Enhancing Diabetic Foot Ulcer Healing with Cardiac Stem Cell Sheets

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Enhancing Diabetic Foot Ulcer Healing with Cardiac Stem Cell Sheets

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Seo In Lee

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This certifies that the Master's Thesis of Seo In Lee is approved.

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ABSTRACT

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A diabetic foot ulcer (DFU), a significant complication of diabetes, is characterized as a full-thickness wound occurring below the ankle in individuals with diabetes. Present treatment protocols for DFU recommend debridement, infection control, revascularization, and pressure off-loading to promote healing. However, complications like ischemia, infection, neuropathy, and metabolic conditions frequently impede the healing process, posing a major challenge for both patients and healthcare professionals. Stem cells secrete various cytokines that promote cell recruitment, modulate the immune response, remodel the extracellular matrix (ECM), support angiogenesis, and aid in neuro-regeneration, all of which play crucial roles in the healing of wounds. This study reports the efficacy of cell sheets created using an encapsulation method utilizing cardiac stem cells (CSCs), which possess unique abilities to promote angiogenesis, immune regulation, anti-inflammation, and ECM formation. Additionally, the CSCs-based cell sheets promote wound healing by enhancing oxygen and nutrient delivery to the damaged tissue, thereby aiding in blood vessel formation. Unlike commonly known detachment techniques such as thermo-responsive, electro-responsive, pH-responsive, and magnetic-induced methods, we employ the encapsulation method. In this approach, cells are cultured on PET(polyethylene terephthalate) films to create cell sheets, which are then treated with fibrinogen and thrombin to form a gel. Incubation at 37°C for 30 minutes transfers the cell sheet into the fibrin gel. This process is repeated to achieve layering, and the effects of the layered cell sheets are evaluated through various in vitro methods.

Key words : Diabetic foot ulcer, Cardiac stem cells, Cell sheet, Cytokines

1. Introduction

1.1. Diabetic foot ulcers

A DFU is a severe complication associated with diabetes. The Global Diabetes Foot Care Consortium defines it as a deep wound occurring below the ankle in individuals with a diabetes diagnosis. It is often linked to diabetic neuropathy and peripheral arterial disease.[1] Beyond pain, infection, amputation, and reduced mobility, DFUs can lead to significant economic, social, and psychological consequences. Every 30 seconds, a diabetes-related amputation occurs, and 84% of these cases are linked to a preceding diabetic foot ulcer (DFU).[2, 3] The current recommendations for treating diabetic foot ulcers (DFUs) include debridement, controlling infections, restoring blood flow, and relieving pressure on the affected area to promote healing. However, factors such as oxygen deprivation, microbial contamination, Nerve damage, and Metabolic imbalances often hinder wound healing, presenting a notable challenge for both patients and healthcare providers.[4] During the process of wound healing, stem cells secrete cytokines that activate cell recruitment, regulate the immune system, reshape tissues, stimulate blood vessel formation, and promote neural repair.[5]

1.2. Cell-based therapy

Cell therapy marks the latest advancement in the biotechnology revolution within the medical profession.[6] Cell-based therapy, which taps into the restorative potential of stem cells from organs, Vascular fluid, and Marrow tissue, has become a highly promising strategy to treat various tissue or organ failures by directly injecting a dissociated cell suspension.[7] The primary focus of regenerative medicine is tissue regeneration and cellular replacement, which is pursued through the use of various types of stem cells.[8] CSCs secrete paracrine mediators that promote angiogenesis, exhibit anti-inflammatory effects, and enhance ECM production. Therefore, they have the potential to regenerate ischemic wounds associated with peripheral vascular diseases, such as diabetic ulcers.

1.3. Cell sheet technology

Recently, a novel approach has been introduced to eliminate the need for biodegradable scaffolds. Cell sheet engineering, an innovative approach in tissue engineering, was first developed by Yamato and Okano.[9, 10] This technology is emerging as an innovative strategy for cell-based therapy. In many instances, the injected single-cell suspensions fail to remain near the target tissue, making it challenging to regulate the size, shape, and positioning of the inserted cells. The main benefit of cell sheet technology lies in its capacity to preserve cell-cell connections and the ECM while achieving high cell density. Okano's group has shown that cell sheet transplantation is superior to cell injections for cardiac repair.[11] Tissue engineering has been utilized for various applications, including the epidermis, bone, cartilage, blood vessels, and heart valves.[12] Cell sheet technology has been applied in initial human clinical studies across seven fields: the cardiovascular system, corneal tissue, digestive tract, gum tissue, ear reconstruction, joint cartilage, and lung tissue regeneration.[13]

1.4. Cell sheet detachment method

The cell sheet harvesting method enables the transfer of cell sheets for diverse applications in tissue engineering. While most current studies utilize thermo-responsive techniques for detaching cell sheets, other innovative methods have shown promise as well. These include electro-responsive, pH-responsive, and magnetic-induced approaches for harvesting cell sheets.[14] In this study, cell sheets were harvested using an encapsulation method. Cells are commonly encapsulated in hydrogels by combining a cell suspension with hydrogel precursors, followed by the crosslinking of the resulting network. A technique involves forming a fibrin gel by combining fibrinogen and thrombin on the surface of the cell sheet film, enabling the detachment of the sheet along with the ECM.[15] Fibrin is a viscoelastic polymer with properties that combine both elasticity and viscosity. During the polymerization process, finite clot stiffness is initially observed when the network forms at the gel point, and this stiffness continues to increase over time.[16]

1.5. Objective of this study

In this study, the development of cell sheets using cardiac stem cells aims to treat diabetic foot ulcers, a significant complication of diabetes. Current drug therapies for these ulcers often exhibit limited efficacy compared to conventional treatment methods, and healing rates are particularly slow. The development of new blood vessels, known as angiogenesis, is essential for processes like organ growth, embryonic development, and the healing of wounds. These newly formed blood vessels, which are lined with endothelial cells, provide oxygen and nutrients to tissues, enhance immune surveillance by supporting hematopoietic cells, and facilitate the removal of waste products.[17] Thus, inducing angiogenesis to enhance blood circulation at the wound site is crucial. Cardiac stem cells have the unique ability to promote angiogenesis, immune regulation, anti-inflammation, and the formation of extracellular matrix. This research focuses on creating cell sheets enriched with extracellular matrix and growth factors derived from these cells. An encapsulation method is being explored for detaching the cell sheets, in contrast to traditional thermo-responsive methods that require specialized temperature-sensitive plates. This approach seeks to provide a simpler and more accessible technique for producing cell sheets, even in environments lacking such equipment. The effectiveness of both stacked and non-stacked cell sheets will be evaluated to determine whether the increased density of stacked sheets enhances therapeutic outcomes.

