

Establishment of chicken bone marrow mesenchymal stem cells  
and evaluation of stem cell capacity  
雞骨髓間葉幹細胞之建立與幹細胞能力評估

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Eggs provide essential nutrients for humans. Only hens are needed for egg production, males are discarded after hatching. The aim of this study was to collect bone marrow cells from culled male chickens, study and confirm the characteristics of the stem cells after culturing and purification into bone marrow mesenchymal stem cells for subsequent research applications. Bone marrow cells were collected from the femur and tibia of 1-day-old male chickens using a syringe with a needle. The cells were filtered and centrifuged, added to  $\alpha$ -MEM medium, counted and cultured. According to the adherence property of stem cells, every 12 hrs of culture, the floating cells were aspirated, and half of the original culture medium was replaced with fresh medium. After changing the culture medium within 72 hrs, the cells were returned to the normal culture mode. After cell subculture (P2), the cells can be frozen for future experiments. Chicken bone marrow mesenchymal stem cells (cBMSCs) of different passages were collected and subjected to colony forming unit (CFU) assay to observe the number of cell colonies and the efficiency of stem cells. cBMSC were induced to differentiate into adipocyte and osteocyte to evaluate the differentiation ability of cBMSCs. Reverse transcriptase PCR (RT-PCR) was used to confirm the surface antigens specific for mesenchymal stem cells. The CFU results showed a decline in cBMSCs growth and proliferation as cell generation increased from P3 to P7, indicating a decrease in the stem cell's capability for passages development. The cell differentiation assay showed, cBMSCs were able to differentiate into adipocytes and osteocytes through appropriate induction, indicating that cBMSCs share similar traits with mammalian stem cells. RT-PCR results showed that cBMSCs exhibited CD29, CD31 and CD44 surface antigens, while lacking CD34 and CD45, thereby consistent with mesenchymal stem cells. The results demonstrate that the technique purifying cultured chicken bone marrow cells using the stem cell adhesion properties, could be utilized as a method of establishing cBMSC. Such a technique has the potential to aid in future development and application of cBMSCs.

**Key words:** Chicken bone marrow mesenchymal stem cells, adipocyte and osteocyte differentiation, colony forming unit, reverse transcriptase-PCR