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INTERNATIONAL ADVANCED RESEARCHES and ENGINEERING JOURNAL

Journal homepage: www.dergipark.org.tr/en/pub/iarej

e-ISSN: 2618-575X

International Open Access

Volume 07
Issue 02

August, 2023

Research Article

The promoter effect of laminin-derived IKVAV peptide on three dimensional HUVEC microtissue

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ARTICLE INFO

Article history:
Received 31 December 2022
Accepted 30 May 2023
Published 15 August 2023

Keywords:
Huvec cell line
Ikvav peptide
Microtissue

ABSTRACT

Tissue engineering research is recently a popular field but the vascularization process of existing methods limits the study area. Human Umbilical Vein Endothelial Cells (HUVEC) are essential cell models for vascularization study in vitro. Although studies about vascular biomaterial are mostly performed in traditional 2 Dimensional (D) cell culture, the system has some disadvantages. However, 3D scaffold-free microtissue can be used to overcome these disadvantages for the identification of the optimum concentration of biomaterials. IKVAV is an active unit of laminin which is an effective protein in the extracellular matrix. IKVAV may increase cell adhesion, proliferation, migration, and cellular differentiation. Since IKVAV directly affects endothelial cells, the definition of the optimum concentration of IKVAV is critically important for HUVEC growth and viability during vascularization. Thus, the study aimed identification of the optimal IKVAV peptide concentration for the production and viability of 3D HUVEC SFM. After peptide synthesis, 3D SFM was fabricated. 0.5 mM and 1 mM concentrations of IKVAV peptide were treated with SFM. The control group was incubated without any peptide concentration. Diameters and viabilities of SFMs were evaluated. 1 mM concentration showed the highest diameter and viability. The increasing concentrations may support HUVEC growth and viability so it may induce vascularization in vivo conditions.

1. Introduction

Tissue engineering has an important field that has recently become popular. Tissue engineering aims to provide restoration and regeneration of biological tissues by using cells, engineering science, and biomaterials. Vascularization is one of the major challenges in tissue engineering [1]. During vascularization, endothelial cells produce new blood vessels [2]. One of the model cell lines in vasculogenesis is Human Umbilical Vein Endothelial Cells (HUVEC) [3, 4]. Endothelial cells are cells that can progress through a cell-matrix adhesion complex mediated by integrin and laminin in the extracellular matrix in vascular development [3, 4]. Prevascularization may be supported by biomaterials such as growth factors, cytokines, peptides, and proteins to generate new vessels [1, 2].

Many different studies can be performed with traditional cell culture techniques to understand the effectiveness of vascular biomaterials. Traditional cell culture systems give molecular cues to define the efficacy of biomaterials in vitro conditions [5]. As traditional 2 Dimensional (D) cell culture has low cost and easy handling, it is a preferable technique for the research of biomaterials. Thus, the primarily studies in tissue engineering are performed on traditional 2D cell culture. However, 2D cell culture has some limitations [3, 6, 7]. 2D cell culture cannot mimic the natural tissues [3, 8]. 2D cell culture outrightly presents communications between cell to cell [5, 8]. Moreover, it may show limited cell to matrix [3, 8]. Since the definition of the optimum dosage of biomaterials or drugs in tissue engineering research is critical, the traditional 2D cell culture may be limited. Thus, new techniques are needed to evaluate the efficacy of the components. 3D scaffold-free cell culture system may mimic physical and biochemical features of natural tissue in vitro conditions [5, 7, 8]. Since 3D scaffold-free microtissue (3D SFMs) consists of cell clusters, it constructs tissue depth. Thus, the therapeutic agents pass through the tissue depth. 3D SFMs create the penetration field for therapeutic agents [9]. 3D culture has a higher in vivo mimicking capacity than 2D cell culture [3, 4, 8, 9].

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DOI: 10.35860/iarej.1227443

Thus, 3D SFM may be one of the qualified techniques for the measurement of the optimal concentration of biomaterials.

