A STUDY OF TRACHEAL PROSTHESES PRODUCED BY COMPOSITE LAMINATE FABRICATION METHOD: *IN-VITRO* BIODEGRADATION AND *IN-VIVO* IMPLANTATION EXPERIMENTS

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ABSTRACT

Natural tracheal may become defective due to, among other things, abnormal narrowing or fistulas, direct or indirect result of accidents or narrowing caused by inflammation. In an acute situation when direct anastomoses (cross-connection) cannot be made, tracheal prostheses are often needed to bridge the defects. This study reports *in-vitro* biodegradation and *in-vivo* implantation studies on prosthetic tracheal composite specimens fabricated by filament winding. The tracheal prostheses fabricated with biocompatible polymeric fibre and matrix materials that were either biodegradable or non-biodegradable, were used and assessed for their performance. The study revealed that the characteristics of the tracheal prostheses were controlled by the fibre volume fraction and the proportion and combination sequences of the biodegradable and non-biodegradable components of the mixed matrix. The *in-vivo* implantation showed fairly satisfactory results in satisfying the stringent mechanical requirements imposed by nature in natural tracheas. Although relatively short survival periods were observed in the animals used, after eliminating mechanical design as a cause of failure, failure could be said to have occurred due mainly to insufficient tissue growth onto the prostheses.

Keywords: Trachea, Biocompatibility, Filament Winding, *in-vitro* Degradation, *in-vivo* Implantation, Block Copolymer.

INTRODUCTION

Trachea, also referred to as the windpipe, is an unpaired, short, almost rigid organ, surrounded by major vessels and of limited stretch ability. It is flexible enough to allow for basic motion but rigid enough to prevent collapse, even under pressure. The trachea is nearly but not quite cylindrical, being flattened posteriorly. The average adult trachea is 11 cm in length with a range of 9 to 15 cm. On the average, the outer diameter is 21-27 mm, the internal diameter measures about 2.3 cm laterally and 1.8 cm anteroposteriorly while the average cross sectional area of a male adult trachea is approximately 2.8 cm^2. (Bronchoscopy International, 2012; Griscom and Wohl, 1986). The trachea is lined by ciliated columnar epithelium, which is adherent to the tracheal wall composed of incomplete cartilaginous rings approximately two rings per centimetre anterolaterally and a membrane posteriorly. A thin layer of connective tissue separates the membranous portion of trachea from the oesophagus. (Bergman *et al.*, 2012).

A natural trachea is a complex composite structure made up primarily of collagen and elastin fibres, smooth muscles and endothelium as in most other biological tissues. The natural design of trachea makes it different from most other tissues because of the need for easy movements along both the longitudinal and hoop directions. The natural trachea is reinforced with 'ox-bow-like' rigid rings of stiff tissues which are joined at their ends by more elastic tissue to make for easy expansion and thus prevent collapse. A successfully designed artificial trachea must therefore endeavour to satisfy this fundamental mechanical design requirement imposed by nature. A report of the study of human trachea has previously been carried out (Croteau and Cook, 1961).

To make a replacement tracheal prosthesis be able to avoid collapse and still possess considerable longitudinal elastic properties, one of the easiest option that comes to mind is to incorporate a helically wound stiff fibre for additional reinforcement of the softer part (e.g. Bottema and Wildervuur, 1986).

Meanwhile, natural tracheal may become defective due to, among other things, congenital or acquired stenoses (i.e. abnormal narrowing), fistulas or abnormal passage between a hollow organ and the body surface. Also, narrowing may

be a direct or indirect result of accidents or inflammatory changes caused by inflammation (Greve *et al.*, 1988). In an acute situation when direct anastomoses cannot be made, tracheal prostheses are often needed to bridge tracheal defects. However, the major problems in the development of tracheal prosthesis are anastomotic dehiscence and stenosis, caused by poor epithelialization of the prosthetic graft (Kim *et al.*, 2004).

