Use of an experimental model to evaluate infection resistance of meshes in abdominal wall surgery

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ABSTRACT

Background: Staphylococcal species are the most common organisms causing prosthetic mesh infections, however, infections due to rapidly growing mycobacteria are increasing. This study evaluates the resistance of biomaterial for abdominal wall prostheses against the development of postoperative infection in a rat model.

Material and methods: In 75 rats, we intramuscularly implanted three different types of prostheses: (1) low-density polypropylene monofilament mesh (PMM), (2) high-density PMM, and (3) a composite prosthesis composed of low-density PMM and a nonporous hydrophilic film. Meshes were inoculated with a suspension containing $10^8$ colony-forming units of Staphylococcus aureus, Staphylococcus epidermidis, Mycobacterium fortuitum, or Mycobacterium abscessus before wound closure. Animals were sacrificed on the eighth day postoperatively for clinical evaluation, and the implants were removed for bacteriologic analyses.

Results: Prostheses infected with S aureus showed a higher bacterial viability, worse integration, and clinical outcome compared with infection by other bacteria. Composite prostheses showed a higher number of viable colonies of both M fortuitum and Staphylococcus spp., with poorer integration in host tissue. However, when the composite prosthesis was infected with M abscessus, a lower number of viable bacteria were isolated and a better integration was observed compared with infection by other bacteria.

Conclusions: Considering M abscessus, a smaller collagen-free contact surface shows better resistance to infection, however, depending on the type of bacteria, prostheses with a large surface, and covered with collagen shows reduced resistance to infection, worse integration, and worse clinical outcome.

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Introduction

An increasing number of hernia repairs are performed each year in Spain. The use of prostheses has become the preferred method for abdominal wall reconstruction in primary and incisional hernia repair. Repairs of incisional hernias are considered as contaminated surgery, due to the significantly higher infection rates described with these repairs. According to the Spanish Society of Epidemiology, the rate of postoperative surgical infections in Spain is 4.6%. The presence of a foreign body reaction caused by the implanted device predisposes to postoperative clinical infections by smaller numbers of bacteria of a given species. Acute superficial and late deep infection is well known after mesh abdominal wall reconstruction causing patient disability, hospital costs, and the chance of recurrence, frequently making the surgical removal the only solution to an infected prosthesis.

The overall clinical outcome of such persistent infections depends on the virulence of the contaminating pathogen, the microenvironmental factors of the wound site, and the type of surgical mesh material. The well-known key steps in the pathogenesis of infection are bacterial adhesion to implanted biomaterial surfaces, followed by proliferation of bacteria and biofilm development. The adsorption or binding of serum proteins and formation of biofilms can be promoted by means of factors including chemical composition of biomaterial, electrostatic interaction with potential pathogens, hydrophobicity, and surface roughness or physical configuration of the prosthesis.

Surgical site and implant contamination could occur during surgery and in the early postoperative period. Staphylococcus aureus and Staphylococcus epidermidis are prevalent microorganisms of skin flora, ones responsible for over 90% of surgical site infections. Infections due to rapidly growing mycobacteria (RGM) such as the Mycobacterium fortuitum and Mycobacterium abscessus complexes are growing interest as an example of chronic infection associated with biomaterial-related surgical procedures, such as orthopedic prostheses, peritoneal dialysis catheters, vascular catheters, prosthetic heart valves, and also abdominal wall prostheses. It may be due to RGM are difficult to eradicate with common decontamination practices when forming biofilms adhered to the biomaterial and are also relatively resistant to standard disinfectants.

Surgeons commonly apply polypropylene monofilament mesh (PPM) and dual-facing mesh made of PPM and a non-adherent film (composite prostheses [CP]) in repair of abdominal wall hernias. The influence of biomaterial of abdominal wall prostheses on the development of postoperative infection by S aureus and S epidermidis has been widely investigated, indicating that meshes with large pores, low-density meshes have a reduced contact area and may be therefore less prone to bacterial colonization than high-density meshes. Moreover, there is evidence to suggest that CP can provide an adequate environment for bacterial adherence, niche formation, and biofilm development due essentially to the large surface area provided by the non-adherent film, thus precluding their use in contaminated surgical fields. However, prosthetic mesh infections due to RGM as a paradigm of chronic infection resistant to common antimicrobial treatments have not received enough attention, a point of concern which is the inspiration for our work. In a previous in vitro study, we evaluated the bacterial adherence on these meshes. Subsequently, an in vivo experimental study was conducted as described in the following section to examine the infection resistance of a contaminated mesh after an abdominal wall reconstruction at the site where the prosthesis was implanted. To our knowledge, this is the first model in vivo of foreign body infection by mycobacteria.

