SILICONE COVERED POLYPROPYLENE MESH FOR LAPAROSCOPIC VENTRAL HERNIA REPAIR

PhD. Thesis

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Abbreviations

BMA	Butylmethacrylate
ePTFE	expanded Polytetrafluoroethylene
EtO	Ethylene Oxide
HA	Hyaluronic Acid
HE	Haematoxylin Eosin
IP	Intraperitoneal
IPOM	Intraperitoneal Onlay Mesh
LVHR	Laparoscopic Ventral Hernia Repair
MDI	Methylene Diphenyl Diisocyanate
NVP	N-vinyl pyrrolidone
PAS	Periodic acid Schiff
PDS	Polydioxinone polymer
PG	Polyglactin
PE	Polyesther
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PU	Polyurethane
SEM	Scanning Electronmicroscopy
Si	Silicone
TAPP	Transabdominal Preperitoneal
TEP	Totally Extraperitoneal
TDI	Toluene Diisocyanate
TI	Titanium

1. Introduction

From the date 1804, when Astley Cooper first defined the abdominal hernia, the technique of the reconstruction of abdominal wall hernia changed a lot. Prosthetic material was introduced with steel mesh in the ‘40s. Usher et al. was the first who reported the use of polypropylene mesh for incisional hernia repair. A minimally invasive approach was applied to the ventral hernia repair with the expection of earlier recovery, fewer postoperative complications, and decreased recurrence rate. The first reference of laparoscopic hernia repair with ePTFE mesh was published in 1993.

This technique allows the intraperitoneal organs to get direct contact with the prostheses, which leads to adhesion formation, because there is still no mesh available avoiding adhesion.

A monofilament polypropylene mesh (Marlex®, Davol Inc, Cranston, RI) was in 1958 available on the market, and Usher has reported a successful incisional hernia repair with this mesh. Since then, the mostly used basic commodity of prosthetic surgical meshes is the polypropylene. Besides its benefits – tensile strength, tissue ingrowth, non carcinogen, chemically inactive, can be sterilized without changes in characteristic– short- and long-term complications are reported. The first late complication of intraperitoneal placed mesh (fistula formation caused by Marlex® mesh) was reported in 1981 by Kaufman et al. In 1998 Leber and his colleagues reported a retrospective review of incisional hernias repaired with different prosthetic materials. Early complications (seroma/haematoma, wound drainage, cellulites, postop. ileus) occurred in 18%, while the incidence of long term complications (recurrence, small bowel obstruction, enterocutaneous fistula) was 27%. Enterocutaneous fistula as complication was reported also after open and laparoscopic hernia repair using Marlex® mesh.

Laparoscopic ventral hernia repair (LVHR)

Surgical treatment of ventral hernias has changed over the past decades by introducing the laparoscopy and prosthetic materials for the reconstruction of the abdominal wall.

There is increasing tendency of acceptance of LVHR (laparoscopic ventral hernia repair) that is superior to open repair in terms of postoperative infectious complications, length of hospital stay, recurrence, blood loss, and cosmetic outcome. Insertion of a prosthetic material for tension free closure of the hernia defect is standard for laparoscopic repair.

There are many meshes on the market for LVHR, and the reported complications allow us to conclude that specific mesh materials are related to specific complications.

The ideal mesh

The ideal mesh for LVHR has yet to be found. There is a definition of it from a theoretical point of view, which is well known, that non-carcinogenic, chemically inert, causes no inflammation and change in mesh characteristics after tissue contact, cause no allergic reaction, it is resistant to physical manipulations and can be resterilized.

From a surgeon point of view the optimal mesh should have certain characteristics such as minimal adhesion formation, excellent tissue ingrowths, no shrinkage, no infection or fistula formation and promote minimal pain and seroma formation. And it is also important that the mesh causes no change in abdominal wall compliance, has a low price and easy to manipulate.

Products available on the market

There are more than 70 different meshes used for hernia repair available on the market. They can be classified into different categories. The meshes are made in average one of the 3 prosthetic materials: polypropylene (PP, listed in Table 1), polyester (PE), and expanded polytetrafluoroethylene (ePTFE). The pure PP and PE meshes are not recommended for the LVHR. It is generally accepted that the PP or PE meshes must be covered with a protective membrane or film against the viscera.

