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## ESTIMATION OF THE EFFECT OF PLATELET RICH PLASMA PRODUCTS IN THE INTEGRATION OF POLYPROPYLENE MESH IMPLANT IN BIOLOGICAL TISSUES. EXPERIMENTAL MODEL IN RATS

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### Abstract

**The aim of the research** is to determine morphological changes in the area of implantation of the polypropylene mesh implant and to determine the effect on the integration of the prosthesis of locally introduced adipose tissue and platelet rich plasma.

**Materials and methods.** The experiment was performed on 36 sexually mature males of the Wistar line rats. The experiment simulated, studied and quantified local morphological responses and changes in developing in biological tissues that are in contact with implanted highly porous lightweight (80 g/m<sup>2</sup>) mesh implant in isolation and also in conditions of local administration of fatty graft and platelet rich plasma.

**Results:** Assuming introduction of adipose tissue and platelet rich plasma in the zone of integration of mesh alloprosthesis under the influence of introduced regenerative cytokines as well as stromal stem cells activated by them there is an earlier activation of regenerative processes, enhanced angiogenesis which determines the optimal nature of the integration of the prosthesis with the formation of thin collagen fibers in more early terms minimizing excess peri-prosthetic fibrosis. Isolated introduction into the implantation zone of fatty suspension determines similar changes that have a slightly less pronounced character. These changes are quantitatively studied and the results obtained are statistically significant.

**Conclusions:** Applying a fatty graft together with platelet rich plasma in the area of implantation of the lung polypropylene prosthesis, there was an accelerated tissue reaction from the integration of the prosthesis.

Mesenchymal stem cells of adipose tissue that is a target for plasma cytokines enriched with thrombocytes have a more pronounced effect in stimulating reparative processes provided that they are simultaneously administered with PRP compared with isolated administration without PRP. The use of platelet rich plasma and adipose tissue design has a significant positive effect on local

angiogenesis. Under conditions of improved angiogenesis and other stimulating factors in the conditions of introduction of adipose tissue and PRP, the integration of the prosthesis occurs with significantly lower peri-prosthetic fibrosis.

**Keywords:** platelet rich plasma, fatty graft, regenerative cytokines, multipotent stem cells, polypropylene mesh allograft.

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## 1. Introduction

At the present stage, surgical interventions for ventral postoperative hernias remain among the most common according to many authors [1, 2]. Operative introductions have become the gold standard of modern herniology, which in various variations adhere to the principles of non-aggressive plastics with the use of mesh prostheses [2, 3]. System polypropylene implants have been widely used in hernioplastic operations due to significant biological inertia, high strength and sufficient elasticity [4], which allows the use of polypropylene mesh implants in alohernioplasty with good results [2, 5]. However, inevitably, the integration of any materials in biological tissues develops non-specific inflammatory reactions by the type of “foreign body reaction” [3, 4] in the early alterative phase, resulting in excessive exudation. Such a mechanism explains the development of postoperative seromas in the zone of alohernioplasty. In the long-term period, the prolonged proliferative phase of inflammation determines excess peri-prosthetic fibrosis, which is realized in biomechanical complications, such as the sensation of an extraneous body in the plastic zone and local pathological impairment of mobility [5, 6].

Platelet rich plasma (PRP), as a donor of regenerative cytokines and matrix proteins, is used in various branches of medicine to optimize regenerative reparative processes [7–8]. The main growth factors are: TGF $\beta$ 1 (transforming growth factor  $\beta$ 1), PDGF-AB (platelet-derived growth factor), and VEGF (vascular endothelial growth factor), as well as matrix proteins: fibronectin, vitronectin and thrombospondin [9, 10]. Such a substrate has a positive effect on most regenerative processes, mainly due to the stimulation of local inflammatory and local proliferative processes, mainly due to the action on the connective tissue structures [9, 11]. The effective target for cytokines in tissues is multipotent stromal stem cells of adipose tissue [12, 13]. In turn, such a substrate in isolation has a high therapeutic effect, which is expected to be potentiated in the context of local introduction of PRP products jointly and simultaneously with adipose tissue [14, 15].

