IN VITRO STUDIES OF BIOFILMS ON THE SURFACE OF SYNTHETIC MACROPOROUS ENDOPROSTHESES FOR ABDOMINAL WALL PLASTY

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The aim of the investigation is to study the bacterial growth characteristics in vitro on the surface of synthetic macroporous endoprostheses used in modern surgery for abdominal wall plasty in case of hernias, and biofilms formation.

Materials and methods. We studied endoprostheses made of polypropylene biofilms (standard, light), polyvinyliden fluoride, reperen, composite materials (polypropylene and polyvinyliden fluoride) used in hernia surgery. Meshes were contaminated by Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa. After incubation the preparations were studied under microscope in dark field, and biofilm formation was assessed using special scale.

Results. Microbial biofilm forms within 48 h in vitro on endoprostheses surfaces. Ps. aeruginosa has the maximum capacity to form a microbial biofilm, St. aureus — small capacity, St. epidermidis — the minimum capacity (p=0.027). Ps. aeruginosa significantly low contaminates light polypropylene meshes than any other meshes (p=0.009), and colonizes more intensively standard polypropylene meshes than smooth surface of reperen endoprostheses (p=0.024). There is no such relation for St. aureus. Reperen is maximally contaminated by St. epidermidis (p=0.044).

Conclusion. Biofilm formation is a universal mechanism of mesh infection, and it can be realized in vitro on any endoprosthesis. The mechanism characteristics depend on the material, mesh type, surface microrelief, and microbial strain. To perform operations using synthetic materials in bacterial contamination conditions it is necessary to design special endoprostheses capable to resist colonization and biofilm formation.

Key words: mesh, biofilm, tension-free plasty, hernia, synthetic endoprosthesis, emergency surgery.

Modern surgical procedures for abdominal wall repair by hernias are based on the implantation of synthetic endoprosthesis. The use of mesh according to principles of tension-free plasty was an important step in resolving the problem of hernia recurrence. Life quality is significantly high in patients after abdominal wall reconstruction with endoprosthesis compared to the cases of hernia repair without mesh implantation [1–3]. Today the feasibility and usefulness of tension-free technique in urgent surgery are proved [4, 5]. Implants are also recommended in patients with strangulated hernias [6].

However, the possibility of using mesh in contaminated areas is discussed. There are no recommendations for choice of endoprosthesis to implantation in compromised wound [7, 8]. Chronic paraprosthetic infection is referred to as unresolved problem of surgery [9, 10].

In experimental study the reparative process after mesh implantation with bacterial contamination of wound was found to occur differently, its phase remaining the same, but the speed of reparation being slower [11]. The growth of microorganisms is likely to depend on the implant type. Microporous mesh (polytetraftorethylene) are widely known not to be used in infected area [12]. But successful application of macroporous mesh in urgent surgery was confirmed in clinical study [13].

The resistance of mesh to infection development in wound has special importance in those cases, when synthetic materials are used by relative indications — in patients with small hernia (W1), and in preventive plasty of abdominal wall (in cases without hernia).

The question of mesh use in critical high contamination of implantation area is not solved today, for example in patients with peritonitis [14–16]. There are only a few works describing in detail the situation after tension-free plasty in infected operative field [17–19].

A number of problems may be associated with the formation of microbial biofilm on the surface of the prosthesis and the suture material. Biofilm is known as the basis for chronic infection development [20]. No definitive data on the dependence of bacterial growth on material of macroporous implants are presented. The colonization is known to be associated with hydrophobic properties of endoprosthesis, its multifilament construction and the availability of niches in mesh fibers [21].

Recently was presented, that in 90 days after mesh implantation into infected area of rabbit abdominal wall

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was found no macroscopic signs of inflammation, but the bacterial contamination was confirmed [22]. This pattern is established in experimental study with standard and lightweight polypropylene mesh having 0.8–3.6 mm pore size. The use of tension-free technique in a compromised area of operation relates to topical issues of abdominal wall repair. Infection development after implantation of synthetic endoprosthesis yet has no final solution. This problem requires fundamental research, both clinical and experimental.

