We are investigating protein combinations that enhance differentiation of pre-cardiac mesoderm

We **analysed** proteins in an **array** format to study the effects on pluripotency and the differentiation efficiency of cardiac mesoderm.

Defining differentiation conditions of human embryonic stem cells derived cardiomyocytes with arrayed cellular microenvironments

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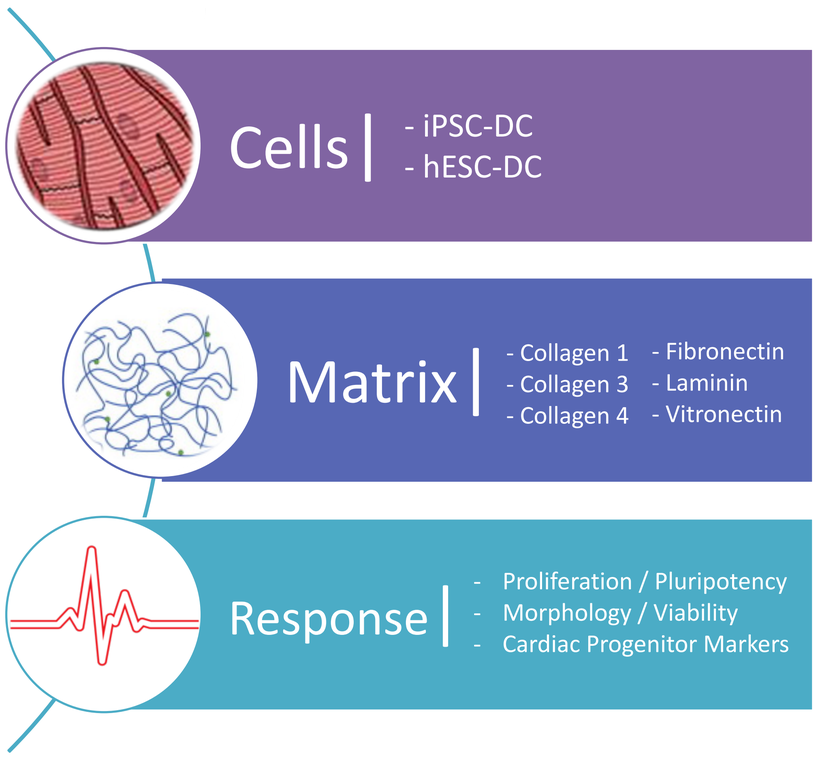
Introduction

- Pluripotent stem cell derived cardiomyocytes (PSC-DC) are a promising cell source for myocardial repair and regeneration (1).
- iPSC-DC have immature phenotypes and many researchers are trying accelerated iPSC-DC maturation (2).
- ECM plays a significant role in cardiomyocyte differentiation and cell fate in vivo (3).
- The desire to develop myocardial differentiation and maturation is for cardiac modeling and pharmacological screening (2).

Aims

- Determine the effects of ECM combination on pluripotency and stemness.
- Assess how ECM proteins can affect cell morphology and viability
- Find ECM combinations that promote cardiac mesoderm and cardiac progenitor differentiation.



Methods

96 Well configuration

	1	2	3	4	5	6	7	8	9	10	11	12
A	C1	C1F	C4L	C1C3V	C3C4L	FLV	C1C4FV	C1C3C4FL			Plastic	Matrigel
B	C3	C1L	C4V	C1C4F	C3C4V	C1C3C4F	C1C4LV	C1C3C4FV			Plastic	Matrigel
C	C4	C1V	FL	C1C4L	C3FL	C1C3C4L	C1FLV	C1C3C4LV			Plastic	Matrigel
D	F	C3C4	FV	C1C4V	C3FV	C1C3C4V	C3C4FL	C1C3FLV			Plastic	Matrigel
E	L	C3F	LV	C1FL	C3LV	C1C3FL	C3C4FV	C1C4FLV			Plastic	Matrigel
F	V	C3L	C1C3C4	C1FV	C4FL	C1C3FV	C3C4LV	C3C4FLV			Plastic	Matrigel
G	C1C3	C3V	C1C3F	C1LV	C4FV	C1C3LV	C3FLV	C1C3C4FLV			Plastic	Matrigel
H	C1C4	C4F	C1C3L	C3C4F	C4LV	C1C4FL	C4FLV				Plastic	Matrigel

Tables 1. Schematic of how the ECM proteins were allocated to the wells of a 96 well plate.

