The ability of usage various RGD configurations for the biodegradable vascular graft modification

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Abstract. The results of modifying non-woven matrices based on polyhydroxybutyrate / valerate and polycaprolactone with various RGD peptides (peptides: RGDK, AhRGD, c[RGDFK]) attached to the surface of matrices using hexamethylenediamine were studied. The structure of the surface of the matrices before and after the modification was not significantly changed. After 7 days of cultivation, the largest number of adherent and viable human umbilical vein endothelial cells of a person were detected on the surface of matrices with AhRGD or c[RGDFK]. However, cell proliferation was practically absent on the matrices with AhRGD. Thus, the modification of the surface of matrices with the AhRGD peptide is most suitable for products requiring rapid endothelialization.

1. Introduction

The surgery needs vascular grafts to be used due to high risk and widespreadness of cardiovascular diseases. Autologic blood vessel grafts are the clinical standard of todays for CABG and small blood vessel reconstruction. But they may be not available. Dacron or ePTFE grafts may partly solve this problem but with diameter less 5 mm they may cause thrombosis or hyperplasia of intima.

A functionally active biodegradable vascular prosthesis of small diameter, which has high permeability, atrombogenicity and biocompatibility can become an alternative to arteries and veins. Such strategies as immobilization of specific proteins and bioactive peptides can make the polymer surfaces highly biocompatible and non thrombogenic. One of the most perspective method is to immobilize RGD peptides (arginine-glycine-asparagine acid) on the surface of polymer vascular grafts as a sites of cellular adhesion which the most proteins of extracellular matrix have. This RGD peptides is one of the the key ligands for integrines – the receptors that are responsible for cellular adhesion, cellular migration, differentiation and existence. The RGD which contains peptides have simple structure and are more chemically stable than proteins.

Today in literature the usage of wide specter of peptides containing RGD parts is described, but there are no supports of any preferences for exact peptides.
2. Research methods and equipment
The polymer matrices had been produced by Nanon-01A using electrospinning method from PHBV and PCL composition in chloroform. Hexamethylenediamine, glutaric dialdehyde, ninhydrin, ascorbic acid, cyclic peptides c[RGDFK], RGDK and AhRGD had been used for surface modification. The quality of the modification were measured using ninhydrin test and by arginine-contain peptide finding.

The structure of matrices was studied by SEM before and after modification. Cellular adhesion, viability and proliferation of HUVEC which were cultivated for 7 days on matrices surface with and without RGD were studied using confocal laser scanning microscopy and fluorescence microscopy.

The obtained data was processed using the STATISTICA 6.0. The significance of differences was determined using the non-parametric Mann – Whitney test for disconnected pairs. Differences between groups were statistically significant at p <0.05. The Bonferroni amendment on multiple comparisons was also applied. Data are presented as medians (Me) and 25% and 75% percentiles.

3. Results
The surface modification of non-woven PHBV / PCL matrices with peptides containing the RGD fragment was carried out in two stages: at the first stage, partial aminolysis of the ester bonds of the graft material under the action of diamines, at the second stage, the introduction of the RGD peptide by means of cross-linking reagent.

The presence of peptides on the polymer surface was confirmed using the Sakaguchi test for the presence of arginine [8, 9].

Thus, 3 samples of polymer matrices were obtained, with a different structure of the peptide containing the RGD fragment (Table 1).

| Peptide 1 (RGDK) | Peptide 2 (AhRGD) | Peptide 3 (c[RGDFK]) |
|------------------|-------------------|----------------------|
| Hexamethylenediamine Amine 1 | A1P1 | A1P2 | A1P3 |

Scanning electron microscopy showed that non-woven PHBV / PCL matrices, both before and after modification with various RGD configurations, had a homogeneous highly porous structure formed by randomly arranged curved filaments (Figure 1).

Table 1. Samples of polymeric grafts obtained during the modification.

Figure 1. Scanning electron microscopy of the surface of non-woven matrices PHBV / PCL with and without RGD.
Modifying RGD peptides did not lead to structural changes in the surface of the matrices, but unlike unmodified samples, the matrices with RGD had bulges on the polymer filaments that were clearly noticeable at high magnification. These irregularities, presumably, are RGD peptides immobilized on the surface.

There was no adhesion and proliferation of HUVEC on the unmodified PHBV / PCL matrices, due to the lack of cell adhesion sites, which are mandatory for HUVEC adhesion.

The performed matrix modification with RGD peptides ensured the presence of cell adhesion sites on the polymer surface. On all presented types of modified matrices, the adhesion process of HUVEC proceeded with similar dynamics (Figure 2).

![Figure 2. Absolute amount of adhesion of HUVEC on the surface of non-woven matrices PHBV/PCL/RGD.](image)

The following results were obtained by the number of cells adhered on the matrixes: A1P2: 64.7 (52.9; 94.1)> A1P3: 58.8 (38.2; 132.4)> A1P1: 47.1 (20.6; 67.6). There were no statistically significant differences in the total number of cells per 1 mm² between the groups.

The assessing the relative number of viable and dead cells on various matrices with RGD showed that A1P2 matrices have more than 50% viable cells (range from 53% to 84%), whereas in A1P1 and A1P3 matrices, on the contrary, more than 50% of dead cells (from 54% to 77%) (Figure 3).

![Figure 3. The relative number of viable and dead cells on the surface of non-woven matrices PHBV/PCL/RGD.](image)
The largest relative number of proliferating cells was detected on A1P1 matrices: 11.1 (7.1; 23.1)%. While on the A1P3 matrices this amount was 5.0 (0.0; 22.2)%. The smallest relative number of proliferating cells was found on the A1P2 1.4 (0.6; 11.1)% matrices (Figure 4).

![Figure 4. Relative number of proliferating cells on the surface of non-woven matrices PHBV/PCL/RGD.](image)

4. Discussion
The results of the modification of non-woven matrices based on PHBV / PCL with different configurations of RGD peptides were studied: A1P1 - RGDK peptide; A1P2 - AhRGD peptide; A1P3 is a cyclic peptide c [RGDFK] sewn using hexamethylenediamine.

A1P3 matrices showed the lowest results of cell viability, against the background of satisfactory cell adhesion. Dead cells prevailed on the surface of these matrices, and proliferation of endothelial cells was reduced.

Polymer grafts with A1P1 have had high proliferation of endothelial cells on the surface, which may be due to the effective tropism for endothelial cells of a selected configuration of the A1P1 peptide itself.

Matrices with A1P2 showed the highest number of adherent and viable cells in comparison with A1P1 and A1P3. Also, more than 50% of viable cells were found on A1P2 matrices. However, according to the number of proliferating cells, the matrices with this peptide showed the worst result.

5. Conclusion
It can be concluded that the AhRGD peptide is the most favorable for cell adhesion and cell viability: the PHBV / PCL samples modified by it exceeded the matrices with RGDK and c [RGDFK] in the number of adhered and viable cells. The results show that the AhRGD peptide is most suitable for modifying the surface of grafts for their quick endothelialization.

Funding: This study was supported by the Complex Program of Basic Research under the Siberian Branch of the Russian Academy of Sciences within the Basic Research Topic of Research Institute for Complex Issues of Cardiovascular Diseases No. 0546-2015-0011 “Pathogenetic basis for the development of cardiovascular implants from biocompatible materials using patient-oriented approach, mathematical modeling, tissue engineering, and genomic predictors”.


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