Experimental Study Comparing Structural Changes Induced by Biologic Versus Synthetic Mesh Implants in Nephropexy

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Abstract

BACKGROUND: Long-term chronic inflammatory response when using meshes for nephropexy may lead to kidney fibrosis, renal failure, and chronic pain syndrome. Research on biological properties of various materials used for nephropexy, as well as the search for new implant materials is a pressing problem, as it greatly affects patients' quality of life. The present study suggests that the use of the extracellular bovine-derived peritoneum matrix as a new biological implant opens up new prospects for nephropexy.

AIM: This study aims to evaluate the structural changes at the interface of the renal tissues and paranephron with the extracellular bovine-derived peritoneum matrix and the UltraPro mesh after nephropexy in an experimental animal.

MATERIALS AND METHODS: Experimental nephropexy was performed in 64 white shorthaired adult rats, divided into two groups: Extracellular bovine-derived peritoneum matrix and UltraPro mesh. Implants were 1.5 × 1.5 cm per one animal. Observation periods were 7, 21, 30, and 180 days. The tissue was stained with hematoxylin and eosin, Van Gieson's with Pikro-Fuchsin. Cellular infiltrate was evaluated by counting granulocytes, mononuclear cells, and foreign body giant cells on five high-magnification images for each stained section (×400).

RESULTS: The use of the extracellular bovine-derived peritoneum matrix induced less intense and less prolonged chronic inflammatory response, as well as intense production of new collagen fibers more similar to the native connective tissue in terms of their histologic structure. The UltraPro mesh induced moderately persistent chronic inflammatory response throughout the 6-month study period. By this period, the level of multinucleated foreign body giant cells in the UltraPro group was 1.0 ± 0.2, it was statistically significantly higher than in the group with extracellular bovine-derived peritoneum matrix – 0.6 ± 0.1 (p < 0.05).

CONCLUSION: Histologic evaluation demonstrates high biocompatibility of both the extracellular bovine-derived peritoneum matrix and the UltraPro mesh implants. The results of using new biological material are not worse than synthetic mesh.

Introduction

Research on biological properties of various materials used for nephropexy, as well as the search for new implant materials is a pressing problem, as it greatly affects patients' quality of life. Meshes have been routinely used in surgery for quite some time. Histologic observations describe long persistent chronic inflammation with foreign body granulomas forming at the mesh/tissue interface. The chronic inflammatory process impedes both adequate wound healing and tissue regeneration. It may still be acceptable in light of the suggested function of the permanent implants aiming primarily at restoring mechanical functionality. However, long-term chronic inflammatory response when using meshes for nephropexy may lead to kidney fibrosis, renal failure, and chronic pain syndrome [1], [2].

The aim of this study is to evaluate the structural changes at the interface of the renal tissues and paranephron with the extracellular bovine-derived peritoneum matrix and the UltraPro mesh after nephropexy in an experimental animal.

Materials and Methods

Implants and animals

Extracellular bovine-derived peritoneum matrix is a new domestic developed biological implant (Kazakhstan), acquired through 2 cycles detergent-enzymatic decellularization with gamma-irradiation sterilization [3]. The UltraPro mesh is a partially absorbable lightweight monofilament mesh comprised of non-absorbable polypropylene fibers (Prolene) and absorbable poliglecaprone fibers (Monocryl) with large pores (1–4 mm) [4]. The UltraPro mesh was purchased

from an authorized distributor of Hernia System Ethicon (USA).

An experimental study was performed on 64 white shorthaired adult rats of both sexes (180–220 g). The rats were randomly assigned to two groups and four subgroups, eight rats in each combination. Groups correspond to different types of implants, while subgroups correspond to the duration of the experiment (7, 21, 30, and 180 days), after which the rats were euthanized.

All animals were kept and cared for in accordance with the basic principles established by the Guide for Care and Use of Laboratory Animals. Eight edition. ILAR publication, 2012, National Academy Press. Furthermore, experimental work with laboratory animals was carried out in accordance with the order of the Ministry of Health No. 348 dated May 15, 2015, "On approval of the rules for preclinical studies, biomedical experiments, and clinical trials in the Republic of Kazakhstan" and in compliance with international principles established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose.

