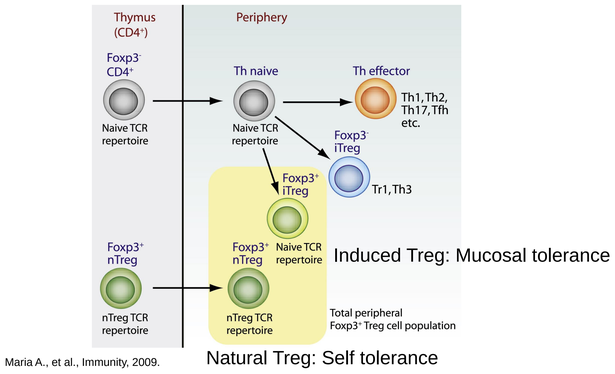
Substrate Stiffness Modulates Human Regulatory T Cell Induction and Metabolism

Lingting Shi[1], Jee Yoon Lee[1], and Lance C. Kam[1]

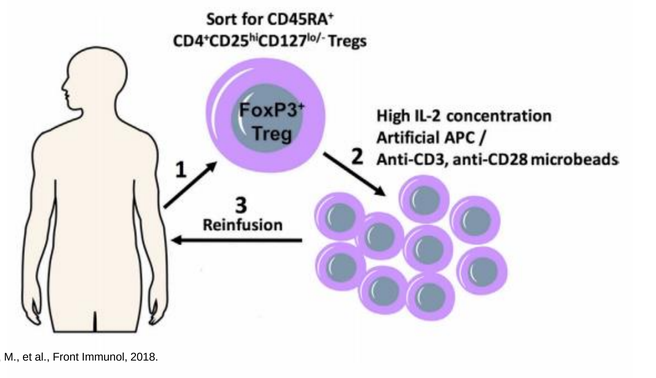
[1]Department of Biomedical Engineering, Columbia University, New York, NY, USA

I: INTRODUCTION



Maria A., et al., Immunity, 2009.

Treg adoptive therapy for autoimmune diseases



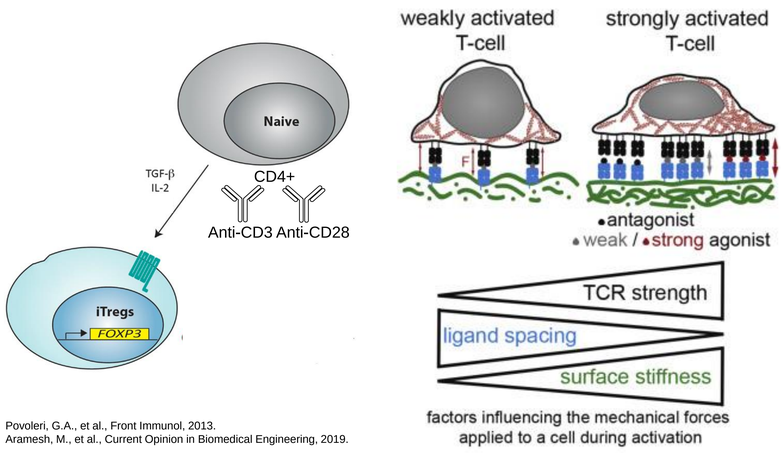
Biswas, M., et al., Front Immunol, 2018.

Limitation of natural Tregs[1]

- Unstable and dysfunctional in the inflammatory environment
- Potentially altered phenotype during expansion
- Intrinsic defect in patients with autoimmune diseases

Thus, *ex vivo* Treg induction from conventional T cells may alternatively generate sufficient amount of functional Tregs.

Treg induction requires activating signals and cytokines

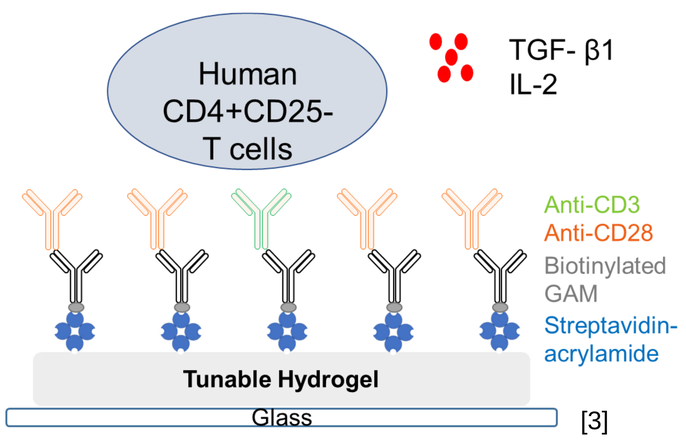


Povoleri, G.A., et al., Front Immunol, 2013.
Aramesh, M., et al., Current Opinion in Biomedical Engineering, 2019.

