

Seeding Efficiency and Distribution of Primary Osteoblasts in 3D Porous Poly(L-lactide) Scaffolds

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INTRODUCTION: Three-dimensional porous polymer scaffolds can be fabricated to convey spatial information to seeded cells. As the initial step in tissue engineering utilising 3D constructs, cell seeding efficiency and distribution is of paramount importance. Resultant construct cellularity, matrix composition and mechanical integrity are greatly influenced by cell distribution¹. In this study, the effects of initial seeding conditions and culture techniques were investigated with respect to porous poly(L-lactide) scaffolds.

METHODS: The cylinder shaped (ϕ 9 x 4 mm) porous scaffolds were made from medical grade poly(L-lactide) (PLLA) using a salt-leaching technique, resulting in 250-350 μ m pore size and 90% porosity². To encourage cell attachment, the 3D scaffolds were coated by collagen type I (Sigma). Cell seeding efficiency in the scaffolds was investigated for both static and dynamic seeding techniques by counting the number of cells remaining in the culture media in which constructs were suspended. Primary bone cells were labelled with PKH26 red fluorescent linker (Sigma) prior to seeding, allowing cell distribution within the construct to be observed using confocal microscopy. In long-term experiments, constructs were cultured statically for 4 weeks or perfused for the last week of the 4-week culture period, then embedded in Technovit 7100 resin. Cross-sections were taken from the cylindrical constructs and cell distribution calculated.

RESULTS: Dynamic seeding, where 1 million primary osteoblasts suspended in 50 μ l of media were seeded directly onto the scaffold surface, placed in a conical tube and rotated at approximately 60rpm on a cortical shaker for 3 hours, resulted in 91-92% cell seeding efficiency.

Static seeding techniques, where the same amount of cell suspension was applied in the same manner, but constructs were not agitated in media, resulted in lower seeding efficiency. Cell distribution throughout the scaffold was more uniform when using the dynamic seeding technique, where cells penetrated throughout the construct. Static seeding

resulted in cell distribution being limited to the upper portion and middle of the scaffold, with few osteoblasts observed at the lowermost face of the construct.

Long-term experiments, where osteoblasts were initially applied to the scaffold by static seeding, indicated cells penetrated through scaffolds, however the distribution was asymmetrical, with the majority of cells contained within the upper portion of the construct, supporting the cell seeding observations at early times under static conditions. Furthermore, constructs subjected to perfusion had improved cell distribution when compared to static control.

DISCUSSION & CONCLUSIONS: A 91-92% seeding efficiency for primary osteoblasts in porous PLLA 3D scaffolds was recorded for dynamic seeding techniques after 3 hours. Static seeding techniques resulted in lower efficiency and less well distributed cells throughout the construct at the same time period. Long-term experiments, where primary osteoblasts were seeded using the static technique, and cultured for 4 weeks demonstrated that the cells migrate into the centre of the scaffolds, but do not penetrate the lower portion of the scaffold, resulting in asymmetrical distribution. With 1 week of perfusion culture following the 3-week static culture, the asymmetrical distribution was improved, suggesting that in perfusion culture, where nutrient and gas diffusion is enhanced, osteoblasts can migrate and proliferate more readily than cells in static culture conditions.

REFERENCES: ¹ G. Vunjak-Novakovic et al (1998) *Biotechnol. Prog.* 14, 193-202. ² Y. Yang et al (2002) *Biomaterials* 23, 2119-2126. ³ S. Saini & T. M. Wick, (2003) *Biotechnol. Prog.* 19, 510-521.

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