411 PROGRAMMING IMMUNOGENICITY OF BACTERIAL CANCER THERAPY WITH BIOSENSOR-DRIVEN ENCAPSULATION SYSTEMS

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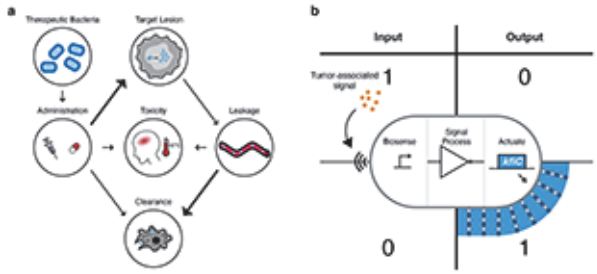
Background More than a century after the development of Coley's toxins, bacteria are re-emerging as a platform for cancer immunotherapy. In addition to advances in microbiology and immunology, the advent of synthetic biology has enabled the construction of complex genetic circuits to program bacteria for modulating the immune system. Systemic delivery is necessary for therapeutic bacteria to reach inaccessible tumors and colonize immune-privileged tumor microenvironments (TME). However, this intentional establishment of bacteremia can result in serious toxicity due to infection of healthy tissues and recognition of bacteria in the circulatory system as pathogens. To ensure safety, bacteria have been engineered to reduce virulence, but the attenuation can unintentionally diminish the ability of bacteria to colonize TME and stimulate the immune system, rendering the bacterial cancer immunotherapy ineffective. Therefore, more sophisticated modulation of bacterial immunogenicity is necessary to retain their effectiveness while reducing their toxicity (figure 1a).

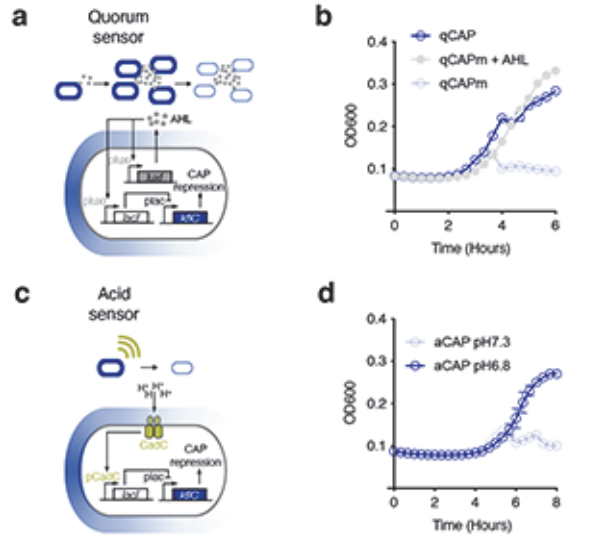
Methods Biosensor-driven encapsulation systems were constructed with a NOT gate to modulate immunogenicity of probiotic strain, *E. coli* Nissle 1917, based on environmental pH or bacterial density that can be found in TME (figure 1b). The genetic circuits were characterized using phage that target and lyse the encapsulated bacteria. The complement-mediated lysis of bacteria was used to test the ability of biosensor-driven encapsulation systems to resist clearance depending on environmental pH and bacterial density *in vitro*. A pharmacokinetic model was developed and analyzed *in silico* to elucidate the mechanism of the encapsulation systems for modulating biodistribution.

Results The encapsulation systems modulated the phage- and complement-mediated lysis upon change in environmental pH and bacterial density as programmed (figure 2 and 3). A dual-track, 3-compartment pharmacokinetic model was constructed to inform and analyze biodistribution of future *in vivo* studies (figure 4).

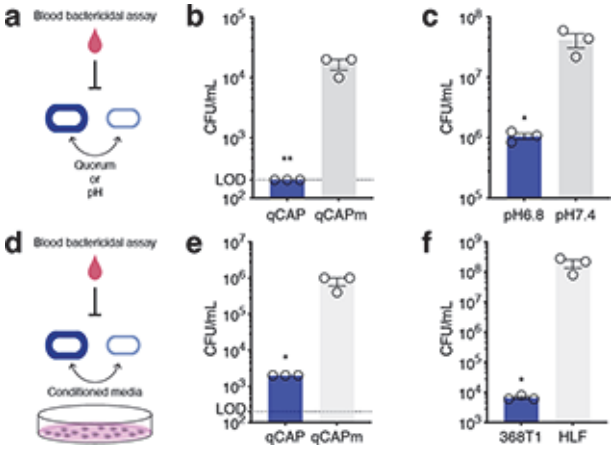
Conclusions The biosensor-driven encapsulation systems function as intended *in vitro* to lower immunogenicity outside of tumor by hiding immunogenic molecules of bacterial outer membrane within the thick capsular polysaccharide. Upon sensing TME, either through lower pH or immune-privileged environment that facilitate bacterial growth, the engineered bacteria shut down the production of capsular polysaccharide and become more susceptible to recognition by the immune system, which may have profound impact on safety and efficacy of bacterial cancer immunotherapy.



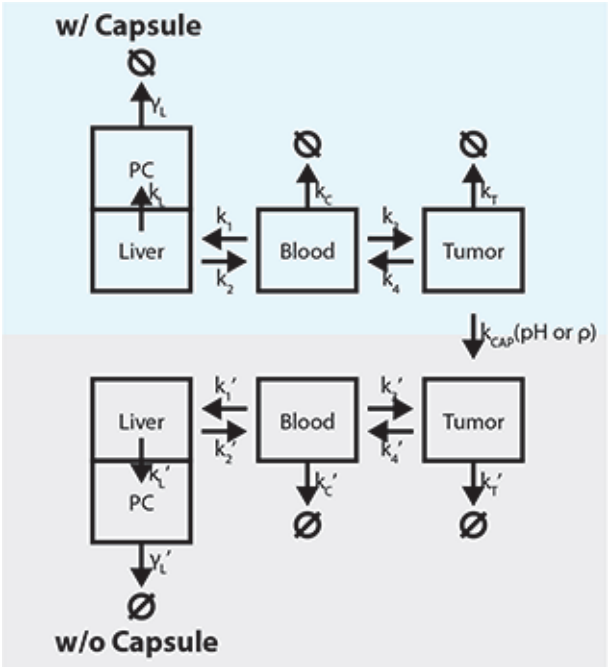
Abstract 411 Figure 1



Abstract 411 Figure 2



Abstract 411 Figure 3



Abstract 411 Figure 4

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0411