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Laminin Türevli YIGSR Peptidinin HUVEC Mikro Doku Oluşumuna Etkisinin Değerlendirilmesi

Evaluation of the Effect of Laminin-Derived YIGSR Peptide on HUVEC Micro-Tissue Formation

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***Özetçe*-Damarlanmanın doku mühendisliği çalışmalarında önemi oldukça fazladır. İnsan Kordon Damarı Endotel Hücre (HUVEC) 'leri, 3 boyutlu (3B) doku çalışmaları ile uygunlukları ve damar oluşturma yeteneklerinden dolayı en çok tercih edilen endotel hücrelerden biridir. Peptidler mikro doku oluşumunda aktif rol almaktadır. Özellikle laminin türevi peptidler mikro doku çalışmalarında sıklıkla kullanılmaktadır. Bu çalışmanın amacı laminin türevi peptidlerin 3B HUVEC hücre kültüründe etkinliğini belirlemektir. Bu projede, 1.5 mM laminin türevli tirosin-izolösin-glisin-serin-arjinin(Tyr-Ile-Gly-Ser-Arg(YIGSR)) peptidinin HUVEC mikro-doku oluşumunu artırabildiği gözlenmiştir.**

Anahtar Kelimeler — YIGSR peptit, HUVEC mikrodokusu, katı faz peptit sentezi.

***Abstract*—The vascularization is a quite significant process in tissue engineering studies. Human Umbilical Cord Vein Endothelial Cells (HUVEC) are one of the most preferred endothelial cells due to their suitability for 3D tissue studies and their ability to form vessels. Peptides have an active role in micro-tissue formation. Especially laminin-derived peptides are frequently used in micro-tissue studies. The aim of this study is to determine the efficacy of the laminin-derived peptides in 3D HUVEC cell culture. In this project, it was observed that the laminin-derived 1.5 mM tyrosine-isoleucine-glycine-serine-arginine (Tyr-Ile-Gly-Ser-Arg (YIGSR)) peptide can enhance the formation of HUVEC micro-tissue.**

Keywords — YIGSR peptide, HUVEC micro-tissue, solid state peptide synthesis.

I. INTRODUCTION

Cell culture studies are an important part of popular researches. One of the preferred techniques is the 3D culture method. 3D culture method exhibits behaviors that are closer to those of the cells in vivo environment [1]. Thanks to the 3D culture, cell phenotypes of physiological tissues are protected by minimizing the differences between in vivo and in vitro tissues [2].

Endothelial cells are frequently used in micro-tissue studies. Endothelial cells are a flat epithelial tissue covering the inner surface of the vessel, located between blood and vessel walls [3]. HUVEC is one of the most used cell types in vascular researches. They have the basic properties of endothelial cells and also easily available. HUVECs have capillary vascularity and are often used on organ cultivation [4]. These cells can be appropriated for the development of alternative therapies for vessel diseases.

The extracellular matrix includes many proteins such as laminin, fibronectin and integrin. Laminin has many effects on the cells such as growth, differentiation, metastasis, adhesion, proliferation, spreading. Tyr-Ile-Gly-Ser-Arg(YIGSR) sequence is one of the essential parts of laminin. It has several effects on the cells when using alone or with other peptides. Suitable concentrations of YIGSR peptide in proliferation, adhesion and migration were demonstrated to rise. These effects may provide endothelization.

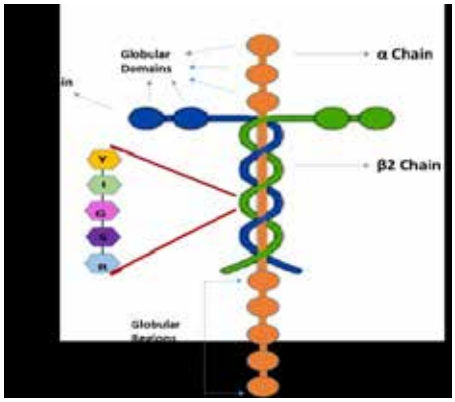

Figure.1. YIGSR configuration on laminin structure

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II. MATERIAL AND METHODS

YIGSR peptide synthesized by using solid-state peptide synthesis (SSPS) method [5].

According to 2D cell culture process, HUVECs cultured (passage 17). When the cells proliferation accesses the sufficient level, they were transferred to three-dimensional culture. During the 3D culture process of HUVECs, the peptide was added to cells.

On the 1st, 4th and 7th days. Micro-tissue images were taken at 4X, 10X and 20X magnifications using inverted microscope The rate of live or dead cells in micro-tissues was indicated by using Double Staining Kit (Dojindo, Molecular Technologies, Inc, Japan). This protocol was applied as described in [6].