## 2. Aim of the research

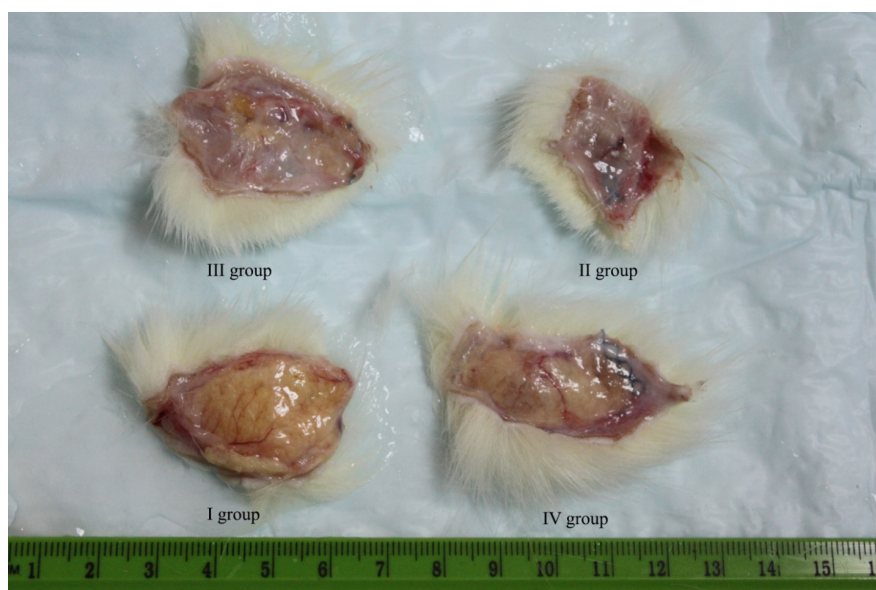
To evaluate the local reaction of biological tissues in the zone of integration of the facilitated polypropylene mesh implant (PMI), as well as to evaluate the features of integration of the implant in conditions of local administration of adipose tissue and platelet rich plasma.

## 3. Materials and methods

The experiment was modelled on 36 sexually mature males of the Wistar line rats weighing 160–180 g. The study was carried out in compliance with the rules of pathophysiological experiment, under the conditions of vivarium and the department of pathomorphology of the Center for Reconstructive and Restorative Medicine (University Clinic) of Odessa State Medical University. Taking into account the anatomical features, preparation of rat's fatty autotransplant technically was not possible. As previously proved, the fatty substrate in experimental studies in rats may be in the form of a xenotransplant that does not have a significant statistical error. The adipose tissue was removed during liposuction in healthy patients and subjected to standard treatment using the method described by Sydney R Coleman, MD [14, 16]. Platelet rich plasma were obtained by modified double centrifugation (DoubleSpin) using syringe-containers of reduced size [10, 12]. The principle is the blood collection of animals of one genetic line [17]. Blood for centrifugation was obtained by means of puncture aspiration of the contents of the heart chambers of rats. The substrate meets the requirements for platelet rich plasma. The control of the quantitative characteristics of the substrate was carried out by determining the level of haemoglobin and platelet count using a semi-automatic analyzer HTI MICROCC-20PLUS. Comparison was done between the increases in the number of platelets with the indicators of whole

blood of animals. Satisfactory was the increase in the number of platelets 4 times or more, taking into account the reference values of platelet count for rats. [18] Activation of PRP was carried out by introducing a calcium chloride solution of 0.01 mg/ml in a volume ratio of 1:10. In the syringe, the previously prepared fatty tissue with activated PRP mixed in a volumetric ratio of 9:1, was mixed. [16,17]. We expect a positive effect on the integration of mesh prosthesis in tissues due to better neoangiogenesis [19] and the acceleration of the course of reparative regenerative processes under the action of regenerative cytokines, the direct target of which are stem cells of adipose tissue [8, 15]. Thus, the expected tissue reactions of the soft tissues of the rat's back will have a character that is very similar to the structures of the abdominal wall [20, 21].

