



# Human Muscle-Derived Stem Cells

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## INTRODUCTION

Many scientists engaged in looking for other homogeneous origin of stem cells, such as fat tissue,<sup>1</sup> amniotic fluid<sup>2</sup> or skeletal muscle<sup>3</sup> etc. In studies using rat models, it was found that stem cells originated from skeletal muscles have the ability to differentiate into myogenic, osteogenic and chondrogenic cell lineages,<sup>4-6</sup> or even differentiate into tissue of different organs, such as hematopoietic system.<sup>7,8</sup> Furthermore, stem cells originated from skeletal muscle can be used as vector in gene therapy and reveal its effects in many diseases treatment, such as muscular dystrophy,<sup>5</sup> myocardial infarction (MI),<sup>9</sup> and stress urinary incontinence (SUI)<sup>10</sup> etc. Human muscle derived stem cells have been isolated successfully using modified preplating technique.<sup>11</sup> These cells have also been purified using Dynal cell selection system. These purified cells were proven to have the ability of differentiation.<sup>12</sup> Using the purified human muscle derived stem cell, we can test whether property of differentiating into various kind of tissue existed or not. Changing the microenvironment of the cells, laboratory data showed that different proportion of differentiate medium and different duration would successfully differentiate these purified cells into skeletal muscle, adipocytes, osteocytes, and chondrocyte,<sup>12</sup> smooth muscle cells and neurogenic cells (Lu SH et al, unpublished data). These results proved again that we have successfully isolated pluripotent stem cell from human skeletal muscle tissue.

In earlier study, mice muscle derived stem cells have been transplanted to small intestinal submucosa (SIS) and it was successfully differentiated into contractile

muscle tissue<sup>13</sup> which also made more compliant biomaterials for management.<sup>14</sup> Next, to obtain the high differentiation rate, we have to increase the growth stability of these kinds of cells, and incorporate the cells into 3D scaffold. If we could make it grow successfully and differentiate into different cellular tissue, in the future, the cell transplantation of tissue reconstructive engineering using human muscle derived stem cells would offer further benefits to people for bladder reconstitution, treatment of SUI and erectile dysfunction.

### ■ Where are the stem cells in skeletal muscle?

Some studies discussed about the satellite cells and muscle derived stem cells. However, the definition of these cells has not been completely elucidated. Actually, the human muscle stem-like cells are normally quiescent and niche in between the matrix regions of myoblasts and the quantity of the cells is scanty.<sup>15-18</sup> The IHC study in our laboratory found that a small amount of stem cells are found in between the matrix regions of myoblasts from the human limb skeletal muscle (Fig. 1). However, these cells proliferate in response to muscle injuries, producing myoblasts to repair the injured muscles.<sup>19,20</sup> Some study suggested that, after the transplantation of cultured skeletal muscle cell into injured muscle area, there would be massive apoptosis of transplanted cell, but small amount of cells would process rapid cell division, this showed that skeletal muscle cell might contain the cell group with stem cell-like.<sup>21</sup>

### ■ Stem cells isolated from human skeletal muscle

Though the studies about the human adult stem cells are not much, human muscle derived stem cells have been successfully isolated using modified preplating technique.<sup>11</sup> The doubling time of these isolated cells is

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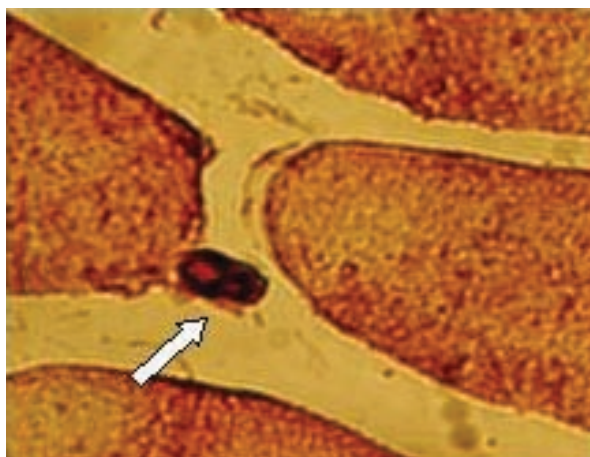


Fig. 1. Immunohistochemistry study revealed that the human muscle stem-like cells (arrow) are normally found in between the matrix regions of myoblasts from the human limb skeletal muscle and the quantity of the cells is scanty (200X magnification).