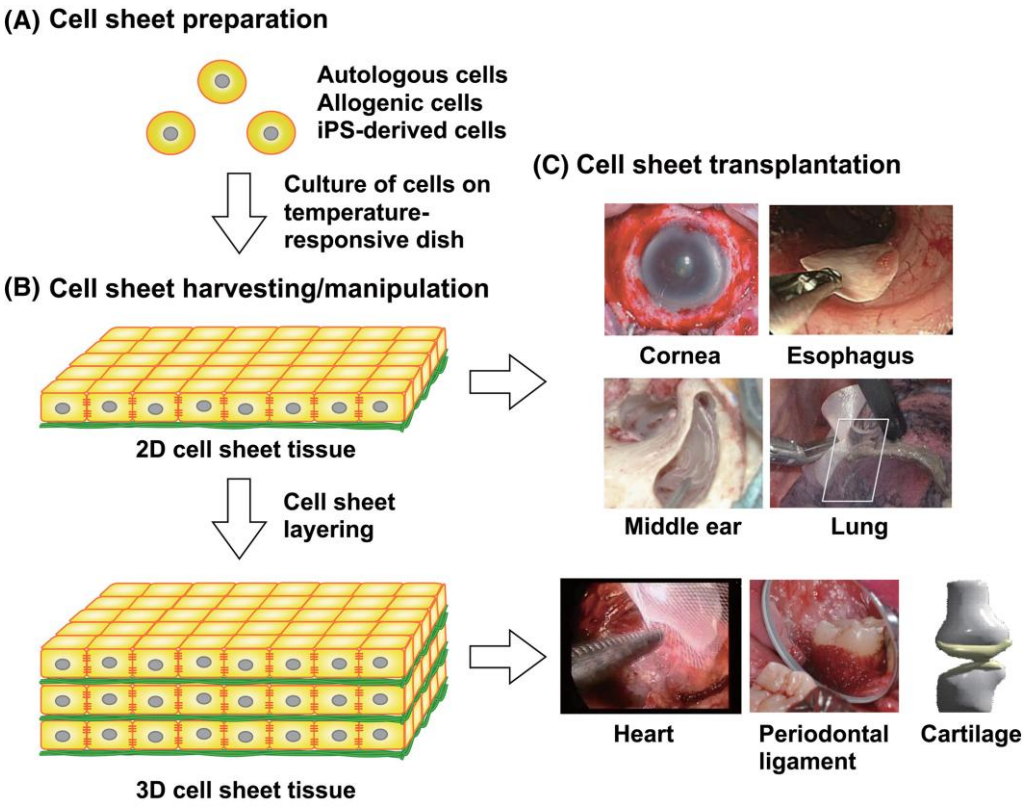


Figure 1. The proposed framework for cell sheet technology. (A) Preparation of cell sheets, (B) harvesting and handling, and (C) transplantation.[13]

2. MATERIALS AND METHODS

2.1. Cell cultivation and preparation of the cell sheet

CSCs(Innostembio, Busan, Korea) were grown in DMEM/F12 medium containing 10% FBS, 10ng/ml EGF, 10ng/ml IGF, 2ng/ml bFGF, and 15μg/ml gentamacin (Wellgene, Korea The cells were cultured in a incubator set at 37°C and 5% CO_2, and the media is replaced every three to four days, and when the cells reach 90% confluency in the flask, they are detached using Trypsin and saline. Experiments were conducted using cells at passage 13 or lower. Additionally, cells were plated onto PET films at a concentration of $5x10^4$ cells per cm^2 and allowed to glow and generate a cell sheet over a period of 4 days.

2.2 Fibrin gel preparation

Fibrin gel assists in transferring the cell sheet. To prepare the fibrin gel, fibrinogen and thrombin are used. Fibrinogen is mixed at a concentration of 10 mg/ml, and thrombin at 50 U/ml to create the gel.

2.3 Cell sheet transplantation process

Cells were plated onto PET films at $5x10^4$ cells per cm^2 and permitted to proliferate, forming a cell sheet over the course of 4 days. For laboratory-based transfer of the cell sheet, the film with the cell sheet was initially positioned onto a fibrin gel without removing the cell culture medium. Fibrinogen and thrombin were incorporated into the cell sheet to create a fibrin gel. The sample was kept at 37°C for 30 minutes, after which the PET film was carefully eliminated. A fibrin gel was formed as the first layer, and a cell sheet with its cell sheet film was placed on top. The construct was incubated for 1 hour, followed by the removal of the PET film. This procedure was carried out multiple times to create a 3-layered cell sheet.

2.4 Cell viability and cytotoxicity assay

To evaluate the viability of the cell sheet layers, the LIVE/DEAD Kit (Molecular Probes) was utilized. The cell sheets were placed onto the fibrin gel and incubated for 30 minutes with a solution containing 2 μM calcein-AM and 4 μM ethidium homodimer-1 (EthD-1). Following the incubation, the cell sheets were rinsed with phosphate-buffered saline (PBS, pH 7.4) and analyzed under a fluorescence microscope (IX71/DP71, Olympus, Tokyo, Japan).

2.5 Immunocytochemistry

To evaluate the preservation of the extracellular matrix during cell sheet detachment, we analyze the presence of Laminin and Fibronectin. First, the cells are fixed by treating them with 0.5 mL of 4% paraformaldehyde at room temperature. After fixation, the cells are rinsed three times with PBS. To allow permeability the cells, 0.3% Triton X-100 dissolved in PBS is applied at ambient temperature for 15 minutes, and then the cells are washed three times with PBS. Blocking is performed by incubating the cells with 0.5 mL of 1% bovine serum albumin (BSA) for 1 hour at ambient temperature. Laminin, diluted 1:100 in 1% BSA is carried out for 24 hours at 4°C. After three washes, a secondary antibody labeled with fluorescence (diluted 1:500 in 1% BSA) is added for 1 hour at ambient temperature. Nuclear staining is performed using Hoechst 33258. For Fibronectin detection, a fluorescently labeled antibody solution is prepared by diluting 1:100 in 1% BSA, incubated for 1 hour at ambient temperature, followed by nuclear staining. Laminin and Fibronectin are visualized using fluorescence microscopy. For Collagen detection, cells are fixed using 0.5 mL of Kahle fixative, washed with PBS, and stained with 0.2 mL of staining solution for 30 minutes at ambient temperature. Following the removal of the staining solution and washing with distilled water, the cells were exposed to 1 ml of stain extraction buffer. Absorbance was then measured at 605 nm using a microplate reader.

