

Effects of Resterilization on the Surface-Structure of Lightweight Polypropylene Hernia Meshes-An *in vitro* Study

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Abstract: The frequency of reports about postoperative complications after implantation of hernia meshes seems to point to a deficit in their biocompatibility. A simple experiment was designed to show the effect of resterilization which is allowed by some manufacturers of the meshes on their surface structure that should be important for biocompatibility. Two common lightweight polypropylene hernia meshes (Optilene® LP, Atrium® prolite) were examined with an electron microscope before and after incubation with MRC 5 cells which are used as a model for human fibroblasts in this experiment. Atrium® prolite which was additionally resterilized due to the manufacturers recommendation in a steam autoclave for 20 min at 121°C was treated to the same procedure as the two native meshes. Then the images made with the electron microscope were compared to each other. The sterile hernia meshes showed differences in respect to their surface structure when taken out of the packaging. Besides irregularities in their filamentary structure (Atrium® prolite, Optilene® LP) they showed artefacts (Optilene® LP) which were not attributable to the mesh and possibly were due to the manufacturing process. The Atrium®-mesh treated in the autoclave showed multiple fissures on the micrometer level and small bubbles which were not shown by the Atrium®-mesh not treated with the autoclave. After 72 h of incubation with MRC 5 cells and cell culture medium a progress was discernible in respect to the morphological change. Light weight polypropylene hernia meshes were developed to improve their biocompatibility. Some manufacturers offer the possibility of resterilization of lightweight hernia meshes which were taken out of their packaging for one time. That means a clear reduction of the cost for material. This experiment which was deliberately kept simple shows that treatment with the autoclave results in visible changes on the filament surface of light weight hernia meshes which are increased after incubation with human connective tissue cells and cell culture medium. Because hernia meshes are supposed to remain in the human body for life there could be an increase of changes of the surface structure in the course of time which would probably have a negative effect on the biocompatibility of the meshes.

Key words: Hernia meshes, *in vitro* study, biocompatibility, irregularities, packaging, postoperative

INTRODUCTION

The use of hernia meshes to improve stability of the abdominal wall is common all over the globe and has become indispensable in especially incisional hernia surgery. This is underlined by the fact that every year a seven digit number of hernia meshes is implanted. With rates of relapses of 20-50% conventional methods of hernia treatment without mesh do not deliver satisfying results (Amid *et al.*, 1996, 1994; Paech *et al.*, 2008). With the increased use of synthetic meshes the rate of recurrences could be lowered to 10% (Langer and Christiansen, 1985) but parallel to that there was an increase in the rate of complications. Postoperative seroma or haematoma occurred in the area of the

implantation in 30-50% of cases, a paraesthesia in 10-20% of cases and 25% of patients reported a limited abdominal mobility (Lichtenstein *et al.*, 1993).

After explantation of the meshes in a part of the cases there was a massive shrinking and folding of the implants. (Shulman *et al.*, 1990) to improve biocompatibility lightweight hernia meshes were developed by different manufacturers. They are characterized by a lower weight and a reduced surface.

This experiment is to show whether a 72 h incubation of lightweight, sterile and especially resterilized hernia meshes with human cells under physiological conditions results in structural changes on the surface of the mesh. It is designed to become an idea of biocompatibility of different meshes.

MATERIALS AND METHODS

Meshes: For raster electron microscopy we chose the following implantable synthetic meshes: Optilene® LP-a lightweight non resorbable monofil polypropylene mesh with a weight of 36 g m^{-2} . The size of pores is 1.0 mm. The manufacturer is BBD Aesculap GmbH PO box 31, 78532 Tuttlingen, Germany. Atrium® prolite, a monofilamentary, non resorbable lightweight polypropylene mesh. Its weight is 85 g m^{-2} , the size of pores is 0.8 mm. If the sterile original packaging is opened or damaged by mistake, the Atrium® prolite net can be resterilized once using ethylene oxide or steam. The manufacturer is Atrium Medical Corporation, 5 Wendworth Drive, Hudson, New Hampshire 03051 USA.

Primary cells: The MRC 5 cells used for the *in vitro* tests were cultivated in 1966 out of lung tissue of a 14 week old male fetus of a 27 year old woman. The cell morphology is described as fibroblast-like and is used as a model for human fibroblasts in the experiment.

Experimental setting: Because we wanted to study the effect of incubation with medium and MRC 5 cells on lightweight propylene hernia meshes, 3 different groups are formed. Mechanical changes in the cover of Optilene® Mesh LP (Group 1) and Atrium® prolite nets (Group 2) were supposed to be shown in raster electron microscopy before and after the incubation with MRC 5 cells. The resterilized Atrium® prolite formed the third group and was treated to the same procedure.

