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Research Article

Improved endothelial cell proliferation on laminin-derived peptide conjugated nanofibrous microtubes using custom made bioreactor

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ABSTRACT

Cardiovascular diseases (CVD) are currently considered as one of the major reasons for death worldwide. The blockage of minor vessels such as the coronary arteries may be linked to more severe occurrences that might be fatal. The gold standard approach involves the transplantation of secondary vessels or the use of synthetic vascular grafts. Electrospun nanofiber (NF) based grafts produced with synthetic polymers might be simply modified to resemble the original structure of vessels providing desirable physical features and potentially improving cellular behavior including cell attachment, growth, and differentiation. Although poly lactic-co-glycolic acid (PLGA), is well-known, commercially available, degradable synthetic, has good mechanical and biocompatibility properties, PLGA is inadequate in terms of cell recognition signals. To overcome the bioactivity problem of PLGA, bioactive peptides are the most extensively utilized approach for surface modification. On the other hand, seeding and cultivation of tube-like conduits are challenging due to their shapes, and dynamic seeding and culture are considered beneficial for these grafts. Herein, we attempted to enhance the Endothelial Cells (ECs) attachment and proliferation on PLGA electrospun NF-based vascular grafts by both the conjugation of laminin-derived peptide IKVAV and perfusion culture with the custom-made bioreactor system. The bioreactor and its flow and pressure were simulated and decided using COMSOL Multiphysics 5.4. Human umbilical vein endothelial cell (HUVEC) adhesion and proliferation were increased by both functionalization of PLGA graft with IKVAV and using a custom-made perfusion bioreactor for cell seeding and cultivation within 7 days (d). This tubular vascular graft could be a potential tissue-engineered scaffold for the restoration of the venous system.

1. Introduction

Cardiovascular disease (CVD) is one of the most common reasons for mortality and disability in the world [1]. Surgical intervention using a bypass graft is a common method used to repair a damaged blood artery. Nevertheless, these vasculatures are generally inadequate in availability due to the condition of patients. Tissue-engineered grafts are an alternative to autografts due to their unlimited availability, a wide variety of material choices, and ability to be modified their properties to provide the mechanical features of natural vessels, their potential to outperform autografts in the future [2].

The use of biodegradable synthetic grafts, including poly(lactic acid), poly(glycolic acid) copolymer poly lactic-co-glycolic acid (PLGA), as a new generation of higher efficiency vascular grafts with small diameter are being investigated [3]. Although PLGA has an adjustable degradation profile, it is easily molded into the required shape, and has good mechanical strength. Despite these benefits, scaffolds made of synthetic polymers are inadequate in terms of cell recognition signals. Surface functionalization may be required in some circumstances to increase cell adherence to the surface. Due to their efficiency in cell attachment, proliferation, adhesion, migration, growth, and differentiation, peptides are the most extensively utilized approach for surface modification [4]. The diversity of peptides aids applications to mimic the native tissues. One of the first appearing proteins of the extracellular matrix (ECM) during embryogenesis is laminin which is essential for embryo development and organogenesis; angiogenesis;

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cell adhesion, migration, and differentiation. The laminin-derived IKVAV peptide sequence induces capillary-like structures in endothelial cells (ECs) to encourage vascularization. It is also essential in promoting EC migration and proliferation [5].

Electrospinning is a versatile approach that allows you to quickly control the mechanical and biological features of nanofibers (NF) by changing the composition of a combination, which is not achievable with other scaffold manufacturing techniques [6]. Electrospun NF characteristics might be simply modified to imitate the natural vessel structure with desirable physical features including high porosity and a large surface area, potentially improving cellular behavior such as cell attachment, proliferation, and differentiation [7]. ECs cultivation on various electrospun NFs has previously been investigated and demonstrated the ability of the electrospinning technology to produce fibers that can be employed in natural and synthetic polymers that have been treated with various growth factors [8]. These studies show that it is possible to make a structure comparable to that of a native blood artery. PLGA electrospun NFs generally are insufficient due to their hydrophobic properties and lack of cell-recognition signals. To overcome this problem, Kim *et al.* covalently immobilized Gly-Arg-Gly-Asp-Tyr (GRGDY), a cell adhesive peptide, on PLGA and found that conjugation by surface-amine groups on PLGA NFs resulted in improved attachment of NIH 3T3 cells [9]. RGD peptide surface modification not only boosted cell adhesion but also resulted in increased proliferation.

