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

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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. Upon binding of a target biomarker to the aptamers, a conformational change takes place allowing the nanorobots to release their payloads (dyes or drugs). To demonstrate modularity of the approach, aptamers for a variety of biomarkers related to diseases will be conjugated to the nanorobots and tested. To scale-up the manufacturing 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 research goal of this project is to determine 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 manufacturing of nanorobots in nanomedicine.

A cyclic peptide nanotube used for antitumor drug delivery *in vitro* has been fabricated through this support. We also have designed and fabricated the self-assembled cyclic peptide nanotube through optimizing multiple parameters including 1) length of PEG, 2) density of PEG, 3) cyclic peptide sequence, 4) concentration, 5) purification methods. We currently are fabricating and validating a stimulus-responsive hybrid nanorobot with controllable sizes using cyclic peptide nanotube and AS1411 DNA aptamers for *in vivo* sensing and disease diagnosis. Meanwhile, tunable cyclic peptide nanotubes as visible fluorescent biomarkers for *in vivo* Sensing are investigated and characterized.

Methods and Results

1. Cyclic Peptide Design

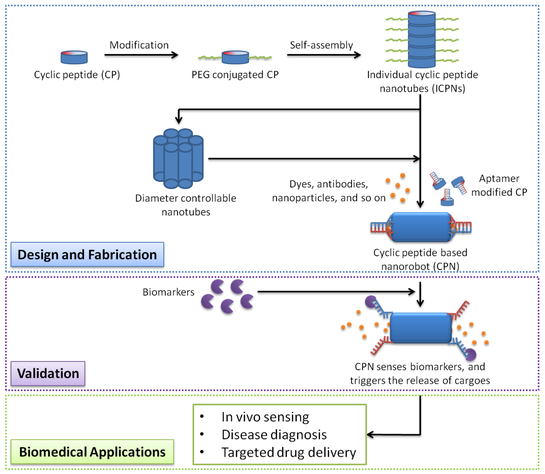

Fig. 1 Overview of the design, fabrication, validation and biomedical applications of the cyclic peptide nanotube based nanorobots.

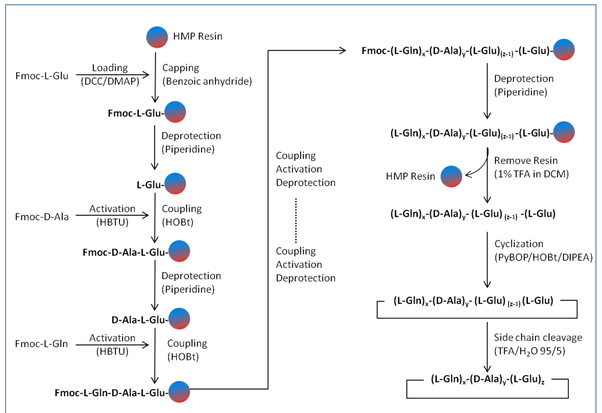

Fig. 2 Peptide synthesis and in-solution peptide cyclization procedures. The HMP resin was used for the linear peptide synthesis and PyBOP/HOBt/DIPEA method was used for the cyclic peptides synthesis.

2. Characterization of the Cyclic Peptide Nanotubes (CPNs) Self-assembled using "Phase Equilibrium Method"

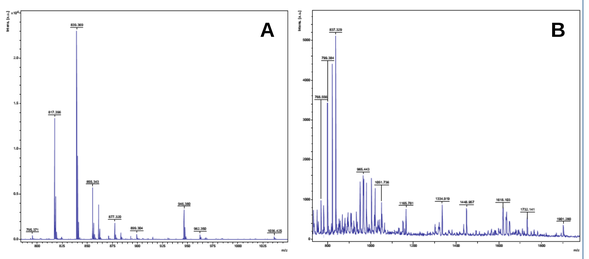

Fig. 3 Mass spectrometry characterization of the cyclic peptides. The MALDI mass spectrometry characterization of the linear (A) and cyclic peptides (B).

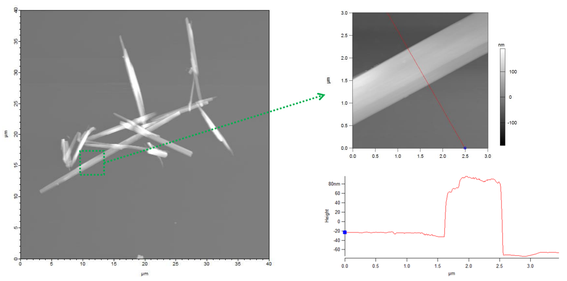

Fig. 4 AFM characterization of CPNs self-assembled using "phase equilibrium method".

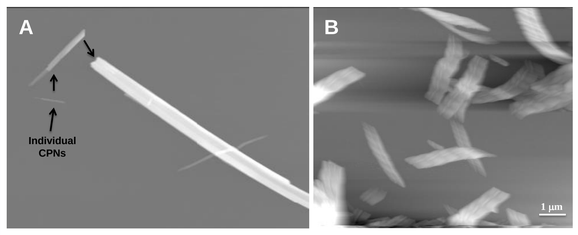

Fig. 5 The potential self-assembly process from individual CPNs to CPNs bundles (A) and AFM characterization of CPNs self-assembled using "phase equilibrium method" with a low cyclic peptides concentration (B).

3. Characterization of the Cyclic Peptide Nanotubes (CPNs) and PEG-modified CPNs using pH-driven Method

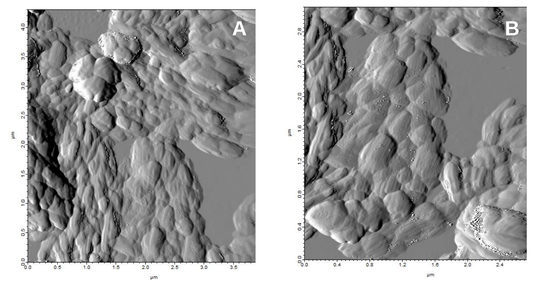

Fig. 6 AFM characterization of CPNs self-assembled using pH-driven method.

