Establishing an *ex vivo* Glioblastoma Brain Slice Model for Tumor Growth and Behaviour Analysis

Katharina Fuchs[1,*], Clara Keller[1], Bianca Layer[1], Katja Nadler[2], Ellaine Salvador[1], Tobias Weigel[2], Bastian Christ[2], Sofia Dembski[2], Ralf-Ingo Ernestus[1], Mario Löhr[1], and Carsten Hagemann[1]

[1] *University Hospital Würzburg, Department of Neurosurgery, Section Experimental Neurosurgery, Würzburg, Germany*
[2] *Fraunhofer Institute for Silicate Research, Translational Center for Regenerative Medicine, Würzburg, Germany*

BACKGROUND

Organotypic cultures of brain slices are well-established and important 3D models in neuroscience, especially in cancer research. Unfortunately, many 3D models require adult animals or focus on distinct parts of the brain, e.g. the hippocampus. Therefore, we established a whole brain slice model that conserves the complex cellular brain-architecture with its different cell types. In addition, we reduce required animal numbers by sectioning the brain of 6-to-9-day postnatal old mice into several adjacent 300 µm thick slices.

OBJECTIVE

This study aimed to establish a 3D organotypic GBM model, which is stable for a minimum of 7 days to investigate tumor growth and invasion.

METHODS

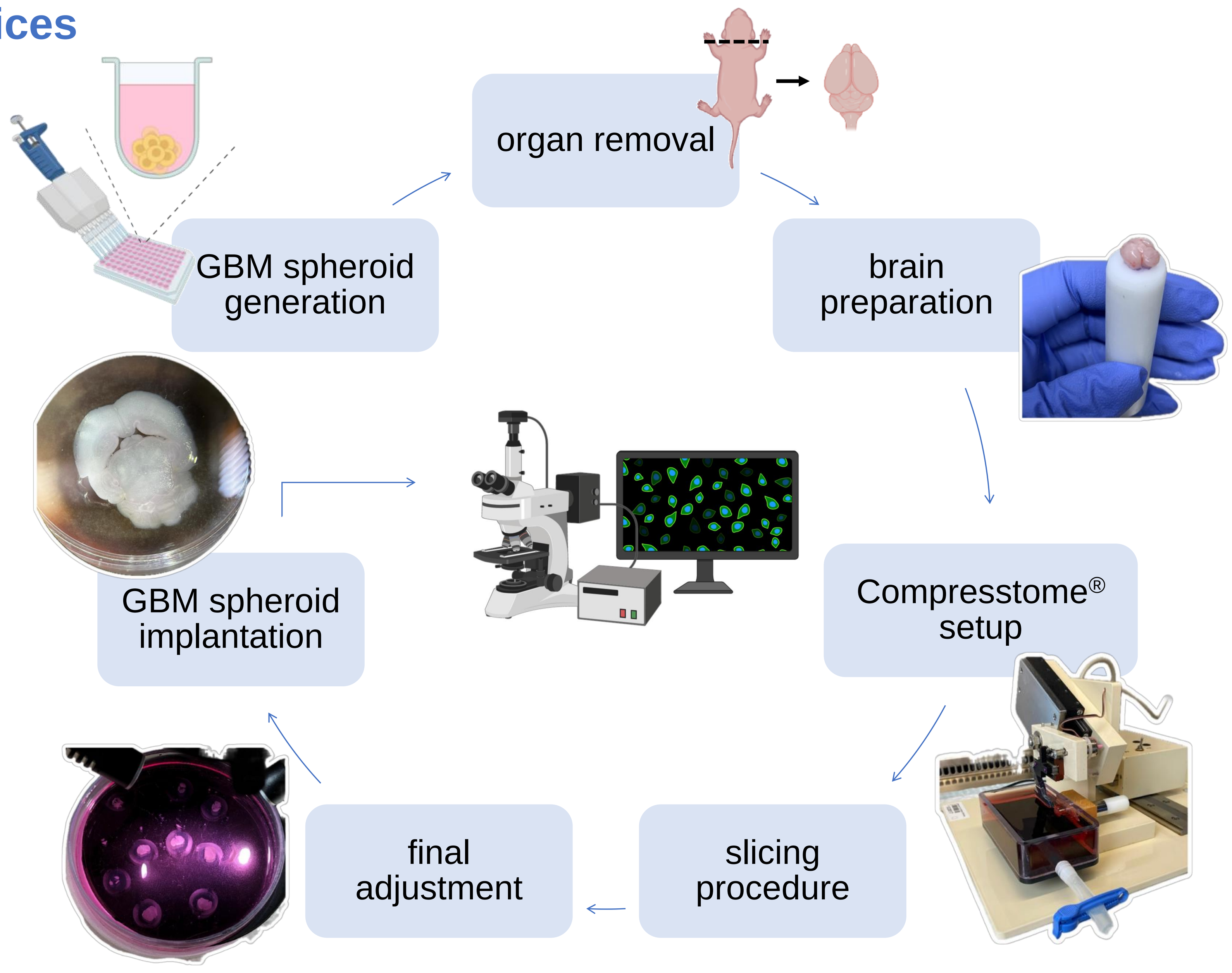
The *ex vivo* 3D GBM model was assembled as follows:

