

Supplementary Material

Multicellular spheroid based on a triple co-culture: a novel 3D model to mimic pancreatic tumor complexity

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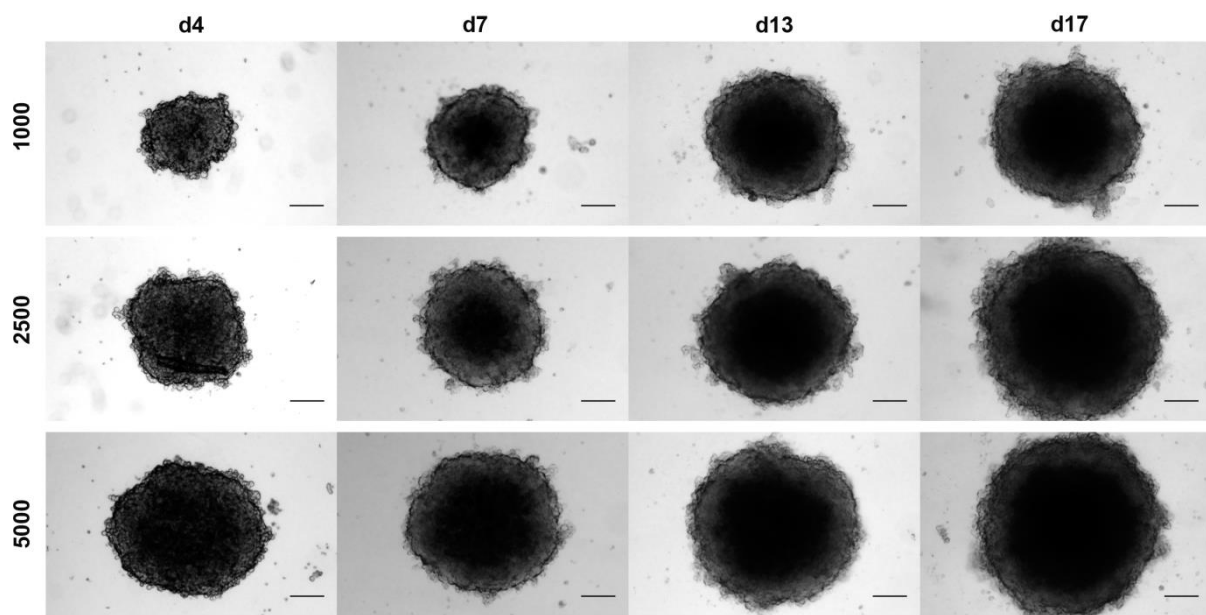


Fig. S1. Representative optical imaging of PANC-1 spheroids (seeding density of 1000, 2500 or 5000 cells per well) at days 4, 7, 13 and 17 post seeding. Scale bars: 200 μm .

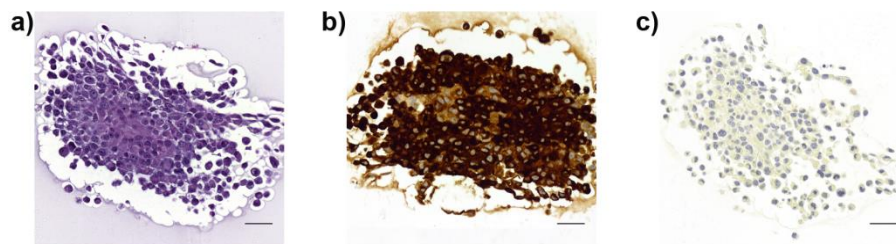


Fig. S2. MCTS_#1 characterization. Histological analysis of 5 μm sections at day 17 post seeding: (a) haematoxylin eosin staining, (b) cytokeratin AE1/AE3 and (c) fibronectin immunostaining. Scale bars: 50 μm .

