Assessment of a Smooth Muscle Cell Maturation Protocol for the Analysis of Contractile Properties

Matthias Gossmann[1], Jan Hunker[1], Peter Linder[1], Sonja Stoelzle-Feix[2], Bettina Lickiss[1], Elena Dragicevic[2], Ulrich Thomas[2], Michael George[2] and Niels Fertig[2]

[1]innoVitro GmbH, Juelich, Germany, [2]Nanion Technologies GmbH, Munich, Germany

ABSTRACT

Cardiovascular diseases (CVDs) are the leading cause of death globally with 1.3 billion people of the world's population suffering from hypertension, causing a tremendous public health issue. Despite this global burden, preclinical drug development lacks a human relevant cell-based assay system employing human smooth muscle cells for the assessment of CVD-related hypertension.

Here we assessed two aortic smooth muscle cell (HAoSMC) cultivation protocols and compared the maturation process of the cells on phenotypic level with immunostainings and on functional level with the FLEXcyte 96 technology. Primary HAoSMCs were cultured in T25 flasks for several days in recommended maintenance medium. After preculture, the cells were dissociated and plated on flexible membranes of the FLEXcyte 96 plates to ensure a human heart related physiological environment. Subsequently, the cells were cultured for 5 days in either maturation or maintenance medium before functional assessment of contractile properties. Here, compounds with positive inotropic effect were used. For phenotypic characterization, the cells were fixed and stained with Alexa Fluor 488 Phalloidin directly within the plate.

The results demonstrate the effect of the maturation protocol on HAoSMCs with a stronger contraction (+30% compared to non-maturated control cells) and more pronounced actin structures. The assessment of an optimal preculture protocol for HAoSMCs ensures ideal conditions for further development. The use of HAoSMCs on the FLEXcyte 96 as standard cell model in near future will yield in a new standard cell-based assay system for the analysis of CVD related hypertension.

METHODS

The assay is based on a special 96-well plates that contains high-precision, ultra-thin and hyper-elastic silicone membranes instead of stiff plastic surfaces for culturing smooth muscle cells, cardiomyocytes and other cell types. The cells are grown as monolayers to the flexible substrates. The deflection of the membranes is controlled by the contractile force of the cells and recorded by means of capacitive distance sensing with the FLEXcyte 96 device (Nanion Technologies).

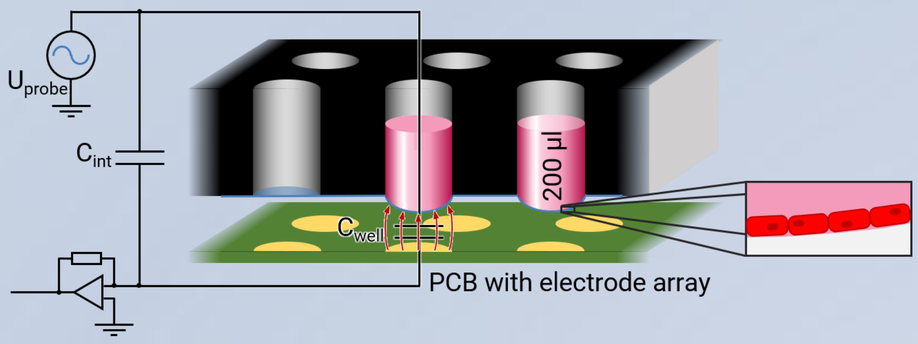

Figure 1. Measurement principle. The weight of the cell culture medium deflects the cell-seeded membranes. The contractile force of the cells controls the extent of deflection, measured by means of capacitive distance sensing.

Primary HAoSMCs (PromoCell GmbH, Heidelberg, Germany) were precultured for 7 days in standard T25 flasks using the recommended maintenance medium. Subsequently, the cells were dissociated and seeded into FLEXcyte 96 plates at a density of 12k cells per cm². After reaching confluence, the medium was replaced with maturation medium (basal medium, FCS 2%, heparin 750 U/mL) and the cells were cultured for another 6 days. Contraction force was measured after acute application of phenylephrine (0.1, 1, 10 µM).

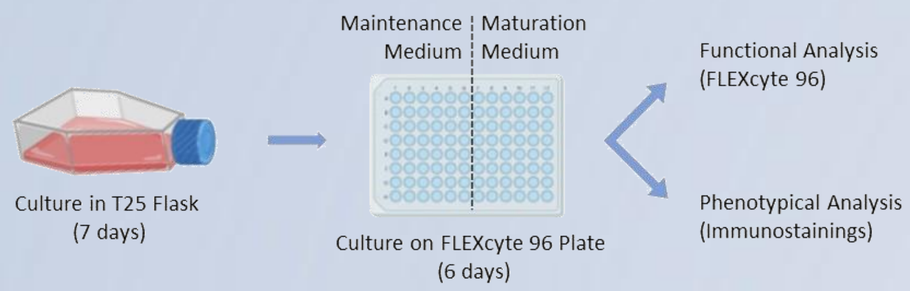

Figure 2. Cell culture protocol. HAoSMCs were precultured for 7 days in T25 flasks before seeding into FLEXcyte plates. After six days in the respective cell culture medium, contraction force was measured and cells were stained for actin with phalloidin.