Up to date, substitution or entire removal of sections from natural trachea still faces considerable difficulties (Rimpler *et al.*, 1990). Attempts made at employing fresh or conserved grafts of diseased persons as substitutes for the tracheal wall failed just as alloplastic materials, glass, metal and durable plastics had (Greve *et al.*, 1988).

Tracheal reconstruction and outright replacement with either a synthetic and/or natural materials have for long engaged the interest of researchers around the world as it remains a serious challenge in healthcare delivery (Delaere, *et al.*, 2001; Delaere and Hardillo, 2003). Reconstruction of the patient's own tracheobronchial tissue by direct end to end anastomosis showed that it was possible to reconstruct the trachea after resection of up to 6.4 cm of its length (Grillo, 1988, 1989; Grillo *et al.*, 1964). This involved complex surgical manoeuvres and no non-tracheal material was used for the reconstruction. However, reconstruction of extensive circumferential tracheal defects larger than 6.4 cm remained a serious challenging problem in tracheal surgery (Kim *et al.*, 2004).

Different types of prosthetic and tissue grafts have been used in attempts to repair tracheal defects but with limited success due to chronic infections, which stimulate granular tissue formation, anastomotic disruption and erosion of major blood vessels (Neville *et al.*, 1990; Cull *et al.*, 1990; Suh *et al.*, 2000; Scherer *et al.*, 1986; Papp *et al.*, 1985; Messineo *et al.*, 1991; Cohen *et al.*, 1986; Lobe *et al.*, 1991; Fleischer *et al.*, 1989; Eliachar *et al.*, 1984; Rimpler *et al.*, 1990; Nakayama, 1990; Letang *et al.*, 1990). Several of these, including reconstruction by allotransplantation (Spinazzola *et al.*, 1969; Balderman and Weinblatt, 1987; Zalzal *et al.*, 1989; Moriyama *et al.*, 1989) also failed due to rejection; even though only patches in the tracheal

wall were replaced as they did not involve circumferential replacement.

Analysis of experimental data and experiences that have accumulated during testing of prospective materials for prosthetic use at the trachea had found that ideal prosthesis for trachea repair should possess specific requirements (Scherer *et al.*, 1986; Greve, 1988; Wildeuvuur *et al.*, 1984; Matsubara et al., 1990). These include airtightness to prevent infection from contaminated air, compatibility with the surrounding living tissue and geometrically stable with simultaneous flexibility for sufficient rigidity to prevent collapse during inspiration and easy movement of the neck while always maintaining a satisfactory lumen. The ability to prevent fibroblast proliferation which could cause obstruction and quick overgrowth of respiratory epithelium to line the luminal surface are critical. They must be non-toxic, non-carcinogenic and readily available (Scherer *et al.*, 1986).

The most important requirements for materials to be used for tracheal reconstruction or entire replacement are biocompatibility with the host tissues, mechanical stability and non-toxicity. A typical non-porous material that has been relatively used successfully in tracheal reconstruction and replacement is silicone, developed and intensively studied by Neville and co-workers and other investigators (Neville, 1982; Neville and Bolanowski, 1976; Neville *et al.*, 1990, 1992; Matsubara *et al.*, 1990).

Incorporation of porous material in natural tissue with the advantages of granulation tissue and epithelial in-growth for better incorporation in the host tissue have been found to be firmly invaded by the host tissue around the implant (Merritt *et al.*, 1979). However, incidence of infection in the presence of a porous implant was found to be greater than in the presence of the implant if the bacteria are present before the implant was invaded by host tissue.

A prosthesis made of a porous, rigid cylinder of titanium fibres, coated with polymer that provides an initial air seal in which the polymer later absorbed to permit subsequent fibrous tissue in-growth and epithelisation was reported with different polymers (Mendak *et al.*, 1984). The tracheal prosthesis, fabricated from Marlex mesh

(Hayashi, 1991) and reinforced with a continuous Teflon spiral, was grafted and coated with collagen to promote connective tissue infiltration and provided air-tightness (Okumura *et al.*, 1991). Another type is continuous polypropylene spiral, grafted and coated with porcine collagen to increase its biocompatibility and provide an airtight seal (Okumura *et al.*, 1993).

