Piatnochka V. I. Morphometric characteristics of changes in the anterior abdominal wall muscles after implantation of polypropylene meshes of different types in experiment. Journal of Education, Health and Sport. 2018;8(5):325-334. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1493496
http://ojs.ukw.edu.pl/index.php/johs/article/view/6310

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 02.04.2018. Revised: 12.04.2018. Accepted: 31.05.2018.

Morphometric characteristics of changes in the anterior abdominal wall muscles after implantation of polypropylene meshes of different types in experiment

V. I. Piatnochka

Department of Surgery of Academic and Research Institute of Postgraduate Education of I. Horbachevsky Ternopil State Medical University, Ternopil, Ukraine

Abstract

A widespread introduction of modern alloplastic materials into the surgical practice has significantly improved the results of surgical treatment of the patients with ventral and postoperative ventral hernia. However, the implantation of synthetic material into the anterior abdominal wall tissues is accompanied by different local reactions, especially when using 'heavyweight' meshes that often cause development of complications. The analysis of the results determines the topical issue of this study.

Objective. The aim of the study was to conduct a morphometric evaluation of changes in the anterior abdominal wall muscles after the implantation of 'heavy' and 'light' polypropylene meshes as well as 'light' ones in combination with the PRF membrane.

Materials and methods. Morphometric study involved analysis of anterior abdominal wall muscles after implantation of 'heavy', 'light' polypropylene meshes, as well as the 'light' ones in combination with the PRF membrane, into the retro-muscular space in light-optical investigation of microslides. The experiments were carried out on 18 mature male Vietnamese pigs, which were divided into 6 groups.

Results. In the group of animals 2m significant structural changes of muscles were revealed. The diameter of myocytes increased from (13.50±0.15) to (14.20±0.18) μm. These

morphometric parameters statistically significantly ($p<0.05$) differed among themselves. In this case, the last morphometric parameter exceeded the previous one by 5.2%. The diameter of nuclei of these structures changed more or less similarly. Thus, the diameter of myocytes in the control group of animals was (5.10±0.04), and in the modeled experimental conditions it was (5.68 ± 0.06) μm. The relative volume of stroma in the muscles in the study groups with a high degree of statistical significance ($p<0.001$) increased by 16.6%. Herewith, stromal-myocyte ratio significantly changed. Thus, in the control group this morphometric parameter reached (0.168±0.003), and in the 2nd group of animals – (0.202±0.006). The quantitative morphometric parameters of anterior abdominal muscles in the experimental animals of the 5th group (3m) proved that they changed significantly in response to the experiment; thus, in this case the diameter of myocytes statistically significantly increased from (13.50±0.15) μm to (14.10±0.15) μm, i.e. by 4.4%. The diameter of the studied cells in the modeled experimental conditions statistically significantly ($p<0.001$) increased from (5.10±0.04) to (5.60±0.05) μm. The last morphometric parameter exceeded the previous one by 9.8%.

Conclusions. The quantitative analysis of morphometric evaluations of changes in the muscular layer of anterior abdominal wall, in cases of implantation of the polypropylene meshes of different types, in the late period of the experiment proved that during the implantation of the 'light' mesh, in this period of the experiment, the structural changes of anterior abdominal wall muscles were the least; and the most favorable features of structural changes of anterior abdominal wall were in the experimental animals, where a 'light' polypropylene mesh in combination with the PRF membrane was used for its plastics.

Key words: implantation, polypropylene mesh, morphometric study, muscles.

Introduction

At present, with no doubts an operation of choice in the surgical treatment of ventral and post-surgical ventral hernias is the aloplasty. Nevertheless, allohernioplastics does not always ensure reliability of surgical intervention [3, 5, 11]. In a group of patients with postoperative ventral hernias and other concomitant diseases accompanied by anorexia, surgical interventions using mesh implants does not always allow achieving the expected result. Taking into account the high frequency of postoperative complications 11.8-50% and relapses 6.5-15%, surgeons consistently develop new methods of allohernioplastics [2, 4, 8].

The morphometric picture of the tissue reaction in the area of polypropylene mesh implantation [6, 9] is studied insufficiently.

A thorough study of the combined use of polypropylene mesh with PRF membrane consisting of platelets-enriched fibrin in the surgical treatment of postoperative ventral hernias, above all for the patients at risk, is thought-provoking. The biocompatible PRF membranes rich in growth factors have already been used in surgical dentistry, periodontology, implantology, bone plastics, and maxillofacial surgery [7, 12, 13]. We have not found any information about their use in surgical treatment of hernias. When the PRF membranes of blood plasma are used, an active increase of new capillaries is stimulated, blood flow improves, metabolic processes in tissues are accelerated, collagen, hyaluronic acid formation increases sharply, and the inflammatory process in tissues is significantly reduced [10]. Information about the dynamics of these changes helps cope with some tactical tasks in the patients with postoperative ventral hernia.

