Influence of steam sterilization induced surface changes of polyester materials on its biocompatibility

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Abstract. Steam sterilization is conventionally used for the sterilization of materials used in surgery and medicine. When subjected to repeated steam sterilization, oligomers of polyethylene terephthalate are produced on the surface of the polyester materials. The consequences of the production of such oligomers on the biocompatibility of the polyester material have been investigated. Growth inhibition of L929 fibroblast cells has been observed on the repeatedly sterilized materials in the *in vitro* cell-material contact studies. *In vivo* implantation of the repeatedly sterilized materials elicited a hostile tissue response comprising enhanced macrophage activity, which could have an adverse effect on the long term stability of the polyester materials.

Keywords. Steam sterilization; polyester; surface; oligomer; biocompatibility.

1. Introduction

Steam sterilization by autoclaving has traditionally been the most widely used method for medical instruments. The process should be used with caution, while sterilizing polymers, as both heat and steam can drastically alter the properties of materials. The main deleterious effect arising due to steam sterilization is while the hydrolysis of the polymer takes place. Haberman and Waitzova (1985), observed the release of contaminating substances when PVC containing aqueous suspensions were autoclaved. Seemingly stable polymers can also undergo changes in surface morphology especially if the glass transition temperature of the polymers is exceeded while autoclaving. Conway *et al* (1979) in a scanning electron microscopy study of different types of arterial prostheses observed a layer of oligomers on autoclaving, which resulted in an increase of haemolysis. Studies of Nair and Srinivasan (1984) and Nair (1990) on the effect of steam sterilization of polyester fabrics used in medical applications, indicated that the cyclic trimer oligomer increased on repeated autoclaving. The present study reports the consequences of the oligomer formation on the biocompatibility of the polyester materials.

2. Materials and methods

2.1 Materials

Polyethylene terephthalate (PET) films (Garware, India) were used after soxhlet extraction of the films by methanol, water and ether to remove residual oligomers, sizing and finishing agents. The cleaned PET films were further wiped with methanol

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before packing for steam sterilization. The samples were steam sterilized by autoclaving for 15 min at 121°C, 137.9 kNm⁻¹ pressure and subjected to repeated sterilization cycles of 30 and 60 min.

2.2 Cell line

Primary cell line, L929 fibroblast was maintained at 37°C in Eagle's medium 199 (Gibco) supplemented with 5% foetal calf serum (Gibco) and 1% antibiotic-antimycotic solution in an incubator with 5% CO₂/95% air atmosphere. The cell line was detached when required using trypsin-EDTA (Gibco) in phosphate buffered saline. The detached cells were further grown as a monolayer to a cell count of 1×10^5 after 72 h.

2.3 Cytotoxicity

2.3a Indirect contact test: The cytotoxicity of aqueous extracts of the PET films after the different sterilized cycles was assessed using a 3-(4, 5 dimethyl thiazol-2-yl)-2, 5 diphenyl tetrazolium bromide, (MTT) assay (Nair *et al* 1997). The aqueous extracts of PET films were prepared by immersing 50 × 10 mm strips in 10 ml of sterile water at 80°C for 2 weeks.

MTT assay: The MTT assay was carried out according to the modified procedure for polymeric films (Nair *et al* 1997). Briefly, the extracts were used in preparing the culture medium and introduced into at least 12 wells of a 96 well tissue culture plate in which monolayer of mature L929 fibroblasts were grown. After 24 h incubation, the supernatant in each well was replaced with 50 µl of MTT (10 mg/ml in sterile phosphate buffered saline) and incubated for a further 4 h. The blue formazan product formed by the reduction of the MTT by the live cells was measured spectrophotometrically (570 nm) to assess the cytotoxicity and/or activity of the cells.

2.3b Direct contact test: Cytotoxicity was also assessed by the direct contact test. PET film strips (10 × 5 mm) were fixed onto tissue culture petri dishes by a Silastic medical grade adhesive (Dow Corning) and the growth of L929 fibroblasts in contact with films was compared microscopically to the controls.

2.4 In vivo studies

The PET films sterilized for the different cycles were intramuscularly implanted in black hooded Lister rats (Liverpool strain) for 1 month. After explantation, the tissue around the implant was immunostained (Vince *et al* 1991) using monoclonal antibodies for the macrophages (ED2) to specifically stain the macrophages. Specific stains for T cells, B cells and neutrophils were also used. The cell number and distribution were estimated using a computer aided image analysis system.

3. Results and discussion

Commercial polyethylene terephthalate (PET) is known to contain cyclic and linear oligomers as natural impurities which are formed as byproducts during polymerization

and texturing. The content of cyclic trimer, a major oligomer component, is actually a criterion for the quality of PET. Studies of Nair and Sreenivasan (1984) and Nair (1990) on the morphological changes induced in PET on repeated steam sterilization revealed the degradation of amorphous regions and increase of cyclic trimer on the surface of the PET. While the total oligomer content may vary for PET samples from different sources, the studies of Nair and Sreenivasan (1984) and Nair (1990) have shown that the surface oligomer content increases on repeated steam sterilization.

Scanning electron micrographs of the surface of the polyester films which were subjected to different cycles of steam sterilization are presented in figures 1 a–d. The bare surface of the polyester which was initially cleaned by soxhlet extraction with the solvents (methanol, water, ether) and further wiped with methanol, shows hardly any

Figure 1. a–b.