RESULTS

After 6 days of incubation with maturation medium, HAoSMCs responded to acute application of phenylephrine with an increase in contraction force to 130% of control, indicating the positive inotropic effect of the alpha-1 adrenoceptor agonist. In contrast, when the cells were kept in maintenance medium, no inotropic effect could be detected.

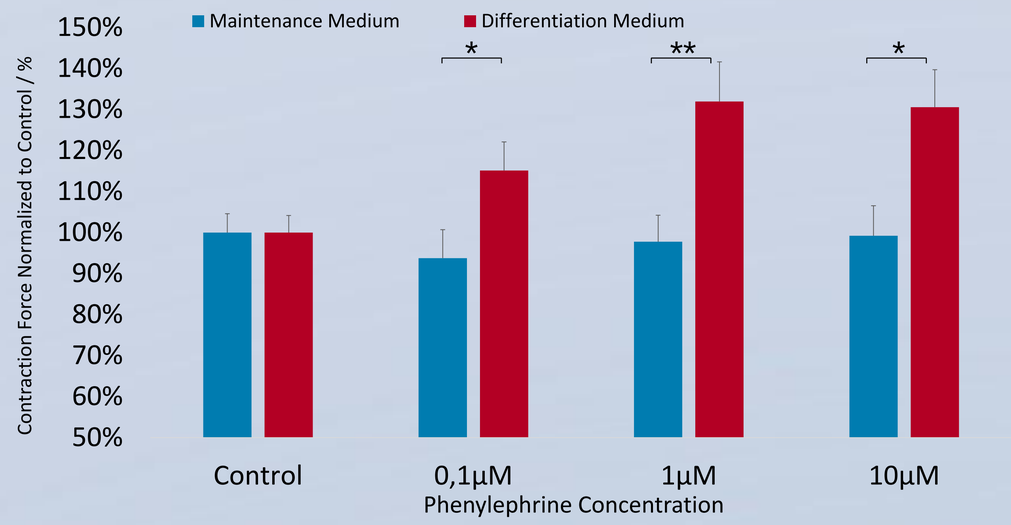

Figure 3. Inotropic effect of phenylephrine in different media. When incubated with maturation medium for 6 days, the cells responded to phenylephrine with an increase in contraction force to 130% of control. Statistical significance was assessed using the Student's t-test and P-values are given as *P < 0.05, **P < 0.01 and ***P < 0.02, n=11.

Staining of the actin cytoskeleton revealed a pronounced organization after culturing the cells in maturation medium, underlining the cellular transition from the synthetic phenotype (proliferation, migration, ECM production) to the contractile phenotype. While the actin molecules are distributed evenly throughout the cell bodies in maintenance medium, culture in maturation medium induces the organization of actin in filaments, promoting contractile function of the cells.

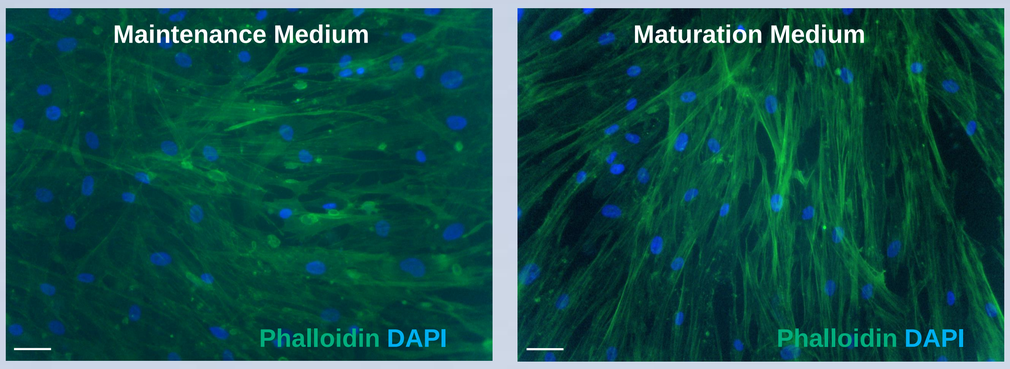

Figure 4. Comparison of HAoSMC phenotype via phalloidin staining. Cells were fixed and stained with Phalloidin (actin marker) and DAPI (nuclei marker) after 6 days in culture with either maintenance or maturation medium. White bar indicates 20µM.

Summary

We present a method to induce HAoSMCs maturation by application of a starvation medium containing heparin and reduced serum. The effect was validated both on a functional level as well phenotypically by immunostainings. While HAoSMCs showed no response to alpha-1 adrenoceptor agonist phenylephrine in maintenance medium, maturation with starvation medium resulted in an increase in contraction force to 130% of control. Cytoskeletal staining underlines the transition from synthetic to the contractile phenotype.

Conclusions

While primary human SMCs are commercially available, their application in drug development and safety assessment is limited due to the immature state of proliferating cultures. Additionally, a high throughput cell-based assay system employing SMCs is needed to fill the gap for modern preclinical risk assessment for diseases like CVD-related hypertension. We present a robust method to improve the functionality of human SMCs and integrate them into drug development and preclinical safety processes.