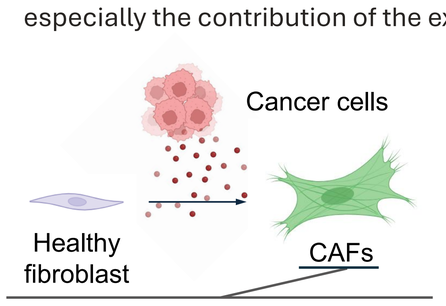
Harnessing cancer cell-derived extracellular vesicles to improve the biomimetism of a 3D tissue-engineered bladder cancer model

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Introduction

- Cancer models, both *in vitro* and *in vivo*, are essential to assess the effectiveness of new potential cancer treatments. However, up to 95% of treatments validated on existing models fail during clinical trials.
- Current models fail to capture the complexity of the human tumor microenvironment, especially the contribution of the extracellular matrix (ECM).



- Highly heterogenous population
- High ECM deposition and remodeling
- Contractile phenotype
- Expression of α-smooth muscle actin (αSMA)

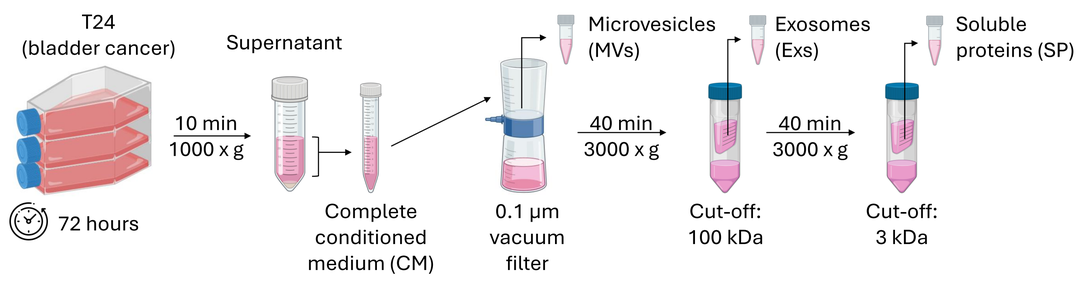
- Tumors are not only stiffer than comparable healthy tissues, but also progressively stiffen with tumor progression. This change in stiffness modulates important biological processes, including cancer cell proliferation,
- This stiffening is primarily driven by cancer associated fibroblasts (CAFs), which are responsible for the increased collagen deposition and crosslinking.
- The majority of CAFs stems from the activation of healthy neighboring fibroblasts. This activation can be mediated by several factors such as a stiff ECM, TGF-β and extracellular vesicles secreted by cancer cells (microvesicles ranging from 100 nm to 1 µm; exosomes ranging from 30 nm to 150 nm).

Objective

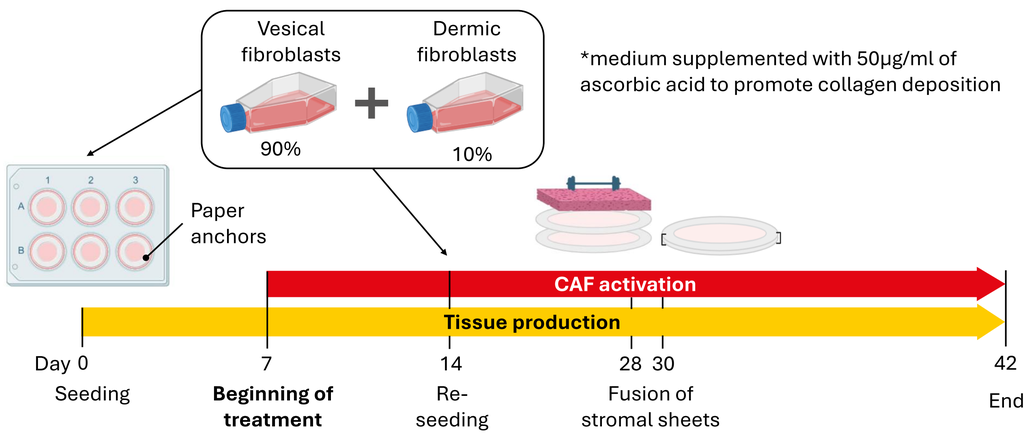
- Our team has developed a 3D tissue-engineered bladder cancer model using conditioned media from aggressive bladder cancer cells to activate healthy fibroblasts into CAFs.
- **We aim to assess how different cancer-conditioned media fractions affect the induction of healthy fibroblasts into CAFs and the biophysical properties of the resulting engineered tissues.**

