Development of a 3D system to study endothelial cell-smooth muscle cell interactions

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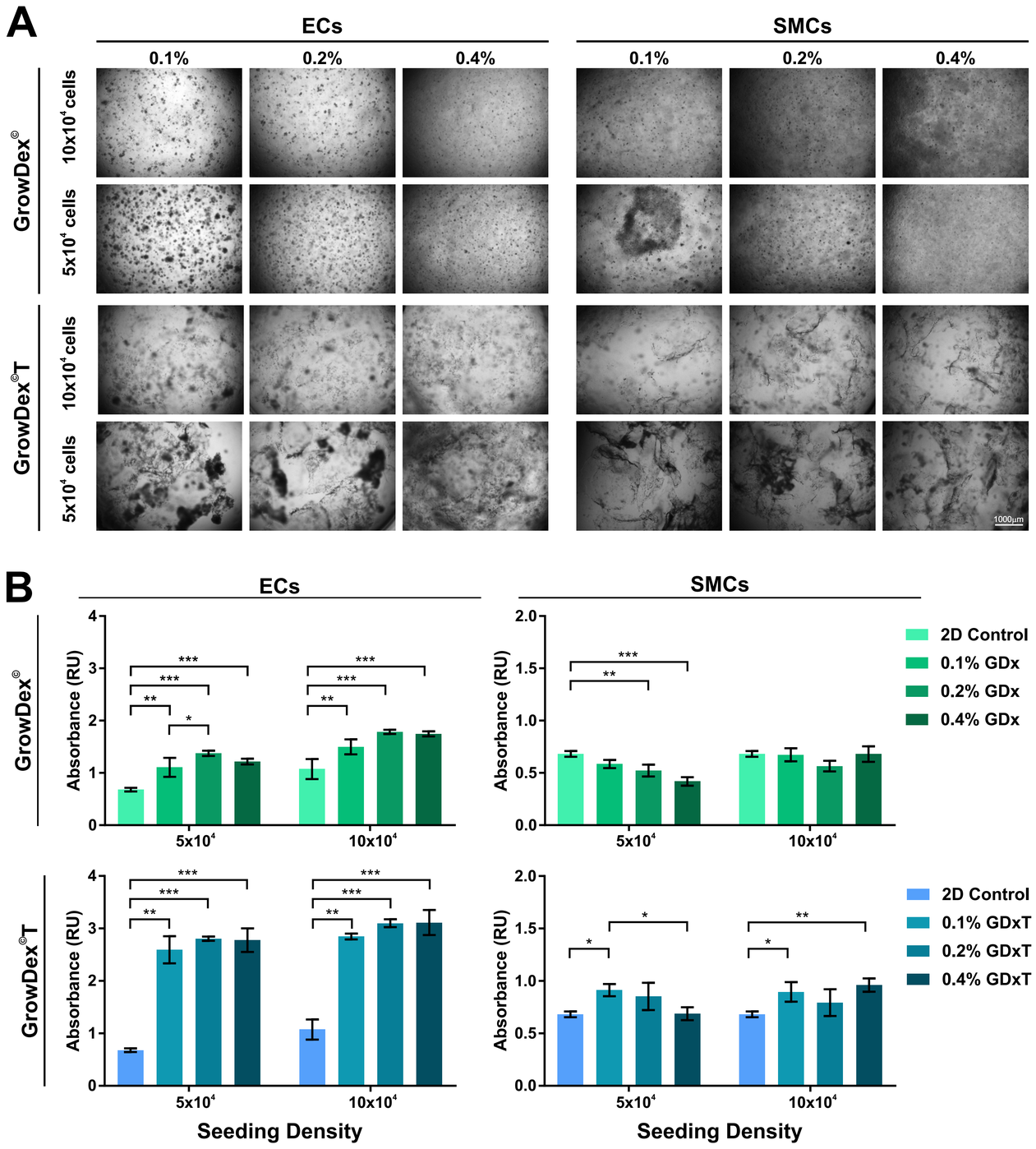
Introduction. Blood vessels are composed of two main cell types, endothelial cells (ECs) and smooth muscle cells (SMCs), which make up a dual layered structure. An elastic membrane, (internal elastic lamina) separates a single concentric layer of ECs from the secondary surrounding layer of SMCs. **Aim.** We hypothesise that ECs not only communicate via extracellular vesicles (EVs) over intermediate and long distances but also signal to neighbouring SMCs in the myoendothelial junction (MEJ) using the same paracrine mechanism. The overall goal of this project is to use nanofibrillar cellulose hydrogels (NFC) to create a physiological model of the EC and SMC layers, whilst studying EV-mediated communication between these ECs and SMCs.