Surgical procedures

Extracellular bovine-derived peritoneum matrix and the UltraPro mesh implants were fixed by suture to the renal tissue and paranephron in experimental animals. For this, under general ether anesthesia (diethyl ether, Rahim, Russia; exposure time: 5 min), rats were subjected to a linear dorsal cut, continuing layer-by-layer down to the kidney and paranephron. Test materials sized 15 × 15 mm were fixed in the renal capsule with Prolene 4/0 on an atraumatic needle. After the intervention, antibacterial treatment with enrofloxacin (0.1 ml/10 mg) (Enforex, World-Vet, Turkey) was administered over 5 days to prevent wound infection.

Histologic evaluation

Animals were euthanized after 7, 21, 30, and 180 days of implantation through decapitulation under general anesthesia, and histologic material was explanted. The explants comprised tissue fragments at the interface of renal tissue and paranephron with the implant, outside suture line. Tissue explants were fixed with 10% neutral buffered formalin solution, treated with isopropyl alcohol and xylene, and embedded in paraffin in a carousel-type tissue processor following the established protocol. After orientation in the processing, cassettes samples were embedded into paraffin and formed into blocks for further sectioning (3 µm). Histologic sections were stained by hematoxylin-eosin (BioVitrum, Russia) and Van Gieson's picrofuchsin (BioVitrum, Russia) using standard techniques.

To evaluate tissue response to implants in compliance with the recommendations of ISO 10993 [5], the system of semi-quantitative histologic evaluation by Zheng [6] and Valentin [7] was adapted (Table 1). Microscopic evaluation was conducted for semi-quantitative evaluation of cellular infiltrates, neovascularization, and fibrosis.

Cellular infiltrate was evaluated by counting granulocytes, mononuclear cells, and foreign body giant cells on five high-magnification images for each stained section (×400) (Zeiss Axioplan 400, Oberkochen, Germany). These images were randomly selected at the interface of the implants with the surrounding native renal tissues and paranephron.

The evaluation system was expanded by the histologic evaluation of the organization, composition, and amount of collagen, adapted from Junqueira [8] and Badylak [9] scales. Collagen organization was evaluated on a scale from completely disorganized to well-organized scar tissue (from 0 to 3). Scar collagen composition was evaluated on a scale of absent (0), cellular (1), mixed (2), and (nearly) acellular (3). Collagen deposition was assessed on the whole surface of a histological section on a scale of absent (0), minimal/below 10% (1), moderate/below 30% (2), and abundant/over 30% (3).

When evaluating neovascularization, the number of blood vessels was evaluated in the same fragments of tissue as in the case of the cellular infiltrate. Blood vessel distribution was assessed on the whole surface of the histological section.

Statistical analysis

Mean value (X) and standard deviation (SD) were calculated for each quantitative data group. Group differences were determined using the statistical methods of non-parametric tests. The intergroup comparison was done using the Mann–Whitney U-test, while the intragroup comparison was done using the Kruskal–Wallis test. The level of statistical significance is taken at $p \leq 0.05$. Software used for statistical analysis was IBM SPSS Statistics 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

Results

Table 2 presents the results of a comparative histomorphometric analysis of the cellular infiltrate and tissue response in representative tissue fragments at the interface of the extracellular bovine-derived peritoneum matrix and the UltraPro mesh with the renal tissues and paranephron.

Table 1: Semi-quantitative histologic evaluation system

Criteria	Scores			
	0	1	2	3
Cellular infiltrate** [4]				
Polymorphonuclear cells	0	1–5	6–10	>10
Mononuclear cells	0	1–5	6–10	>10
Foreign body giant cells	0	1–5	6–10	>10
Relative quantities of fibroblasts and inflammatory cells	Inflammatory cells only, no fibroblasts	More inflammatory cells, few fibroblasts	More fibroblasts, few inflammatory cells	Fibroblasts only, no inflammatory cells
Neovascularization [4], [5]				
Number**	0 or 1	2–5 blood vessels	6–10 blood vessels	Over 10 blood vessels
Distribution	Indeterminable	Blood vessels are located along the edge of the implant	Blood vessels are located on the periphery of the implant without reaching its center	Blood vessels reach the center of the implant
Collagen [6], [7]				
Organization	Completely disorganized	Relatively disorganized	Relatively organized	Well-organized scar tissue
Composition	–	Cellular	Mixed	Nearly acellular collagen scar
Quantity	Absent	Minimal	Moderate	Abundant