According to the formulated tasks of the study, rats had subcutaneously implantation in 4 segments of the spine: 2 ml of a fatty tissue suspension (segment I), a fragment of lightened polypropylene (80 g/m<sup>2</sup>) synthetic implant 10×10 mm (segment II), an implant fragment of 10×10 mm in combination with 2 ml of fat tissue (segment III), 10×10 mm implant fragment is surrounded by a 2 ml layer of fatty tissue from PRP (9: 1) (segment IV). The duration of the observation was 90 days. After implantation, the animals were withdrawn from the study at 30, 60 and 90 days. A section of soft tissues from the back of animals was isolated containing transplantat and underlying tissues (**Fig. 1**), and cuts were prepared according to the standard procedure. All received cuts were studied and evaluated morphologically. In order to objectify and implement quantitative control, the main available assessments of morphometric criteria were determined: evaluation of the area of fibrosis - by determining the proportion of connective tissue in the zone adjacent to the prosthesis, estimating the intensity of growth of the vessels of the vascular bed (neoangiogeosis) [19]. Standardized techniques were used (Avtandilov GG, 2002) with a microscope Leica DM 750 (Germany). Statistical processing followed by visualization was graphically executed using standard functions and algorithms of MS Office Excel 2013. The mean square deviation was calculated by determining Student's reliability ( $p<0.05$ ).



**Fig. 1.** Macroscopically: Implants in the soft tissues of the rat's back. The animal was withdrawn from the experiment after 60 days

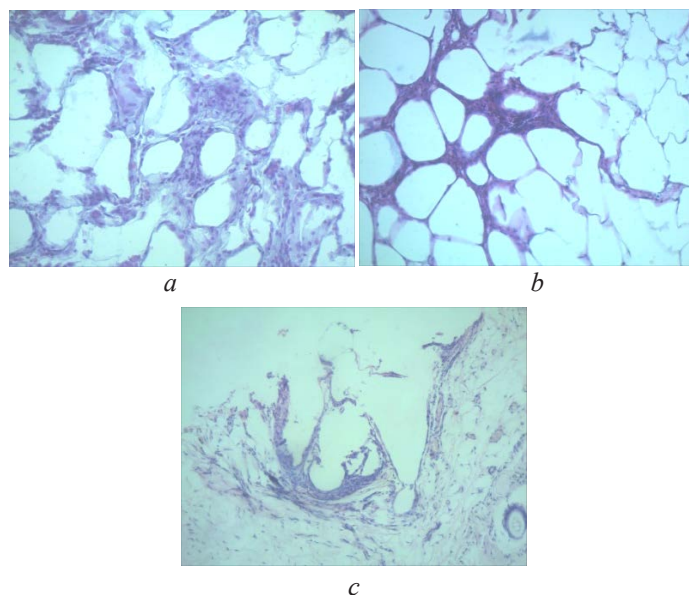
## 5. Results of the research

According to the results of the morphological study, it was determined that during the first stage of the study (30 days), the most pronounced infiltration by lymphocytes and plasmocytes was observed in the area of implantation of the polypropylene mesh implant in combination with adipose tissue and PRP; the number of cellular elements in other observational groups was significantly less than indicating an earlier and active cellular immune response. In turn, the lower level

of the cellular immune response in the zones of the isolated introduction of adipose tissue and fatty tissue with a polypropylene implant as compared with the main study group as well as a group of isolated implantation of polypropylene alloprosthesis indicates a fairly low immunogenicity of the fatty substrate as a xenotransplant, which confirms the possibility and feasibility of using foreign fatty tissue as a donor of stem cells in the experiment.