The use of peptides is quite common in tissue engineering and regenerative medicine studies as biomaterials. Peptides are the effects of short active sequences in long protein chains [3, 4]. It is known that peptides are effective biomaterials in cellular events such as cell growth, proliferation, migration, adhesion, and differentiation [10-12]. However, these effects can be stimulatory or suppressive depending on the cell and peptide types [12]. Laminin is a large cross-shaped glycoprotein commonly found in the extracellular matrix of basement membranes [11]. Laminin has 15 different units which consist of a unique combination of three subchains [13]. In case of mutation of laminin, cells may face death. Thus, laminin is critically important for cell viability. Moreover, it also has biological activities such as cell differentiation, cell polarity, and angiogenesis [14-16]. As an extracellular matrix protein (ECM) laminin may provide adhesiveness for donor cells and subsequently mediate cellular signaling events [17]. In addition, laminin-derived peptides may increase the interaction of cells for angiogenesis [18]. IKVAV (Ile-Lys-Val-Ala-Val) is an active sequence of laminin-1 located at the C-terminal end of the long arm of the α1 chain [19, 20]. IKVAV promotes cell adhesion, proliferation, migration, cellular differentiation, and collagenase IV production. The peptide interacts directly with endothelial cells in vitro and elicits certain biological activities, including attachment, migration, and invasion, which are critical during vessel formation [21]. IKVAV peptide promotes HUVEC growth, differentiation, angiogenesis, re-epithelialization, and collagen deposition [22, 23].

IKVAV peptide may enhance adhesion, differentiation, proliferation, and angiogenesis [24-26]. It is critically important to identify the optimum concentration of the IKVAV peptide for HUVEC proliferation and viability. However, it has not been evaluated that the effects of IKVAV sequence on the fabrication of 3D HUVEC SFM, yet. Therefore, the novelty of the study is the first research for the identification of the optimal IKVAV peptide concentration for the production and viability of 3D HUVEC SFM (as seen in Figure 1.).

2. Materials and Method

2.1 Materials

While all peptide products were purchased from Aapptec (Louisville, KY, USA), all cell culture products were taken from Sigma (St. Louis, Missouri, USA). Endothelial Basal Medium-2 (EBM-2) was taken from Lonza, (C3156, Basel, Switzerland). Live and Dead Cell Double Staining Kit was purchased from Dojindo (CS01-10, Munich, German).

2.2 Solid Phase Peptide Synthesis

The solid phase peptide synthesis of IKVAV was performed by following previous studies [3, 4, 27, 28]. Rink Amide MBHA resin in peptide synthesis and 2 eq O-Benzotriazole-N,N,N',N'-tetramethyluronium-hexafluoro-phosphate (HBTU), 4 eq N,N-Diisopropylethylamine (DIEA), 2 eq Hydroxybenzotriazole monohydrate (HOBt), 4 eq of amino acids were mixed in chromatography tube. Then, N,N-Dimethylformamide (DMF) was added and mixed at 320 rpm for 30 minutes to swell the resin. DMF was removed and added 20% cyclohexylamine for the removal of F-moc protecting groups.

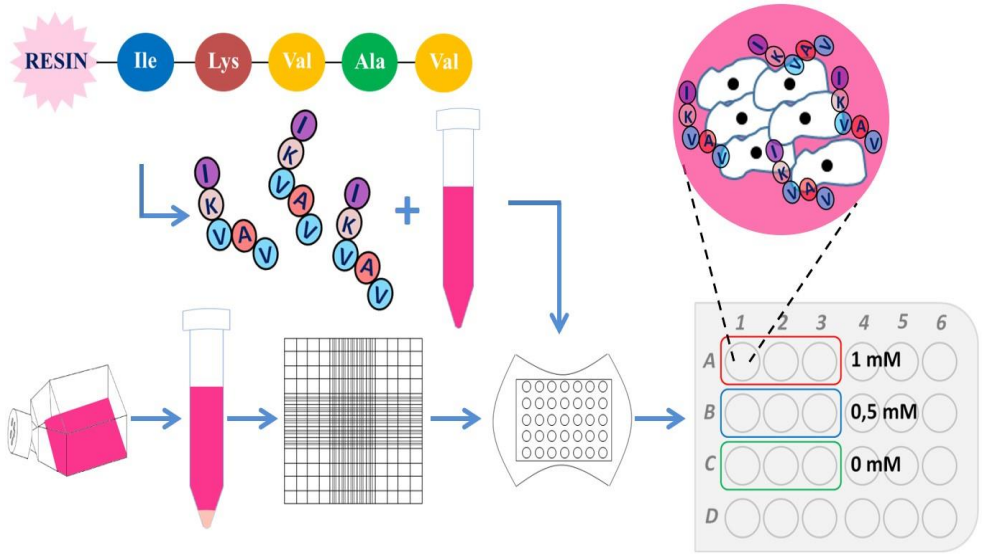

Figure 1. Treatment of HUVEC microtissues with 0.5 mM and 1 mM concentrations of IKVAV peptide. After IKVAV peptide was synthesized on resin, counted cells were added agarose mold to construct microtissue. The 0.5 mM and 1 mM concentrations of IKVAV were mixed with media and added into HUVEC microtissues