**Histopathologic evaluation was conducted on five high-magnification images for each stained section (×400).

Table 2: Histologic evaluation of the cellular infiltrate and tissue response in the representative fragments at the interface of the extracellular bovine-derived peritoneum matrix (ECM) and the UltraPro mesh with the renal tissues and paranephron

Days	Group	Cellular infiltrate				Neovascularization		Collagen		
		Polymorphonuclear cells	Monocytes	Multinucleated foreign body giant cells	Relative quantity of fibroblasts and inflammatory cells	Number of blood vessels	Blood vessel distribution	Organization	Composition	Quantity
7	ECM	2.4 ± 0.0	2.5 ± 0.2	1.1 ± 0.1	0.6 ± 0.1	2.1 ± 0.1	1.4 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.5 ± 0.1
	UltraPro	2.1 ± 0.1*	2.4 ± 0.1	1.3 ± 0.2	0.7 ± 0.1	1.6 ± 0.1	1.2 ± 0.2	0.1 ± 0.2	0.3 ± 0.1	0.6 ± 0.2
21	ECM	1.9 ± 0.2	2.8 ± 0.1	1.8 ± 0.2	1.1 ± 0.1	2.4 ± 0.1	1.2 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.9 ± 1.1
	UltraPro	1.6 ± 0.2	1.9 ± 02*	1.9 ± 0.3	1.2 ± 0.2	2.2 ± 0.2	1.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	1.4 ± 0.2*
30	ECM	0.6 ± 0.4	1.2 ± 0.2	2.1 ± 0.2	2.4 ± 0.2	1.9 ± 0.2	2.2 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.8 ± 0.1
	UltraPro	0.6 ± 0.3	1.1 ± 0.2	2.8 ± 0.2*	2.2 ± 0.1	2.1 ± 0.2	2.0 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	2.2 ± 0.2*
180	ECM	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	2.8 ± 0.2	1.4 ± 0.2	2.2 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	1.9 ± 0.2
	UltraPro	0.2 ± 0.2	0.3 ± 0.1	1.0 ± 0.2*	2.6 ± 0.1	2.1 ± 0.2*	2.4 ± 0.2	2.0 ± 0.3	1.6 ± 0.1*	2.7 ± 0.2*

The data presented are mean values and standard deviations. *p<0.05 (comparing the ECM and the UltraPro mesh of the same term) (Mann–Whitney U-test).

Extracellular bovine-derived peritoneum matrix

Morphological pattern was characterized by pronounced acute inflammatory response with the prevalence of granulocytes at peak level after 7 days, their subsequent decrease by 30 days, and their complete absence after 180 days. Necrotic inflammation with microabscesses was observed in one experimental animal.

After 7 days, moderate infiltration by lymphocytes, plasma cells, and macrophages, as well as the formation of the young granulation tissue rich in newly formed thin-walled vessels were observed at the implant/tissue interface. Cellular infiltrate showed higher concentration of granulocytic and lymphocytic cells over other cellular elements, which is typical of the exudate phase of tissue response to an implant or a surgical wound. Connective tissue was characterized by dispersed formation of filamentary reticular fibers and occupied less than 10% of the image. After 7 days, angiogenesis was characterized by the formation of thin-walled hyperemic small-sized vessels with sparse enlarged endothelial cells, which is typical of active capillary budding process (Figure 1).
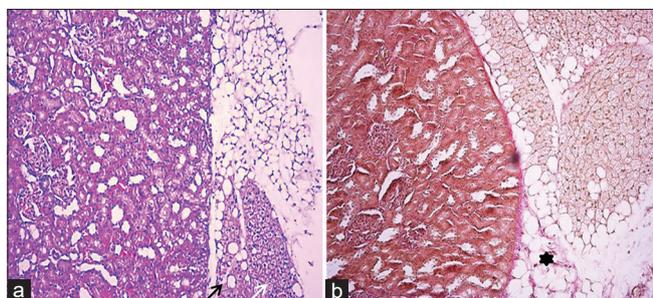

