

## Cell Therapy Products in Menopausal Medicine

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The incidence of postmenopausal diseases increases with the age of women. In this review, we introduce cell therapy products, a new treatment for postmenopausal osteoporosis, which often occurs in postmenopausal women. We also figure out the trends of research on cell therapy products and emphasize the necessity and importance of this research for researchers and postmenopausal women. Finally, we suggest the direction for improvement of postmenopausal osteoporosis and research on cell therapy products. We investigated which medication have been used so far. We also examined the development and technical problems of technologies that are currently in use. (**J Menopausal Med 2016;22:71-75**)

**Key Words:** Cell- and tissue-based therapy · Menopause · Osteoporosis, postmenopausal · Stem cells

### Introduction

The average life expectancy has been increasing constantly due to technical developments in medicine, industry, and science. The average life expectancy appears to be longer in women than in men. The average life expectancy of Korean women was 85.5 years in 2014, which was 0.84 years higher than in 2012. Consequently, the incidence of diseases related to aging in women and interest in studying and finding treatments for these diseases have been also increasing.<sup>1</sup> Especially, postmenopausal osteoporosis, the main disease related to aging in women, is a high-risk disease that decreases bone strength and makes it easier for patients' bones to be fractured.<sup>2</sup> Currently, most medications for postmenopausal osteoporosis suppress activity of osteoclasts and cause adverse effects.<sup>3,4</sup> Most widely used medications for postmenopausal osteoporosis are

oral and intravenous bisphosphonates, such as alendronate, ibandronate, risedronate, and zoledronate. However, despite their proven safety and efficacy, adverse events such as upper gastrointestinal, impaired renal function, fever, osteonecrosis of jaw, uveitis, and atrial fibrillations have been reported, and thereby their use is limited.<sup>5</sup> However, cell therapy products could be a new treatment which reduces the possibility of adverse effects and used widely, unlike existing medication treatments. We reviewed history, research and development (R & D) of cell therapy products for postmenopausal osteoporosis, and examined current problems. We also suggest how cell therapy could be improved.

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## Development of Cell Therapy Products for the Treatment of Postmenopausal Osteoporosis

In 1976, Friedenstein proved that there is a possibility that mesenchymal stem cells exist in bone marrow. In the late 1990s, technology to differentiate mesenchymal stem cells from human bone marrow into osteoblasts was established, and many studies based on this technology have been conducted.<sup>6</sup> Several studies have improved the osteoblast differentiation method using various active substances. There have also been some studies on bone formation by extracting mesenchymal stem cells from tissues other than bone marrow and an attempt to replace the cells alone to existing biological tissue engineering method. However, applying these methods to clinical research is still in the early stages.<sup>7</sup>

## Technologies and Principles of Cell Therapy Products in Postmenopausal Osteoporosis

Most studies on stem cell applications likely aim to advance fundamental knowledge. However, phased studies that aim to develop a cell therapy product are in progress and bring them to the market.

### 1. Mechanism of differentiation of mesenchymal stem cells from adult bone marrow into chondrocytes and adipocytes

Technology to induce differentiation of mesenchymal stem cells from human bone marrow into chondrocytes or adipocytes was developed. Differentiation of mesenchymal stem cells into osteoblasts is decreased without nuclear factor 1-C (NF1C). Bone formation and bone mineral density (BMD) are also decreased because mesenchymal stem cells differentiation into adipocyte is increased.<sup>8</sup> Postmenopausal osteoporosis could be treated by controlling gene and promoting the differentiation of osteoblasts. When controlling stem cell genes, localized growth factors, cytokines and endocrine signals are needed for bone formation. Therefore, modifying stem cells genetically would

