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Deformation Behavior under Static and Cyclic Tension of Polymer Grafts without and after Modification by RGD Peptides

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Abstract. The structure, mechanical properties, and deformation behavior under static and cyclic tension of biofunctionalized biodegradable vascular grafts based on polyhydroxybutyrate/valerate and polycaprolactone were studied. It is shown that the modification gives rise to an almost twofold decrease of the elongation at break as well as the ultimate strength. It is shown that under cyclic loading the modification of grafts results in decreasing cyclic durability by more than twice. In doing so, the level of deforming stress decreases to a much lesser extent and is practically inferior to that for unmodified material. The analysis of principal strain ε_1 and ε_2 component distribution patterns in grafts of both types is carried out while the reason for the observed changes is discussed.

1. Introduction

To date, clinical practice still lacks ready-to-use vascular prostheses (grafts) of small diameter suitable for in situ regeneration of blood vessels. They are of actuality for carrying out shunting operations with replacing damaged vessels. However, the use of synthetic non-biodegradable polymers for manufacturing vascular grafts of small diameter was not successful due to development of chronic inflammation, thrombosis and neointima hyperplasia [1, 2].

For manufacturing vascular prostheses in situ many natural and artificial biodegradable polymers are used [3]. Natural polymers possess high biocompatibility and in vivo are completely subjected to gradual degradation to non-toxic substances. However, they give rise to a pronounced immune response; also, they have poor manufacturability while their mechanical properties are far distinct from blood vessels [4]. On the other hand, synthetic polymers do not usually cause a strong immune response; possess appropriate mechanical characteristics and high processability while their disadvantage is low biocompatibility: the absence of sites which promote cell adhesion [3].

Recently, a number of strategies have been developed to provide polymer surfaces the ability to selectively adhere endothelial cells in order to accelerate endothelization. Most of them are based on immobilization or fixation on the surface of specific cell adhesion proteins and bioactive peptides [3].

Arginine-glycine-asparagine (RGD) is a cell adhesion site that presents in many extracellular matrix proteins [5]. This sequence is one of the key ligands for integrins — receptors that are responsible for cell adhesion, migration, proliferation, differentiation, and survival [6]. The RGD peptide can be considered as a common integrin binding motif. Compared to proteins, bioactive RGD-containing peptides possess a simpler structure and higher chemical stability. Due to artificial synthesis, it is possible to develop a variety of configurations of RGD peptides with low immunogenicity and different potential for interaction with cells.

Biofunctionalization of synthetic materials allows one to create biomimetic surfaces capable of generating specific cellular responses. The incorporation of biologically active peptides into the tubular scaffolds during the electrospinning process in combination with providing the created structure with functional activity can change the architectonics of surface. The latter should affect their deformation behavior under cyclic loading. The aim of the work was to study the deformation behavior under static and cyclic loading of biofunctionalized biodegradable vascular grafts based on polyhydroxybutyrate/valerate and polycaprolactone without and after modification by RGD peptides.

2. Materials and research methods

Tubular polymer matrices with a diameter 1.5 mm were fabricated by electrospinning with the use of a "Nanon-01A" device (MECC Co. Ltd, Japan). The composition of polyhydroxybutyrate/valerate (PHBV) polymers with 10 % valerate (Sigma-Aldrich, USA) and polycaprolactone (PCL) with a molecular weight of 80,000 kDa (Sigma-Aldrich, USA) were employed. Nonpolar organic chloroform ChP (Vecton, Russia) was used as a solvent. The ratio of polymers in solution made 1:2.

Hexamethylene diamine, glutaraldehyde, ninhydrin, ascorbic acid (Sigma-Aldrich, USA) and RGD (NanoTech-S, Russia) were used for surface modification of the polymer tubular frameworks made of the PHBV/PCL with the RGD peptides. First, the frameworks were treated with a 10 % solution of hexamethylenediamine in a water solution of isopropanol (1:1 ratio). The treatment was conducted at 37 °C for 30 min which reduced the modification time twice [7, 8]. For the further treatments, each graft was put horizontally in a separate reactor (with a volume of 15 ml) with constant swaying.

