

An Evaluation of the Effect of Polycaprolactone/Gelatin (Pcl/Gt) Nanofiber Scaffold on the Therapeutic Function of Hematopoietic Stem Cells

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J Babol Univ Med Sci; 20(10); Oct 2018; PP: 7-13

Received: Feb 19th 2018, Revised: May 10th 2018, Accepted: Jun 11th 2018.

ABSTRACT

BACKGROUND AND OBJECTIVE: Hematopoietic stem cells are one of the new therapeutic options for treating skin ulcers. Recently, a new perspective has been developed to better utilize stem cells using nanofiber scaffolds. The present study was conducted to investigate the effect of polycaprolactone/gelatin (PCL/GT) nanofiber scaffold on the therapeutic function of hematopoietic stem cells.

METHODS: In this experimental study, 15 male BALB / c mice were divided into three groups of five, including the control group, the group receiving stem cells in the wound site (cell group) and the group receiving cell + PCL nanofiber scaffold. PCL/GT nanofiber scaffold was prepared by electrotherapy. After hematoxylin and eosin staining, the parameters of epidermal repair and hair follicle formation in the wound site were evaluated by fluorescence microscope and Image J and SPSS programs.

FINDINGS: On day 28 after transplantation, the highest and lowest epidermal thicknesses were observed in the cell + scaffold group and control group, which were 10.5 ± 0.3 and 27.3 ± 0.9 μm , respectively, which was significant ($p < 0.05$). Moreover, the highest and lowest number of hair follicles were observed in the cell + scaffold group and control group, respectively; 5.2 ± 0.2 and 4.2 ± 0.3 . The difference between the two groups was significant ($p < 0.01$)

CONCLUSION: Polycaprolactone / gelatin (PCL/GT) nanofiber scaffold significantly increases the therapeutic function of hematopoietic stem cells in the wound site.

KEY WORDS: Hematopoietic Stem Cells, Nanofiber, Wound Healing.

Please cite this article as follows:

Zafari F, Moghanloo E, Sadeghi M, Khafaei M, Bakhtiyari M, Teimourian Sh. An Evaluation of the Effect of Polycaprolactone/Gelatin (Pcl/Gt) Nanofiber Scaffold on the Therapeutic Function of Hematopoietic Stem Cells. J Babol Univ Med Sci. 2018;20(10):7-13.

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Introduction

One of the most important limitation of the therapeutic use of stem cells is the lack of control over the differentiation of these cells into specific tissues, and this limitation is associated with the physical difference between the external environment and the internal environment of the body. In body tissue, the natural 3D environment creates a network of nanoscale fibers that makes contact between cells and, on the other hand, facilitates the access of cells to oxygen, hormones and food. To overcome this problem, tissue engineering has been used in recent years to use artificial scaffolds to simulate a natural 3D environment within tissues (1, 2). The tissue engineering framework is the combination of cells with a natural or artificial matrix structure to create a live 3D structure similar to the natural environment inside the target tissue (3, 4). In tissue engineering, four main parameters are considered in order to achieve the proper structure and function: 3D scaffold, cell growth factors, extracellular matrix and cell type (5, 6). Among these factors, 3D scaffold plays the role of growth organization and the differentiation and protection of cells and the material and structure of the scaffold plays an important role in this. By imitating and simulating the extracellular matrix, scaffolds provides a suitable environment for various cellular functions such as adhesion, migration, enhancement and differentiation (7). Polycaprolactone nanofiber scaffolds are biodegradable and biocompatible amphiphilic polyesters that are used for tissue engineering due to their lower toxicity to cells (8 – 11). It has been reported in several studies that stem cell culture on an artificial 3D scaffold leads to better differentiation and longevity of these cells due to the structure and material of the scaffold (12, 13). Recent reports indicate that hematopoietic stem cells exist in some tissues of the adults, including bone marrow, spleen, and blood (14, 15). On the other hand, these cells have the ability to differentiate into epithelial cells during wound healing and, with a significant increase in the production of collagen matrix at the site of the wound, induce angiogenesis and improve blood supply, which results in early closure of the wound and improvement of the treatment process (16, 17).

