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SHORT COMMUNICATION

# *In vivo* Biofilm on the Surface of a Surgical Mesh Implant

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Abstract

Mesh hernioplasty is among the most frequently performed surgical procedures. The introduction of mesh implants has decreased recurrence rates, but the use of synthetic materials carries the risk of infection and biofilm formation. This paper presents the course of the disease in the case of biofilm formation on the surface of an implanted surgical mesh. Antimicrobial therapy and partial removal of the implant were unsuccessful. Recurring surgical site infection could be managed only through total excision of the infected implant.

Key words: bacterial biofilm, hernia, synthetic implants

The availability of biomaterials for use in diagnostics and therapy is a major achievement of modern surgery. One of the most frequently performed procedures is hernioplasty with the use of a synthetic implant. Theoretically, the ideal hernioplasty biomaterial should be strong yet flexible, should allow for tissue ingrowth with minimal foreign-body response and be resistant to infection. (Harrell *et al.*, 2006). Despite ongoing research, such a material has not yet been found, and the infections accompanying surgical mesh implantation often necessitate their removal, possibly leading to a recurrence of the hernia (Petersen *et al.*, 2001).

Based on *in vitro* studies, the bacteria responsible for surgical mesh infections have been found to form a biofilm on their surface (Bellón *et al.*, 2001; Engelsman *et al.*, 2008). It serves to protect bacterial cells against the host's immune reactions and antimicrobial agents. It can become fragmented and detached, potentially forming new foci of infection (Lindsay and von Holy, 2006).

The presence of a biofilm on the surface of a mesh implant was confirmed in a 41-year-old male patient admitted to the Department of General and Endocrine Surgery of the Bydgoszcz University Hospital in January 2009 complaining of skin ulceration with purulent fistulas located in the right epigastrium.

In May 2008, the patient had been diagnosed with an incisional hernia in a post-laparotomy scar. The laparotomy had been performed due to peritonitis. A hernioplasty was performed and a multifiber polypropylene mesh was implanted. The early postoperative period was uneventful and the patient was discharged on the fifth postoperative day. On the twelfth day, a mixed purulent/serous discharge appeared, accompanied by a regional edema and reddening of the skin above the implant. Treatment included local application of gentamycin and antiseptic agents, as well as amoxicillin – clavulanate given intravenously and later orally. Subsequent attempts at conservative treatment and partial excision of the implant yielded unsatisfactory results.

Upon admission, the patient presented in good overall condition, with no abnormalities in physical examination, aside from a few purulent fistulas within the scar. Laboratory findings were normal. The fistula smear culture revealed the presence of methicillin susceptible *Staphylococcus aureus* (MSSA), susceptible also to erythromycin, clindamycin, gentamycin, tobramycin, amikacin, netilmicin, tetracycline, ciprofloxacin, trimetoprim/sulphamethoxazole, as well as vancomycin, teicoplanin and linezolid. The strain was identified by slide test for the presence of bound coagulase

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(clumping factor). Drug susceptibility was tested using the disk diffusion test, following the guidelines of the National Reference Center on Microbial Drug Susceptibility (Hryniewicz *et al.*, 2007). Methicillin resistance was tested using a cefoxitin disk (30 μg). The results were interpreted following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2009).

The patient was subjected to elective surgery, whereupon the fistulas were excised along with the unincorporated polypropylene mesh surrounded by necrotic and inflamed tissues. A few specimens were harvested intraoperatively from the implant for microbiological evaluation. A simple closure of the hernial defect was performed by suturing the muscular fasciae.

After cleaning the implant of blood and residual tissues, the presence of a biofilm was confirmed with the use of Richards' technique was used (Gallimore *et al.*, 1991). The method was based on the assessment of the reduction of colorless 2,3,5-triphenyltetrazolium chloride (TTC, POCH) to red formazan by metabolically active microorganisms. The colored metabolite of TTC was observed after approximately 50 minutes incubation at 37°C, and the intensity of the color increased over time (Fig. 1). A quantitative analysis of the biofilm present on the removed implant was then performed. The biofilm was detached from the surface of the biomaterial by shaking in 0.5% saponin (Fluka). Serial 10-fold dilutions of the obtained suspension were performed, with subsequent inoculation on solid substrates. After a 24-hour incubation in 37°C, a growth of $1{,}8\times10^8$ colony forming units per square centimeter of mesh ($CFU/cm^2$) was observed in the biofilm present on one implant sample (an average of three measurements). The etiological factor of mesh implant infection was identified as *S. aureus.* Pulsed field gel electrophoresis was performed. The DNA isolation of *S. aureus* strains was performed with Kit1 (Bio-Rad). The DNA was separated with the CHEF Mapper (Bio-Rad) apparatus for 24 hours, initial pulse 1 s, final pulse 30s. After separation, the gel was stained with ethidium bromide. The results of chromosomal DNA separation were archived using the GEL DOC 2000 (Bio-Rad) gel documentation system and Quantity One software (Bio-Rad). The criteria of Tenover *et al.* (1995) were used for result interpretation. According to those criteria, strains are considered identical if they present identical chromosomal DNA patterns (no differences in the number and distribution of the bands). Based on drug susceptibility testing and pulsed-field electrophoresis it has been ascertained that the *S. aureus* strain isolated from the surgical mesh implant bore identical drug-resistance and chromosomal DNA patterns as the strain obtained from the wound smear culture (Fig. 2).