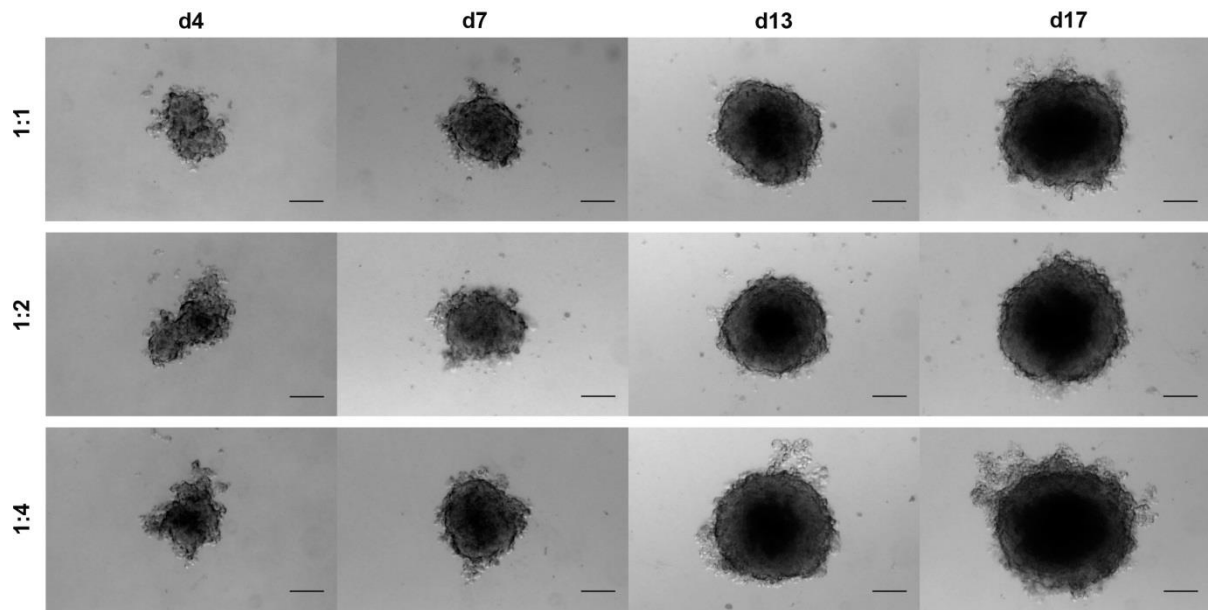


Fig. S3. Representative optical imaging of PANC-1:MRC-5 spheroids (ratio 1:1, 1:2 and 1:4) at days 4, 7, 13 and 17 post seeding. Scale bars: 200 μm

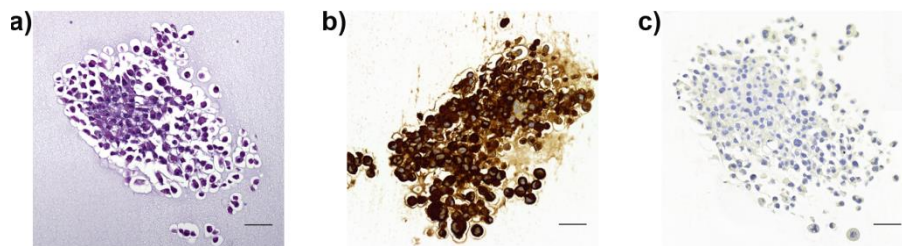


Fig. S4. MCTS_#2 characterization. Histological analysis of 5 μm sections at day 17 post seeding: (a) haematoxylin eosin staining, (b) cytokeratin AE1/AE3 and (c) fibronectin immunostaining. Scale bars: 50 μm .