2.6 Identification of cytokines involved in angiogenesis by ELISA

The cytokines associated with angiogenesis, such as Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor(EGF), Fibroblast Growth Factor(FGF), and Angiopoietin-1(Ang-1), were quantified using an enzyme-linked immunosorbent assay (ELISA). Cardiac stem cell sheets were layered into 1, 2, and 3 layers and cultured in basal media for 24 hours. Once the incubation was complete, the culture media were centrifuged, and the supernatant was collected for sampling. For each ELISA kit, washing solutions and substrate solutions were prepared in advance, and standard solutions for each cytokine were also prepared. The following describes the pre-treatment procedures for each cytokine analysis. After applying the appropriate cytokine to each well, add 200 µL of reaction solution to each well and keep at ambient temperature, stored away from light, for 30 minutes. Then, add stop solution to each well. Analyze the samples within 30 minutes using an ELISA plate reader at 450 nm. Optical concentrations were calculated using a microtiter plate spectrophotometer. A standard was generated by plotting the logarithmic values of the average absorbance for each standard against the logarithmic values of the cytokine concentration.

2.7 Tube-formation assay

To assess tube formation, Matrigel Matrix was thawed overnight at 4°C and gently mixed with a chilled pipette to prevent bubble formation. 125 µl of Each well of a 48-well plate was filled with Matrigel, which was then incubated at 37°C for 30 minutes to facilitate the solidification process. Afterward, any excess liquid was discarded. HUVECs were plated at a density of $3x10^4$ cells per well and incubated for 2 hours. Cardiac stem cell layers were then introduced, and the cells were cultured for an additional 48 hours. Subsequently, 150 µl of Calcein AM fluorescent dye solution (8 µl/ml in HBSS) was added to each well and incubated for 30 minutes. Following this period, the staining reagent was discarded, and HBSS was used to rinse the wells twice. The tube formation was visualized using a fluorescence microscope. Images were taken and processed with ImageJ software to quantify the number of junctions, mesh count, total mesh area, and the overall length of the tube-like structures.

2.8 Anti-inflammatory

The anti-inflammatory effects of the CSCs cell sheet were assessed by analyzing the levels of Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), and Interleukin-6 (IL-6). THP-1 cells were seeded in a 48-well plate at $1x10^6$ cells per well and allowed to adhere for 24 hours. Afterward, the medium was exchanged for 0.5 ml of cardiac stem cell sheet overlay solution. After a further 1 hour, the The cells were exposed to 10 ng/ml of Lipopolysaccharide(LPS) and cultured for another 24 hours. Subsequent to the treatment, the culture supernatants were harvested and centrifuge at 3,000 rpm for 10 minutes. to eliminate cell remnants. The resulting clear supernatant was then utilized for ELISA analysis. For the ELISA procedure, the washing buffer and substrate solution were prepared according to the manufacturer's guidelines. Standards were also prepared as per the kit instructions. The experiment was performed as per the guidelines provided with the ELISA kit. The study included a standard group, an untreated control group, a control group treated with LPS only, and groups treated with LPS and conditioned media from CSCs sheets with 1, 2, and 3 layers. The anti-inflammatory effects of the CSCs sheets were evaluated by comparing the concentrations of inflammatory markers between the LPS-treated control group and the groups treated with 1-, 2-, and 3-layer CSCs sheets.

2.9 Skin wound healing for cell proliferation

HaCaT cells were seeded at a concentration of $1x10^4$ cells per well in 48-well plates. CSCs cell sheets were applied in 1, 2, and 3 layers and cultured in basal media for 24 hours. Following the incubation, the growth medium was centrifuged, and the supernatant was collected for further analysis. HaCaT cells were cultured for 24 hours at 37°C, after which the medium was replaced with conditioned media. After 24 and 48 hours, the supernatants were discarded, and an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay was conducted. MTT reagent was combined with Endothelial Growth Medium-2(EGM-2) and incubated in the dark for 4 hours. The MTT solution was taken up by the cells, and dimethyl sulfoxide(DMSO) was added to dissolve the cells and release the purple formazan crystals. The solution was subsequently placed in a 96-well plate, and absorbance was measured at 570 nm using a microplate reader (Molecular Device, CA, USA).

2.10 Data assessment

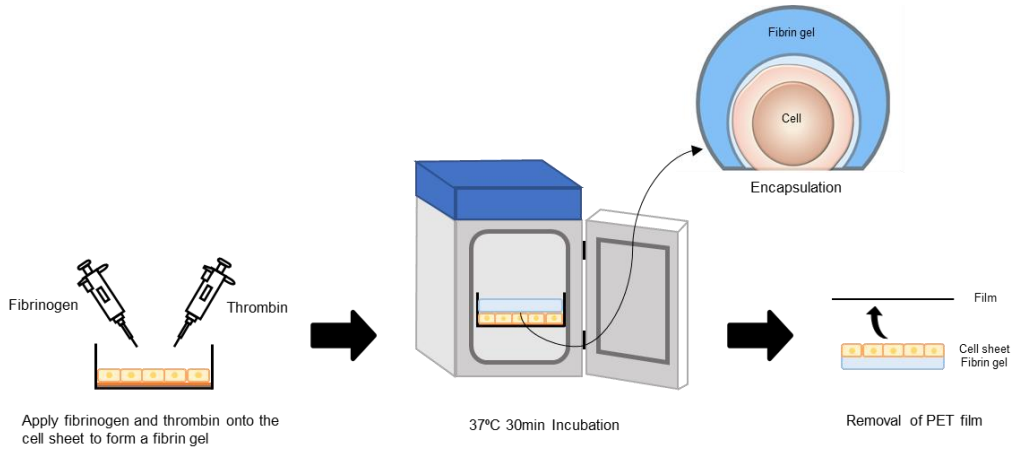
The data are expressed as the mean ± standard error of the mean (SEM). Statistical evaluation was performed using SPSS version 23.0. To compare the group means, a one-way analysis of variance (ANOVA) was applied. A p-value below 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Confirmation of CSCs sheets transfer to fibrin gel

After seeding the cell sheets (CSCs) at a density of $5x10^4$ in $1cm^2$, incubation was performed for 4 days to allow for cell sheet formation. Fibrinogen and thrombin were introduced to the cell sheet to promote the creation of a fibrin gel. The sample was kept at 37°C for 30 minutes, after which the PET film was carefully eliminated. The first layer was formed using fibrin gel. A live/dead staining test was performed, where viable cells were stained green and non-viable cells were stained red. The pictures illustrate that no cells were observed on the film following the culturing period, while the fibrin gel displayed the successful transfer of the cell sheets without any presence of dead cells.

