FACULTY OF MEDICINE AND HEALTH SCIENCES

HETEROCELLULAR 3D SCAFFOLDS AS BIOMIMETIC OF PERITONEAL METASTASIS *IN VITRO* AND *IN VIVO*

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Introduction

Peritoneal carcinomatosis is a major source of morbidity and mortality in patients with advanced abdominal neoplasms. Intraperitoneal chemotherapy (IPC) is an area of intense interest given its efficacy in ovarian cancer. However, large peritoneal metastasis with adequate blood flow have high interstitial fluid pressure, which inhibits intratumoral drug distribution. To study drug penetration and its influencing factors reliable *in vivo* models are crucial that mimic peritoneal metastasis.

Heterocellular 3D scaffolds: *in vitro* culture

Poly-lactic acid scaffolds of 0.1cm^3 were coated by gelatin and were seeded by combinations of ovarian cancer cells (SK-OV-3 Luc eGFP, 2.10^6) with cancer-associated fibroblasts (CAF, 8.10^6). Viability of these tumor scaffolds was longitudinally monitored by bioluminescent imaging (BLI) and assessed by end-point live/dead staining. Cancer cell-CAF organization in the scaffold was visualized by histology, scanning electronic microscopy (SEM, fig.1a) and confocal microscopy (fig.1b). CAFs are essential for 3D organized spheroid growth in the scaffold.

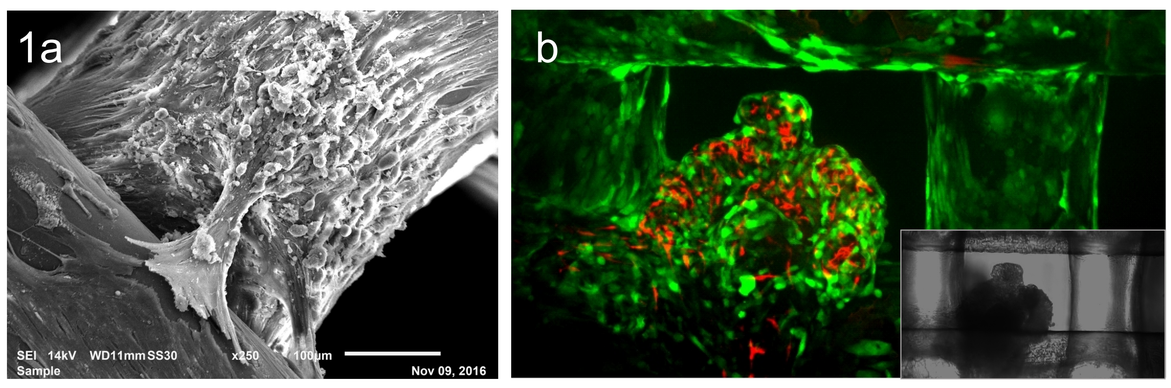

Fig.1: *in vitro* culture of heterocellular 3D scaffolds

a) SEM; Cells adhered to the 3D scaffolds are follow the print direction of struts. b) Confocal microscopy; CAFs (red) are organized into spheroids between the pores. While SK-OV-3 Luc eGFP (green) cancer cells grow on top the scaffolds struts and on the CAFs spheroid.

In vivo implanted scaffolds biomimic peritoneal metastasis

After 3 weeks of *in vitro* culture the tumor scaffolds were intraperitoneally implanted to the peritoneal wall (fig.2a). Tumor scaffolds become vascularized and show cancer cell growth proportional with vascularization as evidenced respectively by contrast-enhanced µCT (fig.2b) and bioluminescence monitoring (fig.2c and e). Histological analysis revealed infiltration of host fibroblasts, inflammatory cells, small and large pericyte-covered blood vessels; all histological aspects which show remarkable similarities to size-comparable peritoneal metastasis in ovarian cancer patients (fig.2d).