Methods

1. Isolation of different cancer-conditioned medium fractions



2. Production timeline of tissue-engineered bladder cancer stromas



Validation

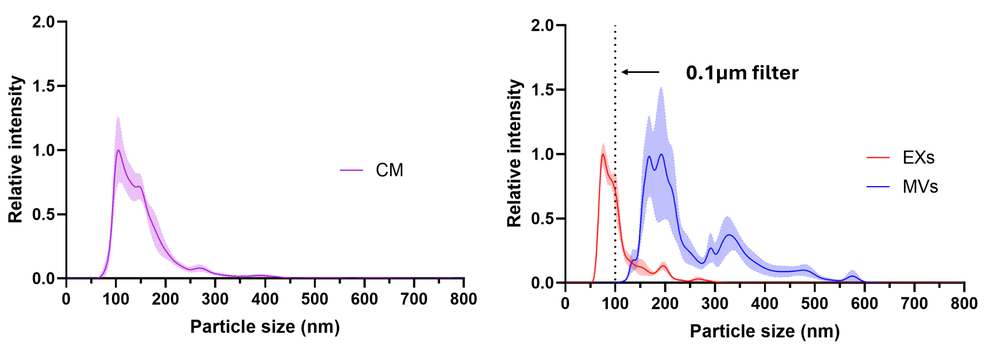


Fig 1. The filtering methods proved effective althought further characterization is needed. Particle size distribution of different conditioned medium fractions measured by nanoparticle tracking analysis (NTA).

Results of 2D vesical fibroblast activation

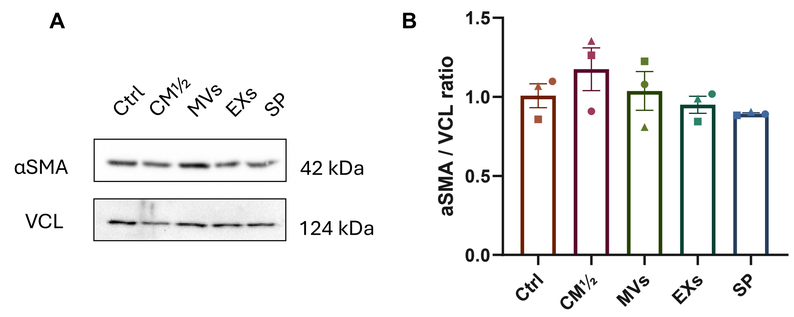
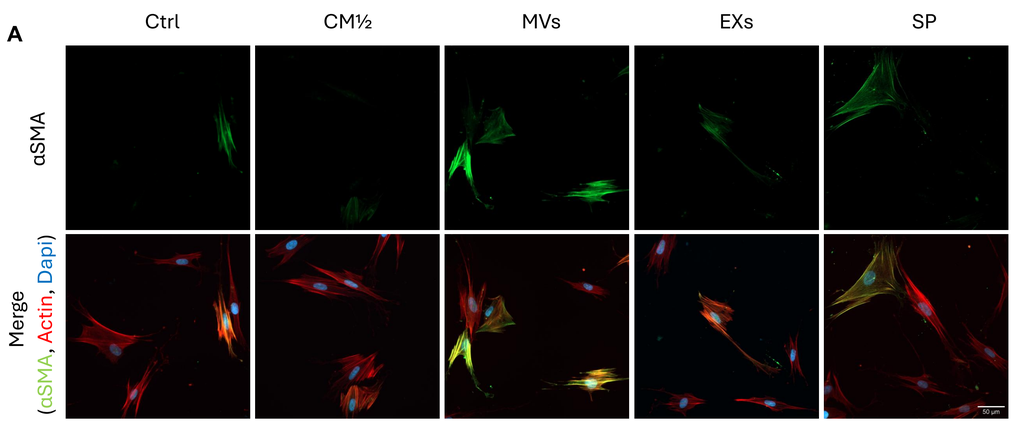


Fig 2. Complete conditioned medium seems to increase overall αSMA expression. A) Expression and B) quantification of alpha smooth muscle actin (αSMA) measured by Western blot in FHU5 primary vesical fibroblasts treated for 10 days with half of complete conditioned media (CM½), the microvesicle fraction (MVs), the exosome fraction (EXs) or the soluble proteins (SP). N = 3; ns p>0.05