The CD93 stem cells are a new subgroup of hematopoietic stem cells that can be isolated from the blood and bone marrow of adults, and its ability of conversion to nerve cells and skin cells has recently been reported (18, 19). Considering the possibility of purification of CD93 cells from the blood of adults and the role of nano-scaffolds in improving the therapeutic function of stem cells, conducting a study to evaluate the effect of nano-scaffolds on the therapeutic function of these cells seems necessary. Therefore, the present study was performed to evaluate the effect of PCL / GT nanofiber scaffold in the therapeutic function of CD93 stem cell cultures on the skin ulcers in the mice.

Materials and methods

This experimental study was conducted after approval by the Ethics Committee of Qazvin University of Medical Sciences with the code of ethics IR.BMSU.RBC.177. The CD93 stem cells were previously isolated by the researchers from the red bone marrow of the mice and confirmed by the FACS SORTER device (20). StemSpan™ Serum-Free Expansion Medium (SFEM) was used to culture the cells and after culturing, cells were kept in incubator at 37 °C with 5% CO₂.

Polycaprolactone (PCL) nanofiber scaffold: Polycaprolactone nanofiber scaffold was prepared by electrospinning at a velocity of 0.5 ml / h and a voltage of 25 kV. After the construction of the scaffold, reactive oxygen species were induced on the surface of the polymers by Plasma Generator (3230, Germany) by plasma treatment method to increase the adhesion and growth of the cells. At the final stage, the diameter of the constructed scaffold fibers was measured by electron microscope (SEM S-416 Hitachi, Japan) and the scaffolds were sterilized for 45 minutes under UV exposure (Image 1).

Preparation of experimental animal: In this study, 15 adult mice weighing 25-30 g were divided into three groups of five, including Group I: the control group, Group II: the mice receiving HSC on the wound site (2×10^6 cells), Group III: mice transplanted with Cell + PCL nanofiber scaffold. After anesthesia, the hair of the

animals' back was shaved and after washing with betadine, a piece of skin was separated from the underlying layer in a circular form by a punch with a diameter of six millimeters and a depth of two millimeters with full thickness and after transplantation, the wound site was dressed. The mice were then transferred to animal propagation and care center and kept in standard and similar conditions.

Hematoxylin Eosin (H&E) staining and Immunohistochemistry (IHC): To observe and evaluate the connective tissue cells to measure the thickness of the epidermis, the number of hair follicles and the number of blood vessels, Hematoxylin Eosin (H&E) staining (Merck, Germany) was performed according to the standard protocol (21). In order to verify the presence of implanted CD93 cells in the wound site, the immunohistochemistry test was performed according to the standard protocols of Sigma Company. After the preparation of tissues, the presence of marked CD93 cells in the tissue of the restoration site was directly confirmed by the fluorescence microscope (Olympus BH2-RFL-T3) and OLYSIA Bio Report Soft Imaging System Software. Data were analyzed by t-test and $p < 0.05$ was considered significant.

Results

Survival Rate of CD93 Stem Cells in the Wound Site:

Fluorescence microscope images of examining and detecting CD93 hematopoietic stem cells on days 7, 14, and 28 after cell transplantation indicated the presence and survival of a balanced population of over 50% of CD93 stem cells in the wound site and these cells were observed at the wound site until 28 days after transplantation.

Epidermal thickness measurement: On day 14 after transplantation, the thickness of the epidermis in the control group, the cell group and the cell + scaffold group were 8.3 ± 0.2 , 13.8 ± 0.6 , and 14.2 ± 0.3 μm , respectively, and the difference in control and cell + scaffold groups was significant ($p < 0.05$). On day 28, despite an increase in the thickness of the epidermis in all groups, the highest epidermis levels were observed in the cell group and the cell + scaffold group, which

was 26.06 ± 0.5 and 27.03 ± 0.9 μm , respectively. Among all the groups, the highest epidermis thickness was related to the cell + scaffold group (27.03 μm) (Image 2, Fig. 1 and 2).

