

# Application of CD34<sup>+</sup> Human Peripheral Blood Stem Cell is Able to Increase Fibroblast and Collagen in Skin of a Male Ultraviolet B Exposed Rat

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**Abstract:** *The decrease of fibroblast cells and collagen is one of the symptoms of skin degeneration caused by ultraviolet exposure. The aim of this research is to find out the effect of CD34<sup>+</sup> Human Peripheral Blood Stem Cell (HPBSC) application subcutaneously towards the amount of fibroblast cells and collagen on a male ultraviolet B exposed Wistar rat. This is an experimental research using Randomized Posttest Only Control Group Design method. Two groups of male wistar rats, each consisting 18 rats were exposed to ultraviolet ray for 4 weeks. The first group was given subcutaneous injection of Phosphate Buffered Saline (PBS) solution, meanwhile the second group was given CD34<sup>+</sup> HPBSC subcutaneously. After 4 weeks, a histopathology – anatomy slide of the rat skin was observed to find out the amount of fibroblast cell and collagen. The result came out to be finding regeneration of fibroblast cell and collagen. Independent t test on the amount of fibroblast is 5,53; p= 0,001 and on amount of collagen is 7,05; p= 0,001. The application of CD34<sup>+</sup> HPBSC subcutaneously is able to increase the amount of fibroblast cell and collagen on male wistar rat's skin which has been exposed by ultraviolet B ray. Clinical tests still has to be carried out before applying this to human skin.*

**Keywords:** CD34<sup>+</sup>, HPBSC, Fibroblast, Collagen, Skin Regeneration

## 1. Introduction

Ultraviolet ray (UV) until now is still considered as one of the factors that plays a big part in skin premature aging [1]. The damage of skin because of UV-B on the dermis of skin has a meaning of the decrease of fibroblast cell and amount of collagen [2].

Fibroblast is a cell that forms the connective tissue of the skin. Fibroblast has the ability to repair damaged tissue and to increase the amount if scar were formed. If damages were done to the dermis because of UV exposure, then the amount of collagen will decrease. Clinical signs would be like wrinkly skin, expression lines and loose skin. Collagen is a protein that is very unstable and there are many factors that are able to affect it in its process of forming as well as degradation [3,4].

The new treatment that is very promising is skin tissue engineering with the use of stem cell. Stem cell is a cell that has the ability to take shape and form into body tissues. Stem cell is the beginning cell of life that is able to develop into other cells and to form other tissues in the body (*multipotent*). The characteristics of stem cell is *Undifferentiated*, able to multiply itself (*self renewal*), and able to differentiate to become more than one type of cell (*Multipotent/Pluripotent*) [5].

Mesenchymal stem cell is a potential source for tissue engineering but its application is constrained by its extraction and characteristics [6]. Its characteristics such as amount, age, proliferation and differentiation decreased as the age of the person increases [7]. Other drawback is that it requires culture, delivery, differentiation initiator, as well as its regeneration has a risk of resulting in tissue forming and integration [6].

Khan suggests that there is a need for alternative whereby the extraction is simple, its complication is at its lowest, high cell concentration, proliferation and differentiation is well without being affected by the age of the person. According to Terayama (2011) that alternative is Hematopoietic stem cell, which is CD34<sup>+</sup> stem cell [8].

Hematopoietic stem cell has a *plastic* character, which means it is able to form other cells within the same offspring line, which can be explained through various mechanisms, such as double offspring model, somatic model, transdifferentiation model and dedifferentiation redifferentiation model [9].

Transdifferentiation is a mechanism of change and forming of cells that are different from its offspring line. Tissue stem cell transdifferentiates according to where it belongs. Bone marrow stem cell or stem cell belonging to the blood flow forms cell and not blood. Bone marrow stem cell, hematopoietic and mesenchymal are able to move towards

specific tissue and take form into the cell of the tissue. It happens through transdifferentiation, fusion or inflammation signal [10].

The cell's characteristics makes hematopoietic stem cell able to form heart cells, hepar, pancreas, skin, muscle and bone. The mechanism is through transdifferentiation model or fusion. To prove the plastic property, so stem cell has to be identified in the beginning of isolation and when it becomes a new cell. The cell has to be proven that it is able to integrate and fusion as well as exert protein that is according to the new tissue [11].

CD34<sup>+</sup> cell is a hematopoietic stem cell which is positive towards the CD34 cell marker. CD34 is the best hematopoietic stem cell marker. CD34 antigen is able to be found in pluripotent blood stem cell, unipotent myeloid cell, endothel of blood vessels, nerve membrane structure, and human skin follicular cell [12].

CD34<sup>+</sup> cell character gives plasticity research chance, which is the change of hematopoietic stem cell to become non hematopoietic stem cell [13].