Table 1: Some polypropylene meshes with their features

Company	Name	Material	Weight (g/m^2)	Thickness (mm)	Pore size (μm)
Bard	Marlex	PP	95,1	0,6	100-800
Ethicon	Prolene	PP	82,5	0,6	1000-2000
	Vypro II	PP + PG	30	0,39	3000-4000
	Ultrapro	PP + PG	28	0,5	3000-4000
BBraun Aesculap	Premilene	PP	82	0,48	800
	Optilene LP	PP	36	0,39	1000
	Optilene elastic	PP	48	0,55	3600-2800
Medizintechnik	Ti mesh	PP + Ti	35	0,3	1000
	TiMesh extralight	PP + Ti	16	0,2	1000

PTFE meshes: The first ePTFE mesh was put on clinical practice in 1993 first, the GoreTex®. There are MycroMesh®, DualMesh® and MotifMesh® the most known ones.

Composite PE meshes: The Parietex Composite® mesh is composed of multifilament PE with a resorbable collagen oxidized film against the viscera.

Composite PP meshes: some of them are listed in Table 1. TiMesh®, Parietene Composix® mesh, this is a woven PP mesh with a protective collagen-oxidized film on the visceral side. Composix® mesh is a Marlex® (PP) with a thin ePTFE film, Sepramesh® is coated with an absorbable barrier of sodium hyaluronate and carboxylmethylcellulose. Proceed® is a Prolene® (the first PP mesh in the practice) encapsulated in a polydioxinone polymer film (PDS®).

Biological meshes: Biological meshes are cellular materials derived from humans or animals with an intact extracellular matrix. The acellular porcine dermal collagen and porcine small intestine mucosa the so called Surgisis® mesh makes a good impression also on the LVHR repair, used in a big international, multicenter randomised trial (Lapsis) where the Department of Surgery (University of Pécs, Medical School) is involved.

Mesh pore size

Prosthetic meshes are divided into macro- and micropore meshes according to their pore size. The pore size describes the size of fenestrations in the mesh. Macropore meshes (>75 μm) gives better tissue ingrowth/host integration whereas a mesh with small pore size (10-75 μm) or no pores carries a risk of encapsulation thus resulting in decreased integration into the abdominal wall. On the other hand micropore meshes are traditionally known of as causing a minimal adhesion formation, while macropore mesh may result in a disorganized neoperitonealization and therefore potentially cause more adhesions.

Based on experimental data, the logic approach in LVHR is to place a macro porous mesh against the parietal peritoneum and a micro- or "no pore" side against the viscera.

Strength of ingrowth

The majority of tissue ingrowth and strength take place within 2 weeks after mesh implantation and thereafter increase slowly until 3 months postoperatively. The biological response to hernia meshes can be characterised morphologically by the formation of collagenous tissue, inflammation, foreign body reaction, neoperitoneum formation and neovascularization. The tissue response depends on the material and the pore size of the surgical mesh. Tensiometric tests have been used to determine the strength of ingrowth at the interface between the mesh and the parietal peritoneum. All data based on experimental animal studies which have defined a required maximum limit of tensile strength of 16 N/cm^2 to overcome physical demands.

Different experimental studies have shown the superiority of PP meshes to all other mesh material regarding strength of ingrowth to the surrounding tissue. It has been documented that ePTFE materials have tendency to encapsulate instead of being integrated into the host abdominal wall.

Adhesions

After intraabdominal insertion of a prosthetic mesh, adhesions between the mesh and the peritoneum and /or organs may be formed until neoperitonealization of the mesh is complete, which lasts about 1 week.
Adhesions are often measured in terms of grade (% of mesh surface covered by adhesions) and type of adhesions (filmy, blunt/sharp dissection, solid organ or peritoneal adhesion).
Different animal studies, including small animals (rats, rabbits), and large animals (porcine) supports a tendency towards fewer adhesions when using composite meshes or ePTFE for LVHR. Harrel et al. implanted 4x4 cm pieces of mesh in 30 rabbits and adhesion formation was assessed after 1, 4, 8 and 16 weeks with sequential laparoscopy. DualMesh® had significantly less adhesions than Proceed®, Composix® and Marlex® at all investigated times. There were no differences in adhesions between Proceed® and Composix® mesh. Another newly published study in rabbits showed significantly lower adhesion degrees with Proceed® and ePTFE mesh compared with Mersilene®, Prolene®, and Vypro® mesh in 4 weeks post implantation.

Finally we can conclude that the literature clearly points in the direction of using a covered mesh/composite mesh, or ePTFE for LVHR in humans although it is important to clarify that there are no human data at the moment to support this.

