Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

An *in vitro* study assessing the infection risk of low-cost polyethylene mosquito net compared with commercial hernia prosthetics

David L. Sanders, MBCHB, MRCS, BSc, PG Dip Med Ed,[a,*]
Andrew N. Kingsnorth, FRCS, FACS,[b] *Roy Moate, PhD,*[c] *and Jane A. Steer, MSc, FRCPath*[a,d]

[a] *Peninsula College of Medicine and Dentistry, Plymouth, UK*
[b] *Department of Surgery, Derriford Hospital, Plymouth, UK*
[c] *Plymouth Electron Microscopy Centre, Plymouth University, Plymouth, UK*
[d] *Department of Microbiology, Derriford Hospital, Plymouth, UK*

ARTICLE INFO

Article history:
Received 27 December 2012
Received in revised form
27 December 2012
Accepted 24 January 2013
Available online 15 February 2013

Keywords:
Hernia
Rural
Mesh
Mosquito net
Infection
International

ABSTRACT

Background: The innovative use of sterilized mosquito net as a cheaper alternative to commercial mesh for hernia repair has gained increasing recognition. Developing health care systems have inherently higher surgical site infection rates, and concerns regarding the introduction of untested prosthetic hernia meshes have been raised. This *in vitro* study assesses the infection risk of polyethylene (PE) mosquito net mesh compared with commercial hernia prosthetics by assessing the essential (first) step in the pathogenesis of mesh infections.

Materials and methods: Individual meshes were inoculated with *Staphylococcus epidermidis* and *Staphylococcus aureus* with a bacterial inoculum of 10^2 bacteria. Inoculated meshes were incubated for 18 h in tryptone soy broth and then analyzed using scanning electron microcopy. The final fraction of the bacteria adherent to each of the meshes was compared. One-way analysis of variance was performed on the bacterial counts. The Tukey test was used to determine the difference between the different biomaterials in the event the one-way analysis of variance was significant.

Results: There was no significant difference in the mean number of adherent bacteria to PE mosquito net compared with the monofilament polypropylene-based meshes (Prolene and Bard Soft Mesh). Multifilament Vypro mesh had significantly greater mean bacterial adherence compared with PE mosquito net ($P < 0.001$ with *S aureus* and $P = 0.003$ with *S epidermidis*).

Conclusions: In vitro infection risk of PE mosquito net is not significantly different from commonly used monofilament polypropylene commercial prosthetics and is in fact lower than a commonly used commercial multifilament mesh. This study adds to the growing body of evidence that indicates that these meshes can be safely deployed.

* *Corresponding author*. Department of Upper GI Surgery, The Royal Cornwall Hospital, Truro TR1 3LJ, UK. Tel.: +44 7815319851; fax: +44 1872 241203.
E-mail address: dsanders@doctors.org.uk (D.L. Sanders).
0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.01.047

1. Introduction

Recognition of surgery as a global health priority is increasing [1,2]. In economically developing countries, the health burden of untreated hernias is substantial and has significant effects on their economy [1,3]. In Sub-Saharan Africa alone, it has been estimated that 6.3 million adult males have untreated inguinal hernias [4]. The use of prosthetic mesh to reinforce the abdominal wall in inguinal hernia repair is now accepted as the gold standard and has reduced recurrence rates to below 5% [5–7]. However, in most low-income, resource-poor developing countries, a traditional sutured repair, with significantly inferior results, is still commonplace as commercial mesh is either unavailable or unaffordable [8]. An innovative frugal technology that has been reported with positive short-term clinical outcomes is the use of sterilized mosquito net as an alternative to commercial meshes [9–13]. The Indian surgeon RR Tongaonkar has popularized this technique (although he attributes the idea to his co-author Reddy), and the surgical charity *Operation Hernia* uses mosquito net exclusively in the low- and middle-income countries where it works [14].