Static and dynamic seeding and growth of cell-substrate constructs are available. A shaker, spinner flask, rotator, or perfusion device could be used for dynamic culture. Dynamic seeding and culture are considered beneficial for conduits and tube-like constructions in the literature [10, 11]. The perfusion system, on the other hand, better simulates the physiological state of blood vessels. Although there are some studies which modified electrospun NFs with peptides and showed the effect of dynamic culture and seeding on cell proliferation, separately, no study exists which demonstrated the effect of dynamic culture and seeding on ECs proliferation on IKVAV peptide conjugated electrospun PLGA tubular grafts. Herein, we attempted to improve the ECs attachment and proliferation on PLGA electrospun NF-based vascular grafts by both the conjugation of laminin-derived peptide IKVAV and perfusion culture with the custom-made bioreactor system. The effects of static and dynamic cell seeding and culturing of PLGA vascular grafts were compared on HUVEC (Human umbilical vein endothelial cell) attachment and proliferation. HUVEC attachment and proliferation were increased by both functionalization of PLGA graft with IKVAV and using custom-made perfusion bioreactor for cell seeding and cultivation. This tubular vascular graft could be a potential tissue-engineered scaffold for the regeneration of the venous system.

2. Materials and Methods

2.1 Peptide Synthesis

All chemical agents utilized to synthesize the peptide were obtained from AAPPTEC (Louisville, KY, USA). The peptide of isoleucine-lysine-valine-alanine-valine (IKVAV) was synthesized on 4-methylbenzhydrylamine (MBHA) resin (0.67 mmol/g loading capacity) [12]. The resin was added to DMF (Dimethylformamide) and swelled for 30 minutes. and rinsed with DMF two times. Then, Fmoc-protected amino acids (2 equiv.), diisopropylethylamine (DIEA; 4 equivalents), hydroxybenzotriazole (HOBt; 2 equivalents), and O-Benzotriazole-N,N,N’,N’-tetramethyluronium-hexafluoro-phosphate (HBTU; 2 equivalents) added into DMF and mixed for 6 h on an orbital shaker [13]. The incidence of unreacted amine groups was tested by Ninhydrin test which is applied to the resin solution [12]. If the positive result was obtained, the resin solution was rinsed with DMF and amino acid was coupled until the result of the Kaiser test was negative. If the result is negative, the resin was rinsed with DMF (3x3ml). Then, deprotection solution was added into resin solution for the elimination of Fmoc protecting groups. After that, Kaiser Test was applied again to check that Fmoc groups were detached. When a negative result was obtained, the deprotection step was applied again until obtaining a positive result. Finally, the resin solution was rinsed with DMF and filtered. The same procedure was applied until the targeted peptide sequence was obtained. By adding 95% trifluoroacetic acid (TFA), 2.5% distilled water and 2.5% triisopropylsilane (TIPS), the peptide was cleaved from the resin and thrown into cold-diethyl ether [14]. The solution was centrifuged at 4500 rpm and the supernatant was removed [15]. Then, the peptide was obtained after freeze-drying of pellet.

2.2 Fabrication of PLGA Vascular Grafts

The 3 wt % PLGA (85:15; PURASORB PDLG 8531; Corbion Biomaterials, the Netherlands) containing electrospinning solution was prepared in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP; Matrix Scientific; Columbia) [16]. The solution was transferred into a syringe and the syringe was placed on the syringe pump. The needle was linked to a positively charged electrode of a high voltage source. Nanofibers were ejected with 20 kV electrical potential and 1 ml/h injection rate. The ejected PLGA nanofibers were collected by a special collector designed by our group in 3 mm inner diameter to produce microtubular structures [17]. PLGA nanofiber sheets were also produced by using an aluminum rotating wheel covered with circular glass coverslips with 20 kV electrical potential, 1 ml/h injection rate, and 1200 RPM rotation speed parameters.

