The Importance of Factorial Design in Tissue Engineering and Biomaterials Science: Optimization of Cell Seeding Efficiency on Dermal Scaffolds as a Case Study

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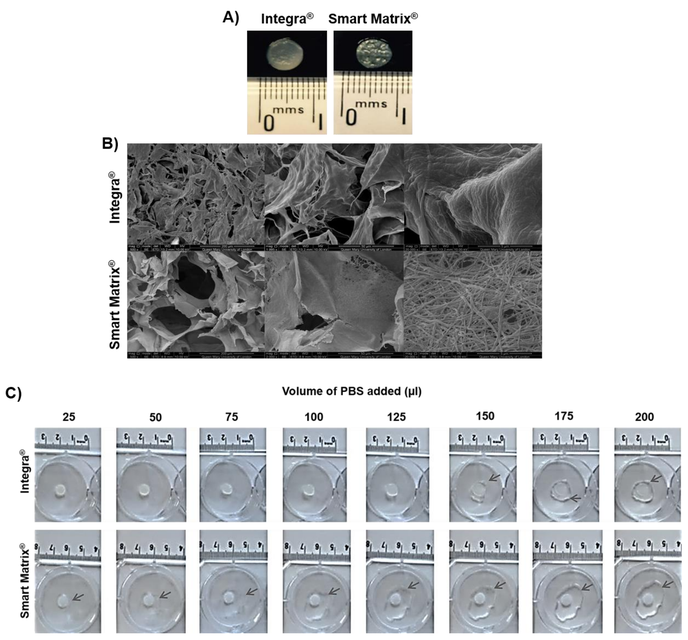
Background: We present a case study to show the usefulness and importance of using experimental factorial designs in tissue engineering and biomaterials science.

We used a full factorial design (2x2x2x3) to solve a routine query in biomaterials research: the optimisation of cell seeding efficiency for pre-clinical *in vitro* cell studies, the importance of which is often overlooked. Moreover, tissue-engineered scaffolds can be cellularised to form implantable tissue constructs, where the cell seeding method must be reliable and robust.

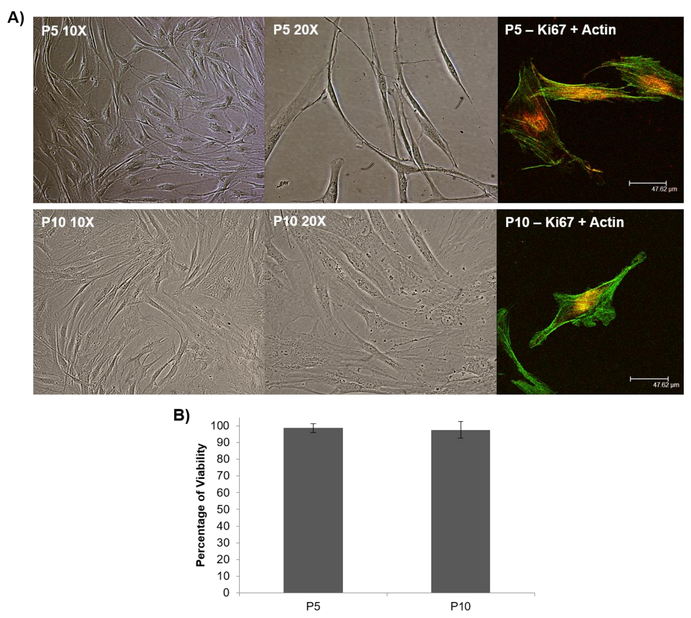
Aim: to optimise cell seeding efficiency on dermal scaffolds for in vitro pre-clinical studies using full factorial design

Method & Results

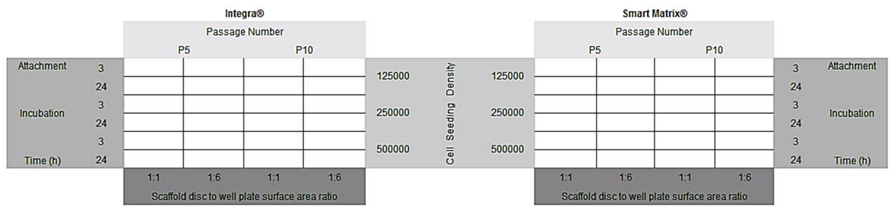
Dermal scaffolds: Integra® (collagen/GAG+silicone) and bovine Smart Matrix® (fibrin/alginate) cut to 6mm diameter.
- Homogenous structures of open, interconnected macro and micro-pores (A,B).
- Nano-pores and densely packed nano-fibres only observed for Smart Matrix® (B).
- Integra® is mechanically stronger and retains larger volumes of liquid (~125 µL) than Smart Matrix® (~25 µL)due to the silicone backing layer (C).



