Introduction: Trabecular bone including bone marrow contains mesenchymal stem cells (MSCs). MSCs are usually isolated from bone marrow aspirates and form single cell-derived colonies. However, the low incidence of MSCs in bone marrow aspirates and donor- and sample- variations could be major limitation for study and clinical applications. Recently, medullary vascular network was described as the closest in vivo application to the MSCs. Here we digested human trabecular bone in a collagenase solution and collected the cells referred as collagenase-released cells (CR cells). CR cells should be derived from soft connective tissues containing a number of endothelial cells, one of candidates for ontogeny of MSCs. The purpose of this study was to investigate the characteristics of CR cells as stem cells in comparison with bone marrow aspirate-derived MSCs (BM cells).

Materials and Methods: Trabecular bone fragments and bone marrow aspirates: Bone fragments were harvested during the knee operations of 12 donors; 6 young patients (24 ± 4 years) with anterior cruciate ligament injury and 6 elderly patients (69 ± 5 years) with osteoarthritis. The study was approved by an Institutional Review Board and informed consent was obtained. Isolation and culture of CR cells and BM cells: Trabecular bone fragments were washed thoroughly to remove hematopoietic cells. The fragments were then digested in collagenase solution for 3 h. The filtered cells obtained from a 0.5 mg of the original trabecular bone were plated in a 145 cm² culture dish in α MEM containing 20% fetal bovine serum. The pelleted bone marrow was also plated and cultured in a similar method (1). Flow cytometry: One million cells at passage 1 were suspended in 20 μg/ml of antibody labeled with FITC or PE, incubated for 30 min, and cell fluorescence was evaluated by flow cytometry. RT-PCR: Total RNA was prepared from 40 million CR cells just after collagenase digestion and 1 million CR cells and BM cells 14 days after plating Passage 0, and extracted. RNA was converted to cDNA and amplified by the Titan One Tube RT-PCR System. Statistical analysis: Two-factor ANOVA and Student’s t-test were used for assessing differences. A value of p<0.05 was considered significant. Results: Culture of CR cells and BM cells: CR cells and BM cells showed similar morphology and most of cells were spindle-shaped. Cell number harvested at Passage 0 was 47 ± 49 folds higher in CR cells than in BM cells. There were no significant differences of cell number between young and elderly donors both in CR cells and BM cells (Fig. 1). Fold increases of CR cells and BM cells at Passage 1 were similar in each donor. Fold increases of cells in young donors were higher than in elderly donors (Fig. 2).

Discussion: We have shown that (1) more MSCs at P0 could be harvested from trabecular bone than MSCs from same weight of bone marrow aspirate and (2) CR cells at P1 had a similar ability for differentiation and multilineage differentiation as BM cells (3) these two cell populations had similar phenotypes except VCAM-1 and ICAM-1. Recently, adult human osteoblastic cells derived from explant cultures of collagenase-pretreated trabecular bone fragment were reported to be similar to bone marrow derived MSCs. These indicates small piece of trabecular bone harvested with biopsy needle will be advantageous source of MSCs.