Materials and methods

Animals

Seventy-five Wistar white rats weighing 350-500g were used in this study. Animal testing will be performed according to current Spanish legislation regarding the use, protection, and care of experimental animals (Royal Decree 1201/2005) and in accordance with those recommended procedures by the Ethics Committee of our institution. The study was conducted with the approval of the local ethics committee for experimental studies.

Ethical approval details

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Mesh materials and study design

An established rat infection model by Bellows et al. with some modifications considering our previous research in vitro was used to evaluate three different types of abdominal wall prostheses: (1) low-density PPM (LD-PPM) (Parietene; Sofradim Production, Trévoux, France), (2) high-density PPM (HD-PPM) (SurgiproUnited States Surgical, Norwalk, CT), and (3) dual-facing prostheses made of PPM and a resorbable hydrophilic film (CP) (Parietene composite; Sofradim Production). Rats (n = 5 per mesh type) were assigned randomly to undergo intramuscularly implantation of patches of size 1 × 1 cm of each abdominal wall prostheses. Meshes were prepared as instructed by the manufacturers and were cut into uniform strips at the time of surgery using a precut plastic sterile template. Each patch was implanted in a different rat and was inoculated with 1 mL bacterial suspension of 10⁶ colony-forming units (CFU) of each strain in phosphate-buffered saline (PBS), into the surgical wound after mesh implantation but before to closure the internal edges of superficial incised muscles and skin closure to mimic contaminated conditions. Control (noncolonized) animals received 1 mL of PBS instead of the bacterial suspension (n = 5 rats per mesh type). At eighth day after surgery, the animals
were sacrificed and explants underwent microbiologic analyses.16

Bacterial inoculum preparation

The following four known in vitro biofilm-forming collection strains were studied: S aureus 15,981,17 S epidermidis ATCC 35984, M abscessus DSM 44196, and M fortuitum ATCC 13756.12 Both the staphylococci strains were methicillin susceptible. The growth media and incubation conditions are described in our previous in vitro study.18 Briefly, mycobacterial strains were grown at 30°C on Middlebrook 7H10 agar (Becton Dickinson, Franklin Lakes, NJ) supplemented with glycerol for 7 d and were later grown in Middlebrook 7H9 broth (Becton Dickinson) supplemented with Tween-80 0.01% v/v for 5 d at 30°C. S aureus and S epidermidis were grown overnight at 37°C on tryptic soy agar containing sheep blood 5% v/v and were grown subsequently in tryptic soy broth (bioMerieux, Marcy d’Etoile, France) at 37°C for 1 d. Culture concentration was determined by spectrophotometry (OD 600) and compared to a predetermined growth curve for each strain. Each culture was brought to 10⁸ CFU in PBS and verified by plating serial 10-fold dilutions (in triplicate) of the final solutions used during surgery.

Surgical procedure

All rats were subjected to surgical creation of an abdominal wall defect using a previously described model.19 The surgical procedure of mesh implantation was performed using a general anesthesia, intraperitoneal application of ketamine (40 mg/kg). The surgical site was prepared by disinfection with betadine solution. Under sterile conditions, a 3-cm midline skin incision was made, and the surrounding subcutaneous tissues were dissected. We created a defect in the abdominal wall by longitudinal incisions on both sides of linea alba (2 cm each) and dissected the muscular plane in both incisions until the preperitoneal plane. After confirming the integrity of the preperitoneal layer, the defect was repaired by suturing a size-matched test mesh into the defect site using (4/0) polypropylene sutures at each of the four corners of the prostheses.