A



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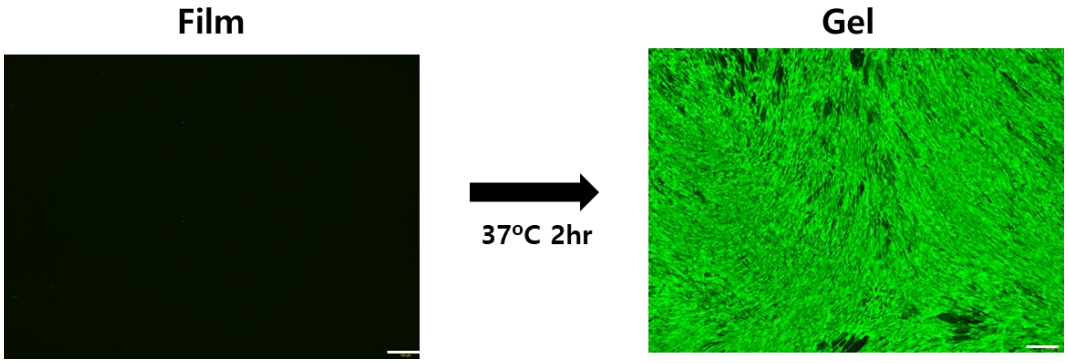

Figure 2. Cell sheet transfer on fibrin gel. (A) Schematic depiction of the CSCs sheet transfer procedure onto the fibrin gel. (B) Positioning of the CSCs sheet on the fibrin gel and assessment of the viability of the detached cell sheet. The scale bar represents 1.0 mm.

3.2. Detachment of CSCs sheets alongside extracellular matrix

To verify if the ECM accompanies the cell sheet during its placement onto the fibrin gel, immunostaining was performed. Laminin and fibronectin were examined, and the results proved that both the cell sheet and ECM were successfully moved to the fibrin gel, as shown in the accompanying figures.

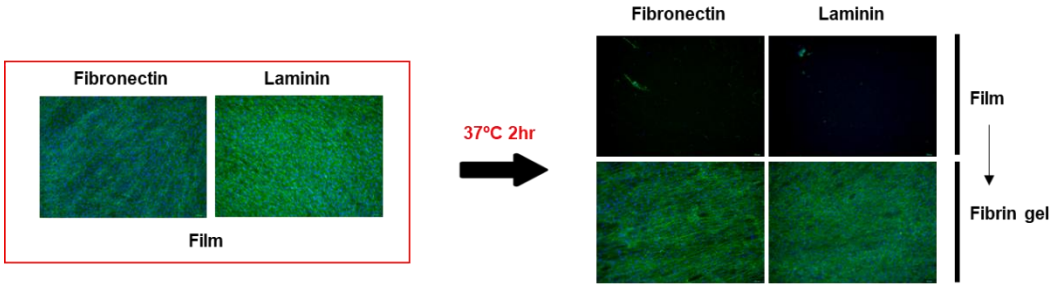

Figure 3. ECM Retention in Detached Cell Sheets Transferred onto Fibrin Gel. Fluorescent staining images of CSCs sheets transfer with ECM proteins fibronectin (green), laminin (green) and nucleus (blue).

3.3. Stacking of CSCs sheets

A multi-layered CSCs cell sheet was fabricated using our proposed in vitro technique, which involved the repeated layering of cells. In this process, each new layer of cells was placed onto the previous one on a fibrin gel, with the procedure done three times to create a 3-layered structure. To distinguish the layers, each cell sheet was labeled with a different CellTracker stain: CellTracker Violet BMQC for the 1 layer, CellTracker Green CMFDA for the second, and CellTracker Orange CMRA for the third. Imaging was conducted at 1.21 μm intervals along the Z-axis to capture the stacking of the layers, with each cell sheet becoming progressively more distant from the fibrin gel in the images. The sectional images offered a clearer view of the three-layered structure. These findings show the potential of using the encapsulated cell sheet transfer method to fabricate 3D tissue constructs and effectively deliver cell sheets to specific locations.