- Foxhead box P3 – transcription factor, common marker for Tregs
- Regulate Treg development, function, and homeostasis[2]

This work explores how substrate stiffness impacts Treg induction.

II: METHOD



[3]

III: RESULTS

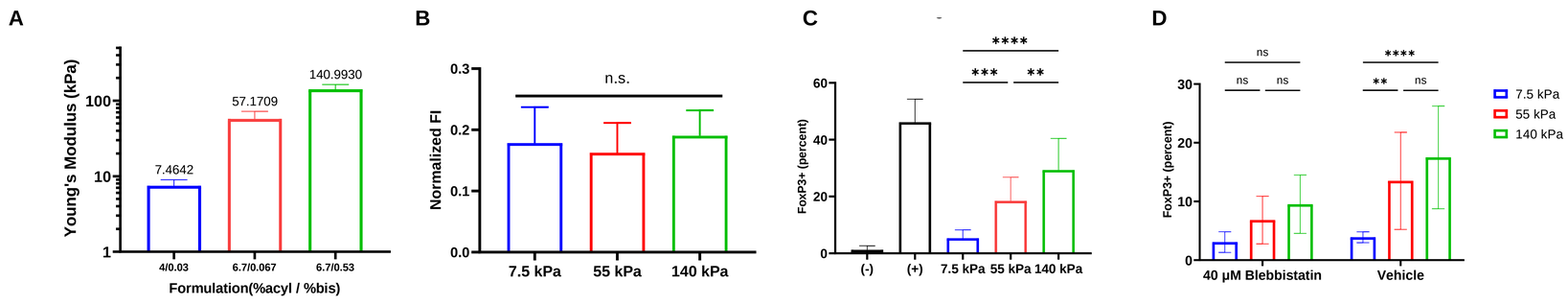


Figure 1. (A) Mechanical testing via indentation. (B) Streptavidin-acrylamide concentrations on gels. (C) Treg induction rate on gels. (D) Treg induction with Blebbistatin and vehicle control.

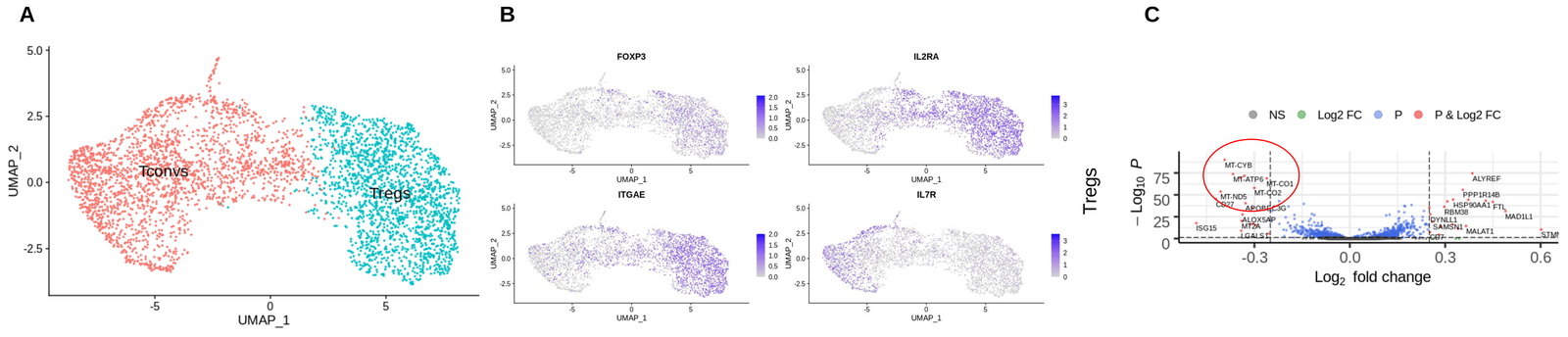


Figure 2. (A) Single-cell RNA Seq data from 7.5 kPa and 140 kPa dimension reduced with UMAP and clustered with Louvain algorithm. (B) Treg markers expression. (C) Differential expression analysis comparing Tregs induced on the soft and hard substrate.