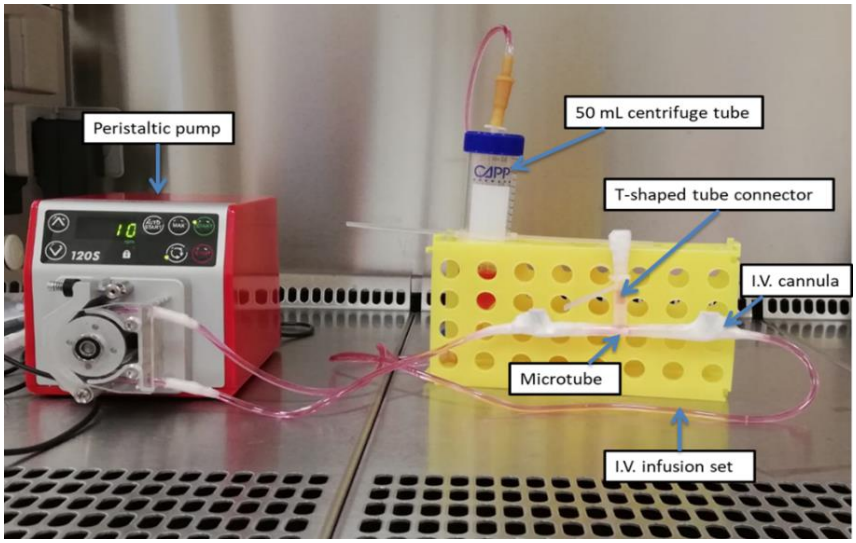

Figure 1. Perfusion Based Bioreactor System

2.3 Scanning Electron Microscopy

Scanning electron microscope (SEM; Carl Zeiss Microscopy, Germany) technique was performed for characterization of PLGA nanofibers by using with 3 kV accelerating voltage to determine the NF morphology and the wall thickness and diameter of the microtubes. The samples were coated with gold (QUORUM; Q150 RES; East Sussex; United Kingdom) at 20 mA for 60 sec and then, characterization was started [12]. The scale bars of images were measured by the software of SEM.

2.4 Peptide Conjugation of Nanofibers

Fabricated NFs were washed with deionized water. Then, they immersed in 0.1M MES (2-Morpholinoethanesulfonic acid buffer) solution with 2 mM EDC and 5 mM NHS to obtain a carboxyl-rich surface for 45 minutes at 37°C. Then, the NFs were reacted with 1 mM peptide in PBS 24 hours at 4°C.

2.5 Bioreactor Design and Simulation

Dynamic cell culture was performed using a bioreactor system developed by our group as shown in Figure 1. Microtube nanofibers were placed inside the bioreactor. All parameters were determined using COMSOL simulation, which is bioreactor flow and pressure. The system was started with a 10 RPM speed, which is approximately 4 ml/min flow rate. The custom-made bioreactor used in dynamic cell-culture was simulated COMSOL Multiphysics Simulation 5.4 to find essential parameters in terms of velocity and pressure. Table 1 summarized the input and calculated parameters for simulating the custom-made bioreactor in which the scaffold was placed.

2.6 Static and Dynamic Cell Culture

HUVECs (kindly donated from Ege University Research Group of Animal Cell Culture and Tissue Engineering Laboratory) were cultured with F12 DMEM (Dulbecco's Modified Eagle Medium) containing 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% Fetal Bovine Serum (FBS) and incubated in 5% CO_2 at 37 °C [18]. The scaffolds were sterilized with UV radiation. HUVECs ($5x10^6$ cells/cm^2) were seeded on the planer NFs and the lumen of the microtubular nanofibers. The seeding volume and the seeding are were optimized as 150 µl and 20 mm^2. The cell concentration was calculated as $6.6x10^6$ cells/ml. For static seeding, 150µl cell suspension ($6.6x10^6$ cells/ml) were inserted in the lumen and incubated for 1.5 hours. Then, the microtube was placed in the bioreactor system and the culture media was perfused for 7 days. For the dynamic seeding, the microtube was placed in a bioreactor and 10 ml cell suspension ($6.6x10^6$ cells/ml) was perfused for 1.5 hours. Then, the basal media was inserted into the bioreactor system and cultivated for 7 days. For the static culture, the planer NFs and microtubes were inserted in the cell culture medium and statically cultured. For dynamic seeding, the cell culture media was perfused in the bioreactor system after the microtube was placed in the system. For each experimental group, the growth medium was replaced every three days.