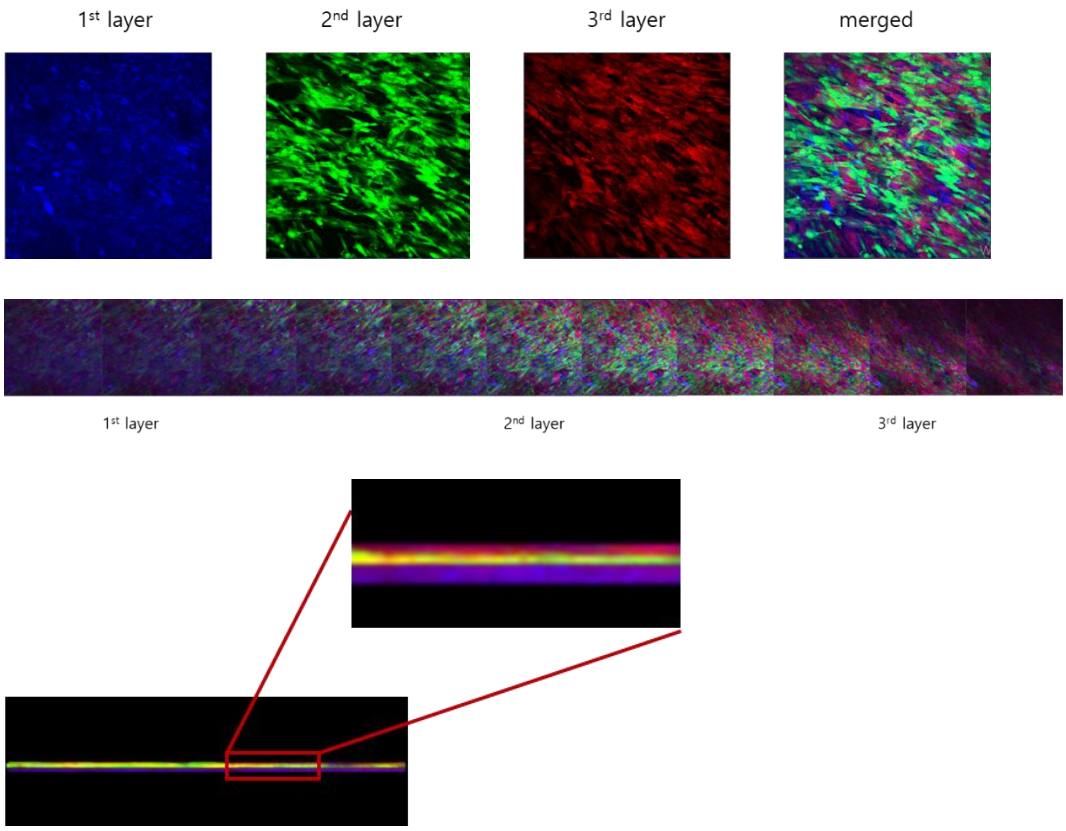

Figure 4. Confocal image. The stacked CSCs sheets, labeled with CellTracker Violet, CellTracker Green, and CellTracker Orange, were analyzed using a fluorescence microscope.

3.4. Confirmation of the release of cytokines

CSCs were cultured on a 1 cm^2 PET film at a density of $5x10^4$ cells for 4 days. Following this, the cells were transferred to a fibrin gel to create cell sheets, which were then layered in 1, 2, and 3 layers. The fibrin gels with the cell sheets were transferred to basal media and placed at 37°C for 24 hours period. After culturing period, the media were collected for sampling. The collected samples from the 1-layer, 2-layer, and 3-layer constructs were analyzed for cytokine secretion using ELISA. In this study, we measured the cytokines secreted by CSCs, specifically focusing on VEGF, EGF, FGF and Ang-1. These factors are crucial for angiogenesis, tissue repair, and cell proliferation. In the ELISA assay, all materials markedly elevated in comparison to the control in the 1, 2, and 3 layer stacked CSCs cell sheets. Each cytokine also exhibited a notable rise. The levels of VEGF, EGF, and FGF in the second and third layers were significantly different compared to the first layer. Additionally, the levels of VEGF, EGF, and FGF in the third layer showed significant differences when compared to the second layer. Furthermore, Ang-1 levels showed a marked difference between the second and third layers in comparison to the first layer.

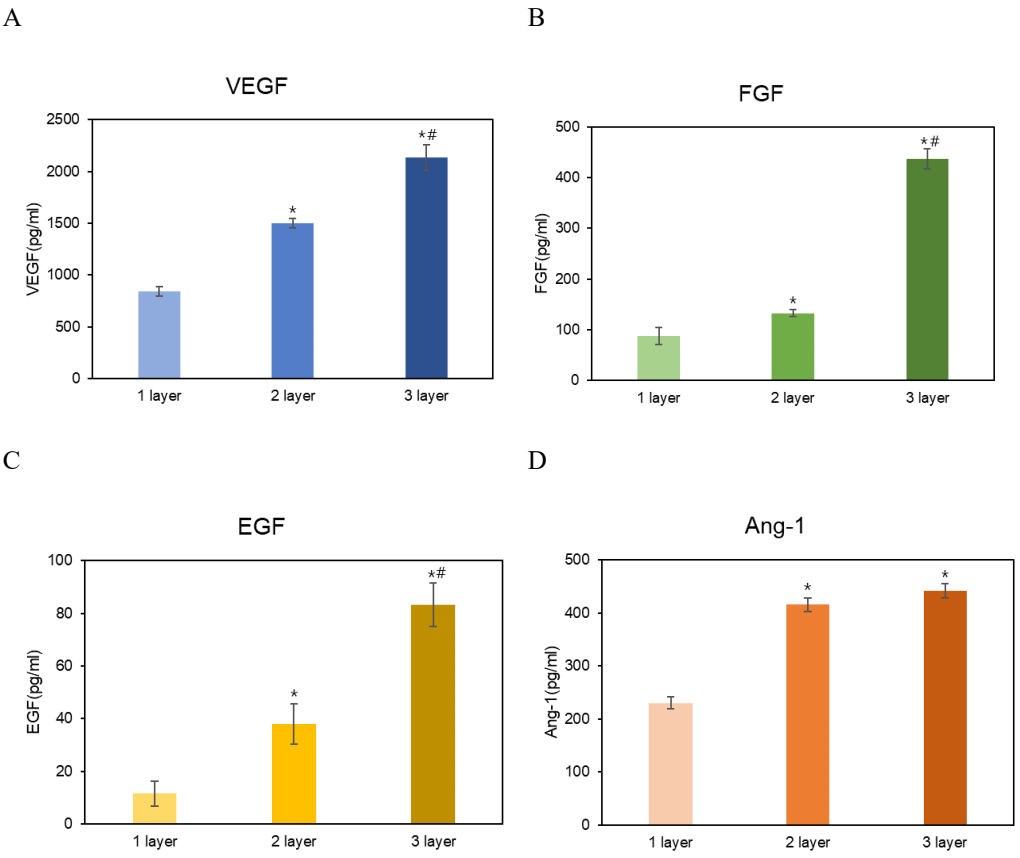

Figure 5. Comparison of cytokine secretion levels in 1, 2, and 3- Layer CSCs sheets. (A) Human VEGF, (B) Human FGF, (C) Human EGF, (D) Human Ang-1 levels were quantified using an ELISA assay. The data are presented as the mean ± SD. *$p < 0.05$ vs. 1 layer, #$p < 0.05$ vs. 2 layer.

