RGD PEPTİDİN VASKÜLER KEMİK MİKRO DOKU FORMASYONU ÜZERİNE ETKİSİ

THE EFFECT OF RGD PEPTIDE ON VASCULAR BONE MICRO-TISSUE FORMATION

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***Abstract*— The aim of this study is to investigate the effect of RGD peptide sequence on micro-tissues formed by BMSC-HUVEC co-culture. With this aim, the RGD peptide was synthesized in the solid phase. BMSCs and HUVECs were co-cultured on agarose gel, totally 40,000 cells were used. 4 experimental groups were prepared by preparing the culture medium to 0 mg / ml, 0,5 mg / ml, 1 mg / ml and 2 mg / ml RGD. On the 1st, 3rd, 5th and 7th days, micro-tissue images were taken under different magnification scales (4x, 10x, 20x) of a microscope and viability and size analyzes were performed. Image-J software was used for size analysis. In the results of viability and size analyzes, it was determined that the optimum RGD value which effect of the micro-tissue formed by BMSC-HUVEC co-culture was 0,5 mg / ml RGD.**

Keywords — BMSC, HUVEC, Co-culture, RGD peptide Sequence.

***Özetçe*— Bu çalışmada BMSC-HUVEC ortak kültürüyle oluşturulmuş mikro dokulara RGD peptit sekansı etkisinin incelenmesi amaçlanmıştır. Bu doğrultuda, RGD peptidi katı fazda sentezlenmiştir. BMSC ve HUVEC toplamda her bir kuyu için 40.000 hücre olacak şekilde agaroz jel üzerinde ortak kültüre alınmıştır. Oluşturulmuş ortak hücre kültürüne 0 mg/ml, 0,5 mg/ml,1 mg/ml ve 2 mg/ml RGD olacak şekilde besi yerleri hazırlanarak 4 deney grubu oluşturulmuştur. 1., 3., 5. ve 7. günlerde farklı büyütme ölçeklerinde (4x, 10x, 20x) mikroskop altında görüntüleri alınarak canlılık ve boyut analizleri yapılmıştır. Boyut analizi için Image-J programı kullanılmıştır. Canlılık ve boyut analizleri sonuçlarında BMSC-HUVEC ortak kültürüyle oluşturulmuş olan mikro dokuya optimum düzeyde etkiyi 0,5 mg/ml RGD değerinin sağladığı tespit edilmiştir.**

Anahtar Kelimeler — BMSC, HUVEC, Ortak kültür, RGD Peptid Dizisi.

I. INTRODUCTION

Stem cells are the main cell types that make up the tissues and organs of multicellular organisms, and they can also make tissue repairs by self-renewing. Bone Marrow Mesenchymal Stem Cells (BMSCs) have the feature of self-renewal, which is the general feature of tissue and stem cells. Besides, they can differentiate into many connective tissues, so they are used in bone regeneration studies [1]. Vascularization has an important role in terms of nutrient and oxygen transport to the cells. Endothelial cells are in the inner part of the vascular structure [2]. Endothelial cells communicate with similar cells in prolonged and causes angiogenesis. This contact is due to cell-cell adhesions between endothelial cells [3]. HUVEC is a highly suitable model for the endothelial layer in vivo. It has basic properties of endothelial cells and it is easy to find and cheap since it can be obtained from a tissue removed immediately after birth. With the BMSC differentiation and self-renewability, it is aimed to obtain 3D micro-tissue with ossification in the co-culture with HUVEC. The integrin molecules, which are called attachment receptors, bind to Arginine-Glycine-Aspartate (RGD) peptide sequence and provide cell binding, and cell binding is an important factor in cell proliferation and differentiation [4]. RGD peptide is one of the most studied and used peptides for bone tissue studies as it can bind to multiple integrins to promote cell adhesion and differentiation [5]. In this way, the RGD sequence is expected to affect cell attachment and proliferation positively in the HUVEC-BMSC co-culture. The aim of this study is to determine the optimal RGD peptide value for pre-ossification micro-tissue formation in BMSC-HUVEC co-culture.

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II. MATERIALS & METHODS

A. GRGDS Peptide Synthesis

Synthesis of Gly-Arg-Gly-Asp-Ser (GRGDS) peptide sequence was synthesized in the solid phase on Rink Amide NovaGel resin [6].

