Functional assessment of bladder smooth muscle grown in 3D co-culture

Clara I. Chamorro[1,2], Amy Wu[1,3,], Sema Latic[1], Dennis Rootsi[1,] Magdalena Fossum[1,2,4]

1. Laboratory of Tissue Engineering, Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden, 2. Laboratory of Tissue Engineering, Rigshospitalet, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 3. Department of Anesthesiology, Columbia University, New York, NY, USA. 4. Division of Pediatric Surgery, Department of Surgery and Transplantation, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.

Conclusion

We demonstrated, for the first time, observable contractility of human bladder smooth muscle cells (BSM) co-cultured with stromal cell lines in a self-assembled construct. This model enables investigation of paracrine signaling within a physiologically relevant context for disease modeling and drug discovery. In addition, stromal cells co-cultured with epithelium at an air–liquid interface retained canonical lineage markers.

Introduction

A key function of hollow visceral organs is contractility, and its dysfunction underlies many pathological conditions. For example, abnormal contraction of the urinary bladder can lead to incontinence, while in the lung it can cause bronchospasm. However, there is still no ex-vivo human engineered tissue model that adequately captures this function. Recent advances in self-assembled, scaffold-free tissue constructs have aimed to bridge the gap between cellular assays and whole-tissue models, but smooth muscle has not yet been effectively incorporated.

Research question: Can BSM be incorporated into self-assembled constructs while retaining contractile responsiveness to native ligands?

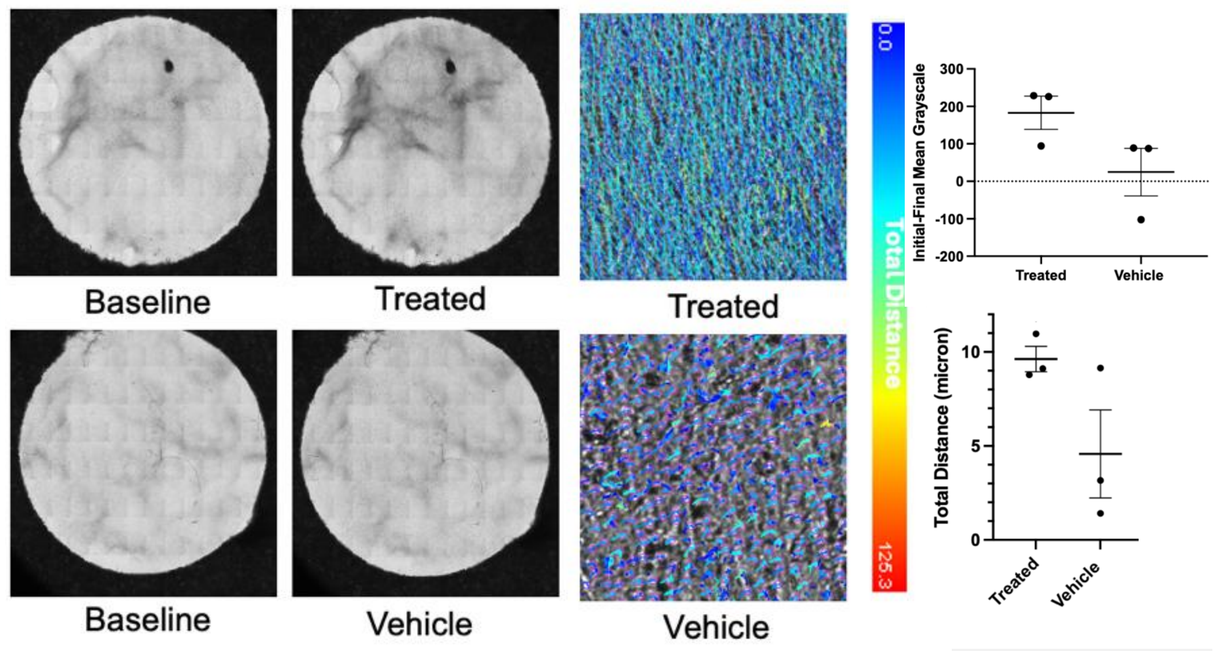
Aims

1) Create self-assembled constructs with human BSM, fibroblast, and vascular endothelium with or without epithelium
2) Assess physiologic contractility of these self-assembled constructs.

