Introduction: Autologous platelet rich concentrate (PRC) has an established history of clinical use in dental and orthopaedic surgery. However, there is some controversy in the scientific and clinical literature regarding the effectiveness of PRC in augmenting bone healing. There are a number of reasons for the different results reported; the methods used to prepare the PRC, inter-patient variation in platelet and growth factor content and the use of different bone grafts. Ranly et al (2007) suggested that PRC negatively interacts with demineralised bone matrix to suppress bone formation. In light of these results and the widespread clinical use of PRC with demineralised bone matrix (DBM) we sought to determine the interaction between PRC and DBM and its effect on human bone marrow stromal cell (hBMSC) proliferation, differentiation and bone nodule formation under osteo-permissive conditions in vitro.

Materials and Methods: PRC was produced from the blood of 10 healthy volunteers using Caption (Smith & Nephew Inc), a fully disposable device that utilizes gravity fed filtration based technology to produce PRC with a mean platelet concentration 4 times that of whole blood.

hBMSC and DBM were prepared from bone marrow and trabecular bone taken from femoral heads donated by patients undergoing total hip replacement due to osteoarthritis. Cells were isolated following standard protocols and expanded in monolayer using Cambrex MSC media supplemented with Cambrex MSC growth supplements. DBM was prepared using 0.5M HCL to remove the mineral phase. Fresh acid was removed and the DBM neutralised prior to being lyophilised. Cells and DBM from the same donor were not combined in the same experiment.

Stromal cells were recovered at passage 2, washed and plated in to 24 well cell culture plates at a density of 5 X103 cells/cm2 and cultured for 24hrs to enable the cells to adhere and recover. The media was exchanged for low serum (1%)FCS overnight prior to addition of the biological agents and osteogenic supplements; 50microg/ml ascorbic acid 2-phosphate and 3mM beta glycerol phosphate. To model the in vivo delivery of PRC and DBM to bony defects, PRC and PRC+DBM constructs were clotted by addition of bovine thrombin (1000U/ml). All materials (PRC, DBM and PRC+DBM) were retained in well inserts to enable the growth factors delivered by these agents to be slowly released into the culture media. Untreated cultures also received a well insert and thrombin, thrombin was also added to the DBM alone treated group. Cultures were supplemented with ascorbic acid and beta glycerol phosphate for the duration of the experiments.

Cell proliferation was assessed by pico-green analysis to determine DNA content and alkaline phosphatase (ALP) activity was determined by pNP reactivity after 3 and 5 days. Mineralisation was determined by alizarin red staining and assessed visually and spectophotometrically. To determine the release kinetics for PDGF-AB, TGFbeta1 and VEGF from PRC and PRC+DBM, 60microL of PRC +/- 65mg of DBM were clotted in a well insert and placed in 3ml of culture media. Growth factor release was determined at 24hrs, 3 days and 6 days by ELISA (R&D systems).

Results: Combination of PRC with DBM resulted in a significant (p<0.05) reduction in ALP activity compared to DMB alone at 3 and 5 days and PRC alone at 5 days (p<0.05). ALP activity was greatest in the DBM treated group at 3 days but there was no difference between PRC and DBM treatment at 5 days. PRC+DBM treatment significantly (p<0.05) increased proliferation over DBM alone at 3 and 5 days and over PRC alone at just 5 days. Over multiple experiments there was no consistent difference in the level of proliferation between PRC and DBM at both time points.

To determine if the suppression of osteoblastic differentiation (ALP activity) observed in the PRC+DBM treated groups at 3 and 5 days lead to a long term suppression of bone formation, mineralisation was analysed at 23 days. The effect of combining PRC with DBM resulted in a significant (p<0.05) increase in mineralisation compared to DBM alone. However, when compared to PRC alone, PRC+DBM resulted in decreased mineralisation. Greatest mineralisation was seen in the PRC alone treated group.

To determine if the cellular effects of combining PRC with DBM resulted from altered release kinetics of growth factors from the composite clot, we analysed growth factor release from PRC alone and in combination with DBM. When PRC was combined with DBM, PDGF-AB release from the clot was significantly (P<0.05) reduced compared to PRC alone. No effect was observed on TGF beta1 or VEGF release.

We next sought to determine if the altered cellular responses were specific to DBM, or could be induced by use of any collagen scaffold. Using a commercially available type I collagen sponge, no effects on PRC induced cell proliferation, ALP activity or mineralisation were observed.

Discussion: The data presented demonstrate that the combination of DBM with PRC significantly modulated hBMSC response compared to the individual biological agents. When compared to DBM alone, PRC enhanced DBM induced cell proliferation and bone formation. However, when compared to PRC alone the PRC+DBM induced cell proliferation but was less effective at inducing bone formation. These differing effects suggest that the variance in bone formation is not solely due to the increased cellular proliferation observed, and that other cell and molecular pathways are involved.

The data we provide also suggest that the combination of the two agents can significantly impact on the release kinetics of growth factors from the graft material, as demonstrated by the decreased release of PDGF-AB. This observation offers a potential mechanism for the altered cellular responses observed. However, the effects of DBM on PRC induced hBMSC proliferation and differentiation appear to be DBM specific as no effects on hBMSC responses were observed when a collagen sponge was added to the PRC.

When taken together the data presented here and those reported by Ranly et al 2007, although not directly comparable, highlight the central role that the microenvironment into which activities are delivered has on the final outcome. In our study we placed the composite grafts into an osteo-permissive environment whereas in Ranly et al (2007) the grafts were placed into a muscle environment and thus lacked the additional osteogenic stimuli. We propose that additional microenvironmental signals are essential in stimulating progenitor cell differentiation towards the osteoblastic lineage and yielding enhanced bone formation in response to exposure to PRC.

In conclusion the data presented here suggest that, under osteo-permissive conditions, PRC can enhance DBM induced bone formation and supports the clinical use of PRC with DBM for bone repair.