

## Original Article

# Influence of different culture media on isolation of murine BMSCs

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Received November 26, 2015; Accepted February 13, 2016; Epub June 15, 2016; Published June 30, 2016

**Abstract:** Various studies involve bone marrow stromal cells (BMSCs) have been carried out in pre-clinical and clinical studies, which leads to an increasing demand for extensive expansion of BMSCs in vitro. Efficient isolation of BMSCs, especially from small experimental animals such as mouse from which it is difficult to obtain adequate volume of bone marrow sample, becomes a necessity. In this study, BMSCs isolated from C57BL/6J mice were cultured in n three different media:  $\alpha$ -MEM, DMEM and DMEM/F12, each was supplemented with 10% FCS. The growth condition of BMSCs in different culture media was observed by inverted microscope and the number of adherent cells was counted at 5 days and 15 days of culture. Results demonstrated the number of adherent cells increased obviously in  $\alpha$ -MEM and DMEM media but just a few in DMEM/F12 medium at 5 days of culture. 15 days later, cells in  $\alpha$ -MEM medium grew far faster than that in DMEM medium and there was no living cell in DMEM/F12 medium. Cell counting results showed there were more adherent cells in both  $\alpha$ -MEM and DMEM medium than in DMEM/F12 medium at 5 days of culture but there was no difference between  $\alpha$ -MEM and DMEM group. At 15 days of culture, adherent cells cultured in  $\alpha$ -MEM medium was significantly more than that in DMEM medium. In conclusion,  $\alpha$ -MEM medium has the potential to be the most appropriate cell culture medium for prolonged culturing of BMSCs.

**Keywords:** Bone marrow stromal cells, isolation, culture medium, influence

### Introduction

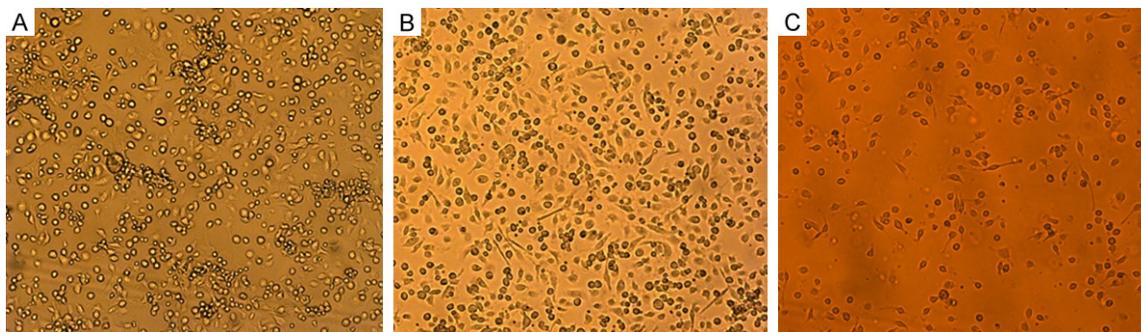
As a type of pluripotent mesenchymal stem cells, bone marrow stromal cells (BMSCs) are capable of self-renewal and differentiation into tissues of, at least, mesodermal origin such as fat, bone, cartilage, ligament and more. And no evidence has been showed that BMSCs may result in any primary tumor or other serious side effects. Therefore, BMSCs have been considered as an effective and safe resource for tissue engineering and stem cell-based therapy. Various studies involve BMSCs have been carried out in pre-clinical and clinical studies, which leads to an increasing demand for extensive expansion of BMSCs in vitro which are derived from different species [1-3].

However, the major disadvantage for BMSCs availability is the extremely low yield of MSCs isolated from the bone marrow. In bone marrow, BMSCs represent a mere 0.001% to 0.01%

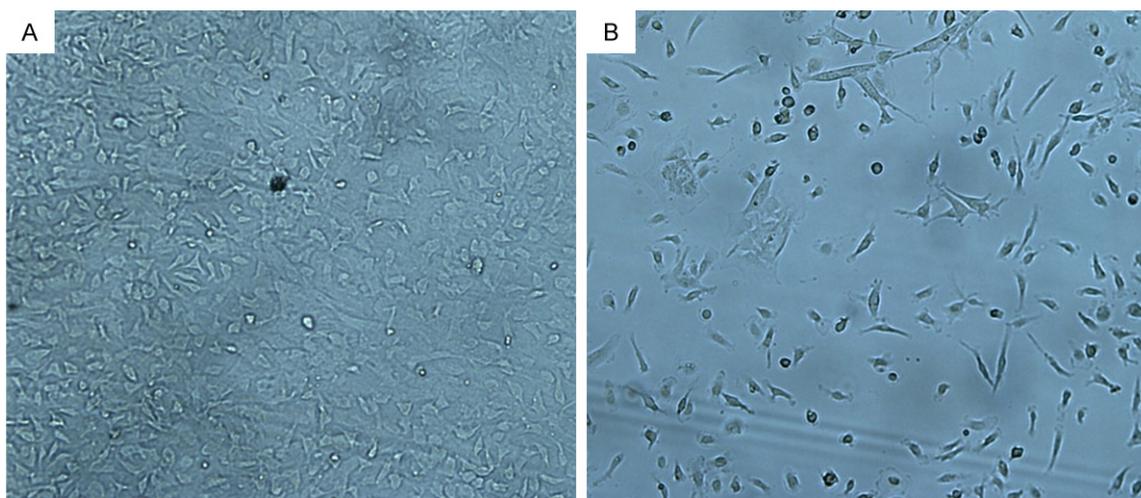
of the total bone marrow mononuclear cells and nearly 10% of the hematopoietic stem cells (HSCs) [4]. Thus, efficient isolation of BMSCs, especially from small experimental animals such as mouse from which it is difficult to obtain adequate volume of bone marrow sample, becomes a necessity for exploring the biology characters and therapeutic use of BMSCs.

