IN VIVO VISUALIZATION OF VESSEL FORMATION IN PERITONEAL TUMOR SCAFFOLDS WITH MICRO-CT

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Introduction

Peritoneal carcinomatosis is a major source of morbidity and mortality in patients with advanced abdominal neoplasms. Intraperitoneal chemotherapy is an area of intense interest given its efficacy in ovarian cancer. However, large peritoneal metastases (>0.5cm^3) with adequate blood flow have high interstitial fluid pressure, which inhibits intra-tumoral drug distribution. To study drug penetration and its influencing factors, reliable *in vivo* models that mimic peritoneal carcinomatosis are crucial. By applying tissue engineering we successfully bio-mimicked a peritoneal ovarian tumor and its environment in mice. Functional blood vessel formation was evaluated with contrast enhanced µCT.

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.

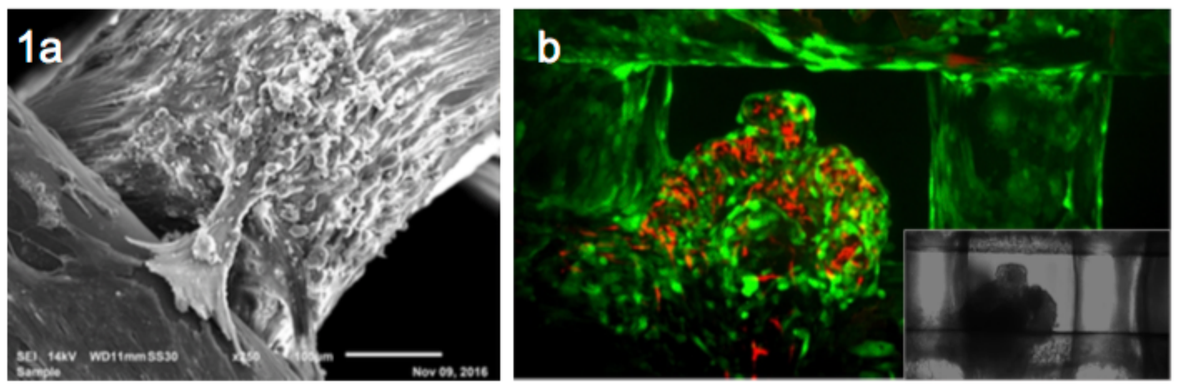

*Figure 1: in vitro culture of heterocellular 3D scaffolds. a) SEM; Cells adhered to the 3D scaffolds follow the print direction of struts. b) Confocal microscopy; CAFs(red) are organized in spheroids between the pores, while cancer cells (green) grow on top of the scaffolds struts and on the CAFs s*pheroid*.*

In vivo implanted scaffolds become vascularized

After 3 weeks of *in vitro* culture, the tumor scaffolds (TS) and blank scaffolds (BS) were intraperitoneally implanted to the peritoneal wall (fig. 2a). Tumor scaffolds became vascularized as evidenced with Exitron12000 (Miltenyi Biotec) enhanced high-resolution µCT (X-CUBE, MOLECUBES NV) with an acquisition time of 4 minutes. Functional blood vessels from the peritoneal wall enter the tumor scaffolds, visible *in vivo* on µCT and *ex vivo* after dissection (fig. 2b, 2c, respectively). 3D renders of the dissected TS were obtained. Quantification was performed by calculating vessel volume (mm^3) on *in vivo* µCT data (2e). More vessel formation in TS compared to BS was observed.

