytotoxicity against MCF-7/ADR cells; (b) Cellular uptake by MCF-7/ADR cells; (c-d) intracellular distribution of free DOX(c) and the bundles (d) in MCF-7 cells. (e-f) intracellular distribution in free DOX (e) and the bundles (f) in MCF-7/ADR cells.

IV. Results

In this study, a new eight-residue cyclic peptide that contains a cysteine residue and a glutamic acid residue at the symmetric positions on the peptide unit was used to form the CPNTs (**Fig.1a**). A pH-driven self-assembly of CPNT bundles was elucidated. Using the phase equilibrium method, the CPNT bundles with a diameter of ~10 nm and a length of ~50-80 nm, self-assembled in a micro-scaled aggregate, were first prepared (**Fig.1b**). In order to explore the potential application of these supramolecular structures as a nanocarrier for cancer therapy, PEG-modified DOX-loaded CPNT bundles were fabricated, and showed a high drug encapsulation ratio and good dispersion with a diameter of ~50nm and a length of ~200-300nm (**Fig.1c-d**). More importantly, compared to free DOX, the PEG-modified DOX-loaded CPNT

bundles demonstrated higher cytotoxicity (**Fig. 2a**), increased DOX uptake (**Fig. 2b**), and altered intracellular distribution of DOX in human breast cancer MCF-7/ADR cells *in vitro* (**Fig. 2c-f**). An enhanced inhibition of P-gp activity was observed in MCF-7/ADR cells by the PEG-modified DOX-loaded CPNT bundles, which shows their potential to overcome the multidrug resistance in tumor therapy. These findings indicate that using cyclic peptide-based supramolecular structures as nanocarriers is a feasible and a potential solution for drug delivery to resistant tumor cells.

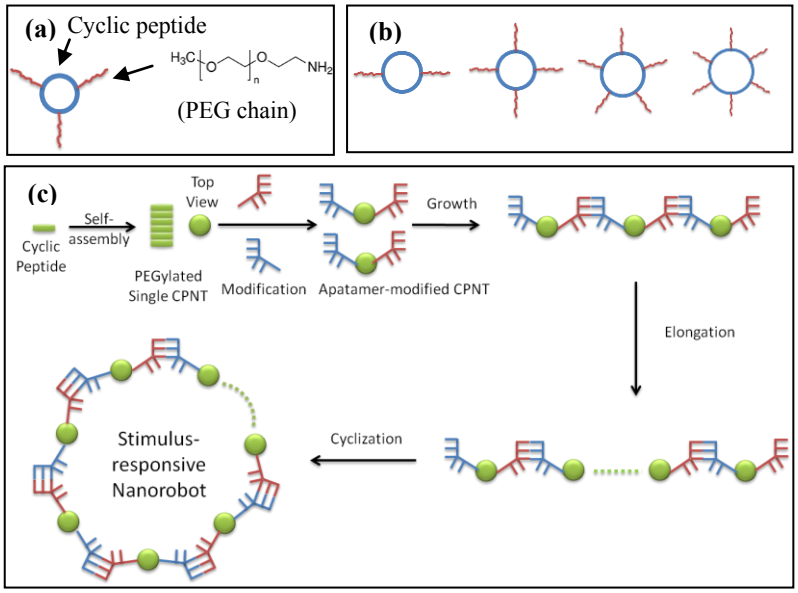

Fig. 3 Overview of current effort: (1) PEGylated single CPNTs, and (2) fabrication of a stimulus-responsive nanorobot. (a) A PEGylated cyclic hexapeptide; (b) other architectures of cyclic peptides, including octapeptide, decapeptide and dodecapeptide; (c) Using PEGylated single CPNTs and DNA-based adaptors, a stimulus-responsive hybrid nanorobot with controllable sizes will be fabricated.

V. On-going Work

Currently, we are fabricating a stimulus-responsive hybrid nanorobot with controllable sizes using DNA-based adaptors and single CPNTs for *in vivo* sensing and disease diagnosis. According to the previous studies, significant challenge remains for single cyclic peptide nanotubes (CPNTs) fabrication [1]. Most of the synthesized CPNTs were bundled together at several levels due to the hydrophobic surfaces of the CPNTs. In order to fabricate the hybrid nanorobots, single CPNTs as basic building blocks are needed for developing this complex nanostructure. Therefore, two studies related to this project are examined underway: (1) preparation of single CPNTs using site-specific PEGylation; and (2) fabrication of the stimulus-responsive hybrid nanorobot with controllable sizes using single CPNTs and DNA-based adaptors. The overall description of the on-going studies is depicted in **Fig. 3**.

References:

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