be appropriate.<sup>9-11</sup> There are some potential candidate genes for treating bone defects. These genes promote osteoblast proliferation and survival; the proteins they encode synthesize extracellular matrix components and accelerate mineral acquisition. Transient transfection with adenovirus is used to control genes. For long-term expression, an adeno-associated virus, retrovirus, or lentivirus vector capable of genomic recombination is required.<sup>12</sup> To express a particular protein in mesenchymal stem cells, it should be expressed mainly in osteoblasts. Generally, adenovirus vector targeting the expression of a cellular virus receptor or type 1 collagen, or the activity of osteocalcin promoter is used.<sup>12,13</sup> Recently, it was shown that myocyte regeneration into adipocytes is controlled by peroxisome proliferator-activated receptor-gamma (PPAR- $\gamma$ ), CCAAT/enhancer-binding protein-alpha (C/EBP- $\alpha$ ), the adipocyte transcription factors. The phenotype induced in chondrocytes was shown due to over-expression of Sox-9 cDNA in rodent mesenchymal stem cells.

### 2. Differentiated rate originated from bone marrow in adults and technology for mesenchymal stem cell isolation and culture

The expression of hematopoietic stem cell gene in mesenchymal stem cells is low, whereas hematopoietic stem cells have a high level of the cell surface antigen CD34. These cells can be isolated not only from bone marrow, but also from peripheral blood and umbilical cord blood.<sup>12,13</sup> Studies of cell surface antibody suggested that mesenchymal stem cells could be purified by using magnetic-activated cell sorting (MACS) or fluorescence-activated cell sorting (FACS). However, more studies of representative surface antigens are needed.<sup>14</sup> Changes in cytomorphological features, reduction in differentiation rate, and cell proliferation were reported in subcultures of mesenchymal stem cells isolated from adult bone marrow by using a Ficoll technique. Therefore, separating single-cell clones from a heterogeneous population is used to solve this problem. However, it takes a long time to acquire a large quantity of cells from a single-cell clone.<sup>14</sup> Using a surface antibody such as stro-1 or sca-1 or separating cells by changing medium composition has been proved to efficiently purify mesenchymal stem cells originated from bone marrow.<sup>13</sup>

### 3. Activation of existing stem cells

New technology to treat postmenopausal osteoporosis by activating stem cells was invented recently. BMD could be increased by activating mesenchymal stem cells with Velcade, the proteasome inhibitor, a therapeutic agent for bone marrow disorders. This study used a recovery mechanism in humans not by applying an established technique uses adoptive transfer of stem cells into human body, but by activating existing stem cells in human tissue using medication. Stimulating differentiation of immature cells and make it specific cell structure is a new approach that applies established technology. The use of stem cell-targeting medication is a groundbreaking and useful technology.<sup>13,14</sup> However, finding stem cells for medication therapy and developing medication with no or low toxicity are the first things to do before any further progress can be made. In this respect, there are a few reports that the level of alkaline phosphatase in blood is increased by Velcade injection in patients with multiple myeloma. In another preliminary study, Velcade increased human osteoblast differentiation.<sup>14</sup> There is a study verifying whether Velcade injection would restore bone damage irrelevant to cancer. The researchers transplanted mesenchymal stem cells into mice and then injected low doses of Velcade. Heterotopic ossification was observed.<sup>15</sup> Similar results were obtained with cultured mesenchymal stem cells. Osteogenesis and BMD were improved significantly as a result of injecting Velcade into mice with postmenopausal osteoporosis but not when Velcade was injected into mesenchymal stem cells that had been differentiated into a specific cell type.<sup>15,16</sup> According to the results of a follow-up study, Velcade injection affected mesenchymal stem cells and activated osteoblasts but did not affect rather differentiated osteoblast precursor cells. The researchers found that Velcade injection controlled runt-related transcription factor 2 (Runx-2), a transcription factor involved in bone formation, and allowed mesenchymal stem cells to differentiate into osteoblasts.<sup>16</sup>

Current treatments for postmenopausal osteoporosis have a limitation since it targets differentiated cells such as osteoblasts or osteoclasts. For example, when osteoclast activity is impeded, osteoblast activation is hindered as well. Recent studies reported that bisphosphonate led to osteonecrosis of jaw. However, if the differentiation of

mesenchymal stem cells could be controlled directly, bone loss related to postmenopausal osteoporosis would be cured more effectively. These technologies have become a basis for developing stable cell therapy of bone. In addition, unlike medical treatments, which is likely to cause adverse effects, cell therapy would cure the fundamental cause of postmenopausal osteoporosis while having few adverse effects and reducing pain.

