TIPTEKNO'18
TIP TEKNOLOJİLERİ KONGRESİ
8-10 Kasım 2018 / Gazi Magosa / KKTC

HUVEC Mikrodokusu Büyümesi Üzerinde IKVAV Peptidinin Etkisi
Efficacy of IKVAV Peptide On HUVEC Microtissue Growth

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***Özetçe*— Vücuttaki hücrelerin bulunduğu üç boyutlu (3B) ağ örgüsünden oluşan hücre dışı matris (ECM) yapısı hücresel olaylarda önemli rol oynamaktadır. 3B hücre kültürü sistemlerinde *in vivo* sistemlerdeki gibi hücre-hücre ve hücre-matriks etkileşimleri sağlanabilmektedir. Bu sebeple iki boyutlu sistemlere kıyasla 3B kültürlerde hücre fonksiyonlarının kaybı daha düşüktür. İnsan göbek kordonu damar endotel hücreleri (Human Umbilical Cord Vein Endothelial Cells (HUVEC)) kolay izole edilebilen ve 3B kültürlerde damarlanma çalışmalarında sıklıkla kullanılan model hücre türleridir. Lamininin en etkili ünitelerinden olan izolösin-lizin-valin-alanin-valin sekansı (Ile-Lys-Val-Ala-Val (IKVAV)) ECM' de destek materyali olarak kullanılır. IKVAV peptidi adezyon, proliferasyon, migrasyon ve metastaz gibi birçok hücresel fonksiyona etki edebilmekte ve hücre kültürü sistemlerinde kullanılmaktadır. Bu çalışmada IKVAV peptidinin HUVEC mikrodoku oluşumundaki etkileri incelenmiştir. 0,5 ve 1 mM peptid konsantrasyonları ile inkübe edilen mikrodokularda çap artışı gözlemlenmiştir. Ayrıca peptid kullanılan mikrodokular, peptid kullanılmayanlarla karşılaştırıldığında IKVAV peptidinin hücre canlılığını arttırdığı görülmüştür. 0,5 ve 1 mM karşılaştırıldığında ise 1 mM peptid konsantrasyonunda daha yüksek hücre canlılığı ve çap değerleri görülmektedir. Elde edilen bu bilgiler literatürdeki monolayer çalışmalarda yapılan sonuçları destekler niteliktedir.**

Anahtar Kelimeler — IKVAV peptidi; HUVEC hücre hattı; mikrodoku.

***Abstract* — The extracellular matrix (ECM) structure of the three-dimensional (3D) network of cells in body play an important role in cellular events. Cell-cell and cell-matrix interactions can be achieved in 3D cell culture systems as *in vivo* systems. For this reason, the loss of cell functions is less in 3D cultures than in two dimensional systems. Human umbilical cord vein endothelial cells (HUVECs) which are model cell types, are frequently used because of easily isolation and using vasculogenesis research in 3D culture. The isoleucine-lysine-valine-alanine-valine (Ile-Lys-Val-Ala-Val (IKVAV)) sequence, one of the most effective units of laminin, is used as a support material in the ECM. IKVAV peptides can affect many cellular functions such as adhesion, proliferation, migration, metastasis, and are used in cell culture systems. The effects of IKVAV peptide on HUVEC microtissues were examined in this study. Increase in diameter was observed in microtissues incubated with concentrations of 0.5 and 1 mM peptides. Also when comparison of microtissues with and without peptide concentrations, has been shown to the IKVAV peptide increase cell viability. Compared with 0.5 and 1 mM, higher cell viability and diameter values are observed at 1 mM peptide concentration. This information supports the results of monolayer studies in the literature.**

Keywords — IKVAV peptide; HUVEC cell line; microtissue.

I. INTRODUCTION

Cell culture systems are the basic method of conducting advanced research applications in tissue engineering, drug discovery and stem cell research. Certain cellular characteristics, such as cell morphology, cell proliferation and cell cycle phase, show differences in 2D and 3D cell culture systems [1, 2]. It is accepted that 3D cell culture models serve more meaningful results than traditional 2D culture due to improved cell-cell interactions, cell-ECM interactions, cell populations and structures resembling *in vivo* architecture [3]. For this reason, 3D systems help close the gap between 2D culture studies and *in vivo* pre-clinical animal models [4].

The most commonly used cell line is human umbilical cord vein endothelial cells (HUVEC) in terms of proliferation frequency, ease of isolation and low cost among endothelial cells in vasculogenesis. HUVEC cell lines are used for important researches such as angiogenesis and differentiation in 3D culture models. Peptides are involved in many biochemical processes such as cell-cell interaction, metabolism, immune response and reproduction [5]. Significant micro environmental cues, such as cellular signals and phenotypic differentiation *in vivo*, have been obtained in 3D cell cultures using laminin protein [6].

The IKVAV peptide in the α1 chain of the laminin promotes cell adhesion, proliferation, migration and cellular differentiation and affects cellular events such as angiogenesis and metastasis [7]. Previous studies have found that laminin-

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derived IKVAV peptide affects the binding, migration and morphology of endothelial cells. IKVAV is known to be effective on the growth and differentiation of HUVECs [8, 9].

The aim of this study is to determine the effect of different concentrations of laminin-derived IKVAV peptide in the HUVEC microtissue in terms of microtissue size and viability.

