An investigation into cell seeding efficiency on dermal scaffolds for *in vitro* pre-clinical studies

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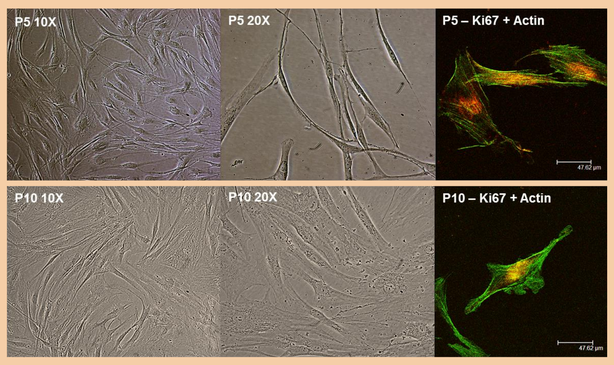
BACKGROUND

- The demand for tissue-engineered dermal scaffolds for full thickness skin wounds continues as current treatments are inefficient.
- Dermal scaffolds undergo rigorous *in vitro* and *in vivo* testing to fine-tune their optimal properties for efficient wound healing.
- During *in vitro* cell studies the percentage of seeded cells that adhere to the scaffolds is low and its importance overlooked.
- Inefficient cell seeding slows *in vitro* experiments and are costly in terms of resources and time.

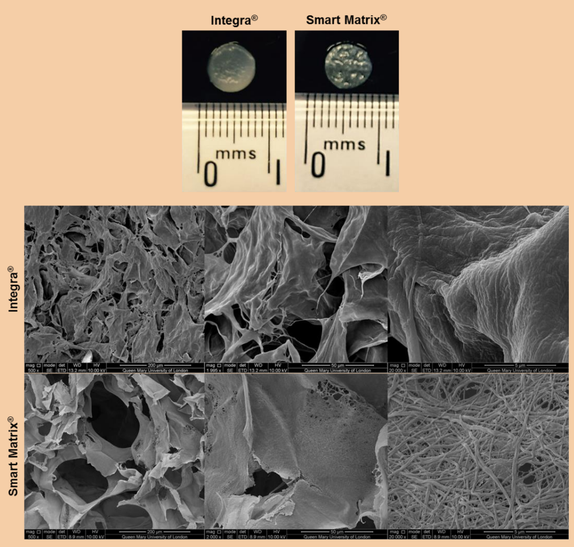
AIM

- **To investigate optimum conditions to improve cell seeding efficiency on dermal scaffolds for *in vitro* pre-clinical studies.**
- **We hypothesised that synergy of variable affects cell seeding efficiency.**

MATERIALS AND METHODS



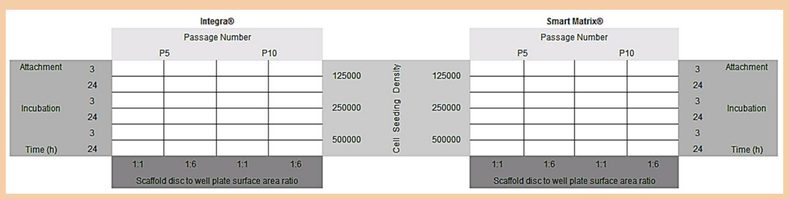
Cells: primary normal human dermal fibroblasts (pnHDFs), main cell type in the dermis.



Dermal scaffolds: Integra® (commercially available) and Smart Matrix® (under development).

Cell Seeding Variables

1) Cell passage number (5 and 10)
2) Cell seeding density ($1.25x10^5$, $2.5x10^5$ or $5x10^5$ in 200µl) per scaffold
3) Scaffold disc to well plate surface area ratio (1:1 or 1:6)
4) Attachment incubation time at 37°C with 5% CO_2 (3h or 24h)



- Matrix of variables to study synergy.
- For each individual set of conditions n=3.

