Impedance Analysis of Phenotypic Variation in Vascular Smooth Muscle Cells

C. Bradley [1], C. McCormick [1], M.E. Sandison [1]
[1] *Wolfson Centre, University of Strathclyde, Glasgow, G4 0NW*

Introduction

Vascular smooth muscle cells (vSMCs) play a central role in the development of atherosclerosis, with vSMCs switching from a contractile to a proliferative, migratory phenotype that contributes to plaque development[1]. Similar phenotypic changes occur when vSMCs are exposed to standard culture conditions[1]. Recent findings have also shown significant heterogeneity within vSMC populations, both between cells obtained from different vascular beds and at the single cell level, with a spectrum of vSMC phenotypes possible. With the ever increasing interest in developing label-free, real-time, impedance-based cellular assays, including for characterising vascular cell behaviours[2], a better understanding of how different SMC phenotypes alter impedance signatures is required. We have therefore performed impedance spectroscopy (EIS) time-courses to characterise vSMC populations obtained from different vascular beds, employing prolonged culture times and serum starvation to alter vSMC phenotypes.

Methods

Primary aorta, carotid artery (CA) and saphenous vein (SV) vSMCs were isolated from male Sprague-Dawley rats by enzymatic digestion[1]. The SMCs were then seeded (1×10^5 cells/cm^2) within culture dishes patterned with sputter-coated gold electrodes (1mm wide working electrodes). Impedance spectra (1Hz-100kHZ, 50 mV, PalmSens4 analyser) were acquired at a series of fixed time points: when cultures first reached confluence (established by imaging); then 7 days post-confluence; then after a 24-72h period of serum starvation; and finally during pharmacological stimulation (fixed frequency of 10kHz) with the vSMC agonist phenylephrine (PE, 10μM).

Results and Discussion

Impedance spectroscopy measurements (background-corrected impedance magnitude, $|Z|-|Z_{media}|$) showed differences between confluent vSMC cultures derived from the different tissues, with aorta and SV showing significantly different impedance profiles when compared to CA cultures ($p < 0.05$ for certain frequencies). Within the spectrum of vSMC behaviours from a proliferative, migratory to a mature, contractile phenotype, confluent vSMC cultures are known to exhibit a more contractile-like behaviour than sub-confluent cells[3]. Prolonged culture beyond confluence could further promote this. A decrease in $|Z|-|Z_{media}|$ for all cultures (aorta, CA and SV) between initial confluence and 7 days post-confluence was observed, indicating a phenotypic shift, with notable differences between all three at 1kHz. Serum starvation also promotes a more contractile-like phenotype[4] and 24h starvation resulted in a further decrease in $|Z|-|Z_{media}|$ for all cell types, with the greatest decrease being observed for aorta, where prolonged serum starvation (72h) also further decreased the measured impedance. 24h serum-starved cultures were stimulated with PE, an agonist that elicits contraction in mature vSMCs. Although all cultures responded with a transient decrease in impedance, there were significant differences in the magnitude of the response, with aorta cultures showing the greatest fold-decrease in $|Z|-|Z_{media}|$, followed by CA then SV cultures.

Conclusion

Clear differences in spectra between vSMC cultures from different vascular beds were observed using EIS, with EIS capable of detecting phenotypic shifts as culture conditions were altered.

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References

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