2. Objectives

The aim of our investigations was to find the best barrier which can ward off the adhesion formation on the visceral surface of the PP mesh, specifically:

1. investigation of antiadhesive behaviour of different non absorbable materials on the visceral surface of the polypropylene mesh

2. evaluation the biological behaviour of composite mesh in decreasing adhesion formation using polyurethane as non-absorbable and hyaluronic acid as absorbable barrier on the visceral side of polypropylene mesh

3. comparing the biological behaviour of three different light-weight meshes with or without polyurethane covering on the visceral side

4. evaluation of the biological behaviour of the silicone covered polypropylene mesh

5. investigation of the silicone covered polypropylene mesh

6. immunohistochemical analysis of incorporation and adhesion prevention of different polypropylene meshes

3.1. Investigation of antiadhesive behaviour of different non absorbable materials on the visceral surface of the polypropylene mesh

All animal experiments was executed in accordance to rules and regulations regarding the use of animals in medical research, and the study was approved by the Committee on Animal Research of Pécs University (BA02/2000-1/2004).
All animals were allowed to adapt for at least a week prior to surgery. The animals were given rabbit chow and water ad libitum during the acclimatization period and throughout the rest of the study except the day of surgery.

A total of 12 New Zealand White rabbits (weighing 2,00-3,2 kg) were anaesthetized with intramuscular ketamine hydrochloride (200 mg), after premedication with diazepam (10 mg), and as antibiotic prophylaxis, the rabbits were given cephalosporin.

A midline incision was carried out, and two, 3x4 cm big artificial hernia was made by cutting all the abdominal layers including the peritoneum on both side of the linea alba. The abdominal wall defects were covered with a 4x 5cm sized Prolene® mesh on the left side, while the right side defects were covered with "composite" meshes (Table 2). Meshes were fixed with running sutures (Prolene® 4/0, monofilament, polypropylene, non absorbable suture, Johnson & Johnson Medical Ltd. Sommerville NJ, USA). The skin and subcutaneous tissues were closed also with running sutures (Vicryl Rapid® 2/0, monofilament, polyglactin, absorbable suture, Johnson & Johnson Medical Ltd. Sommerville NJ, USA).

Table 2: Grouping the different meshes over the defects of abdominal wall

	Left side	**Right side**
Group I.	Prolene®	PU covered Prolene®
Group II.	Prolene®	HA covered Prolene®
Group III.	Prolene®	Si covered Prolene®

- Adhesion formation

Although the sample size of this primary study was small, we could clearly prove the aggressive adhesion formation tendency generated by the polypropylene. In 10 cases out of 12, the Prolene® mesh was covered on visceral surface with peritoneal adhesions and large intestines, while the composite meshes showed no adhesions in six cases.
The adhesion formation was scored according to the grade (% of mesh covered by adhesions). The silicone covering has prevented the adhesion formation 6 weeks long, and after this only minimal adhesion formation was detected.
The polyurethane layer showed different tendency, by having intact surface on the 9th and12th week postoperatively and the hyaluronic acid on the peritoneal surface was manifested the same.

- Complications

Seroma formation was detected only in 2 cases (from different groups –silicone and polyurethane covering), which can be explained with the longer follow up period. There was 1 abscess detected, and in 1 case the mesh ground the skin, causing an ulcerated defect.
At last the shrinkage of the polyurethane layer must be mentioned, because all of the cases a shrunk, rumpled layer was detected, causing palpable resistance.

3.2. Evaluation the biological behaviour of composite mesh in decreasing adhesion formation using polyurethane as non-absorbable and hyaluronic acid as absorbable barrier on the visceral side of polypropylene mesh

The animal model was the same defined previously. There were 12 New Zealand White rabbits operated (weighing 2,00-2,8 kg). The rabbits were divided into 3 groups according to the different meshes covering the right side defects (see Table 3). The left side defect was covered with a 4x5 cm big Surgipro® mesh, while in Group I. the right side defect was covered with Prolene® mesh, in Group II. with a polyurethane covered Surgipro® and in Group III. with hyaluronic acid creamed Surgipro®. The meshes were removed 1, 2, 3 and 4 month after surgery, in a way, that 1 animal from each group were euthanized in each period.

Table 3: Grouping the different meshes over the defects of abdominal wall

	Left side	**Right side**
Group I.	Surgipro®	Prolene®
Group II.	Surgipro®	PU covered Surgipro®
Group III.	Surgipro®	HA covered Surgipro®

- Adhesion formation

There was only 1 case where both meshes were found intact on the peritoneal surface. The polyurethane layer could inhibit the adhesion formation and it was effective also in 3 months, because this result was found after 90 days. The remaining 3 cases had also fewer adhesions over the right side, where the composite mesh was used.
The Prolene® mesh alone caused same adhesion formation as the Surgipro® except 1 case where ascites was found intraabdominally. The peritoneal surface of the meshes was intact, and only to the suture line was peritoneum adhered.

The hyaluronic acid cover was worsted then we've expected, due to the last experiment. In all of the cases the composite was covered with peritoneal adhesion, large intestinal loops, and also a part of the stomach wall was adhered to it.