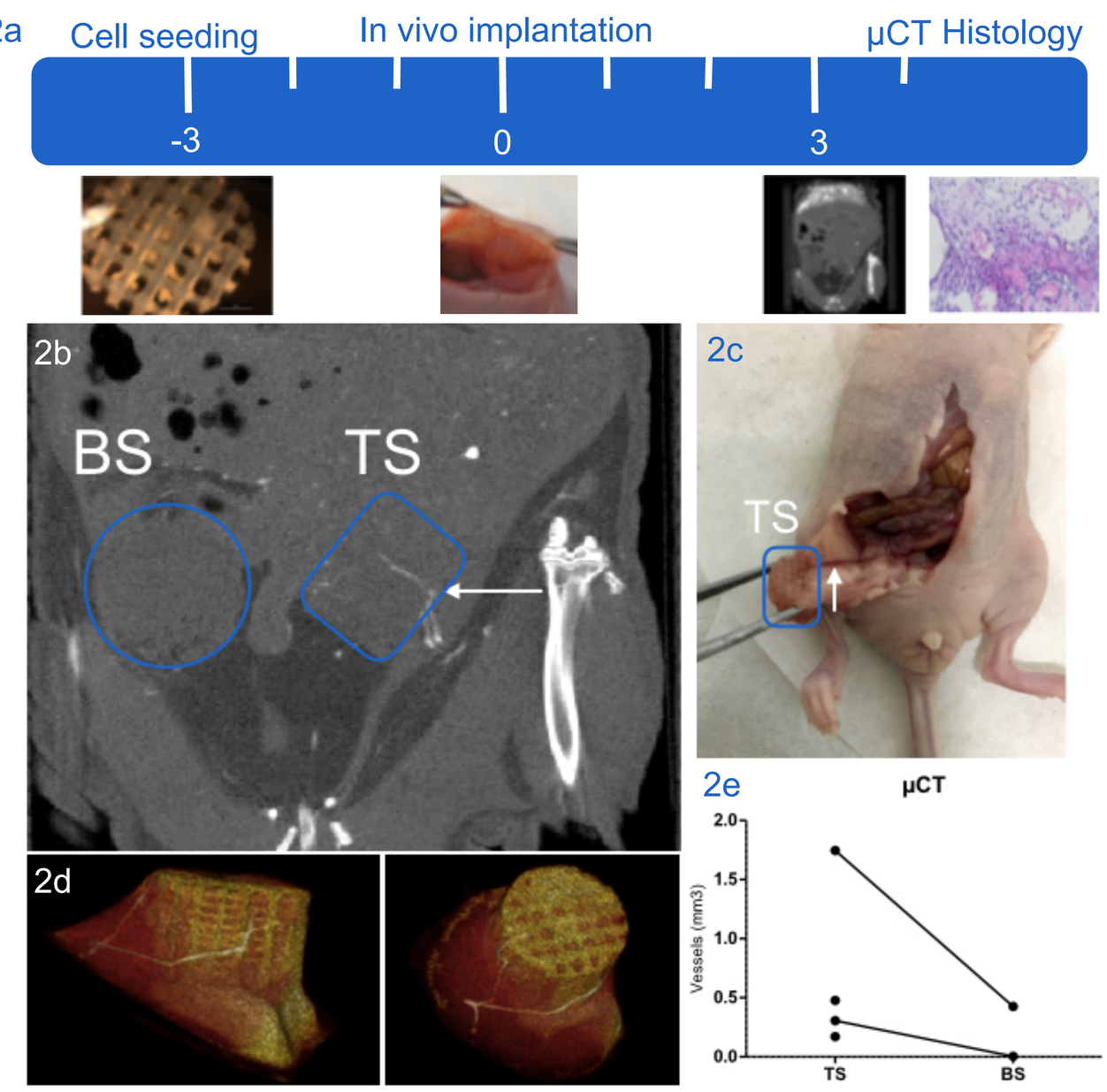

Figure 2: In vivo implantation and monitoring of heterocellular 3D scaffolds. a) Timeline of in vivo experiments; b) contrast enhanced µCT 4 weeks post implantation; c) corresponding animal after dissection; d) 3D render of dissected TS; e) vessel volume of TS compared to BS.

In vivo implanted scaffolds bio-mimic peritoneal metastasis

Histological analysis revealed infiltration of host fibroblasts, inflammatory cells, small and large pericyte-covered blood vessels. All histological aspects show remarkable similarities to size-comparable peritoneal metastasis in ovarian cancer patients. However, few similarities can be detected with the classical animal model with IP tumor, in which less blood vessels and necrosis in the center is observed (fig. 3).

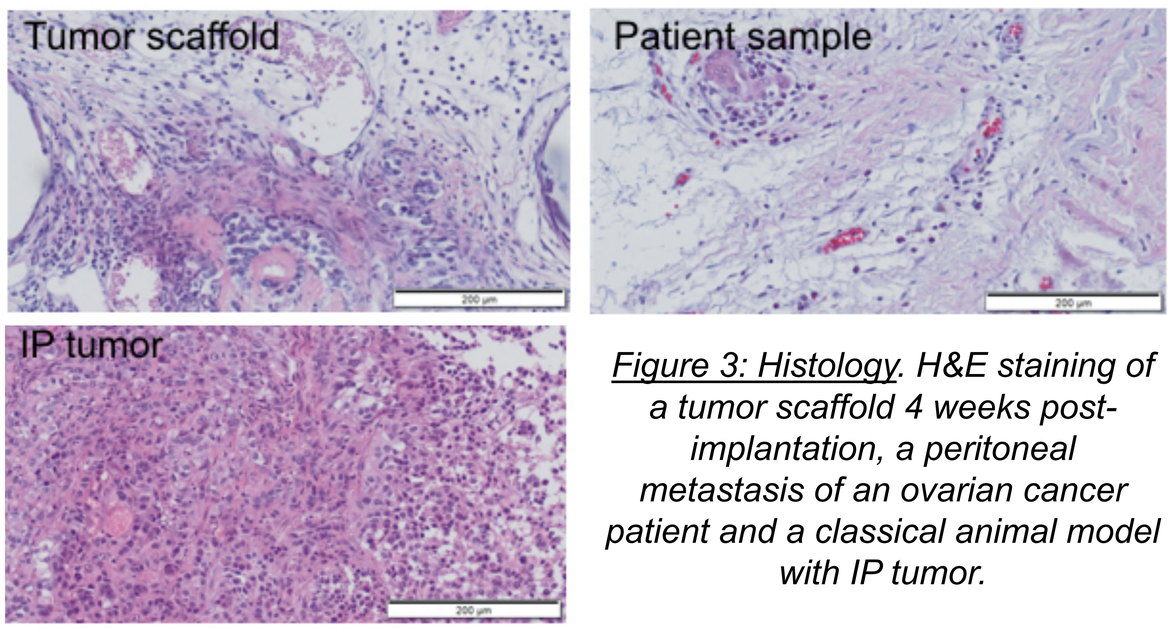

Figure 3: Histology. H&E staining of a tumor scaffold 4 weeks post-implantation, a peritoneal metastasis of an ovarian cancer patient and a classical animal model with IP tumor.

Conclusion

We can conclude that µCT imaging is a useful tool to assess vessel formation in tumor scaffolds. The tumor scaffolds become vascularized and show cancer cell growth proportional with vascularization as evidenced by contrast-enhanced µCT. This model opens new opportunities for therapy evaluation of peritoneal carcinomatosis and its tumor environment.

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