- ECM proteins were chosen based on there prevalence in the human heart through the early stages of differentiation.
- 63 permutations of the 6 ECM proteins were physically absorbed into the wells of a 96 well plate.

Proteins	Abbreviation	Brand/Product #	Host Source
Collagen 1	C1	Advanced Biomatrix #5007	Human
Collagen 3	C3	Advanced Biomatrix #5021	Human
Collagen 4	C4	Advanced Biomatrix #5016	Human
Fibronectin	F	ThermoFisher #33016015	Human
Laminin	L	ThermoFisher #23017015	Mouse
Vitronectin	V	ThermoFisher #A14700	Human

Tables 2. Protein names, abbreviated names, brand information, and host animal the protein was derived from.

Results 1: PSC Proliferation and Pluripotency

- Generating global index values using the intensity of biomarker stains allows for the visual comparison between multiple groups.
- Protein combinations that contain Laminin and vitronectin promote more efficient proliferation than control groups in multiple repeats.
- There is a strong correlation between the cell density and the use of laminin and vitronectin.

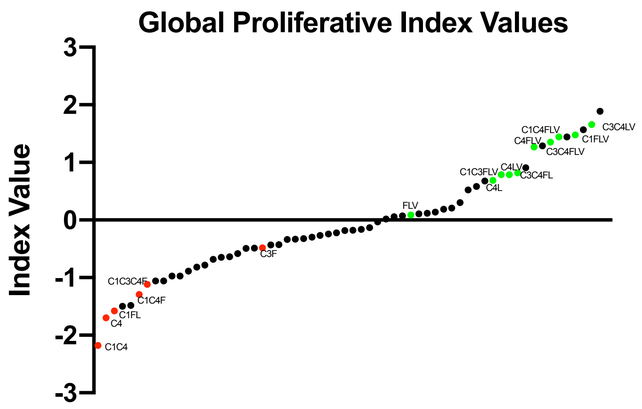
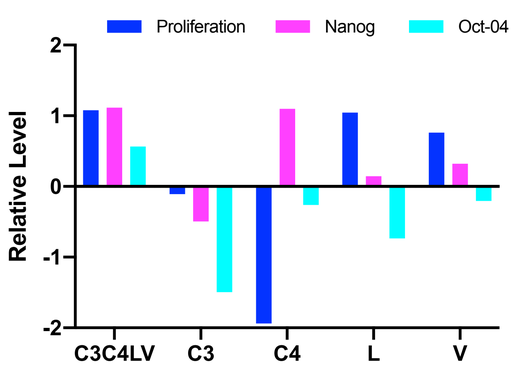
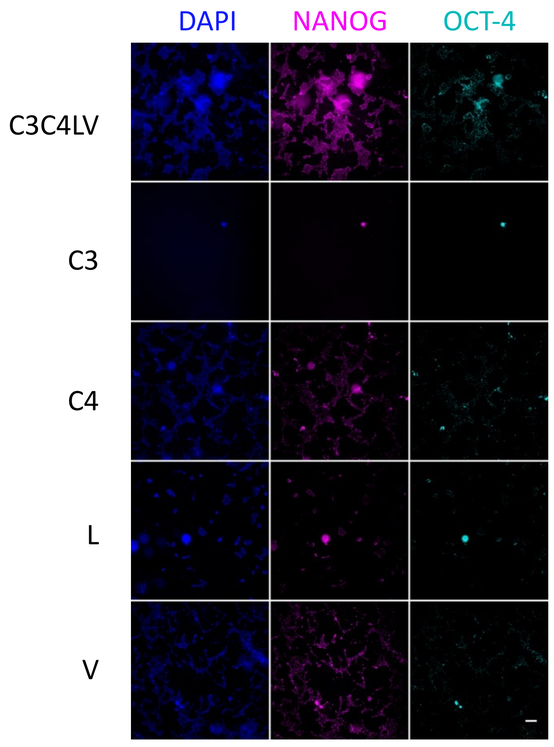


Figure 1: Distribution of global mean index values from each ECM combination. Green indicates repeatable high proliferation groups while red indicates repeatable low proliferation groups.



