CELL LYTIC ENZYME-BASED NANOSCALE COMPOSITES FOR ANTIMICROBIAL APPLICATIONS

By

Ravindra Chandrakantrao Pangule

A Thesis Submitted to the Graduate
Faculty of Rensselaer Polytechnic Institute
in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY
Major Subject: Chemical and Biological Engineering

Approved by the
Examining Committee:

____
Prof. Jonathan S. Dordick, Thesis Adviser

____
Prof. Ravi S. Kane, Thesis Adviser

____
Prof. Cynthia H. Collins, Member

____
Prof. Steven M. Cramer, Member

____
Prof. Robert J. Linhardt, Member

Rensselaer Polytechnic Institute
Troy, New York
July 2011
(For Graduation August 2011)

ABSTRACT

Pathogen–mediated infectious diseases constitute a growing societal problem. As a result, demand for hygienic living conditions has increased and hence created a need for antimicrobial agents and coatings. Taking inspiration from nature, we have designed cell lytic enzyme-based nanoscale systems for such applications. In the first part of this work, we have developed enzyme-based antimicrobial paint nanocomposites that are highly effective against methicillin-resistant *Staphylococcus aureus* (MRSA) – a pathogenic bacterium responsible for thousands of hospitalizations and deaths per year. We generated active conjugates of lysostaphin, a cell wall-degrading enzyme, by interfacing it with carbon nanotubes. The enzymatic activity of these conjugates was further enhanced by attaching the enzyme onto carbon nanotubes with a poly(ethylene glycol) spacer, which provided mobility and substrate accessibility to the immobilized enzyme molecules. We found the enzyme–based nanocomposites, prepared by incorporating these conjugates in paint matrix, to be highly effective against four different strains of MRSA. The surface characterization using scanning electron microscopy helped us study the coverage of conjugates on the surface of paint nanocomposites. The results from antimicrobial assays suggested that the conjugate-based paints show such bactericidal effect through a contact-active or non-release mechanism. Additionally, we evaluated the long-term storage stability of these enzyme-based paint nanocomposites. Based on these promising results, we further extended this work in collaboration with Prof. Linhardt's group, and developed a biocompatible and anti-infective bandage system that utilizes electrospun cellulose fibers and lysostaphin. The biocompatibility and anti-infective properties of lysostaphin-cellulose fiber mats were studied using a keratinocyte-based reconstructed *in vitro* epidermis model. The results strongly suggest that lysostaphin-cellulose fiber mats could potentially be used in wound-healing applications.

Next, we extended the applicability of our approach by preparing the enzyme-based formulations that are effective against vegetative cells and dormant species, *i.e.*, spores of *Bacillus cereus* (a lab model of *B. anthracis*). Hydrolytic activity of heterologously expressed and purified cell lytic enzyme, PlyPH, on the peptidoglycan fragments extracted from spore cortex, suggests that the combination of PlyPH and spore coat-permeabilizing agents may potentially result in development of an effective spore

neutralization formulation. We exploited the species-specific nature of cell lytic enzymes (PlyPH, lysostaphin, and lysozyme) and luciferase-mediated detection of ATP, in demonstrating a rapid and selective detection of *B. cereus*, *S. aureus*, and *Micrococcus lysodeikticus*, when present in a mixed population. In a final study, we have prepared biocatalytic paint nanocomposites based on cell wall-, protein- and polysaccharide-degrading enzymes, with the objective of designing a surface that limits the surface fouling by bacterial cells. In summary, we have developed cell lytic enzyme-based systems for their potential uses in antimicrobial, antifouling and pathogen-sensing applications.