We have recently documented the material characteristics of a widely available polyethylene (PE) mosquito net for standardized use in resource-limited setting and demonstrated substantial equivalence in terms of morphologic features to large pore, lightweight commercial hernia meshes [15]. The drawback of PE is that its melting point is 122°C, which is unsuitable for use in high-pressure steam sterilizers, in which the temperature rises to at least 134°C. However, as the vast majority of rural hospitals in developing countries use bench top vertical autoclaves, in which the temperature only reaches 121°C, the PE mosquito mesh can be sterilized without damage. Although this may cause anxiety and scepticism, steam sterilization at 121°C for at least 15 min is a well-established and accepted method for sterilizing medical devices; however, it is not recommended by the Medical Device Agency in the United Kingdom, who advocate sterilization at 134°C. Consequently, there remains concern about the infection risk of the PE mosquito net polymer. This is especially important in the developing world where postoperative surgical site infection (SSI) remains high [16].

Bacterial adhesion to biomaterials is the essential (first) step in the pathogenesis of mesh infections, and we have previously validated a method for quantitative enumeration of bacteria on porous material using scanning electron microscopy (SEM) [15]. The usual causative organisms associated with cases of mesh infection are *Staphylococcus aureus* and coagulase negative staphylococci such as *Staphylococcus epidermidis* [17,18].

The aims of this experimental study were to compare the infection risk of steam sterilized PE mosquito net with commercial hernia prosthetics made of polypropylene (PP) by assessing bacterial adherence *in vitro*.

2. Materials and methods

Autoclave (121°C) sterilized PE mosquito net obtained from Amsa Plastics, Karur, India, and used by the non-profit charitable organisation *Operation Hernia* for hernia repair in developing countries was compared with three commercially sourced PP-based flat hernia meshes. These were Prolene (Ethicon), Bard Soft Mesh (Bard, Bard-Davol Inc, RI), and Vypro (Ethicon, Johnson & Johnson company, Somerville, NJ) (a PP and polyglactin-910 multifilament mesh). Individual meshes were inoculated with *S epidermidis* and *S aureus* with a bacterial inoculum of 10^2 bacteria, and each study was repeated three times. We have previously shown that this size of inoculum is appropriate for comparing mesh subtypes and allows for quantifiable enumeration using SEM [19].

2.1. Preparation of bacteria

S epidermidis (Winslow and Winslow - Evans - ATCC 12228) and *S aureus* (Winslow and Winslow - Rosenbach - ATCC 25923) were maintained as frozen stock. Bacteria were subcultured on blood agar plates (Columbia agar horse blood lot 1186593, Oxoid Ltd, Basingstoke, UK) kept at 37°C, 95% air/5% CO_2 for 18-h overnight incubation. Bacterial stock solution was created from a single colony added to phosphate-buffered saline (PBS) and vortex mixed for 30 s. Serial dilution of the stock solution was performed using PBS as described by Collins [20] to produce solutions with dilution of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} to generate the desired inoculum of 10^2 bacteria. From a previous study, we estimated that the 10^{-4} dilution would yield an inoculum of 10^2 bacteria; however as a quality control check to ensure the correct size of inoculum, the number of viable bacteria in each dilution was calculated using a spot plate technique; this was conducted immediately before inoculation of the meshes to accurately reflect the bacterial inoculum (repeated three times for each dilution) [21]. A sterility check of PBS was performed on blood agar plates at incubation at 37°C, 95% air/5% CO_2 for 18-h overnight incubation.

2.2. Substrate preparation

Meshes were prepared in an operating theatre using sterile technique and were cut into 5-mm square samples using a sterile predesigned punch. Each sample was placed individually into an autoclave sterilized prelabelled glass tube, which was sealed with a lid. An extra sample of each mesh was prepared for electron microscopy analysis to confirm the sterility of the mesh.

2.3. Inoculation, rinsing, and preparation for SEM

Each mesh sample was inoculated with 1 mL of 10^{-4} dilution of bacterial suspension (which equated to a 10^2 inoculum) and 3 mL of tryptone soy broth (TSB) (CM0129; Oxoid Ltd) added using an automated pipette. A sterility check for TSB was performed using the same method that is described previously for PBS sterility check. Meshes were cultured for 18-h overnight incubation at 37°C, 95% air/5% CO2. Mesh samples were then rinsed individually in a sterile water bath to remove nonadherent bacteria. Rinsing was performed four times with 15 mL of PBS solution dispensed from a 20-mL sterile syringe over 10 s and directed at the edge of the rinsing bath rather than directly onto the mesh to reduce the shearing force