Introduction

Bone marrow stromal cells derive from a common precursor, commonly identified as mesenchymal stem cell, also known as skeletal stem cells and/or stromal stem cells. These progenitors are able self-renew, and differentiate into osteoblasts, chondrocytes, adipocytes, and smooth muscle cells, both in vivo and in vitro. Several studies indicate these progenitors are rare in the marrow cavity, ranging from 10 to 100 units per million of nucleated cells. However, recent data showed higher frequency of these progenitors in the bone marrow, and suggested FBS batch may interfere with progenitors adherence performance in vitro. In the present work, our main objective was to show that stromal progenitors frequency in the bone marrow is higher than previously described, and that FBS may cause misquantification due to cell proliferation, but not cell adhesion. Additionally, we show that a higher frequency of progenitors may reside in the subendosteal niche.

Materials and Methods

Bone marrow samples were collected from patients (20-60 year-old, male or female, with no osteometabolic disease) undergoing total hip arthroplasty. Nucleated cells were plated, and after three days, nonadherent cells were washed out and adherent cells were cultured up to 14 days. Cell morphology was evaluated, and phenotype analyses were performed by flow cytometry and RT-PCR. Osteogenic, chondrogenic, and adipogenic potentials were tested. Nine FBS different batches were tested for adhesion and proliferation of progenitors. Trabecular bone washed (TW) marrow and subendosteal fraction (from collagenase treated bone fragments) were also evaluated.

Results

Stromal cells presented myofibrobastic morphology in vitro. CFE average was 200 (30 – 720) per million of nucleated cells. Cells were positive for CD13, CD44, and CD105. RT-PCR revealed that cells expressed osteogenic, chondrogenic, and myogenic related genes, but no adipogenic related genes. FBS batch influenced cells expansion, but not their adhesion to plastic. CFE in TW marrow was similar to reaming debris derived cell suspension. A higher number of progenitors was observed in the subendosteal derived fraction.

Discussion and Conclusions

Although it is still low when compared to hematopoietic stem cells, our data indicate that stromal progenitors frequency in the marrow cavity is higher than previously described. Serum may influence cell proliferation, but not cell adhesion, and the higher frequency herein observed is the result of small blood contamination. A great number of progenitors reside in the subendosteal niche, and this should be better explored in order to improve mesenchymal cells isolation.

References


Acknowledgments

CNPq, Brazilian Health Ministry

Disclosures

Authors have nothing to disclose.