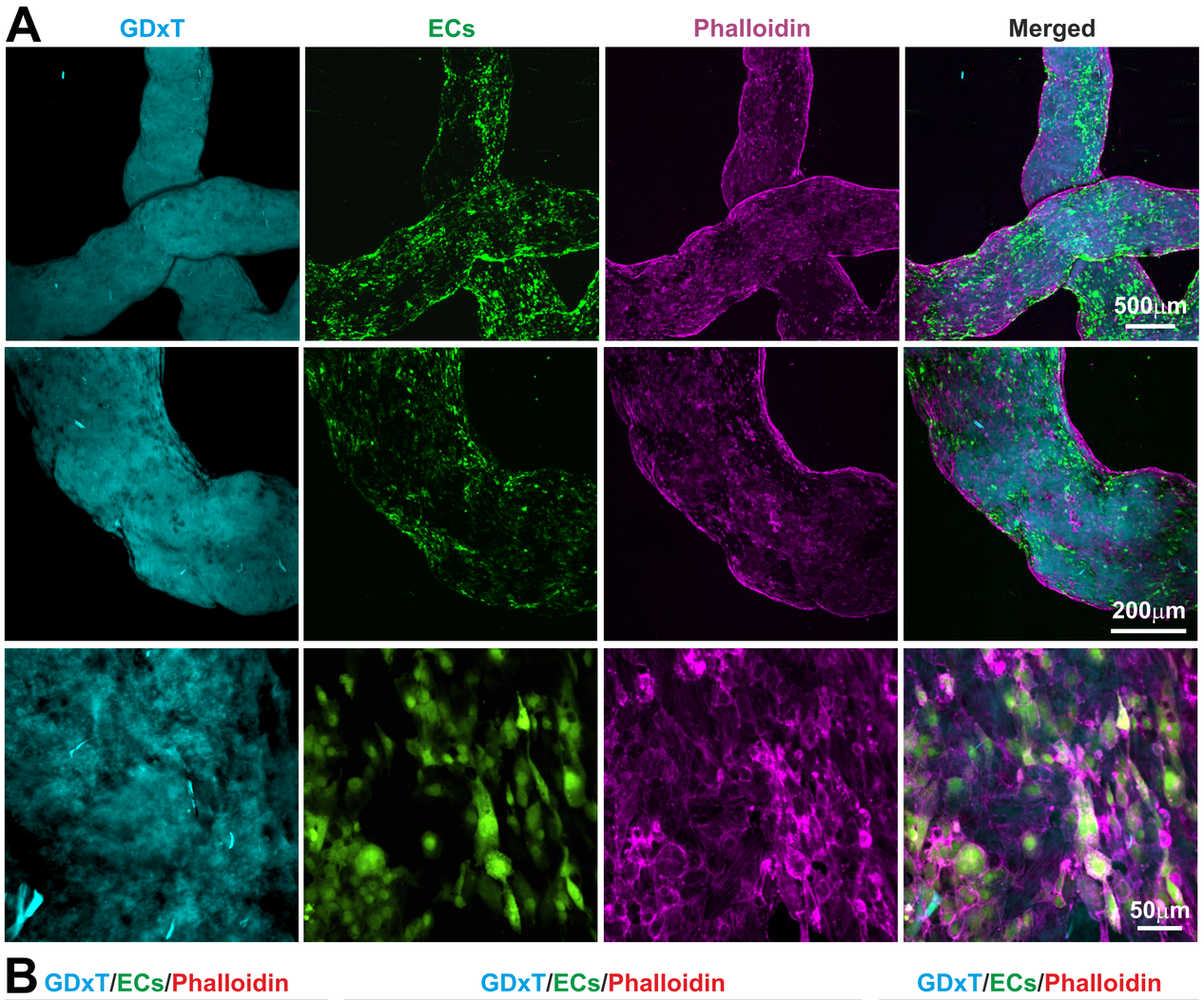
Results. We used GFP expressing HMEC-1 cells as model ECs and A10 cells as model SMCs. We have been able to determine the optimal 3D cultivation conditions and viability of ECs and SMCs within and on the surface of GrowDex® and GrowDex®T. We have successfully transduced A10 cells (SMCs) with an RFP construct for better identification when in co-culture. Finally, we have used GrowDex®T to form long tube like constructs, coated the outer layer of the constructs with a thick layer of ECs and cultured them for up to 4 weeks. **Summary.** In summary, we have been able to develop a novel co-culture model of GFP-ECs and RFP-SMCs to study the cell-cell interactions on an NFC based scaffold.

Endothelial Cells and Smooth Muscle Cells are viable and grow within GrowDex® and GrowDex®T in 3D



ECs and SMCs show distinctly different morphologies when seeded within either GrowDex® or GrowDex®T whilst maintaining viability compared to 2D. ECs and SMCs were seeded at different seeding concentrations ($5x10^4$ or $10x10^4$ cells/well) within different densities of GrowDex® (GDx) or GrowDex®T (GDxT- 0.1%, 0.2% and 0.4%). Cell morphology **(A)** was observed and cell viability **(B)** tested with XTT assays following 48hrs of culture and it was noted that ECs are more viable in GrowDex®T. Bar: all images 1000µm.