2.7 Cell Proliferation Analysis

MTT assay ((Vybrant, Invitrogen, Grand Island, NY) was applied to both nanofiber sheets and microtubes on 1 d, 4 d, and 7 d to assess cell proliferation. 10% MTT solution was prepared with bare F12 DMEM and incubated with the cell-cultured nanofibers for two hours. Then, the MTT solution was replaced with DMSO (Sigma Aldrich, St. Louis, MO, USA) and formazan cyristals was dissolved after 5 minutes. Finally, the incubated DMSO was transferred into a 48-well plate to measure optical densities. The optical densities were calculated by measuring at 570 nm with SynergyTM HTX Multi-Mode Microplate Reader (BioTek, Epoch 2). The obtained absorbance values were related with cell numbers based on a calibration curve.

Table 1. Input and calculated parameters for the custom-made bioreactor conditions using COMSOL

Input parameters		Calculated parameters	
Scaffold size		**Scaffold geometry and strain**	
Scaffold diameter (cm)	5	Scaffold area (mm^2)	19.63
Scaffold height (cm)	12	Tortuosity	1.05
Scaffold porosity/pore size		Equiv.particle diam. (micron)	13.04
Porosity (%)	92	Compression time (sec)	0.50
Pore diameter (micron)	100	**Flow-related parameters**	
Compression parameters		Reynold's number	1.10E-01
Compression frequency (Hz)	1	Scaffold permeability (m^2)	1.38E-10
Applied compression strain (%)	5	Brinkman constant	1.27E+00
Other parameters		Perfusion pore fluid velocity (mm/sec)	0.92
Bioreactor perfusion rate (ml/min)	1	Compression pore fluid velocity (mm/sec)	1.30
Medium density (gr/ltr, typical 1000)	1	Total pore fluid velocity (mm/sec)	2.23
Medium viscosity (mPas, typical 0.7)	0.8	**Volumetric flow due to compression**	
Bioreactor perfusion rates		Flow (mm^3/sec)	2.36E+01
Typical low value (ml/min)	0.1	Flow (ml/min)	1.41
typical high value (ml/min)	1	**Wall shear stress due to flow**	
Culture medium density and viscosity		Shear stress from perfusion (Pa)	0.080
Density (gr/ltr)	1000	Shear stress from compression (Pa)	0.113
Viscosity (mPas)	0.7	Total shear stress (Pa)	0.193

2.8 Cell Morhology Analysis

Actin filaments and cell nuclei were dyed with phalloidin and DAPI (Merck Millipore, Actin Cytoskeleton and Focal Adhesion Staining Kit, Catalog No. FAK100), respectively, in accordance with manufacturer's instructions for the purpose of observing cell morphology on planar NFs [19]. First, cell-seeded NFs were washed twice in PBS before being fixed at 4 °C for 20 minutes with 4% paraformaldehyde (Sigma Aldrich, St. Louis, MO, USA). After that, samples were permeabilized for 5 minutes with 0.1% Triton X-100 in PBS and blocked for 30 minutes with 1.5% bovine serum albumin (BSA) in PBS. Then, samples were incubated with DAPI for 5 minutes and phalloidin in PBS for 1 hour at 4 °C [12]. To examine cell morphology, images of the stained samples were captured using an inverted fluorescence microscope.

2.9 Statistical Analysis

We performed the experiments with at least three repetitions. All the obtained data were statistically analyzed with two-way analysis of variance (ANOVA) (SPSS 12.0, SPSS GmbH, Germany) and the Student-Newman-Keuls method as a post hoc test. Significant differences among groups were defined at p values at least less than 0.05. (*p<0.05, **p<0.01, ***p<0.001) [20].

3. Results and Discussion

The tubular vascular graft was successfully produced and SEM imaging was used to investigate the morphology of the PLGA nanofibers and determine the diameter and wall thickness of the microtubes (Figure 2). The inner diameter of the tubular graft was measured as 2.5 ± 0.2 mm and the thickness of the wall is 370 ± 20 nm. Since the grafts were produced by custom-made collector, the graft in various diameters might be also produced with the electrospinning setup. Integrating different scaffold fabrication methods with the development of vascular scaffolds might offer a patient-specific grafts [20]. Therefore, our strategy which allows fabricated patient-specific vascular grafts in different diameters might be used for clinical applications.

COMSOL Multiphysics was assessed to improve the quality of the custom-made bioreactor. The designed scaffold, velocity, and pressure parameter of the bioreactor were simulated and the results are shown in Table 2. Permeability and Brinkman number were estimated based on porosity and equivalent particle diameter.