3.5. Tube formation

CSCs were cultured on a 1 cm^2 PET film at a density of $5x10^4$ cells for 4 days. Following this, the cells were transferred to a fibrin gel to create cell sheets, which were then layered in 1, 2, and 3 layers. The fibrin gels containing the cell sheets were transferred to basal media and placed at 37°C for 24 hours period. After culturing period, the media were collected for sampling. The collected samples from the 1-layer, 2-layer, and 3-layer constructs were analyzed for tube formation. HUVECs were plated at a concentration of $3x10^4$cells per well in 48 well plates on the matrigel and cultered for 2 hours. After this, samples were added, and the cells were placed for an additional 48 hours. Tube formation was assessed with the help of ImageJ software to assess the number of junctions, number of meshes, total mesh area, and total branching length. all materials markedly elevated in comparison to the control in the 1, 2, and 3 layer stacked CSCs cell sheets. Each level also exhibited a significant increase. And all materials in the second and third layers were significantly different compared to the first layer. Additionally, the levels of all materials in the third layer showed significant differences when compared to the second layer. Furthermore, the number of junctions, total mesh area, and total branching length of the three-layer structures exhibited no considerable variation when compared to the positive control, VEGF. This indicates that the tube formation observed in the three-layer system closely resembles that of the favorable control

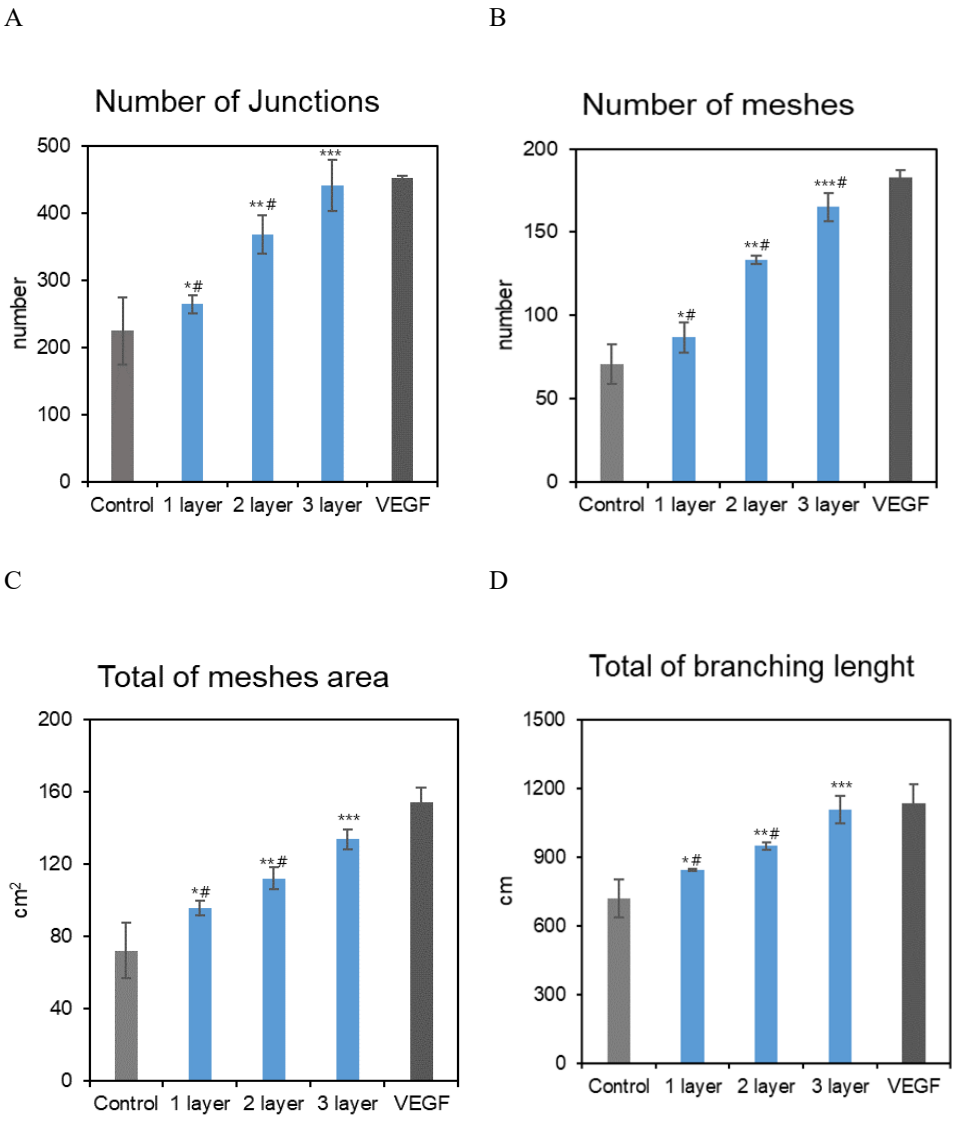

Figure 6. Comparison of tube formation levels in 1, 2, and 3- layer CSCs sheets. (A) Number of junctions, (B) Number of meshes, (C) Total of meshes area, (D) Total of branching lenght levels were measured by Image J. The data are presented as the mean ± SD. *$p < 0.05$ vs. control, **$p < 0.05$ vs. 1 layer, ***$p < 0.05$ vs. 2 layer, #$p < 0.05$ vs. VEGF.

3.6. Measurement of anti-inflammatory cytokine

CSCs were cultured on a 1 cm^2 PET film at a density of $5x10^4$ cells for 4 days. Following this, the cells were transferred to a fibrin gel to create cell sheets, which were then layered in 1, 2, and 3 layers. The fibrin gels containing the cell sheets were placed in basal media and kept at 37°C for 24 hours period. After culturing period, the media were collected for sampling. The collected samples from the 1-layer, 2-layer, and 3-layer constructs were analyzed for anti-inflammatoryTHP-1 cells were plated in a 48-well plate at a concentration of $1x10^6$ cells per well and cultured for 24 hours. To induce an inflammatory response, samples were treated with LPS, and the anti-inflammatory effects were assessed using ELISA. As a result, our observations revealed a proportional decrease in anti-inflammatory cytokines corresponding to the layered CSCs sheets throughout the study period(Figure 7, 8, 9).