A biodegradable tracheal prosthesis, consisting of hydroxyapatite rings as artificial tracheal cartilage and a carbon fibre tube coated with collagen (Takahama *et al.*, 1989) and collagen-coated poly(L-lactic acid) mesh, have also been reported (Ike *et al.*, 1991). Polytetrafluoroethylene has also been extensively studied as a material of choice for tracheal prosthesis. Thin-walled venous (Gore-Tex) prosthesis, reinforced with rings (Trojan *et al.*, 1985), incorporating a microporous Teflon tracheal prosthesis in rabbits (Bottema *et al.*, 1986), ringed PTFE graft for dog's tracheal reconstruction (Shaha *et al.*, 1988) and expanded PTFE prosthesis reinforced with a continuous silicone spiral, was implanted in the rabbits (Jorge *et al.*, 1990). Tissue-engineering techniques have also been used in the construction of a mucosal prosthetic lining from skin cells for its usefulness in tracheal replacement (Kim *et al.*, 2004; Nakamura *et al.*, 2009).

Dacron cloth mesh is another material that has been investigated for use in porous tracheal prosthesis. Dacron cloth mesh stiffened with heat-curing polyetherurethane coated and uncoated with Biolite™ carbon were compared for dogs tracheal reconstruction (Leake *et al.*, 1985). The presence of the carbon coating, which conferred the chemical characteristics of the carbon without altering the mechanical properties of the implant, enhanced biocompatibility with a consequent decrease in connective tissue. However, granulation tissue encompassed the prosthesis and the lumen was narrowed.

Polyurethane prostheses have also been of interest to investigators in attempts to find acceptable solution to tracheal reconstruction and replacement (Nelson *et al.*, 1979). A two-layered prosthesis was developed in which the inner layer was to prevent penetration of bacteria through the prosthesis wall and the outer layer thereby favouring incorporation of the connective tissue for the fixation of the prosthesis within the surrounding tissue (Planck *et al.*, 1985).

In spite of a multitude of options and the availability of very different materials for tracheal reconstruction and replacement, none of the reviewed methods were successfully introduced into the clinical routine (Rickert, 2009). However, one of the commercially available tracheal prosthesis is that made of Silastic®, a non-biodegradable silicone rubber with Dacron® cuffs on the ends to permit suturing and fibroblast growth (Neville *et al.*, 1992; Spinazzola *et al.*, 1969) though with limited success.

The arterial prosthesis fabrication by filament winding, which was previously described (Gershon *et al.*, 1990), has been adopted in this study for the fabrication of tracheal prostheses for *in-vitro* biodegradation and *in-vivo* implantation experiments. Tubes were fabricated from medical grade polymer matrices and fibres.

This paper therefore reports fundamental *in-vitro* biodegradation and *in-vivo* implantation study of a tracheal prosthetic device fabricated by filament winding with biocompatible polymeric fibres and either biodegradable or non-biodegradable matrices. This is with a view to understanding their applicability for complete replacement in human where neither repair nor reconstruction of the trachea could be carried out.

MATERIALS AND METHODS
The *in-vitro* degradation study was carried out in a Harmann's solution (obtained from the Hadassah Medical School, En-Kereim, Jerusalem), a physiological fluid, for each of the tracheal prosthesis with the container maintained in a deionised water bath at 37 C - 38 C for periods of up to 60 days.

List of Materials and Manufacturers
i.   A non-biodegradable T336/44Dtex Lycra® elastomeric yarn (Dupont, USA);
ii. A non-biodegradable polyurethane 2363-80A also referred to as Pellethane supplied by Dow Chemical in a granular form;
iii. Polyethylene oxide (PEO) and Polylactic acid (Aldrich, Germany);
iv. PELA block biodegradable copolymer produced in-house;
v.   250Dtex Dacron®, a polyester yarn (Dupont, USA).

vi. Hytrel® 4056 fibre (Israel Fibre Institute, Jerusalem).

vii. Nylon cord (0.6 mm, 1.00 mm).

viii. Polypropylene fibre (1.00 mm)

ix. 300mg/kg Intra-venous Nembutal®.

