Effect of Vascular Endothelial Growth Factor (VEGF) on Cells Isolated from Pericardial Fluid

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***Özetçe*— Perikardiyal sıvı (PF) kalp ile kalbin etrafını çevreleyen perikardiyum arasındaki boşlukta bulunmaktadır. Bu sıvı içinde mezoteliyal hücreler ve monosit, lenfosit, makrofaj, nötrofil gibi lökositler yer almaktadır. Mezotheliyal hücreler farklılaşma potansiyeline sahip olması ve karakteristik özellikleriyle mezenkimal kök hücrelere benzemektedir. Mezoteliyal hücrelerin sayısı kalpte meydana gelen hasarla artmakla kalmayıp bu hücreler büyüme faktörleri de salgılayarak doku onarımını destekler.**

Bu çalışmada sığır perikardiyal sıvısından izole edilen hücreleri kullanılarak, *in vitro* kültür ortamına 1, 10, 20, 50 ng/mL miktarlarında vasküler endoteliyal büyüme faktörü (VEGF) eklenmesiyle bu büyüme faktörünün hücrelere olan etkisi incelenmiştir. Büyüme faktörü eklenmiş ve büyüme faktörü eklenmemiş hücrelerinin birinci ve beşinci günlerdeki ışık mikroskobu görüntüleri incelendiğinde 50 ng/mL VEGF'nin perikardiyal sıvı hücreleri için etkili konsantrasyon olduğu sonucuna ulaşılmıştır. Ayrıca, görüntünün komplekslik, çarpıklık (skewness) ve görüntü histogramı komplekslik bilgisine gore 10 ng/mL VEGF'nin etkili konsantrasyon olduğu değerlendirilmiştir.

Anahtar Kelimeler — Perikardiyal sıvı; perikardiyal sıvı hücreleri; VEGF; doku mühendisliği

***Abstract*— Pericardial fluid (PF) is located in the space between the heart and the pericardium surrounding the heart. PF contains mesothelial cells and leukocytes such as monocytes, lymphocytes, macrophages, and neutrophils. Mesothelial cells are similar to mesenchymal stem cells with their differentiation potential and characteristic features. The number of mesothelial cells not only increases with damage to the heart, but also secrete growth factors to support tissue repair. In this study, 1, 10, 20, 50 ng/mL vascular endothelial growth factor (VEGF) was added to the *in vitro* cultured cells isolated from bovine pericardial fluid.**

For this purpose, images of control and VEGF groups on the first, fifth and tenth days were examined by light microscope and it was concluded that 50 ng/mL VEGF is the effective concentration for pericardial fluid cells. 10ng/mL VEGF was determined as effective concentration according to the complexity of the image, skewness and complexity of the histogram.

Keywords — Pericardial fluid; cells of pericardial fluid; VEGF; tissue engineering.

I. INTRODUCTION

PF is located in the space between the heart and the pericardial membrane surrounding the heart as a non-adhesive barrier. Content of pericardial fluid changes according to the dynamic structure of the body and tissue damage. Also it plays an important role in the diagnosis and treatment of diseases.

PF has heterogenous cell population including mesothelial cell, lymphocytes, glanulocytes, macrophages, eosinophils, and basophils, and has important roles for repairing heart damages [1,2]. Mesothelial cells have differentiation potential and the number of these cells increase with tissue damage. They also synthesize their ESM molecules and growth factors (VEGF, FGF-2, PDGF, TGF-b) to restore tissue [3].

Vascular endothelial growth factor (VEGF) is a homodimeric protein 34–42 kD molecular weight. VEGF induces angiogenesis, formation of new vascular tissue, and has heparin binding activity for proliferation and migration of vascular endothelial cells.

In a study endothelial /mesenchymal stem cells were treated with VEGF, FGF and PDGF and 5 ng/mL was concluded as effective concentration [4]. In another study the capacity of VEGF to induce differentiation of endothelial progenitor cells was investigated in varying concentrations (0, 10, or 50 ng/mL) [5]. Furthermore, differentiation of endothelial cells from

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mesenchymal stem cells was performed by applying 50ng/mL VEGF [6]. In this study, VEGF, which also plays an important role in angiogenesis, was added to the attached cells of pericardial fluid 1, 10, 20 and 50 ng/mL *in vitro* culture medium. The effects of VEGF on pericardial fluid cells were examined by light microscopy.