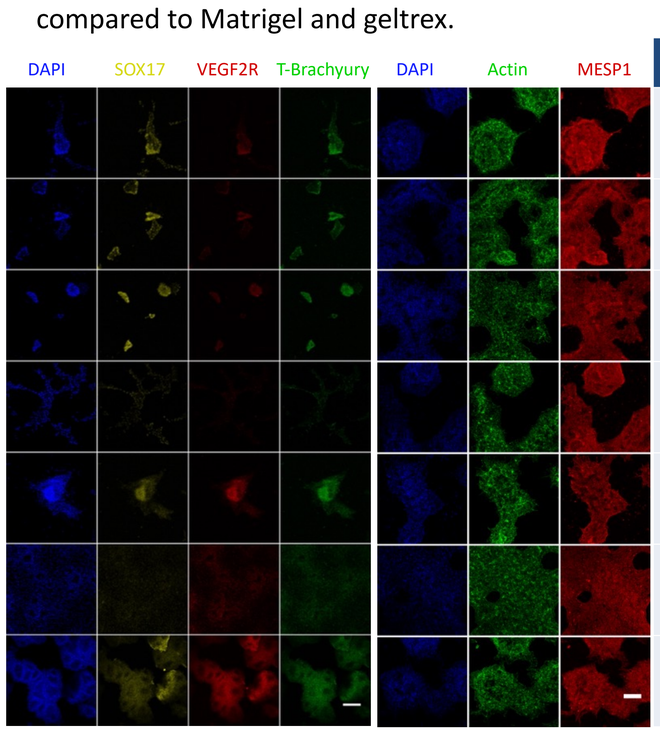


- The combination of collagens 3 & 4 with laminin and vitronectin promotes pluripotency in multiple repeats and aligns with literature sources (4, 5).
- Proliferation doesn't correlate with pluripotency when comparing DAPI to Nanog and OCT-4 (P=0.2806 and P=0.3516 respectively).

Figure 2: Relative level of biomarker expression compared to the global average index value of 0 (top left).
Figure 3: Fluorescent images of the relative biomarkers represented in figure 2 (right) scale bar = 100um.

Results 2: PSC Differentiation and Cardiac Progenitors

- Global average index values highlight 7 unique protein combinations that promote germ layer differentiation with a higher efficiency of differentiation in the mesoderm linage (T-brachyury) and decreased differentiation in the endoderm linage (Sox17).
- These 7 unique protein combinations also highlight an increased expression of cardiac mesoderm markers (VEGF2R and MESP1) and an increased density of actin filaments when compared to Matrigel and geltrex.



DAPI	SOX17	VEGF2R	T-Brachyury	Actin	MESP 1
0.376909	0.202453	0.58581	0.65607	0.43731	0.35031
0.431226	0.354008	1.222792	0.695799	1.24512	1.19951
0.461448	0.087821	0.361296	0.225646	0.81517	0.70414
0.837553	0.40379	1.290722	0.632681	1.43842	1.24637
0.965312	-0.049321	0.846805	0.246825	0.63301	0.86023
1.644378	-0.472429	0.079766	0.204892	2.16856	1.98300
1.684781	-0.75551	0.051571	0.002681	1.07874	0.84620

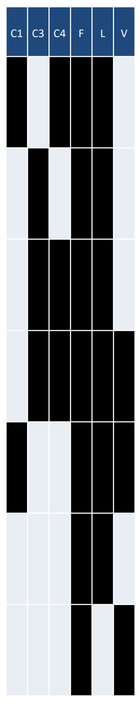


Figure 4: Confocal images of biomarker expression on 7 unique ECM combinations with the respective global average index values and the specific ECM protein combination.

Conclusion

- Here, we demonstrate a unique method of using an array of ECM proteins to analyze the differentiation efficiency of Pluripotent stem cells.
- We found the combination of collagens 3 and 4 with laminin and vitronectin could maintain pluripotency over multiple passages better than pluripotent cells on Matrigel
- We identified 7 unique ECM protein combinations that enhanced development of cardiac progenitor cells compared to traditional methods on Matrigel and Geltrex.

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Acknowledgments and funding

I would like to acknowledge UNSW for funding my PhD through the University international postgraduate award.
The imaging component of this study was carried out using instruments situated in, and maintained by, the Katharina Gaus Light Microscopy Facility (KGLMF) at UNSW.
I would also like to acknowledge the support of my supervisor Dr Kristopher Kilian, and my colleague Pallavi Sirvastava for her influence and wisdom in this field.