Static tension tests were carried out with the use of a Zwick/Roell universal testing machine (Zwick GmbH, Germany). The force gauge of a nominal 50 N capacity was employed with cross head velocity 10 mm/min. The tensile strength was estimated as the maximum tensile stress (MPa) before the onset of fracture. Elastic properties were estimated by the relative elongation at break (%) and Young's modulus (MPa).

The structure of the inner grafts' surface as well as wall thickness of non-woven tubular PHBV/PCL frameworks (at observation area of 0.5 × 0.5 mm) without and after the RGD peptides modified was studied using S-3400N scanning electron microscope (Hitachi, Japan) under high vacuum. For the observation, specimens were sprayed with gold and palladium foils with the help of a SC-7640 vacuum unit (Quorum Technologies, England).

The Digital Image Correlation technique (DIC) was used to analyze the deformation behavior of loaded vascular grafts. The VIC 3D system (Correlated Solutions) was employed. Specimens in the initial and modified state were loaded at static tension with the use of an Instron 5582 electromechanical testing machine. For this, fragments 60 mm long were cut out from tubular blanks. Then fine paint particles were sprayed with the use of an airbrush to form a contrast speckle pattern. The flattened (initially tubular shaped) specimens after clamping in grips had a strip-like shape. This is motivated by the fact of fiber damaging being cut along the axis. For this reason they became extremely heterogeneous and their elongation at failure was noticeably reduced. Then, such specimens were clamped into wedge grips and stretched at the loading rate 10 mm/min. Surface images were recorded every 10 seconds with the help of Canon EOS 700 digital SLR camera equipped with a telescopic lens. Further, using the DIC technique strain fields were calculated. Then dependencies of the average principal strains ε_1 and ε_2 versus tensile strain were constructed.

To study the deformation behavior of specimens under cyclic tension a tubular graft was cut along the axis. Dog-bone shape specimen had the dimension shown in figure 1a. One of the folds oriented

along the specimen axis remained in the center (Figure 1b). However the latter did not affect their deformation behavior. A speckle pattern consisted of black alkyd paint spots over white background and was deposited to the outside surface of the specimen (Figure 1b).

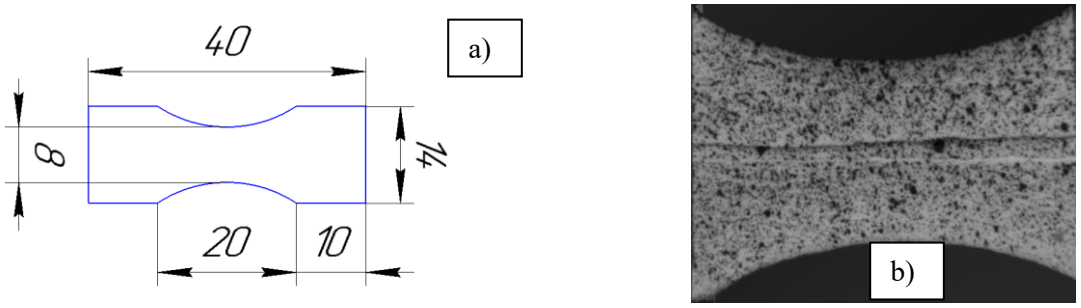

Figure 1. Dog-bone shape specimen sketch (a) and speckle pattern photograph (b) of a vascular graft.

The tests were carried out with the use of a Makron servo-hydraulic machine (Biss, India). The maximum cross head displacement is 60 mm with the accuracy of 1 μm. Specimens were tested by blocks of 100 cycles with the control over the grip movement (control by deformation). The amplitude of the cross head displacement was equal to 2 mm. After each cyclic loading block the surface was imaged. Then, the cross head was displaced to compensate the specimen loosening that took place during the cyclic loading. The average elongation of the specimens during 1 block of cycling was 0.25 mm, i.e. for every 100 cycles the specimen was extended by ~ 1.25 %. Canon EOS 700D camera with an EF 70-300 mm telephoto lens was used. The necessary zoom was ensured by two macrolenses (+1, +2 diopters).

