Armored microbes - Controllable assembly of nanoparticles/polyelectrolyte shells

Glenn Johnson[2], Shantanu Balkundi[1], D. Matthew Eby[2], Yuri Lvov[1].
[1]Institute for Micromanufacturing, Louisiana Tech University, Ruston, LA
[2]Microbiology and Applied Biochemistry, AFRL/RXQL, Tyndall, FL

Statement of Purpose: Various encapsulation techniques effectively form nanoshells on cores material templates. The process of using layer-by-layer (LbL)[1, 2] for coating living cells has been of great interest due to the advantages of LbL assembly over other encapsulation techniques including: controllable coating thickness in nanometers, surface modification, selective control over permeability, simplicity, and cost effectiveness. In the present work we have used bacterial spores as templates for forming a nano capsule. We have successfully coated spores with various polymers and have shown that they can be adsorbed with bentonite clay coating and Si nanoparticles. Germination assays showed that the spores retained viability after coating. The "nanothick" engineered coatings may act as an armored shell that also offers control over spore germination.

Methods: Spores of *Bacillus subtilis* ATCC 23857 served as the core template for LbL process. The coating materials used in LbL included: polystyrene sulphonate (PSS), poly (dimethyldiallyl ammonium chloride) (PDDA), polyethyleneimine (PEI), polyglutamic acid (PGA), polylysine (PLL), albumin, lysozyme, gelatin A, protamine sulphate ($ProtSO_4$) and chondroitin sulphate ($ChSO_4$), silica particles and bentonite clay. All materials were from Sigma Aldrich and were used without further purification. The polymers (2 mg/mL) were deposited on the surfaces using alternate charge methods established previously in our laboratory[3, 4]. The spore samples were washed three times with deionized water before the assembly process. Characterization tools that were used for our study included: confocal microscopy, and zeta-potentiometry.

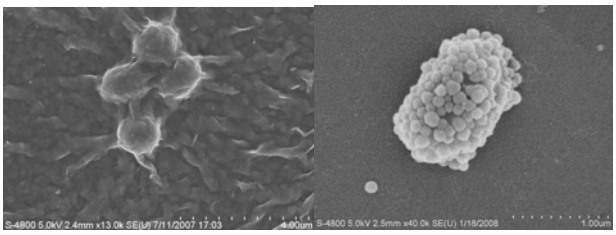

Figure 1. SEM Images of *B. subtilis* coated with bentonite and Si nanoparticles.

Results: Figure 1 shows polymer-encapsulated bacterial spores with final coats of bentonite or silica nanoparticles. The SEM image revealed that the structure of the individual spore did not change after encapsulation using various polymers. Figure 2 shows the surface charge reversal of encapsulated microbes for various combinations of polymers. The zeta potentiometer readings confirmed alternation of charges. The magnitude of recharging was high enough to yield a stable complex. Confocal images showed evidence of the encapsulated spores. The encapsulated spores were intact even after a period of about 15 days.

Figure 2 shows that PDDA/PSS and PLL/PGA assembled very well over the spores giving a high magnitude on the ζ- potentiometer. There was full reversal for all the other combinations of synthetic and also biopolymers. Confocal microscopy was used as a characterization technique to provide visual evidence for the layer formation and encapsulation of the bacillus spores. The kinetics and extent of spore germination was monitored by measuring release of dipicolinic acid, a specific component expelled from endospores at onset of germination. Cell germination and proliferation studies showed that the coated spores are viable, but germination kinetics is changed compared to the native spore.

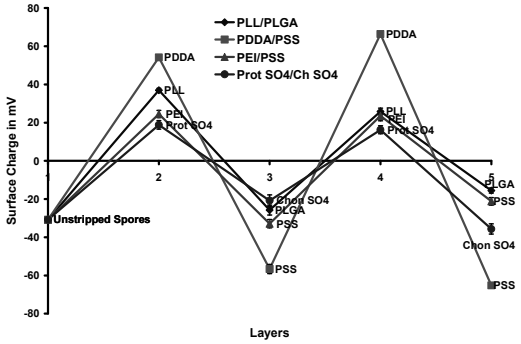

Figure 2. Zeta-potentiometer readings showing reversal of charges for various combinations of polyelectrolytes.

Conclusions: We successfully demonstrated that bacterial spores can be used as templates for LbL assembly. We achieved individually coated microbes with a protective shell. The spore germination assay showed that the spores were viable even after encapsulation using various polyelectrolytes. The use of biocompatible polymers and the viability of the cells after encapsulation make the encapsulation noncytotoxic as far the cell activity is concerned. The multi-coated spore acts as a dormant cell and the number of layers can be changed to affect the dormancy period. Encapsulation of bacterial spores may be further explored to study fundamental questions of spore biology and in practical agricultural applications.

References:

1. Decher, G. *Science* 1997, 277, 1232.
2. Lvov, Y.; Agria, K.; Ichinose, I.; Kunitake, T. *J Am. Chem. Soc*. 1995, 117, 6123.
3. Ai, H.; Fang, M.; S. Jones, S.; Lvov, Y. *Biomacromolecules*, 2002, v.3, 560-564
4. Shutava, T.; Balkundi, S.; Lvov, Y. *J. Colloid Inter. Sci*, v.330, in press, 2009