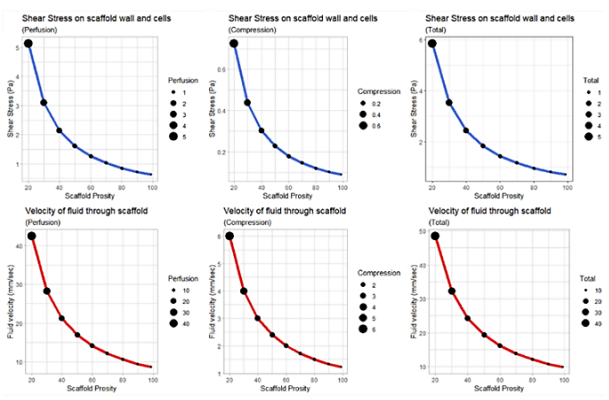

Figure 2. SEM image of PLGA vascular graft microtube produced by electrospinning with A) Higher magnification B) Lower magnification. Scale bar represents 100μm

Table 2. The simulation result of the scaffold and the custom-made bioreactor using COMSOL

				Fluid velocity (mm/sec)			Shear stress (Pa)		
Porosity (%)	**Particle Diameter (μm)**	**Permeability (m^2)**	**Brinkman**	**Perfusion**	**Compression**	**Total**	**Perfusion**	**Compression**	**Total**
20	600	3E-11	0.944876592	4.244132	6	10.24413	0.585724	0.828048	1.413773
30	350	4.5E-11	1.050277406	2.829421	4	6.829421	0.354393	0.501012	0.855405
40	225	6E-11	1.116333336	2.122066	3	5.122066	0.244662	0.345883	0.590546
50	150	7.5E-11	1.167020032	1.697653	2.4	4.097653	0.183015	0.258731	0.441746
60	100	9E-11	1.206426184	1.414711	2	3.414711	0.143925	0.20347	0.347395
70	64.28571	1.05E-10	1.236111092	1.212609	1.714286	2.926895	0.117024	0.165438	0.282462
80	37.5	1.2E-10	1.256873227	1.061033	1.5	2.561033	0.097391	0.137684	0.235075
90	16.66667	1.35E-10	1.269167702	0.94314	1.333333	2.276474	0.082417	0.116515	0.198932
99	1.515152	1.485E-10	1.273198891	0.8574	1.212121	2.069522	0.071665	0.101314	0.172979

The fluid velocity (mm/s) and shear stress (Pa) resulting from perfusion and compression were calculated. The graph for shear stress and velocity on scaffold wall and cell due to perfusion and compression and the total were drawn according to the parameter used in our experiment and illustrated in Figure 3. The velocity and pressure simulation result of the bioreactor in which the scaffold was placed was demonstrated in Figure 4. As a result, the simulation findings and the proposed model are condired as accurate and suitable for predicting system behavior. Validating the results with experimental data will indirectly establish the velocity and pressure as a hydrodynamic model. It is observed that there is a direct relationship between the velocity and pressure of the bioreactor and scaffold porosity. Increasing porosity of scaffold causes a decrease in total shear stress and fluid velocity.

Cell proliferation on PLGA NFs produced on glass slides after static seeding-static culture (SS-SC) and vascular grafts after static seeding-static culture (SS-SC), static seeding-dynamic culture (SC-DC), dynamic seeding-dynamic culture (DS-DC) was evaluated by MTT assay in at 1, 4, and 7 d (Figure 5). According to MTT analysis, IKVAV conjugated PLGA NFs enhanced the cell proliferation for 7d in all experimental groups. Moreover, the seeding and cultivation techniques by using perfusion-based bioreactor were developed for optimization for efficient coating of HUVECs on grafts. The proliferation was improved by dynamic seeding compared with static seeding, while dynamic culture helped to obtain better proliferation results compared to static culture. Similarly, the rotating EC seeding used in various research for many 3-D cell-material designs has favored dynamic culture [10, 21]. Çelebi-Saltık *et al.* coated polyurethane NFs with fibronectin and heparin and added NFs between media layers and the tunica intima to mechanically strengthen the grafts [22]. They cultured HUVECs on the graft using the cell sheet engineering approach with a designed bioreactor system and demonstrated that the dynamic culture system preserved the vascular graft, aided in the differentiation of primary human hematopoietic cells into thrombocytes, and exhibited anti-thrombogenic properties.

