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Engineering Tunable Cyborg Bacterial Vaccines Using Intracellular Hydrogelation

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Abstract:

Whole-bacteria vaccines are primary human tools to tackle infections ranging from recalcitrant urinary tract infections to respiratory and blood infections. Classical whole-bacteria vaccines use two main states of living systems: killed bacteria; live but weakened bacteria. Here, we enable a third bacterial state for vaccine applications: “non-replicating but metabolically active” or Cyborg bacteria. Cyborg bacterial vaccines are created through a tunable intracellular hydrogelation approach to control the phenotypic and physical state of Cyborg bacterial vaccines, using *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as infection models. Through flow cytometry and CFU assays, we confirm that Cyborg bacterial vaccines are non-proliferative but metabolically active. In addition to metabolic activity, Cyborg bacteria exhibit significant resistance to oxidative stress, maintaining a low intracellular ROS level. Secretome data reveal the retention of key immunogenic and secreted proteins, while high-toxicity effectors are not detected under our conditions. We also demonstrate that Cyborg bacteria remain stable in the presence of immune cells, resulting in the durable and tunable activation of immune systems. Immunological studies demonstrate that Cyborg bacterial vaccines effectively activate antigen-presenting cells and enhance T-cell response. In vivo vaccination and infection challenge in C57BL/6 mice demonstrate ~70-80% survival rates compared to heat-killed bacteria. Our Cyborg bacterial vaccine platform leverages the novel non-replicating-but-active state of bacteria, resulting in tunable, superior, and safe immune activation. Our platform may enable the rapid discovery and creation of Cyborg bacterial vaccines against emerging antibiotic-resistant bacteria.

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