

## The Proliferation of hMSCs on PLA Scaffolds

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**INTRODUCTION:** Biodegradable synthetic polymers including poly(lactic acid) (PLA) are suitable for biocompatible scaffold constructs but are known to undergo *in vitro* degradation<sup>1</sup>. This may limit their potential for use in long-term cultures or loading regimes. This investigation determines whether it is advantageous to culture cells on scaffolds prior to mechanical compression.

**METHODS:** Human MSCs (hMSCs) were obtained commercially from Poietics™. P4 cells were seeded at  $1.5 \times 10^6$  cells onto cylindrical PLA scaffolds ( $\phi$  9mm x 4mm). The constructs were cultured for either 3 hours, 4 days or 7 days in D-MEM supplemented with 10% fetal calf serum (FCS) with slow rotation. Scaffolds were then divided into 8 sections and a cell proliferation assay was performed on each section using the Promega CellTiter 96® AQueous One Solution (n=5) and cell distribution was also determined with 16 $\mu$ m cryo-sections stained with haematoxylin. SEM was used to image scaffold integrity.

**RESULTS:** There was no significant proliferation of the MSCs on the scaffolds during culture. The distribution of cells throughout the scaffolds after the initial 3 hour seeding period and subsequent cultures remained similar and the cells were mainly found in the top half of the scaffolds, shown in Figure 1. This was also confirmed by haematoxylin staining of scaffolds cultured for 3 hours shown in Figure 2A. SEM images showed that during the 7 day culture period the scaffold integrity decreased, shown in Figures 2B-2D.

**DISCUSSION & CONCLUSIONS:** This data suggests that hMSCs do not proliferate during 7 days of culture on PLA scaffolds. This is consistent with an initial lag phase in cell proliferation observed in monolayer cultures<sup>2,3</sup>. The cells appear to remain in the upper half of the scaffold which suggests that the seeding methods used do not result in uniform distribution and that the cells do not migrate throughout the scaffold. This data suggests that there are no advantages in culturing the constructs prior to loading due to a lack of cell proliferation and degradation of the scaffolds which may ultimately affect their mechanical properties.

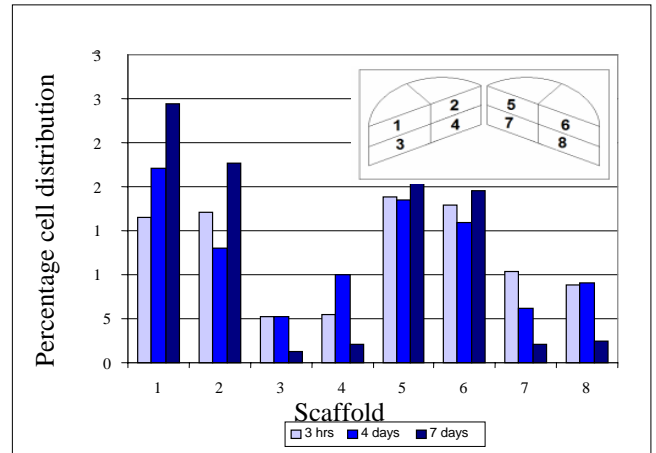


Figure 1: The percentage distribution of P4 MSCs in PLA scaffolds after 3 hrs, 4 days and 7 days

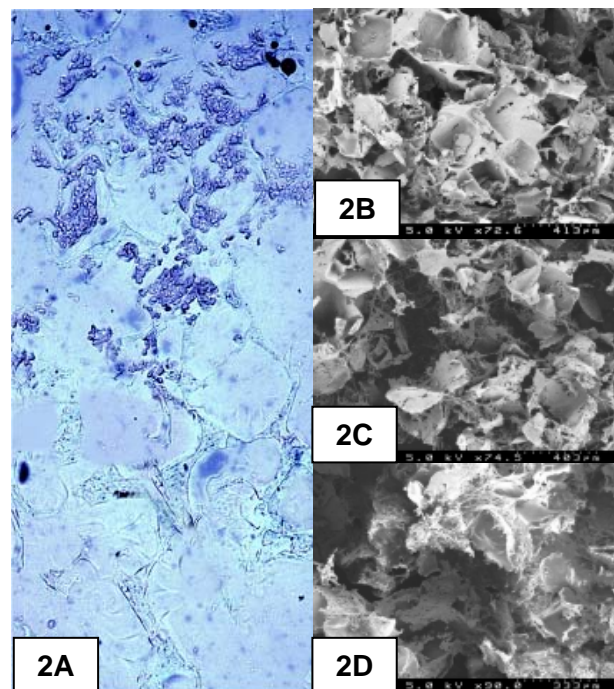


Figure 2: 16 $\mu$ m cryo-section stained with haematoxylin after 3 hrs (2A), and SEM images showing scaffold integrity after 3 hrs (2B), 4 days (2C) and 7 days (2D)

**REFERENCES:** <sup>1</sup> A Pathiraja, R Adhilar (2003) *Eur Cells and Mat* 5:1-16; <sup>2</sup> S Bruder et al (1997) *J Cell Biochem* 64:278-294; <sup>3</sup> D Colter et al (2000) *PNAS* 97:3213-3218

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