Cells: primary normal human dermal fibroblasts (pnHDFs) from a single donor.
- Cells maintained their spindle-shaped morphology throughout the study (A).
- Immunostaining of cells for Ki67 (red) and actin (green) suggests cells were proliferative at the time of the experiments (A).
- Cells were viable at the time of the experiments (B).



Experimental design: Variables and levels investigated (see matrix below), based on our experience with these materials, were:
1) cell passage number (5 or 10)
2) cell seeding density ($1.25x10^5$, $2.5x10^5$ or $5x10^5$ cells in 200 µL)
3) scaffold disc to well plate surface area ratio (1:1 or 1:6)
4) attachment incubation time (3 h or 24 h).
- Full factorial experimental design (2x2x2x3).
- For each individual set of experimental conditions n=3.
- Cell seeding efficiency was quantitatively assessed using alamarBlue®, a metabolic redox assay and calculated as percentage of cells remaining on the scaffolds.
- A standard curve was created for each passage number and attachment incubation time.
- Cell seeding was qualitatively assessed by histological processing and microscopy.

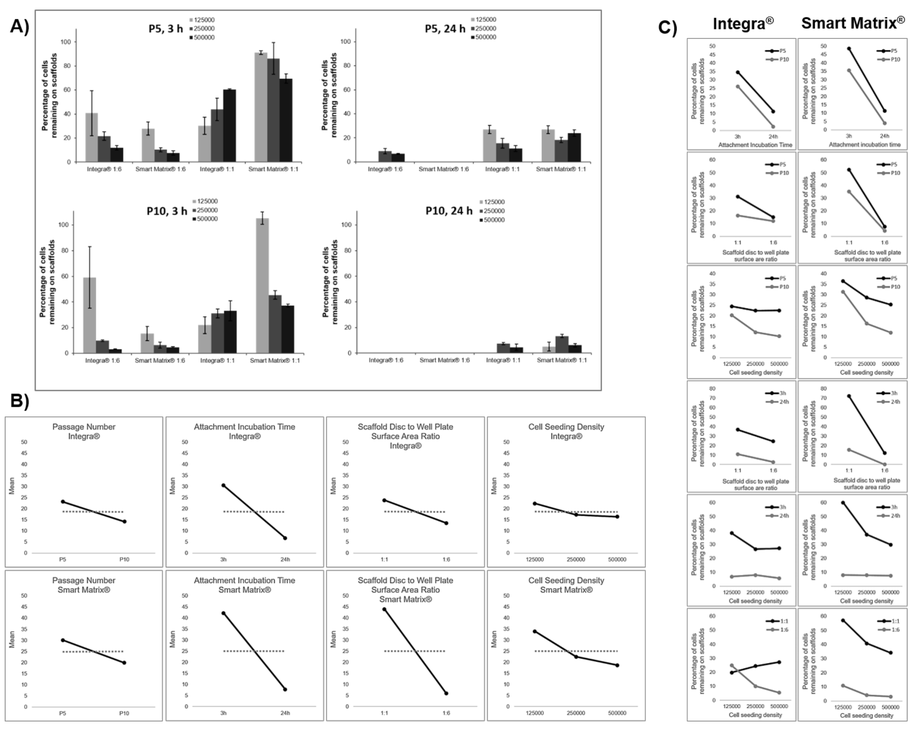


Visual representation of results: in order to more clearly observe the effects and interactions of the different variables and find the optimum combinations that should be used for each scaffold, we propose 2 different visual representations of the data:
1) 3D graphs (A),
2) the matrix above, was filled with results and a colour key was assigned to values (B).