The objectives of this study are to investigate the degenerative rat skin on fibroblast using the hematopoietic stem cell CD34<sup>+</sup> and to investigate the degenerative rat skin on collagen using the hematopoietic stem cell CD34<sup>+</sup>

## 2. Methods

This research is *Randomized Posttest Only Control Group Design* (Gliner, 2000). There are 2 groups, consisting of 18 Wistar rats each.

The sample that is selected is divided into 2 groups, which are control group whereby 18 rats were exposed to UVB and given subcutaneous injection of PBS while the other group of 18 rats were exposed to UVB and given subcutaneous injection of CD34<sup>+</sup> stem cell.

The independent variable in this research is suspension of human peripheral blood CD34<sup>+</sup> stem cell while the dependent variable are amount of fibroblast with *Picro-Sirius Red* and *Hematoxylin* and amount of collagen with *Sirius Red* staining.

The operational variable definition are :

- 1) CD34<sup>+</sup> stem cell is a hematopoietic stem cell that is positive towards CD34<sup>+</sup> cell marker.
- 2) PBS (*Phosphate Buffered Saline*) is a saline buffer solution in which per 1 litre consists of 800ml of distilled water, 8g NaCl, 0,2g KCl, 1,44g Na<sub>2</sub>HPO<sub>4</sub>, 0,24g KH<sub>2</sub>PO<sub>4</sub>, with pH 7,4.

- 3) Number of fibroblast cell is the number of cells that is specifically stained with *Picro-Sirius Red* and *Hematoxylin*. *Image Raster* software is used to count the number of fibroblasts.
- 4) Amount of collagen are the collagen that are specifically stained with *Sirius Red*, counted quantitatively with software Adobe Photoshop 3.0 image JPEG.
- 5) Injection of PBS by injecting PBS 0,5 cc into a insulin syringe subcutaneously into the back of the rat.
- 6) Injection of CD34<sup>+</sup> suspension to PBS 0,5 cc in an insulin syringe subcutaneously into the back of the rat.
- 7) UVB exposure is done by exposing it to the back of the rat which has been shaved, using UV Bioditech for 4 weeks:  
Week 1 : 3 times per week at 50mJ/cm<sup>2</sup>  
Week 2 : 3 times per week at 70 mJ/cm<sup>2</sup>  
Week 3 : 3 times per week at 80 mJ/cm<sup>2</sup>  
Week 4 : 3 times per week at 80 mJ/cm<sup>2</sup>
- 8) Rat skin that has been shaved off with gilette, which is then exposed to UVB for 4 weeks with dosage of 840 mJ/cm<sup>2</sup>.

## 3. Results

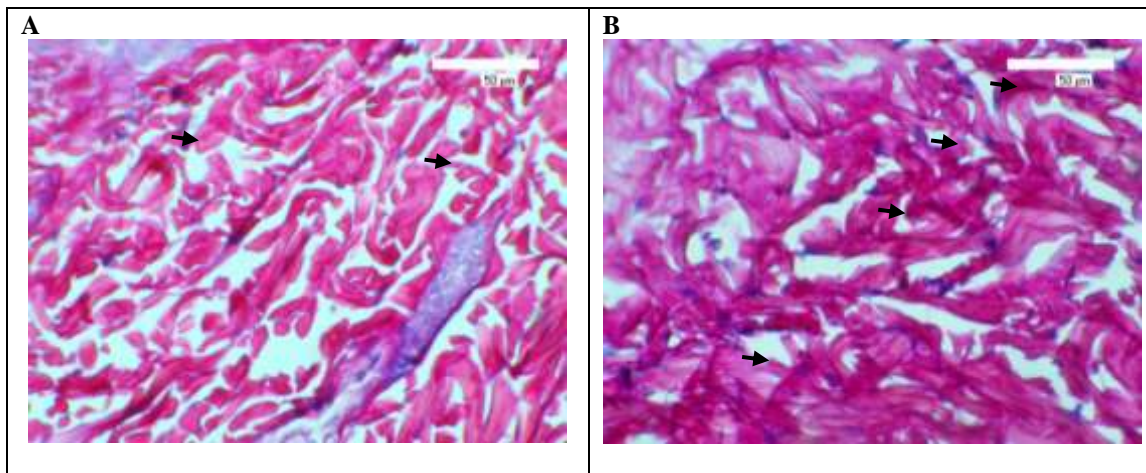
Data normality test: Fibroblast and collagen data after treatment is tested of its normality using the test of *Shapiro-Wilk*. The result shows that the data is normally distributed ( $p > 0,05$ )

Data homogeneity test: Amount of fibroblast cell and collagen after treatment is tested of its homogeneity by using *Levene's Test*. The result shows that the data is homogeneity ( $p > 0,05$ )

