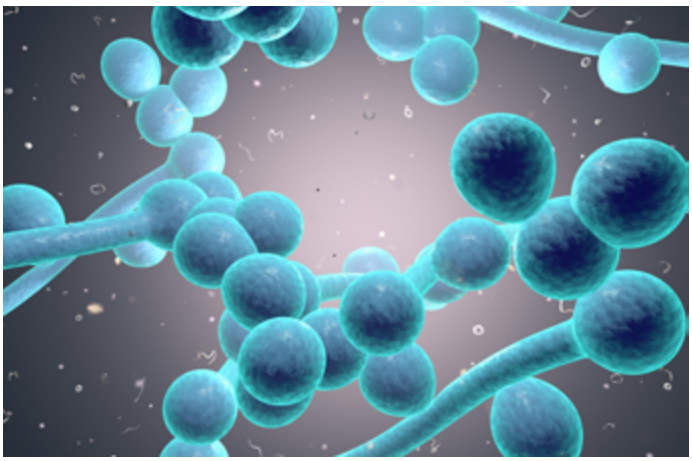

In vivo visualization of *Candida albicans* infections

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Introduction

Candida yeasts are commensals of human skin and mucosal surfaces but can also cause fungal infections. *Candida albicans* is a major fungal pathogen and causes life-threatening diseases especially but not limited to immuno-compromised patients. Candidiasis most often develops in the mouth or throat (called oropharyngeal candidiasis or thrush), the vagina (yeast infection) or as invasive candidiasis after entering the bloodstream and thus spreading throughout the body (Brown et al., 2012).

The efficacy of drugs to combat fungal infections is limited, as 40 – 60 % of infected patients still die from the disease. There is a need for novel therapeutic approaches, and this necessitates a detailed knowledge of this fungal pathogens and of its pathogenesis (Sanglard and Odds, 2002).

Our group not only takes action into the discovery of novel antifungal agents but also participates into a better understanding of *C. albicans* pathogenesis. One of the key aspects in this research is to be able to undertake animal experimentations to test novel drugs or to follow the fate of infectionover time. This requires the implementation of *in vivo* imaging technologies like Bioluminescence Imaging (BLI) in animal facilities and to minimize the risk of secondary infections for the research models.

Material and Methods

Mice experiments

For all mice experiments, female BALB/c mice (7 weeks old; Charles River France) were housed in ventilated cages with free access to food and water. For mice infections, *C. albicans* was grown for 16 h under agitation at 30 °C, in yeast extract complete medium supplemented with 2 % glucose. Overnight cultures were washed twice with phosphate buffer saline (PBS) and resuspended in 5 ml PBS. The concentration of each culture was measured through optical density, and each strain was diluted to 2 x 10^6 cells/ml. Mice were injected through the lateral tail vein with 250 µl of a cell suspension.

Bioluminescence reporter

The Bioluminescence Imaging (BLI) reporter was constructed from a codon-optimized firefly luciferase (Mut0) (Jacobsen et al., 2014) and was mutated at site S284T and L295F. S284T is known to result in a red-shifting in light emission, while L295F contributes to thermal stability (Branchini et al., 2005; 2007). The rationale behind this idea was to reduce light absorption by tissues and hemoglobin, as reported (Papon et al., 2014). In a second round of mutations, 2 other substitutions were introduced (T214A and A215L) that were known to confer enhancement of luciferin-dependent reactions and a better resistance to body temperature as described (Branchini et al., 2007). The modified BLI reporter (Mut2) was expressed in C. albicans under the control of a strong constitutive promoter.

Non-invasive *in vivo* Imaging

For *in vivo* BLI, the In-Vivo Xtreme II (Bruker BioSpin MRI GmbH, Germany) was used. The system is equipped with a deeply-cooled CCD camera (-90 °C absolute) and a high-end lens with an f-stop of 1.1-16. For anatomical references, molecular BLI signals could be overlayed onto respective RGB-white-light or high-resolution, low-dose X-ray images. BLI signal intensities were quantified using ROI tools and given out as photons/second/millimeter square (ph/sec/mm²). BLI parameters were f/1.1, binning 8 x 8, 5 min exposure, and 19 cm x 19 cm FOV (field of view). X–ray imaging was performed with 5 second acquisitions at f/2.8, 19 cm x 19 cm FOV, and binning 2 x 2. A special Sealed Animal Tray was used to host SPF animals during imaging. HEPA filters provided ultra-filtered air and anesthesia inflow as well as filtered waste gas (WAG) exhaust. In the Sealed Animal Tray, an Equaflow Anesthesia nose-cone delivered equal Isoflurane amounts to all animals. A feedback regulated temperature probe controlled the temperature to secure animal welfare.

Details on the handling of the Sealed Animal Tray Setup can be watched also in this video.

The luciferase substrate used per mouse, 100 µL of D-luciferin firefly (18 mg/ml; Biosynth, Switzerland), was injected intraperitoneally 10 min before BLI.

Results and Discussion

The Bruker In-Vivo Xtreme II instrument offered the flexibility of multimodal *in vivo* imaging as well as a temperature controlled and sealed environment appropriate for containing mice with (fungal) pathogens (Figure 1). Moreover, BLI and X-ray imaging provided non-invasive, high-throughput imaging of pathogenic animals.

