Platelet-Derived Growth Factors In Vascular Smooth Muscle Cells On Compliance Mismatched Polymer Film Under Cyclic Stretching

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Introduction

Background:

- Currently available synthetic small diameter vascular grafts (SDVGs), defined as smaller than 6mm in diameter [1], have a **failure rate as high as 82%** [2], with the biggest factor contributing to the failure is **intimal hyperplasia** [2].
- One of the potential explanations for the unsuccessful long-term SDVGs is **compliance mismatch** [3].
- Compliance in vascular graft engineering refers to circumferential elasticity of either the blood vessel or graft.
- Compliance of native blood vessels have compliance of 6.6±1.3% per 100 mmHg, whereas currently available sSDVG have 1.2±0.3 % per 100 mmHg [4].
- Suture-line effects have been studied, but the effects of **compliance mismatch on continuous polymer** is not yet well understood [5], especially with **application of cyclic stretch**.
- Cyclic stretching is used to simulate pulsatile flow of blood *in vitro*. Exposure to cyclic strain resulted in increased vascular smooth muscle cell (VSMC) proliferation [6].
- **Increased SMC proliferation** can cause intimal hyperplasia [7].
- **Platelet-derived growth factor (PDGF)** has been shown to affect SMC proliferation [7].

Hypothesis:

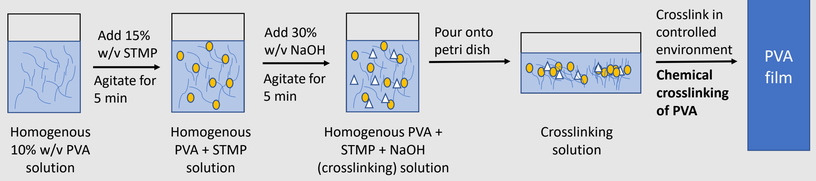
- **Compliance mismatch will result in higher PDGF signal after cyclic stretching.**

Objective:

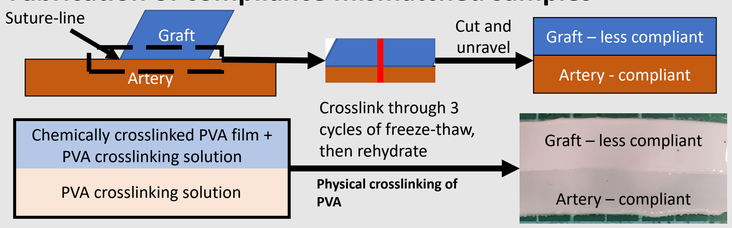
- Develop a platform to expose SMCs cultured on a continuous compliance mismatched films to cyclic stretching.
- Visualize and quantify biological markers and its distributions.

Methods and Materials

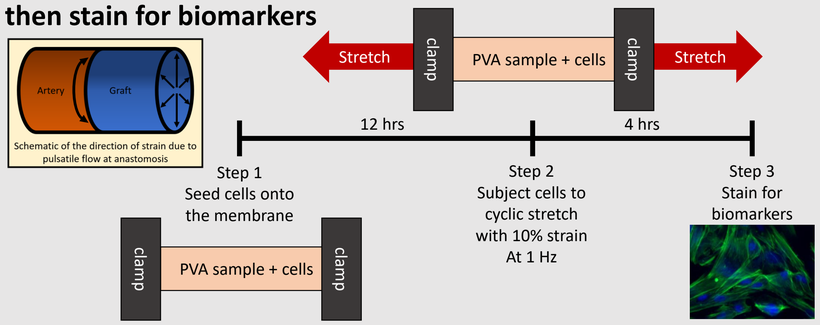
1. Preparation of crosslinking solution and making PVA film



2. Fabrication of compliance mismatched samples



3. Seed cells on the samples and expose to cyclic stretching, then stain for biomarkers



Results and Discussion

Verification of cell adhesion

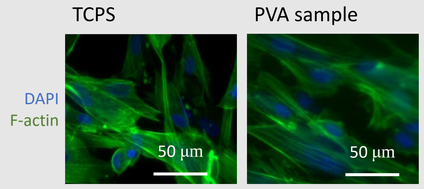


Fig 1. PVA samples seeded with human umbilical smooth muscle cells. PVA samples resulted in good cellular attachment. Tissue culture polystyrene (TCPS) was used as control.

