Comparison of the adherence of *E. Coli* and *S. Aureus* to ten different prosthetic mesh grafts: *In vitro* experimental study

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Received: 10 November 2009 / Accepted: 9 December 2009
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Abstract

Background The prosthetic mesh grafts used to repair the abdominal wall may become infected, primarily by *S. aureus* and *E. coli*. This study sought to provide a rational basis for the choice of mesh used to repair a hernia when there is a likelihood of infection or contamination.

Methods *S. aureus* and *E. coli* were incubated with ten types of prosthetic mesh graft (Table 1) in liquid growth medium. After sequential dilution of samples from the prosthetic mesh grafts, the colony forming units of adherent *S. aureus* and *E. coli* were counted.

Results There was no significant difference in the numbers of *E. coli* and *S. aureus* adherent to simple polypropylene mesh grafts. Significantly more of both species were adherent to the polyester, expanded polytetrafluoroethylene (ePTFE), and composite prosthetic mesh grafts, except for *E. coli* on graft 5. Significantly fewer *E. coli* were adherent to composite mesh grafts 5, 8, and 10 than *S. aureus*.

Conclusion *S. aureus* and *E. coli* adhere to polypropylene similarly. *In vitro*, fewer *S. aureus* and *E. coli* adhere to simple polypropylene mesh grafts than to polyester, ePTFE, or composite prosthetic mesh grafts.

Keywords Bacterial adhesion · Bacterial infection · Prosthetic mesh graft · Hernia repair · Sequential dilution · Laparoscopy

Introduction

With the increasing popularity of laparoscopic ventral hernia repair, many combination meshes have been designed for intraperitoneal placement [1–4]. However, the prosthetic mesh grafts used in the repair of abdominal wall and inguinal hernias may become contaminated during the perioperative period or it may be necessary to place the prosthesis in an infected body compartment. In addition, infection may complicate the implantation of a prosthetic material after clean surgery [3, 5–8]. The repair method or type of mesh used to repair the abdominal wall after intestinal resections, cholecystectomies, the drainage of intra-abdominal abscesses, or resection of cancer remains controversial [2, 9, 10].

Escherichia coli is the major bacterial species colonizing the gastrointestinal tract. When repairing an incarcerated or strangulated hernia, the risk of contamination of the repair with *E. coli* increases. The adherence of this species to the prosthesis may also trigger systemic infection and sepsis, especially in immunosuppressed patients [11, 12].

Staphylococcus aureus, the main species in the skin flora, commonly contaminates hernia surgery. It is also difficult to treat in patients with prostheses, catheters, and vascular grafts [3, 10, 11, 13].

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The virulence of *S. aureus* and *E. coli* depends on the patient's tissue, structural and molecular properties of the prostheses, secretory products, and the surface properties of the bacteria [14–16]. Several studies have examined the *in vivo* and *in vitro* interactions between prostheses and bacteria [15, 17, 18]. In this study, we examined the adherence of *E. coli* and *S. aureus* to ten different prosthetic mesh grafts using a sequential dilution method to determine which graft is better for use in infected or contaminated situations.

Materials and methods

Ten different simple and composite prosthetic mesh graft materials were studied: 1) Bard Mesh (polypropylene; Davol and Bard, USA); 2) Parietene (polypropylene; Sofradim, France); 3) Parietex (polyester; Sofradim, France); 4) Gore-Tex (expanded polytetrafluoroethylene (ePTFE); DualMesh, Arizona, USA); 5) Vypro II (polypropylene + polyglactin; Ethicon, USA); 6) Sepramesh (polypropylene + hyaluronic acid + carboxymethyl cellulose; Genzyme, Cambridge, MA, USA); 7) Bard Composix Mesh (polypropylene + ePTFE; Davol and Bard, USA); 8) Parietex Composite (polyester + collagen + ethylene glycol + glycerol; Sofradim, France); 9) Parietene Composite (polypropylene + collagen + ethylene glycol + glycerol; Sofradim, France); and 10) Colekin (polypropylene + oxidized regenerated cellulose + PDS (polydioxanone; Textile Hi-Tec, France). Each sample of prosthetic mesh was cut into six discs 1 cm in diameter. Three discs each were incubated with *E. coli* ATCC 25922 and *S. aureus* ATCC 43300 in liquid growth medium (tryptic soy broth) at 37°C for 24 hours. Then, 500 µl of tryptic soy broth were added to sterile 1-mL dilution tubes to perform sequential dilution. The respective mesh discs were then put in liquid growth medium containing 0.5 Mc Farland *E. coli* solution or 0.5 Mc Farland *S. aureus* solution, respectively, and incubated for 16 h at 37°C. Then, the samples were removed from the tubes using a sterile

pin. The samples were washed thoroughly under a stream of 0.09% isotonic NaCl solution at a velocity of 1200 cm/min for 1 minute. Then, they were put in sterile tubes containing 1 ml of liquid growth medium and vortexed for 30 minutes. Sequential dilutions of 1/10, 1/100, 1/1000, and 1/10000 were prepared from the vortexed solutions. Then, the *E. coli* samples were incubated in sterile eosin methylene blue (EMB) and the *S. aureus* samples were incubated in sterile 5% sheep blood nutrient agar at 37°C for 24 h. The bacterial growth after a 24 h incubation was calculated as the colony-forming units/ml (CFU/ml).