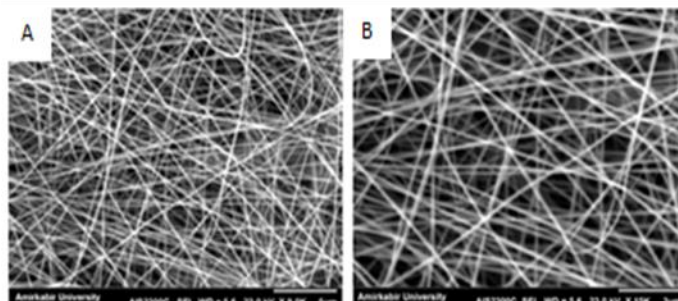


Image 1. The image of PCL / GT nanofiber scaffold by electrospinning, imaging by scanning electron microscope (S-416 FESEM model) at 15 kV voltage.

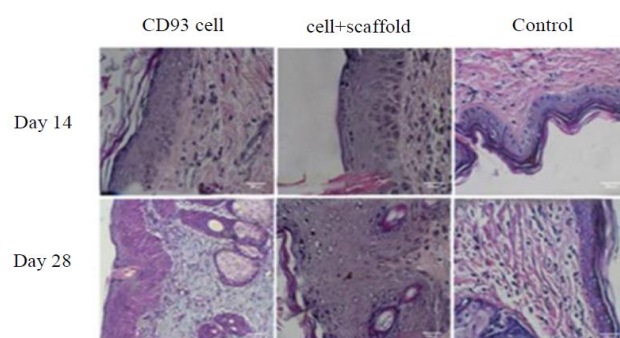


Image 2. Assessment of the epidermis and stratum corneum restoration by H & E staining in the study groups. The proper and complete epidermal formation in the cell + scaffold group on day 28 can be observed.

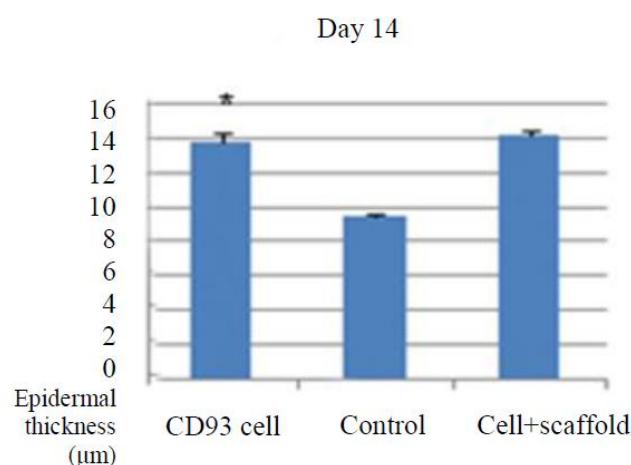


Figure 1. Epidermal thickness measurement in the study groups on day 14 after wound creation

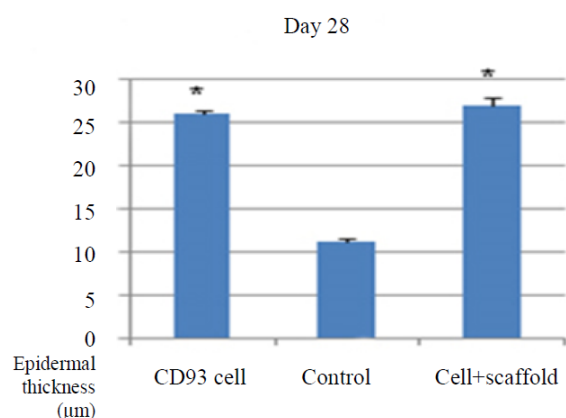


Figure 2. Epidermal thickness measurement in the study groups on day 28 after wound creation

Evaluating the number of hair follicles: In the analysis of stained slides by Masson's trichrome, hair follicles were visible from the second week in all samples. On day 14, the number of hair follicles in control group, cell group and cell + scaffold group was 3 ± 0.7 , 3.6 ± 0.4 and 4.2 ± 0.3 , respectively. On day 28, the number of hair follicles in control group, cell group and cell + scaffold group was 4.2 ± 0.3 , 4.05 ± 0.6 , and 5.2 ± 0.2 , respectively, while the highest number of hair follicles was in the cell + scaffold group on day 28 (Table 1).