Objective

The aim of our study was to conduct morphometric evaluation of changes in the anterior abdominal wall muscles after implantation of 'heavy' and 'light' polypropylene meshes as well as the 'light' ones in combination with the PRF membrane in experiment.

Materials and methods

The experimental study was carried out at the Department of Operative Surgery and Topographic Anatomy of I. Horbachevsky Ternopil State Medical University. 18 mature male Vietnamese pigs were used in the experiment, which were divided into 6 groups. The 1st group consisted of 3 almost healthy intact animals, which were kept in normal vivarium environments; the 2nd group (2m) – 3 pigs with a 'light' mesh, which was taken out together with the adjacent tissues on the 14th day of the experiment, the 3rd group (2m2) – 3 experimental animals with a lightweight mesh, which was taken out together with the adjacent tissues on the 28th day of the experiment, the 4th group (2m + PRF) involved pigs with a 'light' polypropylene mesh (PPM) + PRF membrane taken out on the 28th day of the experiment, the 5th group – 3 pigs with a 'heavy' mesh, which was taken out together with the adjacent tissues on the 14th day of the experiment , the 6th group (3m) – 3 experimental animals with a 'heavy' PPM mesh, which was taken out together with the adjacent tissues on the 28th day of the experiment. The study was conducted according to the "General Ethical Principles of Animal Experiments" (Ukraine, 2011), consistent with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1985). No exclusion of animals out of the experiment was conducted, as they were later used for educational purposes at the Department of Operative Surgery and Topographic Anatomy.

After sampling the fragment of muscular aponeurotic layer of anterior abdominal wall in the area of the implanted mesh in the experimental animals, the pieces were anchored in a 10% neutral formaline solution, put through ethyl alcohols of increasing concentration and placed in paraffin. The 5-7 μm thick slices obtained on the sledge microtome MS-2, after being deparaffinized, were stained with hematoxylin and eosin according to Van Gison, Melori. Light-optical histological microslides were investigated in microscopes MBD-6, MBD-15, Luman P-8. Morphometry was performed on histological microslides using the ocular micrometer MOV-1.5. The experiment was conducted according to the main principles of G.G. Avtandilov guidelines (2002) [1]. On each microslide, 50 measured tests were made.

Results

As is seen in Table 1, in the group of animals 2m significant structural changes of muscles were revealed. Thus, the diameter of myocytes with that increased from (13.50±0.15) to (14.20±0.18) μm. These morphometric parameters statistically significantly ($p<0.05$) differed among themselves. In this case, the last morphometric parameter exceeded the previous one by 5.2%. The diameter of nuclei of these structures changed more or less similarly. Thus, the diameter of myocytes in the control group of animals was (5.10±0.04), and in the modeled experimental conditions (5.68±0.06) μm.

Table 1. Morphometric characteristics of the anterior abdominal muscles of the experimental animals (M±m)

Indices	Group of animals		
	The 1st group	The 2nd group (‘light’ mesh, 14th day)	The 3rd group (‘light’ mesh, the 28th day)
Diameter of myocytes, μm	13.50±0.15	14.20±0.18*	13.90±0.18*
Diameter of nuclei, μm	5.10±0.04	5.68±0.06***	5.34±0.06*
Nuclear-cytoplasmic ratio	0.145±0.003	0.160±0.005***	0.148±0.005
Relative volume of stroma, %	14.4±0.12	16.8±0.15***	18.70±0.15***
Relative volume of myocytes, %	85.6±1.2	83.2±1.2	81.3±1.5*
Stromal-myocyte ratio	0.168±0.003	0.202±0.006***	0.230±0.006***
Relative volume of damaged myocytes, %	2.40±0.04	18.70±0.21***	5.30±0.06***

Notes: * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$, compare to the 1st group of study.

A statistically significant difference ($p<0.001$) between these morphometric parameters was proved; the latest quantitative morphometric parameter exceeded the previous one by 11.4%; nuclear-cytoplasmic ratio in the studied structures with that increased by 10.3% ($p<0.01$). The relative volume of stroma in muscles of the study group with a high degree of statistical significance ($p<0.001$) increased by 16.6%. Stromal-myocyte ratio changed significantly with that. Thus, in the control group the morphometric parameters reached (0.168±0.003), and in the 2nd group of animals – (0.202±0.006). These morphometric parameters statistically significantly ($p<0.001$) differed and the last quantitative morphometric index exceeded the previous one by 20.2%. The increase of the last morphometric parameter proved a significant increase in stroma in the studied structures. The relative volume of the damaged myocytes in the studied muscles with a high degree of statistical significance ($p<0.001$) increased in 7.8 times.