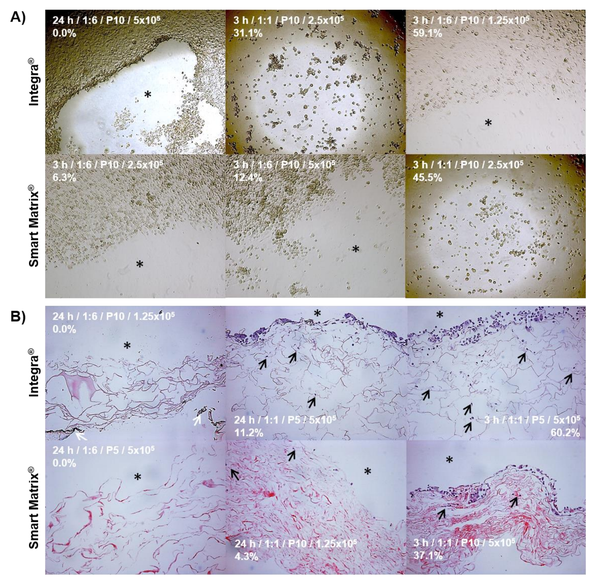
For Integra®, highest efficiencies were found when:
1) $5x10^5$ cells at P5 where seeded on scaffolds placed in 96 well plates (1:1) and incubated for 3h (60.2%),
2) 1.25 $x10^5$ cells at P10 were seeded on scaffolds placed in 24 well plates (1:6) and incubated for 3h (59.1%).

For Smart Matrix®, highest efficiencies were found when:
1) $1.25x10^5$ cells at P10 where seeded on scaffolds placed in 96 well plates (1:1) and incubated for 3h (105.3%),
2) 1.25 $x10^5$ cells at P5 were seeded on scaffolds placed in 96 well plates (1:1) and incubated for 3h (91.1%).

Main effects and interactions: attachment incubation time had a strong negative effect on seeding efficiency (B).
- Scaffold disc to well plate surface area ratio had a strong negative effect for Smart Matrix.
- Increasing passage number and cell seeding density had a negative effect for both scaffolds
- Statistical analysis showed that for Smart Matrix® the main effects of attachment incubation time and scaffold disc to well plate surface area ratio were statistically significant and so was their interaction (C).
- For Integra® only the main effect of attachment incubation time was statistically significant.



Microscopy: Phase-contrast light microscopy of empty wells revealed a ring of cells left behind following scaffold removal from 24 well plates (A).
- Fewer cells were left behind in 96 well plates: using a 96 well plate restricts cell seeding adhesion to the scaffold.
- H&E staining of seeded scaffolds revealed a layer of cells at the top of the scaffold where they were seeded (B).
- Qualitatively fewer cells were observed as the seeding efficiency decreased.



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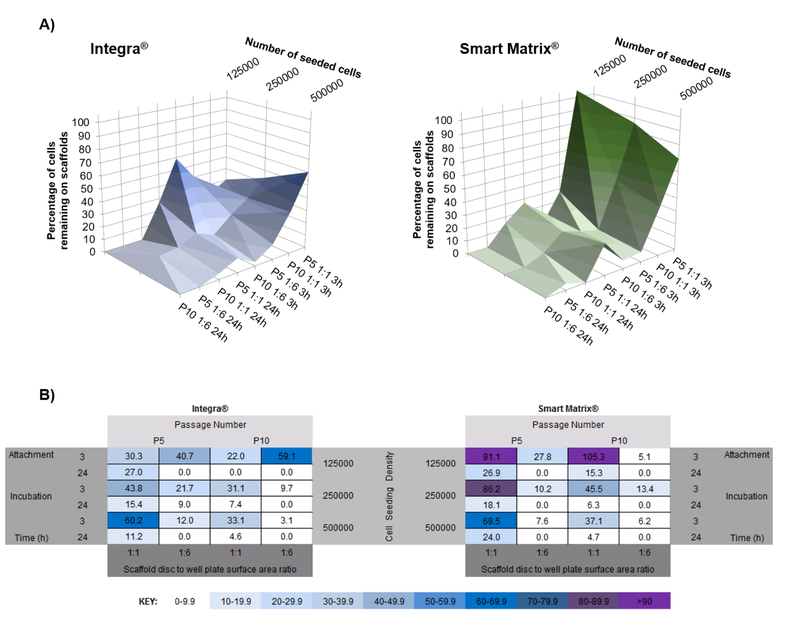

Conclusion:

- Our study design could save time and resources and the optimum seeding conditions should be investigated for individual scaffolds.
- Our study can be easily translated to other cell types and biomaterials, where multiple interacting variables can be thoroughly investigated for better understanding cell-biomaterial interactions.

References & Acknowledgements:

Levin *et al*. 2018. J Tissue Eng 9:1-14; Sharma *et al*. 2016. Biomed Mater 11:055001.

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