DONOR VARIATION AND LOSS OF PLURIPOTENCY DURING IN VITRO EXPANSION OF HUMAN MESENCHYMAL STEM CELLS FOR BONE TISSUE ENGINEERING

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Introduction
We use human mesenchymal stem cells (hMSCs) as a source for autologous bone tissue engineering. The donor variation in growth, differentiation and in vivo bone forming ability of hMSCs is a bottleneck for standardization of therapeutic protocols. In this study, we isolated and characterized hMSCs from 19 independent donors, aged between 27 and 85 years and investigated the extent of heterogeneity of the cells and the extent to which hMSCs can be expanded without losing multipotency.

Methods
hMSCs were isolated as the adherent fraction of bone marrow from donors with written informed consent. Culturing and analysis were done as described (1). ALP expression was analyzed by FACS (BD cytometry). The total calcium deposition was assayed using a calcium assay kit (Sigma). Gene expression profiles were analyzed by quantitative RT-PCR on a Lightcycler (Roche). To investigate in vivo bone formation, hMSCs from 4 different donors were seeded onto 2-3 mm porous biphasic calcium phosphate particles, cultured for one week on the scaffold and implanted subcutaneously in immune-deficient mice. Bone formation was analyzed 6 weeks after implantation by histological analysis.

Results
1. Donor variation in response to dexamethasone
Dexamethasone-induced ALP expression varied between 1.2 and 3.7-fold. The percentage of ALP positive cells in the untreated group varied between 1 % and 33 % indicating varying amounts of ALP positive cells in the initial culture. Upon dexamethasone treatment, the percentage of ALP positive cells varied between 3 % and 50 % (Figure 1). No statistical correlation was found between ALP expression and age, gender or source of isolation (data not shown). The cells from donors with a higher percentage of ALP-positive cells in control and dexamethasone-induced groups showed more calcium deposition than cells with lower percentage of ALP positive cells (data not shown).

![Figure 1. Percentage of ALP positive cells in untreated (Con) and dexamethasone-treated (dex) cell populations. Donors are sorted in an ascending order of percentage of ALP positive cells in the dexamethasone-treated group.](image1)

qPCR studies show that, despite the variability in osteogenic gene expression among the donors tested, ALP, Collagen type1, osteocalcin and S100A4 showed similar trends during the course of osteogenic differentiation (data not shown).

2. Loss of pluripotency during in vitro expansion.
In vitro expansion studies showed that hMSCs lose their differentiation ability during in vitro expansion. The hMSCs were able to mineralize in vitro up to 3 passages and cells were able to differentiate into adipocytes up to 5 passages (Figure 2).

![Figure 2. Loss of pluripotency during in vitro expansion of hMSCs.](image2)

3. Variation in in vivo bone formation by hMSCs
To access the variation in in vivo bone formation by hMSCs, the cells from four different donors were tested for their ability to form bone in vivo. As shown in Figure 3, cells from donor 16, 17 and 18 formed bone in vivo while cells from donor 19 did not emphasizing donor variation in in vivo bone formation. Further comparative analyses with in vitro ALP expression suggest a correlation between in vitro ALP expression and in vivo bone formation.

![Figure 3. In vivo bone formation by hMSCs. The arrow indicates newly formed bone. S, Scaffold material. F, Fibrous tissue.](image3)

Discussion
Our studies demonstrate that irrespective of age, gender and source of isolation, cells from all donors showed osteogenic potential. The variability in ALP expression appears to be a result of sampling method and cellular heterogeneity among the donor population. In vitro expansion studies demonstrate that cells can effectively expanded in vitro up to 4 passages (approx. 10-12 Population Doublings from P1 Culture) while retaining their pluripotency. The resolution of in vitro ALP expression is not indicative enough to assess the in vivo bone formation. Therefore our current research is focused on identifying new diagnostic markers which predict the bone forming capacity of hMSCs. We approach this by isolating hMSCs from 60 donors, analyzing their gene expression profiles and in parallel assessing their bone forming capacity in vivo.

References
(1). de Boer et al. Bone 34:818-26; 2004