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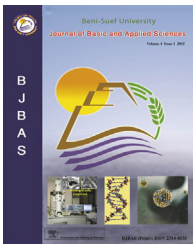

Full Length Article

Comparison of the osteogenic differentiation potential of mesenchymal cells isolated from human bone marrow, umbilical cord blood and placenta derived stem cells

Shymaa Maher [a,1], *Eman Kolieb* [b,1], *Nagwan A. Sabik* [a], *Dalia Abd-Elhalim* [b], *Ahmed T. El-Serafi* [a,c,*], *Yasser El-Wazir* [b]

[a] *Medical Biochemistry Department, School of Medicine, Suez Canal University, Egypt*
[b] *Physiology Department, School of Medicine, Suez Canal University, Egypt*
[c] *Basic Medical Sciences Department, College of Medicine, University of Sharjah, United Arab Emirates*

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ABSTRACT

Bone marrow has been considered for long time as the main source for mesenchymal stem cells. However, bone marrow aspiration is an invasive process that can be associated with morbidity as well as few numbers of obtained cells. Umbilical cord blood and placental tissues are other potential sources for the same type of cells. These sources are abundant, accessible and associated with no harm to the donor. This study aimed at determining the differentiation of the three cell types towards the osteogenic lineage in short term culture and in classical osteogenic conditions. The gene expression profile showed that bone marrow derived cells were the most responsive to the culture conditions while umbilical cord blood derived cells were next, as shown by the expression by the osteogenic key transcription factors 'Runx-2' and osterix. At the meantime, umbilical cord blood and placenta derived cells showed significant enhancement of the gene expression over the study course, which denoted potential response of the cells. Based on these results and the availability of these two sources, umbilical cord blood and placenta should still be considered as potential sources for mesenchymal stem cells in osteogenic research program. However their differentiation potential will need further enhancement.

* *Corresponding author*. M27-138, College of Medicine, Sharjah University City, Sharjah, United Arab Emirates. Tel.: +971 553798583; fax: +971 65585879.
E-mail addresses: ahmed_elserafi@med.suez.edu.eg, ahmserafi@yahoo.co.uk (A.T. El-Serafi).
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[1] These two authors contributed equally to this work.
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1. Introduction

Many patients all over the world are suffering from bone associated problems that cannot be satisfactorily treated by the current management modalities and need physiological replacement, such as non-united fractures, bone cysts and bone defects, which could be congenital or acquired due to blast injuries (Dawson and Oreffo, 2008). The efficient bone regeneration is a hot topic of research based on using different types of stem cells in order to establish the most efficient protocol for induction and consequently could be applied in the future for tissue regeneration programs (Ichinose et al., 2013; Nikukar et al., 2013). Bone marrow derived stem cells (BM-MSCs) are considered as multipotent stem cells that of mesodermal origin (mesenchymal stem cells) that are still considered as the golden standard for this purpose. These cells can preferentially differentiate into mature cells of their lineage, including the osteoblasts (Brooke et al., 2008; Ichinose et al., 2013).

However, the difficulty of obtaining BM-MSCs represents a significant problem. The current approach is to apply a drill in the hip bone under anesthesia; a technique which is invasive, associated with pain and the yield of the cells is variable (Bain, 2001, 2003). Thus looking for other sources of such multipotent cells is an attractive goal of research. These sources include the umbilical cord blood (UCB) and the placental core derived cells. UCB contains a cell population that has the characteristics of mesenchymal stem cells, which have similar properties to BM-MSCs. The differentiation ability of these cells is controversial in different research papers (Jeong et al., 2005; Roobrouck et al., 2008). At the same time, the abundance and accessibility of UCB would make it a promising source of stem cells for research and clinical applications if those cells showed potent differentiation ability (Ali and Bahbahani, 2010).