In the third group of study, the diameter of myocytes statistically significantly ($p<0.05$) increased by 2.9%, and the diameter of nuclei – by 4.7% ($p<0.05$) compare to the control indices. Nuclear-cytoplasmic ratios in the studied structures did not significantly ($p>0.05$) differ from the control morphometric parameters and were (0.148±0.005). The relative volume of stroma with that was (18.70±0.15)%. This morphometric parameter exceeded the same one in the control group by 29.8% ($p<0.001$). The relative volume of myocytes in the studied muscles statistically significantly ($p<0.05$) decreased from (85.6±1.2) to (81.3±1.5)%, i.e. by 5.0%.

Stromal-myocyte ratios in the studied experimental conditions statistically significantly ($p<0.001$) increased from (0.168±0.003) to (0.280±0.006), i.e. by 36.9%.

The relative volume of the damaged myocytes decreased significantly with that from (18.70±0.21)% to (5.30±0.06)%, i.e. in 3.5 times ($p<0.001$). It should be noted that the last morphometric parameter statistically significantly ($p<0.001$) exceeded the same control index in 2.2 times.

The morphometric parameters of anterior abdominal wall muscles of the 4th group of animals (lightweight mesh + PRF membrane) in 28 days from the beginning of the experiment are presented in Table 2.

As is seen from the Table 2, the diameters of myocytes, the diameters of nuclei, the nuclear cytoplasmic ratios of the 4th group of study did not differ from the control indices. With that, the relative volume of stroma statistically significantly ($p<0.001$) increased in this groups from (14.40±0.12)% to (19.60±0.15)%, i.e. by 36.1%.

Table 2. Morphometric characteristics of anterior abdominal wall muscles of the experimental animals (M ± m)

Indices	Group of animals	
	The 1st group	The 4th group ('light' mesh+PRF, the 28th day)
Diameter of myocytes, μm	13.50±0.15	13.60±0.18
Diameter of nuclei, μm	5.10±0.04	5.19±0.008
Nuclear-cytoplasmic ratio	0.143±0.003	0.146±0.005
Relative volume of stroma, %	14.40±0.12	16.8±0.15***
Relative volume of myocytes, %	85.6±1.2	80.4±1.5*
Stromal-myocyte ratio	0.168±0.003	0.244±0.006***
Relative volume of damaged myocytes, %	2.40±0.04	2.80±0.09*

Notes: * – $p<0.05$; *** – $p<0.001$, compare to the 1st group of study.

The relative volume of myocytes in these experimental conditions decreased from (85.6±1.2)% to (80.4±1.5)%, i.e. by 5.2% ($p<0.05$).

Stromal-myocyte ratios statistically significantly ($p<0.001$) increased with that from (0.168±0.003) to (0.244±0.006), i.e. by 45.2%, that proved a significant increase of stromal elements.

The relative volume of the damaged myocytes in the studied experimental conditions increased ($p<0.05$) from (2.40±0.04) to (2.80±0.09)%, i.e. by 16.6%.

The quantitative morphometric parameters of anterior abdominal muscles in the experimental animals of the 5th group (3m) are presented in Table 3. The comprehensive analysis, which is presented in this table, proved that these parameters significantly changed in these experimental conditions, thus, the diameter of myocytes with that statistically significantly increased from (13.50±0.15) μm to (14.10±0.15) μm, i.e. by 4.4%. The diameter of the studied cells in the modeled experimental conditions statistically significantly ($p<0.001$) increased from (5.10±0.04) to (5.60±0.05) μm. The last morphometric parameter exceeded the previous one by 9.8%.

In these experimental conditions, nuclear-cytoplasmic ratios in the studied cells were disrupted. Thus, in the control group the nuclear-cytoplasmic ratios in the myocytes were (0.143±0.003), and in the study groups – (0.158±0.003). A statistically significant difference

was established ($p<0.05$) between these morphometric parameters and the last morphometric parameter exceeded the previous one by 10.5%.

Table 3. Quantitative morphological characteristics of anterior abdominal wall muscles in the experimental animals of the group 3m (M±m)

Indices	Group of study	
	The 1st group	The 5th group (3m) ('heavy' mesh, the 14th day)
Diameter of myocytes, μm	13.50±0.15	14.10±0.15*
Diameter of nuclei, μm	5.10±0.04	5.60±0.05***
Nuclear-cytoplasmic ratio	0.143±0.003	0.158±0.003*
Relative volume of stroma, %	14.40±0.12	17.1±0.15***
Relative volume of myocytes, %	85.6±1.2	82.9±1.5
Stromal-myocyte ratio	0.168±0.003	0.206±0.005**
Relative volume of damaged myocytes, %	2.40±0.04	16.50±0.24***

Notes: * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$, compare to the 1st group of study.