Figure 1: Seven days. The microscopic area of implantation into the fibrous capsule of the kidney. Early granulation tissue (white arrow) with a large number of newly formed microvessels (black arrow) is determined. Connective tissue is represented by single filamentous reticular fibers (black asterisk) (a) – hematoxylin-eosin stain; (b) – Van Gieson's picrofuchsin stain, 200 times magnification

On the 21ˢᵗ day after the implantation, morphology is characterized by moderate lymphocytic and macrophagal infiltration with fibrous connective tissue forming at the implant/renal tissue interface. Cellular composition at the implant/tissue interface is characterized by a higher concentration of lymphocytic cells as compared to granulocytes, albeit the concentration of the inflammatory cells is on the decrease, while that of the stromal cells (fibroblasts/fibrocytes) is on the increase, which is the evidence of active stroma growth, intense production of collagen, and active fibroblast growth. At this time point, formation of connective tissue was characterized by focal formation of mature connective tissue fibers, comprising 10–50% of the image.

On the 30ᵗʰ day, the test group shows predominance of mature connective tissue and the ingrowth of the collagenic fibers both into the implant material and into the renal capsule. This means that connective tissue structures predominate over cellular infiltrate. There is a statistically significant decrease of cells of the exudative inflammation and proliferative phase of tissue response in the cellular infiltrate as compared to the initial terms of observation, with a significant predominance of stromal cells. This shows that persistent inflammatory response does not accompany scar formation.

By 180ᵗʰ day after the implantation, stromal cells predominate. They form firm connective tissue contact between the implant materials and the renal tissues with no signs of chronic inflammation or rejection of the implant (Figure 2)
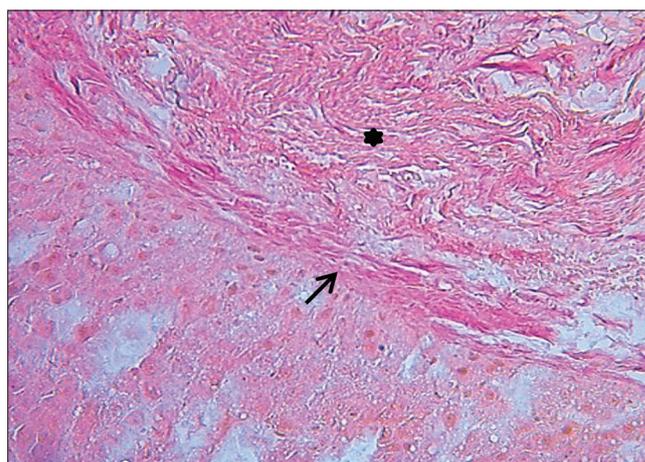

Figure 2: One hundred and eighty days. connective tissue contact between the extracellular bovine-derived peritoneum matrix and the renal tissues. A well-organized connective tissue (black asterisk) is attached to the fibrous capsule of the kidney (black arrow), which forms an almost acellular collagen scar. There are a lot of moderately crimped collagen fibers, located mainly in parallel, with even distance between the collagen fibers (black asterisk). Van Gieson's picrofuchsin stain, 200 times magnification

Giant cell response with multinucleated foreign body giant cell formation gradually subsided. Active neovascularization was observed over the whole period of the experiment. Van Gieson staining showed that the extracellular bovine-derived peritoneum matrix induced less intense collagen production.

UltraPro mesh

The morphological pattern was characterized by the predominance of granulocytes after 7 days. After 21 days, granulocyte count decreased, and by 30 and 180 days, it decreased almost to zero. After 7 days, granulocytes and lymphocytes predominated in the cellular infiltrate (Figure 3). In histologic testing, the mesh itself was represented in the form of porous spherical and elliptical structures surrounded by fibrous tissue.