The bacterial inoculum (1 mL suspension of 10⁸ CFU S aureus, S epidermidis, M abscessus or M fortuitum) or PBS (1 mL) was then pipetted onto the top of each implanted mesh before the internal edges of superficial incised muscles were sutured at midline with a continuous polypropylene (4/0) sutures (Fig. 1). Finally, the skin was also closed with continuous polypropylene (4/0) sutures. Animals were distributed into four randomized groups containing five prostheses of each type:

- Group 0 (15 animals), prosthesis were implanted as a control group.
- Group 1 (15 animals), prosthesis with the bacterial inoculum of S aureus was placed.
- Group 2 (15 animals), prosthesis with the bacterial inoculum of S epidermidis was placed.
- Group 3 (15 animals), prosthesis with the bacterial inoculum of M abscessus were implanted.
- Group 4 (15 animals), prosthesis with the bacterial inoculum of M fortuitum were implanted.

Postoperative care

After recovering from anesthesia, rats were returned to individual cages for the remainder of the study, allowed normal ambulation and diet for the remainder of the study and evaluated daily for signs of local infection, sepsis, pain or distress, or wound complications.

Explantation and analyses

At eighth day, rats were sacrificed and qualitative assessment of the integration of the prosthesis into abdominal tissue was done using one of four grades, as inspired by the work of Brown et al.20: grade 1, no integration; grade 2, minimal integration; grade 3, moderate integration; and grade 4, complete integration. A numerical assessment for the degree of integration was done during the autopsy, and the mean was calculated for each group. The derived means of the groups were then compared as described under data analysis.

After euthanasia, the patches were excised carefully under sterile conditions, placed in a tube containing 2.5 mL of PBS and were sonicated in an ultrasonic cleaning bath USC100 T (VWR, Leuven, Belgium) at 45 kHz with a power output of 300 W for 5 min to evaluate the bacterial biofilm formation. The protocol described by Zamora et al.18 was modified as described in the following section. Removed patches were

Fig. 1 – Surgical technique. (A) Midline incision in the skin and subcutaneous dissection plane, (B) preperitoneal planes were dissected in both side of abdominal midline to place the prosthesis, (C) contamination with bacterial inoculum, and (D) the defects were closed with continuous suture. (Color version of figure is available online.)
weighed and the number of CFU obtained for each mesh was referenced to their weight, so that we obtain the CFU/g.

Data analysis

Statistical multiple comparisons with EPI-Info software, version 3.5.1 (CDC, Atlanta, GA) were performed by means of Mann–Whitney/Wilcoxon (two species) or Kruskal–Wallis test (more than two species).

Results

General appearance and clinical response of animals

Postoperative recovery of all animals followed a normal course and all animals survived until sacrifice. Normal eating, drinking, urination, and bowel movements were shown by all animals throughout the study.

Results of integration of prostheses

Table 1 shows the scores of integration. All control rats exhibited the maximum level of integration. The inoculation of bacteria in the abdominal significantly reduced the integration. This occurred for the different type of prosthesis ($P < 0.05$).

Group 1
LD-PMM inoculated with S aureus showed a firm integration removable only with sharp instruments; however, CP presented an extremely labile integration ($P = 0.0086$, Mann–Whitney test). There were no differences between HD-PMM and other prostheses ($P = 0.2375$ and 0.1202 for LD-PMM and CP, respectively).

Group 2, group 3, and group 4
There were not observed statistically differences between LD-PMM, HD-PMM, and CP when were infected with S epidermidis, M fortuitum, and M abscessus ($P = 0.1546$, 0.6941, and 0.1943, respectively, Kruskal–Wallis test).

Between groups
Considering the integration of each material, only between groups 1 and 3, there were statistically differences. LD-PMM and CP showed worse integration in the presence of M abscessus and S aureus, respectively ($P = 0.0104$ and $P = 0.0495$).

Results of biofilm formation

RGM showed lower biofilm formation than strains of Staphylococcus spp. For all abdominal wall prostheses, as we can see in Table 2, except in the case of low-density PMM for M abscessus. The biofilm formation of Staphylococcus spp. In low-density PMM was reduced such that reaches the same levels of M abscessus.

Group 1
Figure 2A shows that lower CFU of S aureus were isolated from LD-PMM compared with HD-PMM and CP ($P = 0.0090$ for both).

Group 2
LD-PMM exhibited a higher bacterial resistance to S epidermidis compared with other prostheses but the difference was statistically significant difference only compared with CP ($P = 0.0090$, Mann–Whitney test).

