Comparative study of a dynamic seeding method and osteogenic supplementation in Mesenchymal Stem Cells

Ilona Bognar, supervised by Areli Munive Olarte and Prof. Gwendolen Reilly

INSIGNEO Institute for *in silico* Medicine

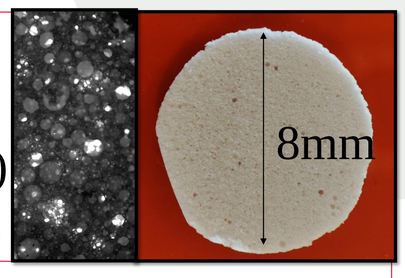
Background

Development of *in vitro* bone models that resemble the *in vivo* physiological bone structure remain a challenge. In this study, Mesenchymal Stem Cell (MSC) proliferation on a 3D porous material under static and dynamic conditions was compared. In addition, the effect of dexamethasone (DEX) withdrawal on MSC cultures was studied as it is shown to induce osteogenic differentiation, but timing exposure is not well clarified.

Methods

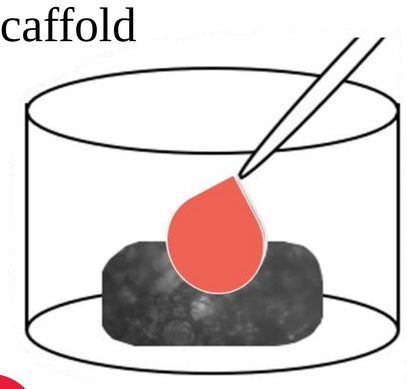
I Scaffold preparation

- Synthesis of IBOA & EHA scaffolds of 3 different % porosities (81 > 77 > 72)
- Plasma coating & hydration of scaffolds



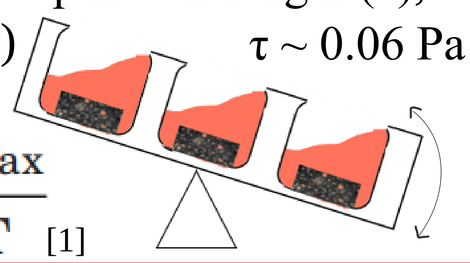
II Cell seeding - Y201 human MSCs

Static - cell suspension passively pipetted onto scaffold



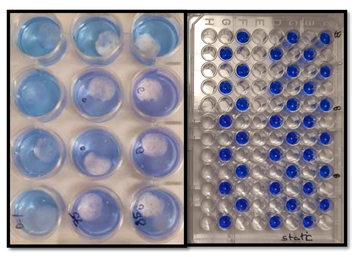
Dynamic - after suspension, plate was rocked for 1hr at 80 rpm. Modeling fluid shear stress at bottom of well(τ) with variables: fluid viscosity(μ), flip angle(θ), fluid depth/well length (δ), cycle length(T)

$\tau \sim 0.06$ Pa



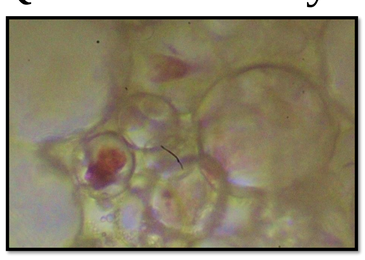
$$|\tau| = \frac{\pi \mu \theta_{max}}{2\delta^2 T}$$ [1]

III Measurements

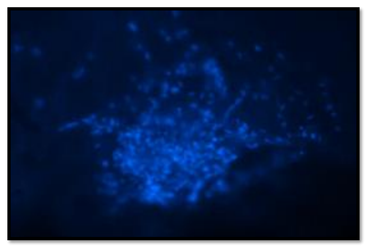


Resazurin assay
Fluorescent dye to quantify cell viability

Qualitative analysis of cell distribution:



Neutral red
Lysosome stain



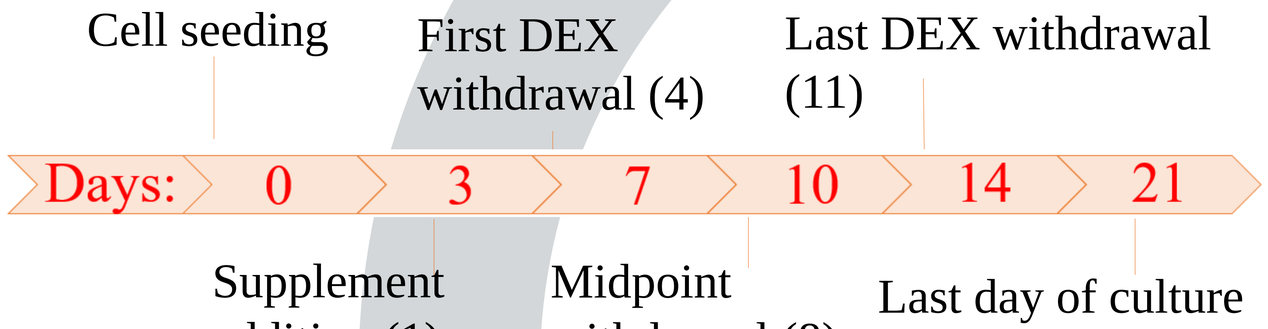
Hoechst 33342
Fluorescent DNA stain

Effects of DEX on osteogenic activity

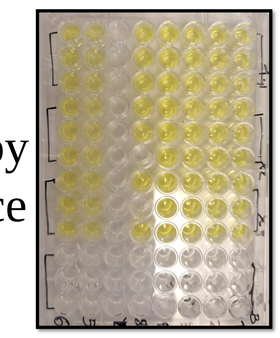
Sample	[Dex] (nM)	Time of withdrawal (days)
A	10(-)	4(-)
B	100(+)	11(+)
C	10(-)	11(+)
D	100(+)	4(-)
E	50(o)	8(o)
control	100(+)	

Table of 5 different conditions; maximum(+), minimum(-), midpoint(o) and a control

Minitab factorial design was used to create DOE



Osteogenic activity quantification, alkaline phosphatase (ALP) assays: Cell lysate + substrate. Breakdown of p-nitrophenyl phosphate indicated by yellow colour, measured over 30mins. Fluorescence vs Time graph plotted and slope used to determine ALP activity ($= \frac{Max\ Slope \times K \times V_{Sample}}{V_{Measured}}$).



ALP assay

Results

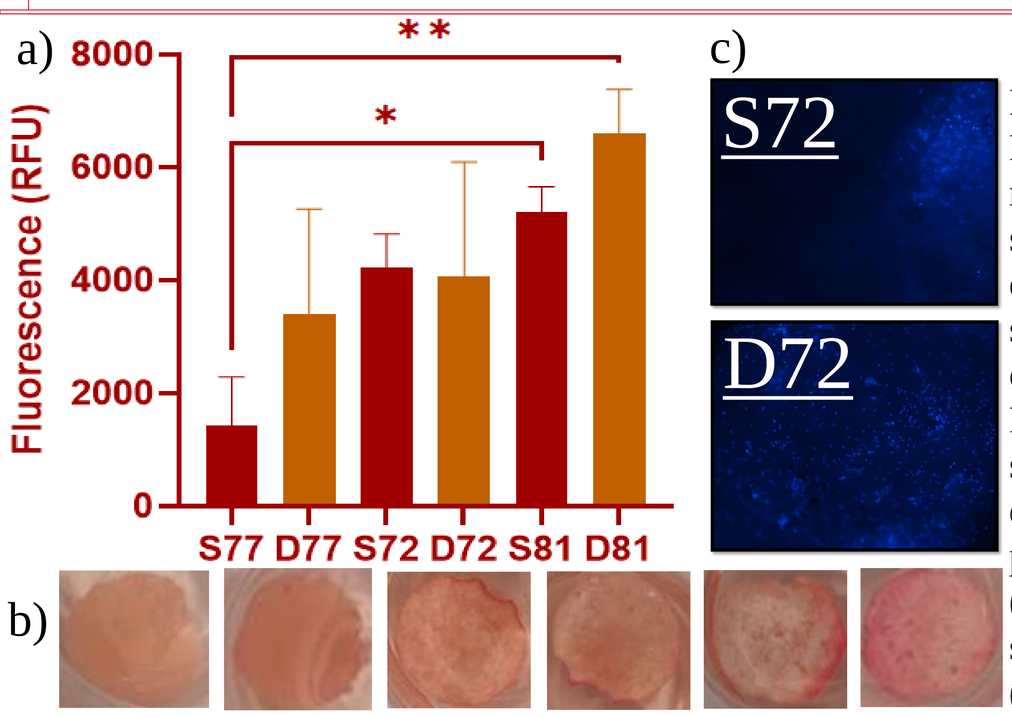
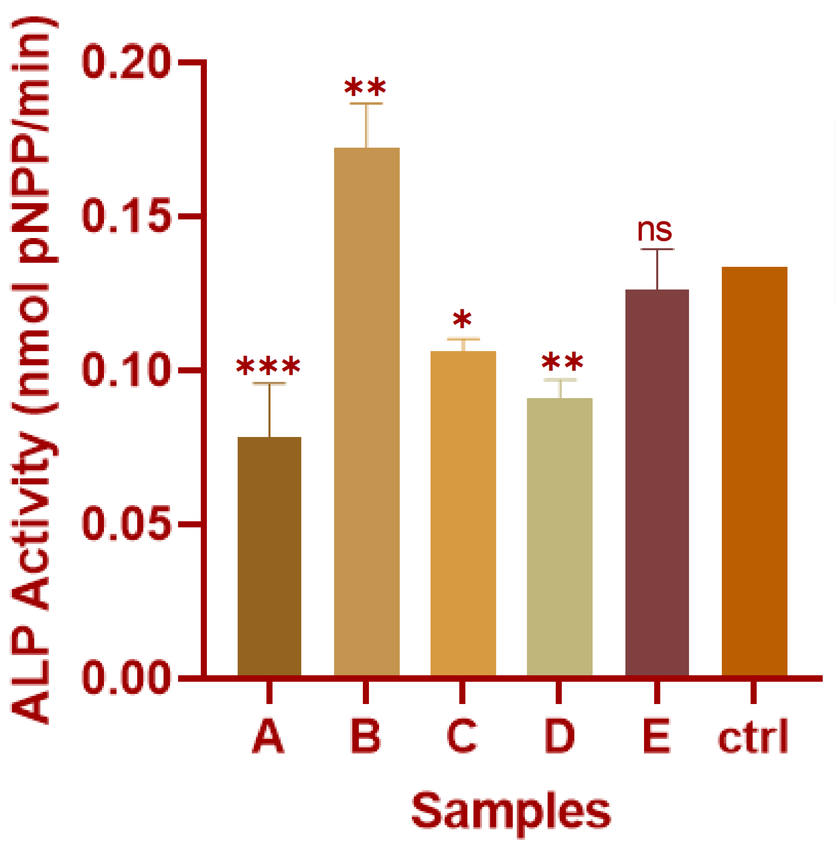