3. Results and discussion

Modification density of the prostheses' surface with the RGD peptides at the treatment for 30 minutes was 6.3 nM/cm^2. The presence of peptides on the polymer surface was confirmed with the use of the Sakaguchi test over arginine presence [7, 8]. The specimen was remained colored after their washing that indicated covalent bonding of the peptide. A control specimen containing no RGD peptide stained light yellow; its coloring removed upon washing. Thus, the efficiency of the RGD peptide modification of the PHBV/PCL tubular framework surfaces at the modification time 30 minutes was confirmed.

The PHBV/PCL grafts in both states possess a highly porous structure with a wall thickness 170–250 μm consisting of homogeneous fibers (Figure 2). The average fiber diameter and average pore area in the grafts were 2.63 ± 1.14 μm and 47.13 ± 23.0 μm^2, respectively, with the average porosity of ~50.0 %.

Stiffness is a particularly crucial indicator for the subsequent vessel compliance into which the prosthesis is planned to be implanted. Significant discrepancies in these parameters of the prosthetic vessel and vascular one may subsequently provoke neointima hypertrophy or thrombosis. Therefore, the stiffness diminishing was one of the main missions at PHBV/PCL based vascular grafts modification by the RGD peptides. It is shown that modification of the PHBV/PCL grafts with the RGD peptides gives rise to variation of their physic-mechanical properties (Table 1): a 1.7-fold decrease in elongation at break takes place against a 1.6-fold decrease in stiffness in regard to the unmodified analogues ($p < 0.05$).

Table 1. Physico-mechanical properties of biodegradable vascular grafts without and after RGD modification

Specimen	Tensile strength, MPa	Young's modulus, MPa	Elongation at break, %
PHBV/PCL	7.1	28.0	431.3
PHBV/PCL + RGD	1.8	17.8	248.9

