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ABSTRACT

***Background*: Prosthetic mesh repair for abdominal wall hernias is widely used because of its technical simplicity and low hernia recurrence rates. The most commonly used material is pure polypropylene mesh, however newer composite materials are recommended by some centers because of their advantages. However, these meshes are more expensive than pure polypropylene meshes. Resterilisation of a pure polypropylene mesh has been shown to be quite safe, and many centers prefer slicing a large mesh into smaller pieces that suitable for hernia type or defect size. Nevertheless there is no data about the safety after resterilisation of the composite meshes.**

***Objective*: To search the effects of resterilisation and *In vitro* degradation in phosphate buffered saline solution on the physical structure and the mechanical properties of partially absorbable lightweigth meshes.**

***Design*: Laboratory-based research.**

***Subjects*: Two composite meshes were used in the study: One mesh is consisted of monofilament polypropylene and monofilament polyglecaprone -a copolymer of glycolide and epsilon (ε)-caprolactone- (Ultrapro®, 28 g/m², Ethicon, Hamburg, Germany), and the other one consisted of multifilament polypropylene and multifilament polyglactine (Vypro II®, 30 g/m2, Ethicon, Hamburg, Germany). Two large meshes were cut into rectangular specimens sized 50x20 mm for mechanical testing and 20x20 mm for In vitro degradation experiments. Meshes were divided into control group with no resterilisation and gas resterilisation. Ethylene oxide gas sterilisation was performed at 55°C for 4.5 hours. In vitro degradation in 0.01 M phosphate buffered saline (PBS, pH 7.4) solution at 37 ± 1°C for 8 weeks was applied to one subgroup in each mesh group. Tensiometric measurements and scanning electron microscopyic evaluations were completed for control and resterilisation specimens.**

***Results*: Regardless of resterilisation, when meshes were exposed to *In vitro* degradation, all mechanical parameters decreased significantly. Highest reduction in mechanical properties was observed for Ultrapro due to the degradation of absorbable polyglecaprone and polyglactin parts of these meshes. It was observed that resterilisation by ethylene oxide did not have significant difference on the degradation characteristics and almost similar physical structures were observed for resterilised and non-resterilised meshes. For Vypro II meshes, no significant mechanical difference was observed between resterilised and non-resterilised meshes after degradation while resterilised Ultrapro meshes exhibited stronger characteristics than non-resterilised counterparts, after degradation.**

***Conclusion*: Resterilisation with ethylene oxide did not affect the mechanical properties of partially absorbable composite meshes. No important surface changes were observed in scanning electron microscopy after resterilisation.**

INTRODUCTION

Prosthetic mesh repair for abdominal wall hernias is widely used because of its technical simplicity and low hernia recurrence rates (1). The most commonly used material is pure polypropylene mesh, however newer composite materials are recommended by some centres because of their advantages shown in the clinical and laboratory studies (2-5). Meshes comprising nonabsorbable and absorbable materials together have been presented to be strong enough to protect the recurrence and lighter to provide less complications related to biocompatibility (2, 3, 6, 7).

Two types of composite meshes have frequently been used in the literature: a composition of monofilament polypropylene and monofilament polyglecaprone, and a composition of multifilament polypropylene and multifilament polyglactine. These two meshes are more expensive than pure polypropylene meshes. Besides, resterilisation of a pure polypropylene mesh has been shown to be quite safe (8, 9), and many centers in developing countries in Africa, Asia and the Middle East prefer slicing a 30x30 mesh into smaller pieces that suitable for hernia type or defect size. Nevertheless there is no data about the safety after resterilisation of the composite meshes.

In the product manual of the all commercial meshes an offical warning states that "do not sterilise". We previously reported that pure polypropylene mesh can be resterilised with autoclave or ethylene oxide at least ones without significant changes in their mechanical properties and physical structures, however gas sterilisation with ethylene oxide should be the preferred method (8). In continuation of our previous study we aimed to search the effect of ethylene oxide resterilisation on the composite meshes.