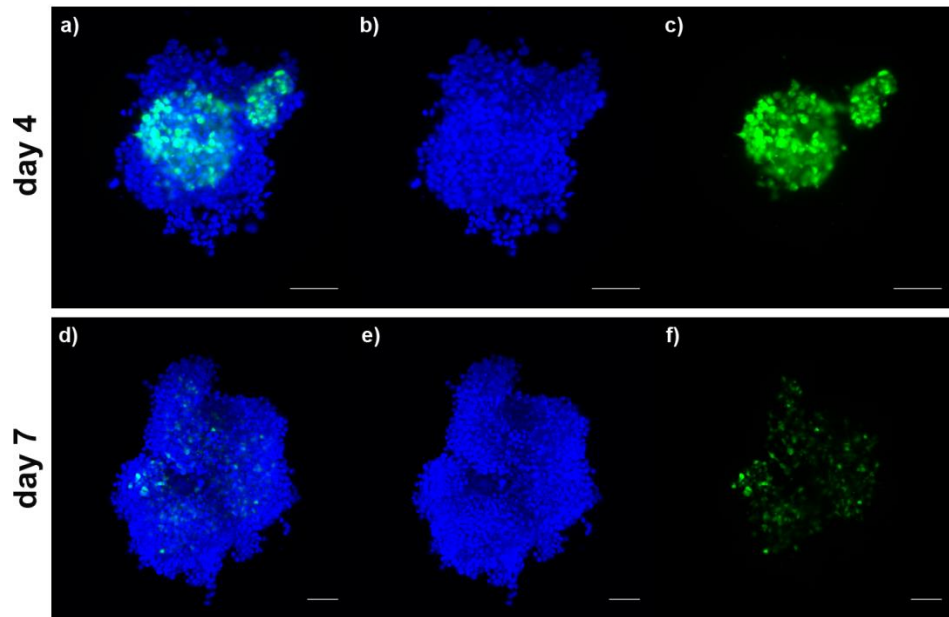


Fig. S5. SPIM 3D topography of MCTS_#2 at day 4 and day 7 post seeding. (a,d) Overlay of blue (Hoechst 33342, nuclei) and green (GFP-expressing MRC-5 fibroblasts) fluorescence; (b,e) single blue channel (Excitation/emission 405/440 nm) showing all cell nuclei; (c,f) single green channel (Excitation/emission 488/525 nm) for detection of GFP-expressing MRC-5 fibroblasts. Scale bars: 100 μm .

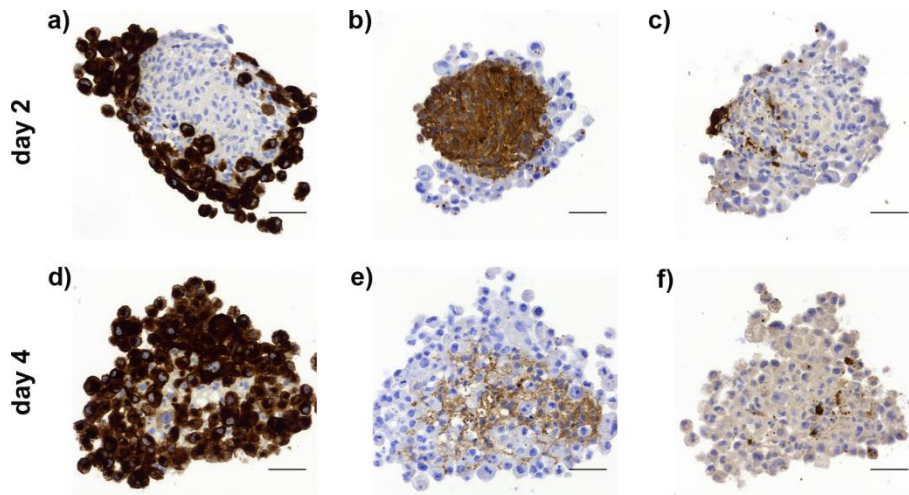


Fig. S6. Histological analysis of 5 μm sections of hetero-type PANC-1:MRC-5:HUVEC MCTS (ratio 1:9:4) created according to the conventional liquid overlay technique. Upper panel: day 2 post seeding. (a) cytokeratin AE1/AE3, (b) fibronectin and (c) CD31 immunostaining. Scale bars: 50 μm . Lower panel: day 4 post seeding. (d) cytokeratin AE1/AE3, (e) fibronectin and (f) CD31 immunostaining. Scale bars: 50 μm

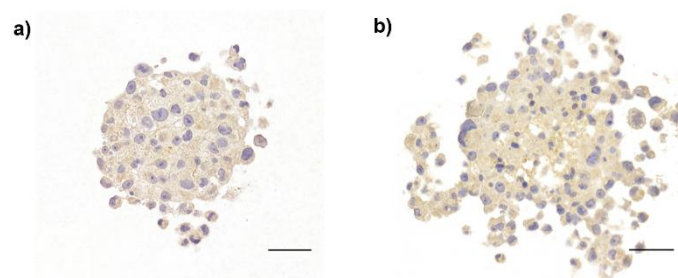


Fig. S7. CD31 immunostaining (negative controls). Histological analysis of 5 μm sections at day 4 post seeding: (a) MCTS_#1 and (b) MCTS_#2. Scale bars: 50 μm .