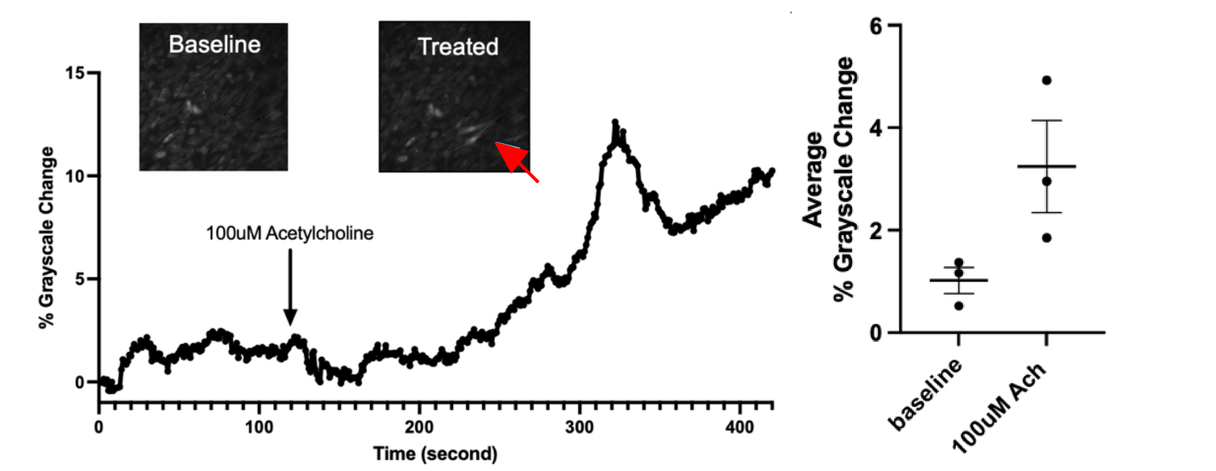
Materials and Methods

Human BSM cells were co-cultured with human dermal fibroblasts for ~4 weeks. Contractile activity in response to parasympathetic stimulation (acetylcholine) was evaluated using optical density measurements under light microscopy and particle motion tracking (TrackMate, FIJI). Constructs were loaded with FLUO-4 calcium dye to visualize calcium oscillations under stimulation. Immunohistochemical staining of paraffin-embedded sections was performed to confirm presence of organized (αSMA-positive) cells a matrix containing HDF and BSM.

References:
(1) Chabaud S and Bolduc S. Dermatol Res Prac 2016 (2) Saba I et al. Biomed Res Int 2018. (3) (2) Serdinsek T et al. Sci Rep 2020. (4) Mikami M et al. AM J Respir Cell Mol Biol 2017.



Figures 1: Self-assembled constructs exposed to acetylcholine exhibited qualitatively increased optical density (n = 3) and particle motion (n = 3, NS).



Figures 2: Self-assembled constructs exposed to acetylcholine exhibited qualitatively increased calcium oscillations (n = 3, NS). Example of intracellular calcium flux post-treatment is highlighted (red arrow).

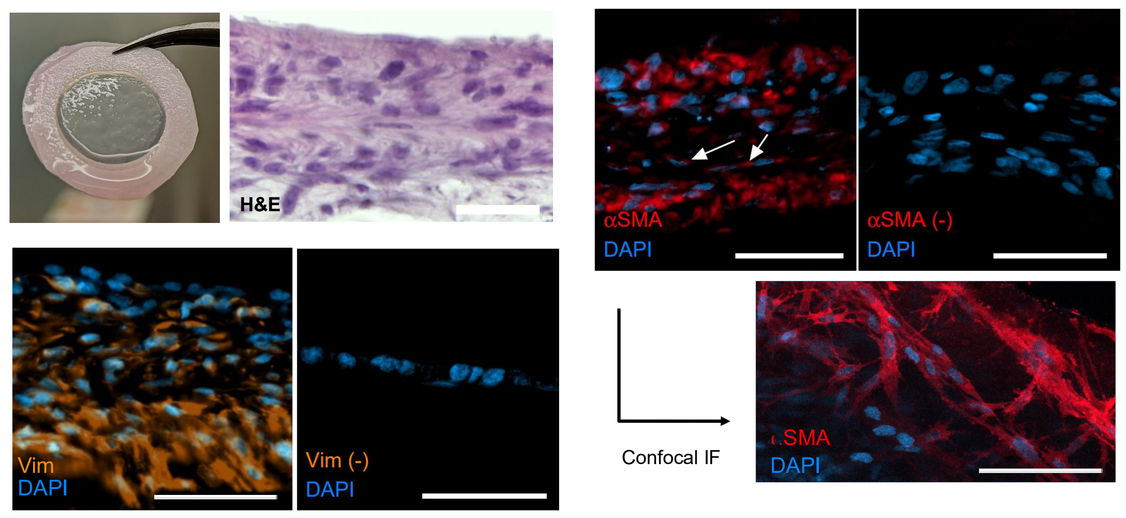


Figure 3: Representative images of the self-assembled stromal construct showing its macroscopic appearance, H&E staining, and confocal immunofluorescence staining for stromal markers (vimentin and αSMA). Stromal cells were present and displayed an organized fiber-like structure (n=3). Arrows indicate positive staining in confocal images. Scale bar 100 μm.

Clara Ibel Chamorro, PhD
Laboratory of Tissue Engineering
KBH
Karolinska Institutet
Email: clara.chamorro.jimenez@ki.se

linkedin.com/in/clara-ibel-chamorro-jimenez-b0ba0249

Amy D. Wu, MD
Department of Anaesthesiology
Columbia University
Email: amy.wu@columbia.edu

Karolinska Institutet