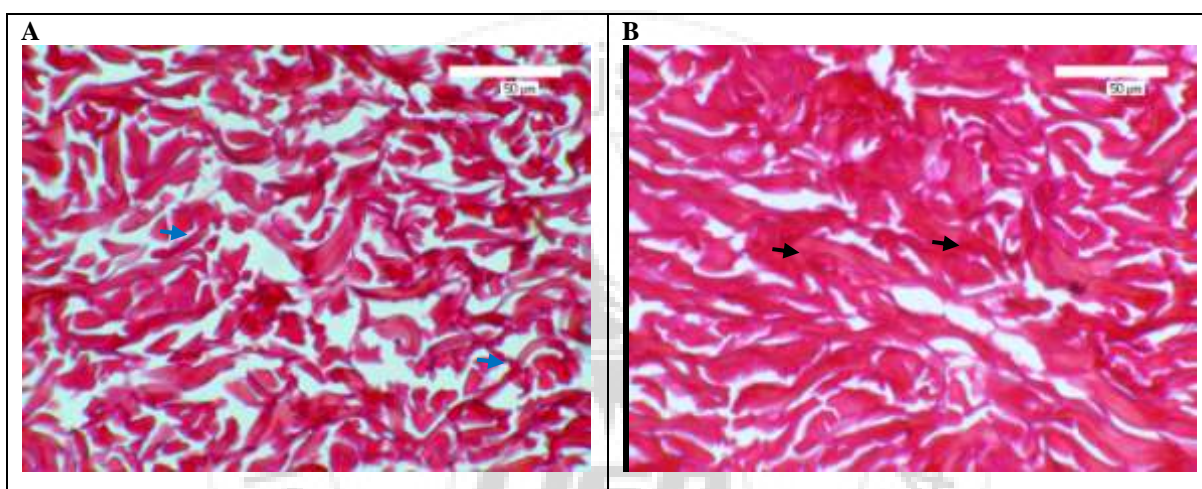
It is showed that the average number of fibroblast of the control group is  $9,67 \pm 2,81$  and average of the treated group is  $16,28 \pm 4,23$ . The analysis with the use of *t-independent* showed that the value of  $t = 5,53$  and value of  $p = 0,01$ . This shows that the average number of fibroblast cell in both groups after treated are different significantly.

It is showed that the average amount of collagen in the control group is  $70,76 \pm 6,15$  and the average in the treated group is  $83,96 \pm 5,03$ . The analysis with the use of *t-independent* test showed that the value of  $t = 7,05$  and value of  $p = 0,01$ . This means that the average amount of collagens in both groups are different significantly ( $p < 0,05$ ).

Histopathology result amount of fibroblast and collagen on dermis tissue before and after treatment (figure 1 and figure 2).



**Figure 1:** Amount of fibroblast cell on dermis tissue of male wistar rat with HE staining; A: UV Group + Placebo. Shows amount of fibroblast cell with decreased blue coloured nucleus. The black arrow shows fibroblast cell; B: UV Group + CD34. Shows amount of fibroblast cell with increased blue coloured nucleus. The black arrow shows fibroblast cell.



**Figure 2:** Amount of collagen on dermis tissue of male wistar rat with Picro-Sirius red staining; A: UV Group + Placebo. Structure of damage is observed as well as collagen structure with collagen fiber red coloured that looks thin. Blue arrow shows collagen fibre which is not whole; B: UV Group + CD34. Amount of collagen with red coloured collagen fibre looks bigger and thicker. Arrow shows a whole collagen fibre.

#### 4. Discussion

Based on the research above, it is found that on the treated group, there is an increase of fibroblast cell by 68,39% and increase of collagen by 18,66% as compared to the control group. This occurred because of the transdifferentiation mechanism as well as fibroblast and collagen regeneration.

Transdifferentiation is a mechanism of change and forming of cell that is different from its offspring line. Tissue stem cell differentiates according to where it belongs. Bone marrow stem cell or stem cell in the blood flow forms cell not blood. Bone marrow stem cell hematopoietic and mesenchymal is able to move to specific tissue and transforms into the cell in the tissue. This happens because of transdifferentiation mechanism, fusion or inflammation signal [10]. Regeneration of fibroblast cell and collagen is determined by CD34<sup>+</sup> stem cell, a micro scaffolding and signalling molecule tissue. Whereas the most dominant molecule signal are keratinocytes stem cells (KSCs) and cytokeratine (CK5/14/15), p63, α6β4<sup>-</sup> and α3β1-integrins and transport ATP-binding cassette (ABC). KSCs holds a play in

continuity of keratin cell regeneration in epidermis, be it in normal case or post trauma from UV ray exposure [14]. The complex relationship is shown by EGFR molecule signal, Notch, Insulin-like Growth Factor (IGF-1)/IGF-R1, immunoglobulin-like domain 1 (Lrig1), Myc, Transforming Growth Factor - β (TGF-β) and Polycomb-group protein BMI-1. All of those molecule signal strengthens the role of KSCs in fibroblast and collagen regeneration [14].

#### 5. Conclusion

Application of HPBSC CD34<sup>+</sup> stem cell subcutaneously increases the fibroblast and collagen on a male wistar rat that has been exposed to UVB.

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