Endothelial Cells bind to 3D GrowDex©T tubules

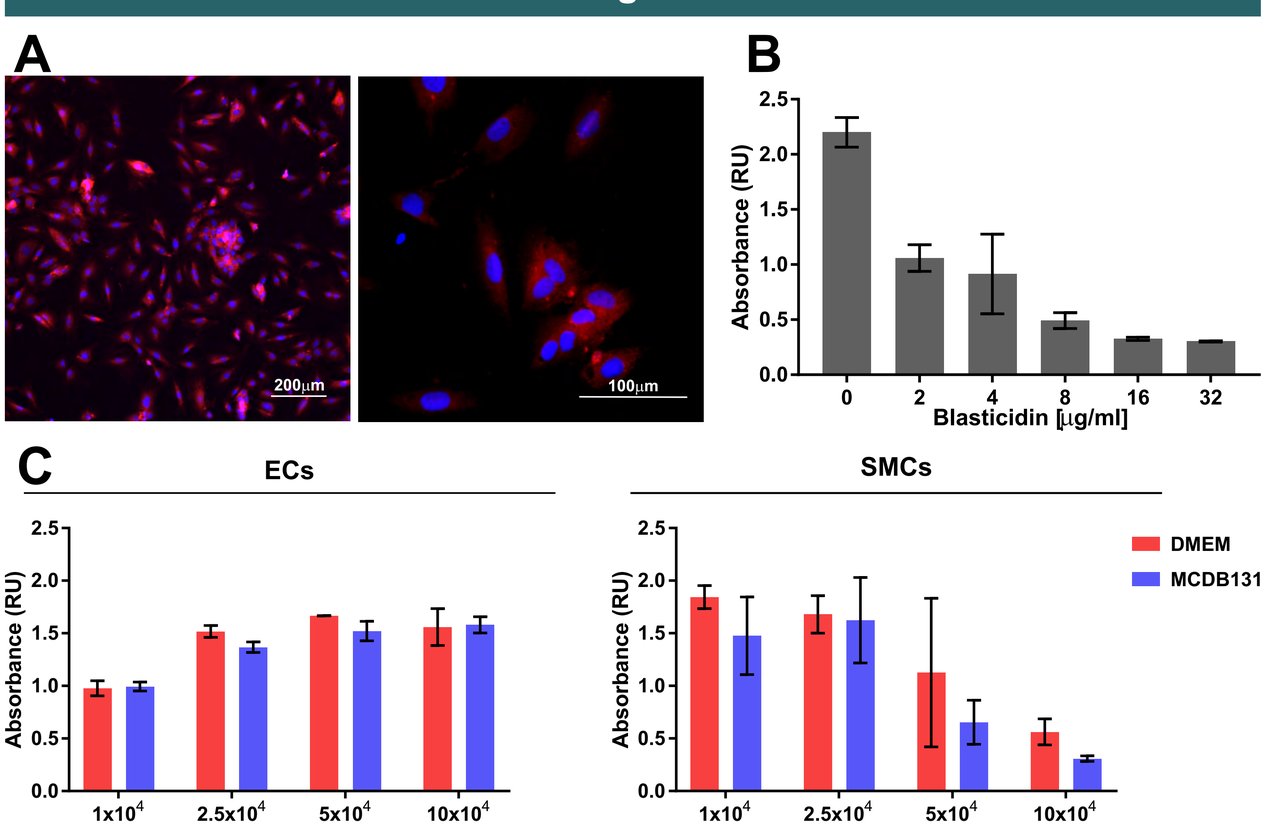


ECs adhere to GrowDex®T tubules, forming a confluent monolayer of cells. ECs were seeded onto tubules formed from GrowDex®T (GDxT 1.0%). Confocal imaging shows detailed tubule morphology, cell morphology, cell-cell organisation, as well as actin fillament organisation (phalloidin) in 2D maximum intensity projection images **(A)** and 3D reconstruction images **(B)**. ECs did not migrate into the GrowDex®T tubule.

Endothelial Cells and Smooth Muscle Cells are viable and grow ON TOP of GrowDex© and GrowDex©T in Semi 2D

ECs and SMCs were cultured as semi 2D cultures ON TOP of either GrowDex® or GrowDex®T. ECs and SMCs were seeded at different seeding concentrations ($1x10^4$, $2.5x10^4$, $5x10^4$ or $10x10^4$ cells/well) on top of either GrowDex® (GDx 1.5%) or GrowDex®T (GDxT 1.0%). Cell morphology **(A)** was observed and cell viability **(B)** tested with XTT assays following 48hrs of culture and it was noted that ECs and SMCs are more viable on GrowDex®T than GrowDex®. Bar: all images 1000µm.

Viral Transduction of SMCs with and RFP protein allows easier identification during EC/SMC co-culture



SMCs were transduced with an RFP-blastocidin resistance lenti virus, whilst EC and SMC viability was tested within alternative culture conditions. Blastocidin resistant (B) SMCs express RFP (A) allowing easier identification within co-culture conditions. XTT viability was measured from ECs and SMCs seeded at different seeding concentrations within either MCDB131 (ECs basal media) or DMEM (SMCs basal media).