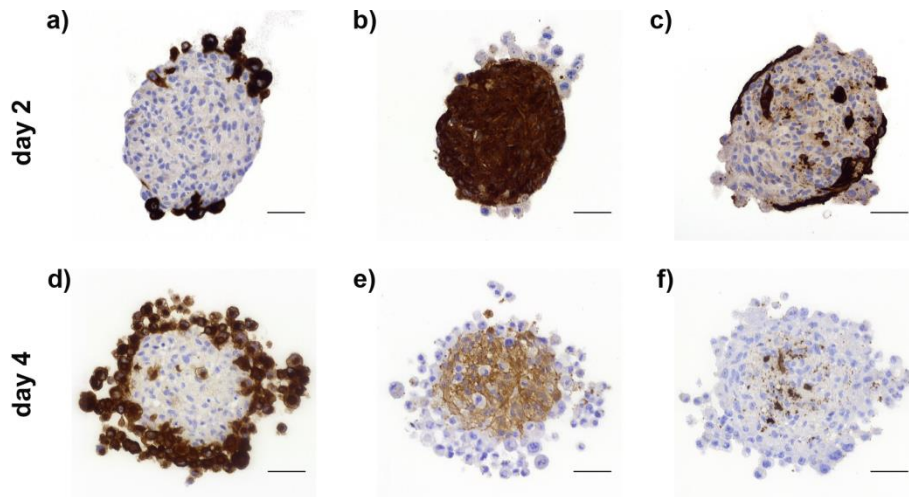


Fig. S8. Histological analysis of 5 μm sections of hetero-type PANC-1:MRC-5:HUVEC MCTS (ratio 1:9:4) created using FN-G-coated fibroblasts. Upper panel: day 2 post seeding. (a) cytochrome oxidase (COX), (b) fibronectin and (c) CD31 immunostaining. Scale bars: 50 μm . Lower panel: day 4 post seeding. (d) COX, (e) fibronectin and (f) CD31 immunostaining. Scale bars: 50 μm .

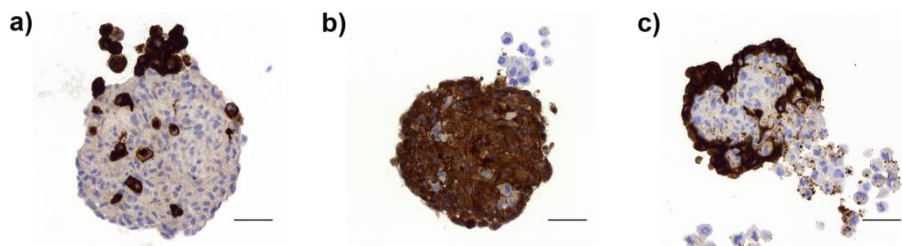


Fig. S9. Histological analysis of 5 μm sections of hetero-type PANC-1:MRC-5:HUVEC MCTS_#3 (ratio 1:9:4) at day 2 post seeding: (a) COX, (b) fibronectin and (c) CD31 immunostaining. Scale bars: 50 μm .

