Visualizing the colonization dynamics of pathogenic bacteria labelled by upconverting nanoparticles inside the gut

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Abstract: This study intends to show the use of upconversion photoluminescence imaging to investigate the colonization and infection dynamics of a natural murine intestinal pathogen, *Citrobacter rodentium (C.rodentium)*, which induces inflammation in mice.
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Inflammatory Bowel Diseases (IBDs) including Crohn's disease (CD) and ulcerative colitis (UC) are associated with the chronic inflammatory state of gastrointestinal tract. The exact causes of IBD are still unknown, however, the recent studies show that gut microbiota is a contributing factor to IBD [1]. Although the role of intestinal microbes in the development of IBD in animals is revealed, the interaction mechanisms between the host and these microbes are yet not precisely defined. To understand these interactions *in vivo*, *C.rodentium* infection model has been studied. *C.rodentium* is the most widely used model to study host-pathogen interactions as it shows high genetic similarities to human pathogens, including enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E.coli* (EHEC) [2]. These pathogens are associated with the development of IBD in human. *C.rodentium* infection model has been studied by different *in vivo* imaging techniques, including fluorescence imaging (FI) and bioluminescence (BL) [3, 4]. FI employs custom designed fluorophores as contrast markers, whereas BL rely on genetically modified pathogen strains that are able to catalyse luciferin molecule by luciferase enzyme. However, using fluorescent probes *in vivo* studies has some limitations such as tissue autofluorescence, photobleaching and photodamage. In case of BL, the process is dependent on the metabolic state of the pathogen and the oxygen content in the environment (*i.e.* requires *in vivo* studies with blood circulation). In addition, it is not always possible to genetically engineer all types of bacteria for BL use.

In this study, lanthanide based upconverting nanoparticles (Ln-UCNPs), a class of photoluminescent nanomaterials, are being exploited as labelling agent as well as contrast marker to visualise infection dynamics of *C.rodentium*. Ln-UCNP are promising candidates to overcome the aforementioned limitations of FI and BL. Ln-UCNPs are capable of photon

upconversion, *i.e.* the conversion of incoming photons with lower energy into emitted photons with higher energy. The photon upconversion is as a result of two or multiple sequential photon absorption and has anti-Stokes nature. In this work, near infra-red (NIR) laser light at 976nm is used as an excitation source and Ln-UCNPs emits upconverted photons at 804 nm, which also falls in NIR region. In NIR region, most of the tissue molecules have relatively low absorption and scattering coefficients, which results in higher light penetration depths, low autofluorescence, and high signal-noise ratio. Thereby, *C.rodentium* infected mice is labelled by means of Ln-UCNPs conjugated with anti *C.rodentium* antibody. 10^8-10^9 colony forming unit (CFU) of *C.rodentium* are orally administered to mice at different time points to quantify *C.rodentium*. The infection kinetics of *C.rodentium* is consisted of three distinct phases. Phase one, from day 0 to day 3 is known as initiation of infection. Phase two is the peak of infection, from day 7 to day 10. Third phase is known as resolution phase, begins after day 10, where *C.rodentium* is cleared out from the host. Upconversion photoluminescence from *C.rodentium* labelled with UNCP-antibody in mice is imaged by a custom modified commercial *in vivo* imaging system (IVIS Lumina III, *Perkin Elmer*) through these three distinct phases. Herein, it should be noted that major optical design modifications has been done to IVIS Lumina III to be employed in NIR region. For this purpose, fiber coupled multi-mode NIR laser is attached to the system. An optical cage has been designed particularly for this system, in which laser beam is expanded and its spot size is selectively varied in a range from 5 mm up to 38 mm. Wisely selected spectral filters are also placed to overexpose the existing camera inside the imaging system. These Ln-UCNPs are chemically stable, *i.e.* no photo degradation is observed, as compared to widely used organic fluorescent dyes. In addition, they show high photo stability, no photoblinking is observed, as compared quantum dyes that are commonly used in FI. Thereby, Ln-UNCPs can be used for studies in which long/repeated exposure times are required, *e.g.* bacteria dissemination studies.

The upconversion photoluminescence is being compared with the BL in terms of light intensities. Moreover, the success of the bio-conjugation between UCNP-antibody and *C.rodentium* is being investigated. In addition the monitoring of the bacterial colonisation dynamics inside the gut, biodistribution and pharmacokinetics of Ln-UCNPs are being studied.

References:

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