Preparation of meshes: After taking them out of their packaging we welded the Atrium® prolite meshes in using autoclaving film (SBW, Self Seal Sterilisation, Pouch, Smith Brothers, Whitehaven) and sterilized them according to the guidelines of the manufacturers at 121°C for 20 min. in a steam autoclave (Webeco Model CS-V, Webeco, Bad Schwartau). Native, resterilized Atrium® prolite and native Optilene® LP were then cut down to a size of 1 cm^2 under sterile conditions.

***In vitro* test:** For the cultivation of MRC 5 cells we used the basis medium Minimal Essential Medium Eagle (Sigma Aldrich, Deisenhofen). Inactivated Fetal Calf Serum (FCS), L-glutaminey, non essential amino acid and penicillin-streptomycin-solution for prevention of bacteria growth were added. The cells were now filled into the deepenings of standard 12 corrugated boards (Nunc Brand Products, Denmark) in a density of 3×10^4 cells per deepening and incubated for 72 h with the different meshes of the three groups (Fig. 1). After incubation time the meshes were removed from the boards and dried.

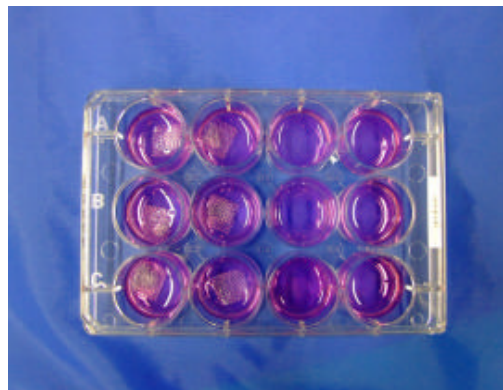


Fig. 1: *In vitro* test arrangement; in a standard 12 corrugated board meshes cut to a size of 1 cm^2 were put in together with standard cell medium and MRC cells. Then 72 h of incubation under physiological conditions followed. For better clearness meshes were put in only 6 of 12 deepenings

Raster electron microscopy: All samples of the meshes were now dried in a critical point dryer (E 300, Polaron) and applied to an aluminium board. After sputtering the meshes with platinum-palladium (SCD 040, Blazes) images were made using a raster electron microscope (SEM 505, Philips) at 10-15 kV. The results were documented on APX 100 films (Agfa).

RESULTS

Group 1-native Optilene® LP before and after incubation with MRC 5 cells: Figure 2a shows the native Optilene® LP magnified 312 times. The lengthways parallel filament structure of the polymere thread is clearly shown. In a fine netlike structure (Fig. 2b) balls with a diameter of $10 \mu\text{m}$ are stuck. After incubation with the primary cells chosen by us (Fig. 2d) there are no significant tears visible. At the point where both polypropylene threads meet (Fig. 2c) residue of cell culture medium has crystallized.

Group 2-native Atrium® prolite before and after incubation with MRC 5 cells: Magnified 1250 times (Fig. 3a, b) the Atrium® prolite which was taken sterile out of the packaging shows the typical groove-like structures parallel to the longitudinal axis which could be observed in all nets examined by us. At the meeting points of the filaments where the material is compressed the surface appears corrugated and irregular. After 72 h of incubation with MRC 5 cells there were no significant changes on the

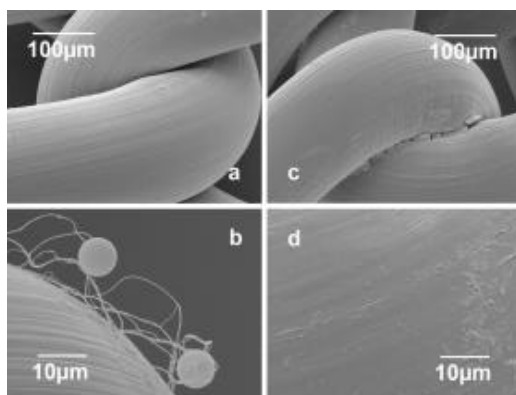


Fig. 2: Native (a, b) Optilene® LP that was incubated with medium and MRC 5 cells (c, d) magnified 1250 times (b, d) and 312 times (a, c). The lengthways paralleld structure of the polypropylene filaments of the native (a) and incubated Optilene® LP is clearly visible. Beside that we saw the balls of about 10 µm in diameter in the native Optilene® LP shown in illustration 2b which were caught in fine threads of an unknown material

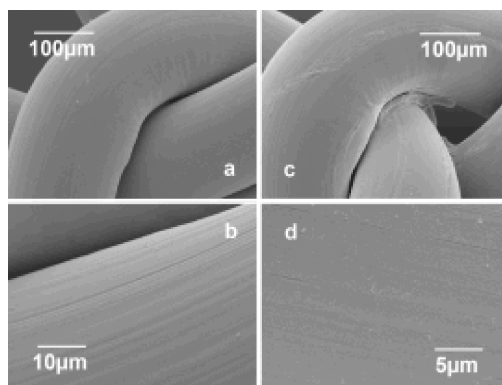


Fig. 3: Native (a, b) Atrium® prolite and Atrium® prolite incubated with medium and MRC 5 cells (c, d) magnified 312 times (a, c) 1250 times (b) and 2500 times (d); these images, too, show the lengthways grooves already observed on Optilene® LP; tears and other changes are not recognizeable on this mesh neither before nor after incubation

surface of the non resterilized Atrium® prolite meshes. Only residue of cell culture medium is visible at the meeting points of the meshes (Fig. 3c, d).

