

# *Stem cell implantation in treatment of peripheral vascular ischemia*

*By*

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## **Thesis**

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## **ABSTRACT**

There is recent evidence from clinical trials that implantation of stem/progenitor cells improve limb ischemia. In the present study implantation of autologous peripheral blood mononuclear cells (PBMNCs) mobilized by granulocyte-colony stimulating factor (G-CSF) - investigated in patients with chronic limb ischemia.

Twenty-four patients with chronic lower limb ischemia were enrolled and randomized (1:1) to either the implanted group or the control group. In the implanted group, the patients received subcutaneous injections of recombinant human G-CSF (300µg/day) for 5 days to mobilize stem/progenitor cells, and their PBMNCs were collected and implanted by multiple intramuscular injections into ischemic limbs while control group injected by sterile saline and receive medical treatment. All of the patients were followed up after at 12 week.

At the end of the follow-up period, the main manifestations were significantly improved in the patients of the implanted group compared to the control group. Mean of rest pain decreased from  $6.42 \pm 2.15$  to  $1.67 \pm 3.89$  ( $P < 0.001$ ). Mean of pain free walking distance increased from  $25 \pm 29$  to  $409 \pm 204$  ( $P < 0.001$ ). Mean ankle-brachial pressure index increased from  $0.45 \pm 0.32$  to  $0.79 \pm 0.38$  ( $P = 0.005$ ). A total of 7 of 9 limb ulcers and wounds (77.8%) of implanted patients healed after cell implantation. Two lower limb amputations occurred in the implanted patients. In contrast, eight control patients had to receive a lower limb amputation.

**Key Words:** G-CSF -Chronic limb ischemia-PBMNC-stem/progenitor cells.

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## **List of Abbreviations**

| <b>Abbreviation</b> | <b>The full term</b>                                         |
|---------------------|--------------------------------------------------------------|
| ABI                 | Ankle-brachial index                                         |
| AECs                | Amniotic epithelial cells                                    |
| BM                  | Bone marrow                                                  |
| BMMNCs              | Bone marrow mononuclear cells                                |
| BMP                 | Bone morphogenic protein                                     |
| BMSC                | Bone marrow stem cells                                       |
| C                   | Complement                                                   |
| CD                  | Cluster of differentiation                                   |
| CE                  | Corneal epithelial                                           |
| CLI                 | Critical limb ischemia                                       |
| CRP                 | C-reactive protein                                           |
| CSFs                | Colony stimulating factors                                   |
| CTA                 | Tomographic angiography                                      |
| DM                  | Diabetes mellitus                                            |
| DNA                 | Deoxyribonucleic acid                                        |
| DNMT3B              | DNA methyltransferase 3 beta                                 |
| EBAF                | Endometrial Bleeding-Associated Factor                       |
| ECM                 | Extracellular matrix                                         |
| ECs                 | Endothelial cells                                            |
| EGCs                | Embryonic germ cells                                         |
| eNOS                | Endothelial nitric oxide                                     |
| EPCs                | Endothelial progenitor cells                                 |
| ESC                 | Embryonic stem cells                                         |
| FGF                 | Fibroblast growth factor                                     |
| FITC                | Fluorescein iso thiocyanate                                  |
| Flk-1               | Fetal liver kinase                                           |
| FSCs                | Fetal stem cells                                             |
| G-CSF               | Granulocyte-colony stimulating factor                        |
| GFR                 | Glomerular filtration rate                                   |
| Hct                 | Hematocrit                                                   |
| HDAC                | Histone deacetylase                                          |
| HESC                | Human embryonic stem cell                                    |
| HGF                 | Hepatocyte growth factor                                     |
| HIF-1               | Hypoxia-inducible factor-1                                   |
| HMGB1               | High-mobility group box 1                                    |
| HSCs                | Hematopoietic stem cells                                     |
| HSPC                | Hematopoietic stem/progenitor cells                          |
| hTERT               | Human active subunit of the telomerase reverse Transcriptase |