II. MATERIAL AND METHOD

A. Peptide Synthesis

The IKVAV peptide was synthesized by solid phase peptide synthesis using rink amide resin as descripted in [10]. During the synthesis step, the amino acids were added respectively to the isoleucine-lysine-valine-alanine-valine. After the last amino acid was attached, the resin was cleaved with the synthesized peptide and the peptide was dried.

B. Cell Culture

In 2D cell culture, P17 HUVECs (Ege University, Bioengineering) incubated with Dulbecco's Modified Eagle Medium (DMEM, D2429, Sigma, St. Louis, Missouri, USA) nutrient media were trypsinized. Cell incubation were made with agarose plates of 330 µl to be 60,000 cells. 500 µl of Endothelial Basal Medium-2 (EBM-2, C3156, Lonza, Basel, Switzerland) nutrient media with 0 mM, 0.5 mM and 1 mM peptide concentrations was added. The generated 3D cell culture was allowed to incubate at 36.5°C and 5% CO_2.

C. Microtissue Size and Cell Viability Analysis

Microscope images were taken using an Olympus brand microscope-computer interface. Microtissue diameters were calculated using the ImageJ program. A Double Staining Kit [11] (Dojindo, Molecular Technologies, Inc, Japan) was used to determine the proportion of live and dead cells on day 7 of the generated microtissues.

III. RESULTS

The mean values of the diameters obtained from the microtissues in the media with 0 mM, 0.5 mM and 1 mM peptide concentration on day 1 were 227,15 ± 8,35 µm, 239,78 ± 5,34 µm and 283,09 ± 8,03 µm (p *** <0,001), which were statistically significant. The mean values obtained on day 4 were 223,96 ± 2,34 µm, 246,95 ± 5,16 µm (p *** <0,001) and 272,34 ± 2,94 µm (p *** <0,001) and statistically significant. Finally, mean diameter values at the 7th day were 204,85 ± 11,04 µm, 274,12 ± 2,25 µm (p *** <0,001) and 292,12 ± 2,63 µm (p *** < 0.001) and statistically significant (Figure. 1.).

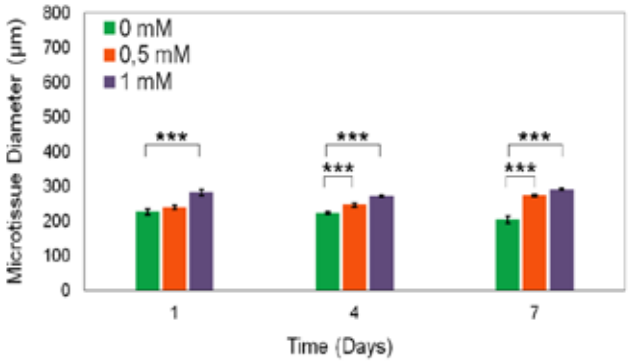

Fig. 1. The effect of different IKVAV concentration on HUVEC microtissue diameter

Microtissues developed in nutrient media at 0 mM peptide concentrations were found to be reduced to day 7. It was observed that nutrient media at concentrations of 0.5 and 1 mM peptides increased the diameters of microtissues used up to day 7.

On day 7, microtissues were analyzed for viability. Images were taken from at least 3 different microtissue as living (green) and dead (red) cells (n = 3). Then the images of the live and dead cells were combined (Figure. 2.). According to the obtained results, it was observed that cell viability was increased in microtissues where 0.5 and 1 mM peptides were used.

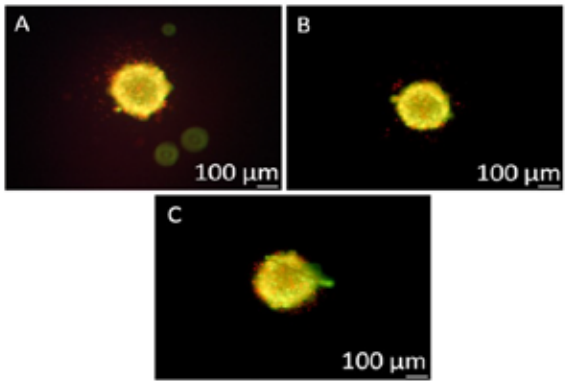

Fig. 2. Viability of IKVAV peptide on HUVEC microtissue on day 7. (A) Microtissue with 0 mM peptide concentration. (B) Microtissue with 0.5 mM peptide concentration. (C) Microtissue with 1 mM peptide concentration.

It has been experimentally proven that IKVAV peptide enhances microtissue size by promoting cell proliferation and positively affects cell viability. In previous studies, IKVAV peptide was found to be effective on endothelial cells and to increase proliferation, adhesion and cell viability.

IV. DISCUSSION

In this study, effects of 0.5 and 1 mM concentrations of IKVAV peptide on HUVEC microtissue were examined. When 0.5 and 1 mM peptide was used, microtissue diameter and cell viability was observed to increase vascular cell proliferation. When 0.5 and 1 mM are compared, microtissues cultured with

1 mM peptide have higher diameters and cell viability. Our results show that the IKVAV peptide from monolayer studies in the literature is compatible with information on cell proliferation and cell viability.

In future studies with HUVEC microtissue, optimum concentration values can be found by testing different peptide concentrations. The optimal peptide value affects vascular cells interaction and matrix communication, hence it can also affect vascular process.

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