Plenty of studies demonstrate different culture media such as  $\alpha$ -MEM (Alpha Minimal Essential Medium), DMEM (Dulbecco's modified Eagle's medium) and DMEM/F12 have been employed for the isolation and culture of murine BMSCs [5-7]. However, to our knowledge, no research on optimization of culture media for primary culture of murine BMSCs has been reported. In this study, we compared the isolation efficiency of murine BMSCs in different culture media including  $\alpha$ -MEM, DMEM and DMEM/F12 in order to optimize the appropriate medium for primary isolation of murine BMSC.

## BMSCs and bone culture media



**Figure 1.** Cell culture at 5 days: A.  $\alpha$ -MEM medium; B. DMEM medium; C. DMEM/F12 medium (100 $\times$ ).



**Figure 2.** Cell culture at 15 days: A.  $\alpha$ -MEM medium; B. DMEM medium (100 $\times$ ).

### Materials and methods

#### *Culture media*

Three different culture media including  $\alpha$ -MEM, DMEM and DMEM/F12 (Hyclone) supplemented with 10% fetal calf serum (FCS; Hyclone) were employed in this study.

#### *Isolation of BMSCs*

The animal experimentation was conducted in conformity with the Animal Care and Use Committee of Qingdao University (Qingdao, Shandong Province, China). BMSCs were isolated from C57BL/6J mice (6-8 weeks old) as described previously [2]. Briefly, the animals were sacrificed and the femur and tibias were removed aseptically. Then the bone marrow was flushed with phosphate-buffered saline (PBS) to a cell culture dish by using a sterile needle. The bone marrow samples were further dispersed by gentle repeated pipetting with a

sterile pipet and filtered to get single cell suspension. Cells were plated at a density of  $1 \times 10^6/\text{cm}^2$  in 25  $\text{cm}^2$  culture vessel and cultured in three different media:  $\alpha$ -MEM, DMEM and DMEM/F12, each was supplemented with 10% FCS. The vessels were placed at 37 $^\circ\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$ . The first medium change was performed two days later: the medium was carefully removed and the dish was washed with PBS to remove the non-adherent hematopoietic cells. The culture medium was replaced every 2-3 days. The growth condition of BMSCs in different culture media was observed by inverted microscope.

#### *Cell count*

To assess cell proliferation the adherent cells were counted at 5 days and 15 days of culture. Briefly, the cells were rinsed with phosphate-buffered saline (PBS) and detached with 0.25% trypsin-0.02% EDTA solution (Amresco) and counted with a hemacytometer [8]. The cells

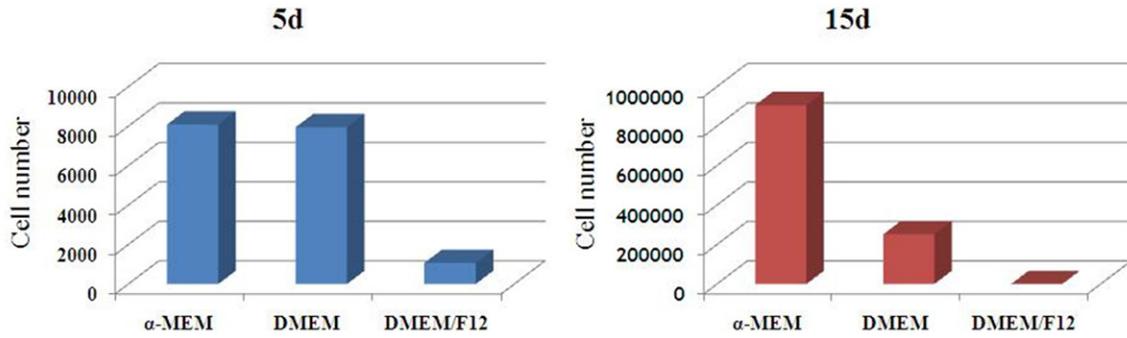


Figure 3. Cell count at 5 days (Left) and 15 days (Right) in the α-MEM, DMEM and DMEM/F12 medium.