Figure 1. Scanning electron micrograph of the surface of PET film (a) bare, (b) steam sterilized for 15 min, (c) steam sterilized for 30 min and (d) steam sterilized for 60 min.

oligomers. However, when the same films were autoclaved, the oligomer content is seen to progressively increase with each cycle of steam sterilization.

3.1 *Influence of the surface oligomer on biocompatibility*

In this study, the MTT assay did not indicate significant cytotoxicity of the aqueous extracts, presumably due to the inability of water to efficiently extract the oligomers. The cells were directly grown on the films in the direct contact test. The cells which were

spindle shaped and in a uniform monolayer in the control petridish, showed differing responses to each of the materials tested. Figure 2a is the control plate consisting of a uniform layer of L929 fibroblasts. The PET sample autoclaved for the normal cycle of 15 min did not cause any changes in cell viability (figure 2b). When the cells were exposed to PET sterilized for 30 min, some of the cells especially those which were immediately on the polymer surface lost their spindle shape and became rounded, some showing lysis. Uniform destruction of the cells is not observed indicating that the oligomer formation is in localized region as is clearly seen in the SEM (figure 1c). A zone of viable and normal cells is also observed in the periphery (figure 2c). The cytotoxic nature of the oligomers is thus evident at higher concentrations of the same.

Figure 2. a-b.

Figure 2. L929 fibroblast cell culture on different samples (a) control cells, (b) on PET film autoclaved for 15 min, (c) on PET film autoclaved for 30 min and (d) on PET film autoclaved for 60 min.

3.2 *In vivo* studies

Sawyer *et al* (1979) observed that the physiological responses (such as healing, intramural thrombus formation, structure and characterization of the inner and outer fibrous capsule) of porous polymeric dacron prostheses are influenced by the final cleaning, crimping and chemical procedures. Gumarglieva *et al* (1982) also suggested that the rapid disintegration of implants under conditions of infection is due to a chemotactic mechanism involving oligomers. In this study, the tissue response to the PET samples sterilized for the various time periods was assessed by the immunohistochemical staining method (Vince *et al* 1991). No T cells, B cells or neutrophils were

Table 1. Effect of autoclaving on the tissue response of PET.

Time of autoclaving (min)	Total number of ED2 macrophages at implant site after 1 month
15	61
30	152
60	487

observed at the implant site at the end of one month. A modified avidin-biotin technique using monoclonal antibody for ED2 macrophages was used to identify and quantify the macrophages. The monoclonal antibody specifically recognizes a membrane antigen present on the tissue macrophages of the rat. Monocytes, dendritic cells, lymphocytes and granulocytes are negative for ED2. An automated image analysis system was utilized to measure the cell number, cell area and distance from the implant. The cell numbers presented as a function of distance from the implant surface is shown in figure 3. The total number of cells at the implant site of each of the PET films are given in table 1. The total number of cells was counted over a 20 frame area, corresponding to a defined location in the section. The tissue response to the PET sample autoclaved for 15 min, produced the minimum number of cells (61) at the end of one month. There is an increase in the number of macrophages (152) when the 30 min sterilized PET sample was analysed in the same conditions. The maximum number of macrophages (487) was observed at the implant site of the 60 min sterilized PET sample.

Figure 3 also depicts the distribution of cells around the vicinity of the implant site, with a distance of 0 indicating the periphery of the implant. The ED2 macrophages around the PET sample sterilized with the normal cycle (1) are lesser in number with counts of 3-5 from the periphery of the implant up to a distance of 312 μ . The area beyond 312 μ shows an absence of the ED2 macrophage counts except for a few macrophages at a distance of 624 μ from the implant site. The tissue reaction is hence comparable to that of a normal wound healing. A higher number of macrophages with counts ranging from 3-5 to 14 can be seen up to a distance of 312 μ in the case of the PET implant which was sterilized in the 30 min cycle. The area beyond 312 μ shows ED2 macrophages with a lesser count of 2. The 60 min sterilized samples seemed to elicit an even distribution with uniformly higher numbers of cells and counts ranging from 4 to 20 up to a distance of 640 μ from the implant site. The higher number of oligomers produced in the 30 min and 60 min cycles in comparison to the normal cycle may be responsible for the enhanced macrophage activity for these samples.

One of the primary roles of the macrophages is to engulf foreign debris and phagocytose them. The increased oligomers may therefore be inducing the macrophages to phagocytose and to detoxify the materials. The increased macrophage number also denotes lesser biocompatibility for the sample sterilized in the 30 min and 60 min cycles. A long term effect of such phagocytosis may adversely affect the integrity of the polyester material by causing accelerated degradation of the matrix material.

Figure 3. Distribution of ED2 macrophages at the implant site of PET sample autoclaved for (1) 15 min, (2) 30 min, and (3) 60 min.

4. Conclusions

Repeated steam sterilization of PET samples is seen to affect the biocompatibility and possible long term stability of the materials. It may therefore be desirable to avoid the repeated steam sterilization of polyester materials especially for long term implant applications, if stability of the material is a matter of concern. The study also highlights the need for the evaluation of each material specifically with relevance not only to the sterilization method but also to the period of the sterilization employed.

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