3D *in-vitro* model to test chemotherapeutic drugs for glioblastoma treatment

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Background

Drug-testing often is performed utilizing two-dimensional (2D) cell cultures. However, 2D models are only suitable to a limited extent because of low histological complexity and functionality. Three-dimensional (3D) cell culture models represent an improvement due to the ability of migration of the cells which enables a more realistic investigation of the efficacy of chemotherapeutic agents. We have developed a 3D cell culture model based on decellularized porcine small intestine submucosa and serosa (SISser) as biological matrix. Suitability of the SISser matrix was tested by assessing the cytotoxic effects of cisplatin on a human glioblastoma (GBM) cell line.

Methods

The porcine intestine was removed, decellularized and the SISser matrix was mounted onto metal crowns (Fig. 1A-C). The human GBM cell line U87 was seeded onto the outer layer of the intestine (serosa). Cells were left to grow for 10 days and treated for 48 h with 5 µM and 10 µM Cisplatin, respectively (Fig 1D).

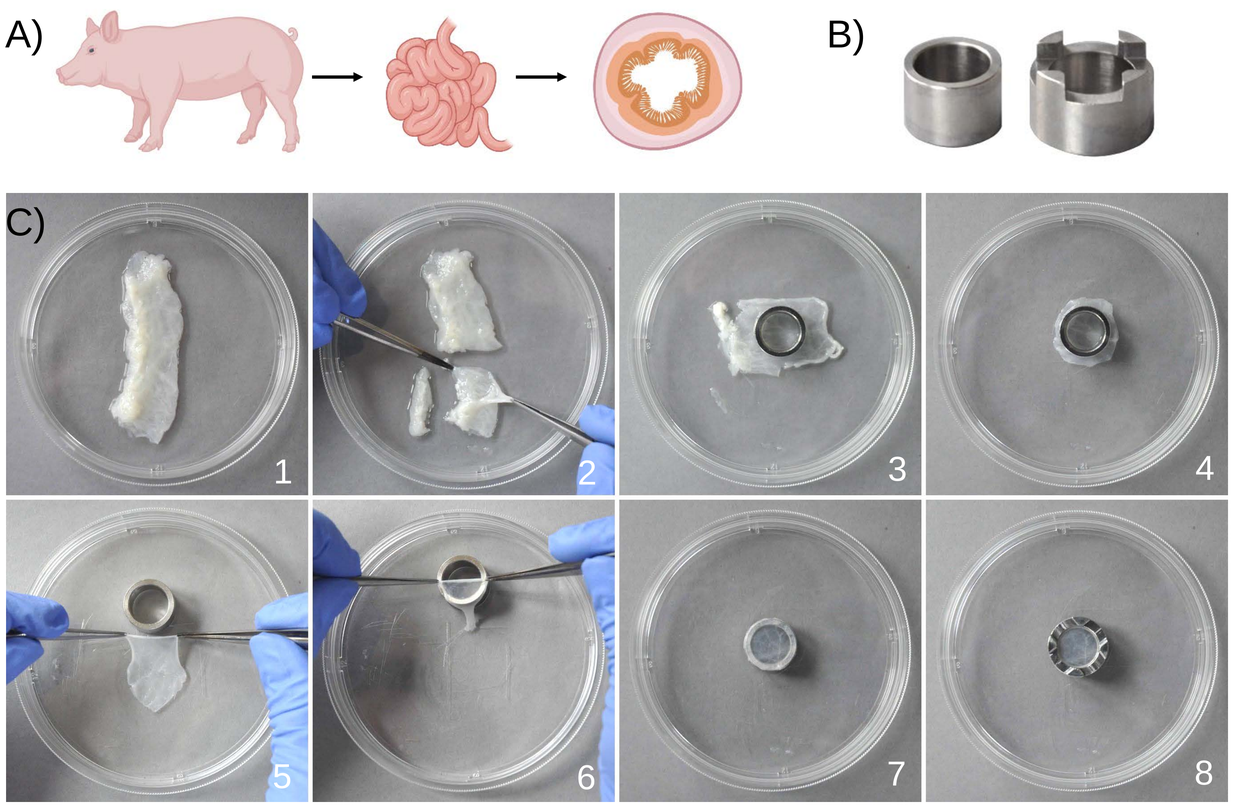

Fig. 1 Preparation of the SISser crowns and experimental setup.
A) Porcine intestine was decellularised and the serosa/ mucosa further used. B) Metal ring and crown. C) Fixation of the SISser between the metal ring and crown. (1) The porcine intestine was spread. (2) The lateral hem was removed so that the intestine could be unfolded. The serosa faces downwards. (3-4) The ring was placed on the intestine and the protruding intestinal matrix was removed. (5-7) The serosa was mounted onto the metal ring. (8) In the last step the crown was placed onto the ring, the serosa was stretched and thus fixed. D) Experimental setup. Seeding of the cells, ingrowth for 10 days, cisplatin treatment for 48 h, and Formalin fixation.

Paraffin sections were prepared prior to immunofluorescence staining. Antibodies against glial fibric acid protein (GFAP) and γH2AX enabled visualisation of cells and cisplatin-induced DNA double-strand breaks. Analysis was performed with a custom-developed macro in Image J.