II. MATERIALS AND METHOD

A. Obtaining pericardial fluid from bovine heart

Pericardial fluid was obtained from healthy bovine, 2-3 years old and weighing 350-450 kg, immediately after slaughtering. PF was aspirated by entering the pericardial cavity with a sterile syringe (50mL) without damaging the pericardium

B. Isolation of pericardial fluid cells

Pericardial fluid samples were taken for approximately 30 min. in room temperature. PF samples were transferred to falcon tubes and centrifuged at 3500x g for 5 minutes. After the centrifuge, pellet was used for cells source. Isolated cells, called as pericardial fluid cells (PRSc), were suspended in completed medium. Alpha-Mem medium was used as medium for PRSc cells [7, 8]. Alpha-MEM was supplemented with 10% fetal bovine serum (FBS), 250 U/mL penicilin, 250 µg/mL streptomisin, 25 mM glutamin, 20 µg/mL bovine insulin [7, 8]. Approximately $1x10^4$ cells/well were cultured in a 6-well plate and incubated in 5% CO_2 at 37^0C.

After 24 hours for waiting cell attachment 1, 10, 20, 50 ng/mL VEGF was added to the culture medium and the cells were visualized by light microscope on days 1, 5 and 10. The analysis of the effect of VEGF on the cells was made by comparing the images of the control cells.

Figure 1 shows the image of attached cells before the growth factor application.

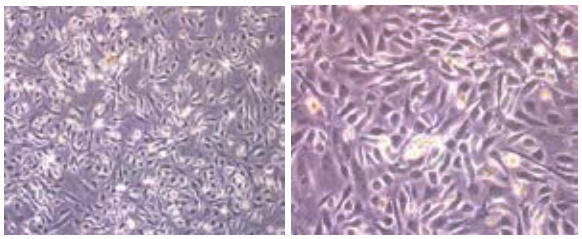

Figure 1. Light microscopy images of attached cells before addition of VEGF at 10x and 20x magnification, respectively.

C. Feature extraction from microscopy images

For numerical analysis, complexity and skewness features from histogram of microscopy image and maximum value of complexity and skewness features from image frames (divided as 256x256 sub images) were used. The complexity property of Hjorth parameters was used to extract the features from the microscopy images. Complexity is defined as a property related to the bandwidth of the array es seen in Eq. 1.

$$Complexity = \sqrt{\frac{Mobility\left(\frac{dx}{dt}\right)}{Mobility}} \quad (1)$$

Skewness is defined as a measure of the probability distribution asymmetry of a random process. In the equation (2); $\bar{Y}$ shows average, s gives the standard deviation and N shows the number of arrays calculated as in equation 2. [9-12]

$$\text{Skewness} = \frac{\sum_{i=1}^{N}(Y_i-\bar{Y})^3}{(N-1)s^3} \quad (2)$$

III. RESULTS

The aim of this study was to investigate the effect of variable VEGF concentrations (1, 10, 20 and 50 ng/mL) on cells isolated from pericardial fluid. Figure 2 shows the light microscopy images of cells applied 1, 10, 20, and 50 ng/mL VEGF on 1^{st}, 5^{th} and 10^{th} days.