The kaiser test (or ninhydrin test) was performed to check whether the F-moc were separated or not. After all, IKVAV amino acids (Isoleucine, Lysine, Valine, Alanine, and Valine) were respectively added the peptide sequence was washed with Dichloromethane (DCM). For each 100 mg of used resin, 1 ml of cyclohexylamine was added and mixed. Then, a Trifluoroacetic acid (TFA) solution containing 2.5% Deionized Water (DIW), 2.5% Triisopropylsilane (TIPS) was prepared. The tube was held upright and uncapped for an hour and gently mixed every 20 minutes. After 1 hour, all of the TFA solution liquid in the tube was precipitated into the cold ether in the falcon tube. The falcon tube was centrifuged 3 times at 4500 rpm and 25°C for 10 minutes. The supernatant was poured out. The peptide was lyophilized at 5 hours.

2.3 HUVEC Cell Culture

Cell culture of HUVEC was performed by following previous studies [4, 8]. Dulbecco's Modified Eagle Medium (DMEM) was used as nutrient medium containing 10% Fetal Bovine Serum (FBS), 1% L-Glutamine and 1% Penicillin streptomycin for HUVEC cell culture. The nutrient medium of HUVEC at passage number 17 (Aegean University, Bioengineering) was kept in 2D cell culture at 5% carbon dioxide and 36.5°C conditions. After washing the cells with Phosphate Buffer Saline (PBS), trypsin was added and incubated for 5 minutes. The cells were separated from the surface and became suspended. Then, cells in suspension were centrifuged at +4°C and 900 rpm for 5 minutes.

2.4 Fabrication of 3D SFM and IKVAV Peptide Treatment

The 3D SFM fabrication process was performed according to previous studies [3, 4, 8]. To construct SFM, agarose molds with a volume of 330 µl were prepared using 2% agarose gel. It was kept in sterile PBS in a 24-well plate. $6x10^4$ /75 µl cells were seeded for each agarose well. After cells were incubated for 30 minutes to construct microtissue. EBM-2 nutrient media without serum were prepared at 0 mM, 0.5 mM and 1 mM peptide concentrations. 0 mM group was used as control group which doesn't contain any peptide or serum concentration. 500 µl nutrient medium was added for each agarose mold. At least 3 SFM were created for each group.

2.5 Measurement of SFM Diameters

All SFM was observed under the inverted microscope to define SFM diameter and form as descripted in previous studies [3, 4, 8]. SFM images were taken on the 1st, 4th and 7th days. Microscope images were taken at 10x magnification by using the Olympus microscope-computer interface (CKX41, Olympus, Tokyo, Japan). Diameter lengths of SFM were obtained from the 3 different microtissues by the ImageJ program (NIH, LOCI, University of Wisconsin).

2.6 SFM Viability Analysis

Double Staining Kit was used to determine the ratio of live and dead cells in the microtissues formed at day 7 [3, 4, 8]. After the nutrient media of the SFM was discarded, each agarose mold was washed with PBS. The washing process was repeated 5 times. After this stage, the protocol specified in [26] was applied. Calcein-AM/DMSO, green fluorescence and propidium iodide/purified water, red fluorescence were stained after 15 min of incubation. Images of live and dead cells were taken under a fluorescence microscope. Green dye indicates live cells and red dye indicates dead cells. The green and red images taken were combined using an Olympus microscope-computer interface.

2.7 Statistical Analysis of the Experimental Set-up

Using the commercial software GraphPad Prism (San Diego, CA, USA), the datas of SFM diameter were statistically analyzed. Each three repeated experiment were recorded to take A standard deviation. One-Way ANOVA method was used and results were evaluated. If P value was less than 0.05, it was accepted as statistically significant.

3. Results and Discussion

3.1 SFM Diameter Measurement

All SFM of all peptide groups was observed under the microscope. Microscope images of SFM were taken at the 1st, 4th and 7th days as shown in Figure 2. All SFM images showed great SFM formation in all peptide treatment groups from the first time point to the end time point.