However, until the second stage of study (60 days), this acute immune tissue reaction becomes roughly the same in all surveillance areas. By the third stage of the study (90 days) there are again significant differences in the number of cellular elements of the immune response in favour of a significant reduction in the monitoring group where the prosthesis was implanted in conjunction with adipose tissue and PRP. As a result of the appearance of numerous fibroblasts in the places of implantation of the prosthesis there are fibrosis fields, their evaluation is shown below.

With isolated implantation of the adipose tissue suspension, a significant lysis of lipoproteins was observed. On the 30th day of the experiment there was a pronounced infiltration of leukocytes, partly necrosis of lipocytes (**Fig. 2, a**). During the 60th day, among large lipocytes, leukocyte infiltration is reduced; the proportion of lipocytes in the total area of the sample increases (**Fig. 2, b**). At the 90th day there was a cyst formation in the zone of dead lipocytes with moderate infiltration by lymphocytes, pericytes, sclerosis, formation of oleogranulomas (**Fig. 2, c**).



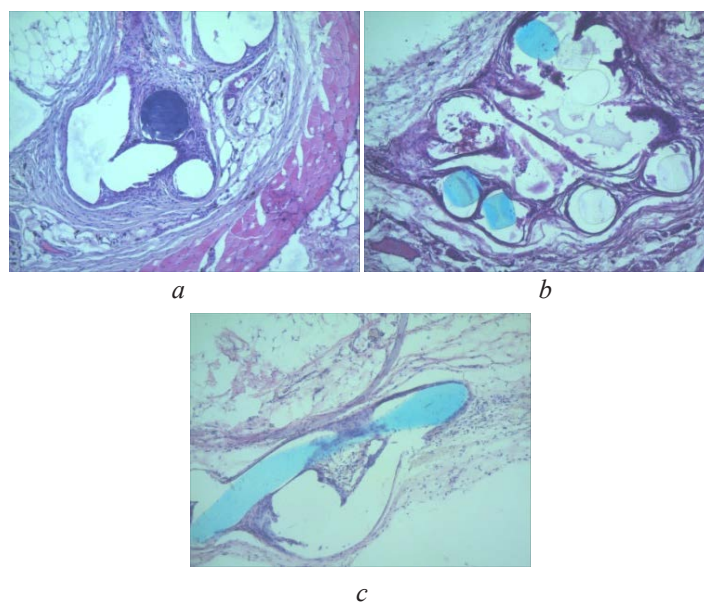
**Fig. 2.** Adipose tissue: *a* – the 30th day. An unambiguous infiltration of leukocytes, necrosis of a part of the lipocytes. Zoom 200. Coloration of this microphoto and further – haematoxylin-eosin; *b* – 60th day. Among large lipocytes, lymphocyte infiltration decreases; the proportion of lipocytes in the total area of the sample increases. Zoom 200; *c* – in the 90th day. Formation of a cyst in the area of dead lipocytes with infiltration of lymphocytes, pericytes, sclerosis. Formation of oleogranulomas. Zoom 100

In the implantation of a lightened (80 g/m<sup>2</sup>) polypropylene mesh implant in isolation, a massive fibrosis of tissues surrounding prosthesis was observed. At the 30th day there is the appearance of connective tissue around the implant, the fiber of predominantly medium and large diameter of the increase in the number of vessels of small and medium caliber (**Fig. 3, a**). At 60 days, there are isolated lymphocytes pericytes in the stroma, an increase in the number of collagen fibers around the net, the appearance of fibroblasts and fibrocytes (**Fig. 3, b**). At the 90th day marked sclerosis and a thin strip of collagen fibers around the implant, large-cell infiltration in the stroma remains (**Fig. 3, c**).