MATERIALS AND METHODS

"Non of the authors has any conflict of interest or disclosure at all"
"The meshes that used in this study were not supplied by any manufacturer"

Mesh: A 30x30 cm composite mesh consisted of monofilament polypropylene and monofilament polyglecaprone -a copolymer of glycolide and epsilon (ε)-caprolactone- (Ultrapro®, 28 g/m2, Ethicon, Hamburg, Germany), and a 30x30 cm composite mesh consisted of multifilament polypropylene and multifilament polyglactine (Vypro II®, 30 g/m2, Ethicon, Hamburg, Germany) were used in the study.

Sample preparation: The two large meshes were cut into rectangular specimens sized 50x20 mm for mechanical testing and 20x20 mm for In vitro degradation experiments.

Ethylene oxide sterilisation: Ethylene oxide gas sterilization was performed in the eto.krt 135 device (Ekol Medical, Ankara, Turkey). Ethylene oxide gas was applied to the specimens at 55°C for 4.5 hours. After the sterilisation phases, aeration was applied to the samples for 12 hours.

Mechanical testing: The specimens were tested mechanically by using the Lloyd LRX5K mechanical testing machine (Lloyd Instruments Limited, Fareham, England). Gauge lengths of the specimens were adjusted to 20 mm. Tensile tests were performed at a strain rate of 20 mm/min (100% strain per minute). Each tensile test ended when the specimen tore completely. For the mesh-structured specimens did not have solid cross-sectional areas and therefore tensile strength and elastic modulus values of the materials could not be calculated. Instead, maximum load before rupture (Fmax, N), elongation at maximum load (ΔL, mm), and quantity of energy required for complete failure of the specimens (E, Nmm) were measured and calculated to investigate the mechanical strength of the specimens.

In vitro degradation: In vitro degradation tests were carried out in 0.01 M phosphate buffered saline (PBS, pH 7.4) solution at 37 ± 1°C [10]. For this purpose, 20x20 mm meshes were prepared and the samples were incubated in PBS up to 8 weeks. At the end of one month and two months, samples (n = 4) were taken out, rinsed with distilled water and dried in vacuum oven at 40°C. Weight loss percentages were calculated from the dried weight obtained before and after degradation using gravimetrical method. Weight loss percentages were obtained using the following equation:
$WL\% = (W0 - Wt) / (W0) \times 100$, where WL is weight loss, W0 and Wt are the dry weights of the samples before and after degradation, respectively.

Scanning electron microscopy: Topographic images of the meshes were obtained by scanning electron microscopy (SEM) (FEI Quanta 400 FEG, USA), after coating the samples with gold-palladium under vacuum.

Statistical analysis: Determination of the significance of the differences for the obtained values for Ultrapro and Vypro II was performed by the Mann-Whitney

U test. Two-way Anova test was used in order to investigate the significance of resterilisation and time effects. Tukey and Tamhane tests were used as post-hoc for time effects. Two-tailed "p" values below 0.05 were accepted for statistical significance.

RESULTS

Tensile properties of Ultrapro mesh and Vypro II mesh after resterilisation and degradation were examined. The maximum load before rupture (Fmax), energy required for complete failure (E) and elongation at maximum load (ΔL) values of each group before and after degradation are given in Figures 1, 2 and 3, respectively. And all the obtained results are summarised in Table 1.

Figure 1

Figure 2

Figure 3

Table 1

No statistically significant differences were observed between the Fmax and E values of Ultrapro mesh and Vypro II mesh control groups. Fmax values of Ultrapro control and Vypro II control groups were found as 113.12 N and 113.28 N (p=0.873), while E values were 1572.83 Nmm and 1504.17 Nmm (p=0.699), respectively. ΔL values of Vypro II mesh were found to be significantly larger than Ultrapro mesh regardless of resterilisation or degradation time ($0.001 < p < 0.030$).

When resterilised meshes were examined; it was observed that resterilisation had no significant effect on Fmax (p=0.779 for Ultrapro, p=0.873 for Vypro), E (p=0.779 for Ultrapro, p=0.109 for Vypro) and ΔL (p=0.708 for Ultrapro, p=0.109 for Vypro) values of both control group meshes. In addition, when resterilised and non-resterilised meshes were compared after degradation processes; there were no statistically significant differences between tensile properties of control and resterilised Vypro II meshes after one month and two months degradation ($0.149 < p < 0.665$ for all mechanical parameters).