Table 1. The number of hair follicles in the study groups on day 14 and day 28 after cell transplantation

Group	Number of hair follicles Mean±SD	Time (day)	P-value
Control	0±3.7	14	0.07
Cell	3.0±6.4	14	
Cell+scaffold	4.0±2.3	14	
Control	4.3±2	28	0.037
Cell	4.0±05.6	28	
Cell+scaffold	5.0±2.2	28	

Discussion and conclusion

In this study, the restoration efficacy of CD93 stem cells increased in the presence of PCL/GT nanofiber scaffold. Among the three groups of the studied mice, the highest epidermal thickness was related to the group in which CD93 stem cells were grown on the PCL/GT

nanofiber scaffold as basic dressing. Ghoroghi et al. reported that stem cell survival increases on the PCL / GT nanofiber scaffold, and this may be due to the thickness and material of polycaprolactone nanofibers. It seems that the polycaprolactone nanofibers constructing this scaffold lead to proper adhesion and growth of the cells on the scaffold by creating suitable 3D structure similar to the 3D environment of the body tissues, in addition to increasing the surface – area – to – volume ratio with their balanced hydrophilic properties (22).

In examining the key parameters of wound restoration, including the thickness of the epidermis and the number of hair follicles, we observed a significant increase in the cell + scaffold group compared to the group that only received CD93 cells (cell group) and the control group. All these findings are consistent with the results of Ghoroghi et al. In another study, Ferreira et al. succeeded in the growth and proliferation of hematopoietic stem cells with the support of stromal cells on a 3D polycaprolactone scaffold. The study of this team showed that the amount of cell proliferation and their attachment to the PCL nano-scaffold at the wound site is much more efficient than other scaffolds (23). Another study by Wang et al. showed that the use of cellular scaffolds along with MSC stem cells significantly increased wound surface closure and the formation of wound layers. These findings are consistent with the findings of the present study (24). One of the requirements for successful tissue restoration and better performance of restorative cells on artificial environments such as scaffolds is the lack of sensitivity of cells to these scaffolds and the lack of toxicity to cells. According to studies, polycaprolactone / gelatin scaffolds are biodegradable and biocompatible and no toxic effects on the cell have been reported so far and this provides an ideal environment for the growth of stem cells (25, 26). The nano-scaffold used in this study was prepared by a high-velocity electrospinning. Our preliminary evaluations in this study showed the survival of appropriate cell population until 28 days after transplantation on the polycaprolactone scaffold in the wound site. Several studies reported that nano-scaffolds made with this method have very tiny pores

and the cells cannot penetrate into these pores, and on the other hand, these nano-fibers increase the surface-area-to-volume ratio and help growth factors reach their targets (27–29). One of the effective factors in overcoming the challenges of extracellular matrix culture system is to optimize the structure and material of scaffolds for proliferation and differentiation of stem cells in each tissue. In this regard, the use of scaffolds with a suitable material and structure without toxic effects on cells are of significant importance. In fact, the more similarity between scaffolds and the extracellular matrix, the better cell growth. In a study, Yoshimoto claimed that among the various types of artificial scaffolds, PCL / GT nanofiber scaffold can be considered as a harmless pharmaceutical product for the cells, and high compatibility of human body with this scaffold is due to non-toxicity, biocompatibility and

appropriate diameter of fibers (30). According to the findings of this study, the use of PCL/GT nanofiber scaffolds as a 3D basis for stem cell culture increases the restorative efficacy of these cells in the wound site. The scaffold does not have any adverse effect on the survival of the CD93 stem cells in the wound site. These findings promise the possibility of complete treatment of wounds and even full hair growth in the site of wound through simultaneous use of PCL/GT nanofiber scaffolds and stem cells in near future.

Acknowledgment

Hereby, we would like to thank the Deputy of Research and Technology of Iran University of Medical Sciences for supporting this study and thank Ms. Ziba Malekshahi for collaborating with the studies on mice.

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