Herein, the use of the tubular vascular graft as a scaffold resulted in lower cell attachment and proliferation on a glass slide at the end of 7d compared to the planar NF scaffold. The 2D planar surface of a flat scaffold is ideal for seeding and optimal EC adhesion [23]. However, 2D planar surfaces cannot fully mimic vascular structures. Therefore, creating scaffolds that can change their morphology from 2D planar structures to 3D tubular shapes, providing 3D endothelialization may be beneficial in the design of vascular grafts. However, the cultivation of ECs on 3D tubular grafts by bioreactors may be a practical technique to facilitate 3D endothelialization. Here, we improved the proliferation of HUVECs on tubular graft with the help of dynamic seeding and dynamic culture.

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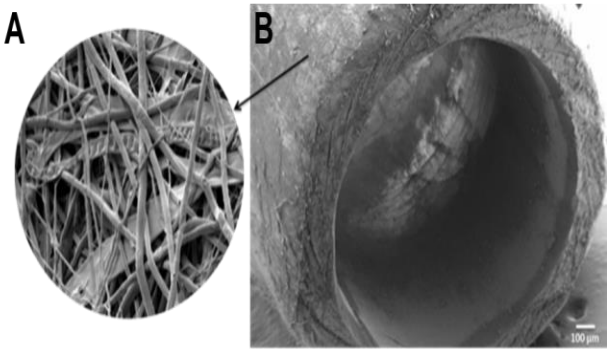

Figure 3. Quantitative results of scaffold and flow rate of the custom-made bioreactor using COMSOL Multiphysics

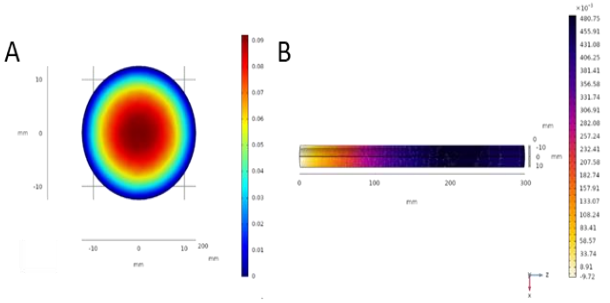

Figure 4. (A) Velocity simulation (B) Pressure simulation result of the bioreactor in which scaffold was placed using COMSOL

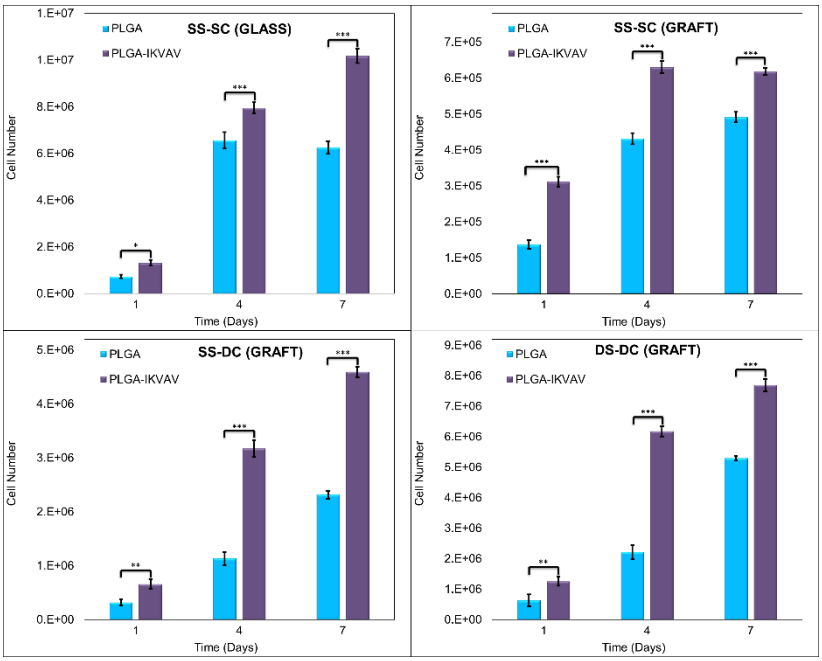

Figure 5. Cell number in vascular grafts after incubation in cell culture medium for 7 days (SS=Static Seeding, SC=Static Culture, DS=Dynamic Seeding, DC=Dynamic Culture)