Statistics

For each species, the adherence to the three discs was calculated in CFU/ml. The mean and median numbers of CFU/ml were calculated for each bacteria species and each graft. After logarithmic transformation of the number of colonies, one-way analysis of variance was used to evaluate the differences among mesh groups. Then, the Tamhane multiple comparison test was used for pairwise comparison of the mean CFUs for each prosthetic mesh graft (Table 1). $P < 0.05$ was accepted as significant. SPSS 13.0 for windows, ver. 13.0.1 (SPSS, Chicago, IL, USA) was used for the statistical analysis.

Results

There was no significant difference between the numbers of *E. coli* and *S. aureus* adherent to the two simple polypropylene mesh grafts. Significantly fewer *E. coli* and *S. aureus* were adherent to both simple polypropylene mesh grafts than to the polyester, ePTFE, and composite prosthetic mesh grafts (except graft 5 for *E. coli*). There were significantly fewer *E. coli* than *S. aureus* adherent to composite mesh grafts 5, 8, and 10.

Table 1 Mean colony forming units/ml of *S.aureus* and *E. coli* for each type of prosthetic mesh grafts

Prosthetic mesh graft	Mean colony forming units/ml	
	<i>S. aureus</i>	<i>E. Coli</i>
1. Polypropylene I	150	150
2. Polypropylene II	200	210
3. Polyester	4100	5000
4. ePTFE	1800	1530
5. Polypropylene + ePTFE	3500	300
6. Polyester + Collagen + Ethylene glycol + Glycerol	5800	6000
7. Polypropylene + Collagen + Ethylene glycol + Glycerol	12950	7560
8. Polypropylene + Oxidized regenerated cellulose + PDS	13460	3260
9. Polypropylene + Hyaluronic acid + Carboxymethylcellulose	2000	1900
10. Polypropylene + Polyglactin	8200	3630

Fig. 1 Median colony forming units/ml of *S.aureus* and *E.coli* for each type of prosthetic mesh grafts

Discussion

The use of synthetic mesh grafts in hernia repair is critical when there is any contamination in the repair area. Examples are abdominal wall defects containing incarcerated, strangulated, or perforated intestinal segments, or the open abdomen after trauma. Morbidity and mortality increase with infection of the prosthetic material. The biomaterial with the least adherent capacity for bacterial adhesion is important.

Bacteria can interfere with the integration of a prosthesis due to their affinity to it [18], which is determined by their attachment or the resistance of the bacteria to detachment. The formation of a biofilm may also permit bacteria to remain on the material surface. Infection is linked to the molecular structure, manufacturer, bacterial properties, and size of each prosthesis [10]. A complex combination of the physicochemical nature of the biological and synthetic surfaces, the structure of the polymer (i.e., flat or rough), and cell adhesion molecule receptors, as well

as the properties of the interstitial fluids, electrostatic, and hydrodynamic effects, determine adhesion [8, 11, 12, 16, 19]. The prosthetic material to which the fewest bacteria adhere is the most desirable.

Our study revealed that the numbers of *S. aureus* and *E. coli* adherent to the polypropylene mesh grafts did not differ significantly. This suggests that adherence to polypropylene is related more to the structure of the polypropylene than to the characteristics of the bacteria. We know that biofilms on polypropylene prostheses [17], surface properties, and the functional structure of the polypropylene determine the extent of adhesion [5, 21]. Polypropylene provides an ideal surface for the formation of biofilms, which are not always easy for contaminating organisms to form [21]. A biofilm helps adhesion, but decreases the growth of microorganisms, resulting in fewer adherent bacteria. In addition, the native properties of the two bacteria leading to resistance to adherence to polypropylene may be similar. These factors may be responsible for the anti-adhesive properties of *S. aureus* and the *E. coli*. If these properties could be identified, less-adherent material might be developed.

