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Key Words: Atherosclerosis • Wnt Signalling Pathway • β catenin • Shear Stress
• Human Umbilical Vein Endothelial Cells (HUVECs) • Angiopoietin-2 •
Thrombospondin-1

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Introduction

Methods

Orbital Shaker

HUVECs were cultured in flasks until ~80% confluent. Cells were then washed with warmed PBS and incubated with 1ml of trypsin until cells thoroughly detached. M199 media was added to the cells, before being transferred to a falcon tube and spun for 5 minutes at 400g. The supernatant was discarded, and cells were re-suspended

37 in M199 media and transferred to 10mm radius 6 well plates. Once confluent, 3ml of
 38 1% DMSO in M199 was added to one half of the plates, and 3ml of 1% Wnt inhibitor
 39 in M199 to the other half. Cells were subjected to flow using a orbital shaker at 210
 40 rpm for 72 hours, with the exception of a static control.

41 mRNA Isolation and qPCR

42 Media was removed from the plates and cells were washed with cold PBS. Cells were
 43 isolated from the periphery and centre of the plates using 350µl of PBS, before cen-
 44 trifuging for 5 minutes at 400g and removing the supernatant. Total mRNA was
 45 isolated using the RNEasy Mini Kit (Qiagen) and concentration was determined us-
 46 ing a spectrophotometer. mRNA was reverse transcribed to cDNA using the Verso
 47 cDNA Synthesis Kit (Thermo Scientific).

48 The oligonucleotide qPCR primers were obtained from Ensembl¹ (Table 1).

Table 1. Oligonucleotide qPCR primers from Ensembl.

Gene	Direction	Sequence
ANGPT2	L	CGGCTGTGATGATAGAAATAGGGA
	R	GTTCCAAGAGCTGAAGTTCAAGTC
AXIN	L	TGTCACCTACTTTTTCTGTGGGGA
	R	TGTCACCTACTTTTTCTGTGGGGA
HPRT1	L	NA
	R	NA
KLF2	L	NA
	R	NA
THSB1	L	AAAGATGGAGAATGCTGAGTTGGA
	R	GGTTCCAAGACAAACCTCACATT

49 Results

50 Discussion

51 Acknowledgements

52 (211 Words) remove headers !!!

53 References

54 1. Howe, K. L. *et al.* Ensembl 2021. *Nucleic Acids Research* **49**, D884–D891 (2020).

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