Fig. 1: GBM spheroids were generated 3-4 days prior to preparation day of the slices and two of the spheroids were seeded onto each slice. (Generated with BioRender)

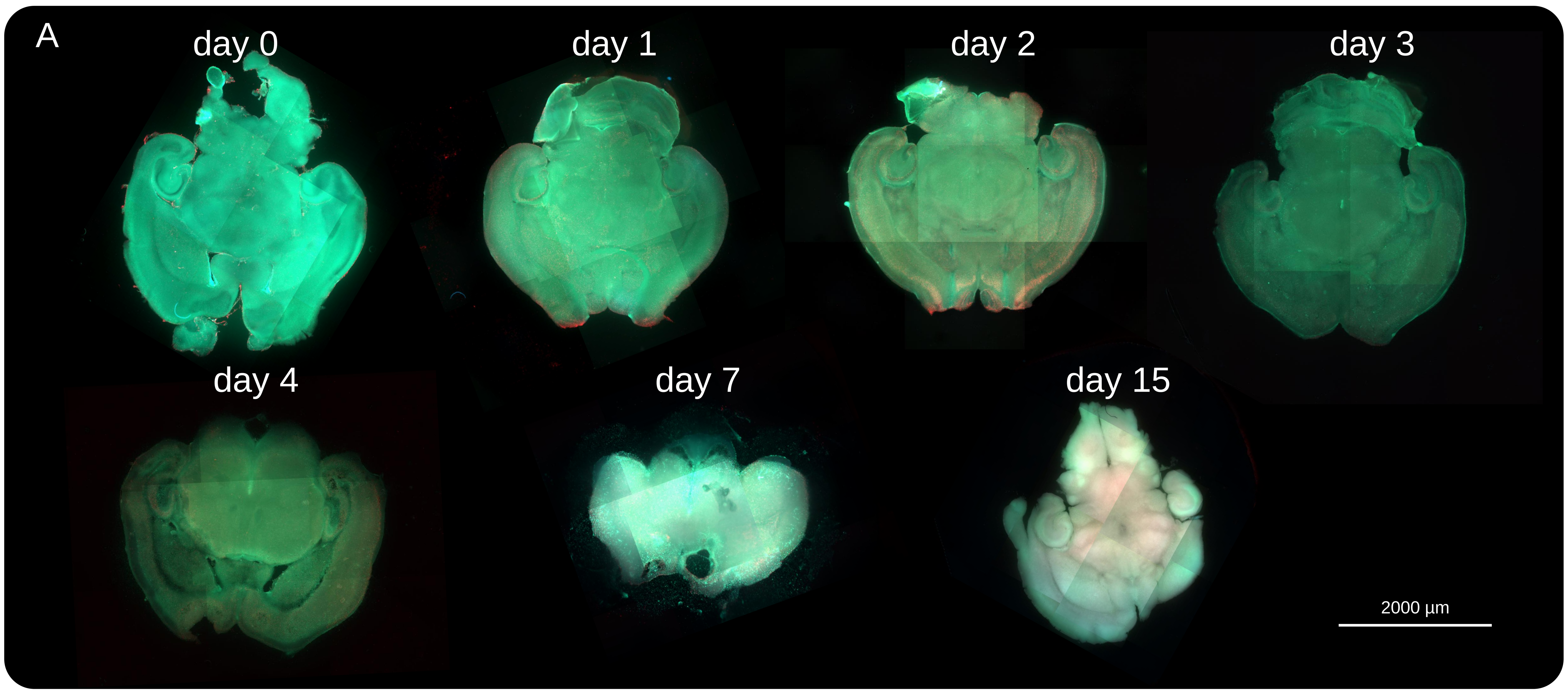
Mice brains 6-9 days post partum were sectioned at 300 µm with a Compresstome®. Tissue viability and tumor invasiveness was analysed by Live/Death staining and alamarBlue™ assay, respectively, based on fluorescence signals.

