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Topic: Microscopy & Advanced Imaging
Subtopic: In vitro & in vivo monitoring

TERMIS25_442 - Real-time imaging of tumor microenvironments implanted in avian embryos by using a platform integrating an imaging window and a synthetic eggshell

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Objectives: The chick embryo is a valuable model for studying the effects of new drugs on the vascular system, a knowledge crucial to understand many diseases as cancer, diabetes, and viral infections[1]. Conventional methods of intravital imaging require upright microscopes and invasive imaging windows, which limit accessibility of the vascular network. We developed a novel platform (Eggs&Beacon) that uses a synthetic eggshell to house avian embryos and allows intravital microscopy using commercial inverted microscopes to study the embryo reaction to external implants along time. In this work, we traced the development of a xenogeneic cancer implant and elaborated a predictive model of its druggability based on real-time nonlinear microscopy acquisitions (multiphoton and second harmonic generation, SHG).

Methods: We fabricated a synthetic eggshell (composed of a permeable membrane, a plastic case and an imaging window) using additive manufacturing techniques. This platform housed embryos on an inverted motorized microscope stage (Ti-2, Nikon). This enabled the spatial orientation of the embryo and the exposure of its blood vessels to the microscope objective, so we repositioned and imaged the same embryo at different time-points (days7-14). The imaging window, a fluidic device that we imaged with a long-working distance objective (40X,NA=1.1,Nikon), gave us the possibility to flow fluids targeting the vessels with drugs. Embryos were implanted at day 4 with human breast cancer cells (MDA-MB-231) and treated with anticancer drugs administered topically. We developed predictive numerical models of embryo oxygenation through the membrane and of drug diffusion through its vasculature (COMSOL).

Results: Working prototypes of permeable membranes were realized with a silicone-like texture and a thickness of 250µm, which enabled the embryos to survive till day14 (n=3). The Eggs&Beacon platform let expose the embryo vasculature to the microscope objective (days 7-14), without compromising the embryo viability and sterility. We imaged and quantified the vasculature of the embryonic foreign body reaction to the cancer implant and the infiltrated tumoral cells by performing nonlinear microscopy (Z=600µm, $\lambda_{exc/em}$=800/450nm). SHG($\lambda_{exc/em}$=900/450) showed the collagen I matrix amount and directionality (days 12-14). The computational model predicted the concentration of anticancer drug diffused in the tumor microenvironment to optimize the dose administered to the organism.

Conclusions: We quantified real-time vascular dynamic parameters, changes in vascularity and collagen production at different time-points in living avian embryos implanted with xenogeneic cancer models. Our platform offered a unique versatile tool to evaluate efficacy and safety of drugs targeting vascular system in vivo. This will be employable in every biological laboratory. The platform reduces the animal replicates needed for in vivo studies, providing reliable readouts related to the systemic and

microvascular response to drugs merging live imaging and computational predictions. Our tests indicate the device as compatible with various inverted fluorescence microscopes. We are working on correcting optical distortions by adding index matching fluids to the imaging window.

References

[1] Conci et al. 2023, DOI:10.1063/5.0165411

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