Group 3-resterilized Atrium® prolite before and after incubation with MRC 5 cells: The surface of the Atrium® prolite which was resterilized at 121°C in the steam

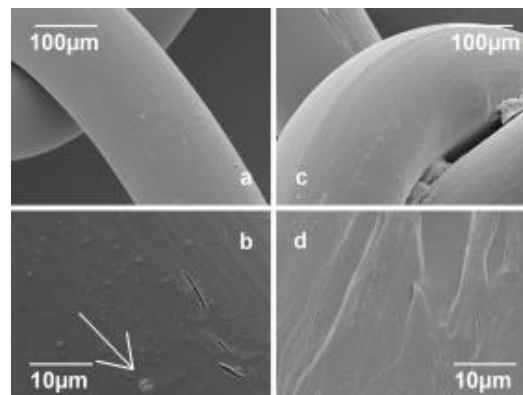


Fig. 4: Atrium® prolite resterilized (a, b) and additionally incubated with medium and MRC 5 cells (c, d) magnified 312 times (a,c) and 1250 times (b, d). Clear changes of the surface are already visible on images a and c and more detailed on image b where an arrow marks a bubble caused by heat. A number of undirected tears is also visible. After incubation the surface appears increasingly rough

autoclave (Fig. 4a, b) showed some changes compared to the native net. Undirected tears and small bubbles were visible. It seems that the heat of the steam autoclave has thermally changed the material. After incubation (Fig. 4c, d) of the resterilized Atrium® prolite mesh there is a rough, clod-like structure.

DISCUSSION

To improve their biocompatibility lightweight hernia meshes were developed by different manufacturers and brought on the market (Weyhe *et al.*, 2007). Some of these manufacturers offer the possibility of resterilization of the meshes in a steam autoclave. Surgery departments that conduct a high number of hernia operations can almost cut their costs up to 50% by cutting a large mesh sterile in smaller pieces and using these resterilized pieces for hernia operations. The resterilization of propylene hernia meshes is common practice in developing countries and is being propagated internationally (Serbetci *et al.*, 2007). Because there is a growing need to cut costs this option is becoming more interesting for the hospitals. But there is a lack of studies to prove the harmlessness of treating lightweight propylene hernia meshes with an autoclave. Nevertheless there is no doubt about the fact that there is an unsatisfying biocompatibility of meshes which is proven by the development of seroma, adhesion and parallel to that intense postoperative pain (Lichtenstein *et al.*, 1993). In this connection a working

group around Duchrow *et al.* (2002) managed to prove in an *in vitro* experiment that the growth and rate of apoptosis of human fibroblasts is influenced unfavourably if they are incubated with resterilized polypropylene meshes. In the model which was kept deliberately simple we wanted to examine the effects of resterilization on the surface structure of mesh filaments after 72 h of incubation with cells of human connective tissue. For that purpose two lightweight polypropylene hernia meshes (Optilene® LP, Atrium® prolite) were examined with an electron microscope. Atrium® prolite which was additionally resterilized due to guidelines of the manufacturers in a steam autoclave for 20 min at 121°C was treated to the same procedure as the two native meshes. Then the images made using the electron microscope were compared to each other. The most significant changes in structure took place in the resterilized Atrium® prolite as expected. The surface appeared to have been distorted thermally. It is known that polypropylene can be heated to 140°C for short periods of time. The melting point is at 160°C and accordingly about 40°C above the temperature in the steam autoclave. The temperature of 121°C and the damp environment in the steam autoclave seem to be enough to cause the described changes in the surface structure. After only 72 h of incubation with human fibroblasts in a watery environment the typical lengthways parallel structure of the polypropylene filaments of the resterilized Atrium® prolite is no more recognizable. For the non resterilized meshes only minor changes in the surface structure can be observed. Beside that small geometrical objects with a diameter of about 15 µm which were considered residue of the manufacturing process were observed in the Optilene® LP which was taken sterile out of the packaging. Those artefacts are sure to get into the human body in case of implantation.

CONCLUSION

The results of this study emphasize that the morphological characteristics of light weight meshes are influenced unfavourably by treatment with the autoclave. The described changes in surface structure could be a reason for the problems in biocompatibility of hernia meshes. Tears and irregularities as found on the surface

of resterilized Atrium® prolite meshes offer an increased surface for the foreign body reaction following implantation. The resterilization of lightweight hernia meshes, which were developed in respect to an improved biocompatibility could therefore be a step in the wrong direction.

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