Preparation of Pellethane Matrix

The Pellethane matrix was prepared in batches by dissolving 20 g of the granule in Dimethylformamide (DMF) for about 3-4 hrs per batch. The DMF was extracted by washing in Methanol for between two and four times followed by a thorough washing in water. The dissolved Pellethane was dried for a minimum of 24 hrs at the temperature of between 50 % and 60 C. The prepared Pellethane was cast into a thin film by dissolving in 50 ml of Tetrahydroforan (THF) and allowed to evaporate in a glass Petri-dish. The films were then kept in a desiccator for a minimum of 3 days before use.

Polyethylene Lactic Acid (PELA) biodegradable block copolymer matrix materials were synthesized through polyesterification reaction of lactic acid in the presence of PEO chains covering 1500 and 3400 molecular weights using an antimony trioxide Sb_2O_3 catalyst and phosphoric acid (as detailed in Younes and Cohn, 1987; Cohn and Younes, 1988; Younes *et al.*, 1988; Cohn and Younes, 1989). The required amount of PELA for each matrix concentration was then dissolved in THF for 1-2 hours.

Preparation of Prostheses for Implantation

A typical tracheal prosthesis for implantation was prepared by taking the following parameters into consideration: the desired thickness, the Lycra fibre weight fraction or concentration, the diameter of the prosthesis which is determined by the mandrel diameter, the proportions of the biodegradable and non-biodegradable matrix materials, the winding angle and the concentration of the matrix. The required amount of matrix materials and the number of revolutions of the Lycra fibre to be wound, which would make up the weight of the fibre in the composite, were then computed.

The volume of the composite was estimated from the thickness, diameter of the mandrel and fibre weight fraction. The weight of the composite, volume of the matrix materials and the volume of the solution required for mixing the matrix material were calculated from fundamental definitions. The specific number of revolutions of the Lycra fibre, which would be equivalent to the weight of fibre based on the winding angle, was wound and weighed accurately. The appropriate equations were then manipulated based on the required arrangement of the biodegradable and non-biodegradable matrix or their mixture for each specimen.

Specimens were prepared by winding Lycra® fibres of the required diameter at specific angles with the combination of the biodegradable (PELA 1500 or 3400) and non-biodegradable (Pellethane) as the matrix and Dacron®, polypropylene or nylon fibre for hoop (or helical) reinforcement.

Experimental Procedure

In-vitro Biodegradation Studies

7.00 mm diameter specimens were prepared with double tow Dacron® yarn for helical reinforcement and used for this study. The degradation rate, measured by the loss in the total weight of the tracheal prosthesis over time, the effect of Lycra fibre concentration and Pellethane masking (or shielding) were investigated. Three types of specimens were studied for the different concentrations:

1. Pellethane-PELA mixture;

2. PELA-Pellethane-PELA interlayer (i.e. Skin of PELA 'shielding' or 'masking' Pellethane; also referred to as 'Pellethane sandwiched by PELA'); and

3. Pellethane-PELA-Pellethane interlayer (i.e. Skin of Pellethane 'shielding' or 'masking' PELA; also referred to as 'PELA sandwiched by Pellethane').

Three Lycra fibre weight fractions or concentrations of 25 %, 40 % and 60% constituting 60% of the total weight of the prosthesis at the winding angle of 45 were considered. Extra layers of the Lycra fibres were wound on the Dacron fibre helical reinforcement while the matrix layer immediately after the helical reinforcement was also doubled for structural stability. The matrix material was a combination of the non-biodegradable Pellethane and biodegradable PELA in the ratio of either 20:80 or 50:50 respectively making up 40% of the total weight of each s.