The average diameter graphics of 3D SFM were created by measuring the diameter values of SFM images by using the ImageJ program as shown in Figure 3. The results of the peptide treatment group diameter were compared with the results of the control group.

Mean values of diameters obtained from SFM of 0 mM, 0.5 mM, and 1 mM IKVAV peptide were 227.15 ± 8.35 µm, 239.78 ± 5.34 µm, and 283.09 ± 8.03 µm (p *** <0.001) on day 1, respectively. Statistically significance was observed between 0.5 mM and 1 mM concentrations. The average diameter values obtained from 0 mM, 0.5 mM, and 1 mM IKVAV peptide were 223.96 ± 2.34 µm, 246.95 ± 5.16 µm (p *** <0.001), and 272.34 ± 2.94 µm (p *** <0.001) on day 4, respectively. Statistically significance was observed between 0.5 mM and 1 mM concentrations. Finally, average diameter values obtained from 0 mM, 0.5 mM and 1 mM IKVAV peptide were 204.85 ± 11.04 µm, 274.12 ± 2.25 µm (p *** <0.001), and 292.12 ± 2.63 µm (p *** < 0.001) on the 7th day. Statistically significance was observed between 0.5 mM and 1 mM concentrations.

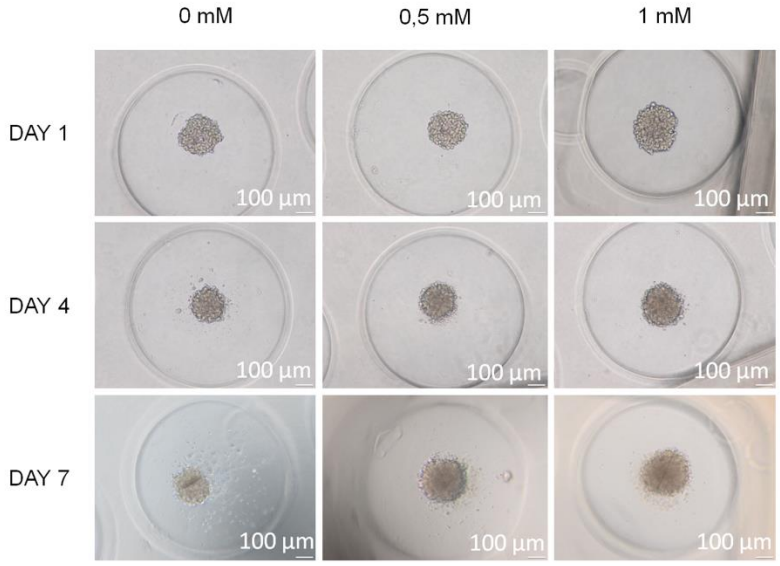

Figure 2. Microscope images of 3D HUVEC SFM on days 1, 4 and 7

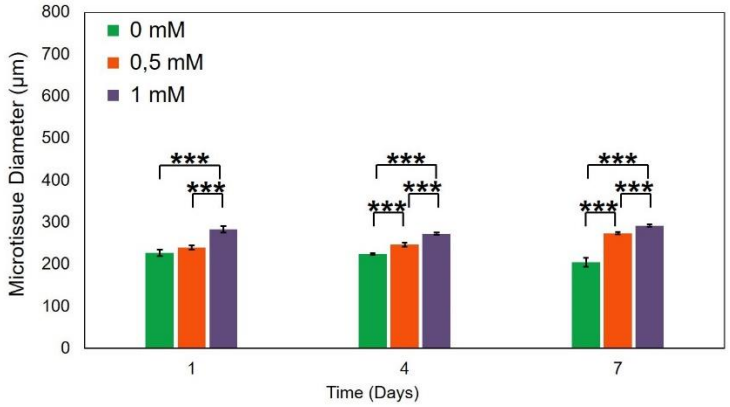

Figure 3. Changes of 3D HUVEC SFM diameters depending on time points

The average diameter results of SFM showed that IKVAV peptide treatment was higher SFM enlargement than the control group. 0.5 mM and 1 mM IKVAV peptide concentrations were effective on the proliferation of 3D HUVEC SFM even on day 1. Diameters of all SFM increased depending on time points. It was shown that 1 mM concentration of IKVAV peptide supported more SFM enlargement than the control and 0.5 mM concentration of IKVAV. The enlargement was observed significant. Moreover, 0.5 mM concentration of IKVAV peptide was also effective in terms of SFM enlargement compared with the control group. However, shrinkage was observed in the peptide-free control group (0 mM) SFM until the 7th day.