Even though the introduction of surgical meshes has led to a decrease in hernia recurrence rates (Luijendijk *et al.*, 2000), the use of synthetic implants is associated with a risk of infection (Petersen *et al.*, 2001; Rios *et al.*, 2001; Taylor and O'Dwyer, 1999). The etiological factors of hernioplasty implant infections include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Acinetobacter baumannii* and *Streptococcus pyogenes* (Petersen *et al.*, 2001; Rios *et al.*, 2001). Reports exist of implant infections caused by *Proteus*, *Peptostreptococcus* (Taylor and O'Dwyer, 1999) and *Mycobacterium* species (Celdrán *et al.*, 2007).

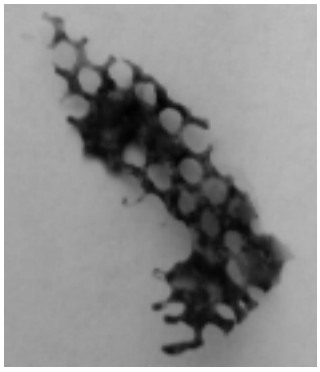

Fig. 1. Formazan on the removed mesh implant.

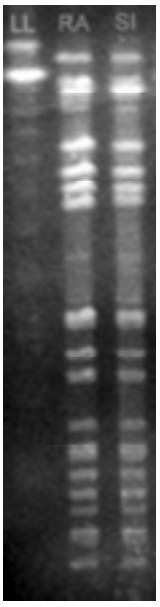


Fig. 2. Chromosomal DNA profiles of *S. aureus* strains isolated from wound smears (RA) and mesh cultures (SI) obtained using the PFGE method after enzymatic cutting with *Sma*I. LL – lambda ladder marker.

A surgical mesh may become infected upon implantation or through colonization by endogenous bacteria (Petersen *et al.*, 2001; Taylor and O'Dwyer, 1999). In the case presented here, the implant became infected by a *S. aureus* strain. Considering that *S. aureus* is a part of the skin flora and that the interval between the initial procedure and the onset of the signs of infection was rather short, one must conclude that the biomaterial had probably become contaminated upon implantation.

The infection of the implant by *S. aureus* interfered with the ingrowing of the host's tissues through the mesh, as evidenced by the presence of free, unattached mesh fragments at the implantation site. Similar observations were made by Bellón *et al.* (2004) in their studies on rabbits. The lack of biomaterial incorporation results from the fact that *S. aureus* induces fibroblast death (Bellón *et al.*, 2001) and inhibits the proliferation of these cells (Edds *et al.*, 2000).

The consequence of implant colonization by bacteria was the formation of a biofilm on its surface. *In vitro* studies have shown that the most significant factors influencing biofilm formation on the surface of a synthetic implant are: its structure and hydrophobicity and the species of bacteria involved (Engelsman *et al.*, 2008). In our case, a multifilament polypropy-

lene mesh had been used. Polypropylene is considered hydrophobic, which prevents initial colonization and promotes host cell adhesion (Karakeçili and Gümüṭderelioğlu, 2002). However, prolonged exposure of Gram positive bacteria to a hydrophobic material stimulates their growth on the implant surface (Engelsman *et al.*, 2008). As shown by Engelsman *et al.* (2008), multifilament meshes are prone to biofilm formation due to the presence of niches between the fibers, providing a good environment for bacterial growth. Besides, multifilament meshes have a greater surface compared to monofilament implants, leading to greater bacterial adhesion (Engelsman *et al.*, 2008). The properties of the microorganism colonizing the implant are crucial to the process of biofilm formation. The ability to create a biofilm requires at least two properties: adhesion to the surface and creation of multilayer cell colonies. The initial adhesion to the surface is mediated primarily by bacterial proteins associated with the surface, while in the formation of multilayer colonies intracellular proteins and polysaccharides play a pivotal role. Polysaccharide intercellular adhesin (PIA) is crucial in *S. aureus* biofilm formation, as well as PIA – independent factors, including, for example biofilm associated protein (Bap) (Becker *et al.*, 2007), an intercellular adhesin active in adhesion to abiotic surfaces, as well as SasG (*S. aureus* surface protein G) active in intercellular aggregation (Kuroda *et al.*, 2008).

The formation of a biofilm on the surface of the implant was the cause of antibiotic therapy failure. The described temporary improvement of local and general condition of the patient was probably the result of the bactericidal activity of the antibiotic against the planktonic form of *S. aureus* present in the tissues surrounding the implant.

Implantation of a synthetic material is associated with the risk of infection around the implant. It is likely that most of these infections are associated with the formation of a biofilm on the biomaterial surface, which can be a significant factor of treatment failure.

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