

Title of the Invention

A method of enhancing migratory effect of human mesenchymal stem cell

Filed of the Invention

The invention relates to a method of enhancing migratory effect of bone marrow-, adipose tissue-, amniotic fluid-, nucleus pulposus- and Wharton's jelly umbilical cord-derived mesenchymal stem cell.

Background of the Invention

Mesenchymal stem cells (MSCs) are multilineage cells with the ability to self-renew and differentiate into a variety of cell types. They have been used as resources for cell therapy in regenerative medicine. MSCs have a "homing" ability in which they can migrate to the specific sites and differentiate into local cell components. MSCs express many receptors and cell adhesion molecules that aid in migration and homing to target tissues. The present invention aimed to enhance migratory potential of MSCs.

Most of clinical trials using MSCs have been reported, and many others are ongoing. For most of the applications, systemic administration of the cells is a safe and minimally invasive way to deliver MSCs. However, there is a question regarding the distribution of MSCs and as to whether MSCs still perform best if not present at the site of injury. Several studies in animal models and patients have shown that most MSC population entraps initially in the lungs due to lack of site-directed migration and poor engraftment.

In osteogenesis imperfecta patient, less than 1% of the cells were detected in the target organ when MSCs were administered intravascular (IV). Besides, in the absence of *ex-vivo* pretreatment or modification, fewer infused MSCs migrate and actually engraft at damaged tissue. Therefore, there is a need to develop a method for enhancing migratory potential of mesenchymal stem cells.

Summary of the Invention

The objective of the present invention is to provide a method for enhancing migratory potential of mesenchymal stem cells. A method of enhancing migratory effect of mesenchymal stem cells according to this invention comprises the steps of thawing of mesenchymal stem cells (MSCs), treating the thawed mesenchymal stem cells, preparing a predefined medium, culturing a population of the mesenchymal stem cells in a predefined medium, incubating for a predetermined period of time at a predefined temperature, suspension in mesenchymal stem cells (MSCs) wherein the treatment of mesenchymal stem cells comprises the step of adding ozanimod into the predefined medium the range of 1 nM to 10 μ M per ml.

In accordance with one aspect of the present invention, the medium is based on α MEM supplemented 10 % human serum + 2 mM L glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin.

In accordance with another aspect of the present invention, the predetermined period of time is 18-72 hours, preferably 24 hours.

In accordance with another aspect of the present invention, the step of culturing a population of the mesenchymal stem cells in a medium is the culturing a population of the mesenchymal stem cells in a medium till cultures reach 70 to 95% cell confluency, preferably 90 to 95% cell confluency. The present invention also covers Mesenchymal stem cells obtained by any of the aforementioned method.

Brief Descriptions of the Drawings

Figure 1 shows effect of ozanimod on wound healing and expression of chemokine receptors involved in migration of MSCs (N = 5 cell line).

Figure 2 shows Ozanimod-treated MSCs migrate towards SDF-1 α (N = 5 cell line).

Detailed Descriptions of the Invention

The descriptions in more detail of this invention will be provided by way of example in order to make the descriptions clearer. The said descriptions in more detail, however, are provided without any limitation to the scope of the invention.

In this regard, this invention can be implemented or applied in various manners. This disclosure will present the different examples as references for implementations or applications having the same characteristics. Nevertheless, as mentioned above, these are provided without any limitation to the scope of the invention.

A method of enhancing migratory effect of mesenchymal stem cells (MSCs) according to this invention comprises the steps of thawing of mesenchymal stem cells (MSCs) in the beginning. In practice, this further comprise the removal of the vial of Human Mesenchymal Stem Cells from liquid nitrogen and incubate in a 37 °C water bath, treating the thawed mesenchymal stem cells, preparing a predefined medium, culturing a population of the mesenchymal stem cells in a predefined medium, incubating for a predetermined period of time at a predefined temperature, suspension in mesenchymal stem cells (MSCs) wherein the treatment of mesenchymal stem cells comprises the step of adding ozanimod into the predefined medium the range of 1 nM to 10 μ M per ml. In one aspect, umbilical cord derived Mesenchymal stem cells (MSCs) are also preferable for usage according to this disclosure.

In the other aspect, the medium is based on α MEM supplemented 10 % human serum + 2 mM L glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin.

In the other aspect, the predetermined period of time is 18-72 hours, preferably 24 hours.