The biodegradation study was carried out by noting the weight of the specimen before and after immersion in the physiological fluid for up to 60 days. The specimens were removed after the desired length of time, wiped dry, kept in an oven at 50 C for about 24 hrs and weighed again. The weight loss of the prosthesis, which is equivalent to the weight of the degraded component of the mixed matrix in the composite (i.e. PELA 1500 or 3400), was recorded.

The 'shielding' effect of either Pellethane or PELA on the biodegradation of the tracheal prosthesis for each fibre weight fraction of the three matrix combinations was studied for the specimens prepared with 20% Pellethane and 80% PELA matrix. The effect of molecular weight of PELA on the degradation of the prostheses was also investigated by comparing the biodegradation of PELA 1500 to that of PELA 3400.

In-vivo Studies

16 mm diameter trachea prosthesis specimens for the *in-vivo* investigation were prepared with Lycra® fibre of volume fraction 30 % wound at an angle of 45 and Hytrel® non-biodegradable fibre made into a tow of 10 or nylon cord or polypropylene fibre for helical reinforcement (Table 1). The matrix was 80 % biodegradable PELA 3400 mixed with 20% Pellethane non-biodegradable component. The implantation of the prostheses was carried out in seven matured dogs of average weight of 8 kg.

Table 1: The Basic Materials (Fibres and Matrices) Combinations and Input Parameters for each of the Seven Tracheal Prostheses used in the Fabrication by Filament Winding.

Dog #	The Lycra fibre	W_f (%)	W_m (%)	Reinforcement	Matrix PELA/Pellethane
1		30	70	Hytrel® (1.0 mm)	80/20
2		30	70	Nylon (1.0 mm)	Inner 3 layer 80/20 Outer 3 layers 50/50
3		30	70	Nylon (0.6 mm)	Inner 3 layers 80/20 Outer 3 layer 50/50
4	T336/44DTex	30	70	Nylon (0.6 mm)	Inner 3 layer 80/20 Outer 3 layers 50/50
5		30	70	Polypropylene 1.00 mm	80/20
6		30	70	Polypropylene 1.00 mm	80/20
7		30	70	Polypropylene 1.00 mm	60/40

Prior to implantation, each tracheal prosthesis was sterilized with ethylene oxide at a temperature of between 50 C and 60 C in vacuum where the whole process took about 5 hrs and then left for one week. The prostheses looked 'tougher' after the sterilization procedure. The anaesthesia used was 300 mg/kg IV Nembutal®.

The surgical protocol conducted by the surgical team included:
  i) a midline incision was made in neck which exposed most of the cervical tracheal;
  ii) a segment of trachea was excised and anastomosis was carried out with continuous suture of monofilament 4/0 polypropylene ('Prolene®') and was alternately interrupted with stitches of 4/0 polyglactin, 'Vicryl' in some of the dogs; and
  iii) closure of incisions in layers.

This procedure was followed for all the seven dogs. The basic clinical examinations were carried out on each of the implanted prosthesis after removal.

RESULTS AND DISCUSSION
In-vitro Degradation Study
Fig. 1(a - e) show the percentage of matrix degraded from the trachea specimens for fibre weight fractions of 25 %, 40 % and 60 % when the biodegradable (PELA 3400) and non-biodegradable (Pellethane) components of the matrix was mixed (Fig. (1a)), Pellethane sandwiched by PELA (Fig. 1(b)), PELA sandwiched by Pellethane (Fig. 1(c)), mixed PELA 1500-Pellethane for fibre weight fractions of 25 % and 60 % (Fig. 1(d)) and PELA 1500-Pellethane interlayers at the Lycra fibre volume fraction of 40 % (Fig. (e)). The figures show the effect of Lycra fibre concentration on the percentage

degradation of the prostheses.

Fig. 1(f) relates the matrix degradation rate to Lycra fibre concentration, a summary of the data presented in Fig. 1(a e). It was observed that degradation rate, which increased with fibre concentration when Pellethane sandwiched PELA, was the inverse of the anticipated shielding effect of the non-biodegradable on the biodegradable component.

