PRGF Induces MSC Osteoblastic Differentiation via BMP-2 and wnt Non-canonical Pathway
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Introduction
Platelet-rich-plasma (PRP) is autologous plasma that has a platelet concentration above baseline. Upon activation, PRP releases a high concentration of various growth factors. Platelet-released growth factors (PRGF) have been used to support the repair of bone defects, especially in maxillofacial and dental surgery, yet their exact effect on bone healing is not clear.

We previously showed¹ a significant osteoinductive effect of PRGF on Mesenchymal Stem Cells (MSC), as shown by an increased expression of typical osteoblastic marker genes and matrix mineralization. In particular, we showed that PRGF up-regulates BMP2 at both gene and protein level. Furthermore, our observations suggested the possible involvement of the Wnt-signalling cascade. In the present work, we further investigate the intracellular pathways leading to MSCs osteogenic differentiation under PRGF stimulation, compared to the classical osteogenic media (Dexamethasone and BMP2 containing media).

Materials and Methods
Cells isolation: Human bone marrow samples obtained with patient consent (KEK Bern 126/03). BMSC were isolated by Ficoll-Paque density-gradient centrifugation. PRP preparation and activation: PRGF was prepared from thrombocytes (expired thrombocytes bags from Blood Bank Kantonspital Chur) concentrates resuspended in PBS (2x10⁶ platelets/mL) and stored at -20°C until use. MSC culture: “Basic” culture medium consisted of IMDM, 10% FBS, nonessential amino acids, 0.1mM ascorbic acid-2-phosphate (Sigma) and 10mM β-glycerophosphate (Sigma). In the experimental cultures, the basic medium was supplemented with either 10% PRGF, 10mM Dexamethasone (Dex+) (Sigma) or 100ng/mL human recombinant BMP2 (PeproTch). Media were changed twice a week. Cell differentiation was followed by real time RT-PCR. BMP2 and ALP protein level were assayed by ELISA, and enzymatic activity dosage respectively. Matrix mineralization was estimated by Ca⁴⁵ incorporation. BMP2 pathway exploration: The role of BMP2 signalling (both canonical and non-canonical) was assayed by RT-PCR in the different conditions in presence or not of human recombinant Noggin, antagonist of BMP2 (R&D). Smad phosphorylation was detected by immunostaining. Statistical analyses were performed using 1-way ANOVA.

Results
Unlike BMP2, Dexamethasone (Dex+, classical osteogenic medium) induces mineralisation through activation of the runx2, osx and ALP via the wnt canonical pathway. This last involves β-catenin nuclear translocation and activation of LEF/TCF mediated gene transcription. In contrast to Dex+, in PRGF treated cells, runx2 and osx stayed below the detection level and ALP was down-regulated, while BMP2 was up-regulated, at both gene and protein level. Furthermore, with PRGF, the non-canonical Wnt pathway was activated as shown by the up-regulation of wnt7b and the down regulation of β-catenin, LEF and TCF. The same profile of gene regulation was observed with BMP2 treatment, suggesting that PRGF effect mediated through the activation of the BMP2 intracellular pathway. This was further suggested by the fact that addition of Noggin (BMP2 antagonist) to the PRGF treated cells inhibited the BMP2 non-canonical pathway. Moreover, PRGF stimulation induced smad phosphorylation, similar to the BMP2 treatment. Again, this was inhibited by Noggin.

Discussion and Conclusions
Unlike Dex+, PRGF seems to induce human MSC differentiation and matrix mineralization through a non classical pathway which does not involve activation of runx, osx and ALP up-regulation. MSC differentiation under PRGF treatment appears to involve BMP2 protein up-regulation, and the BMP2 non-canonical wnt pathway.

References
1. Meury et al, submitted in revised form Tissue Engineering part A.

Disclosures
The authors have nothing to disclose.