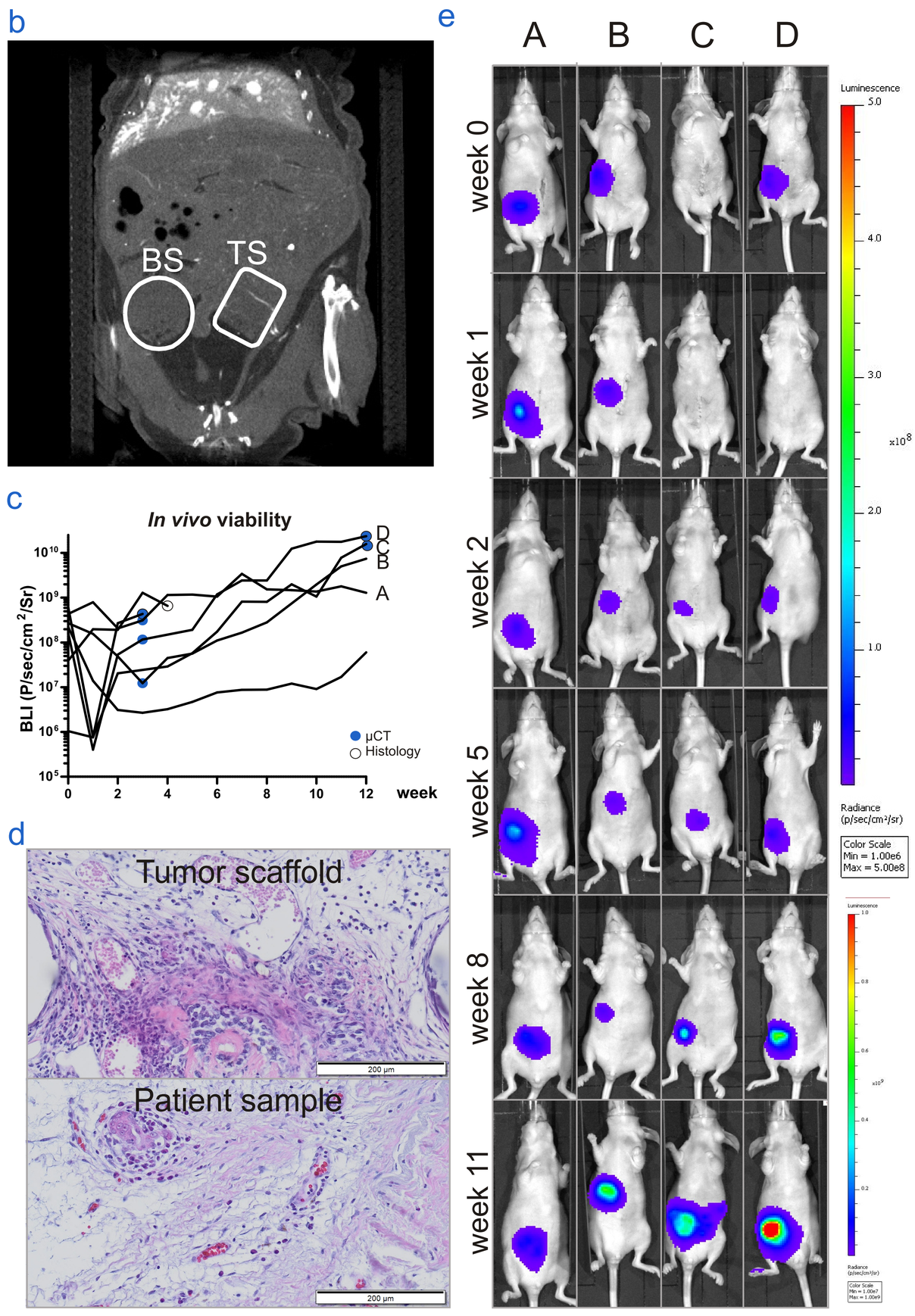

Fig.2 *in vivo* implantanion and monitoring of heterocellular 3D scaffolds

a) Timeline of *in vivo* experiments with images of a scaffolds before and during implantation, µCT, BLI and histology. b) Contrast-enhanced µCT 3 weeks post implantation: the TS (tumor scaffold) but not the BS (blanc scaffold, no cells were seeded) shows functionally active, infiltrated blood vessels. The two scaffolds are differently orientated. Bioluminescence of some individual mice are shown in e) and curves are represented in c) with marked time points of µCT and histology. D) shows H&E staining of a tumor scaffold 4 weeks post-implantation (upper panel) and a peritoneal metastasis of an ovarian cancer patient (lower panel)

Conclusion

In vitro cultured heterocellular 3D scaffolds becomes functionalized *in vivo* by the host. The newly formed tissue remarkably biomimics a peritoneal metastasis of an ovarian patient. This model opens new opportunities for therapy evaluation for peritoneal carcinomatosis and its tumor environment. In a next stage we will explore the possibility of drug penetration/efficacy monitoring in this model by testing intraperitoneal delivery of cisplatin in combination with pazopanib, an experimental VEGFR/PDGFR/FGFR inhibitor showing benefit in phase II/III studies of metastasized ovarian cancer .

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