We also found that fewer bacteria of both species adhered to the two polypropylene grafts than to composite grafts 6 to 10. The porous nature of the composite prostheses may increase the adherence of the bacteria. In composite prostheses, ideal niches for microorganisms to settle and the larger surface area increase the adherence capacity [7, 21]. By contrast, polypropylene offers a limited surface for bacterial adhesion *in vitro*, since it is made from a single interwoven filament. Multi-filament biomaterials enable more bacterial adherence than monofilament materials because of their increased surface area [7]. Bacteria have limited growth and adherence on monofilament polypropylene, especially at nodes or filament crossover regions where the surface available for adhesion is greater [21]. It is apparent that infection is dependent not only on the adherence capacity of the bacteria but also on the biomaterial. Multifilament grafts need to be removed when infected due to strong bacterial adherence, whereas conservative antibacterial treatment may eliminate the bacteria from monofilament grafts [22]. This situation also considers the increased adhesive capacity of bacteria to composite grafts.

Similar to our results, another study reported that significantly fewer *S. aureus* adhered to polypropylene than to polyester sutures [6]. This could be related to the interplay of adhesion, hydrophobicity, surface growth, electrostatic forces, and application of friction of the infecting organisms, as reported in the literature [5, 9, 23]. The adhesion of coagulase negative staphylococcus and *E. coli* is faster on positively charged copolymers than on negatively charged copolymers because of the negative charge on bacteria [24]. However, the growth of Gram-negative bacteria decreased on positively charged surfaces. Biofilm formation is related to the adhesion and growth of microorganisms. This is why

adhesion differs *in vitro* and *in vivo* [24]. Gram-positive bacteria have a comparatively thicker and more rigid peptidoglycan layer, which reduces the effective electrostatic force on Gram-positive bacteria.

Significantly fewer *E. coli* than *S. aureus* adhered to grafts 5, 8, and 10. This may be related to the high surface free energy of these composite grafts. The percentage of attached *E. coli* has been reported to decrease as the surface free energy of the polymer increases [2]. In another study, adhesiveness correlated directly with the surface electronegativity of *Staphylococcus epidermidis*, but inversely for *E. coli* [23]. Graft 10 included polyglactin, and minimal *E. coli* adhesion was observed on polyglactin prostheses used in urology [25].

Grafts 4 and 5 included ePTFE. The surface free energy of ePTFE may be related to the difference observed. ePTFE is more hydrophobic and less porous than polypropylene [26]. The deformation of the internodal filaments and creation of fissures in the ePTFE microstructure by the bacteria increases bacterial attachment [17, 26]. It has been reported that microorganisms adhere to and penetrate between the internodal filaments of PTFE, altering its internal structure. Experimental studies have reported that *S. aureus* formed three-dimensional colonies on the cross-over regions in polypropylene prostheses, and that ePTFE grafts contained zones of erosion, disorganized tissue, hemorrhage, and necrosis [20, 27]. In addition, ePTFE grafts had more bacterial adhesion than did Vicryl grafts [8]. Brown et al. showed that *S. aureus* infected PTFE patches contained 100-fold fewer organisms per square centimeter than did polypropylene mesh implants, but bacteria attached and proliferated on the mesh surface (B). These properties may affect the increased adherence of *S. aureus* and *E. coli* to ePTFE.

Each bacterial species may also have specific adherence properties for any prosthetic graft. *S. aureus* has greater affinity for specific collagen receptors [11], while *E. coli* has greater affinity for prostheses with porcine collagen [5, 10, 11]. The properties of the collagen in our composite grafts should be examined to determine their relationship to bacterial adhesion. Of the collagen-containing grafts, graft 7 had increased *S. aureus* adherence, while graft 6 did not. This difference may be related to the other biomaterials in these grafts. *S. aureus* may have an affinity for one of the structures in the composite mesh grafts. The affinity of *S. aureus* to ePTFE, polypropylene, polypropylene + ePTFE, and polypropylene-carboxymethyl cellulose-hyaluronic acid was similar in an *in vivo* study [28]. The difference seen in our study may result from *in vitro* circumstances. The ability of *S. aureus* to form a biofilm, sialic acid-forming slime, bacterial enzymes increasing adhesion, the molecular properties of the bacteria, and the graft all play important roles in adhesion [14, 18, 29], but we do not know which effects are greater *in vitro* or *in vivo*.

Significantly more *S. aureus* than *E. coli* adhered to graft 8. Oxidized regenerated cellulose has antibacterial

effects against both these species [30]. *S. aureus* adhered to PDS in clusters, whereas *E. coli* adhered individually. The difference in adhesion may be related to the form of colonization.

Conclusion

Simple polypropylene mesh grafts with a monofilament structure seem to be the most resistant to bacterial adhesion. The adherence of *S. aureus* and *E. coli* to polypropylene was similar. *In vitro*, more *S. aureus* and *E. coli* adhered to polyester, ePTFE, and composite prosthetic mesh grafts than to simple polypropylene mesh grafts. Further *in vitro* and supporting *in vivo* studies of the mechanisms of bacterial adherence to recently developed biomaterials could identify the prosthetic materials most resistant to infection. Understanding the interaction between bacteria and prostheses would increase our understanding of the mechanism of infection, allowing optimal biomaterial quality and treatment of infection.

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