Enhancing Extracellular Matrix Deposition in vitro using Macromolecular Crowding
Paula Benny,1,2 Birgitte E. Lane,2 Michael Raghunath1,2
Corresponding Author: g0800072@nus.edu.sg

1Tissue Modulation Laboratory, Division of Bioengineering, Faculty of Engineering, National University of Singapore and 2Epithelial Biology Laboratory, Institute of Medical Biology, Singapore.

Introduction
In vivo, the extracellular milieu (ECM) is an extremely crowded environment. Remarkably, this clear and distinct cell characteristic is not incorporated into customary in vitro culture conditions1. Instead, test molecules are bathed in a non-crowded aqueous solution. To illustrate this issue, we examined one of the bottlenecks in tissue engineering. The rate-limiting enzymatic conversion of procollagen to collagen which occurs in the ECM is notoriously slow in vitro. We hypothesize that this is due to a lack of crowdedness, or an insufficient Excluded Volume Effect (EVE) in the culture media. We hypothesize that macromolecular crowding (MMC), employing EVE, would have an effect on biological reactions in terms of matrix enhancement and ECM deposition in mesenchymal and epithelial cells.

Materials and Methods
Normal embryonic lung fibroblasts (WI-38) and HaCat cells were cultured in the presence2 and absence of crowders; dextran sulfate 500kDa (DxS 500; pK Chemicals A/S) and the novel polysodium-4-styrene sulfonate 200kDa (PSS; Sigma). SDS-PAGE and Immunocytochemistry were carried out for quantitation.

Results
Fibroblasts crowded with DxS500 produced more collagen in the cell layer in 48 hours as compared to the cumulative collagen produced by non-crowded cells monitored for up to 4 weeks (Fig 1). After the technology was developed in fibroblasts, it was applied for the first time to keratinocytes. Using immunocytochemistry, we observed an increase in deposition of Collagen type IV, VII and fibronectin when macromolecular crowders were added in vitro as compared to when no crowders were introduced (Fig 2).

Discussion and Conclusions
The development of an enhanced keratinocyte basement membrane in vitro holds promise for applications in wound healing. Macromolecular crowding opens up avenues in tissue engineering to create tissue faster, as it mimics more closely the physiological in vivo environment.

References

Acknowledgments
My sincere gratitude to my supervisors, Prof. Raghunath and Prof. Lane, for their guidance.

Disclosures
The authors have nothing to disclose.