Step-by-Step preparation of the whole brain slices

Fig. 2: Principle of the slicing procedure.
GBM spheroids were generated 3-4 days prior slicing in a BIOFLOAT™ 96-well plate. On preparation day mice 6-9 days post partum were decapitated and the brain was removed. The tissue was carefully glued onto a holder. Freshly prepared hot agarose solution (55 °C) was poured over the tissue and curing accelerated with pre-cooled metal pliers (-80 °C). The holder was positioned in the Compresstome® and the tub filled to the edge with 4 °C pre-cooled preparation medium. With an oscillation of 6 and an advance between 3 to 4, 300 µm thick sections were cut and transferred carefully into a petri dish containing pre-cooled preparation medium. Under sterile conditions and utilizing a stereo microscope, the agarose was removed from the brain slices, which were transferred onto inserts swimming in culture medium. One to two GBM spheroids were placed onto each hemisphere of each slice, and culture took place at 37 °C, 5 % CO_2 and 95 % humidity.
(Figure partly created with BioRender)



GBM spheroids merge with the organotypic brain slices over a peroid of 15 days



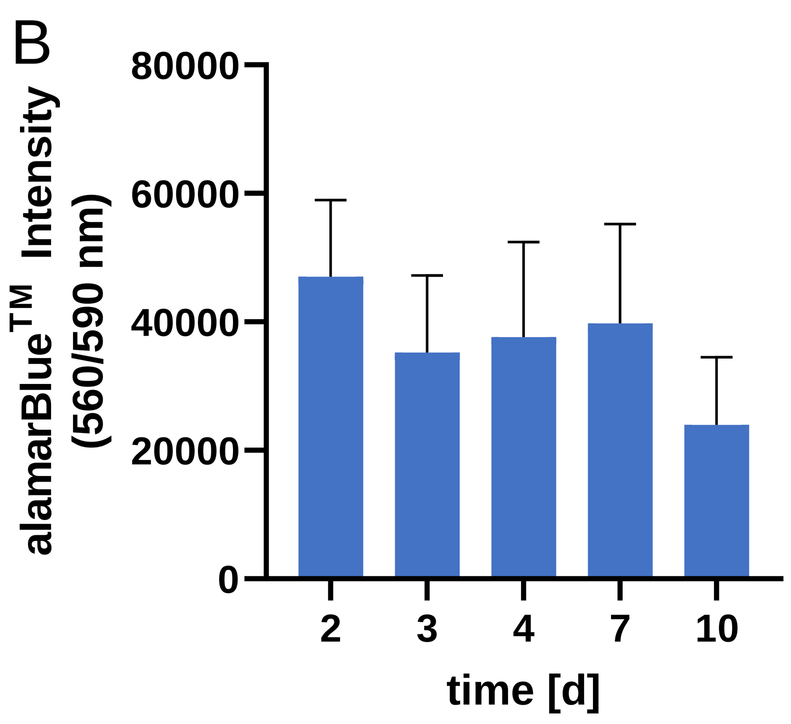
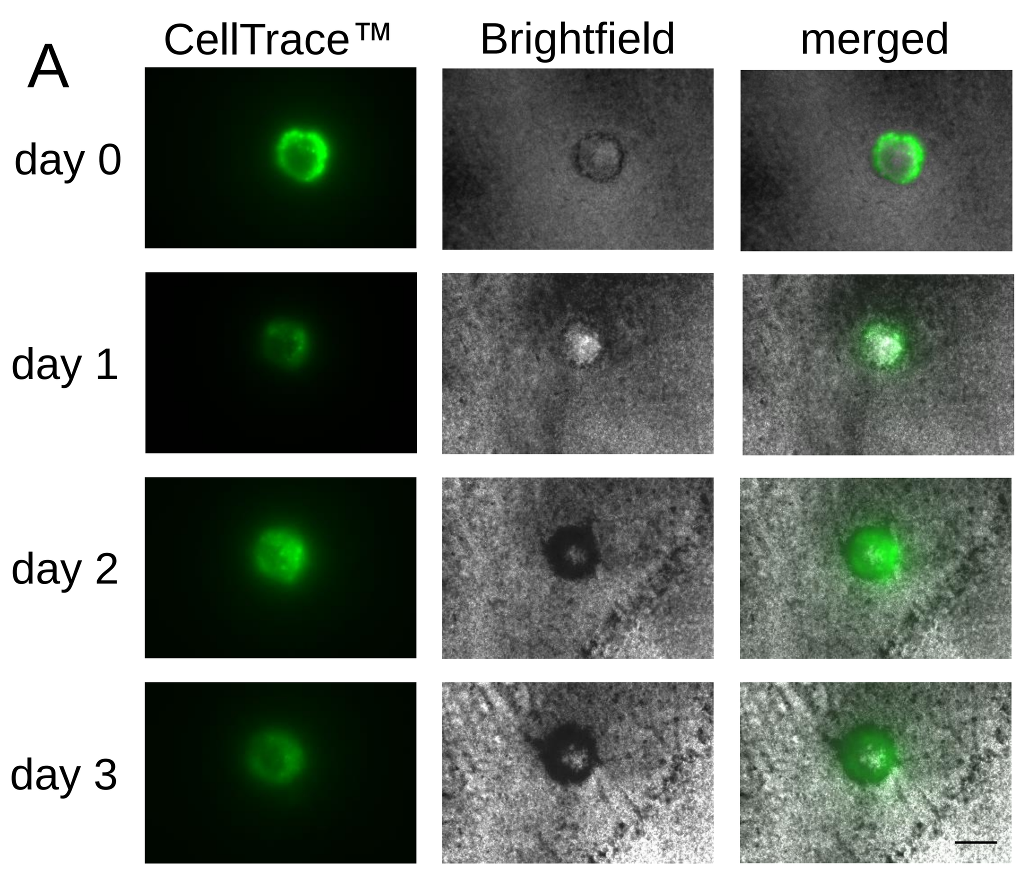