Quantitative: Metabolic assay

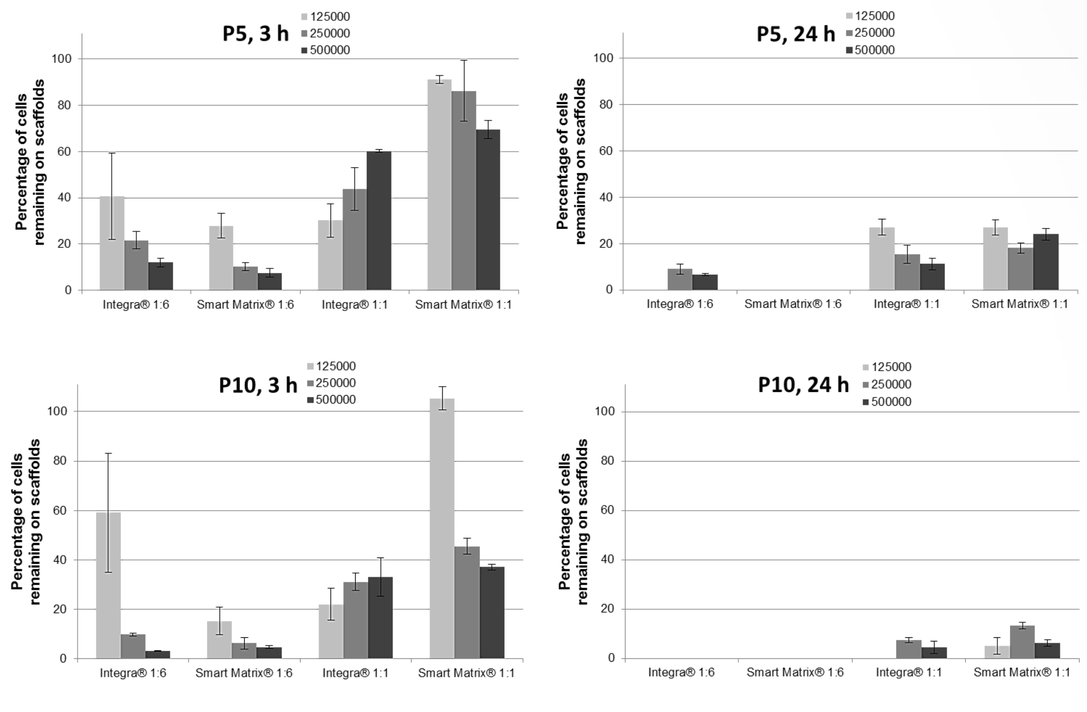
Qualitative: Histology and microscopy

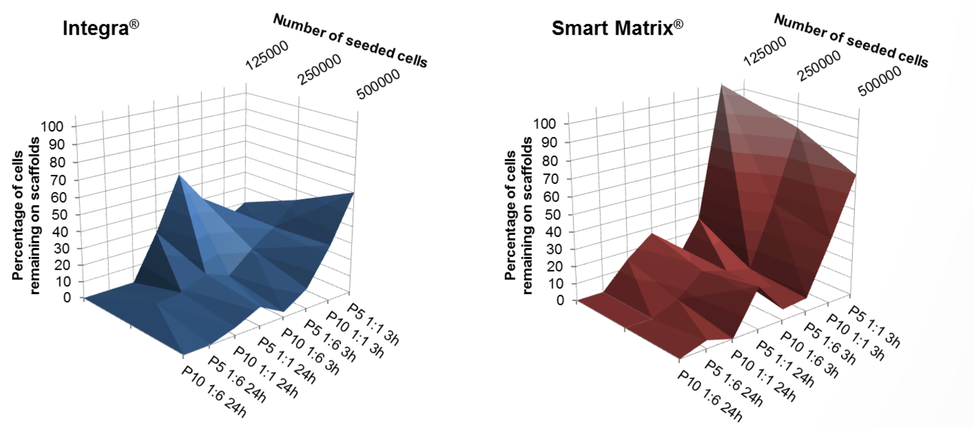
Seeding efficiency calculated as % of cells remaining on scaffolds

- Cells on scaffolds
- Cells left on plates

RESULTS

- Data was plotted in 2D and 3D graphs.
- Attachment incubation time ($p<0.001$ for both scaffolds) and scaffold to well plate surface area ratio ($p<0.001$ for both scaffolds), followed by the cell passage number only for Integra® ($p=0.003$), had the largest effect on seeding efficiency.
- The highest efficiencies were obtained at the lowest density ($1.25x10^5$) for both P5 and P10, which suggests that lower seeding densities may result in less cell wastage.





