Facile Biomolecular Conjugation with Chitosan-Poly(ethylene glycol) Microparticles via Strain-Promoted Alkyne-Azide Cycloaddition Reaction toward Rapid Bioprocess Monitoring Applications

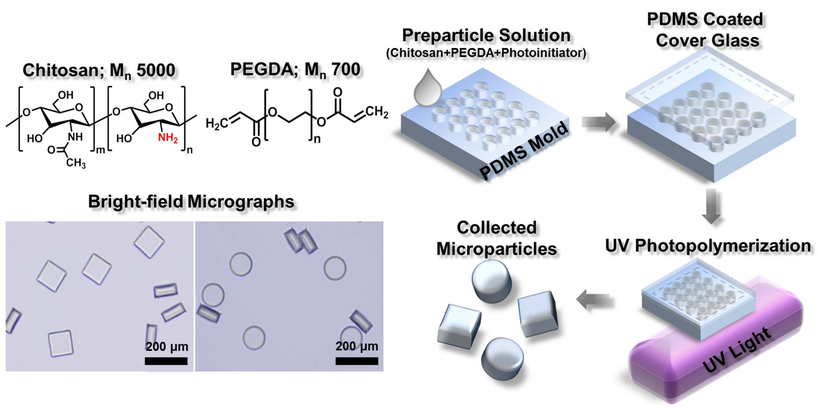
Sukwon Jung and Hyunmin Yi*
Department of Chemical and Biological Engineering, Tufts University, Medford, MA 02155
*hyunmin.yi@tufts.edu
TEL) 617-627-2195

Abstract

Rapid and reliable monitoring of biomacromolecules representing physiological events for facile bioprocess control is an unmet challenge. We strive to address this issue through development of facile fabrication and conjugation schemes for high capacity biosensing platforms that can be enlisted to capture direct variables such as mRNAs and proteins. Specifically, we demonstrate a facile scheme to fabricate chitosan-poly(ethylene glycol) (PEG) microparticles via replica molding for conjugation of biomolecules. Fluorescent labeling and FTIR microscopy results indicate stable incorporation of chitosan within the microparticles as well as chemical reactivity toward anime-reactive chemistries. Next, the as-prepared chitosan-PEG particles are conjugated with proteins via bioorthogonal strain-promoted alkyne-azide cycloaddition (SPAAC) reaction. Fluorescence and confocal micrographs show that the proteins are selectively conjugated near the particle surfaces where mass transfer limitation is minimal. Results on biomolecular conjugation kinetics via the SPAAC reaction show multiple reaction regimes; rapid initial, intermediate, and steady final stage. Selective target protein capture with antibody conjugated microparticles show rapid binding kinetics, indicating the potential of our fabrication-conjugation approach for rapid and reliable monitoring of biomacromolecules in biopharmaceutical processes.

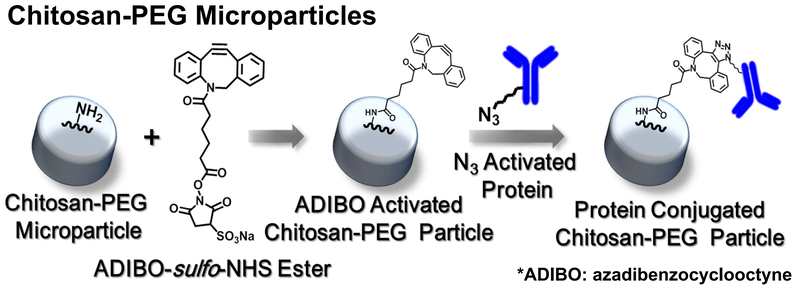
Materials and Methods

▪ Chitosan-PEG Microparticle Fabrication via Replica Molding



- The replica molding method allows fabrication of highly-uniform and well-defined chitosan-PEG microparticles.

▪ SPAAC Reaction for Biomolecular Conjugation with Chitosan-PEG Microparticles



*ADIBO: azadibenzocyclooctyne

-Selective and high yielding conjugation chemistry under mild reaction conditions (i.e. without Cu(I) catalyst resulting in protein damage).