Fig. 3: Viability of the whole brain slices verified by Live/Death staining and alamarBlue™ assay over a time period of 15 days.
A The complex structures of the native brain could be successfully preserved and slices displayed only minimal decrease in viability. FDA (green) stains living cells, GelRed (red) dead cells and Hoechst (blue) cell nuclei. Imaging was performed with the Zeiss Axio Imager M2 with 2.5x magnification and 2D-tile recording. Representative images of n = 8. **B** After an incubation time of 24 h alamarBlue™ intensity was measured in a microplate-reader with an extinction of 560 nm and an emission of 590 nm (n = 6).



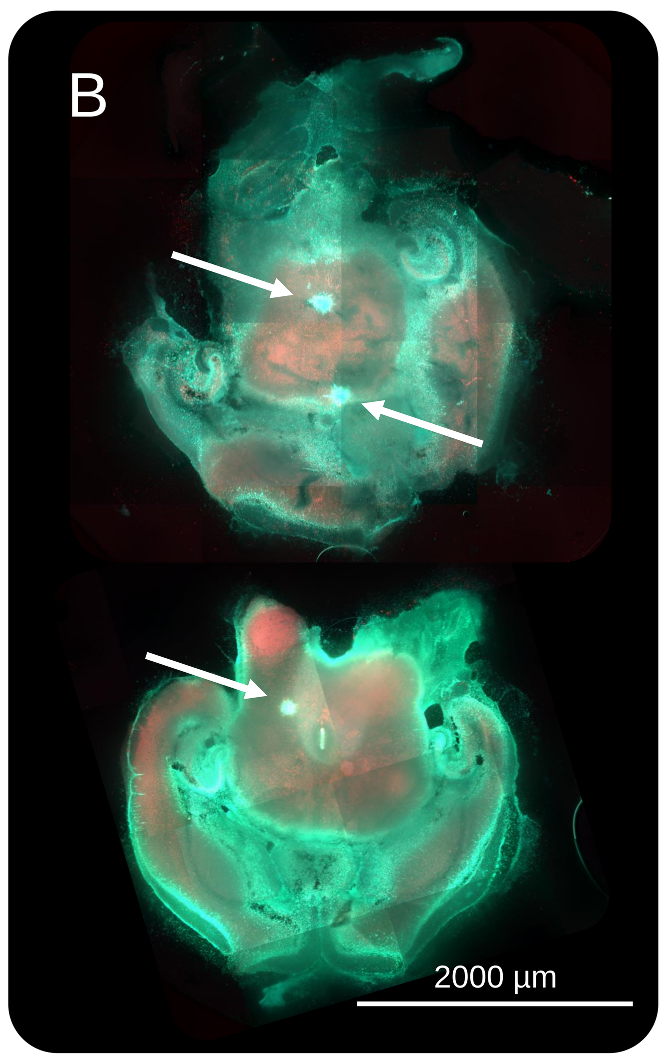


Fig. 4: Viability of the 3D GBM model after 96 h co-culture with GBM spheroids.
A GBM spheroids grown on whole brain slices and stained with CellTrace™ CFSE Cell Proliferation Kit to verify their growth and invasion. Representative images from n = 6. Magnification = 10x. Scale bar = 200 µm. **B** Images show the influence of spheroid growth on whole brain slice viability, which decreased (red area). Arrows point to spheroids. Representative images from n = 8. Magnification = 2.5x with 2D-tile recording.

CONCLUSION

A solid 3D GBM *in vitro* model should preserve the complex organotypic brain and tumor structure. Our model meets these requirements and was stable for at least 7 days. Therefore, it might be a useful tool for brain cancer research.

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Fraunhofer ISC
Uniklinikum Würzburg

*correspondence to:
Katharina Fuchs, M.Sc
fuchs_k6@ukw.de