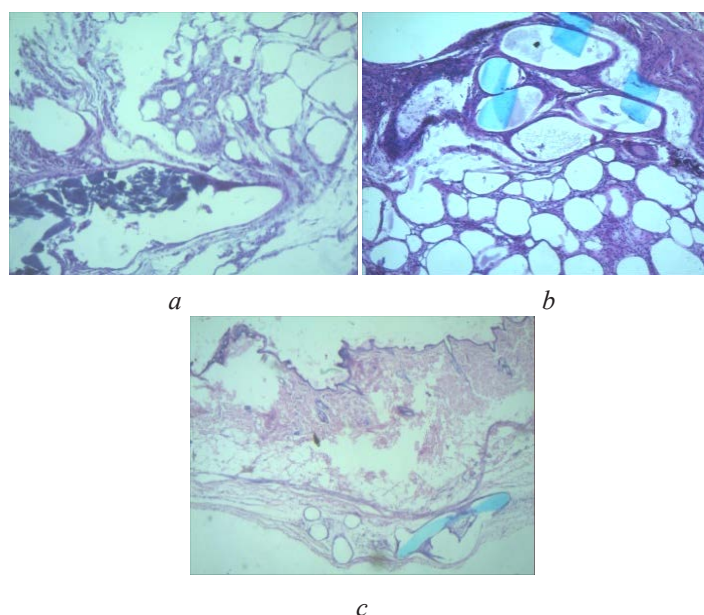
At the implantation of PMI with adipose tissue at the 30th day there was marked focal premature appearance of connective tissue, an increase in the number of capillaries, a combination of large lymphocytes and small multiloculated ones (**Fig. 4, a**). At the 60th day, lymphocytic infil-



tration around PMI, thickening of collagen fibers and an increase in their number, the appearance of small white blood cells in the form of clusters (**Fig. 4, b**) persists. These processes lasted until 90 days (**Fig. 4, c**), then appearance of fibroblasts.

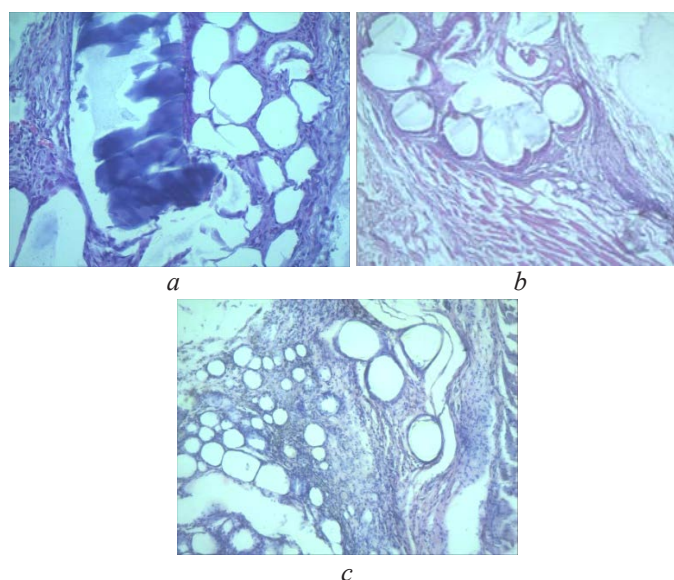


**Fig. 3.** Xenotransplant: *a* – the 30th day. The appearance of connective tissue around the xenotransplant, an increase in the number of vessels of small and medium caliber. Zoom 100; *b* – 60th day. Single lymphocytes and pericytes in the stroma, an increase in the number of collagen fibers around the mesh. Zoom 100; *c* – in the 90th day. Slightly expressed sclerosis and a thin stripe of collagen fibers around the xenotransplant, large cell infiltration in the stroma persists. Zoom 100



**Fig. 4.** Xenotransplant + adipose tissue: *a* – the 30th day. Focal premature appearance of connective tissue, an increase in the number of capillaries, a combination of large lymphocytes and small multiloculated ones. Zoom 100; *b* – 60th day. Lymphocyte infiltration around the xenotransplant, thickening of collagen fibers and an increase in their number, the appearance of small leukocytes in the form of clusters. Zoom 100; *c* – in the 90th day. Zoom 100