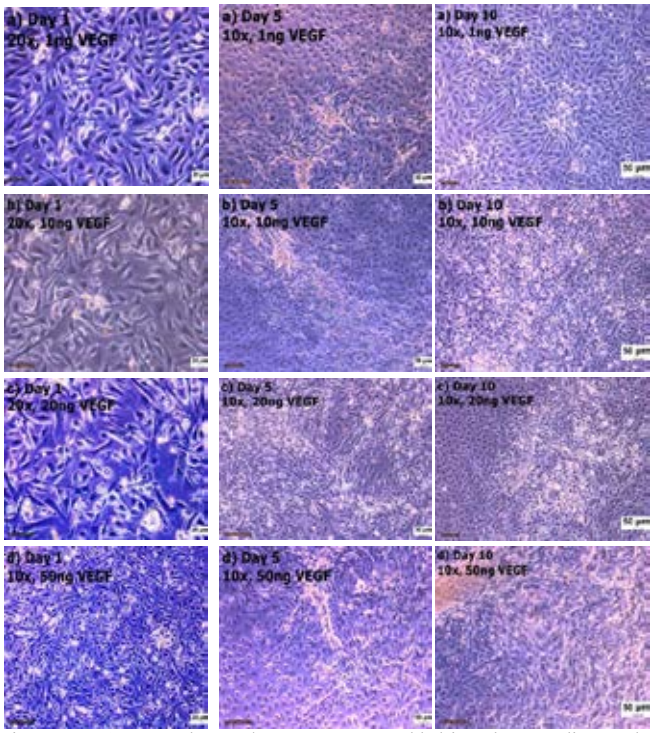

Figure 2. 1, 10, 20 and 50 ng/mL VEGF were added in culture medium and light microscopy images on 1st, 5th and 10th day, at 10x and 20x magnification

Figure 3 shows images of the control cells on the 1st, 5th and 10th days.

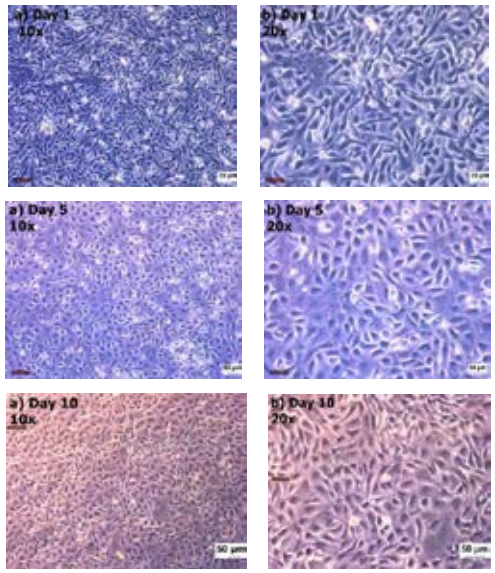

Figure 3. Light microscopy images of 1st, 5th and 10th day cells as control without growth factor at 10x and 20x magnification

TABLE I. IMAGE ANALYSIS DATA AFTER ADDING VEGF

VEGF first, fifth and tenth days	*From Histogram of images*	*From images*	
	Complexity	***Complexity max***	***Skewness max***
1ng/mL-1st day	38.896	95.104	13.414
10ng/mL-1st day	29.591	92.677	0.9401
20ng/mL-1st day	40.193	89.458	0.8447
50ng/mL-1st day	34.557	98.748	10.601
1ng/mL-5th day	56.068	78.407	0.5630
10ng/mL-5th day	148.027	113.096	12.049
20ng/mL-5th day	92.217	58.454	15.191
50ng/mL-5th day	136.457	96.056	13.304
1ng/mL-10th day	55.704	78.299	0.7167
10ng/mL-10th day	47.062	63.132	0.5170
20ng/mL-10th day	49.139	62.768	0.7725
50ng/mL-10th day	70.539	60.188	11.552

IV. DISCUSSIONS

In this study, we investigated the effect of VEGF on PRSc cells isolated from pericardial fluid. In the repair of tissue / organ damage some cells synthesize VEGF and other growth factors to repair tissue.

In this study, the effect of variable VEGF concentration (1, 10, 20 and 50ng/mL) on PRSc cells was examined on the 1st, 5th and 10th days at microscopic level.

In microscopic examination of the cells, a significant difference was observed at all concentrations. However, 10 ng/mL and 50 ng/mL VEGF application on the 5th and 10th days were different than the other concentrations (Figure 3).

Also numerical evaluation of microscopic image as seen from Table 1 that, complexity value of image histogram increased importantly from first day to fifth day for 10ng/mL and 50ng/mL. Also, calculated from image frames, maximum complexity and skewness value increased as histogram complexity for 10ng/mL.

In future studies, the effect of 50ng/mL VEGF which is determined as the ideal amount for pericardial fluid cells will use for tissue engineering application with *in vitro* studies.

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