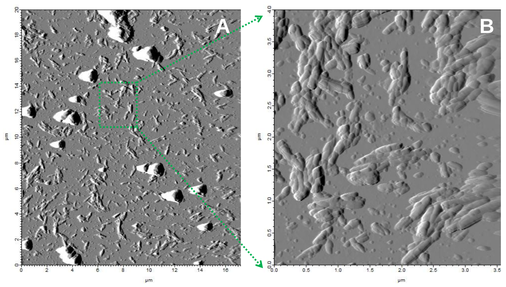

Fig. 7 AFM characterization of PEG modified CPNs self-assembled using pH-driven method.

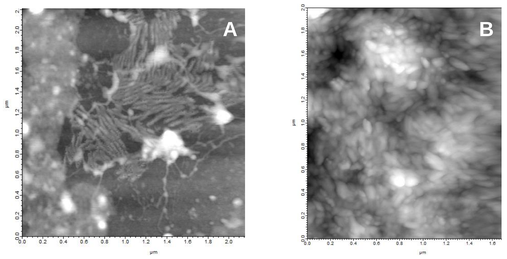

Fig. 8 AFM characterization of PEG modified CPNs (A) and normal CPNs (B) self-assembled using pH-driven method, both of them were treated with sonication and dialysis methods.

4. Conjugation of DNA aptamers to Cyclic Peptides

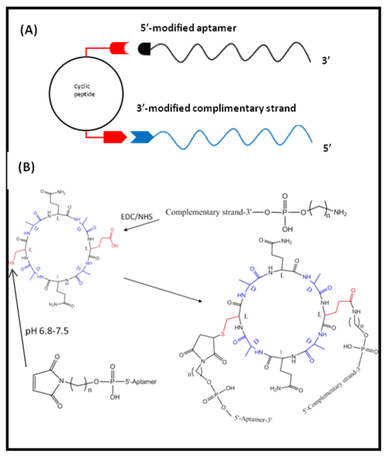

Fig. 9 Directional conjugation of cyclic peptide and DNA aptamers (A). The example of conjugation reaction using a cyclic octapeptide, an aptamer and its complimentary strand (B).

5. Tunable Cyclic Peptide Nanotubes as Visible Fluorescent Biomarkers for *In Vivo* Sensing.

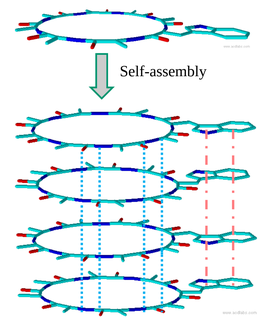

Fig. 10 Self-assembly process of visible fluorescent CPNs.

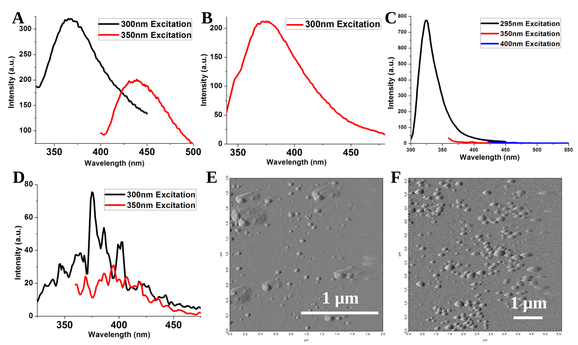

Fig. 11 Fluorescence spectrum and AFM images of different dipeptide based nanoparticles. Dipeptide based nanoparticles with bigger size (A) and (E). Dipeptide based nanoparticles with smaller size (B) and (F). Fluorescence spectrum of Trp (C). Fluorescence spectrum of dipeptide (D).

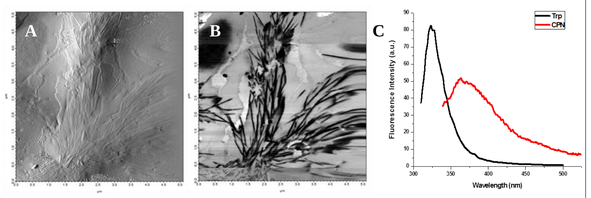

Fig. 12 AFM characterization of cyclic peptide based nanotubes (A) and (B) . Fluorescence spectrum of Trp and CPN in water solution (C).

Conclusions

The effects of the length and density of PEG, cyclic peptide sequence and concentration, self-assembly and purification methods on the association degree of the CPNs bundle were evaluated. Increasing the density of PEG and decreasing the concentration of CP both can decrease the association degree of the CPNs bundle. The pH-driven method showed a smaller size of CPNs bundles than the "phase equilibrium method" . Sonication and dialysis methods both have demonstrated their ability to lower the size of CPNs bundles.

References

Yongzhong Wang, Sijia Yi, Leming Sun, Yujian Huang, Scott C. Lenaghan, Mingjun Zhang. Doxorubicin-loaded Cyclic Peptide Nanotube Bundles Overcome Chemoresistance in Breast Cancer Cells, Journal of Biomedical Nanotechnology, 10, 445-454, 2014.

Leming Sun, Yongzhong Wang, Yujian Huang, Mingjun Zhang. Self-assembly of Individual Cyclic Peptide Nanotubes (ICPNs) for *In Vivo* Sensing. BMES 2014 Annual Meeting. October 22-25, 2014, San Antonio, Texas.