Figure 1

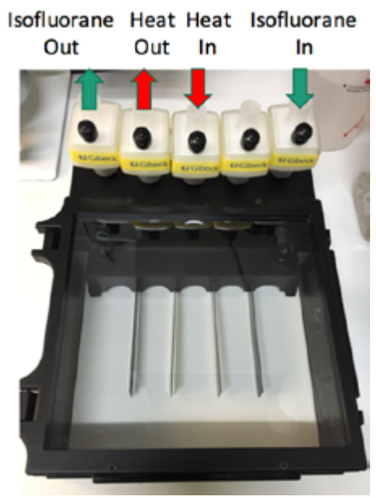

Fig. 1: Sealed Animal Tray for imaging Specific-Pathogen-Free (SPF) or disease animals, equipped with HEPA filters and a 5-fold Equaflow Manifold for homogeneous anesthetic gas support as well as Waste Gas (WAG) exhaust.

Bioluminescence from the firefly luciferase was used as a reporter system to detect *C. albicans* in mice. After intravenous injection of *C. albicans* in the mice bloodstream, animals were recorded repeatedly for BLI at different time points (6 – 72 h).

As shown in Figure 2, the area of bioluminescence emission corresponded to the kidneys: an organ known as being rapidly colonized by *C. albicans* in the systemic infection model. Based on our estimation of the sensitivity of the luciferase system and imaging equipment, we were able to detect down to 200 cells per infected kidney (Fig. 2). This amount of cells corresponds to about 50 photons/sec/mm². Numbers published in the literature for a comparable infection model and a same type of luciferase report 3600 cells per kidneys for 100 photon/sec/mm² (Jacobsen et al., 2014). We suggest, therefore, that the present system coupled with Bruker instrumentation increased sensitivity by a factor of 8, relative to the protocol used by Jacobsen et al., 2014. This is quite remarkable when taking into account that the animal chamber may represent an obstacle for the recording of emitted light by the instrument camera.

Figure 2

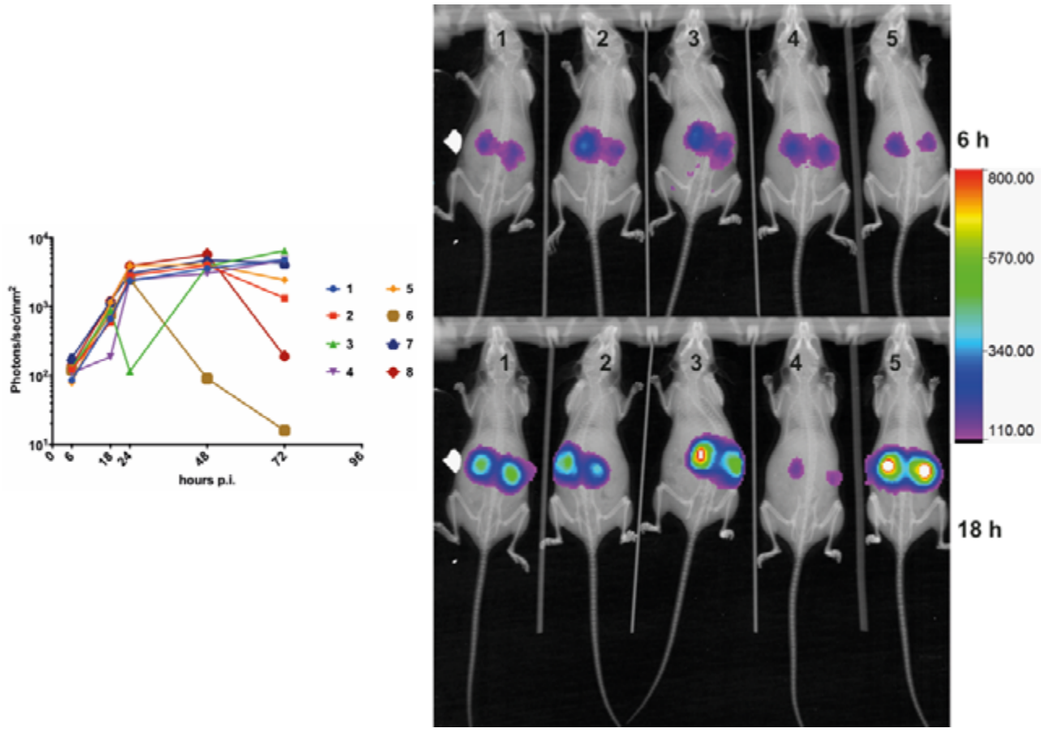

Fig. 2: BLI signals in the kidney of mice derived from infections with *C. albicans*. Animals were imaged out to 72 hours post-challenge, and emitted bioluminescence was quantified. Scale is given in photons/sec/mm². Animals 1-5 are shown at time points 6 and 18 h post-infection. Down to 200 cells per animal injected could be observed *in vivo*. Images originate from Dorsaz et al. (2017).

The model presented here was used to probe the efficacy of antifungal treatments with fluconazole or caspofungin, both of which proved to be successful as judged by decrease of luminescence signals as well as by fungal determination in treated mice (data not shown). The details of all data were recently published in Dorsaz et al., 2017.

Conclusion

The In-Vivo Xtreme II proved to be a robust system to assess the progression of *C. albicans* disease in mice. The use of a Sealed Animal Tray is convenient and decreased risks of cross-contamination in our animal facility, especially when several types of pathogens were tested for BLI. *In vivo* bioluminescent signal intensities were found to correlate well with effective fungal loads. In addition, X-ray imaging provided a means of precisely co-registering molecular signals with anatomical information. On these merits, the In-Vivo Xtreme II now serves our lab as a strong basis for current and future research projects.

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