Results

We successfully established a 3D *in-vitro* cell culture model and compared the results to 2D-cell culture experiments. (Fig. 2) There was no statistically significant difference between 5 µM and 10 µM cisplatin treatment. However, whereas in the 2D cell culture model the treatment led to a 2.2 (5 µM) to 3.4 (10 µM) fold increase in DNA double-strand breaks compared to the untreated control ($p = 0.0031$ and $p = 0.0018$, respectively), the 3D culture model revealed a 7 ($p = 0.0043$), and 7.7 ($p = 0.0025$) fold increase, respectively (Fig 2).

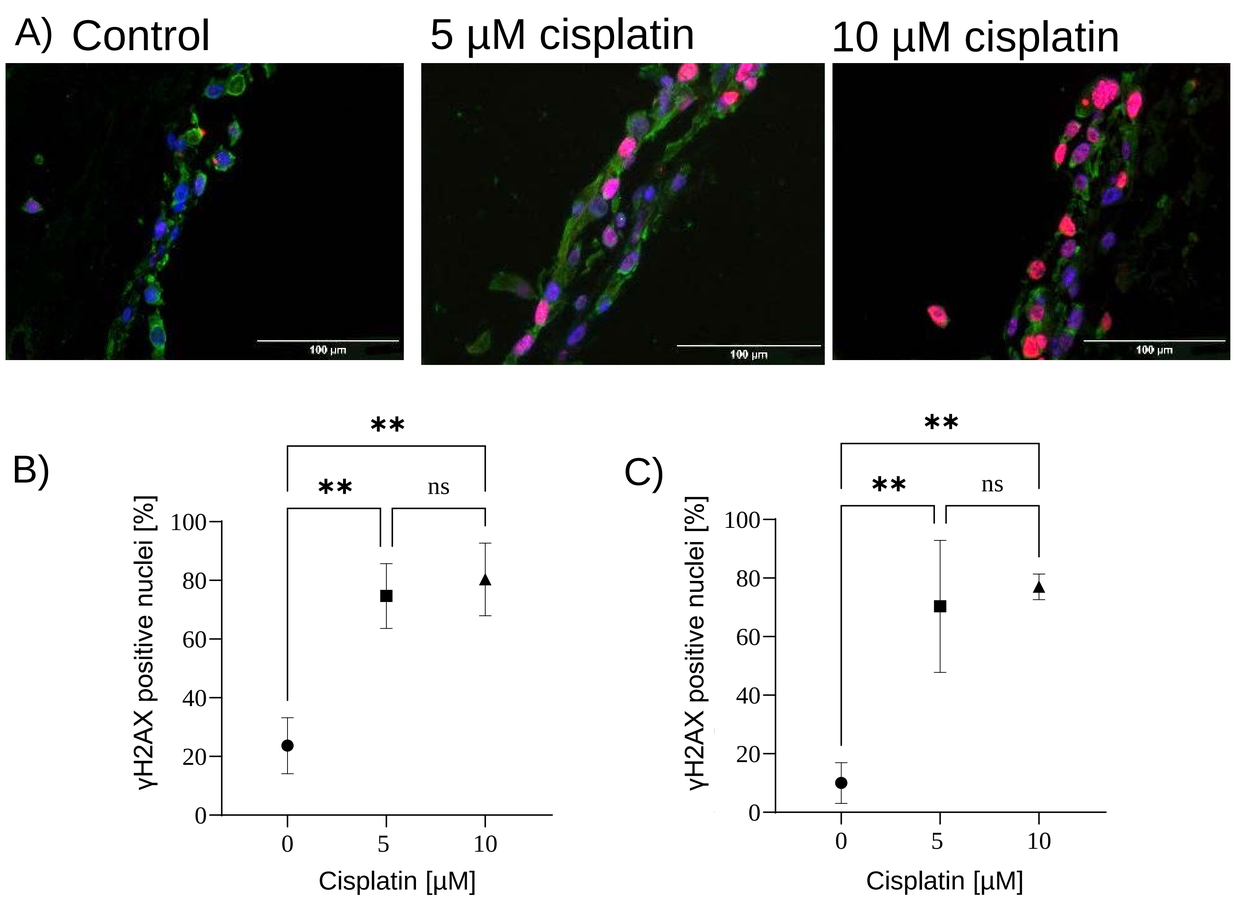

Fig. 2 Treating of U87 GBM cells led to a significant increase in DNA double-strand breaks.
A) Fluorescence micrographs of cells growing in the 3D culture model. DAPI (blue), GFAP (green), γH2AX (red). Representative images of n = 18. Size bar = 100 µm. B) Quantification of γH2AX positive nuclei in 2D cell culture and C) in the 3D cell culture model. ns = not significant, ** $p \leq 0.01$. n = 18.

Conclusion

Our proof of principle experiment indicates that porcine serosa is well-suited as 3D GBM cell culture matrix and that this 3D cell culture model could serve as basis for testing GBM chemotherapeutics. Therefore, the SISser model has the potential to be a supplement to 2D cell culture experiments to confirm results in a 3D setting prior to animal testing.

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