Sample mechanical properties

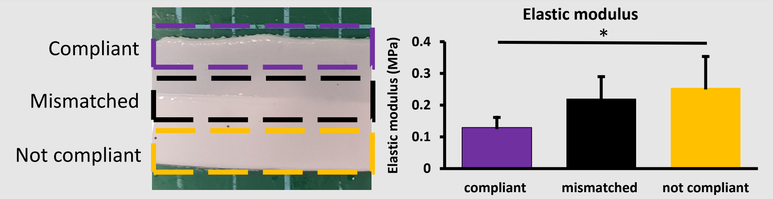


Fig 2. Quantification of elastic modulus of the samples. Not compliant samples were significantly stiffer than the compliant samples. Mismatched samples had elastic modulus between compliant and not compliant samples. *= p<0.05 between indicated groups. Two-way ANOVA used for statical analysis. St. Dev. used for error bars. N=3 per group.

Verification of uniform cyclic stretch

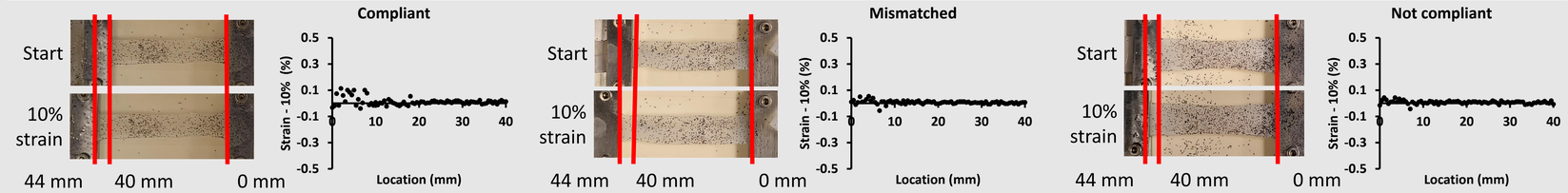


Fig 3. Strain map of individual samples. All of the samples displayed uniform uniaxial 10% strain throughout the sample during stretch.

Compliance mismatch resulted in higher proliferation, concentration of PDGF, and pMLCK