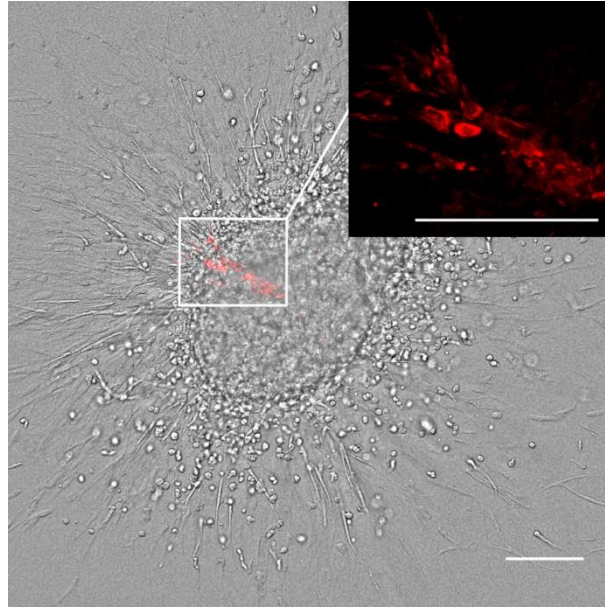


Fig. S10. Confocal imaging of MCTS_#3 spheroids in fibrin matrix at 72 h post embedding. Image of whole embedded spheroid: overlay of brightfield and red channel with RFP-expressing HUVEC. Top-right insert: magnification of the red channel from the white square. Scale bars: 200 μm .

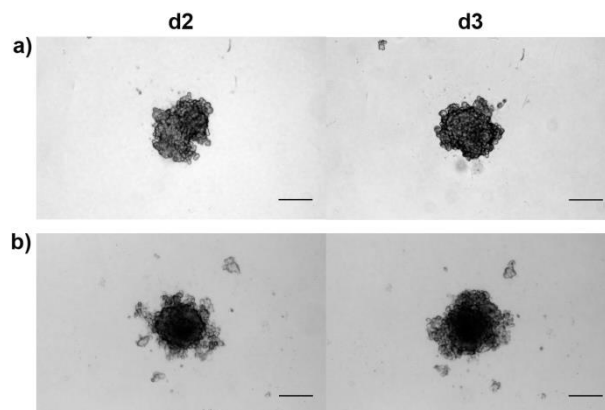


Fig. S11. Representative optical imaging of (a) MCTS_#1 and (b) MCTS_#2 at days 2 and 3 post seeding. Scale bars: 200 μm .

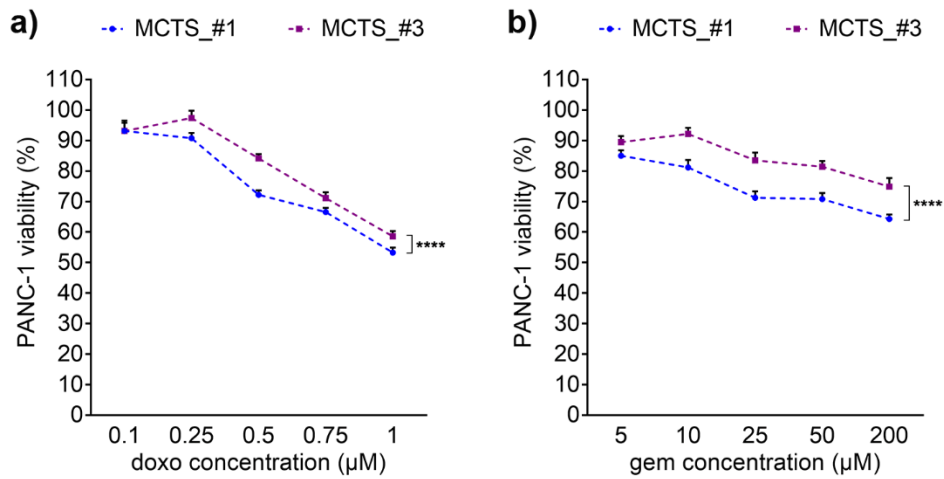


Fig. S12. Doxorubicin (doxo) and gemcitabine (gem) cytotoxicity. PANC-1 cell viability (luciferase activity) in MCTS_#1 and MCTS_#3 following exposure to increasing concentrations of (a) doxo or (b) gem for 72 h. Spheroids have been constructed using luciferase-expressing PANC-1 cells. Data represents mean \pm s.e.m. **** $p < 0.0001$ by a two-way Anova method, with a Sidak's post-test.