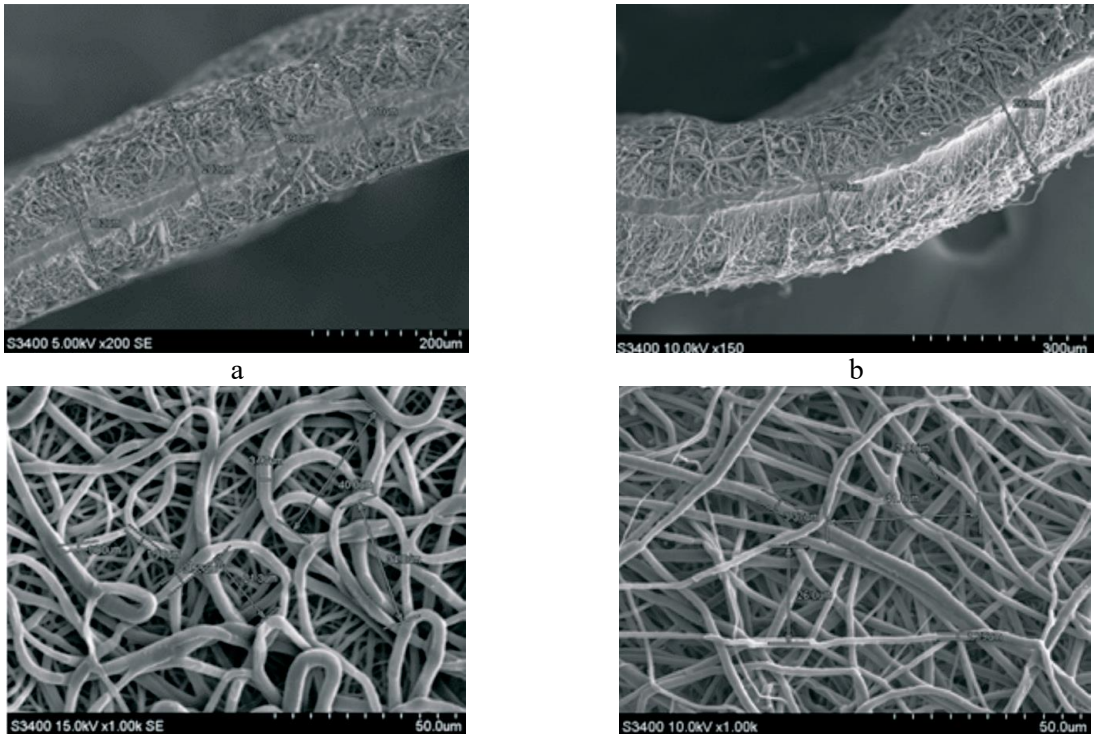

Figure 2. SEM micrographs of PHBV/PCL grafts without and after modification with the RGD peptides: cross section of the wall of the unmodified PHBV/PCL (a) ×200 and PHBV/PCL + RGD (b), ×150; the inner surface of the unmodified PHBV/PCL (c), ×1000, PHBV/PCL + RGD (d), ×1000

3.1. Mechanical behavior of biodegradable grafts under static loading

Figure 3 shows the dependences of the principal strains ε_1 and ε_2 versus tensile strain for the grafts in both states. It is seen that, for the above reason, the elongation at break was reduced less than 2 times while the lowering patter was kept. The graph of the principal strain component ε_1 looks less uniforms; in the case of the nonmodified graft while the inclination angle is slightly lower (Figure 3a). However, the graphs of another component ε_2 differ significantly (Figure 3b). Thus, this deformation parameter turned out to be very sensitive to the structural changes happened. To understand the observed differences the maps of the deformation component distribution were then analyzed.

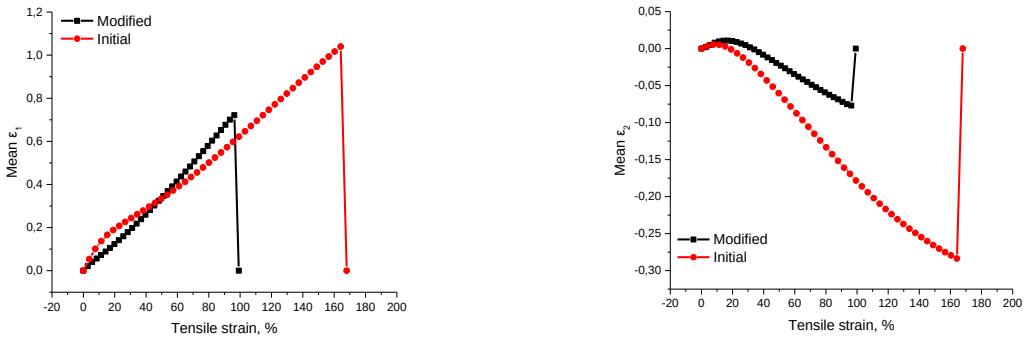

Figure 3. Diagrams of the principal stain ε_1 (a) and ε_2 (b) versus tensile strain at static tension of vascular grafts specimens without (initial) and after the modification (modified)

Maps of the principal strains ε_1 (a) and ε_2 distribution of the initial state and modified grafts at relative strain 20 % are given in figure 4. It is seen that the onset of plastic deformation is not accompanied by any pronounced strain localization (not taking into account the regions in the middle specimen where some extreme values are evident; however, they are related to the shrinkage of the half folded tube). The pattern of ε_2 component distribution shows in the modified graft (which possesses less ductility) exhibits less uniformity (Figure 4d). By the authors opinion the latter is due to the heterogeneous structure of the modified graft.