- Complications

Seroma formation was detected in 1 case 90 days after surgery, in the subcutaneous layer over the polyurethane covered Surgipro® mesh. A gauze pad causing resistentia was found in 1 animal on the dissection. One animal died on the 27th postoperative day in large intestine ileus. There was 1 subcutaneous haematoma, 2 abscesses detected. Ascites in the abdominal cavity was seen in 1 animal.

3.3. Comparing the biological behaviour of three different light-weight meshes with or without polyurethane covering on the visceral side

Three different meshes were evaluated in this study. **TiMESH®** (GfE Medizintechnik GmbH, Germany) is specially designed for all state-of-the-art mesh-surgery techniques.
Premilene® Mesh LP (BBraun Aesculap AG&Co. KG, Germany) is a lightweight mesh, made of pure polypropylene and it is uncoated, non absorbable.
The **Vypro® II Mesh** (BBraun Aesculap AG&Co. KG, Germany) which is a partly absorbable mesh knitted from polypropylene and polyglactin filaments. The combination of polypropylene and polyglactin is supposed to improve the handling of the mesh. As nonabsorbable barrier the same polyurethane (OpSite® Incise Drape, Smith & Nephew Medical Ltd; England) was used as in the previous study.

Table 4: Grouping the different meshes over the defects of abdominal wall

	Left side	**Right side**
Group I.	TiMESH®	PU covered
Group II.	Premilene® Mesh LP	PU covered
Group III.	Vypro® II Mesh	PU covered

There were 12 New Zealand White rabbits operated (weighing 1,97 - 3,14 kg). The rabbits were divided into 3 groups according to the different meshes covering the left side defects (see Table 4). There were 4 animals in each group. The meshes were removed on the 2nd, 4th, 8th and 12th week after surgery, sacrificing 1 animal from each group on each termination.
All the tissue samples were routinely fixed in 4% formaldehyde solution and sent for histological investigations. The specimens were embedded in paraffin. 3 μm thick histological sections were cut, mounted on glass slides, stained with haematoxylin eosin (HE) and periodic acid Schiff (PAS) and evaluated by light microscope to quantify foreign body giant cells, polymorpho-nuclear and mono-nuclear reactive cells, as well as, neo-formed vessels.

- Adhesion formation

TiMESH® has generated strong peritoneal and intestinal adhesion formation also, while the polyurethane covering could successfully prevent this. There were 2 cases with peritoneal

adhesion on the suture line detected, and in 1 case the peritoneal surface of the composite was intact.
Premilene® Mesh LP caused surprisingly in all of the cases adhesions, and in 3 of them were peritoneum, small and large intestinal loop adhered also to the mesh, but the polyurethane covered side was intact in 3 out of 4 cases.
Vypro® II Mesh has generated also aggressive adhesion formation causing peritoneal, small and large intestinal adhesions too except 1 case, where the intraperitoneal positioned mesh was intact. The polyurethane barrier could successfully prevent the adhesion formation caused by the lightweight polypropylene mesh, except 1 case, but there were also peritoneal adhesions detected.
Comparing the three different meshes to each other The Premilene® Mesh LP caused the most adhesions, than the TiMESH® and the best mesh placing intraperitoneal position in relation with adhesion formation was the Vypro® II Mesh.

- Complications

All the TiMESH® (total number of 8 meshes) was bulged and made impression of shrinkage comparing to the other meshes and the polyurethane barrier on its peritoneal surface was crinkled in all of the cases, but it must be point out that it hasn't decreased its efficacy. It has to be mentioned that also in the Premilene® Mesh LP group was 1 mesh shrunk, but also in this case was the crinkled polyurethane barrier intact.
Seroma formation and foreign body reaction was only in the Premilene® Mesh LP group detected. Seroma was found in 1 animal and there was one animal where small, whitish granulomes were found in the abdominal wall surrounding the mesh in a big area.

- Histological investigations

Giving continuance to the macroscopic analysis of the intraperitoneal adhesion formation caused by the different polypropylene meshes, the histological slides showed the signs of foreign body reaction (polymorpho-nuclear giant cells) and sterile inflammatory reaction (lymphocytes). The so called 'granuloma' – concentrically organized connective tissue around the mesh fibres- was also detectable in each type of the meshes.

3.4. Evaluation of the biological behaviour of the silicone covered polypropylene mesh

The surgical meshes used in this experiment were the same characterized in the previous etup (see Chapter 5.2.) which are the followings: **Premilene® Mesh LP**, and the **Vypro® II Mesh**. Creating a silicone layer, the so called NuSil MED-4830 (Politec GmbH, Germany) was used, which is a silicone elastomer with two components.