|        |                                                  |
|--------|--------------------------------------------------|
| ICAM-1 | Intercellular adhesion molecule 1                |
| ICMs   | Inner cell masses                                |
| IFN    | Interferon                                       |
| Ig     | Immunoglobulin                                   |
| IGF    | Insulin-like growth factor                       |
| IL     | Interleukin                                      |
| iPSCs  | Induced pluripotent stem cells                   |
| mAbs   | Monoclonal antibodies                            |
| MAPC   | Multipotent adult progenitor cells               |
| MIF    | Macrophage migration inhibitory factor           |
| MMP-9  | Matrix metalloproteinase 9                       |
| MPCs   | Mesodermal progenitor cells                      |
| MRA    | Magnetic resonance angiography                   |
| mRNA   | Messenger RNA                                    |
| MSCs   | Mesenchymal Stem Cells                           |
| NO     | Nitric oxide                                     |
| Oct    | Octamer-binding protein                          |
| OI     | Osteogenesis imperfecta                          |
| OR     | Odds ratio                                       |
| PAD    | Peripheral arterial disease                      |
| PAOD   | Peripheral arterial occlusive disease            |
| PB     | Peripheral blood                                 |
| PBMNCs | Peripheral blood mononuclear cells               |
| PBS    | Phosphate buffered saline                        |
| PMT    | Photomultiplier tube                             |
| RBCs   | Red blood cells                                  |
| RNA    | Ribonucleic acid                                 |
| RPE    | R-phycoerythrin                                  |
| SC     | Stem cells                                       |
| SCID   | Severe combined immunodeficiency                 |
| SDF-1  | Stromal derived factor-1                         |
| SNS    | Sympathetic nervous system                       |
| SSEA   | Stage-specific embryonic antigen                 |
| TA     | Transit-amplifying                               |
| TACT   | Therapeutic Angiogenesis by Cell Transplantation |
| TASC   | TransAtlantic Inter-Society Consensus            |
| TNF    | Tumor necrosis factor                            |
| TRA    | Tumor-rejection antigen                          |
| UCB    | Umbilical Cord blood                             |
| UCE    | Umbilical cord epithelium                        |
| UKPDS  | United Kingdom Prospective Diabetes Study        |

|        |                                             |
|--------|---------------------------------------------|
| V      | Variables                                   |
| VEGF   | Vascular endothelial growth factor          |
| VEGFR2 | Vascular endothelial growth factor receptor |
| WBCs   | White blood cells                           |
| wt     | Wild-type                                   |

## **Introduction**

Peripheral arterial disease (PAD) is a serious condition that increases individual and population-based risk of heart attack, stroke, death, and amputation and decreases quality of life and functional independence (*Steffen et al., 2008*).

Despite considerable advances in the therapy of patients with peripheral arterial occlusive disease (PAOD) and critical limb ischemia (CLI), a substantial number remain, in whom amputation has to be considered the only and final option (*Lenk et al., 2005*).

Endothelial progenitor cells (EPCs) circulate in adult human peripheral blood and are mobilized from bone marrow by cytokines, growth factors, and ischemic conditions. Vascular injury is repaired by both angiogenesis and vasculogenesis mechanisms. Circulating EPCs contribute to repair of injured blood vessels mainly via a vasculogenesis mechanism (*Murasawa and Asahara, 2005*).

Therapeutic angiogenesis in patients with lower limb ischemic disease was studied by many researchers. Researchers have tried to overcome limitations of the natural angiogenic response by substantially increasing the local concentrations of angiogenic growth factors either by administering recombinant protein for the gene that codes for an angiogenic growth factor or by administering endothelial progenitor cells (EPCs) that will synthesize a cocktail of growth factors in the vicinity of new vessel formation . The EPCs were harvested from peripheral blood, autologous bone marrow, and human umbilical cord blood (*Kim et al., 2006*).

## **Aim of the work**

To assess the application of implantation of autologous granulocyte colony–stimulating factor (G-CSF)–mobilized peripheral blood mononuclear cells (PBMNCs) in the treatment of chronic limb ischemia of patient not eligible for arterial reconstruction or endovascular procedures and to evaluate the safety, efficacy, and feasibility of this novel therapeutic approach.

## Stem cells

Human life begins as single fertilized cell. The journey from single cell to complex being is attributable to the role of the stem cells (SC) (i.e. cells that produce all different types of cells and tissues that make up the Human body) (*Semsarian, 2002*).

In general, embryonic, fetal, and adult stem cells show several common functional properties. Common properties include their high self-renewal capacity and potential to generate differentiated cell progenitors of different lineages under simplified culture conditions in vitro and after transplantation in the host in vivo (*Mimeault and Batra, 2006*).