## Problems

### 1. Lack of technologies for purification and amplification of mesenchymal stem cells

SH-2, SH-3, CD29, and CD44 are used as positive factors for identification of mesenchymal stem cells, whereas CD34 and CD14 are used as negative factors.<sup>14</sup> However, there are no representative markers and these factors are present in differentiated cells as well. Therefore, technology to distinguish differentiated cells and to transplant these cells is required.

### 2. Lack of clinical trials

There have been animal studies that reported transplantation of stem cells originated from bone marrow to treat musculoskeletal system defects such as spondylodiosis and arthritis, and to reconstruct skull bone defects, local defects of joint cartilage, and tendon defects.<sup>13</sup> However, there are no clinical studies about stem cell transplantation from bone marrow in humans, except for the purpose of bone defect reconstruction and in patients with avascular necrosis of femoral head.<sup>12</sup> Therefore, further clinical studies in humans are required.

## Summary

Postmenopausal osteoporosis is a high-risk disease that decreases bone strength and makes it easier for patients' bones to be fractured. There are some therapies and medications such as hormone replacement, complementary and alternative medicine, phytohormones, herbal therapies, homeopathy, antidepressants, anticonvulsants,

dehydroepiandrosterone to treat postmenopausal osteoporosis. However, some adverse effects have been reported and their uses are limited.<sup>17</sup> Therefore, it is important to figure out the trends of research on cell therapy principles and products, and to suggest the direction for improvement and further research on cell therapy for postmenopausal osteoporosis. Mesenchymal stem cells were discovered in 1976, and a method to differentiate mesenchymal stem cells obtained from bone marrow into osteoblasts was developed in the late 1990s. Finally, cell therapy technology for postmenopausal osteoporosis was invented. There are several cell therapy technologies for postmenopausal osteoporosis. Some studies have addressed adoptive transfer of heterogeneous stem cells into humans, whereas others relied on the activation of existing stem cells in human tissue using medication. When heterogeneous stem cells are transplanted into humans, myocyte re-differentiation into adipocytes is induced by PPAR- $\gamma$  and C/EBP- $\alpha$ , the adipocyte transcription factors. However, further studies regarding signal transduction pathways involved in differentiation are needed. There is a separation and culturing technology that is different from the technology mentioned above and purified mesenchymal stem cells with high differentiation rate. In addition, there are several culture technologies such as single-cell clone separation methods from heterogeneous populations, MACS, and FACS. These technologies are able to solve problems such as changes in cytomorphological features and reduction in differentiation rate and cell proliferation. However, there are other methods that use the surface antibodies stro-1 or sca-1, or changes in medium composition. These technologies, adoptive transfer of heterogeneous stem cells into humans, need more studies to become practically useful. Further studies are required since these technologies are at an early phase of development. There are no cases of commercialization of these technologies or their application to patients. Several problems cause stagnation of the development of these technologies: lack of technologies for mesenchymal stem cell purification and amplification, a small number of studies on the mechanism of differentiation of mesenchymal stem cells into adipocytes and chondrocytes, and the lack of clinical trials in humans. Therefore, further studies are needed to apply these technologies to patients.

Once further studies are performed, it would be easier to apply these technologies to postmenopausal patients with gynecological and endocrine-related diseases including osteoporosis.<sup>18,19</sup> Consequently, the quality of life in elderly women as well as postmenopausal women will improve. We perform this study so that scientists as well as physicians pay attention to cell therapy technology and applying cell therapy technology to menopausal medicine.

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## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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