- Procedure of the silicone coating

The aim of the investigations carried out by the team of the Department of Inorganic and Analytical Chemistry of Budapest University of Technology and Economics under the direction of Ödön Wágner was to find the best technology to cover the surface of the filaments of the different surgical meshes.

There are 2 types of silicones from the technical point of view, the so called condensation type with 2 components and the additional type. The additional type seemed to be more practical for our investigations and on the other hand, the condensation polymers are not "medical grade" products on the market.

There were different potentials for the impregnation with silicone investigated, but only 2 of them seemed to be acceptable. The covering can be carried out by dipping the surgical mesh into silicone solution, or the filaments of the mesh can be covered with vaporizing a low viscosity silicone. After the solvent is absconded –using heath- the silicone membrane can be vulcanized or polymerized on the mesh.

In that case the Vypro® II Mesh and the Premilene® Mesh LP were impregnated using the vaporization technique, after the silicone was diluted using hexane solvent. After the solvent was removed the silicone was polymerized on 80-100 ◦C.

- Experimental protocol

There were 12 New Zealand White rabbits operated this time. The implantation of the meshes was carried out the same as before, only the antibiotic prophylaxis was skipped. The defects were covered with a 4x 5cm sized silicone covered Vypro® II Mesh on the right side, and silicone covered Premilene® Mesh LP on the left side one after another. The animals were euthanized with an overdose of potassium injection 7, 20 and 40 days after surgery. Adhesion formation was detected, and the mesh was removed with a surrounding muscle tissue, for histological investigations.

- Adhesion formation

The silicone as antiadhesive barrier was beyond belief. 10 out of 12 cases the silicone covered Vypro® II Meshes were adhesion free on the visceral side. In those 2 cases where strong peritoneal adhesion formation was detected both side were affected.
In the "Premilene-group" were 3 cases when 100% of the mesh surface was covered with large and small intestinal loops, and peritoneal adhesions, and there was 1 case when only the suture line caused intreaperitoneal adhesions The findings not depend on the time of termination, because 7, 20 and 40 days after surgery were the different pathology found. There were in 7 animals perfectly intact visceral side seen both after short term and long term follow up.

- Complications

One animal died 2 days before the planned termination, but according to the observation of the animal nurse, the animal has probably broken the leg in a fight with its "cagemate". There were 3 seromas detected, and in 1 case the mesh was shrunk and crinkled.

- Histological investigations

The specimen were embedded in paraffin, 3 μm thick histological sections were cut, mounted on glass slides, stained with HE and PAS and evaluated by light microscope to investigate inflammation and foreign body reaction.It was well documented, that a new peritoneum (mesothelial layer) was formed over the mesh. The giant cells, polymorpho-nuclear and

mono-nuclear reactive cells, as well as, neo-formed vessels were also represented in almost every slide, as a part of the foreign body reaction, and the intestinal tissue above the mesh filaments demonstrates well the intraabdominal adhesions caused by the surgical mesh.

3.5. Investigation of the silicone covered polypropylene mesh

3.5.1. Sealing procedure with silicone

- Sealing machine – centrifuge

There is a critical and time consuming step during the LVHR, namely to open and position the mesh which was entered scrolled through a trocar. To solve this problem, a rigid but flexible rim was planned to create on the edge of the mesh which helps the mesh to open by "itself" inside the abdominal cavity.
A centrifuge is the machine whereby both procedures can be carried out, namely a flat, smooth silicone layer over the polypropylene filaments, and a uniform rim made from silicone on the edge of the mesh.

The sealing machine takes place in a stainless steel case and consists of controllable temperature heater, and an electro-motor.

- Materials

The **PPKM403 polypropylene mesh** (TDA textile Development Associates, Inc. USA) was used in this experiment, which is a knitted polypropylene mesh with a pore size: 1,3 x 1 mm, weighing 45 g/m^2 and it is 0,43 mm thick.
For the silicone covering the **NuSil MED-6215** (Variachem Ltd., Hungary) was used which is the same 2 components Elastosil RT 601 which has been using since we work with silicone.

- Verifying the physical characteristics of ProSi mesh

After the polymerisation with irradiated heat is finished (polymerisation procedure for 30 minutes on 140 Celsius with the speed of rotation of 20 Hz), the impregnated mesh is carefully removed from the disk. The uniformity of the silicone covering is verified with conventional light microscope, and Scanning Electron Microscope. The SEM investigation was carried out in the Central Electron Microscope Laboratory, University of Pécs under the supervision of Béla Dolgos.

- Sterilisation

According to the established custom of our Central Sterilisation Laboratory, the ProSi meshes were plasma autoclaved, to get them germ free. As a primary study to see the possible changes caused by the sterilisation at all, we have sent the ProSi mesh for 1 cycle of plasma autoclaving.