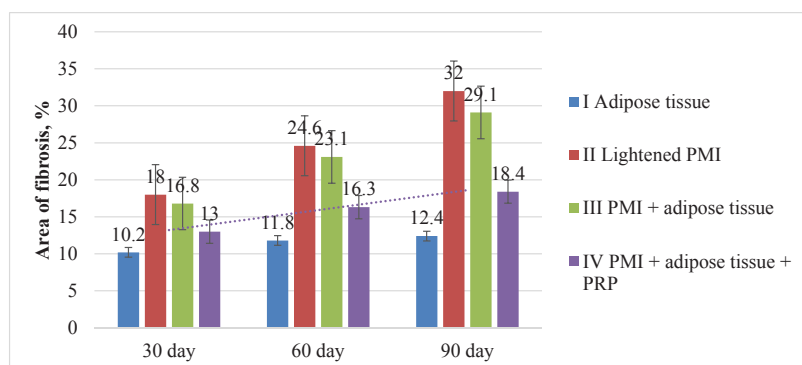
At combined implantation of PMI with adipose tissue and PRP for 30–60 days there is an early appearance and an increase in the number of collagen fibers, mainly due to thin and medium fibers, the early appearance of fibroblasts, an increase in the number of fibrocytes until the 60th day, diffuse moderate lymphocytic infiltration significantly decreases until the 60th day, a large number of capillaries (Fig. 5, *a, b*). On the 90th day after the implantation of PMI with adipose tissue and PRP moderate infiltration by lymphocytes, pericytes, histiocytes, a thin ribbon of mature collagen fibers of predominantly medium and small diameter, moderate fibrosis is predominantly due to fibrocytes with minimal inclusion of fibroblasts (Fig. 5, *c*).



**Fig. 5.** Xenotransplant + PRP + adipose tissue: *a* – the 30th day. Zoom 100; *b* – 60th day. The increase in the number of collagen fibers is mainly due to thin and medium fibers, diffuse moderate lymphocytic infiltration, a large number of capillaries. Zoom 100; *c* – in the 90th day. Preserved infiltration of lymphocytes, pericytes, histiocytes. Thin strip of collagen fibers

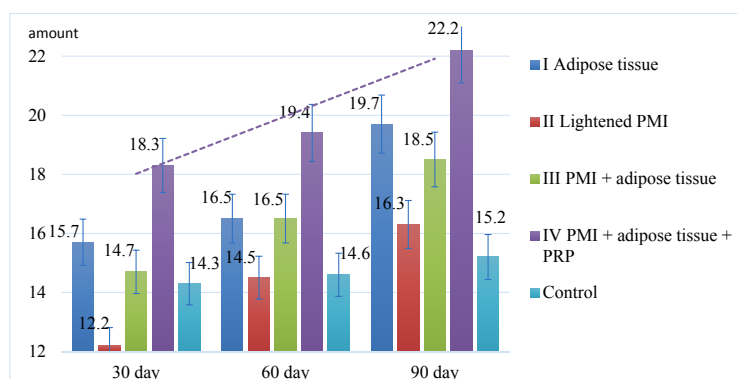
#### Morphometric evaluation

The average area of fibrosis sites in each group was calculated. The obtained results show that in case of combination of polypropylene mesh implant with PRP and adipose tissue, connective tissue occupies a significantly smaller area, in compare to other study groups. The highest level of local fibrosis was in the zone of isolated implantation of the mesh prosthesis. Proximity to the quantitative values was the manifestation of fibrosis in the implant + adipose tissue group. The statistically significant ( $p < 0.05$ ) differences in the index of fibrosis of the main study group (implant + adipose tissue + PRP), in compare to others, were obtained on the 60th day of the experiment. The discrepancy increases at the 3rd stage of the study. The results are shown on the graph (Fig. 6).



