Highly-Branched Poly(N-isopropyl acrylamide) That is Responsive to Bacteria

Stephen Rimmer,[1] Joanna Shepherd,[2,3] Prodip Sarker,[1] Joseba Alonso Villanueva,[1] Linda Swanson,[1] Sheila MacNeil,[2] Ian Douglas.[3]

Departments of Chemistry,[1] Materials Science and Engineering[2] and Dentistry[3]

Statement of Purpose: Control and detection of bacterial infection is a worldwide challenge, which is increasing with the rise of diabetes, advancing age and the progress of antibiotic resistant strains. In order to address some of these issues we have developed highly-branched poly(N-isopropyl acrylamide) (HB-PNIPAM) stimulus responsive materials, which are sensitive to the presence of bacteria. It is well known that PNIPAM materials progress through a solvated coil to desolvated globule transition at a critical temperature. However, the transition can also be driven by the binding of specific ligands, present at chain ends, to cellular targets. The polymers are functionalized at the chain ends with ligands that bind to either Gram+ve or Gram–ve species. We show that binding of bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus)* to the polymers switches the state of the polymer to form insoluble polymer/bacteria assemblies. The highly branched architecture facilitates the binding and collapse process by maintaining the availability of the ligands located at the chain ends. In alternative materials, linear polymers with pendant ligands, the ligands are shielded in the collapsed state and binding is much less efficient.

Methods: HB-PNIPAM was prepared using the RAFT radical polymerization technique using a branching comonomer: a styrene derivative functionalized with a dithionate ester moiety. The polymer chain ends were then modified to produce carboxylic acid groups at all chain ends. A polymer targeted at Gram+ve bacteria was produced by reaction of vancomycin with the N-hydroxy succinimide derivative (HB-PNIPAM-van) and a similar reaction with the polymyxin peptide produced a polymer targeted at Gram-ve bacteria (HB-PNIPAM-pmx). The polymers were added to both the *P. aeruginosa* and *S. aureus* mixtures and studied microscopically.

Results: Figure 1a shows images of HB-PNIPAM-van mixed with *S. aureus*. The image, 5+PBS, shows no features and is derived from a solution of 5 mg ml of HB-PNIPAM-van in PBS. Addition of *S. aureus* to this solution, image 5 produced a mat in which the bacteria were suspended. Reduction of the polymer concentration to 1.25 mg ml gave a looser mat and in the absence of polymer (image 0) the bacteria rolled to the bottom of the well to produce a tight button. Further investigation of the polymer-bacteria aggregates using an anthracene labeled polymer (shown blue in the micrographs) and bacteria labeled with Dylight 649 (red) produced image 1c. Here the bacteria and polymer are clearly seen as intermingled associated structures. Figure 1b shows the bacteria in the absence of polymer and 1d shows the HB-PNIPAM in the presence of the Gram-ve bacteria. The latter image shows that it is necessary to use a polymer that can bind to the cell surface to produce the intermingled aggregates. The aggregates retained the thermal responsive nature of the polymer so that (1e) cooling from 37 °C to 4 °C released the bacteria, which dispersed throughout the medium.

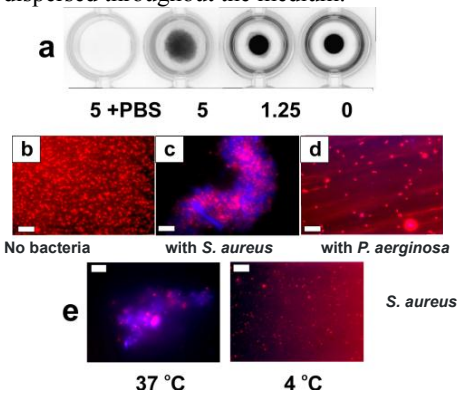

Figure 1. (a) *S. aureus* in contact with HB-PNIPAM-van; (b) *S. aureus* alone dyed with Dyelight 649; (c) *S. aureus* plus HB-PNIPAM-van; (d) *P. aeruginosa* plus HB-PNIPAM-van; (e) S.aureus/HB-PNIPAM-van aggregates disperse on cooling to 4°C.

Similar experiments were conducted with *P. aeruginosa* and HB-PNIPAM-pmx. This polymer also bound to its target, lipopolysaccharide, and produced mats with *P. aeruginosa*, as shown in figure 2a. Cooling the mats released the bacteria, which collected at the bottom of the well. The polymer could be observed, using labeled bacteria and polymer, to form intermingled aggregates as shown in figure 2b and these also dispersed on cooling.

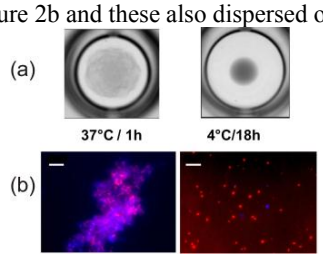

Figure 2. (a) *P. aeruginosa* in contact with HB-PNIPAM-pmx forms a mat at 37° C, which on cooling to 4° C disperses; (b) *P. aeruginosa* (red) intermingled with HB-PNIPAM-pmx at 37 °C. Bacteria re-dispersed at 4 °C.

Conclusions: HB-PNIPAM functionalized at the chain ends with either vancomycin or the polymyxin peptide binds to Gram+ve or Gram-ve bacteria respectively. The binding causes a large perturbation of the solvation of the ligand so that the temperature of the coil-to-globule transition is reduced to below 37 °C. This reduction results in the polymer being driven through the transition so that binding induces the transition. PNIPAM in the globular conformation is adhesive to hydrophobic bacteria and in this state aggregates of bacteria and polymer are formed. However, in the open coil form bacteria do not adhere well to the polymer and, therefore, cooling breaks up the aggregates.