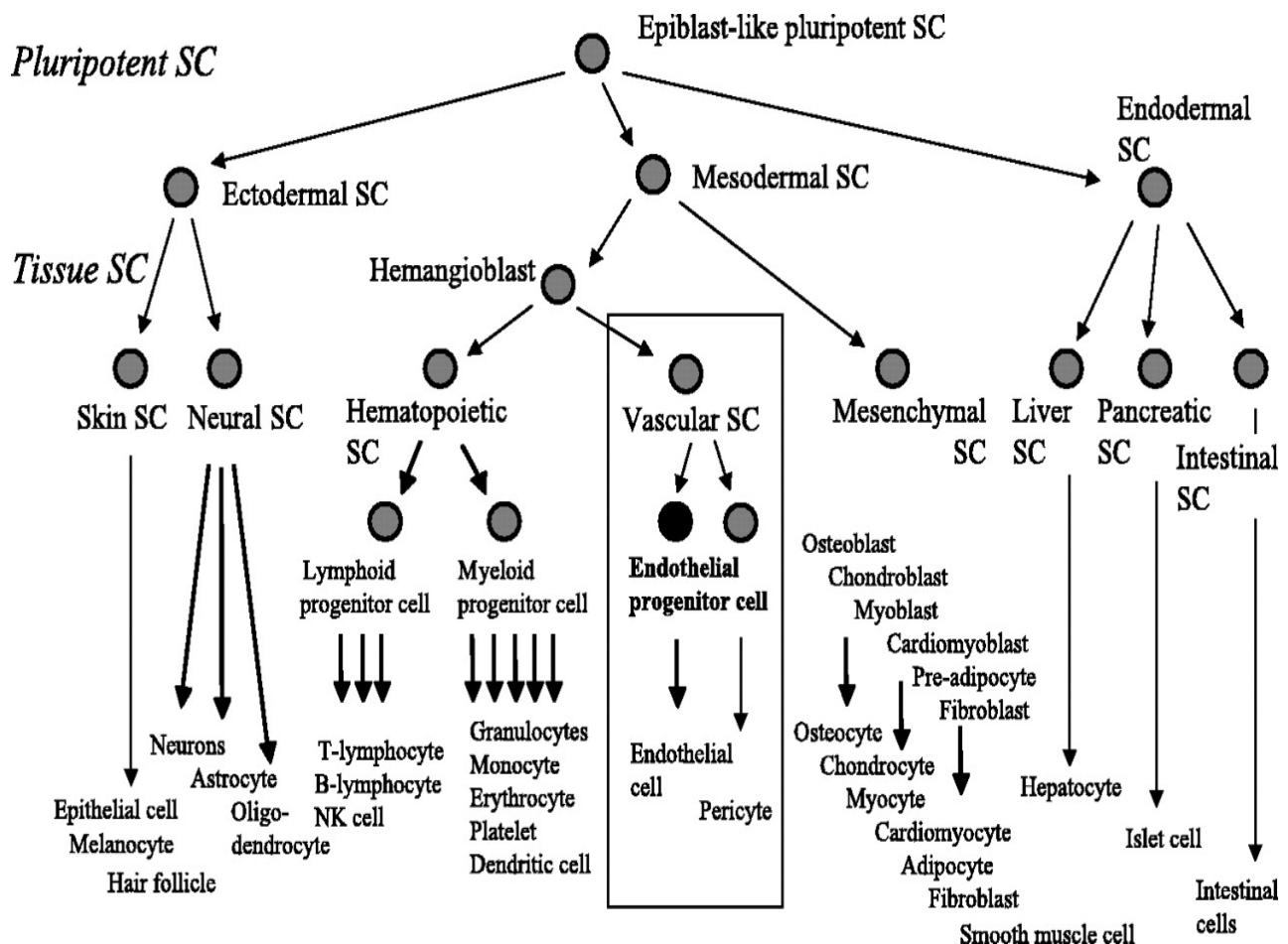
More particularly, the establishment of the functional properties of stem cells and their progenitors in vitro and in vivo has indicated that they may contribute to the regeneration of damaged tissues. Therefore, the use of stem cells and their progenitors is a promising strategy in cellular and genetic therapies for multiple degenerative disorders, as well as adjuvant immunotherapy for diverse aggressive cancer types (*Leri et al., 2005*).

Parkinson and Alzheimer diseases, muscular degenerative disorders, chronic liver and heart failures, and type 1 and 2 diabetes, as well as skin, eye, kidney, and hematopoietic disorders, could be treated by the stem cell-based therapies (Table 1) (*Mimeault and Batra, 2006*).

**Potency definitions:** Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell (Fig.1). A totipotent stem cell (e.g. fertilized egg) can develop into all cell types including the embryonic membranes. A pluripotent stem cell can

develop into cells from all three germinal layers (e.g. cells from the inner cell mass). Other cells can be Multipotent, oligopotential, or unipotent depending on their ability to develop into many, few or one other cell type(s) (*Sell, 2004*).

Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells). (*Lantz and Huff, 1995*).



(Fig.1): stem and progenitor cells. SC, stem cell. (*Asahara and Kawamoto 2004*).