A Novel Mesenchymal Stem Cells and Platelets Concentrate For Bone Reconstruction
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Introduction
Platelets can be isolated in peripheral blood by centrifugations in a small volume of plasma commonly defined as platelet-rich plasma (PRP). Mesenchymal stem cells (MSC) are commonly isolated from the mononuclear cell fraction after separation by density gradient centrifugation of a small volume of bone marrow. PRP and MSC have been used alone or in combination to induce bone repair (1-3). Here we tested whether MSC could be collected from the bone marrow using a system that is used to obtain PRP from the peripheral blood.

Materials and Methods
Peripheral Blood (PB) and Bone Marrow (BM) aspirates were obtained from 6 patients. Platelet Rich Plasma (PRP) samples from BM and PB were prepared using a commercial kit called FIBRINET Autologous Fibrin and Platelet System (Cascade Medical Enterprises Ltd, Plymouth, UK) according to the manufacturer’s instructions developed for peripheral blood. Flow cytometric analysis was performed to assess the cellular composition of the isolated cells. In order to verify whether MSC were present, cells were cultured and CFU assay, cytofluorimetric analysis of cell surface markers and osteogenic ability tests were performed.

Results
The mean concentration of the nucleated cells collected from BM harvested in a 18 ml volume was 55,9 x 10⁶ / ml (SD = 22.2 x10⁶). The number of nucleated cells dramatically decreased after the fibrinet system was used, 0.49 x 10⁶/ml (SD = 0.39x10⁶) nucleated cells were collected. The volume collected after centrifugation of 9 ml of BM was 5,5 ml. Therefore, centrifugation resulted in a 1.6% reduction of the volume and in a 87.7% decrease in the nucleated cell population. Flow cytometry revealed that compared to BM the % of CD45, CD3, CD14, CD15, CD33 and CD34 expressing cells was dramatically decreased after the fibrinet system was used. In contrast, the analysis of the % of CD41 expression was higher. These results are consistent with a selected fraction of the nucleated cells retained in the final product. All samples gave rise to similar cultures with compatible doubling time, cells displayed a distinct profile characteristic of MSC (CD34-/CD44+/CD45-/CD90+/CD105+/CD117-/CD146+/CD166+) and differentiated toward the osteogenic lineage as shown by Alizarin red staining.

Discussion and Conclusions
Our results show that MSC and platelets can be obtained and concentrated at the same time from the bone marrow using a system commonly used to obtain PRP from peripheral blood. This product could be used to treat bone defects clinically, with the advantage that surgeons can prepare and use it within a single surgical procedure.

References

Disclosures
Authors declare they have no conflict of interest.