INTRODUCTION
Platelet-rich plasma (PRP) is increasingly being used as a non-surgical autologous treatment for musculoskeletal disorders because of its simple preparation process, low cost and relative safety. Although a number of basic science studies have demonstrated the in vitro efficacy of PRP in promoting cell proliferation, migration and differentiation, the results from limited clinical trials have been inconclusive to date. One reason for such variable outcomes may be related to the devices used and the resulting variability in the PRP preparations in individual studies. The PRP obtained from different devices differ not only in the platelet concentration and presence of white blood cells (WBCs) but also in its activation state when administered to the injury site. There is an unfulfilled need to understand the scientific impact of each of these factors, which in turn will assist one to optimize the usage of PRP for specific clinical indications. In this study, we used in vitro proliferation and migration models in stem cells to determine the effect of platelet concentration, presence of WBCs and PRP activation.

METHODS
Preparation of PRP: Fresh blood was drawn from seven healthy volunteer donors with platelet counts ranging from 180x10⁶/mm³ to 350x10⁶/mm³. Blood from each donor was aliquoted into 4 Regen™ THt A-PRP tubes (RegenLab, Switzerland) and spun at 1,500 RCF for 8 min to obtain PRP within 24 hrs of collection. Plasma volumes were adjusted to produce 1, 2, 5, and 10-fold platelet concentrations immediately after spin and the platelet and WBCs were resuspended in the remaining plasma. To remove WBCs, the concentrated PRP was spun at 250 RCF for 5 min and the WBC-containing pellet was discarded. Activation of platelet was induced by adding 10% of either bovine thrombin or CaCl₂ solution to the PRP and incubated at room temperature for 1 hr.

Proliferation and migration assays: hMSCs (Lonza) were expanded in DMEM/10%FBS/1% antibiotics until passage 3. For proliferation assay, hMSCs were trypsinized, resuspended in medium and plated into 24-well plate at a density of 1x10⁵ cells per well. After 24 hrs, PRP samples were prepared in a transwell inserts with 8 µm pore size membrane and transferred to the cell-containing plate. Cells were cultured in serum-free medium for 96 hrs before their numbers were quantified using Cell Counting Kit-8 (Dojindo). The migration assay was conducted using QCM 24-well fluorescent cell migration assay kit (Chemicon). PRP samples were plated directly in the well. After activation, hMSC (1x10⁵) suspended in serum-free medium were plated into the insert and allowed to migrate for 24 hrs before evaluation. For both proliferation and migration assay, serum-free DMEM and PPP (platelet poor plasma, 0x) served as negative controls while DMEM containing 10% FBS as positive control.

Statistical analysis: All quantitative data were expressed as mean ± SE. Nonparametric Kruskal-Wallis test was performed using SYSTAT 12 (SYSTAT Software, Chicago, IL). P<0.05 was considered significant.

RESULTS
All PRP samples increased cell proliferation compared to PPP. Among the three variables of PRP evaluated, activation was the only factor that exhibited significant impact on cell proliferation (p=0.05) while platelet concentration (p=0.62) and presence of WBCs (p=0.51) showed little effect (Fig. 1). Activation method did not modulate the proliferation outcome as no difference was observed between thrombin and CaCl₂ activated samples (p=0.28). In comparison, activation did not affect cell migration significantly as non-activated PRP had a similar effect on migration as activated samples (data not shown, p=0.59). On the other hand, platelet concentration exhibited a trend of positively affecting cell migration in the presence of WBCs (Fig. 2. p=0.07, n=12) while showing little effect in the absence of WBCs (p=0.67), when samples from all activation states were combined at each concentration. Furthermore donor variation also appears to play an important role in platelet function in that platelets from different donors promote proliferation and migration to different levels even when the same number of platelets was tested (data not shown).

DISCUSSION
In the present study, we evaluated the effect of platelet concentration, presence of WBCs and platelet activation of PRP on stem cell proliferation and migration. Among the three variables tested, activation and WBCs had the largest effects on cell proliferation and migration, respectively. The impact of increasing platelet concentration was not consistent as it depended on both the presence of WBCs and the activation state of platelets. On the other hand, the positive effect on proliferation that was observed at low PRP concentrations suggested higher PRP concentrations over whole blood might not be needed when other variables are optimized. High concentrations of WBCs have actually been suggested to negatively modulate the biological environment. Since there was no direct contact between PRP and stem cells in our models, it is reasonable to assume that stem cells are regulated by the soluble factors secreted by WBCs and platelets. Whether the direct contact with PRP would have any synergistic effect on cells requires further investigation.

SIGNIFICANCE
Our in vitro study identified the potential benefit of WBCs and activation within PRP compositions while showing the minimal effect of increasing platelet concentration. These results may provide scientific foundation for further clinical investigation, highlighting that different PRP compositions might result in different clinical outcomes based on their modulation of cell behavior.

REFERENCE