**Fig. 6.** Morphometric analysis of the formation of connective tissue (area of fibrosis, %)

The next important factor in the integration of the synthetic implant in the surrounding tissue, in addition to the absence of excess fibrosis, is the degree of vascularization of the formed prosthesis complex – the connective tissue. In the analysis of the data on vascularization of tissues around the prosthesis (10 fields of vision were calculated in diameter of 1 mm). The average value was determined in each group of all three stages of the experiment with the calculation of the mean-square deviation. Statistically proven ( $p < 0.05$ ) is an increase in the units of the microcirculatory channel from 60 days. The dynamics are stored for 90 days, confirming the difference statistically. At the implantation of the lung prosthesis in combination with PRP and adipose tissue, from the first month, markedly increased the number of vessels in the center of the experiment, which significantly increases up to 3 months (Fig. 7). Obviously, the administration of PRP stimulates neo-angiogenesis due to the prolonged action of regenerative cytokines.



**Fig. 7.** Morphometric analysis of the intensity of local neoangiogenesis. The average number of blood vessels in the microcirculation stream around the graft in a field of 1 mm in diameter

## 6. Discussion

Thus, the isolated use of a suspension of adipose tissue only to a small extent reduces the degree of fibrosis of the peri-prosthetic zone and enhances neoangiogenesis in this area. This is due to the weak integration of the lipocytes into the complex “mesh implant – connective tissue” with their significant lysis and resorption. When combined fatty tissue suspension with PRP in the implant zone, multipotent stem cells can also be detected, capable of active differentiation into the various cellular structures of the connective tissue and active cytokines that stimulate this process, which provides for earlier neoangiogenesis. As a result, the integration of the implant takes place faster due to more qualitative vascularization of the zone around the fibers of the prosthesis, less ischemia of the tissues. As a result, chronic inflammation is less pronounced in the distant period after implantation (2–3 months), and a lesser degree of hyperfibrosis. All this creates the conditions for the formation of a thin, elastic, connective tissue with a good blood supply on the facilitated polypropylene implant, which in its properties is as close as possible to the natural undamaged aponeurosis of a person.

In compare to earlier published data, which were modelled similar tissue reactions in rabbits – OscarRubiniÁvila, 2016 [22], the data obtained now correlate with the author’s determined increase in the number of inflammatory cells in the study group. It should be noted that as a target for PRP in the experiment the actual fatty tissue of rabbits performed. The definition by the author of collagen types can also be explained indirectly by the data we received regarding the reduction of the area of fibrosis.

In the work of JeffreyVan Eps 1, 2 2016 [21] demonstrated better incorporation of the biological mesh prosthesis due to the local introduction of PRP. The authors emphasized the intraperitoneal placement of the mesh and the determination of its adhesiveness and the risk of adhesions.

Another job is J. S. Fernandez-Moure, M.D. M.S., 2015 [17] demonstrated a positive effect of PRP on neoangiogenesis and implantation of biological prosthetics. The general idea of the trial is quite close, but the difference is in the material of the mesh implant, which is extremely important in the assessment of local tissue reactions.

## 7. Conclusions

1. After introduction of a fatty graft together with platelet rich plasma in the area of implantation of the lung polypropylene prosthesis, there was an accelerated tissue reaction from the integration of the prosthesis.

2. Mesenchymal stem cells of adipose tissue that are a target for cytokines of platelet rich plasma have a more pronounced effect in stimulating reparative processes upon condition that they are simultaneously administered with PRP in compare to isolated administration without PRP.

3. The use of a platelet rich plasma and adipose tissue construct has a significant positive effect on local angiogenesis.

4. In the presence of improved angiogenesis and other stimulating factors in the conditions of introduction of adipose tissue and PRP, the integration of the prosthesis occurs with significantly less peri-prosthesis fibrosis.

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