

Characterisation and differentiation of porcine bone marrow mesenchymal stem cells in 2D and 3D mPCL-TCP-collagen scaffolds

Chum Zhi Zhen¹ M.A. Woodruff¹ Suman Lal Chirammal Sugunan¹, S. M. Cool^{1,2}, V. Nurcombe^{1,2} D.W. Huttmacher^{1,2}

¹Division of Bioengineering, 9 Engineering Drive 1, National University of Singapore 117576.

² Department of Orthopaedic Surgery, National University of Singapore 119260

INTRODUCTION: This study has several objectives; to study the attachment, growth and differentiation potential of porcine bone marrow mesenchymal stem cells (PBMSC) in 2D and 3D environments, and to investigate the effects of heparin (Hep) on proliferation and osteogenic differentiation in medical grade poly (ϵ -caprolactone) tri-calcium phosphate-collagen (mPCL-TCP-Col) scaffolds. Our results show that scaffolds of mPCL-TCP-Col support multilineage differentiation of PBMSC. Osteogenic differentiation (calcium and collagen I (col I) immunostaining) was evident in cultures stimulated with osteogenic supplements, and areas contained numerous bone nodules (SEM). Furthermore, adipogenic differentiation was also confirmed following stimulation with adipogenic supplements by oil red O staining. Cell attachment and proliferation was also observed in all groups. Notably, stimulation with Hep had no significant effect on the proliferation and osteogenic differentiation of PBMSC cultured on the mPCL-TCP-Col scaffolds. In conclusion, this work showed PBMSC have the capacity to differentiate along osteogenic and adipogenic lineages and that mPCL-TCP-Col scaffolds are suitable supports for cells with potential for tissue engineering applications.

METHODS: Porcine bone marrow was extracted from the iliac crest, plated and cultured in DMEM (low glucose), 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. PBMSC were then sub-cultured until passage 3, then plated into culture wells for the 2D study, or seeded into Col I-lyophilised mPCL-TCP scaffolds for the 3D study and induced with DMEM containing either osteogenic or adipogenic supplements.

RESULTS: 2D Osteogenic Differentiation. H&E staining showed that following osteogenic supplementation, the cellular morphology changed from a fibroblastic state to a flat, cuboidal form. Furthermore, Alizarin Red staining showed increased calcium deposits in induced samples that by day 28 covered most of the cell layer that was

also positive for Col I. SEM analysis showed mineral nodules in only the induced cultures.

2D Adipogenic Differentiation. H&E staining showed cellular morphology change from fibroblastic to flat, oval forms in induced cultures only. Lipid droplets appeared only 28 days post induction; confirmed by Oil Red O staining. SEM analysis and confocal microscopy showed PBMSC attached and spread well on scaffold surfaces, forming cell sheets and bridges. Viability was high in both groups (mPCL-TCP-Col, mPCL-TCP-Col-Hep). Upon induction, ALP activity peaked at day 14 (Fig 1) before decreasing. No significant difference was seen between the two groups ($P>0.05$). Alizarin Red staining using cryo-sectioning showed staining of calcium deposits for both groups (Fig 2). Picogreen Assay showed an increase in cell numbers from day 1-14, demonstrating good cell proliferation on the scaffolds. No significant difference in proliferation was observed between the groups ($P>0.05$). (Fig 3).

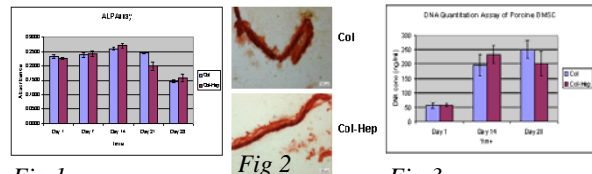


Fig 1

Fig 2

Fig 3

DISCUSSION & CONCLUSIONS: Few studies have characterised the differentiation of PBMSC apart from the study by Ringe (2002). Our results support this study and demonstrate the potential of PBMSC to undergo osteogenesis and adipogenesis following the addition of suitable induction media. Hausser (2004) reported that Hep had a biphasic effect on Saos-2 cells, promoting and inhibiting proliferation and osteogenic differentiation at high ($\geq 5 \mu\text{g/ml}$) and low (5-500 ng/ml) concentrations respectively. However this study was limited to an osteosarcoma cell line. Our results could be due to the osteoconductive TCP/Col I together with the osteogenic induction strongly favouring an osteogenic fate that obscured the effects of additional Hep making differences hard to detect. We are now investigating the use of more specific Hep variants to support the osteogenic process.

REFERENCES: ¹Ringe J.(2002) Cell Tissue Res. 2002 Mar; 307(3):321-7. ²Hausser HJ, (2004). J Cell Biochem 91:1062-1073

ACKNOWLEDGEMENTS: Thanks to Chum Zhi Zhen for experimental contributions.