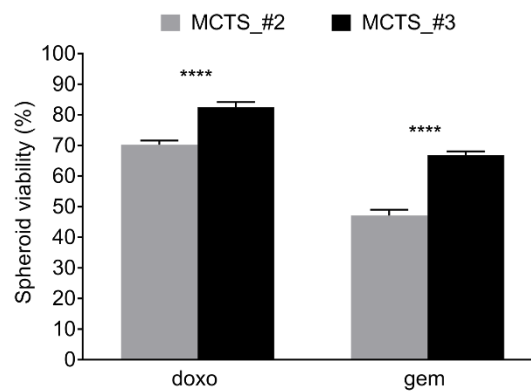


Fig. S13. Spheroid viability (ATP quantification) following exposure to doxorubicin (doxo, 0.5 μ M) or gemcitabine (gem, 50 μ M) for 72 h (n=2). Data represents mean \pm s.e.m. **** $p < 0.0001$ by a two-way Anova method, with a Sidak's post-test.

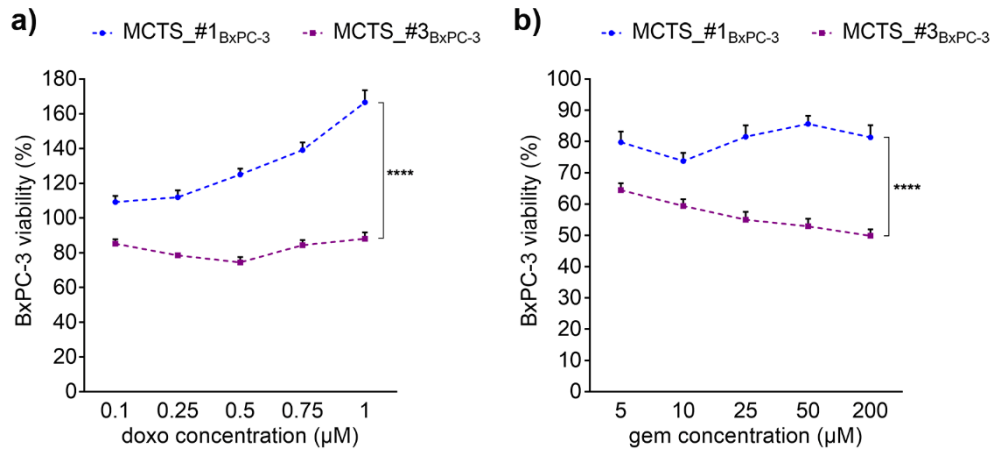


Fig. S14. Doxorubicin (doxo) and gemcitabine (gem) cytotoxicity. BxPC-3 cell viability (luciferase activity) in MCTS_#1_{BxPC-3} and MCTS_#3_{BxPC-3} following exposure to increasing concentrations of (a) doxo or (b) gem for 72 h. Spheroids have been constructed using luciferase-expressing BxPC-3 cells. Data represents mean \pm s.e.m. **** $p < 0.0001$ by a two-way Anova method, with a Sidak's post-test.

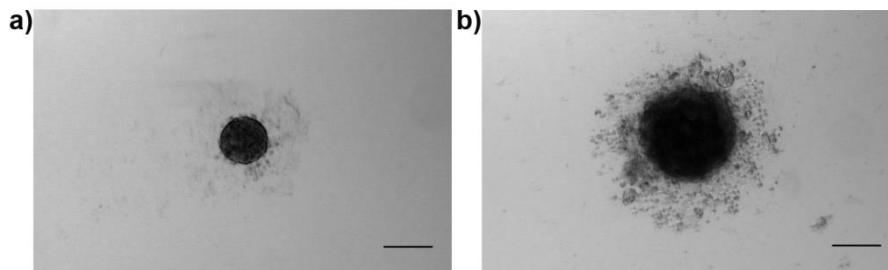


Fig. S15. Representative optical imaging of (a) MCTS_#1_{BxPC-3} and (b) MCTS_#3_{BxPC-3} at day 4 post seeding, before exposure to cytotoxic drugs. Scale bars: 200 μm.