It was understood that the IKVAV peptide supported the HUVEC proliferation. IKVAV was a critically important contribution to HUVEC microtissue enlargement. There was a steady increase in the diameters of the microtissues in 0.5 mM and 1 mM peptide concentrations. It was observed that the diameter of all SFM in 0.5 mM and 1 mM peptide concentrations increased until the 7th day. However, the control group showed diameter shrinkage. The reason is likely lack of serum concentration [29]. Karaman et al. showed that the diameter of SFMs may decrease in case of serum starvation because serum is critical important for cell viability and proliferation. Even though the serum concentration didn't include the media, the diameter of microtissues with peptide concentrations increased. The success of microtissue enlargement may depend on capability of IKVAV. The effectiveness of 0.5 mM and 1 mM peptide concentrations was consistent with the literature information. Previous studies have shown that peptide therapy promoted cellular communications and high cellular density in cell culture [16, 30]. It was concluded that 0.5 mM and 1 mM concentrations of IKVAV peptide may stimulate the growth of cells in all SFM and increase the interactions of HUVEC. However, 1 mM concentration of IKVAV showed more diameter enlargement.

3.2 Viability Analysis of 3D HUVEC SFM

On the 7th day, viability analysis was performed on SFM as shown in Figure 4. Live and dead staining was performed to analyze the efficacy of different IKVAV concentrations by comparing the control group. It was observed that the rate

of dead cells in the control group was higher than including IKVAV concentration. Green cells were also observed in all groups. However, the 0.5 mM IKVAV group showed more green cells than the control group. Similarly, green cells in 1 mM IKVAV peptide were more than the control group and 0.5 mM IKVAV peptide group. All the considerations, 0.5 mM and 1 mM IKVAV peptide concentrations were effective groups that increase 3D HUVEC SFM viability.

As a result, the diameter and viability of microtissues were rather than the control group. The diameter enlargement relates to cell proliferation in SFM [3, 4, 8]. Our results indicated that the viability of SFM including IKVAV was higher than the non-containing (control group). Since the green cell was more than the control group, it was observed IKVAV promoted the viability of SFM.

A study which was conducted by Righi et al. showed that 1.2 mM concentration of IKVAV coatings contributes to the adhesion and proliferation of neural cells after 24 hours. The concentration increased cell differentiation by affecting cell-to-matrix binding [30]. Moreover, IKVAV peptide-coated surface may support selective differentiation in neural tissue [31]. Another study used hydrogels with 0.2 mM concentration of the IKVAV on human melanoma cells. In the study, IKVAV peptide provided cell adhesion and proliferation [32]. Hosseinkhani et al. also used 0.2 mM concentration of IKVAV on dorsal root ganglia cells. It was found that IKVAV was an effective sequence in cell proliferation [33] similar to our results. Parallel to our results Kibbey et al. observed that 3.8 mM concentration of SIKVAV enhanced HUVEC proliferation, migration, and angiogenesis [21]. Grant et al. proved that protein-mimicking peptides may be effective units in terms of the attachment and adhesion of HUVEC on Matrigel. In addition, the IKVAV sequence in the study supported angiogenesis. While the IKVAV peptide with non-sequential amino acid sequences did not affect angiogenesis, the correct sequence peptide showed an angiogenesis-stimulating effect [26].

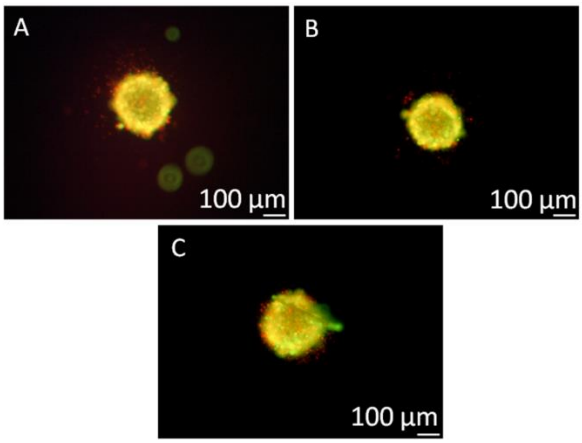

Figure 4. Viability effect of IKVAV peptide on 3D HUVEC SFM at day 7. (A) SFM within 0 mM IKVAV peptide concentration (B) SFM within 0.5 mM IKVAV peptide concentration. (C) SFM within 1 mM IKVAV peptide concentration