The aim of the work was to study the features of bacterial growth on the surface of macroporous synthetic mesh specially designed for abdominal wall repair in patients with hernias.

Material and Methods. We contaminated implants in vitro with certain types of Staphylococci and Pseudomonas aeruginosa, the meshes under study relating to brands widely used in urgent surgery. Clean cultures of Staphylococcus epidermidis (type 178M), Staphylococcus aureus (type 5983/5), and Pseudomonas aeruginosa (type 485) were isolated from clinical sources and identified in laboratory. The colonization of implants was carried out according to accepted standards [23]. Surgical meshes were studied: Standard polypropylene (mesh thickness: 500 mm, thread size: 120 mcm, density: 62 g/m^2), Uniflex (polyvinyliden flouride, mesh thickness: 480 mcm, thread size: 120 mcm, density: 160 g/m^2), Flexilen (composite, PVDF + polypropylene, mesh thickness: 500 mcm, thread size: 120 mcm, density: 90 g/m^2), Lightweight polypropylene (mesh thickness: 390 mcm, thread size: 90 mcm, density: 36 g/m^2), Reperene (3D-sutured polymer from methacryl oligomeres, one side with a smooth surface, and the second — rough, type 1 — a two-layer, type 2 — a three-layer, thickness: 300 mcm).

For contamination the parts of meshes, 1 cm^2 in size, were placed into Petri dishes, 36 mcm in diameter, containing 4 ml of trypsin soya broth, in which the bacteria were suspended at the concentration of 10^7 CFU/ml. The incubation time was 48 hours. The preparations were fixed with 4% formalin, dried, and studied in the dark field with a microscope ("LOMO", St. Petersburg, magnification 1.6x40), documented by photography. To compare biofilm coatings on the surface of mesh, the special score system (0–4) similar to that of Vanderbilt was used, the scale being widely used in herniology to study adhesions between the surface of implant and visceral organs [24]. The results were analyzed statistically using the Mann–Whitney test with Origin Pro 8 for Windows 7 on Emachines.

Results. The colonization indexes of synthetic implants are represented in Table 1. The most important result of study was finding of bacterial biofilm on the surface of surgical mesh.

In a series of experiments with the culture of Ps. aeruginosa the following data were obtained (Fig. 1). On the surface of the Standard polypropylene prosthesis there was revealed a biofilm covering almost all the mesh surface (Fig. 1a). There were no bacteria on the surface of mesh in control series (Fig. 1b). The biofilm was found on the surfaces of mesh from Uniflex and Flexilen (Fig. 1c). The maximal resistance to contamination was noted for Lightweight polypropylene mesh (Fig. 1d) and Reperene (especially the Ultra-smooth surface). The colonization was 2.85 points on the average, PP Std — 3.8; Flexilen — 3.75; Uniflex — 3.67, Reperene — 2.45 (p=0.024), PP Light — 0.67 (p=0.009).

In experimental series with the culture of St. aureus the results were different (Fig. 2). On the surface of the prosthesis from Reperene type 1 a typical biofilm was revealed (Fig. 2a). The surface mesh from Reperene type 2 was covered with colonies of microorganisms, and the smooth side was even more susceptible to bacterial growth (Fig. 2b). The fibers of PP Std were almost completely covered with biofilm of S. aureus (Fig. 2c). The area of biofilm on the surface of Flexilen, Uniflex, PP Light fibers was small (Fig. 2d). The colonization of mesh was 2.52 points on the average: PP Std — 3.67; Reperene — 3.27; Flexilen — 2.5; Uniflex — 1.33; PP Light — 0.5 (p=0.046.)