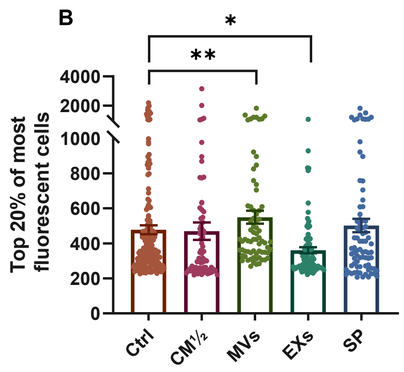


Fig 3. Fibroblasts activated with the microvesicle fraction express more αSMA. A) Representative images of immunofluorescence staining for αSMA (green), actin (red), and nuclei (blue) in FHU5 primary vesical fibroblasts. Fibroblasts were treated with either half of the complete conditioned media (CM½), microvesicle fraction (MVs), exosome fraction (EXs), or soluble protein fraction (SP) for 10 days before seeding on glass coverslips, overnight incubation, and fixation. B) Quantification of mean fluorescence of the top 20% most fluorescent cells. N = 2, scale bar = 500 µm, *p<0.5, **p<0.01, ****p<0.0001

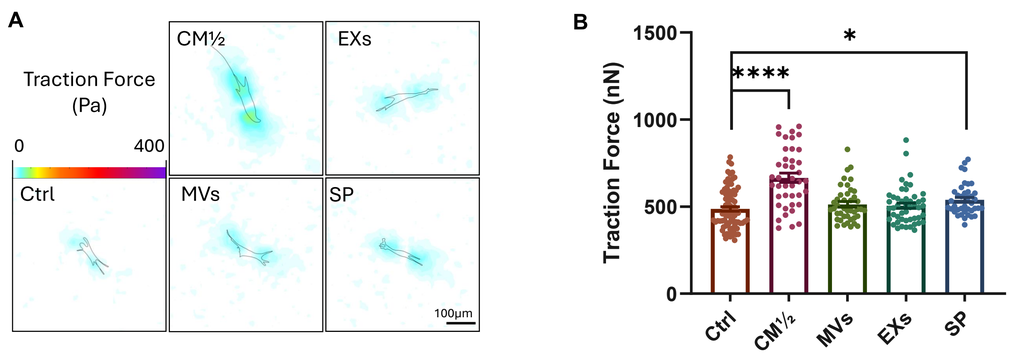


Fig 4. Complete conditioned medium significantly increases cell contractility. A) Representative images and B) quantification of traction forces exerted by FHU5 primary vesical fibroblasts on a 10 kPa polyacrylamide substrate. Fibroblasts were treated with either half of the complete conditioned media (CM½), microvesicle fraction (MVs), exosome fraction (EXs), or soluble protein fraction (SP) for 10 days before seeding on polyacrylamide gel and incubating overnight to allow cells to attach. N = 1, scale bar = 100 µm, *p<0.5, ****p<0.0001

Results of tissue-engineered stroma activation

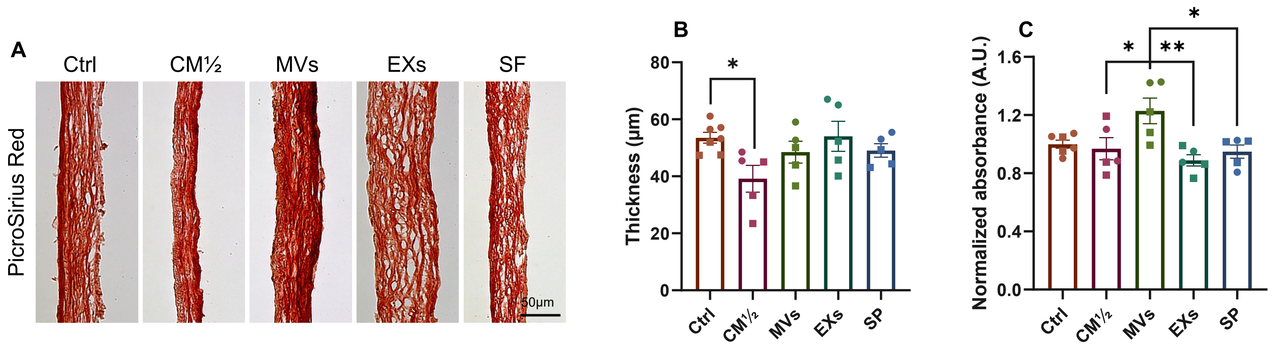


Fig 5. Complete conditioned medium produces thinner tissues, while the microvesicle fraction increases collagen deposition. A) Representative images of tissues cryosections stained with picrosirius red along with the B) measured thickness of tissue cross-sections and the C) normalized mean light absorbance by the picrosirius red dye indicating total collagen concentration. N = 2 , scale bar = 50 µm, *p<0.5, **p<0.01

Conclusions

- Overall, both the microvesicle and exosome fractions appear to significantly impact CAF activation and ECM remodeling.
- CAF heterogeneity significantly complicates the identification of underlying biological processes. Therefore, increased replicates are necessary to validate our findings.

Next : To determine whether the observed differences in CAF activation and collagen deposition lead to altered mechanical properties, we will measure the stiffness of the tissue-engineered stromas using atomic force microscopy (AFM).

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