Nomizu et al. researched the IKVAV sequence, which is one of the most potent active sites of laminin-1, and showed the promotion of cell adhesion. 1 mM concentration of IKVAV peptide increased cell proliferation in the study [34]. Another study used different concentrations ranging from 0.001 mM to 10 mM of IKVAV [35].

It exhibited that IKVAV promoted cell attachment in fibroblasts. Over 1 mM concentration of IKVAV decreased the attachment and migration of fibroblasts. They concluded that the interaction of actin fiber structure was related to the IKVAV peptide so it has a capacity of cells to respond.

4. Conclusion

In this study, the effects of 0.5 mM and 1 mM IKVAV concentrations on 3D HUVEC SFM were evaluated. Although 0.5 mM and 1 mM concentration groups of IKVAV peptide observed higher diameter enlargement and cell viability than peptide free group, 1 mM concentration was the optimum concentration in terms of cell proliferation and viability. 1 mM concentration of IKVAV may be the optimum concentration for vascular research.

Declaration

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

Z.B.Y.Ç. designed, developed, performed analysis, concluded and written. B.K. performed the analysis, reported and written. O.K designed, supported grants and proofread the manuscript.

Acknowledgment

The authors acknowledge Biomedical Test and Calibration Research Center, Katip Çelebi University for laboratory opportunities and thank to Assoc. Prof. Dr. Aylin Şendemir Urkmez who is head of Animal Cell Culture Laboratory, Bioengineering Department, Ege University for HUVEC line.

References

1. Novosel, E.C., C. Kleinhans, and P.J. Kluger, Vascularization is the key challenge in tissue engineering. Adv Drug Deliv Rev, 2011. 63(4-5): p. 300-11.
2. Baiguera, S. and D. Ribatti, Endothelialization approaches for viable engineered tissues. Angiogenesis, 2013. 16(1): p. 1-14.
3. Yaralı, Z.B., G. Onak, and O. Karaman, Effect of Integrin Binding Peptide on Vascularization of Scaffold-Free Microtissue Spheroids. Tissue Engineering and Regenerative Medicine, 2020. 17(5): p. 595-605.
4. ÇEVİK, Z.B.Y., A. Ördek, and O. Karaman, Regulatory effects of laminin derived peptide on microtissue formation for tissue engineered scaffold-free constructs. 2022.

