Abstract

Human mesenchymal stem cells (hMSCs) are pluripotent adult cells that can be isolated from various tissues and used as a valuable source for adipocytes and osteocytes. hMSCs are known to differentiate into adipocytes and osteocytes, and thus they are commonly used as a model system for studying differentiation. The present study addressed the development of a serum-free, xeno-free medium for adipogenesis and osteogenesis of hMSCs using MSCgo™ differentiation media. The quality of the culture medium and differentiation media is particularly crucial with regard to therapeutic applications since multipotent hMSCs and differentiated cells are used in various applications such as tissue engineering and regenerative medicine.

Results

I. Adipogenesis

A. Materials and Methods

Cells

hMSCs (hMSC-AT, hMSC-BM, hMSC-CT, hMSC-WJ, hMSC-PL) were used in this study.

Culture system

hMSCs were cultured in AdipoGenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) and Protein-free Medium (PromoCell) for 15 days. The cells were then harvested and processed for the next step.

II. Chondrogenesis

A. Materials and Methods

Cells

hMSCs (hMSC-AT, hMSC-BM, hMSC-CT) from various sources were cultured in Chondrogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days. The cells were then harvested and processed for the next step.

B. Evaluation of Cartilage Maturation by Histology Examination

Histology examination was performed to evaluate the maturation of the differentiated hMSCs. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

III. Osteogenesis

A. Materials and Methods

Cells

hMSCs (hMSC-AT, hMSC-BM, hMSC-CT) from various sources were cultured in Osteogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days. The cells were then harvested and processed for the next step.

B. Evaluation of Osteogenic Differentiation

Alizarin red-S (ARS) staining was performed to evaluate the mineralization of the differentiated hMSCs. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

IV. Rapid Osteogenesis

A. Materials and Methods

Cells

hMSCs (hMSC-AT, hMSC-BM, hMSC-CT) from various sources were cultured in Rapid Osteogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days. The cells were then harvested and processed for the next step.

B. Evaluation of Rapid Osteogenic Differentiation

Alizarin red-S (ARS) staining was performed to evaluate the mineralization of the differentiated hMSCs. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

Summary

The present study addressed the development of a serum-free, xeno-free medium for adipogenesis and osteogenesis of hMSCs using MSCgo™ differentiation media. The quality of the culture medium and differentiation media is particularly crucial with regard to therapeutic applications since multipotent hMSCs and differentiated cells are used in various applications such as tissue engineering and regenerative medicine. The present study showed that the MSCgo™ AdipoGenic, Chondrogenic, and Osteogenic differentiation media were able to efficiently differentiate hMSCs from various sources into adipocytes, osteocytes, and chondrocytes, respectively.

Evaluation of Differentiation

A. Adipogenesis

hMSCs treated with AdipoGenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days were harvested and stained with Oil Red O for evaluation of lipid accumulation. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

B. Chondrogenesis

hMSCs treated with Chondrogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days were harvested and stained with Alcian Blue for evaluation of cartilage formation. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

C. Osteogenesis

hMSCs treated with Osteogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days were harvested and stained with Alizarin red-S for evaluation of mineralization. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

Materials and Methods

Cells

hMSCs from various sources were used in the study. hMSCs were treated with AdipoGenic, Chondrogenic, and Osteogenic differentiation media for 15 days. The treated hMSCs were harvested and stained with Oil Red O, Alcian Blue, and Alizarin red-S, respectively, for evaluation of lipid accumulation, cartilage formation, and mineralization.

Culture system

hMSCs were cultured in AdipoGenic, Chondrogenic, and Osteogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days. The treated hMSCs were harvested and stained with Oil Red O, Alcian Blue, and Alizarin red-S, respectively, for evaluation of lipid accumulation, cartilage formation, and mineralization.

II. Chondrogenesis

A. Materials and Methods

Cells

hMSCs from various sources were used in the study. hMSCs were treated with Chondrogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days. The treated hMSCs were harvested and stained with Alcian Blue for evaluation of cartilage formation. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

B. Evaluation of Cartilage Maturation by Histology Examination

Histology examination was performed to evaluate the maturation of the differentiated hMSCs. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

III. Osteogenesis

A. Materials and Methods

Cells

hMSCs from various sources were used in the study. hMSCs were treated with Osteogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days. The treated hMSCs were harvested and stained with Alizarin red-S for evaluation of mineralization. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

B. Evaluation of Osteogenic Differentiation

Alizarin red-S (ARS) staining was performed to evaluate the mineralization of the differentiated hMSCs. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

IV. Rapid Osteogenesis

A. Materials and Methods

Cells

hMSCs from various sources were used in the study. hMSCs were treated with Rapid Osteogenic differentiation media (U.S. Patent Number 8,450,735 B2, Promon) for 15 days. The treated hMSCs were harvested and stained with Alizarin red-S for evaluation of mineralization. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.

B. Evaluation of Rapid Osteogenic Differentiation

Alizarin red-S (ARS) staining was performed to evaluate the mineralization of the differentiated hMSCs. The stained sections were analyzed using a light microscope, and the images were captured and analyzed using ImageJ software.