THE EFFECT OF STRAIN ANISOTROPY ON THE INTERPLAY BETWEEN NOTCH SIGNALING AND VASCULAR SMOOTH MUSCLE CELLS

Cansu Karakaya (1,2), Eva van den Hurk (1), Carlijn Bouten (1,2), Cecilia Sahlgren (1,2,3), Sandra Loerakker (1,2)

1. Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands
2. Institute for Complex Molecular Systems, Eindhoven University of Technology, The Netherlands
3. Faculty of Science and Engineering, Biosciences, Åbo Akademi University, Finland

Introduction

Vascular smooth muscle cells (VSMCs) are important regulators of arterial growth and remodeling. They have the ability to switch from a contractile quiescent phenotype to a more proliferative and migratory synthetic phenotype upon mechanical perturbations. Due to the pulsatile blood pressure, VSMCs are mainly exposed to cyclic circumferential strain in the vessel wall, which regulates their phenotypic switching. However, cyclic uniaxial strain has been reported to both up- and downregulate the expression of contractile phenotype markers compared to the static controls *in vitro* [1]. Furthermore, the underlying mechanobiological mechanisms by which strain regulates the phenotype of VSMCs are not fully understood. A better understanding of the interaction between strain and signaling pathways regulating VSMC fate is essential to understand and control the growth and remodeling in (pathological) vascular adaptation and regeneration. The Notch signaling pathway, increasingly recognized as mechanosensitive, may be the link between mechanical strain and VSMC behaviour [2], as the application of equibiaxial strain decreases Notch signaling, which in turn switches the contractile phenotype to a synthetic state in VSMCs [3]. Still, it is not clear if uniaxial strain, which better mimics the *in vivo* strain that VSMCs experience, would have a similar effect on Notch signaling, and to what extent this interplay determines the VSMC phenotype. Thus, the current study aims to explore the interplay between uniaxial strain and Notch signaling on VSMC phenotype, and compare the effects of equibiaxial and uniaxial strain in similar *in vitro* conditions.

Methods

Human coronary artery smooth muscle cells (Lonza) were cultured for 7 days in either smooth muscle cell growth medium (Cell Applications Inc.) to obtain synthetic VSMCs, or smooth muscle differentiation medium (Cell Applications Inc.) to obtain contractile VSMCs. Cells were stretched either equibiaxially or uniaxially with the Flexcell Tension System at 0.5 Hz for 48 hours. The displacement of membranes was analyzed, and the corresponding strains were calculated via digital image correlation. Immunofluorescence staining and quantitative polymerase chain reaction were conducted to characterize the changes in cell phenotype and Notch signaling upon the application of equibiaxial and uniaxial strain.

Results

Synthetic and contractile VSMCs showed their phenotypic characteristics in static conditions, with contractile VSMCs expressing more and fibrous alpha smooth muscle actin (αSMA) compared to synthetic VSMCs (Fig. 1A). The application of uniaxial and equibiaxial strain resulted in a similar decrease in contractility marker *ACTA2* and Notch activating ligand *JAG1* (Fig. 1B). However, in contrast to the equibiaxial strain condition, reduced gene expression was not translated in protein expression in the case of uniaxial strain, as the contractile VSMCs preserved their fibrous organization of αSMA (Fig. 1A).

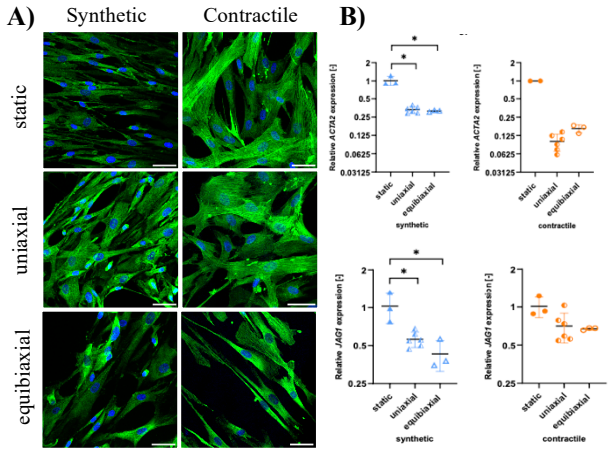

*Figure 1: (A) Immunofluorescence staining of contractility marker αSMA in green and DAPI in blue (scale bar: 100 um) (B) Gene expression of ACTA2 and JAG1 (*p<0,05).*

Discussion

Our results indicate that Notch signaling in 2D *in vitro* cultured VSMCs responds similarly to uniaxial and equibiaxial strain. Therefore, we suggest that Notch signaling is responsive to the maximum principal strain, although there may be some post-translational modifications that affect the functional regulation of VSMC phenotype under uniaxial strain.

References

1. Jensen et al., Cells, 10(9):2209, 2021.
2. Karakaya and van Asten et al., BMMB, 21(1):5-54, 2022.
3. Karakaya et al, Front Cell Dev Biol, 10:910503, 2022.

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