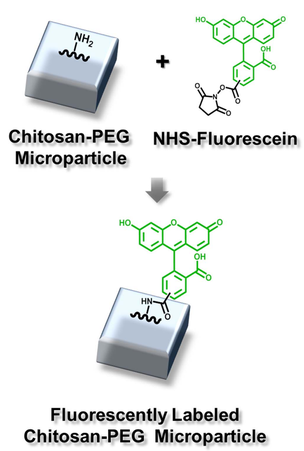
References:

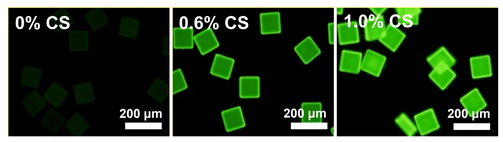
1. Jung, S.; Yi, H., *Langmuir* **2012**, *28*, 17061-17070.
2. Jung. S.; Yi, H., *Biomacromolecules* **2013**, 14, 3892−3902
3. Watkins, A. W.; Anseth, K. S. *Macromolecules* **2005**, *38*, 1326-1334.
4. Lee, A. G.; Arena, C. P.; Beebe, D. J.; Palecek, S. P. *Biomacromolecules* **2010**, *11*, 3316-3324.
5. Orski, S. V.; Sheppard, G. R.; Arumugam, S.; Arnold, R. M.; Popik, V. V.; Locklin, J. *Langmuir* **2012**, *28*, 14693-14702.

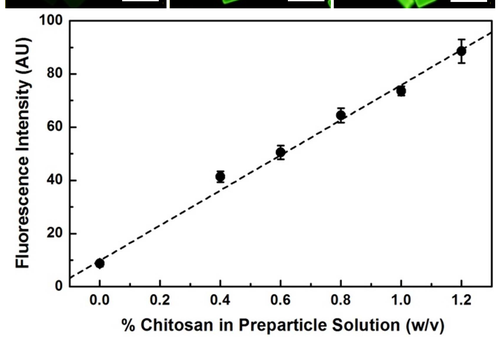
Acknowledgements:

We gratefully acknowledge partial financial support from National Science Foundation (DMR-1006613). We also thank Dr. Chang Hyung Choi (Harvard University) and Professor Chang-soo Lee (Chungnam National University, Korea) for providing the silicon mastermolds.

Chemical Reactivity of Chitosan-PEG Microparticles toward Amine Reactive Chemistry



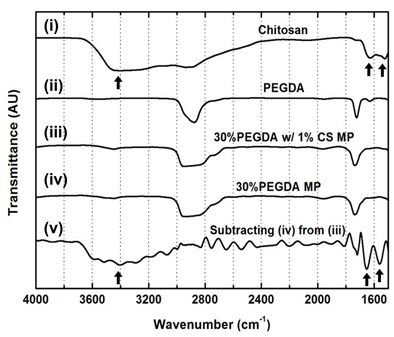




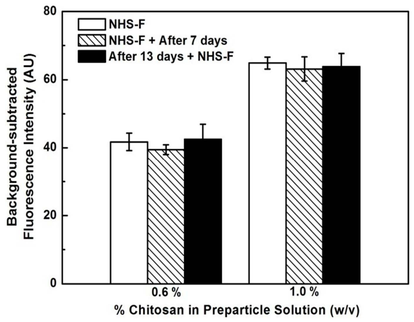
- Fluorescence intensity increases with increasing chitosan concentration, along with negligible fluorescence on the particles without chitosan.

⇒*The chitosan molecules are incorporated within the microparticles with chemical reactivity toward amine reactive chemistry.*

Stable Incorporation of Chitosan Molecules within Chitosan-PEG Microparticles



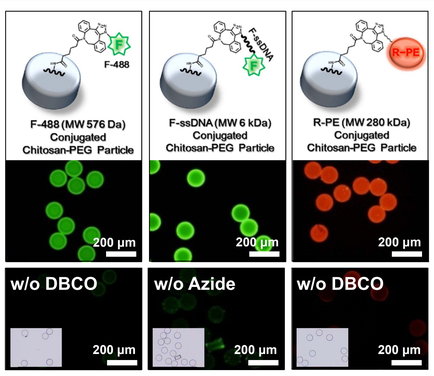
- FTIR microscopy results for the single microparticle after 7 days washing.
- Characteristic peaks of the chitosan in the chitosan-PEG particles are clearly observed as indicated by three small arrows .

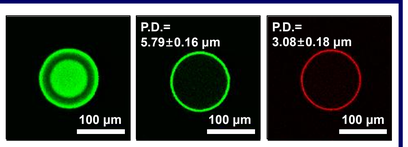


- Negligible difference in fluorescence intensity between the fluorescently labeled particles; right upon fluorescent labeling (white columns), stored for 7 days upon labeling (striped columns), and exposed to NHS-fluorescein upon 13 days particle storage.