In fact, the result suggests that increase in the Lycra fibre concentration encouraged degradation acceleration. It was also noted that when PELA sandwiched Pellethane, matrix degradation rate increased only between 25 % and 40 % followed by a marked decrease above 40 %. It was only when the matrix components were mixed that the degradation rate of the specimen progressively decreased with increase in Lycra fibre concentration. A decrease of about one third was observed as the fibre concentration increased from 25 % to 40 % and 60 %.
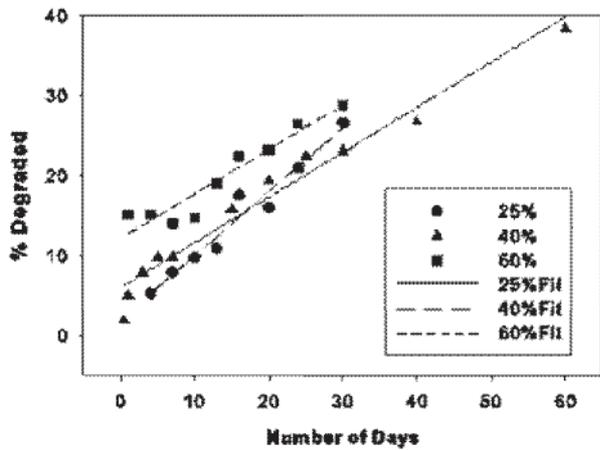
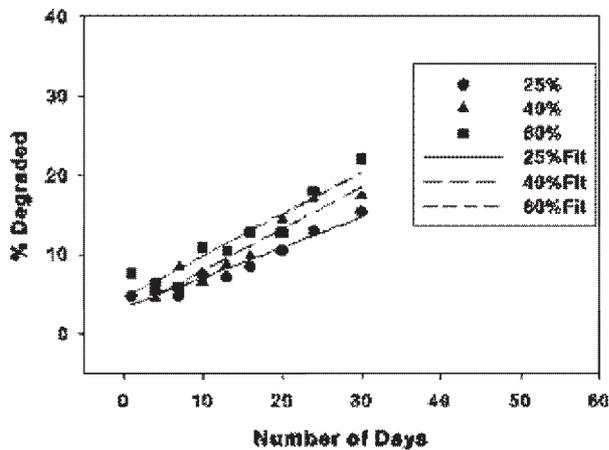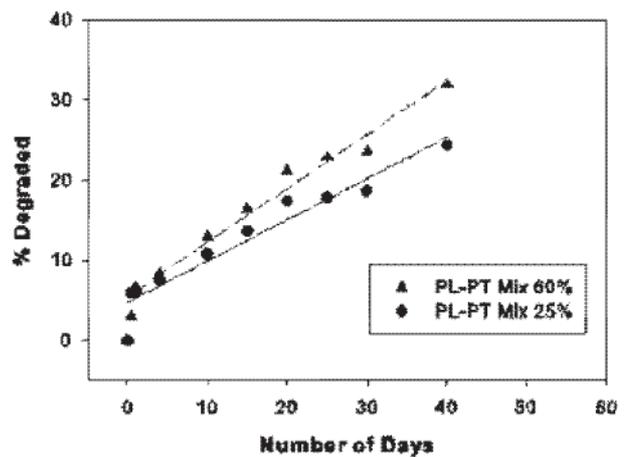
Figure 1(a)
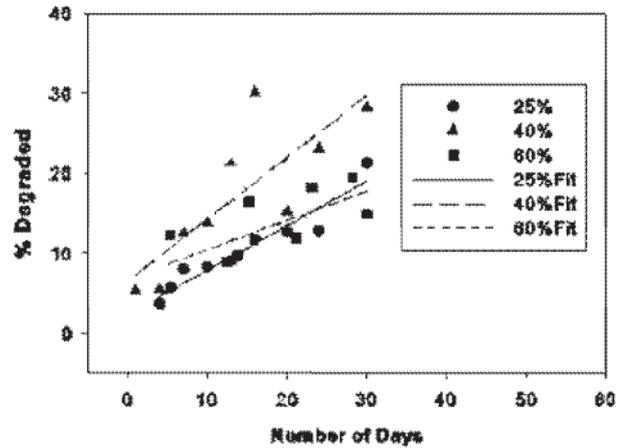
Figure 1(b)