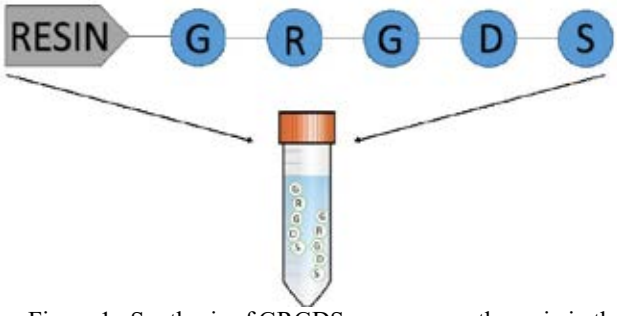

Figure 1.: Synthesis of GRGDS sequence on the resin in the solid phase.

B. 2D Cell Culture

HUVEC are grown in Dulbecco's Modified Eagle's Medium (DMEM), enriched with 10% FBS, 1% L-glutamine, and 0.1% penicillin/streptomycin and its medium was changed every two days, and BMSCs are grown in Alpha Modified Eagle's Medium (α-MEM) enriched with 10% FBS, 1% L-glutamine, 0.1% penicillin / streptomycin, and its medium was changed every 10 days, they were cultured in an incubator containing 37 ° C and 5% CO_2 air.

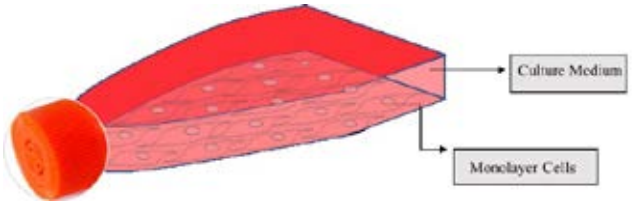

Figure 2.: Monolayer view of cells in 2D cell culture.

C. Co-Culture

Co-culture spheroids of BMSCs with HUVECs were generated statically. BMSCs (10,000 cells each per spheroid) and HUVECs (30,000 cells each per spheroid) were mixed, centrifuged and resuspended in co-culture media of DMEM: ECM/ supplement mix (1:1) containing 10 % FBS and 0.25 % (w/v) methylcellulose. Mixed cells were then seeded into nonadherent round-bottom 24-well plates (Fisher) to generate overnight BMSC/HUVEC spheroids containing 40,000 cells /spheroid.

D. 3D Cell Culture

It was produced using an agarose micro-mold in 1:3 ratios to form micro-tissue with BMSCs and HUVECs. By providing aseptic conditions, 330 µL of molten agarose was placed in a 3D petri dish.

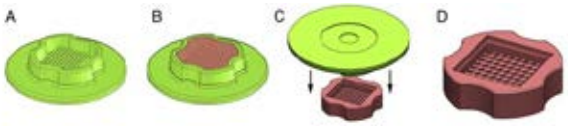

Figure 3.: Micro-tissue formation [7].

E. Micro Tissue Characterization

Microscopic development of cells was observed by adding the synthesized GRGDS peptide sequence to the BMSC-HUVEC co-culture. On the 1st, 3rd, 5th and 7th days, micro-tissue images were taken with different magnification scales (4x, 10x, 20x). Diameter sizes were measured in the Image-J (NIH) software with a 10x magnification scale.

III. RESULTS AND DISCUSSION

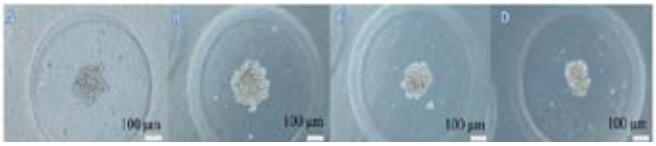

Figure 4.: For 0 mg/ml RGD, A) 1st day 10x image B) 3rd day 10x image C) 5th day 10x image D) 7th day 10x image

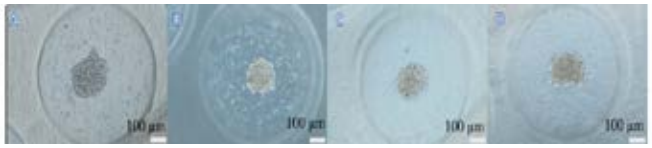

Figure 5.: For 0.5 mg/ml RGD, A) 1st day 10x image B) 3rd day 10x image C) 5th day 10x image D) 7th day 10x image