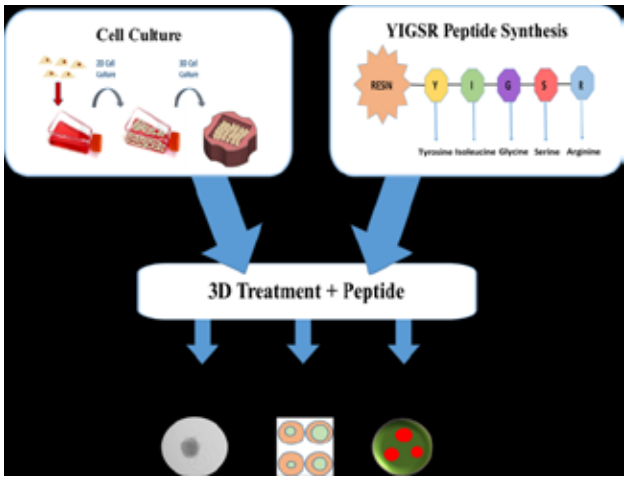

Figure.2. *Steps of YIGSR applications on micro-tissue*

III. RESULTS AND DISCUSSION

On the 1st, 4th and 7th days, very significant differences were observed at 0mM and 3mM YIGSR concentrations (***p <0.001). It is also seen that there is a significant difference at 1.5mM (***p <0.001), on 4th and 7th days. On the 1st day, there was also a significant difference in micro-tissue diameters including 1.5mM YIGSR (**p <0.05). As a result of the analysis, increasing in micro-tissue sizes was observed at 0mM and 1.5mM peptide concentrations. However, 3mM YIGSR decreased micro-tissue diameters.

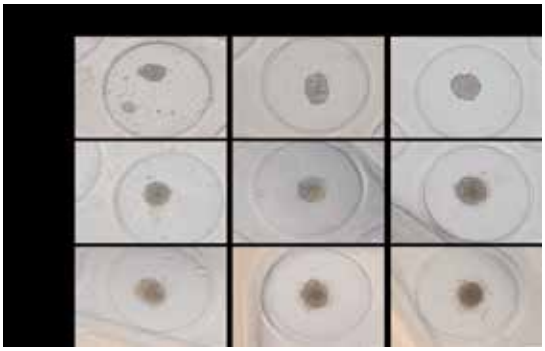

Figure.3. Images of micro-tissues at different concentrations on 1st,4th and 7th days

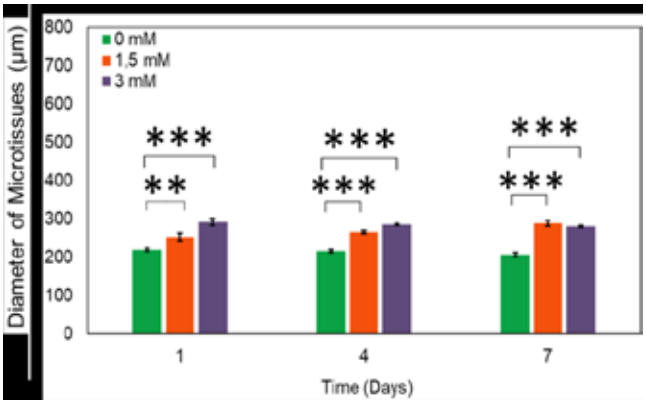

Figure.4. Changing graph of micro-tissue diameters

The viability of micro-tissues containing 3 mM peptide is lower than at 1.5 mM concentration, although it is higher than at 0 mM. Although 3 mM contributed to viability, it caused to decrease on micro-tissue diameters. When all the results are evaluated, it was determined that the most effective amount on HUVEC micro-tissue was 1.5 mM.

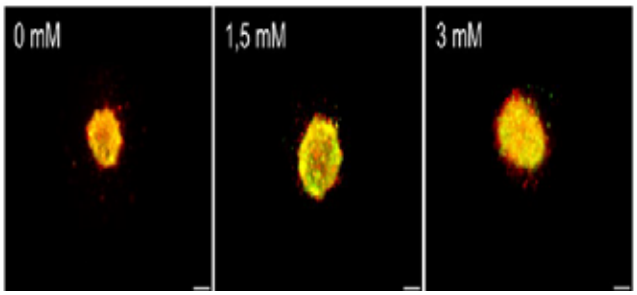

Figure.5. Viability Assay [A] at 0 mM YIGSR concentration. [B] at 1.5 mM YIGSR concentration. [C] at 3 mM YIGSR concentration.

Various studies have indicated that YIGSR has important effects in endothelial cell proliferation, cell-to-cell interactions, adhesion, production of extracellular matrix and migration [7-8]. In the light of previous studies, we have shown that YIGSR peptide at 1.5 mM and 3 mM concentrations have enhanced viability and proliferation potency on HUVEC cells [7]. Because YIGSR peptide mimics laminin, it allows cells to synthesize their own ECMs. Although the proliferation is increased in the two concentrations used, less efficacy was seen at 3 mM compared to 1.5 mM. As seen in previous studies, proliferation was reduced at high YIGSR concentrations [9]. Likewise, YIGSR peptide enhances endothelialization and tube formation [7]. It may be contributed to vascularization.

IV. CONCLUSION

In this study, we aimed to determine the optimal amount of laminin-derived YIGSR peptide for the endothelial cell. When used peptide at 0 mM, 1.5 mM and 3 mM concentrations, it is seen that the optimum amount of YIGSR for HUVECs is 1.5 mM. The amount has positive effects on cellular metabolism,

viability and proliferation of endothelial cells. Thus, 1.5mM YIGSR was found to be the most promising ratio for increasing vascularisation. Generally, the results of this study show that laminin-derived peptide can enhance the formation of HUVEC micro-tissue. The appropriate amount of YIGSR peptide can enhance survival of cells and vascularization. Therefore, we think that YIGSR may be a good option for vascular diseases and artificial organ studies.

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