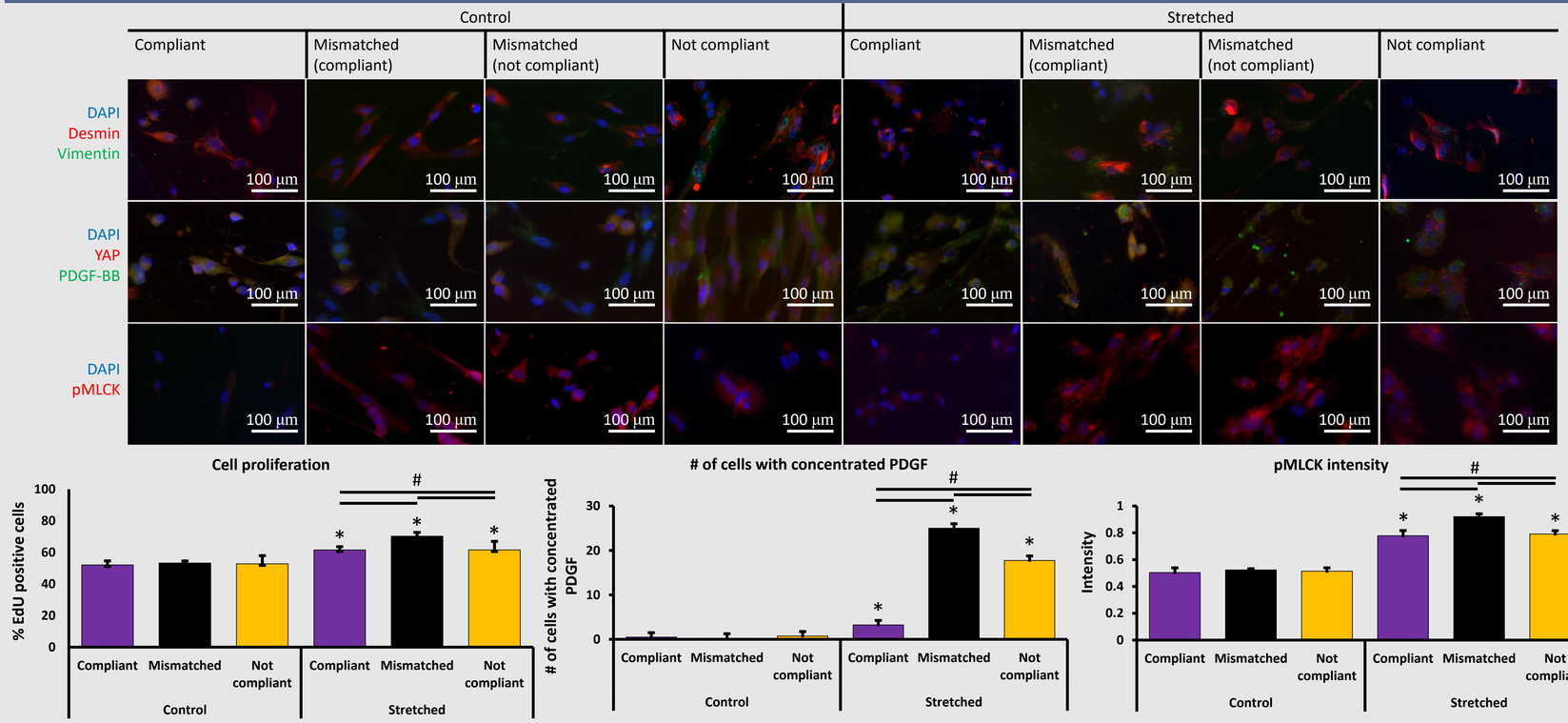


Fig 4. Immunofluorescent staining of different biomarkers and their quantification. Compliance mismatch resulted in higher PDGF-BB signal after stretching with 10% strain. Compliance mismatch resulted in higher phospho-Myosin Light Chain Kinase (pMLCK) signal after stretching with 10% strain. Yes-associated protein (YAP) did not show nuclear localization regardless of cyclic stretch or compliance mismatch. *= p<0.05 between the indicated group and control-compliant group. # = p<0.05 between indicated groups. Two-way ANOVA was used for statistical analysis. St. Dev. used for error bars. N=4, n=100 per group.

Conclusions

- Different PVA crosslinking methods can be used to create continuous compliance mismatched PVA samples to mimic suture-line without suture
- Uniform uniaxial strain can be applied to the compliance mismatched PVA samples to mimic cyclic stretch from pulsatile flow
- Exposure to cyclic stretch resulted in higher SMC proliferation, appearance of concentration of PDGF, and higher pMLCK signal

Acknowledgements

- National Institutes of Health (NIH R01 HL130274-01A1)
- NSERC-CREATE, Training in Global Biomedical Technology Research and Innovation at the University of Waterloo [CREATE Funding 401207296]
- NSERC Discovery RGPIN-2021-03200

References

[1] S. Han, D. Song, and S. Yun, "Long Term Outcomes of Arteriovenous Grafts for Hemodialysis in Lower Extremities.," *Vasc. Spec. Int.*, vol. 32, no. 4, pp. 180–185, Dec. 2016.
[2] Schon, D., Delozier, T., & Patel, N. (n.d.). Investigations Comparison of Procedure Cost for Thrombectomy of Arteriovenous Fistulas and Grafts. https://doi.org/10.1111/sdi.12002
[3] N. R. Tai, H. J. Salacinski, A. Edwards, G. Hamilton, and A. M. Seifalian, "Compliance properties of conduits used in vascular reconstruction," *Br. J. Surg.*, vol. 87, no. 11, pp. 1516–1524, Nov. 2000.
[4] Jeong Y, Yao Y, Mekonnen TH, Yim EK (2021) Changing compliance of poly (vinyl alcohol) vascular grafts through modifying interlayer adhesion and crosslinking density. Frontiers in Materials 7:456
[5] Roussis PC, Giannakopoulos A, Charalambous H (2015) Suture line response of end-to-side anastomosis: A stress concentration methodology. Cardiovascular engineering and technology 6 (1):36-48
[6] Smith P, Janiga K, Bruce M (1994) Strain increases airway smooth muscle cell proliferation. American journal of respiratory cell and molecular biology 10 (1):85-90
[7] Melnik T, Jordan O, Corpataux J-M, Delie F, Saucy F (2022) Pharmacological prevention of intimal hyperplasia: A state-of-the-art review. Pharmacology & Therapeutics:108157