The morphology HUVECs were observed after DAPI and phalloidin staining on 2D planar PLGA and IKVAV conjugated PLGA NF groups (Figure 6). A difference in the cell morphology between the groups was noticed from the images. HUVECs showed spread morphology on the PLGA-IKVAV surface, while scattered morphology was observed on PLGA. Moreover, completely elongated cell morphology was observed on PLGA-IKVAV surfaces. A higher number of nuclei per image in PLGA-IKVAV supports the MTT proliferation assay. IKVAV, a peptide derived from laminin's α-chain, has previously been shown to improve endothelial cell adhesion and tubule formation [24]. Grant *et al*. found that the IKVAV peptide enhanced EC organization, formation of the branched capillary vessel as a result of mouse angiogenesis assay as well as trials with the chick yolk sac/chorioallantois membrane [25]. Vascular ECs enhanced cell migration, attachment, and capillary network development by immobilization of IKVAV on collagen type I hydrogels [5]. IKVAV has also been demonstrated to facilitate ischemic tissue revascularization, making it of significant interest to those attempting to create microvascular networks [26].

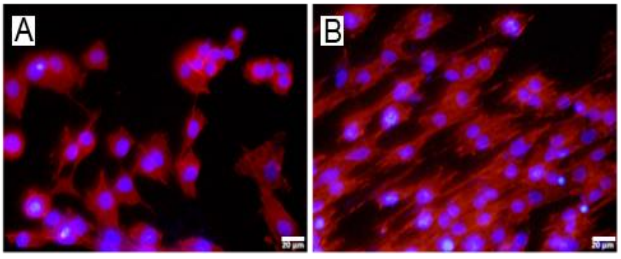

Figure 6. Morphology of human umbilical vein endothelial cell (HUVEC) on 2D planar (A) PLGA (B) IKVAV conjugated PLGA nanofiber. (Cell nuclei and cytoskeletal actin are stained with 4,6-diamidino-2-phenylindole (DAPI; blue) and phalloidin (red) (Scale bar represents 20 μm)

4. Conclusions

Herein, we effectively exhibited improved attachment and proliferation of HUVECs on IKVAV modified PLGA tubular grafts and cultivated in a custom-made dynamic bioreactor system. The custom-made bioreactor system has been designed in such a way that any laboratory can easily install it with low-cost supplies as an alternative to expensive commercially available bioreactors. Furthermore, the design of electrospinning collector for vascular grafts is promising for the researchers to produce polymer-based electrospun grafts with different diameters. Moreover, various peptide molecules might be used to functionalize these grafts according to desired properties. This technology provides a successful technique for increasing pre-endothelialization by seeding small-diameter vascular grafts with ECs, which may dramatically reduce problems in clinical applications. Further improvement and development of this system may result in a commercially available clinical solution to improve implanted tissue-engineered vascular grafts patency.

Declaration

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The author(s) also declared that this article is original, was prepared in accordance with international publication and research ethics, and ethical committee permission or any special permission is not required.

Author Contributions

O. Karaman developed the methodology. G.O. Pulat and A.G. Tatar performed experiments. Y.H. Usta

performed the simulations. O. Karaman supervised and improved the study. All authors wrote the manuscript together.