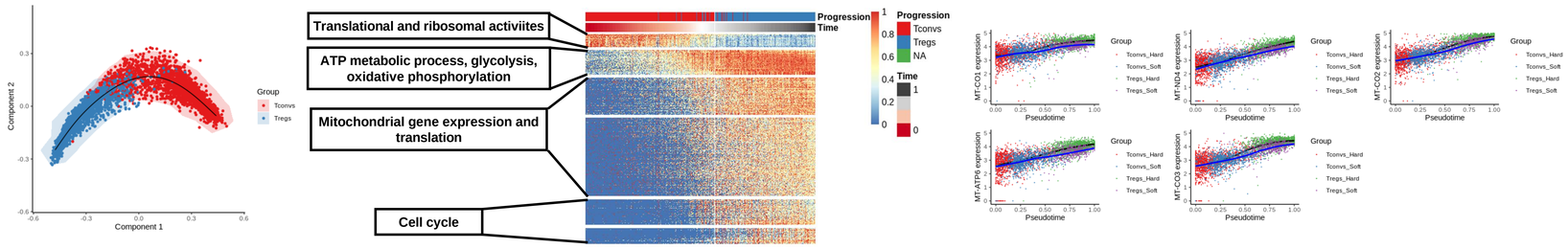


Figure 3. (A) Trajectory analysis was performed with SCORPIUS with T cells from both hard and soft samples combined. (B) Important genes that contribute to Treg induction were organized into different modules. (C) Expression of mitochondrial genes was plotted along the pseudotime projection.

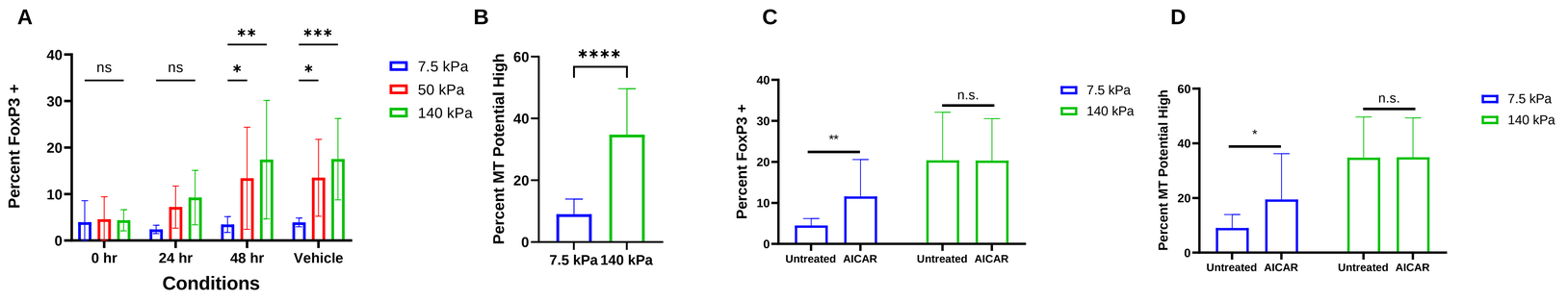


Figure 4. (A) Oligomycin was added in the culture that induces Tregs at 0 hr, 24 hr, 48 hr respectively. (B) 72 hr after induction, mitochondria potential was measured relative to the unstimulated control. (C,D) 250 μM of AICAR was introduced in the culture 18 hr after the induction, and percent FOXP3+ and percent mitochondrial potential were measured, respectively.

IV: SUMMARY AND FUTURE DIRECTIONS

Treg induction on polyacrylamide gels was sensitive to the substrates' elastic modulus, increasing with greater material stiffness. Single-cell RNA-Seq analysis revealed that Treg induction on stiffer substrates involved greater use of oxidative phosphorylation. Inhibition of ATP synthase significantly reduced the rate of Treg induction and abrogated the difference among gels. Activation of AMPK increased Treg induction on the softer sample but not on the harder sample. Thus, Treg induction is mechanosensitive and OXPHOS dependent, providing new strategies for improving production of these cells for cellular immunotherapy. In the future, we will investigate how substrate stiffness affects the stability and functionality of Tregs induced on different substrates.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Lan, Q. et al. *J Mol Cell Biol*, 2012. [2] Gottschalk et al., J Exp Med, 2010. 207(8): p. 1701-11. [3] Judokusumo et al., *Biophys J* 2012. 102(2): p. L5-7.