Fig. 1. a) Resazurin reduction in static(S) and dynamic(D) seeding conditions. PolyHIPE scaffolds of 3 different % porosities (77,72,81) were seeded with (2.5×10^5) cells, which were allowed to proliferate for 3 days. Data presented as mean ± SD (n=3), * indicates $p<0.05$, while ** indicates $p<0.001$ (GraphPad Prism)
b) Neural red: representative stained scaffolds for each condition stated above
c) Static and dynamic 72% porosity samples stained with Hoechst 33342

Fig. 2. ALP activity of Y201 MSCs on tissue culture plates, measured over 30 minutes. Different DEX withdrawal time points at days 4, 8 and 11 of culture, 3 different DEX concentrations of 10, 50 and 100nM (see table above). Data presented as mean ± standard deviation (n=3), * indicates $p<0.05$, ** indicates $p<0.001$ and *** indicates $p<0.0001$ against control (GraphPad Prism)



Conclusions

- It is observed that dynamic seeding methods improve the attachment and distribution of cells with regard to sample porosity. D81 was the sample with the most viable cells, while in D72 the cells were better distributed.
- ALP activity was significantly higher in sample B, suggesting that DEX may be withdrawn at day 11 of culture. In DOE analysis, only time of DEX withdrawal proved to have an effect on the osteogenic activity of Y201 cells.
- Overall, the influence of mechanical stimuli such as shear stress and time of osteogenic supplementation had an impact on MSCs responses and should be taken into account in the development of *in vitro* bone models.

References:
[1] X. Zhou, D. Liu, L. You, and L. Wang, "Quantifying fluid shear stress in a rocking culture dish," Journal of Biomechanics, vol. 43, no. 8, pp. 1598–1602, 2010.

The University Of Sheffield. Sheffield Teaching Hospitals NHS Foundation Trust. Sheffield Children's NHS Foundation Trust.
The Insigneo Institute for *in silico* Medicine is a collaborative initiative between the University of Sheffield, Sheffield Teaching Hospitals NHS Foundation Trust and Sheffield Children's Hospital.