In experimental series with St. epidermidis the following data were obtained (Fig. 3). The bacterial growth on the surface of Light polypropylene mesh was ranged from single colonies to the dense population of staphylococcal surface of the implant. Flexilen and Uniflex were more contaminated. On the polypropylene fibers biofilm was also formed, but the density of bacteria was lower than in the experiment with the culture of St. aureus. On the rough surface of the Reperene type 1 a typical biofilm was found (Fig. 3a), on the smooth surface — there was practically no contamination (Fig. 3b). At the same time on the rough surface of Reperene type 2 many bacterial clusters were found, and on the smooth surface of Reperene a typical biofilm of St. epidermidis was identified (Fig. 3c). The colonization of endoprosthesis was 2.02 points on the

**The bacterial colonization on the surface of surgical mesh**

Material of mesh	Type of bacteria		
	Pseudomonas aeruginosa	Staphylococcus aureus	Staphylococcus epidermidis
Standard polypropylene (PP Std)	3.8	3.67	1.53
Polyvinylidenftoride + polypropylene (Flexilen)	3.75	2.5	1.5
Polyvinylidenftoride (Uniflex)	3.67	1.33	1.5
Light polypropylene	0.67	0.5	1.14
Reperene	2.45	3.27	2.5
Median (M)	2.85	2.52	2.02

