Return of the Jedi: Fighting Antimicrobial Resistance with Nanobiotics

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Tuberculosis (TB), a mycobacterial disease that in humans can be traced back to 9,000 years ago in Atlit Yam, a city now under the Mediterranean Sea, is still today the second deadliest infection after COVID-19 [1]. Being primarily a pulmonary pathogen, the bacteria enters the respiratory tract via inhalation and is readily phagocytosed by alveolar macrophages. In the lungs, the pathogen can either be eliminated by the immune system or persist in a quiescent (latent TB) or active state [2, 3]. The World Health Organization estimates that 1/4 of the world population is infected with latent TB and 10% of those will develop active disease [4]. The discovery of antibiotics in the 40s brought hope for the eradication of the disease, but *M. tuberculosis* showed the ability to develop drug resistance through genetic mutations, not to mention that most first-line antibiotics only target replicating bacteria [2,3].

Inefficient delivery of drugs to the target site is one of the main drivers of the emergence of multi- and extensive drug resistance, as bacilli are exposed to subminimal inhibitory concentrations of drugs. Furthermore, patients must endure long treatments with multiple toxic antibiotics that have numerous side effects, which undermines patient compliance and ultimately plays a role in the rise of resistant strains. Reformulating existent antibiotics in nanocarriers (nanobiotics) is a viable solution to provide targeted drug release, reducing the dosing frequency and the overall systemic toxicity.

In particular, the conjugation of therapeutic molecules to polymers has gained significant attention from the pharmaceutical industry, as it offers the possibility to improve the aqueous solubility and stability of drugs and, consequently, extend their plasma half-life and alter their biodistribution. Such traits are particularly relevant when delivering cytotoxic drugs, which often exhibit poor solubility and rapid clearance. The main setback that the industry is facing relates to the difficulties in achieving a controlled conjugation of the therapeutic agent, which results in polydisperse polymers with wide-ranging drug loadings and sites of modification that fail to meet GMP guidelines [5].

Here, we report the synthesis of a well-defined isoniazid-based polyester with nearly quantitative loading efficiency using a fast lipase-catalysed esterification reaction, followed by hydrazone bond formation (**Figure 1**). Nanobiotics composed of isoniazid-conjugated polymer and encapsulated clofazimine presented lack of toxicity, dose responsiveness, and improved therapeutic efficacy in the treatment of mycobacterial infection in a zebrafish larval model when compared to free drugs (**Figure 2**) [6]. The main advantage of this system is the synthetic simplicity and versatility. The drug is directly conjugated to the polymer without the need for any further chemical modifications. The drug-polymer bond is acid-labile, allowing site-specific drug release, and the polymer itself is hydrolysable facilitating excretion. Polymer size can be tuned without affecting the high drug loading capacity since there is one drug conjugation site per monomeric unit of polymer.

With the slow development of new antibiotics, tunable polymeric nanobiotics offer an opportunity to deliver more effective and more tolerable combination chemotherapy using existing drugs for *M. tuberculosis* and other infectious diseases.

References

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Figures

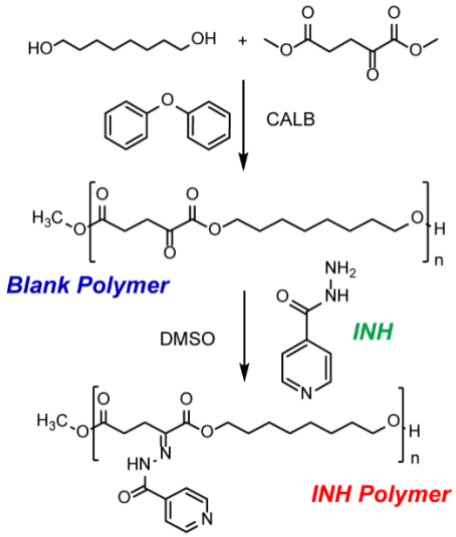

Figure 1. Synthesis of an α-keto polyester by (trans)esterification reaction catalysed by Lipase acrylic resin from *Candida antarctica* and conjugation to isoniazid (INH).

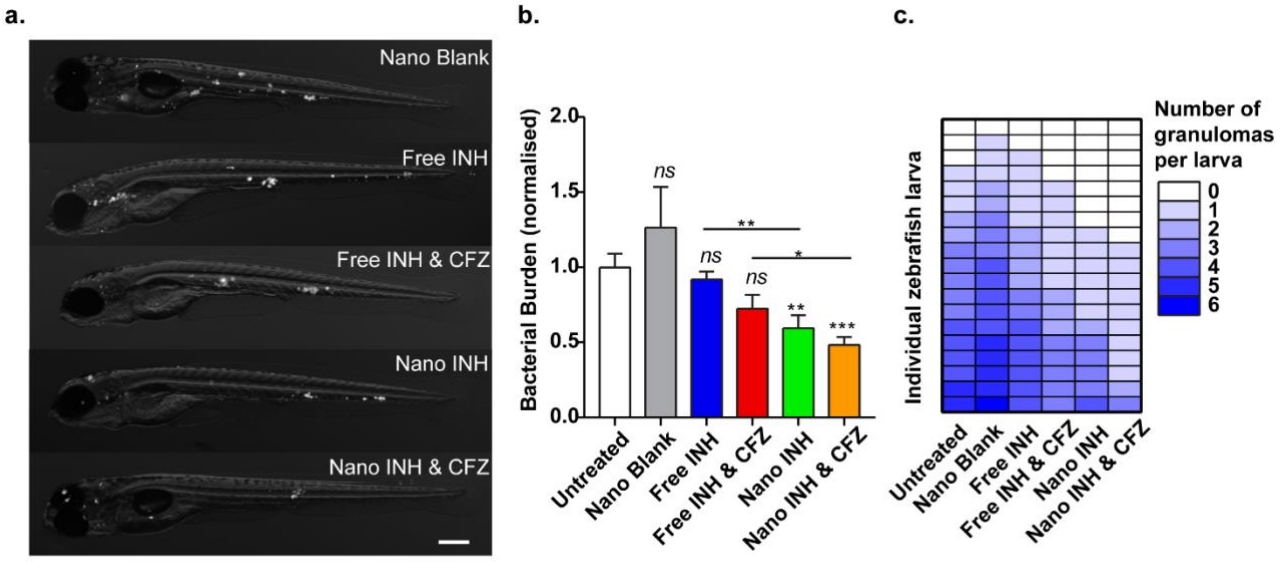

Figure 2. Effect of nanobiotics at 3 days post-infection on zebrafish infected with fluorescently-labelled *M. marinum*. **a.** representative images (scale bar, 200 µm). **b.** quantification of bacterial load (results plotted as mean ± SEM from 2 independent experiments; n=21). **c.** Quantification of granuloma number at 3dpi. Results are plotted as mean ± SEM from 2 independent experiments (n=19).