24 hours. Using immunofluorescence study, these cells were found to express stem cell's specific antigen, such as CD34, CD117, VCAM and VEGFR-2. Besides, specific antigen of muscle precursor cells, such as CD56, CXCR4 and desmin could be expressed too.<sup>11</sup> This showed that, by using preplating method, one might isolate cells that contain stem cell property. Researches have identified populations of muscle derived multipotent stem cells in murine muscle,<sup>22</sup> as well as multipotent stem cells in human muscle that can be isolated with immunoselective techniques.<sup>15,23</sup> The hMDCs isolated by modified preplating technique mimic the muscle-derived myoendothelial cells demonstrated by Zheng et al, which also revealed positive CD56 and CD34 expression.<sup>11,15</sup>

### How do we purify these cells?

Determined by immunofluorescence staining, we have found that large amount of fibroblasts have been found in the isolated human muscles derived cells, this suggests that the cells isolated using preplate technique might not be single cell group, as fibroblast usually do not have the ability of differentiation.

Using flow cytometry analysis, we can recognize specific antigen CD34 of stem cell and specific antigen CD56 of muscle precursor cell, which account for 5.12% and 10.34% from initial isolated cell group separately.<sup>11</sup> This suggests that various cell groups might exist. Therefore, the cell doubling time analyzed previously may be due to coexpression of different types of

cells. As the growing rate of stem cell is relatively slow and possess with high expression of CD34 antigen, highly expressed CD34 cell group can be purified from human adult skeletal muscle derived cells by Dynal CD34 Progenitor cell selection system. The growth doubling time test of this purified cell group is about 35 hours, it suggests that this cell group is more compatible with slow growing property of stem cell, because stem cell is usually in a static state when not being stimulated. The impurity of these cells are also justified by flow cytometry analysis<sup>24</sup>.

### Differentiation capability of human muscle derived stem cells

Laboratory data showed that different proportion of differentiate solution and different duration would successfully make the CD34+ hMDCs differentiate into skeletal muscle, adipocytes, osteocytes, and chondrocyte,<sup>12</sup> smooth muscle cells and neurogenic cells (Lu SH et al, unpublished data). The gene expressions at mRNA and protein levels have been determined by the use of RT-PCR, immunofluorescence, and western blot studies to justify the differentiation capability of these cells. These results proved that the multipotent stem cells have been successfully isolated from human muscle tissue.<sup>12</sup>

### Different kinds of stem cells

Using multipotent stem cell as the materials of tissue reconstructive engineering would be the future trend. Classified by the origin of the stem cells, there are two types of stem cells including embryonic stem cells and adult stem cells. In current cellular tissue regenerative medicine, the most common studies of adult stem cells involve bone marrow mesenchymal stem cells (MSCs), and hematopoietic stem cells. Using these cells, with addition of different growth factors, cytokines and culture medium's stimulus, these stem cells can be successfully differentiated into different required tissue, such as skeletal muscle cells, chondrocytes, adipocytes, smooth muscle cells, osteocytes, and neurons. Since these forms of stem cells belong to human adult stem cells, they contain no immunity and ethical dilemma that embryonic stem cells does. However, when obtaining these materials, it would usually accompany with high risk of invasive injury, low quality of purification, long duration of culture and differentiation, consequently, it would need to spend a high cost to obtain adequate quantity and quality of stem cells with clinical value. Therefore many scientists change to look for other homog-

enous origin of stem cells, such as adipose tissue,<sup>1</sup> amniotic fluid<sup>2</sup> or skeletal muscle<sup>3</sup> etc. In studies using rat models, it was found that stem cells originated from muscles have the ability to differentiate into skeletal muscle, osteocytes and chondrocytes,<sup>4-8,25-31</sup> or even differentiate into tissue of different organs, such as hematopoietic system.<sup>3</sup> Furthermore, stem cells originated from skeletal muscle can be used as vector in gene therapy and reveal its effects in many diseases treatment, such as duchenne muscular dystrophy (DMD), myocardial infarction (MI), and SUI etc.<sup>9,10,32</sup>

## CONCLUSIONS

Although there are only a few studies related to the human muscle-derived stem cells, these kind of stem cell have been successfully isolated and proven that the cells could be differentiated into myogenic, adipogenic, osteogenic, chondrogenic,<sup>11,12</sup> neurogenic cells, and smooth muscle cells (Lu SH et al, unpublished data). In earlier study, the mice muscle derived stem cell has been ever used and transplanted SIS and it was successfully differentiated into contractile muscle tissue which was more complianced tissue.<sup>13,14</sup> Next, to obtain the high differentiation rate, one has to increase the growth stability of these kinds of cells, and transform it into 3D scaffold. If the cells could be grown successfully in the scaffold and differentiated into different cellular tissue, in the future, the cell transplantation of tissue reconstructive engineering using human muscle derived stem cells would offer further benefits to people for urological regeneration.

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