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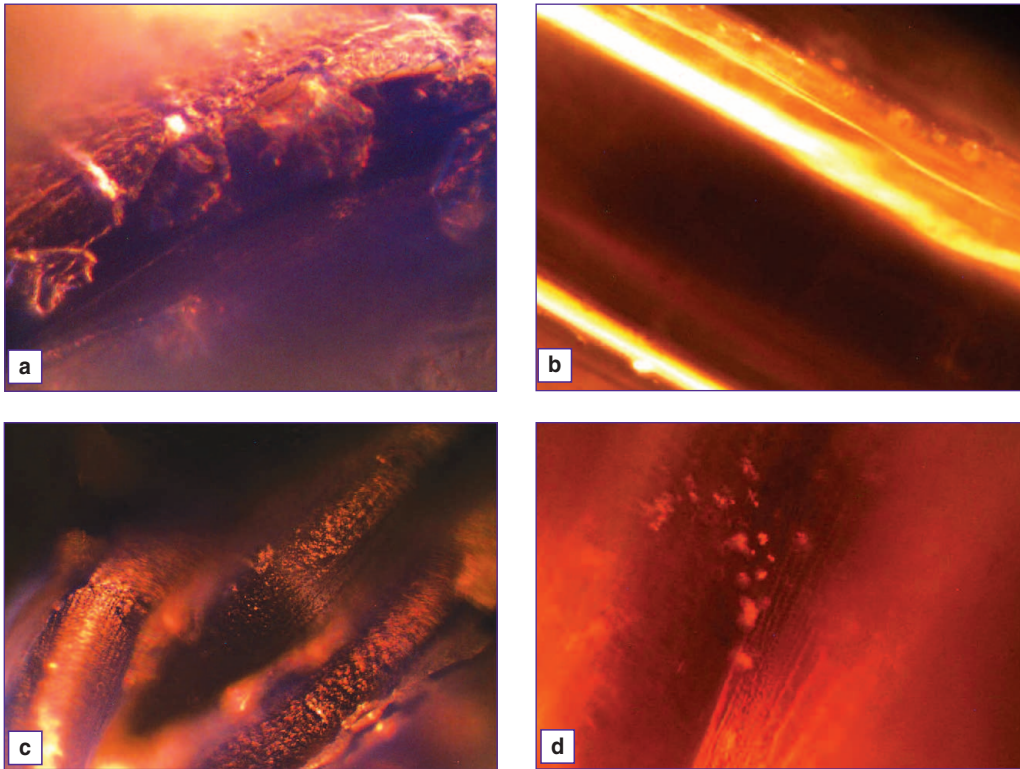
Fig. 1. Contamination of synthetic implants with Ps. aeruginosa: *a* — biofilm on the surface of Standard polypropylene mesh; *b* — Standard polypropylene mesh without contamination (control); *c* — microbial biofilm on the surface of Uniflex mesh; *d* — small colony on the fiber of Lightweight polypropylene prosthesis
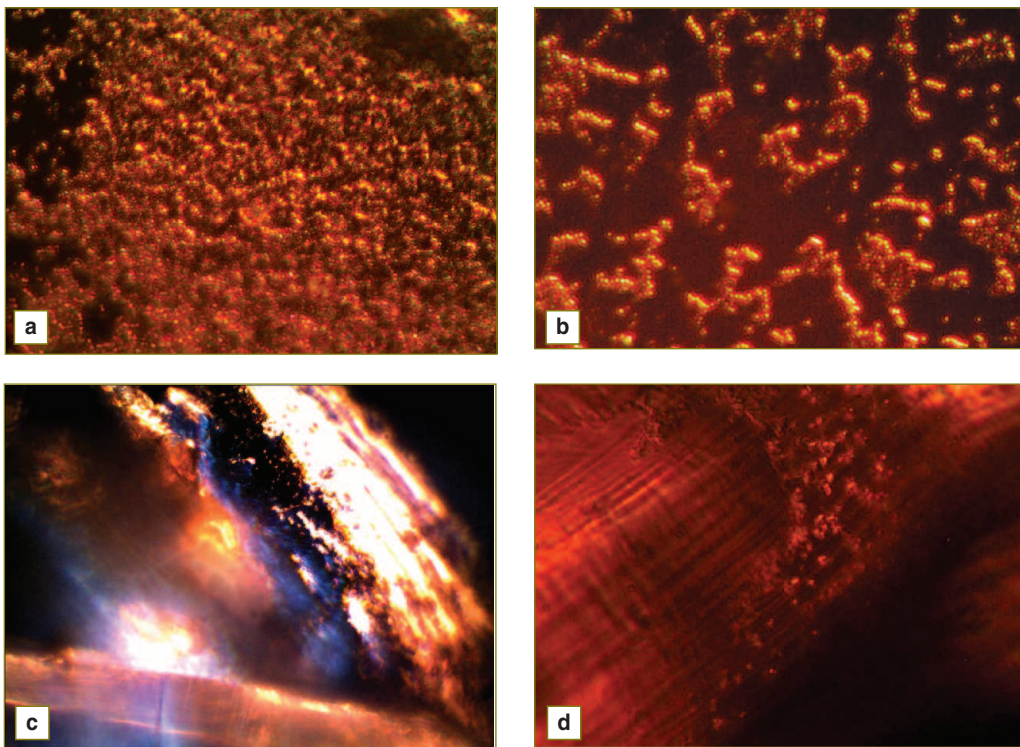
Fig. 2. Contamination of synthetic implants with St. aureus: *a* — biofilm on the surface of Reperene type 1; *b* — clusters on the surface of Reperene type 2; *c* — biofilm on the surface of Standard polypropylene mesh; *d* — area of biofilm on the fiber of Light polypropylene mesh
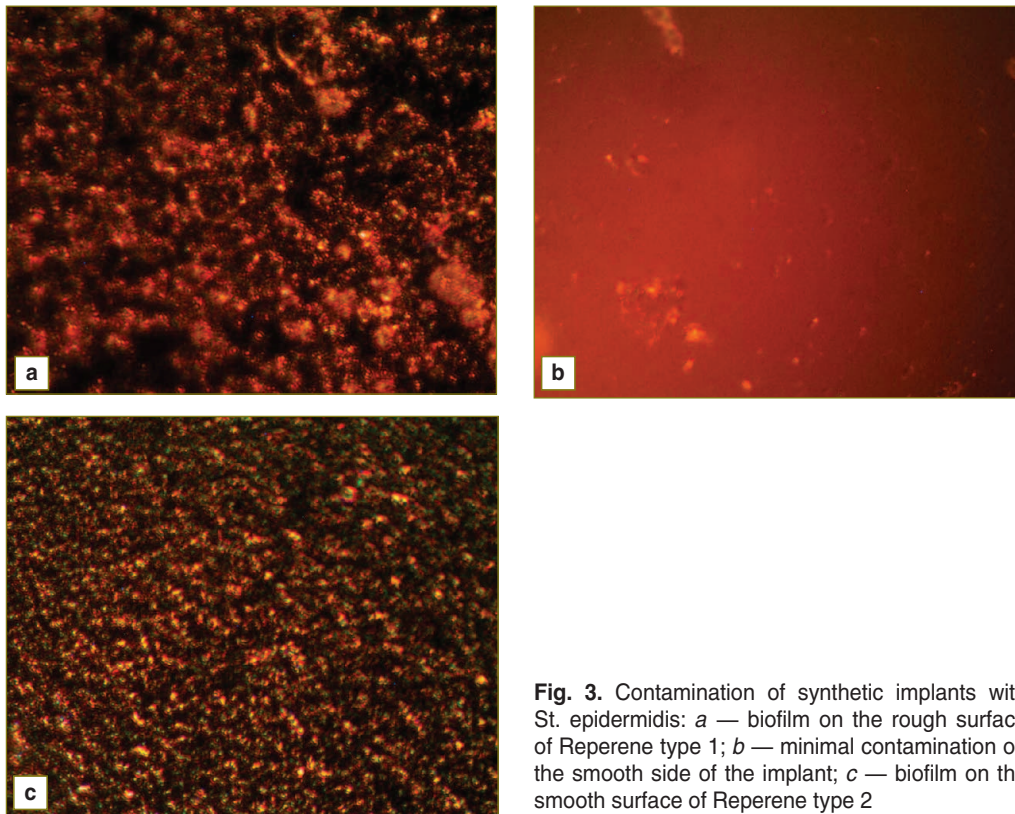
Fig. 3. Contamination of synthetic implants with St. epidermidis: *a* — biofilm on the rough surface of Reperene type 1; *b* — minimal contamination on the smooth side of the implant; *c* — biofilm on the smooth surface of Reperene type 2