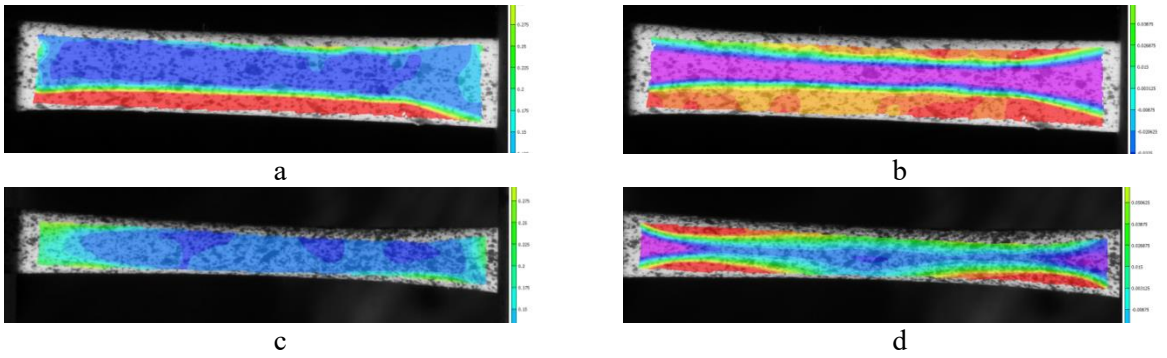

Figure 4. Maps of ε_1 and ε_2 distribution of vascular grafts without and after the modification (c, d); static stretching; strain level 20 %; a), c) ε_1; b), d) ε_2; a), b) without modification; c), d) after modification

With further loading the specimens (in both states) are tightened; in doing so the strain distribution is fairly uniform in the initially half-folded tube specimens. To a greater extent this is related to the ε_1 component distribution. However, another principal strain ε_2 (Figure 5b,d) looks less uniform. Note, that in the modified grafts the average strain level is higher: in the middle part of the specimen it exceeds that for the nonmodified graft up to 2 times.

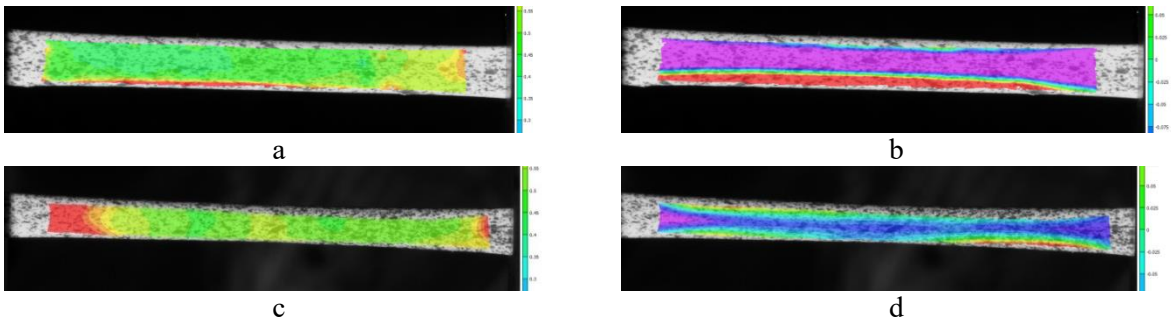

Figure 5. Maps of ε_1 and ε_2 distribution of vascular grafts without and after the modification (c, d); static stretching; strain level 80 %; a), c) ε_1; b), d) ε_2; a), b) without modification; c), d) after modification

Generally, a similar deformation pattern is kept up to critical strains preceding specimens' fracture (Figure 6). Despite high straining (the elongation at break exceeds 100%) bothe types of specimens fail without neck formation or any other form of strain macrolocalization. This is due to both i) tubular shape of the specimens and ii) fibrous structure of the material.

3.2. Mechanical behavior of biodegradable grafts under cyclic loading

The cyclic tension test data evidences for the nonmodied graft failure after 82 blocks of loading (100 cycles each). In so doing, the length of the specimen has enlarged by 202.5 %. The RGD peptides modified graft has failed after applying 33 cyclic loading blocks with relative elongation (at break) 142.5 %. Thus, in contrast to the data of static tension tests (Table 1), the difference in the relative elongation has reduced from 1.73 down to 1.42 times.