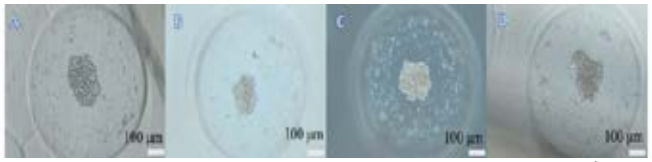

Figure 6.: For 1 mg/ml RGD, A) 1st day 10x image B) 3rd day 10x image C) 5th day 10x image D) 7th day 10x image

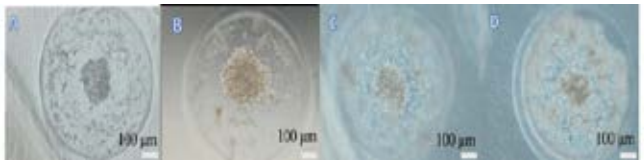

Figure 7.: For 2 mg/ml RGD, A) 1st day 10x image B) 3rd day 10x image C) 5th day 10x image D) 7th day 10x image

As observed in the evaluated micro-tissue images, the micro-tissue form maintained its form for groups containing 0 mg / ml RGD and 0.5 mg / ml RGD from day 1. In the experimental group containing 1 mg / ml RGD, micro-tissue deformations were observed at 5th and 7th days. In the experimental group containing 2 mg / ml RGD, a significant distortion was observed, and a noticeable deterioration was observed in the form of tissue.

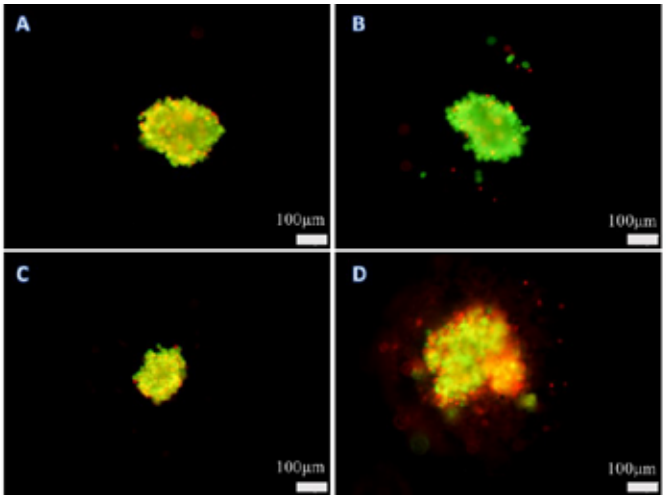

Figure 8.: 10x Combine image of micro-tissues, A) for 0 mg/ml RGD, B) for 0.5 mg/ml RGD, C) for 1 mg/ml RGD, D) for 2 mg/ml RGD.

As observed in Figure 8, in the experimental group containing 0.5 mg / ml RGD, the viability of the cells indicated by green color was quite high and red color was quite low compared to the other experimental groups. However, in the experimental group containing 2 mg / ml RGD, the mortality rate of cells which is indicated by red color was significantly higher than the other experimental groups, while viability rates of cells which is indicated by green color is quite low compared to other experimental groups.

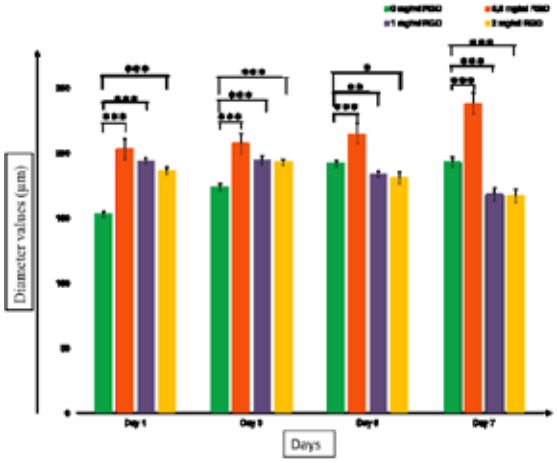

Figure 9.: Diameters of micro-tissues in different RGD concentrations analyzed on different days (p * <0.05, p ** <0.01, p *** <0.001)

IV. CONCLUSION

The optimum value of the RGD peptide, which was added at different concentrations to the micro-tissues formed by the HUVEC-BMSC co-culture, was determined as 0.5 mg / ml RGD as a result of performed size and viability analyzes. It was thought that this study would create an idea for the other investigations in terms of determining the optimum RGD concentration for cell co-culture and observing that 1mg/ml RGD peptide and 2 mg/ml RGD peptide concentrations have negative effects on micro-tissue growth and cell viability.

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