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Low-density PMM</th>
<th>High-density PMM</th>
<th>Composite prostheses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (infected by S aureus)</td>
<td>3.00 ± 0.71</td>
<td>2.20 ± 1.10</td>
<td>1.20 ± 0.45</td>
</tr>
<tr>
<td>Group 2 (infected by S epidermidis)</td>
<td>2.40 ± 0.89</td>
<td>2.20 ± 0.00</td>
<td>1.60 ± 0.55</td>
</tr>
<tr>
<td>Group 3 (infected by M fortuitum)</td>
<td>2.20 ± 0.84</td>
<td>2.20 ± 1.30</td>
<td>1.80 ± 1.30</td>
</tr>
<tr>
<td>Group 4 (infected by M abscessus)</td>
<td>1.60 ± 1.52*</td>
<td>2.20 ± 0.84</td>
<td>3.00 ± 0.71*</td>
</tr>
</tbody>
</table>

*Represents statistically significant difference.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Low-density PMM</th>
<th>High-density PMM</th>
<th>Composite protheses</th>
</tr>
</thead>
<tbody>
<tr>
<td>S aureus versus S epidermidis</td>
<td>0.2506</td>
<td>0.3472</td>
<td>0.4647</td>
</tr>
<tr>
<td>S aureus versus M abscessus</td>
<td>0.1745</td>
<td>0.0090</td>
<td>0.0090</td>
</tr>
<tr>
<td>S aureus versus M fortuitum</td>
<td>0.0090</td>
<td>0.0090</td>
<td>0.0163</td>
</tr>
<tr>
<td>S epidermidis versus M abscessus</td>
<td>0.1745</td>
<td>0.0090</td>
<td>0.0090</td>
</tr>
<tr>
<td>S epidermidis versus M fortuitum</td>
<td>0.0090</td>
<td>0.0090</td>
<td>0.0163</td>
</tr>
<tr>
<td>M abscessus versus M fortuitum</td>
<td>0.3472</td>
<td>0.9168</td>
<td>0.0090</td>
</tr>
</tbody>
</table>
For *S. aureus* and *S. epidermidis*, there were no differences between high-density PMM and CP (*P* = 0.0758 for both, Mann–Whitney test).

**Group 3**

Figure 2B indicates that CP showed higher biofilm formation than other abdominal prostheses for *M. fortuitum* (*P* = 0.0090 with respect both prostheses), and there were no differences between the PMM prostheses (*P* = 0.4647).

**Group 4**

There were no statistical differences between the different prostheses for *M. abscessus* (*P* = 0.4025, Kruskal–Wallis test).

**Between groups**

Only there were statistically differences between group 3 and group 4 for CP. *M. fortuitum* showed higher log CFU/g for CP than *M. abscessus* (*P* = 0.0090, Kruskal–Wallis test).

**Discussion**

The present study showed a clear difference in the infection resistance of PPM and CP against different bacteria tested in a rat model of abdominal body wall repair. We have found that a mesh with a smaller surface area made of a hydrophobic material, such as low-density PMM, is less susceptible to infection compared with a hydrophilic surface of collagen (Parietene composite) for *Staphylococcus* spp. and *M. fortuitum*. However, no differences between the different prostheses for *M. abscessus* were detected. These results are very similar to those obtained in our previous in vitro study, showing different rates of adhesion depending on of the bacterial species and increased surface area of a high-density PPM, which promotes the adherence and persistence of bacteria in the implant bed. Adhesion of bacteria to the surfaces is also influenced by the hydrophobicity of the biomaterial and the bacterial strain. Polymers, such as PPM or PTFE, are hydrophobic, but collagen is hydrophilic. RGM are very hydrophobic organisms and hydrophobic bacteria adhere more eagerly to a hydrophobic surface, so *M. fortuitum* adherence results suggest that this phenomenon was influenced by other factors. The mycobacterial cell wall is highly complex and has lipid content as fatty acids and mycolic acids, which makes them bacteria more hydrophobic. Because hydrophobicity is an important mechanism for attachment to biomaterials, it can be speculated that differences in cell wall lipids among strains or species of *Mycobacterium* spp. could explain the differences detected in the present study. A higher biofilm formation by mycobacteria in the hydrophobic surfaces (PPM) was expected, however, a higher number of CFU for staphylococci in most prostheses was isolated as in previous studies. This finding could be due to a faster replication of *S. aureus* compared with mycobacteria. Moreover, stronger adhesion of bacteria to the surfaces is achieved by the adhesins in the bacterial capsules, as fimbriae and slime. These aspects are known for staphylococci but no specific adherence mechanism has been studied for mycobacteria. Various proteins such as fibronectin, fibrinogen, collagen, laminin, or vitronectin also promote bacterial adherence when they are adsorbed in vitro on polymer surfaces. The higher number of viable colonies of *S. aureus* isolated from CP was correlated with a worse integration of these prostheses compared with others in the presence of the same bacterium. On the contrary, *M. abscessus* was isolated from CP with the lowest CFU counts (despite no statistical differences were found) and CP showed a better integration when infected with this bacterium compared with other bacteria. The integration of the implant in the host tissue depends on the behavior of cells at the tissue-implant interface, and, in particular, on their initial attachment, adhesion, and spreading. If bacterial cells colonize the implant surface first, it inhibits the immune response in the host and prevents integration of the prosthesis in the host tissue. According to this “race for the surface” theory, the postoperative infection susceptibility profile of the prosthesis depends not only on the material but also of the bacterial species.

