Engineering the tumor microenvironment to generate next generation in vitro prostate cancer models

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Previous bladder cancer model:

Ringuette Goulet C, Bernard G, Chabaud S, Couture A, Langlois A, Neveu B, Pouliot F, Bolduc S. Tissue-engineered human 3D model of bladder cancer for invasion study and drug discovery. Biomaterials. 2017 Nov;145:233-241. doi: 10.1016/j.biomaterials.2017.08.041. Epub 2017 Aug 29. PMID: 28888113.

Cancer associated fibroblasts study:

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INTRODUCTION

Prostate cancer (PCa) is one of the most common cancer in developed countries and treatments of advanced PCa are palliative. Research is needed to discover and validate new drugs. Current in vitro and in vivo models for PCa do not accurately recapitulate many critical aspects of its biology and lack potential due to their weak ability to simulate the human tumour microenvironment (TME), which impacts the growth and progression of cancers. Tissue engineering provides an opportunity to overcome the current limitations of cancer models and create a new generation of 3D biomimetics. Compared to 2D cell culture, engineered 3D models display better spatial organization, adequate cell-cell and cell-matrix interactions, and comparable physicochemical and mechanical properties of the tissue of origin. Consequently, such models reproduce essential biological processes, which are critical to adequately reproduce the disease, understand the biology, and propose optimized therapeutic solutions. After our successful story with bladder cancer (BCa) modeling, a world premiere, we firmly believe that we are in the best position to develop a 3D model of prostate cancer (PCa).

METHODS

- CAF induction confirmed by immunofluorescence.
- Cultivation of human prostate fibroblasts with ascorbic acid (vitamin C) in DMEM media supplemented with 10% FBS.
- Stromal sheets reseeded after 2 weeks of culture
- Sheet stacking after 4 weeks
- Stromal sheets / fibroblasts cultured with PCa-conditionned media to assess cancer-associated fibroblasts (CAF) effects on TME properties (Stiffness).
- Prostate epithelial cell spheroids seeded between stromal sheets
- PCa spheroïds deposited onto the stromal sheets and further cultured
- Prostate stromata fixed, cut and stained following Masson's Trichrome protocol

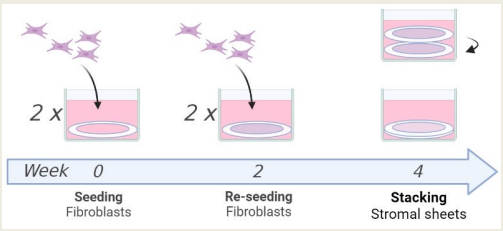


Figure 1. Self-assembly method to produce our 3D prostate stroma

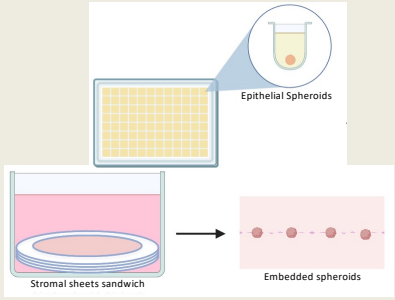


Figure 2. Spheroid embedding method to produce 3D healthy prostate model

CONCLUSIONS

- 3D prostate fibroblast stromata can be produced by the self-assembly technique
- 3D prostate stroma rigidity can be modulated via CAF induction
- PCa cell line invasiveness can be confirmed with collagen gels and our 3D human derived prostate stromata
- Prostate epithelial cell spheroids produce acini in our 3D model
- PSA and citrate secretion, dosage to come

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RESULTS

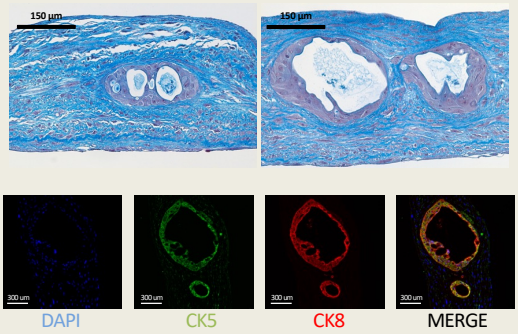


Figure 3. Prostate epithelial spheroids recapitulate in vivo prostate acini 3D structure. Masson's trichrome staining (top) shows general gland structure and lumen presence whereas immunofluorescence (bottom) shows that prostate epithelial acini exibit common epithelial cell makers.

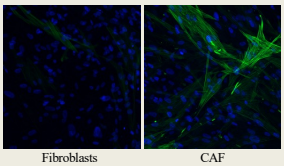


Figure 4. Induced CAFs express more alpha-SMA than fibroblasts: Immunofluorescences against alpha-SMA (green) expressed by prostate fibroblasts cultured with unconditioned (left) or PCa conditioned media (right). DAPI stained nuclei in blue.

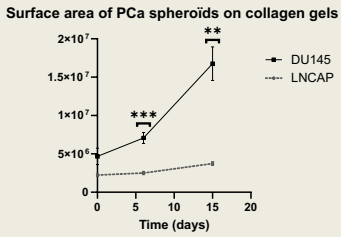


Figure 5. Invasive behaviour of PCa cell into collagen gels recapitulate their biological properties: invasive (DU145) and non-invasive (LNCAP) PCa spheroïd cells were embedded into 2.5 mg/mL collagen gels and followed for 15 days. ** p < 0.01 *** p < 0.001

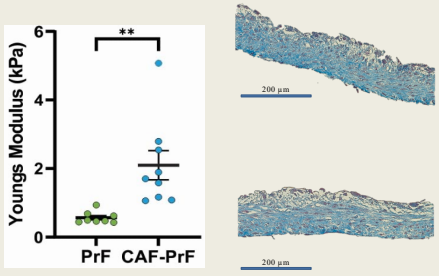


Figure 4. Prostate stroma produced with CAF were stiffer than control stroma produced with normal fibroblasts. AFM mechanical tests of prostate fibroblasts (PrF) and cancer-associated fibroblasts (CAF-PrF) stromata (left). Histological view of 3D reconstructed stromata made from fibroblasts (top right) and CAF (bottom right)

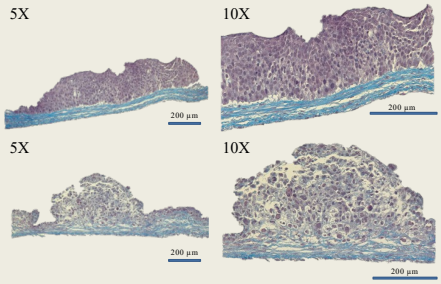


Figure 5. PCa models produced by self-assembly allow recapitulation of invasive phenotype of DU145 and LNCaP cells. Histological view of 15,000 cell PCa spheroids on prostate fibroblast stromata. Non-invasive LNCaP PCa cell line spheroïds (top) and invasive DU145 PCa cell line spheroids (bottom) shown in bright field microscopy after Masson's trichrome staining of tissue slices.

The authors have no potential conflict of interest.

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