For Ultrapro meshes, after one month of degradation, the mechanical properties remained unaffected from resterilisation ($0.060 < p < 0.736$ for all mechanical parameters). But, after two months of degradation, all mechanical properties of resterilised meshes were significantly better than non-resterilised ones ($0.013 < p < 0.031$ for all mechanical parameters). Regardless of resterilisation, when meshes were exposed to In vitro degradation, all mechanical parameters decreased significantly. Significant reduction in mechanical properties was observed due to degradation of absorbable poliglecaprone and polyglactin parts of meshes. Energy values in all groups decreased more than 50%.

After one month and two months degradation regardless of resterilisation Ultrapro mesh was found to be significantly stronger than Vypro II mesh in terms of Fmax and E values ($0.000 < p < 0.005$ for all related Fmax and E values).

After one month degradation, except ΔL values of resterilised meshes, all other mechanical parameters decreased for all control and resterilised meshes ($0.000 < p < 0.05$). After one month degradation, the decrease in ΔL values of resterilised meshes was not significant (p=0.887 for resterilised Ultrapro and p=0.409 for resterilised Vypro). After two months degradation, regardless of resterilisation and the type of the mesh, all mechanical parameters decreased for all control and resterilised meshes compare to initial parameters ($0.000 < p < 0.001$ for all mechanical parameters).

Gravimetric analysis demonstrated that, generally there is no significant difference in percent weight loss values of resterilised and non-resterilised samples ($p > 0.05$ for Ultrapro at 2nd to 8th weeks and for Vypro at 5th to 8th weeks). Although up to five weeks Vypro II resterilised mesh seems to degrade more than Vypro II control group, at the end of eight weeks degradation weight loss values were almost same ($p = 0.343$). In general, it can be concluded that ethylene oxide sterilisation did not affect degradation behavior of Ultrapro and Vypro II meshes. When Ultrapro and Vypro II meshes are compared, in both resterilised and non-resterilised groups, Vypro II degraded slower than Ultrapro up to three weeks. However after four weeks, Vypro II started to degrade faster and at the end of 8 weeks, weight loss of Vypro II EO (57.01%) was higher than weight loss of Ultrapro EO (44.60%).

If the chemical compositons of two meshes are compared Ultrapro consists of polypropylene monofilaments that are closely tangled with a copolymer of glycolide and ε-caprolactone (named as polyglecaprone), and Vypro II is multifilament of polypropylene with glycolide and lactide copolymer (named as polyglactin).

It was observed that Vypro II mesh degraded faster than Ultrapro ($p = 0.001$ at the 8th week). Since polypropylene is rather stable, it is expected that degradation would be controlled by the chains which are polylactide and polycaprolactone for

Vypro II and Ultrapro, respectively. Both of these chains can have a semicrytalline organization, and when semicrystalline materials are immersed in aqueous media the diffusion of water and therefore degradation by hydrolysis take place in two steps. The first step is the diffusion of water in the amorphous parts where the initial and faster degradation by hydrolysis starts; and the second step is the diffusion of water to the more organised crystalline domains where slower degradation continues. The process for degradation occurs as reduction in molecular weight combined with weight loss. In some cases degradation starts from surface and causes a rapid weight loss but not affect the molecular weight, in some other cases degradation results significant decrease in molecular weight but not in total weight. These depend of the initial preparation conditions of the materials such as crystallinity, shape and molecular weight.

In this study, it was observed that, Vypro II mesh degraded faster than Ultrapro mesh indicating that copolymer structure of lactide with glycolide is more sensitive to water hydrolysis than that of polycaprolactone existing in Ultrapro mesh. Percent weight loss of meshes after incubating in PBS is given in Figure 4 and Table 2.

Figure 4

Table 2

SEM: The SEM micrographs of control and resterilised Vypro II mesh before and after degradation are given in Figure 5. Vypro II mesh is made of multifilamentous polypropylene combined with an absorbable component made of vicryl. Significant differences were not observed between resterilised meshes and the control groups. It was observed that fibers of meshes were broken homogeneously after degradation.