Sonication has been an excellent method to isolate microorganisms from infected abdominal prostheses. Bacteria adhered to the prosthesis are occasionally impossible to

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![Fig. 2](image_url)

**Fig. 2** — Results of log CFU/g for (A) *Staphylococcus* spp. and (B) *Mycobacterium* spp. x: The differences with the other materials were statistically significant (*P* < 0.05, Mann–Whitney test); xx: The differences between low-density PMM and CP were statistically significant (*P* < 0.05, Mann–Whitney test).
detect by classical culture methods as periprosthetic tissue culture, sampling from the surface of the implant (direct swab), and surrounding fluids. Moreover, with the use of sonication, potential "contaminants" were not detected and bacteria, with which the prostheses were infected, were recovered from sonicated fluid. Because of these reasons, we have used sonication as a method for study bacterial infection in our experimental model.

However, bacterial adherence is not enough to create a clinically symptomatic infection. Other factors as the virulence are important, as other researchers have suggested. S aureus strains are generally considered to be more virulent to the host than S epidermidis, since S aureus strains produce more toxins and tissue-damaging exoenzymes than S epidermidis. Methicillin-resistant S aureus even have antibiotic resistance factors that made them more difficult to treat, but no differences in other pathogenic factors regarding biomaterial infections have been found, so we tested only one S aureus strain as an example of this species. Moreover, the inadequate vascularization during the early period after implantation of the mesh and a reduced host immunologic response to the site causes that bacterial contamination results in rapid multiplication of the microorganisms and make the foreign body highly sensitive to infection. In this sense, we have used Wistar rats, with a fully operative immune system, so some of these data can be altered by this fact if we consider the immunity of the host as an important factor for infection. However, because most patients with prosthetic meshes have a normal immune system, we consider the election of these animals as adequate for our study.

Another limitation of the study is the period of 8 d before we have sacrificed the animals. It is true that some chronic infection can appear after this period. However, in previous in vitro studies, we have shown that RGM (such as M abscessus and M fortuitum) can develop a biofilm in less than 8 d. Nevertheless, further studies with an increased period could be useful to evaluate the actual development of a chronic infection, together with new microorganisms (gram-negative rods and anaerobes) involved in prosthetic mesh infection.

Conclusions

In conclusion, a direct relationship between isolated CFU from the prostheses, the virulence of the bacteria, and integration has been found. Depending on the type of bacteria, prostheses with a large surface and covered with collagen shows reduced resistance to infection, worse integration, and worse clinical outcome. Moreover, the use of sonication could be an important tool to improve microbiological diagnosis in infections of abdominal wall prostheses.

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Authors’ contributions: R.P.T. was involved in project design, data management, statistical analysis, manuscript writing and experimental part. C.L.L. assisted in manuscript writing and performed experimental part. M.C.I. was involved in the study design and conception and experimental part. M.S.D.M. and C.G.V. performed experimental part. A.C.U. and J.E.M. led project design and assisted in manuscript writing and final editing.

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Disclosure

No conflict of interest for any of the authors regarding this manuscript.

References