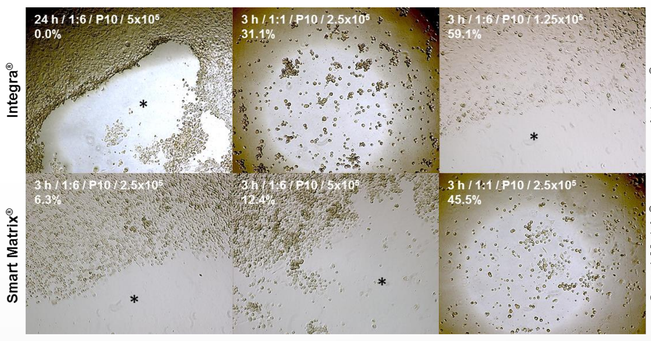
Matrix of variables filled with results (% of cells remaining on scaffolds) to visually observe how synergy of variables affects cells seeding efficiency:

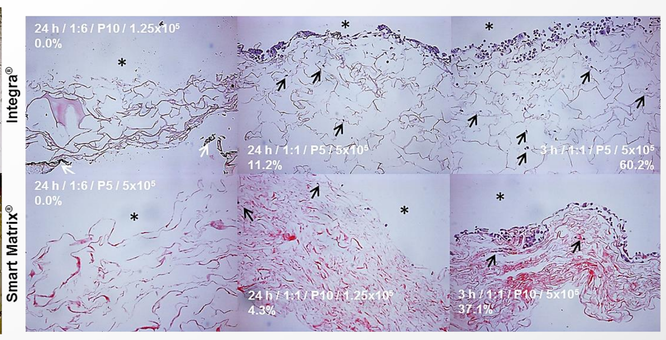
Integra®							Smart Matrix®					
		Passage Number						Passage Number				
		P5		P10				P5		P10		
Attachment	3	30.3	40.7	22.0	59.1	125000	125000	91.1	27.8	105.3	5.1	3	Attachment
	24	27.0	0.0	0.0	0.0			26.9	0.0	15.3	0.0	24	
Incubation	3	43.8	21.7	31.1	9.7	250000	250000	86.2	10.2	45.5	13.4	3	Incubation
	24	15.4	9.0	7.4	0.0			18.1	0.0	6.3	0.0	24	
Time (h)	3	60.2	12.0	33.1	3.1	500000	500000	69.5	7.6	37.1	6.2	3	Time (h)
	24	11.2	0.0	4.6	0.0			24.0	0.0	4.7	0.0	24	
		1:1	1:6	1:1	1:6	Cell Seeding Density		1:1	1:6	1:1	1:6		
		Scaffold disc to well plate surface area ratio						Scaffold disc to well plate surface area ratio					

KEY: 0-9.9 | 10-19.9 | 20-29.9 | 30-39.9 | 40-49.9 | 50-59.9 | 60-69.9 | 70-79.9 | 80-89.9 | >90

Cells left on plates after cell seeding: *highlights the area where the scaffold was placed.

H&E stained seeded scaffolds: * indicates top of scaffold; white arrows point at remaining silicone layer of Integra®; black arrows point to cells that migrated into the scaffolds.





CONCLUSIONS

- **A synergy of different variables affects cell seeding efficiency onto dermal scaffolds, which should be investigated for each individual material.**
- **Optimisation of cell seeding efficiency on dermal scaffolds for pre-clinical *in vitro* studies can save time and resources.**
- **This study can be easily translated to other biomaterials and cell types.**