⇒*Chitosan molecules are incorporated within the microparticles in a stable manner with retained chemical reactivity.*

Covalent Conjugation of Model Biomolecules on Chitosan-PEG Microparticles via SPAAC Reaction

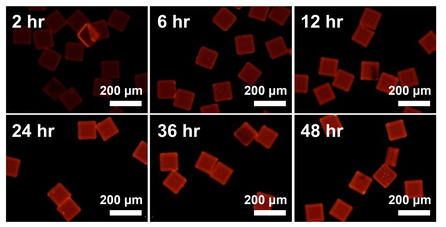


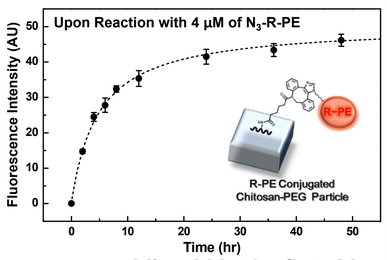


- Uniform fluorescence on ADIBO-activated particles upon 24-hr reaction with azide-activated fluorescent molecules unlike negative control particles.
- Confocal micrographs show that biomolecules are conjugated near the particle surfaces; *diffusion limitation of biomolecules*.

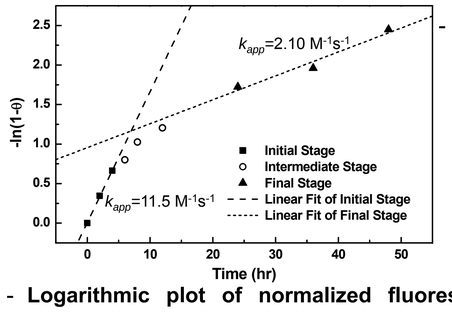
⇒*Facile and selective SPAAC reaction for biomolecular conjugation with chitosan-PEG microparticles.*

Protein-microparticle Conjugation Reaction Kinetics via SPAAC Reaction





- Fluorescence intensity on the particles increases rapidly within the first 4 hr, then the rate of increment slows down approaching saturation.



- The pseudo-first order reaction model for protein conjugation via SPAAC reaction.

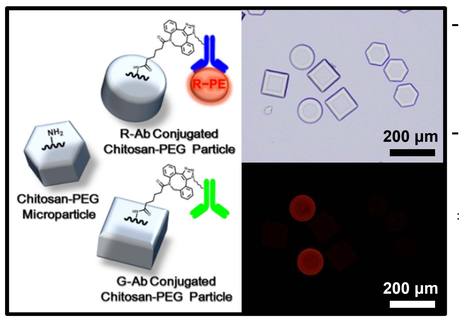
$$\frac{d[A*]}{dt} = k'_{app}[*], \qquad k'_{app} = k_{app}[A]_0$$

$$-\ln(1-\theta) = k'_{app}t, \quad \theta = \frac{[A*]}{[A*]_{max}}$$

- Logarithmic plot of normalized fluorescence intensity, shows that the conjugation reaction rate slows down over time.

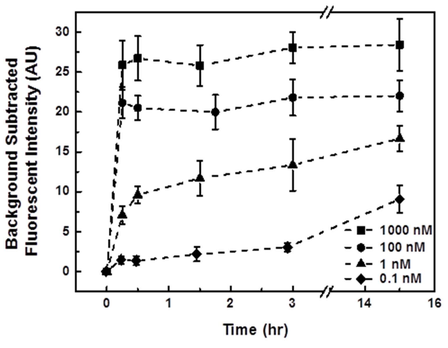
⇒*Three different conjugation reaction phases; rapid initial, intermediate, and steady and slow final stage due to diffusion limitation of R-PEs resulting from small mesh size of particles and as-conjugated R-PEs.*

Antibody Conjugation on Chitosan-PEG Microparticles and Selective and Rapid Target Protein Capture



- Circle: Anti-R-PE antibody conjugated particle, Square: Anti-GFP antibody conjugated particle, Hexagon: Bare particle.
- Selective capture of target proteins, confirming antibody conjugation with particles.

⇒*Shape-based encoding via replica molding readily allows addressable detection of target proteins.*



- Rapid target protein capture within the first 30 min for all target protein (R-PE) concentrations except for 0.1 nM.
- Linear increase in target protein capture at 0.1 nM R-PE with time suggesting readily detected femtomolar quantity of target proteins (10 femtomoles in 100 μL assay volume).

⇒*Potential for rapid protein sensing applications.*

Conclusions

- Replica molding allows for fabrication of well-defined and uniform shapes of chitosan-PEG microparticles with retained chemical reactivity toward amine reactive chemistry.
- Proteins are readily conjugated with the chitosan-PEG microparticles via SPAAC reaction, showing three different conjugation reaction regimes.
- We envision that our overall fabrication-conjugation scheme for protein conjugated platforms can be readily expanded to various protein sensing applications with high capacity and rapid binding kinetics.