Both cell types offer a perfectly natural, controversy-free source for acquiring stem cells and can be considered as one of the most abundant sources of non-embryonic stem cells, bearing in mind that the global birth rate is over 200 million per year (McGuckin et al., 2006; McGuckin and Forraz, 2008). In addition, these cells occupy an intermediate stage between the embryonic stem cells and the adult stem cells, which lead to higher proliferating potential and longer telomeres than adult stem cells that are isolated several decades afterwards (Pipes and Ablin, 2006; Slatter and Gennery, 2006). While there are many reports regarding UCB cells, placental stem cells have not received the same attention (Rus et al., 2011).

The differentiation process of the stem cell into the osteogenic lineage had to go across many stages, starting from the commitment of the cells towards the osteogenic lineage and ending by the formation of the osteogenic matrix, in which the cells should be embedded. Such differentiation is associated with activation of a group of genes. Two transcription factors are essential for osteoblast differentiation and skeletal development during the early stages of embryogenesis which are Runt-related transcription factor-2 (Runx-2) and osterix. Runx-2 – also is known as Cbfa-1 – is the key transcription factor during the early stages of embryogenesis (Ducy et al., 1997, 1999), while osterix is a zinc finger–containing transcription factor that is essential for further differentiation and bone formation. In osterix-null mutant mice, neither endochondral nor intramembranous bone formation occurs, and osteoblast differentiation is arrested (Nakashima et al., 2002). After differentiating to pre-osteoblasts, osterix, and Runx-2 direct the cells to immature osteoblasts, which produce bone matrix proteins, during osteoblast differentiation, Runx-2 up regulates the expression of bone matrix protein genes including type 1 collagen and alkaline phosphatase (Jaiswal et al., 2000; Komori, 2010). Collagen1 is the most abundant protein in animals, makes up about (90–95%) of the organic content of bone; thus collagen is the main constituent of the bone matrix. Mutations in type I collagen gene leads to several forms of bone abnormalities including osteogenesis imperfecta, Ehlers–Danlos syndrome and Marfan syndrome (Kadler, 1995). Alkaline phosphatase (ALP) is an enzyme that is produced in bone during the developmental process. ALP splits pyrophosphate, which is an inhibitor of mineralization, to provide inorganic phosphate that is required as part of the mineralization process. ALP activity typically becomes significantly higher in BM-MSCs cultured in osteogenic conditions compared to cells cultured in basal conditions after 6 days (Mirmalek-Sani et al., 2006).

The aim of the present study is to compare the osteogenic differentiation potential of cells isolated from the UCB and placenta with bone marrow derived stem cells. The verification of such induction was achieved by determining the level of gene expression of the osteogenic master genes Runx-2 and osterix, as well as the osteogenic matrix proteins collagen1 and ALP as the markers of osteogenic induction. The importance of this study was to compare the three sources of stem cells together, and at the meantime to determine which genes should be targeted to investigate early differentiation.

2. Material and methods

2.1. *Sample preparation*

The following samples were obtained: a) cancellous bone segments removed during hip replacement surgery for bone marrow isolation; b) umbilical cord blood collected after the delivery and clamping of the cord of full-term babies of healthy women from the obstetric emergency room; c) placentas, were immediately collected after delivery. All experiments were conducted with passage zero cells. The study was approved by the research and ethics committees in the Faculty of Medicine, Suez Canal University.

Femoral drill is a routine step performed during hip replacement operation, through which a space is created for inserting the prosthesis, associated with removal of cancellous bony segment. Samples were donated from three patients and the cells were separated from the bony structure by repeated vigorous shaking with media (El-Serafi et al., 2011). The cells were cultured in basal conditions, which are minimum essential medium Eagle's alpha modifications (Sigma–Aldrich Ltd) with 10% fetal calf serum (Invitrogen) and 100 U/ml penicillin and 100 U/ml streptomycin (Sigma–Aldrich Ltd).

Cord blood was diluted with phosphate buffered saline and mononuclear cells were separated using the concentration gradient centrifugation and cultured in basal conditions. All placentas were kept in a mixture of phosphate buffered saline