- Tensile strength

The tensile strength measurements were conducted at room temperature using a tensiometer (Pannonlézer Ltd. Pécs, Hungary) with a range of 0–200±0.1 N. The mesh specimens were then mounted on a motorized test stand and held in place using vice clamps. The motorized

test stand gradually moved apart, applying traction at a constant rate of 60 mm/min. The tensile strength was tested by the institutional tensile tester. The original non sterile packed PPKM 403, the silicone covered mesh, and the plasma-autoclaved (Sterrad) ProSi meshes were tested.

- Results

Two important inferences can be drawn from the tensile testing. Firstly that the impregnation procedure alone causes decrease in tensile strength comparing to the non manipulated polypropylene mesh (statistically significant p=0,007), secondly that the sterilization does not decrease significantly the tensile strength as it was suggested by the other manufacturer.

The commercial light microscopic evaluation was used to check the efficacy of the silicone impregnation. We looked for non coloured fibres or parts of the mesh, and examined the rim whether the edge of the mesh sits well in the middle.

After prudently check for silicone defects, the meshes were examined with SEM. There were only a few irregularity of the silicone covering was found on the polypropylene fibres, but both before and after sterilization also. There was no increase in the amount of the silicone leakage detected after "Sterrad" sterilization.

3.5.2. Evaluation of the effect of different sterilization techniques on surgical meshes

In this investigation the effect of the EtO-sterilization as standard was compared to the plasma steam sterilization and formaldehyde gas sterilization.

- Materials

Silicone covered polypropylene mesh (ProSi) which was manufactured in our laboratory. This mesh was non sterile. **Premilene® Mesh** (BBraun, Aesculap AG&Co. KG, Germany), made from monofilament polypropylene, is used for hernia repair or for reconstruction of the chest wall. **Chiralen®** surgical mesh (Chirmax s.r.o. Czech Rebuplic) is a sterile, non absorbable 30x30 cm sized undyed mesh made from polypropylene.

- Experimental protocol

After opening the sterile, ready for use packages of Chiralen® and the Premilene® Mesh, the 30x30 cm big meshes were cut into 35 pieces. Following this all meshes were sent to repetitive sterilizations. All the pieces were separately packed after sterilization and stored on room temperature until they were opened.

Repetitive ethylene oxide gas and autoclave sterilizations were applied to polypropylene meshes up to 2 times. Gas (EtO) sterilization (Siemens, Mediteszt Kft., Hungary) was applied for 4 hours at 50 ◦C for each sterilization process. After the sterilization phases aeration was applied to the samples for 12 hours. For repetitive sterilizations the same procedure was performed on the samples at 1 day interval.
Plasma-sterilization ("Sterrad", Johnson&Johnson, USA) was applied for 55 minutes, on 52 ºC, under 0,3 Hgmm pressure, and the formaldehyde autoclave sterilization (Gattinge, Germany) was applied at 55 ºC, for 300 minutes under 525,42 - 600,048 Hgmm pressure. For repetitive sterilizations the same procedure was performed on the samples at 1 day interval.

The packed samples were kept on the shelf at room temperature until the measurements were started.
Shrinkage and deformity was photographically documented. Brittleness and handling were based on subjective estimation.

- Tensile Strength

There was no decrease in tensile strength detected after the gas and steam sterilizations. Mild decrease was only noted in ProSi mesh after the formaldehyde autoclaving.

The Prosi mesh was tested also after 12 weeks. The sterile packages were kept on room temperature and opened only just before testing. The data show no significant difference in tensile strength either in 12 weeks.

- Structural analysis

There was no structural lesion detected with SEM caused by the different sterilization methods. The silicone covering was impaired in the control group also, which confirms that the impregnation technique may cause structural lesion which is not worsened by sterilization.

In conclusion: resterilizing the polypropylene meshes by ethylene oxide gas-, and steam sterilization do not alter their physical characteristics.

3.6. Immunohistochemical analysis of incorporation and adhesion prevention of different polypropylene meshes

3.6.1. Investigation of the biological behaviour of the pure polypropylene Hi-Tex® mesh

- Experimental protocol

A total of 20 New Zealand White rabbits (weighing 2,05-3,1 kg) were anaesthetized with intramuscular ketamine hydrochloride (200 mg), after premedication with diazepam (10 mg).
A 6 cm long midline incision was carried out, and a 3x4 cm big abdominal wall defect was made by cutting all the abdominal layers including the peritoneum. It was covered with a 4x 5cm sized Hi-Tex® (Textile HiTec S.A. Buenos Aires, Argentina) polypropylene monofilament, knitted structured mesh, and fixed with running sutures (Prolene® 4/0, monofilament, polypropylene, non absorbable suture, Johnson & Johnson Medical Ltd. Sommerville, NJ USA). The skin and subcutaneous tissues were closed also with running sutures (Vicryl Rapid® 2/0, monofilament, polyglactin, absorbable suture, Johnson & Johnson Medical Ltd. Sommerville NJ USA).
Animals were daily checked for complications. 20 rabbits were divided into 2 groups according to the surviving period. Group I (10 animals) was sacrificed 7 days, and Group II (10 animals) 21 days after surgery. The animals were sacrificed with an overdose of potassium injection. Adhesion formation was detected, and the mesh was removed with a surrounding muscle tissue, for histological investigations.