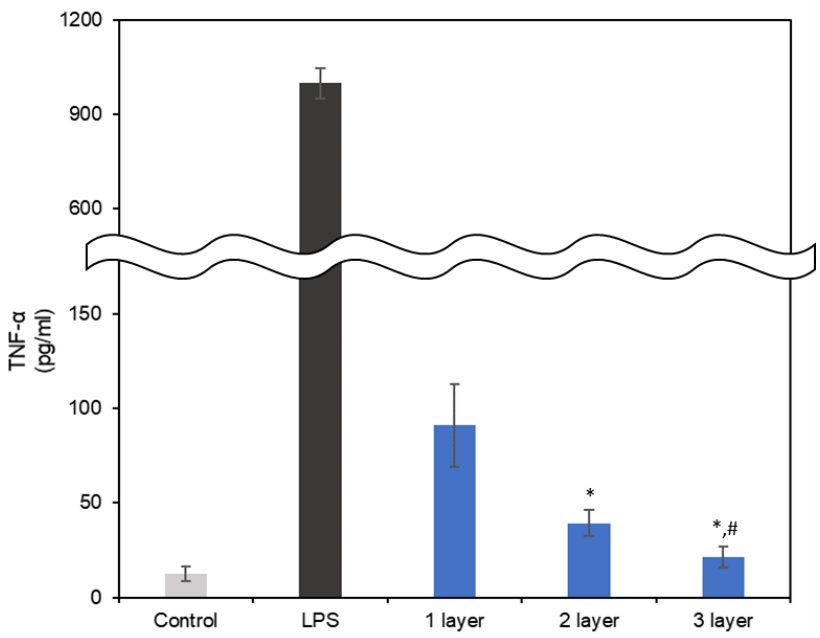

Figure 7. Effect of layered CSCs sheets on TNF-α levels. THP-1 were treated with LPS with layerd CSCs sheets conditioned media. TNF-α level was measured using ELISA. The data are presented as the mean ± SD. *$p < 0.05$ vs. 1 layer, #$p < 0.05$ vs. 2 layer.

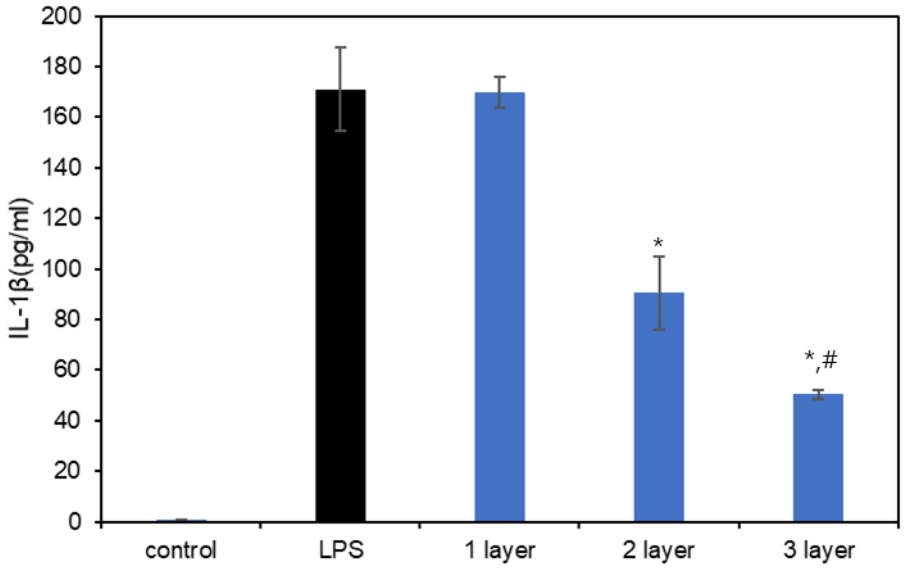

Figure 8. Effect of layered CSCs sheets on IL-1β levels. THP-1 were treated with LPS with layerd CSCs sheets conditioned media. IL-1β level was measured using ELISA. The data are presented as the mean ± SD. *$p < 0.05$ vs. 1 layer, #$p < 0.05$ vs. 2 layer.

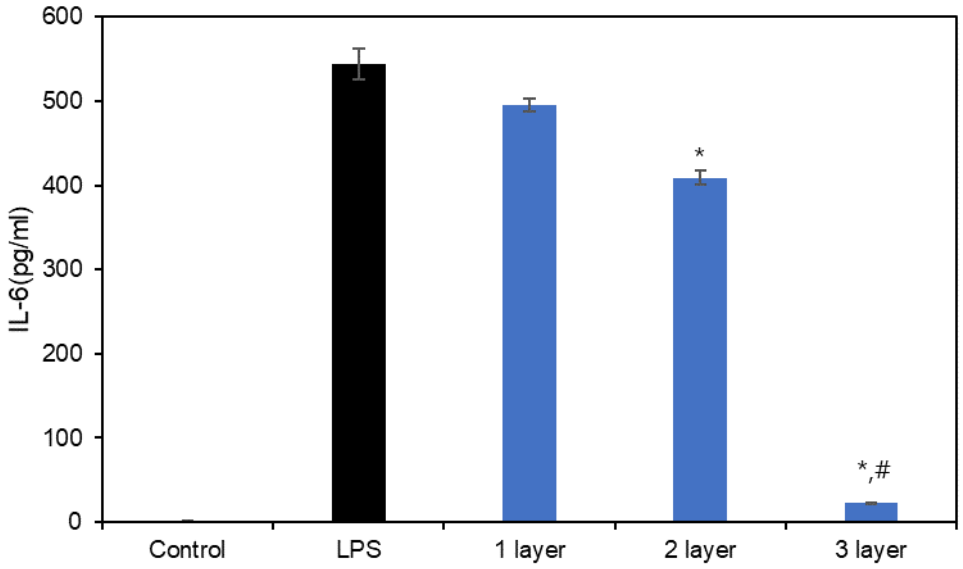

Figure 9. Effect of layered CSCs sheets on IL-6 levels. THP-1 were treated with LPS with layerd CSCs sheets conditioned media. IL-6 level was measured using ELISA. The data are presented as the mean ± SD. *$p < 0.05$ vs. 1 layer, #$p < 0.05$ vs. 2 layer.

3.7. Skin wound healing for cell proliferation

CSCs were cultured on a 1 cm^2 PET film at a density of $5x10^4$ cells for 4 days. Following this, the cells were transferred to a fibrin gel to create cell sheets, which were then layered in 1, 2, and 3 layers. The fibrin gels containing the cell sheets were placed in basal media and kept at 37°C for 24 hours period. After culturing period, the media were collected for sampling. The collected samples from the 1-layer, 2-layer, and 3-layer constructs were used for HaCaT proliferation. HaCat was cultured on 48 well plates at a density of $1x10^4$ cells. And after 24 hours, the cells were treated with a mixture of 30% conditioned media and 70% serum-free media. Observations were conducted at both 24 hours and 48 hours after treatment. The results showed that, after 24 hours of incubation, all three layers showed a statistically significant difference when compared to the control. Furthermore, a distinct difference was observed between the 1-layer and 3-layer structures. After 48 hours of incubation, a notable variation was found between the control and the 1-layer, as well as in comparison with the 3-layer.