5. Kapałczyńska, M., et al., 2D and 3D cell cultures–a comparison of different types of cancer cell cultures. 2018. 14(4): p. 910-919.
6. Mironov, V.A., et al., Design, Fabrication, and Application of Mini-Scaffolds for Cell Components in Tissue Engineering. Polymers (Basel), 2022. 14(23).
7. Anthon, S.G. and K.P. Valente, Vascularization Strategies in 3D Cell Culture Models: From Scaffold-Free Models to 3D Bioprinting. Int J Mol Sci, 2022. 23(23).
8. Çevik, Z.B.Y., et al., Photobiomodulation therapy at red and near-infrared wavelengths for osteogenic differentiation in the scaffold-free microtissues. 2022: p. 112615.
9. Martins, A.M., et al., Efficacy of molecular and nano-therapies on brain tumor models in microfluidic devices. Biomaterials Advances, 2023. 144: p. 213227.
10. Iorio, V., L.D. Troughton, and K.J. Hamill, Laminins: Roles and Utility in Wound Repair. Adv Wound Care (New Rochelle), 2015. 4(4): p. 250-263.
11. Freire, E., et al., Structure of laminin substrate modulates cellular signaling for neuritogenesis. J Cell Sci, 2002. 115(Pt 24): p. 4867-76.
12. Sporn, M.B. and A.B. Roberts, Peptide growth factors are multifunctional. Nature, 1988. 332(6161): p. 217-9.
13. Guldager Kring Rasmussen, D. and M.A. Karsdal, Chapter 29 - Laminins, in Biochemistry of Collagens, Laminins and Elastin, M.A. Karsdal, Editor. 2016, Academic Press. p. 163-196.
14. Aumailley, M., The laminin family. Cell Adh Migr, 2013. 7(1): p. 48-55.
15. Kikkawa, Y., et al., Laminin-111-derived peptides and cancer. Cell Adh Migr, 2013. 7(1): p. 150-256.
16. Yu, X., G.P. Dillon, and R.B. Bellamkonda, A laminin and nerve growth factor-laden three-dimensional scaffold for enhanced neurite extension. Tissue Eng, 1999. 5(4): p. 291-304.
17. Tate, C.C., et al., Laminin and fibronectin scaffolds enhance neural stem cell transplantation into the injured brain. J Tissue Eng Regen Med, 2009. 3(3): p. 208-17.
18. Taubenberger, A.V., et al., 3D extracellular matrix interactions modulate tumour cell growth, invasion and angiogenesis in engineered tumour microenvironments. Acta Biomater, 2016. 36: p. 73-85.
19. Kazemi, S., et al., IKVAV-linked cell membrane-spanning peptide treatment induces neuronal reactivation following spinal cord injury. 2015. 1(4): p. null.
20. Perera, T.H., X. Lu, and L.A. Smith Callahan, Effect of Laminin Derived Peptides IKVAV and LRE Tethered to Hyaluronic Acid on hiPSC Derived Neural Stem Cell Morphology, Attachment and Neurite Extension. 2020. 11(1): p. 15.
21. Kibbey, M.C., et al., Laminin SIKVAV peptide-induced angiogenesis in vivo is potentiated by neutrophils. J Cell Physiol, 1994. 160(1): p. 185-93.
22. Jung, J.P., J.V. Moyano, and J.H. Collier, Multifactorial optimization of endothelial cell growth using modular synthetic extracellular matrices. Integr Biol (Camb), 2011. 3(3): p. 185-96.
23. Chen, S., et al., A laminin mimetic peptide SIKVAV-conjugated chitosan hydrogel promoting wound healing by enhancing angiogenesis, re-epithelialization and collagen deposition. Journal of Materials Chemistry B, 2015. 3(33): p. 6798-6804.
24. Assal, Y., M. Mie, and E. Kobatake, The promotion of angiogenesis by growth factors integrated with ECM proteins through coiled-coil structures. Biomaterials, 2013. 34(13): p. 3315-23.
25. Silva, G.A., et al., Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science, 2004. 303(5662): p. 1352-5.
26. Grant, D.S., et al., *Interaction of endothelial cells with a laminin A chain peptide (SIKVAV) in vitro and induction of angiogenic behavior in vivo*. J Cell Physiol, 1992. 153(3): p. 614-25.
27. Amblard, M., et al., *Methods and protocols of modern solid phase Peptide synthesis.* Mol Biotechnol, 2006. 33(3): p. 239-54.
28. Merrifield, R.B., *Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide.* Journal of the American Chemical Society, 1963. 85(14): p. 2149-2154.
29. Karaman, O. and Z.B.J.T.E.R.J. YARALI, Determination of minimum serum concentration to develop scaffold free micro-tissue. 2018. 4(3): p. 145-151.
30. Righi, M., et al., *Peptide-based coatings for flexible implantable neural interfaces.* Scientific Reports, 2018. 8(1): p. 502.
31. Massia, S.P., M.M. Holecko, and G.R. Ehteshami, *In vitro assessment of bioactive coatings for neural implant applications.* J Biomed Mater Res A, 2004. 68(1): p. 177-86.
32. Reis, E.M.D., et al., *Bacterial nanocellulose-IKVAV hydrogel matrix modulates melanoma tumor cell adhesion and proliferation and induces vasculogenic mimicry in vitro.* J Biomed Mater Res B Appl Biomater, 2018. 106(8): p. 2741-2749.
33. Hosseinkhani, H., et al., *Engineering three-dimensional collagen-IKVAV matrix to mimic neural microenvironment.* 2013. 4 8: p. 1229-35.
34. Nomizu, M., et al., *Structure-activity study of a laminin alpha 1 chain active peptide segment Ile-Lys-Val-Ala-Val (IKVAV).* FEBS Lett, 1995. 365(2-3): p. 227-31.
35. Kasai, S., et al., *Multifunctional peptide fibrils for biomedical materials.* Biopolymers, 2004. 76(1): p. 27-33.