Bone Tissue Engineering Using Proliferation Mesenchymal Stem Cells Towards and PLDLA Scaffolds

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Introduction

The ultimate objective of the present study is to engineer a bone graft by expanding mesenchymal stem cells isolated from bone marrow biopsy onto PLDLA scaffolds. In a first step, we assessed the influence of fluid flow on MSC loaded onto 12 filaments PLDLA scaffolds. In a second step, we determined the kinetics of proliferation and differentiation of MSCs when cultured on 4 or 12 filaments PLDLA scaffolds for a period of 40 days in a bioreactor.

Methods

Isolation of MSCs: MSCs were isolated from rat bone marrow and expanded in alpha-MEM + 10 % FBS supplemented with dexamethasone, Ascorbate2-phosphate and β-glycerophosphate. **Scaffolds:** Knitted 12 or 4 filament Poly-L,D-lactide (PLDLA, L/D ratio 96/4) scaffolds were used. Their characteristics are summarized in Table 1.

	4 fil.	**12 fil.**
Single fibre diameter	80 ±10μm	35±10μm
Distance of the fiber bundles	0.24±0.1mm	0.23±0.1mm
Distance of the single fibers	0.03±0.01mm	0.04±0.2mm
Porosity	38 %	40 %

Influence of fluid flow: At passage P3-P5 12 filament scaffolds were soaked for 1 hour in a MSC cell suspension at 10^6 cells/ml and then placed in 50 ml cell culture tube. Constructs were then cultured either on a stoval low profile roller at 6 rpm or left still. At day 28, DNA content, ALP activity and calcium content were determined.

Influence of PLDLA structure: 4 and 12 PLDLA filament constructs were prepared as aforementioned and DNA content, ALP activity and calcium content were determined every 3 days from day 0 to day 40 (n=3).

Results

Influence of fluid movement: DNA content (87000 ± 23000 versus 56000±13000cells per scaffold), ALP activity (64 ± 20 versus 2±1 UI), and calcium content per scaffold (289 ± 34 versus 21 ± 6 ng /construct), were significantly higher in dynamic culture when compared to static cultures.

Influence of PLDLA structure:

Results are summarized in Figures 1, 2 and 3.

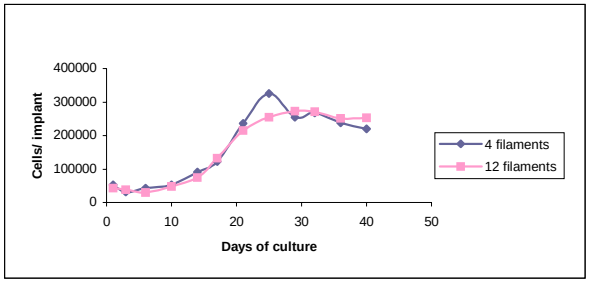

Figure 1: *Kinetics of cell proliferation*

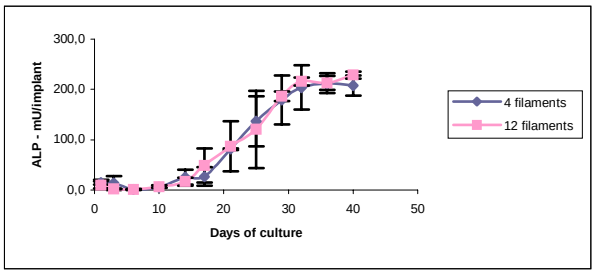

Figure 2: *Kinetics of ALP activity*

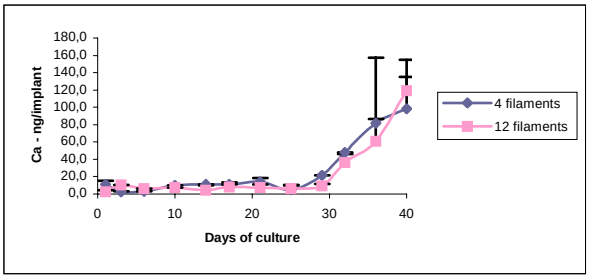

Figure 3: *Kinetics of mineralization*

No significant differences in DNA content, ALP activity or calcium content between the different scaffolds with different fiber thicknesses.

Discussion and Conclusions

MSCs proliferation and differentiation was significantly enhanced when fluid flow was applied to the cells. Thus, a 10 fold increase in calcium content per scaffold was observed when MSCs were cultured in the presence of fluid flow. PLDLA scaffolds were able to support MSC differentiation towards an osteogenic phenotype. Further investigations are now needed to assess the *in vivo* osteogenicity of these constructs.

Acknowledgements

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