- Histology and immunohistochemistry

The tissue samples were fixed in 4% formaldehyde solution and embedded in paraffin. 3 μm thick histological sections were cut, mounted on glass slides, stained with haematoxylin eosin (HE) and evaluated by light microscope.

For immuno-histochemical quantification of proliferating cells, the B56 Ki67-specific mouse monoclonal antibody was used (clone: B56, dilution: 1:200, source: Histopathology Ltd., Pécs, Hungary).

To assess the growth of vascular endothelial cells, vascular endothelial growth factor (VEGF) specific mouse monoclonal antibody (clone: JH121, dilution: 1:200, source: ThermoFisher Scientific/LabVision Corporation, Fremont, California, USA) was applied.

Mesothelial cells were evaluated by immunostaining using a broad spectrum cytokeratin (CK) specific mouse monoclonal antibody (clone: MNF 116, dilution: 1:200, source: Histopathology Ltd., Pécs, Hungary).

- Macroscopic results

All animals survived the operation and no complication was seen during the follow-up period. The average weight of the animals after 7 days was 2,087±0,5 kg, and after 21 days 2, 487±0,5 kg.

Aggressive adhesion formation was observed even after 1 week, with moderately decreasing tendency by the 3rd week. In Group I. the average rate of the mesh surface was 54,9% (20-100%). In Group II (the mean of the adhesion covered surface was 44% (0-85%). As complication the serome formation (4/20, 2 -2-cases from both group) and the sc. haematoma (Group I: 4/10 and Group II: 3/10) is mentionable.

- Histological and immunohistochemical results

There was foreign body generated sterile inflammatory reaction detected with HE staining in each slide.

The Ki-67 positivity was decreased in all the analysed areas after 3 weeks postoperative. The Ki-67 positivity was greater in the ‘granuloma’ zone in each slide. There was no significant difference in relation to the affected area.

According to our macroscopic findings, the VEGF positivity showed significant greater positivity after 3 weeks. The capillaries on the neoformed mesothelial layer were visible after 21 days during section which is in accord with the immunohistochemistry.

A newly formed mesothel layer with small capillaries was detected macroscopically after 3 weeks (Group II). This is well followed up in the MNF 116 stained slides from Group I, where the triangle shaped, swollen positive cells are situated in the granulomatous zone around the foreign body, while in Group II, these positive cells are found on the serosal surface of the tissue, creating a well organised monolayer above the mesh material.

3.6.2. The biological behaviour of Sil Promesh® - a newly developed dual mesh

- Experimental protocol

The used animal model is described above. The same procedure was carried out on 20 New Zealand White rabbits (weighing 3,42-4,59 kg) using the Sil Promesh® (Surgical-IOC Company, France) is a dual-sided, macro perforated, non-woven, polypropylene mesh, with a

non adherent silicone covering on the intraperitoneal side, for the hernia repair. The animals were divided into 2 groups according to the time of removal. All the further investigations were prepared same for histology and all the data were evaluated with the same statistical analysis previously described.

For SEM evaluation the specimens were fixed in a mixture of 2% formaldehyde and of 2,5 % glutare solution for 24 hours. The samples were thereafter carefully washed 3 times in phosphate buffer and dehydrated in different concentrations of alcohol. After the dehydration with absolute alcohol for 20 minutes was finished, the samples were mounted on the worksheet and coated with gold ("4 9"- fine gold) and obtained with electron microscope (JEOL, JSM 6300 Scanning Microscope, Japan).

- Adhesion formation

In Group I. the average rate of the affected mesh surface with adhesions was 30,5%. Half of the cases the visceral surface of the mesh was intact. In Group II. the mean of the adhesion covered mesh surface was 22,2%. There were 7/10 intact meshes detected on the termination but in the remaining two cases the mesh was not visible because of the strongly adhered colon conglomerate, and in 1 case the urinary bladder was adhered to the caudal edge of the mesh.

The seroma formation detected after 7 days was expected, only the amount of the fluid is mentionable (5 – 24 ml). Infectious complications 3 weeks after implantation were surprising for us.