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References

1. Levenson, J.W., P.J. Skerrett, and J.M. Gaziano, *Reducing the global burden of cardiovascular disease: the role of risk factors.* Prev Cardiol, 2002. **5**(4): p. 188-99.
2. Obiweluozor, F.O., et al., *Considerations in the Development of Small-Diameter Vascular Graft as an Alternative for Bypass and Reconstructive Surgeries: A Review.* Cardiovascular Engineering and Technology, 2020. **11**(5): p. 495-521.
3. Mikos, A.G. and J.S. Temenoff, *Formation of Highly Porous Biodegradable Scaffolds for Tissue Engineering.* Electronic Journal of Biotechnology, 2000. **3**(2): p. 23-24.
4. Tallawi, M., et al., *Strategies for the Chemical and Biological Functionalization of Scaffolds for Cardiac Tissue Engineering: a Review.* Journal of the Royal Society, Interface, 2015. **12**(108): p. 20150254-20150254.
5. Nakamura, M., et al., *Construction of multi-functional extracellular matrix proteins that promote tube formation of endothelial cells.* Biomaterials, 2008. **29**(20): p. 2977-2986.
6. Agarwal, S., J.H. Wendorff, and A. Greiner, *Use of electrospinning technique for biomedical applications.* Polymer, 2008. **49**(26): p. 5603-5621.
7. Karkan, S.F., et al., *Electrospun nanofibers for the fabrication of engineered vascular grafts.* Journal of Biological Engineering, 2019. **13**(1): p. 83.
8. Ku, S.H. and C.B. Park, *Human endothelial cell growth on mussel-inspired nanofiber scaffold for vascular tissue engineering.* Biomaterials, 2010. **31**(36): p. 9431-9437.
9. Kim, T.G. and T.G. Park, *Biomimicking extracellular matrix: cell adhesive RGD peptide modified electrospun poly (D, L-lactic-co-glycolic acid) nanofiber mesh.* Tissue engineering, 2006. **12**(2): p. 221-233.
10. Nasseri, B.A., et al., *Dynamic rotational seeding and cell culture system for vascular tube formation.* Tissue engineering, 2003. **9**(2): p. 291-299.
11. Hsu, S.-h., et al., *The effect of dynamic culture conditions on endothelial cell seeding and retention on small diameter polyurethane vascular grafts.* Medical Engineering & Physics, 2005. **27**(3): p. 267-272.
12. Onak, G., U.K. Ercan, and O. Karaman, *Antibacterial activity of antimicrobial peptide-conjugated nanofibrous membranes.* Biomedical Materials, 2020. **16**(1): p. 015020.
13. Zhao, Q., et al., *Programmed Shape-Morphing Scaffolds Enabling Facile 3D Endothelialization.* Advanced Functional Materials, 2018. **28**: p. 1801027.
14. Hamsici, S., et al., *Bioactive peptide functionalized aligned cyclodextrin nanofibers for neurite outgrowth.* Journal of Materials Chemistry B, 2017. **5**(3): p. 517-524.
15. Dicker, K.T., et al., *Spatial Patterning of Molecular Cues and Vascular Cells in Fully Integrated Hydrogel Channels via Interfacial Bioorthogonal Cross-Linking.* ACS Applied Materials & Interfaces, 2019. **11**(18): p. 16402-16411.
16. Onak, G. and O. Karaman, *Accelerated mineralization on nanofibers via non-thermal atmospheric plasma assisted glutamic acid templated peptide conjugation.* Regen. Biomater., 2019. **6**(4): p. 231-240.
17. Usta, Y.H., et al. *Design of electrospinning collector for vascular tissue engineering applications*. in *2017 Medical Technologies National Congress (TIPTEKNO)*. 2017.
18. Onak Pulat, G., et al., *Role of functionalized self-assembled peptide hydrogels in in vitro vasculogenesis.* Soft Matter, 2021. **17**(27): p. 6616-6626.
19. Onak, G., et al., *Aspartic and Glutamic Acid Templated Peptides Conjugation on Plasma Modified Nanofibers for Osteogenic Differentiation of Human Mesenchymal Stem Cells: A Comparative Study*. Scientific Reports, 2018. **8**(1): p. 17620.
20. Best, C., et al., *Toward a patient-specific tissue engineered vascular graft.* Journal of tissue engineering, 2018. **9**: p. 2041731418764709-2041731418764709.
21. Kent, K.C., et al., *An in vitro model for human endothelial cell seeding of a small diameter vascular graft.* ASAIO transactions, 1988. **34**(3): p. 578-580.
22. Çelebi-Saltik, B., M.Ö. Öteyaka, and B. Gökçinar-Yagci, *Stem cell-based small-diameter vascular grafts in dynamic culture.* Connective Tissue Research, 2021. **62**(2): p. 151-163.
23. Zhao, Q., et al., *Programmed Shape-Morphing Scaffolds Enabling Facile 3D Endothelialization.* Advanced Functional Materials, 2018. **28**(29): p. 1801027.
24. Ali, S., et al., *Immobilization of Cell-Adhesive Laminin Peptides in Degradable PEGDA Hydrogels Influences Endothelial Cell Tubulogenesis.* BioResearch open access, 2013. **2**(4): p. 241-249.
25. 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.
26. Grant, D.S. and Z. Zukowska, *Revascularization of ischemic tissues with SIKVAV and neuropeptide Y (NPY).* Angiogenesis, 2000: p. 139-154.