The SEM micrographs of control and resterilised Ultrapro mesh before and after degradation are given in Figure 6. Ultrapro consists of polypropylene monofilaments that are closely tangled with absorbable monocryl, a copolymer of glycolide and ε-caprolactone. Similar type of homogeneously breaks were observed after degradation.

DISCUSSION

Resterilisation of biomedical materials obviously lowers in-hospital care expenses. However, resterilisation process should be reserved for unused medical devices, where the expiration time has been surpassed or which have a damaged package (11). When a mesh has been in contact with the patient tissues during the operation no piece of it should be considered for resterilisation.

Resterilisation can create two problems mainly: infection risk and violation of their mechanical and functional properties. Various allografts and prosthetic materials for dentistry, orthopedics, cardivascular surgery and general surgery were studied for the effects of resterilisation (12-14). Novel sterilisation methods that are supposed to be harmless to mechanical and functional properties of the biomaterials have also been studied (14). There are three studies on the effects of resterilisation of polypropylene meshes that is used in hernia repairs (8, 9, 15).

The only clinical study revealed that a single resterilisation of the mesh with autoclave does not increase infection and recurrence rates in inguinal hernia repair (9). However, at least one central mesh recurrence caused by mesh disruption was reported after repair with a resterilised mesh (16). Our previous laboratory study introduced that single resterilisation with autoclave or ethylene oxide was not resulted in significant changes in mechanical properties or electron microscopic features of the polypropylene meshes.

Dividing a 30x30 cm mesh into smaller pieces for hernia repairs (mostly for inguinal hernia) is an economic way especially in developing countries. Usually pure polypropylene meshes are used for Lichtenstein repair, however some better results with partially absorbable lightweight composite meshes have been reported (17). Although manufacturers strictly warn the surgeons for the use of commercial single use pre-sized materials, scientific data are not completely agree with them (8, 9). A further economic advantage can appear, if newer composite ligtweight meshes are also suitable for resterilisation like their pure polypropylene counterparts.

Pure polypropylene mesh is generally accepted as thoroughly inert and is not affected by the bodily fluids (18). However, Coda *et al* discovered that structural alterations in the size of the mesh pores can be affected by distilled water, saline, blood, as well as in vivo implantation. Prosthetic meshes are, therefore, not the inert materials they are claimed to be and can expand as well as shrink (19). Composite meshes with their absorbable parts are naturally more prone to be affected by water, saline and bodily fluids. These meshes have an absorbable part that contains hydrolytically unstable, linear, aliphatic ester bonds and are resorbed within nearly two months. This is the advantage of this kind of lightweight meshes, but also may render the material susceptible to resterilisation. For this reason, the present study included a two-month in-vitro degradation phase to observe effects of both resterilisation and saline media and and it was

observed that Vypro II mesh degraded faster than Ultrapro. Since polypropylene is rather stable, it is expected that degradation would be controlled by the chains which are polylactide and polycaprolactone for Vypro II and Ultrapro, respectively. Both of these chains can have a semicrytalline organisation, and when semicrystalline materials are immersed in aqueous media the diffusion of water and therefore degradation by hydrolysis take place in two steps. The first step is the diffusion of water in the amorphous parts where the initial and faster degradation by hydrolysis stars; and the second step is the diffusion of water to the more organized crystalline domains where slower degradation continues. The process for degradation occurs as reduction in molecular weight combined with weight loss. In some cases degradation starts from surface and causes a rapid weight loss but not affect the molecular weight, in some other cases degradation results significant decrease in molecular weight but not in total weight. These depend of the initial preparation conditions of the materials such as crystallinity, shape and molecular weight.

Synthetic absorbable suture materials have been in the market for a long time. Experimental and clinical studies in different surgical branches introduced better results in favour of absorbable sutures (20-22). Resterilisation of these suture materials has also been studied. Woods and Nagaraja found no statistically significant difference in the tensile strength after ethylene oxide resterilisation of polyglycolic acid and polyglactin sutures (23, 24). The present study also displayed similar tensile strength measurements for the two meshes before and after ethylene oxide use. In fact, the tensile strengths of the composite meshes mostly rely on nonabsorbable polypropylene part.