- Histological, immunohistomorphological and SEM investigations

The presence of polymorpho nuclear giant cells and the lymphocyte invasion of the tissues are extraordinary with the commercial HE staining. The texture of this mesh was different what we've got used to, not only the connective tissue, but the silicone layer was colonised also with the inflammatory cells. The most surprising was that the decreasing tendency of the foreign body reaction failed and was presented in each slide, also after 3 weeks.

The Ki-67 positivity showed the same tendency as Hi-Tex® (polypropylene) mesh, only the total amount of the cells was little fewer. The number of the proliferating cells decreased after 3 weeks. Statistically significant difference was only detected in case, comparing the cell count of granuloma, to the surrounding zone.

The VEGF scores were just opposite to our expectations, but in accordance to our macroscopic findings, there was no increase in VEGF positivity detected after 3 weeks.

The MNF 116 positive cells representing the mesothelial cells were detected in each slide. The localisation and the get up of the cells after 1 week were the same as what we've detected using Hi-Tex® mesh. The triangle shaped, swollen positive cells were situated in the granulomatous zone around the foreign body, while there was no changes seen during the next 2 weeks. The slides showed the same situation after 3 weeks, which correlates well with the macroscopic findings, meaning no peritoneum was seen. This mesh did not incorporate to the host tissues et all.

- Scanning Electron Microscopy

The laminar silicone layer over the polypropylene mesh is extra punched to avoid the possible seroma formation. There was no tissue ingrowth detected on the mesh, the following pictures

show the texture of the mesh after 1 week. According to the macroscopic findings there was no tissue remodelling or neoperitoneum formation seen even after 3 weeks. The polypropylene thread used for hernia fixation was better integrated than the silicone layer of the mesh.

3.6.3. Investigation of host reaction of the ProSi mesh with immunhistochemisrty

- Experimental protocol

The same procedure was carried out on 20 New Zealand White rabbits. The animals were divided into 2 groups according to the time of removal. All the further investigations were prepared same for histology as already subscribed above. All the data were evaluated with the same statistical analysis. For SEM evaluation the specimens were fixed in a mixture of 2% formaldehyde and of 2,5 % glutare solution

- Adhesion formation

All animals survived the operation and no complication was seen during the follow-up period. In Group I. the average rate of the mesh surface was 37%. There were 3 cases with intact intraperitoneal surface found. In Group II. the mean of the adhesion covered surface was 50,5%. There was 2 intact out of the 10 meshes, with vascularized newly formed good visible neoperitoneum on the intraperitoneal surface.

As complication the serome formation (5/20) and the sc. haematoma (2/20) are mentionable.

- Histomorphological investigations

The conventional HE stained slides foreign body induced sterile inflammation with a decreasing tendency. The polymorphouclear giant cells, the lymphocytes were present in all slides but this mesh induced the least inflammatory reaction in comparison to Hi Tex® and Sil Promesh®.

The total cell count of the Ki-67 positive cells, representing the proliferating cells was significant less compared to the other meshes. The decreasing tendency of the cell turn over was well detectable in each analyzed zone, including the granuloma, and the further surrounding connective tissue. The differences between the groups showed no statistical significance but the tendency is well demonstrated.

The significant increase in the VEGF positivity confirms our macroscopic observation namely the small capillaries on the visceral surface of the surgical mesh, which was well visible 3 weeks after the surgery.

The recreation of serosa in all cases, which was demonstrated with MNF 116, and SEM was over expectation. The electron microscopic evaluations showed an excellent ingrowth of the ProSi mesh. The thin cell layer over the filaments became a 3 dimensional tissue in 21 days.

4. New findings

1. The silicone covered polypropylene mesh “ProSi”, which is a new innovation of our team, is a hernia mesh available for intraperitoneal, therefore laparoscopic use, which is polypropylene mesh with silicone impregnated filaments. This combined mesh is a

macroporous hernia mesh with an excellent tissue ingrowth, minimal foreign body inflammatory reaction, showing acceptable adhesion formation.

2. The new investigational method to visualize the tendency of the tissue response to the foreign material brought a short term follow up New Zealand white rabbit model, in which only 1 type of mesh was implanted into a rabbit, the period of the follow ups was shorten to 7 and 21 days.

3. The creation of the so called neopeitoneum over the intraperitoneal side of the implanted surgical mesh was visualized with the MNF-116 monoclonal mouse antibody which has never been used before for this purpose in a rabbit model.

4. The sealing machine “centrifuge” for the impregnation of polypropylene mesh with silicone was developed only for our experimental work. Creating a dual mesh with that kind of impregnation was never published before.

5. The tensiometer was also designed and developed only for our researches and this machine is also unique in its own category.