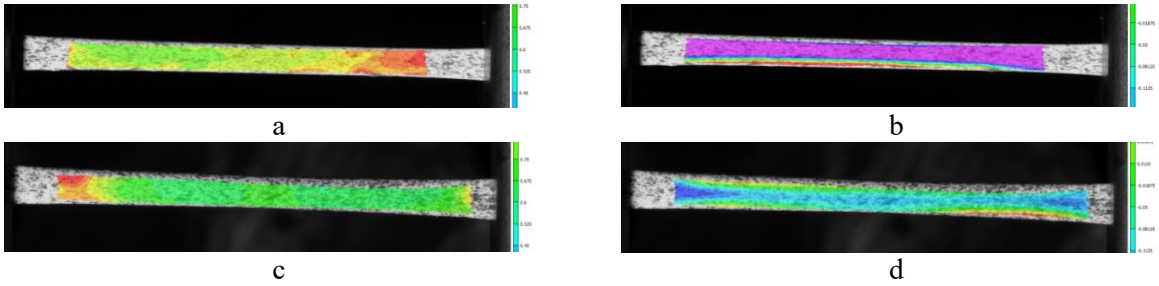

Figure 6. Maps of ε_1 and ε_2 distribution of vascular grafts without and after the modification (c, d); static stretching; a), c) ε_1; b), d) ε_2; a), b) without modification; c), d) after modification; a), b) without modification (deformation degree – 140 %); c), d) after modification (deformation degree – 96 %)

On the other hand, under cyclic tension (at the displacement control loading), the magnitude of the applied load differs insignificantly. This means that regardless less cyclic resistance under used severe testing conditions they possess nearly equal baring capacity. In combination with the fact that mechanical properties of the modified material are closer to native vessels it brings promise of their wide practical applications.

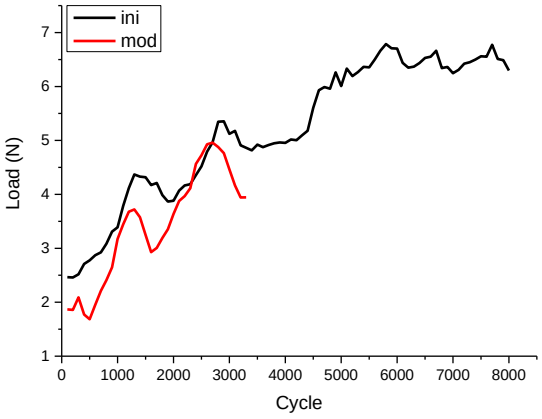

Figure 7. Graph of the applied load versus the number of loading cycles for the nonmodified (ini) and modified (mod) graft specimens

Figure 8 shows patterns of principal strain ε1 distribution with increasing cyclic loading. It is seen that in the nonmodifed graft no signs of strain localization are revealed almost 300 cycles prior to failure N = 8·103 (Figure 8 a,c,e,g). Only at "necking" tearing takes place with cracks arising from opposite sides of the dog-bone shaped specimen to give rise to the main crack propagation (Figure 8i).

In the modified graft a strain macrolocalization is already fixed at the low cycle number $N = 2 \cdot 10^3$. Soon, the tearing started being initiated from one of the edges (Figure 8f). In this case, layered pattern of specimen fracture is observed when the upper layer begins to break while the underlying layers maintain continuity (Figure 8h). Just after, the specimen withstands another 300 loading cycles and

fails (Figure 8j). The reason is obviously related to its heterogeneity induced by the structure modification.