In the other aspect, the step of culturing a population of the mesenchymal stem cells in a medium is the culturing a population of the mesenchymal stem cells in a medium till cultures reach 70 to 95% cell confluency, preferably 90 to 95% cell confluency.

Mesenchymal stem cells obtained by any of the above aspects.

It should be noted that any additional steps according to the regular Mesenchymal Stem Cell Culture Protocols can be included in the method as disclosed herein. For example, the procedure can include the step of thawing of Mesenchymal Stem Cells in the beginning. In practice, this further comprise the removal of the vial of Human Mesenchymal Stem Cells from liquid nitrogen and incubate in a 37 °C water bath. Thereafter, the cells are resuspended with fresh MSC medium in the predefined container and centrifuge the container at 1000 x g for 5 minutes to pellet the cells. Then, the said steps may be followed by suspending the cells in a predefined Mesenchymal Stem Cell Expansion Medium and plating the cell suspension onto a tissue culture plate or a T75 tissue culture flask and subsequently incubating in a 37 °C incubator

Nowadays, the scientists are used the inflammatory cytokines pretreatment, genes and enzymatic modification to enhance the migratory potential of MSCs in *in vitro* study and *in vivo* animal model. However, they have a limitation of usage in clinical trials due to they are unsafe for usage, e.g., cytotoxicity, cell apoptosis, inhibition of tissue regeneration, and teratoma formation. Therefore, the alternative strategies are needed, e.g., small molecules and other chemicals pretreatment, due to they are safety for usage and high efficiency to cure the patients that the hope to enhance the migratory potential of MSCs.

Previous report founded that "Fingolimod" could enhance the migratory potential of bone marrow-derived MSCs (BM-MSCs). It is the various agonist of sphingosine-1-phosphate receptors (S1PRs), S1PR₁ and S1PR₃₋₅. Their biological actions are cell proliferation, apoptosis and cell migration. However, the vignette of boarder actions of fingolimod resulting in many effects of the cells. Therefore, the specific S1PR₁ and S1PR₅ agonists may be needed, "Ozanimod", to enhance the migratory potential of MSCs more than fingolimod.

Figure 1 shows effect of ozanimod on wound healing and expression of chemokine receptors involved in migration of MSCs (N = 5 cell line). A = Wound healing assay, B = Flow cytometry analysis, C = real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) analysis.

Figure 2 shows ozanimod-treated MSCs migrate towards SDF-1 α (N = 5 cell line). A = Transwell chamber assay, B = Wound healing assay.

From the experiment, it has been found that the addition of ozanimod at the concentration of 1nm - 10 μ M per ml for 24 h of the culturing procedure can enhance the migratory potential of MSCs at gene, protein and functional level via CXCR4/SDF-1 α pathway.

Although this invention is described in detail, it will be understood that this invention are intended to cover any modification or solutions provided by the persons skilled in the arts.

The scope of this invention is based on the claims and also cover any characteristics of the invention that may not be specifically covered in the claims, but still cause the same effects as those of this invention.

Best Mode of the Invention

As disclosed in the Detailed Descriptions of the Invention.

Claims**What is claimed is:**

1. A method of enhancing migratory effect of mesenchymal stem cell, the method comprising the steps of:
thawing of mesenchymal stem cells (MSCs),
treating the thawed mesenchymal stem cells,
preparing a predefined medium,
culturing a population of the mesenchymal stem cells in a predefined medium;
incubating for a predetermined period of time at a predefined temperature,
suspension in mesenchymal stem cells (MSCs),
wherein the treatment of mesenchymal stem cells comprises the step of adding ozanimod into the predefined medium the range of 1 nM to 10 μ M per ml.
2. A method of enhancing migratory effect of mesenchymal stem cell according to claim 1 wherein a predefined medium is based on α MEM supplemented with 10 % human serum, 2 mM L glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin.
3. A method of enhancing migratory effect of mesenchymal stem cell according to claim 1 or 2 wherein the predetermined period of time is 18-72 hours, preferably 24 hours.
4. A method of enhancing migratory effect of mesenchymal stem cell according to claim 1, 2 or 3 wherein the step of culturing a population of the mesenchymal stem cells in a medium is the culturing a population of the mesenchymal stem cells in a medium till cultures reach 70 to 95% cell confluency, preferably 90 to 95% cell confluency.
5. Mesenchymal stem cells obtained by any of the above claims.