

**Bone Regeneration with Platelet-rich Plasma, Calcium Phosphate Ceramics and Stem Cells**Philip Kasten<sup>1</sup>, Julia Vogel<sup>1</sup>, Reto Luginbühl<sup>2</sup>, Philipp Niemeyer<sup>3</sup>, Wiltrud Richter<sup>1</sup><sup>1</sup>Department of Orthopaedic Surgery, University of Heidelberg, Germany<sup>2</sup>Dr. hc. Robert Mathys Foundation, Bettlach, CH<sup>3</sup>Department for Orthopaedics and Traumatology, University of Freiburg, Germany

**INTRODUCTION:** Ceramics such as  $\beta$ -tricalcium phosphates ( $\beta$ -TCP, specific surface area  $< 0.5 \text{ m}^2/\text{g}$ ) or Calcium-deficient hydroxyapatite (CDHA, specific surface area  $48 \text{ m}^2/\text{g}$ ) can be combined with expanded mesenchymal stem cells (MSC) and growth factors to accelerate bone healing. In 1998, Marx and coworkers reported that adding platelet-rich plasma (PRP) to an autogenous cancellous bone graft resulted in a faster maturation rate and higher bone formation rate in alveolar defects. The aim of this study was to evaluate whether a combination of expanded MSC with PRP in resorbable calcium phosphate ceramics can promote osteogenesis and enhance ectopic bone formation in a SCID mouse model. We evaluated whether the effects of PRP depend on the type of carrier and the stage of osteogenic differentiation of the applied MSC.

**METHODS:** CDHA and  $\beta$ -TCP ceramic blocks were loaded with human MSC. Half of the specimens were treated with five-fold concentrated PRP. Furthermore, we compared undifferentiated MSC with MSC that were cultured under osteogenic conditions for 2 weeks *in vitro* on the scaffolds. Bone formation and osteogenic differentiation were evaluated by histology, alkaline phosphatase (ALP) enzyme activity, and osteocalcin (OC) content 4 and 8 weeks after ectopic implantation in SCID mice.

**RESULTS:** Ectopic bone formation was enhanced in MSC/CDHA (7/32) compared to MSC/ $\beta$ -TCP (2/30) composites; however, there was only a trend to more bone formation on CDHA after addition of PRP.

The addition of PRP to the composites increased the specific ALP activity significantly ( $P=0.006$ ) on CDHA, but on  $\beta$ -TCP a similar trend did not reach significance.

The specific ALP activity was significantly higher in MSC-loaded samples compared to empty scaffolds ( $p<0.001$ ) in CDHA and  $\beta$ -TCP ceramics. Mean ALP activity values were significantly higher in the undifferentiated MSC/ $\beta$ -TCP group compared with biocomposites subjected to osteogenic induction.

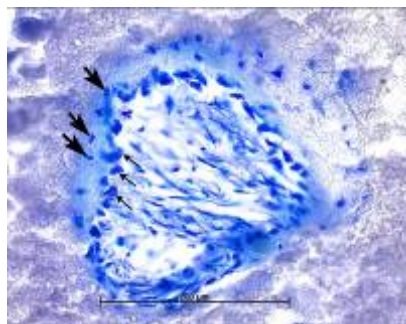


Fig. 1. Toluidine blue staining of a CDHA sample with PRP and undifferentiated MSC after 8 weeks shows bone formation in the outer parts of a peripheral ceramic pore with osteoblasts (small arrows) and osteocytes (large arrows) (200x magnification).

Although higher mean values of OC were obtained in cell-loaded CDHA with PRP versus without PRP, this difference did not reach significance. In contrast to  $\beta$ -TCP biocomposites, MSC/CDHA samples revealed a significantly higher OC content than the empty ceramic ( $P=0.031$ ) but no significant difference was seen between the undifferentiated and induced MSC/CDHA samples.

**DISCUSSION & CONCLUSIONS:**

PRP in combination with MSC loaded on CDHA had a positive effect on osteogenic differentiation regarding ALP activity, but due to a large donor-dependent MSC variability a trend towards better ectopic bone formation did not reach significance. MSC/ $\beta$ -TCP groups failed to profit from the addition of PRP. In conclusion, the effect of PRP depended on the ceramic and the differentiation status of the MSC, however it did not clearly promote osteogenesis of human bone-marrow-derived MSC.

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