By 30 days, cellular response consisted primarily of clusters of mononuclear cells and multinucleated giant cells. After 180 days, cellular
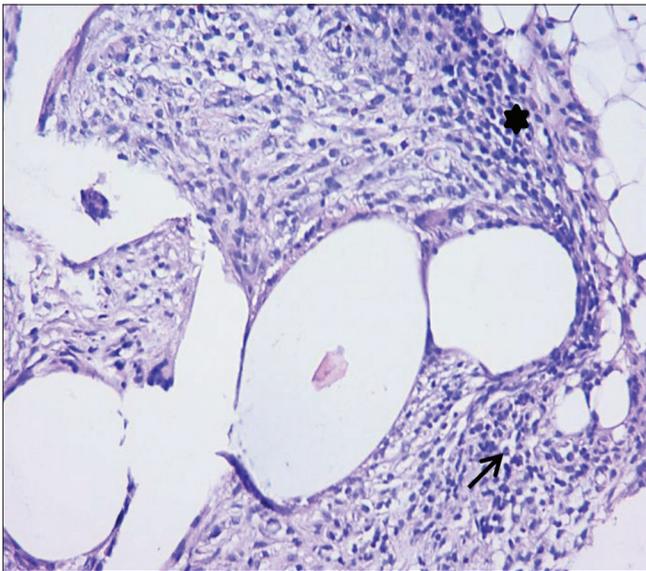
Figure 3: Seven days. Early granulation tissue with diffuse lymphocytic infiltration (black asterisk) and single granulocytes (black arrow). Hematoxylin-eosin stain, ×400 times magnification

infiltrate contained sparse multinucleated cells scattered along the periphery of the implant site. Giant cell reaction with the formation of multinucleated foreign body giant cells consistently subsided (Figure 4).
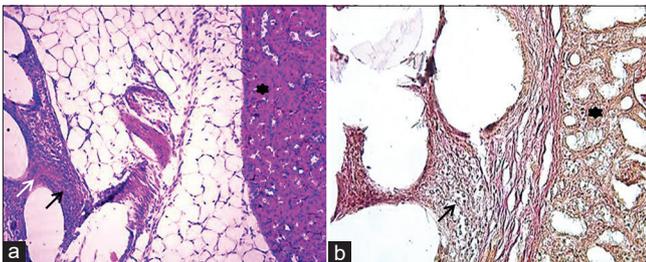

Figure 4: One hundred and eighty days. Formation of coarse fibrous connective tissue with a chaotic arrangement of fibers (white arrow) in the implantation area between the UltraPro prosthesis and renal tissues (black asterisk). There are a lot of organized and mature connective tissue (white arrow) with stromal cells (black arrow) and collagen fibers. (a) Hematoxylin-eosin stain; (b) – Van Gieson's picrofuchsin stain, 200 times magnification

Implant sites are characterized by sparse lymphocytic infiltration with the predominance of stromal cells (fibroblasts/fibrocytes) in the cellular infiltrate, as well as by diffused growth of connective tissue. Van Gieson staining showed that UltraPro mesh induced more intense production of relatively disorganized collagen.

On 180 days, the absorbable poliglecaprone fiber of the UltraPro mesh was absent in all histological sections. Non-absorbable fibers of the UltraPro mesh were surrounded by a moderate amount of fibrous tissue inducing inflammatory response that was quantitatively and qualitatively similar to that observed in the group with the extracellular bovine-derived peritoneum matrix. Yet, significantly larger depositions of more disorganized and less mature collagen fibers were observed. Moderate vascularization was observed both in the surrounding tissue and around the mesh fibers.

Discussion

This animal testing experimental study conducted a comparative histomorphometric analysis of cellular infiltrate pattern and tissue response in the representative fragments at the interface of the extracellular bovine-derived peritoneum matrix and the UltraPro mesh with the renal tissues and paranephron.

The most important finding of this study is that the extracellular bovine-derived peritoneum matrix as compared to the UltraPro mesh induces less intense production of collagen depositions, albeit better organized and structurally more similar to the native tissue, as well as less pronounced chronic inflammatory response at the implant/tissue interface.