Ethylene oxide sterilisation can leave some residues in treated material. Ethylene oxide itself and its breakdown products (ethylene glycol, ethylene chlorohydrin, dioxane) are toxic and sterilized materials should be aired for a period of al least seven days (11). However, as mentioned above, we previously have shown that ethylene oxide resterilisation does not affect the mechanical properties of polypropylene. Hypothetically, similar results are expected for the tensile strength of the composite meshes. In this study, it was observed ethylene oxide resterilisation did not affect the meshes negatively. Furthermore, composite meshes could even displayed stronger parameters after ethylene oxide resterlisation.

The mechanical parameters of resterilised Ultrapro were stronger than non-resterilised Ultrapro during degradation process. The increase in mechanical properties and the resistance to degradation after ethylene oxide resterilisation can be explained with the formation of new intermolecular attractions and new crosslinks between chains. It was reported that degradation of semicrystalline polymers takes place in two steps. First water diffuses into the amorphous regions of the polymer matrix and breaks the ester bonds and then crystalline part becomes susceptible to hydrolytic attack. Upon collapse of the crystalline regions the polymer chain dissolves (25). Slower degradation of Vypro II is compared to Ultrapro at the one to three weeks shows its acceptability to hydrolytic degradation. On the other hand, Vypro II degradation accelerates after 3rd week and weight loss of Vypro II is much higher than that of Ultrapro at the 8th week.

It should remind at this point that lightweight meshes with large pores have shown earlier tissue incorporation and collagen deposition in animal studies (26). The tensile strength values of Ultrapro meshes have been reported to improve after experimental abdominal wall implantation at second and third months (27). An in vivo degradation by bodily fluids is developing, but the strength of the mesh is increasing because of a good tissue integration in spite of an expected decrease in its mass after two months.

It may worth to mention some previous studies on sterilisation of polygycolic acid polymers. Athanasiou and colleagues, in 1996, stated that the majority of currently available sterilisation techniques are not suitable for thermoplastic materials such as polygycolic acid polymers and it may be desirable to develop new sterilization standards (28). The studied techniques in that work were autoclaving, ethylene oxide, and gamma irradiation. Recently, Shearer et al tried to find a new sterilization method that can eliminate the potential problems such as low polymer melting point, complex architectures and hyrolytic degradation mechanisms for the damage of copolymer materials (29). They employed different sterilization techniques (30 min in ethanol solution, 2 h ultraviolet light, and 24 h in antibiotic solution. Although antibiotic solution gave the mildest results, all methods were resulted in surface damage and increase in pore sizes. However, both studies were done for polyglycolic acid scaffolds. The configurations of those scaffolds are quite different from hernia meshes. They are produced for cell culture or as drug carriers with more delicate morphologies.

Another concern about the resterilisation of the meshes was presented by Broll *et al* (15). They found that autoclave resterilisation of polypropylene mesh impaired fibroblast growth after mesh application. The investigators believed that a release of toxic substances from resterilised mesh could have this negative effect. This might be a direct result of autoclaving. Autoclave sterilization subjects the materials to high pressure steam at 121°C or more, for 15 to 20 minutes. On the other hand, ethylene oxide gas is applied to the specimens at 55°C for 4.5 hours. Its heat is less than half of autoclaving, but the duration is much longer. We think it will be useful

to set an in-vivo model for resterilised composite meshes to observe fibroblast proliferation and other components of healing and tissue integration.

According to prospectus information and previous studies, composite meshes lose their absorbable part completely within 60-70 days after implantation (30). A similar pattern was recorded in the present study. The composite mesh with polyglecaprone part lost 44% of its weight at 8th week. The other mesh with polyglactin part also lost 57% of the weight after the same duration. Ethylene oxide sterilization did not accelerate or retard the absorption process. Therefore, resterilisation seemed to be safe in respect of mass effect of these two meshes during the early phase of prosthetic hernia repair.

In conclusion, ethylene oxide resterilisation does not compromise the properties of composite meshes. These meshes can preserve their characteristics even after a degradation process. Eventual decision can be made by studying an in-vivo model. The authors have not used resterilised composite meshes in clinical setting yet. Each center should be setting for an institutional decision for the use of resterilised meshes after evaluating the medicolegal issues.

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