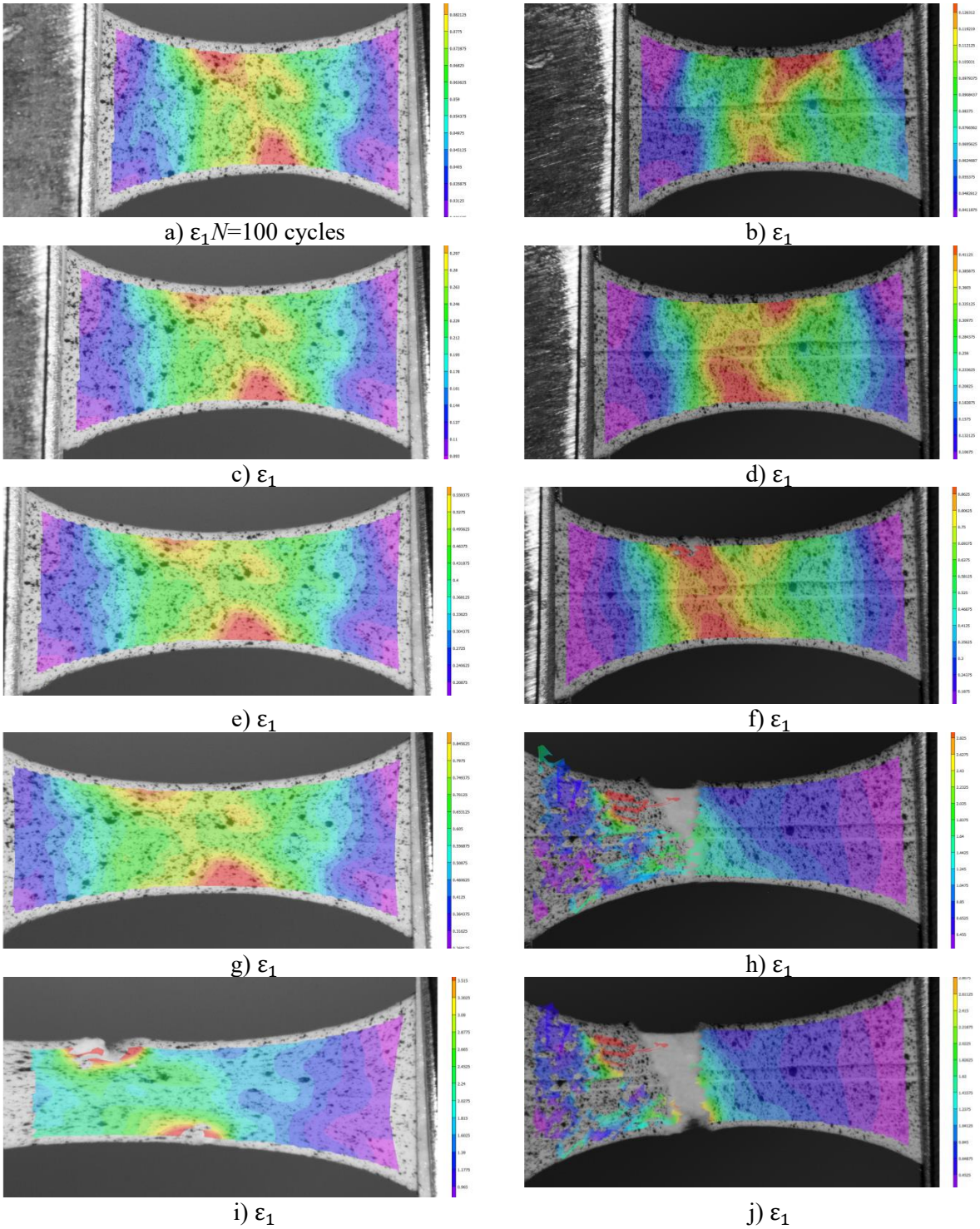

Figure 8. Principle strain ε_1 maps for the vascular graft without and after modification; cyclic stretching; The number of cycles: a, b) 100, c, d) 1000, e, f) 2000, g, h) 3000, i) 8000, j) 3300; a, c, d, g, i) without modification; b, d, f, h, j) after modification

4. Conclusion

The structure, mechanical properties, and deformation behavior of polymer grafts in the initial and modified state were studied. The used modification regime of polymer biodegradable tubular PHBV/PCL grafts with RGD peptides (during 30 minutes) did not result in a statistically significant variation of their surface structure. Moreover, after the modification, the stiffness of grafts and their elongation ability decreased. This has brought the physico-mechanical properties of vascular prostheses PHBV/PCL + RGD closer to ones of native vessels.

Experimental studies on the deformation behavior of biodegradable grafts under static and cyclic tension was carried out. The analysis and visualization of strain fields by means of digital image correlation was conducted. It is shown that principal strain distribution patters correlates well with variation of mechanical properties, namely, the plastic flow stress and elongation at break. It is shown that principal strain component ε2 in the modified graft (possessing lower mechanical properties is distributed less uniformly while its level prior to failure is up to two times higher than that in the unmodified material.

Regardless less cyclic resistance under used severe testing conditions the modified grafts possess nearly the equal baring capacity under cyclic tension. In combination with the fact that their mechanical properties are closer to native vessels it brings promise of their wide practical applications.

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