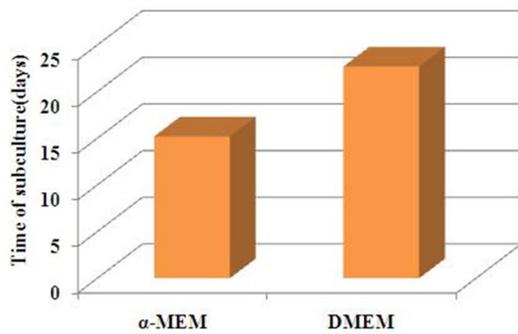


Figure 4. The time of subculture of BMSCs in the α-MEM and DMEM medium.

were counted 3 times for each sample and then the average was taken.

#### Subculture of the primary BMSCs

At 90% confluence, the primary BMSCs were passaged using 0.25% trypsin-0.02% EDTA solution (Amresco) and the first time of subculture was recorded.

#### Statistical analysis

Data analysis was performed using SPSS 18.0 software (IBM, America). Comparison between groups was performed via analysis of Independent-Samples T test. Differences with  $P < 0.05$  were considered statistically significant. The data are expressed as the mean  $\pm$  standard deviation.

### Results

#### Observation under inverted microscope

When the first medium change was performed, a small number of adherent cells which are vari-

ous in shapes were detected in all culture media. At 5 days of culture, the number of adherent cells increased obviously in α-MEM and DMEM media, but just a few in DMEM/F12 medium (Figure 1). 15 days later, the adherent cells appeared clone-like growth and as irregular triangle or shuttle in shape in both α-MEM and DMEM medium (Figure 2). Cells in α-MEM medium grew far faster than that in DMEM medium. There was no living cell in DMEM/F12 medium.

#### Cell count

At 5 days of culture, the cell number was  $8083.3 \pm 147.2$  in α-MEM medium,  $7950 \pm 151.7$  in DMEM medium and  $1066.7 \pm 81.6$  in DMEM/F12 medium. There were more adherent cells in both α-MEM and DMEM medium than in DMEM/F12 medium, but there was no difference between α-MEM and DMEM group ( $P > 0.05$ ). The difference between α-MEM and DMEM/F12 group was statistically significant ( $P < 0.05$ ), and the same between DMEM and DMEM/F12 group. At 15 days of culture, adherent cells cultured in α-MEM medium was significantly more than that in DMEM medium ( $P < 0.05$ ). The cell number was  $906666.7 \pm 68896.1$ ,  $251666.7 \pm 80104.1$  in α-MEM medium and DMEM medium. There was no living cell in DMEM/F12 medium (Figure 3).

#### Subculture of the primary BMSCs

The first subculture of BMSCs cultured in the α-MEM and DMEM medium was at  $(15.2 \pm 0.8)$  d and  $(22.7 \pm 0.8)$  d respectively, which indicated a significant difference ( $P < 0.05$ , Figure 4).

## Discussion

For the sake of self-renewal and multiple differentiation ability and non-oncogenic property, BMSCs have been considered as an effective and safe resource for tissue engineering and stem cell-based therapy. Isolation of BMSCs from different species has been demonstrated in plenty of studies. The previous studies mention different culture media such as  $\alpha$ -MEM, DMEM and DMEM/F12 which have been employed for the isolation and culture of murine BMSCs [5-7]. DMEM/F12 medium has been used in the isolation and culture of BMSCs derived from various species including human, rabbit, rat and murine [6, 9-11]. However, in our study, murine BMSCs had not been successfully gained by cultured in DMEM/F12 medium. In our previously experiments we also succeeded in isolating and culturing rat BMSCs using DMEM/F12 medium as culture medium, but accidentally discovered when seeded living murine BMSCs in DMEM/F12 medium containing 10% FCS the cells could not survive for a long time, which was not consistent with other report [6].

As we know, various studies involve BMSCs have been carried out in pre-clinical and clinical studies, which leads to an increasing demand for extensive expansion of BMSCs in vitro. The extremely low yield of MSCs isolated from the bone marrow especially from the small animals like mice encourages us to find an excellent culture medium for the isolation and culture of BMSCs.  $\alpha$ -MEM and DMEM media have been employed in various cell culture in a lot of studies. In the present study, murine BMSCs were successfully isolated and cultured in both  $\alpha$ -MEM and DMEM media in vitro and showed more rapid proliferation in  $\alpha$ -MEM medium. Therefore,  $\alpha$ -MEM medium has the potential to be the most appropriate cell culture medium for prolonged culturing of murine BMSCs.

However, our research was just carried out on the primary culture of murine BMSCs. Further studies are needed to explore the effect of different culture media on biological characters of subcultured murine BMSCs and find the most appropriate cell culture medium for BMSCs originated from other species.

## Acknowledgements

This study was supported by Foundation for Outstanding Young Scientist in Shandong Province (BS2014YY028) to Quan-Chen Xu.

## Disclosure of conflict of interest

None.

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