According to the preexisting studies, polypropylene mesh behaves like a foreign body in the surgical wound. It, therefore, may induce prolonged (chronic) inflammatory response impeding progress to proliferative healing phase responsible for the scar tensile strength [10], [11].

An experimental study on rats by Klinge *et al*. [12] evaluating cellular response to the polypropylene mesh found that the inflammatory response was at peak level between 7 and 14 days. A different study on rats by the same author reported the presence of the giant cells in the polypropylene meshes 7 days after the surgical intervention. Their number increased by 14 days and continuously increased until the end of the experiment. Klinge also observed full attenuation of the acute inflammatory response and presence of cellular infiltrate after 21 days, which is a sign of chronic inflammation.

This study has demonstrated the presence of neutrophils in the surgical wound until 21 days, which is longer than what was observed in the preexisting studies [13], [14], [15], [16] and shows the persistence of acute inflammatory response.

Notably, the UltraPro mesh group showed the maximum level of the mononuclear cell infiltration 7 days after the intervention, which was consistent with the results of the preexisting studies [11]. It then gradually subsided by 21 days remaining present at low levels up until 180 days. In contrast, in the group with extracellular bovine-derived peritoneum matrix, mononuclear cells persisted until 21 days and subsequently decreased. Giant cells indicating chronic inflammatory response and foreign body reaction appeared after 7 days, but their presence was more pronounced after 21 days remaining such until the end of the experiment in the UltraPro mesh group, which

corresponds to the histologic pattern described in the preexisting studies [17].

This study has shown that the extracellular bovine-derived peritoneum matrix induces a more acute inflammatory response reaching the peak level after 7 days and gradually reduced to the negligible levels by 30–180 days. The UltraPro mesh group showed a more prolonged chronic inflammatory response with a larger number of foreign body giant cells persisting until 30–180 days after the implantation. The UltraPro mesh induced qualitatively and quantitatively more pronounced chronic inflammatory response as compared to the test group, mostly due to a large number of phagocytic macrophages around monofilaments as part of the process of degradation [18]. After 180 days, the UltraPro mesh group showed thicker deposited collagen (Score: 2.7) as compared with the group with the extracellular bovine-derived peritoneum matrix (Score: 1.9). Fibers comprising mesh multifilaments located in the macrophage and giant cell cytoplasm point to intense phagocytosis.

Histomorphometric analysis has shown adequate biocompatibility of both biomaterials used for nephropexy in this study. Extracellular bovine-derived peritoneum matrix shows a short-term acute inflammatory response similar to that in the case of the UltraPro mesh. The important difference is that inflammatory response in the test group is largely attenuated after 30 days preventing prolonged chronic response development and is mostly absent after 6 months. Insofar as extracellular bovine-derived peritoneum matrix completely biodegraded and inflammatory response substantially subsided, well-modeled, and firm connective tissue substituted the implant. The collagen layer surrounding the extracellular bovine-derived peritoneum matrix was thinner but more organized and more structurally similar to the native connective tissue as compared with the UltraPro mesh.

Conclusion

We have thereby found that extracellular bovine-derived peritoneum matrix is a fully biocompatible implant material with total connective tissue ingrowth and a low degree of inflammatory response at the tissue/implant interface in an *in vivo* rat model. The results of the experiment have shown that in contrast to the UltraPro mesh, extracellular bovine-derived peritoneum matrix does not induce chronic inflammatory response and is conducive of production of better-organized collagen structurally similar to the native connective tissue. This result is beneficial because animal testing is a good indication of how the material may behave when used in humans.

Further studies on biocompatibility of the extracellular bovine-derived peritoneum matrix are needed to confirm the above results and to expand the prospects for its use. This biomaterial, derived from bovine peritoneum, has proposed advantages over synthetic materials due to increased biocompatibility and reduced foreign body reaction within human tissues. The lack of clinical trials studies of the implant, call for a medical effectiveness assessment to determine the indications for extracellular bovine-derived peritoneum matrix in parietal and general surgery.

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