average; and on the surface of woven mesh — 1.53 (PP Std, Uniflex), Reperene — 2.5 (p=0.044).

Discussion. These results do not contradict the available data of literature [11, 21]. In our study the phenomenon of bacterial growth in vitro (Ps. aeruginosa, St. aureus, St. epidermidis) on macroporous mesh and the formation of microbial biofilm on each surface was confirmed by the investigated materials within 48 hours and if this critically important time for perioperative antibiotic prophylaxis is lost, further infection control will be ineffective as a biofilm is formed on the implant. Some authors [25] indicate that subsequently in vivo a connective tissue capsule around the infected prosthesis is formed, but a biofilm does not disappear. Our experiment showed the ability to form biofilm on the surface of synthetic prosthesis to be different: maximal for Ps. aeruginosa (2.850), minimal for St. epidermidis (2.02), p=0.027. These results are partially correlated with those of other authors [17, 21]. However, the difference in colonization of mesh between cultures Ps. aeruginosa and St. aureus was not significant (p=0.46).

The literature notes the formation of biofilm to depend on the material, its structure, and the surface characteristic [12, 21, 23]. However, our study showed it to be significantly different for certain types of bacteria. For example, Light polypropylene mesh had high resistance for contamination of Ps. aeruginosa in contrast with other meshes. Ps. aeruginosa colonized the Standard polypropylene mesh more intensively than the smooth surface of Reperene. The experiments with the culture of St. epidermidis showed the opposite effect.

The results are not to be considered as undeniable patterns, as the variety of properties inherent for different types of bacteria should also be taken into account including the ability to form biofilm.

Conclusion. A bacterial biofilm on the surface of macroporous synthetic mesh is formed within 48 h after contamination in vitro. Pseudomonas aeruginosa has the maximal ability, and the lower — Staphylococcus aureus, Staphylococcus epidermidis. The phenomenon may be a condition for complications development, both in immediate and late postoperative period. Experimental evidence suggests the formation of biofilm to be a universal mechanism for mesh infection, and the mechanism can occur in any endoprosthesis. Its peculiarities depend on the material of mesh, structure of implant, features of surface and types of bacteria. To use tension-free technique in terms of bacterial contamination it is necessary to develop special implants resistant to colonization and biofilm formation.

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