The relative volume of stroma with a high degree of statistical significance ($p<0.001$) exceeded with that the same quantitative morphological index by 18.75%. The relative volume of myocytes decreased with that by 3.1%. Stromal-myocyte ratios in these experimental conditions statistically significantly ($p<0.01$) increased by 22.6%. The morphometric studies proved that the relative volume of the damaged myocytes in the modeled experimental conditions with a distinct statistically significant difference ($p<0.001$) increased in 6.9 times.

In the 6th group of study (Table 4), the diameter of the myocytes of anterior abdominal wall muscles was increased by just 1.1% and was (13.65±0.15) μm. The diameter of the nuclei of these cells was with that (5.20±0.05) μm and exceeded the same control index by 1.96%. Nuclear-cytoplasmic ratios in the myocytes of anterior abdominal wall muscles did not significantly differ from this morphometric parameter in the 1st group of study that proved stability of cellular structural homeostasis.

Table 4. Morphometric characteristics of anterior abdominal wall muscles in the experimental animals with a 'heavy' mesh on the 28th day of the experiment (M±m)

Index	Group of study	
	The 1st group	The 6th group ('heavy' mesh, the 28th day)
Diameter of myocytes, μm	13.50±0.15	13.65±0.15
Diameter of nuclei, μm	5.10±0.04	5.20±0.05
Nuclear-cytoplasmic ratio	0.143±0.003	0.145±0.004
Relative volume of stroma, %	14.40±0.12	19.52±0.18***
Relative volume of myocytes, %	85.6±1.2	80.40±1.14*
Stromal-myocyte ratio	0.168±0.003	0.242±0.005***
Relative volume of damaged myocytes, %	2.40±0.04	6.90±0.05***

Notes: * – $p<0.05$; *** – $p<0.001$, compare to the 1st group of study

The relative volume of stroma in the 1st group of study was 14.40±0.17%, and in the 6th study group – 19.52±0.18%. These morphometric parameters statistically significantly ($p<0.001$) differed from each other and the last quantitative morphometric index exceeded the previous one by 35.5%. The relative volume of myocytes in the anterior abdominal wall muscles decreased by 6.1% ($p<0.05$) compare to the control indices. Stromal-myocyte ratios in the studied experimental conditions with a high degree of statistical significance ($p<0.001$) increased by 44.0%, that proved a significant increase of stromal structures in the anterior abdominal wall muscles. The relative volume of the damaged myocytes statistically significantly ($p<0.001$) increased in 2.8 times compare to the same morphometric parameter of the 1st group of study.

In the study of microslides, in which the polypropylene meshes were used, the morphological changes were more or less similar in the studied groups according to the light-optical investigation. Thus, in 14 days of the experiment in these microslides a significant increase of connective tissue around the fragments of the polypropylene mesh was evidenced. The connective tissue in places formed a capsule with a significant amount of fibrocytes and collagen fibers. Rarely, there were elastic fibers with that. During this period of the experiment, edema of tissues and cells, moderate mostly vascular congestion were present. There were also dystrophic processes in myocytes and endotheliocytes of vessels. The

endotheliocytes in the blood vessels were desquamated in places. At times there were also small lympho-histiocytic infiltrates.

In 28 days of the experiment in the studied groups of microslides an insignificant edema of stroma was evidenced. A significant growth of fibrous tissue around the fragments of the polypropylene mesh took place. In places the fibrotic masses formed a capsule around the fragments of the polypropylene mesh, where a large number of fibroblasts and collagen fibers could be localized. Occasionally in the microslides some small cells of early granulation tissue were present. No inflammatory processes around the fragments of the polypropylene mesh were evidenced.

Discussion

The conducted morphological and morphometric studies allowed drawing the conclusion that the most favorable features of structural changes of anterior abdominal wall were in the experimental animals where the 'light' polypropylene mesh in combination with the PRF membrane was used for its plastics.

Conclusions

The quantitative analysis of morphometric evaluations of changes in the muscular layer of anterior abdominal wall, in cases of implantation of different types of polypropylene meshes, in the late period of the experiment proved that during the implantation of the 'light' mesh, in this period of the experiment, the structural changes of anterior abdominal wall muscles were the least.

The use of a 'light' mesh with the PRF membrane for implantation resulted in the least significant structural changes in arteries and the most optimal remodeling of anterior abdominal muscles.

Therefore, the most favorable features of structural changes of anterior abdominal wall were evidenced in the experimental animals where the 'light' polypropylene mesh in combination with the PRF membrane was used for its plastics.

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