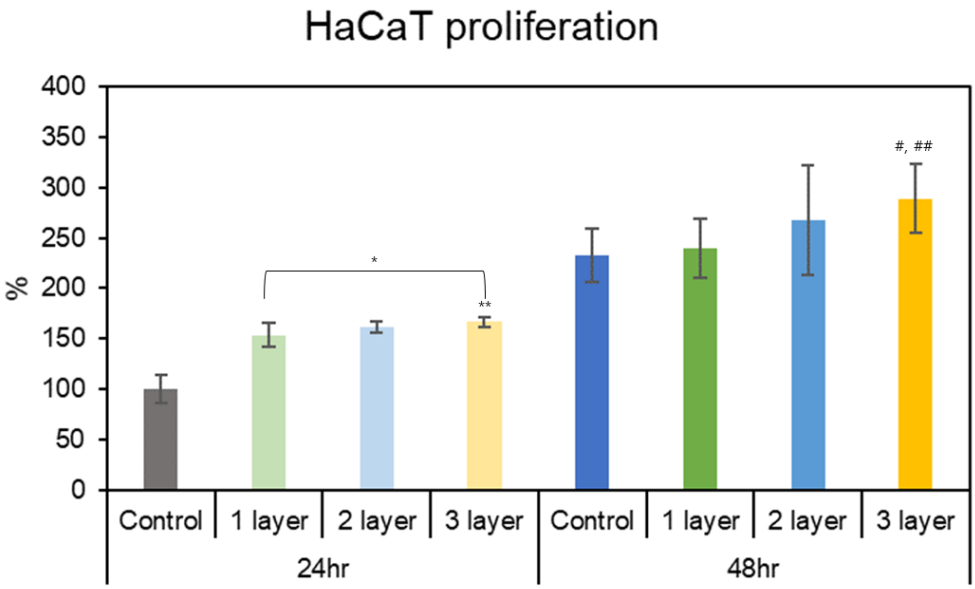

Figure 10. Skin wound healing for cell proliferation. The data are presented as the mean ± SD. *$p < 0.05$ vs. 24hr control, **$p < 0.05$ vs. 24hr 1 layer, #$p < 0.05$ vs. 48hr control, ##$p < 0.05$ vs. 48hr 1 layer.

4. CONCLUSION

This study showed that the encapsulation technique for delivering cell sheets provides an efficient and straightforward approach for layering and transferring cell sheets to specific target areas. In this study, CSCs were cultured on hydrophilic PET films to produce cell sheets, which were then overlaid with a fibrin gel, encasing the viable cells within the gel matrix. This approach is referred to as the encapsulation method. It was observed that the cell sheet transferred to the fibrin gel maintained a rich ECM within the gel. Additionally, no dead cells were found in the transferred cell sheet. After transferring the first layer of the cell sheet to the fibrin gel using the encapsulation method, the adhesion between cells was utilized to stack the second and third layers of the cell sheet. As a result, a 3-layered cell sheet was successfully stacked within the fibrin gel, with minimal dead cells present in the stacked sheets. Through in vitro experiments, it was shown that the 3-layered CSCs cell sheet effectively promotes angiogenesis and exhibits various anti-inflammatory effects. Additionally, it enhances keratinocyte proliferation, indicating its potential application in treating diabetic foot ulcers. Furthermore, these effects can be extended to in vivo studies, highlighting its role in promoting angiogenesis and skin regeneration at wound sites. n conclusion, the encapsulation method was employed to minimize stimulation and facilitate the easy separation of cell sheets. Moreover, the CSCs cell sheets stacked through cell-to-cell adhesion demonstrated angiogenic and anti-inflammatory effects in vitro. Furthermore, applying CSCs cell sheets to diabetic foot ulcers could effectively address slow healing and difficulties in vascular formation, offering a promising therapeutic approach for such challenging wounds.

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Abstract in Korean

당뇨병성 족부궤양 치료를 위한 심장줄기세포 시트의 효과

당뇨병성 족부 궤양(DFU)은 당뇨병의 주요 합병증으로, 당뇨병 환자에서 발목 아래 발생하는 전층 상처로 정의된다. 현재 DFU 치료 가이드라인은 치유를 촉진하기 위해 변연 절제, 감염 관리, 재혈관화 및 압력 분산을 권장한다. 그러나 허혈, 감염, 신경병증 및 대사 장애와 같은 요인이 상처 치유를 방해하여 환자와 의료 제공자에게 중요한 도전 과제가 된다. 줄기 세포는 세포 유인, 면역 조절, 세포외 기질(ECM) 재구성, 혈관 생성 및 신경 재생을 촉진하는 사이토카인을 생성하고 방출하여 상처 치유 과정에 기여한다. 본 연구에서는 심장 줄기 세포(CSCs)를 이용한 캡슐화 방법으로 생성된 세포 시트의 효능을 보고한다. CSCs 는 혈관 생성, 면역 조절, 항염증 및 세포외 기질(ECM) 형성을 촉진하는 독특한 능력을 지닌다. 개발된 CSCs 기반 세포 시트는 상처 부위에 산소와 영양소 공급을 촉진하여 혈관 형성을 가속화함으로써 상처 치유를 향상시킨다. 일반적으로 알려진 분리 기술인 열 반응성, 전기 반응성, pH 반응성 및 자기 유도 방법과는 달리, 캡슐화 방법을 적용한다. 이 방법에서는 세포를 PET 필름에서 배양하여 세포 시트를 생성한 후, 피브리노겐과 트롬빈으로 처리하여 젤을 형성한다. 37℃ 에서 30 분 동안 배양하여 세포 시트를 피브린 젤로 전이하며, 이 과정을 반복하여 층을 형성한다. 층화된 세포 시트의 효과는 다양한 in vitro 방법을 통해 평가된다. 층화된 세포 시트의 효능을 확인함으로써 당뇨병성 발 궤양에 대한 직접 적용의 기초를 제공할 수 있다.

핵심되는 말 : 당뇨병성 족부궤양, 심장 줄기 세포, 세포 시트, 사이토카인