INTRAVITAL OBSERVATION OF BIOENGINEERED AVIAN EMBRYOS USING NONLINEAR MICROSCOPY AND MAGNETIC RESONANCE IMAGING

Claudio Conci [(1)], Leonardo Cherubin[(1)], Matteo Laganà[(2)], Chiara Martinelli[(1)], Domenico Aquino[(3)], Federica Mazzi[(3)], Emanuela Jacchetti[(1)], Manuela T. Raimondi[(1)]

1. Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy; 2. Gemma Prototipi, Italy; 3. Neuroradiology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy.

INTRODUCTION

The chicken embryo is a valuable model for studying the effects of drugs or biomaterials on the vascular system, which is a primary player in diseases as cancer, diabetes, etc. Conventional methods for intravital imaging require upright microscopes, which limit repeatability and accessibility of the vascular network [1]. We developed an innovative platform (Eggs&Beacon) that uses a synthetic eggshell to house avian embryos, and it allows to perform intravital microscopy on inverted microscopes. We tested and confirmed platform efficacy and suitability for culturing avian embryos. In this work, we demonstrated the feasibility of performing multimodal imaging, joining nonlinear microscopy (TPEF, SHG) and MRI at the same time-point.

MATERIALS AND METHODS

We created an artificial eggshell (silicone-like membrane) with a thickness assuring a sufficient oxygen influx and transparency for long-term survival and imaging of embryos. Membranes are supported by a plastic case (stereolithographic printed, Form 3B+, Formlabs) made of a body, a cap and an imaging window (Fig.1a). This platform was designed to fit embryos on inverted microscope stages (e.g. Eclipse Ti-2, Nikon). The orientation of the embryo can be changed in any time to expose blood vessels to the microscope lens. We observed the same embryo at different time-points (EID 7,12). The imaging window is a millifluidic device imaged with a long-working distance water immersion objective (40X, NA=1.1, WD=0.8mm) where fluids can be used to correct optical distortions. We performed also intravital imaging with an MRI setting (Bruker, Ettlingen) on a 7T scanner (BioSpec 70/20), with a 72mm 1H linear radiofrequency coil at EID10-12. T2-RARE weighted sequence echo time=100ms; rep times = 8400ms; FOV=46x46mm; slice=1mm.

RESULTS

Working prototypes of oxygen permeable membrane were successfully realized thanks to additive manufacturing techniques. The realized membranes (thickness=250µm) enabled the embryos to survive until EID 12 (n>10, Fig.1a). The Eggs&Beacon platform allowed to expose the vasculature to the microscope objective, without compromising the embryo's viability and sterility (Fig.1b-e). We imaged and quantified the vasculature with TPEF microscopy (Z=600µm, λexc=800nm, λem<450nm, Fig.1c,e), which allowed us to detect newly formed capillaries. SHG (Z=600µm, λexc=900 nm, λem=450/55nm, Fig.1e,f) showed collagen I matrix development and enabled us to measure the amount and direction of the fibres (EID12 - 14). By MRI, we quantified the microvascular network (Fig.1e) and measured the chicken embryo's body (Fig.1d) at different timepoints.

DISCUSSIONS

Our innovative and patented platform allowed multimodal imaging of a living organism, until advanced stages of development (EID12), in preclinical settings and at multiple time-points. Our platform can potentially reduce the number of animals required for intravital analyses, producing robust data on fibrosis. This platform will help to study systemic and microcapillary responses to anti-angiogenic drugs, etc. Our tests showed the platform worked with inverted microscopes and MRI settings. We are now reducing optical aberrations by exploiting the imaging window. This will enable us to quantify changes in blood vessel density and collagen production in time in untreated conditions and in response to drugs. Our platform offered a fast and versatile way to evaluate the efficacy and safety of new pharma in pathological models, by targeting the vascular system *in vivo*, with drastically reduced use of adult animals.

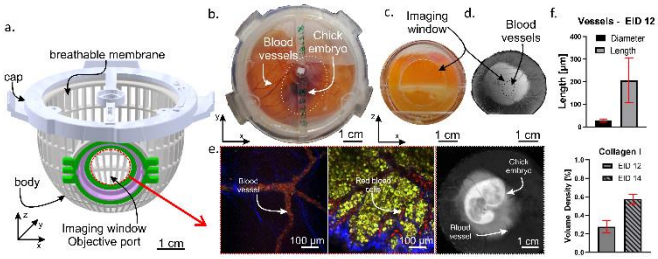

Figure 1: *(a)Eggs&Beacon, made of a plastic eggshell, a permeable membrane, a plastic casing, a cap and an imaging window. (b) Top, Chick embryo at EID12. (c-d) Lateral view photo and MRI through the imaging window. Microscopy/MRI images of the capillaries (TPEF, red – MRI, black) and of the collagen matrix (SHG, blue) Z=600µm, EID12. (f) Data obtained from live images.*

References

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

Acknowledgements

ERC, project BEACONSANDEGG, G.A. 101053122