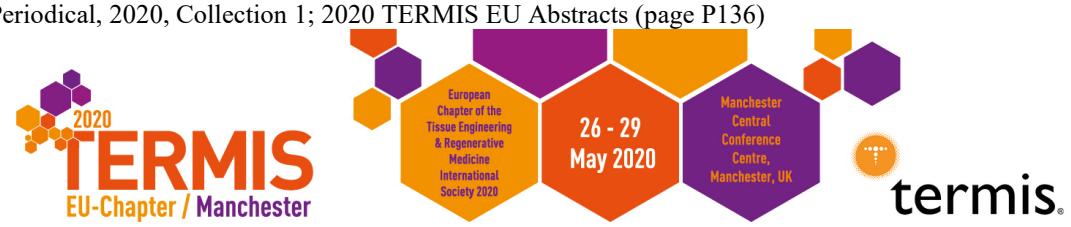
Enhanced bone marrow derived mesenchymal stem cell differentiation when isolated and expanded with human platelet rich plasma and differentiation media is supplemented with vitamin D

Thapa, G., Tirnoveanu, A., Mylona, A. and Webb, R.



Enhanced Bone Marrow derived mesenchymal Stem cell differentiation when isolated and expanded with human Platelet Rich Plasma and differentiation media is supplemented with Vitamin D

Gauri THAPA[1], Alice Nicoleta TIRNOVEANU[2], Athina MYLONA[2], William Richard WEBB[1]
[1]Scrabel Laboratory, Institute of Medical Sciences, Faculty of Health and Wellbeing, Canterbury Christ Church University, Rowan Williams Court, 30 Pembroke Court, Chatham Maritime, Kent, ME4 4UF.
[2]Discovery Park Laboratory, Canterbury Christ Church University, Discovery Park Limited, Innovation House, Ramsgate Road, Sandwich CT13 9FF

INTRODUCTION:Vitamin D3 is well known to be involved in bone formation during foetal development and has been shown to be actively involved from gestational day 13 during foetal rat development [1]. Vitamin D3 and its associated enzyme 1-alpha-hydroxylase a member of the cytochrome P450 super family and encoded for by the gene CYP27B1. In this study we supplemented osteogenic media with vitamin D at a concentration of 10-4M and differentiated for 21 days with samples analysed by means of quantitative alizarin red assay and qPCR for the bone markers RUNX2, ALPL and HPRT1

METHODS:Lonza bone marrow aspirate was seeded at 104 cells per CM3 and isolated and expanded in either FBS or human platelet rich plasma with high glucose glutamax™ DMEM supplemented with 1% non-essential amino acids, 1% penicillin, streptomycin and amphotericin C. once isolated hMSCs were characterised using hMSC antibody panel (R&D systems). hMSCs were then seeded for tri-lineage differentiation with time points of 0, 3,6,9,12,18, and 21 days.Osteogenic media was further supplemented with vitamin D at a concentration of 10-4M. Osteogenic differentiation was quantified using quantitative alizarin staining along with qPCR.

RESULTS:Preliminary results have shown no significant difference was observed over the first 18 days between any of the samples. On comparing FBS directly with hPRP isolated and expanded then differentiated mesenchymal stem cells. Basal differentiation media and osteogenic differentiation media showed no significant differences between FBS versus hPRP over the 21 days. Conversely, on comparing FBS and hPRP when differentiated with osteogenic media supplemented with vitamin D, hPRP had a significantly higher calcification of 2.75 ± 0.276 ($p=0.0032$) compared to FBS calcification of 0.043 ± 0.013 for day 18. For day 21 hPRP calcification had a value of 2.75 ± 0.275 which was significantly higher ($p=0.0003$) when compared to the FBS values of 0.34 ± 0.08. qPCR results are still being evaluated with early results showing hPRP isolated cells showing up regulation of RUNX2 and associated bone markers by day 9, conversely FBS cultured expanded cell still show lower expression of ALPL and RUNX2.

DISCUSSION & CONCLUSIONS:From our early results we have shown clearly that calcification occurs earlier with hPRP expanded cells when differentiated in the presence of Vitamin D, which is also observed when hMSCs are isolated and expanded in FBS. Our early data indicates that vitamin D may be vital for bone differentiation.

References:1. Bikle, D.D. Vitamin D and Bone. Curr Osteoporos Rep, 2012. 10(2): p151-9

Keywords: Cell therapy, Developmental biology