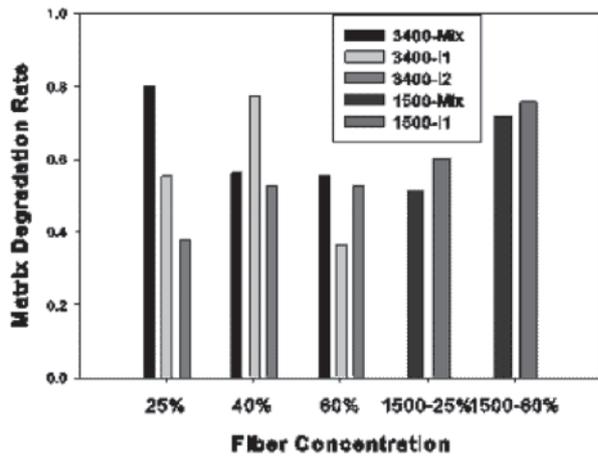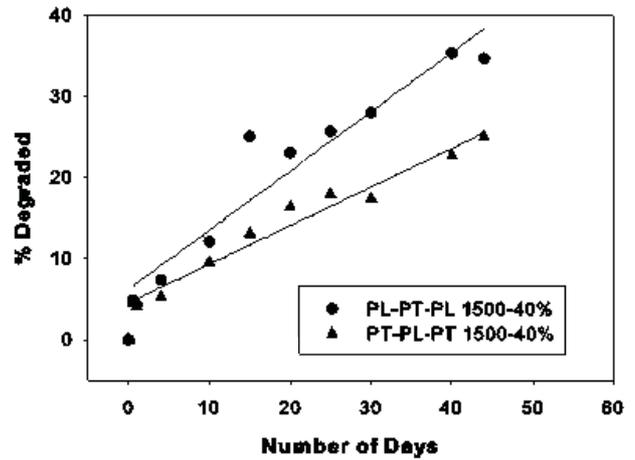
Figure 1(c)

Figure 1(d)

Figure 1(f)

Figure 1(e)

Figure 1: Percentage Of Matrix Degraded Versus Number of Exposure Days in Physiological Solution for PELA 3400 and the Lycra Fibre Volume Contents of 25%, 40% and 60% for:
(a) PELA-Pellethane Mix; (b) PELA sandwiched Pellethane; (c) Pellethane sandwich by PELA.
(d) The biodegradable component is PELA 1500 and the Lycra fibre volume contents are 25% and 60% mixed together; (e) The biodegradable component is PELA 1500 and the Lycra fibre volume contents are 40% with interlayers of PELA and Pellethane. (f) Matrix degradation rate (% degraded/time) versus the fibre concentration for different matrix combination.

The findings also showed that PELA 1500, whether the matrix components were mixed or interlaid, the degradation rate increased with fibre concentration (Fig. 1(f)).

Fig. 2(a - d) relate the percentage degradation of the prosthesis to the number of days of immersion in the physiological fluid showing the effect of matrix combination sequence on degradation for Lycra fibre concentrations of 25 % (Fig. 2(a)), 40 % (Fig. 2(b)) and 60 % (Fig. 2(c)) respectively. The comparative degradation rates for the different Lycra fibre concentrations are as shown in Fig. 2(d) for PELA 3400. It can be observed that when PELA sandwiched Pellethane, there was a sharp increase in the degradation rate as the fibre concentration increased from 25% to 40% followed by another equally sharp decrease as the concentration increased to 60 %.
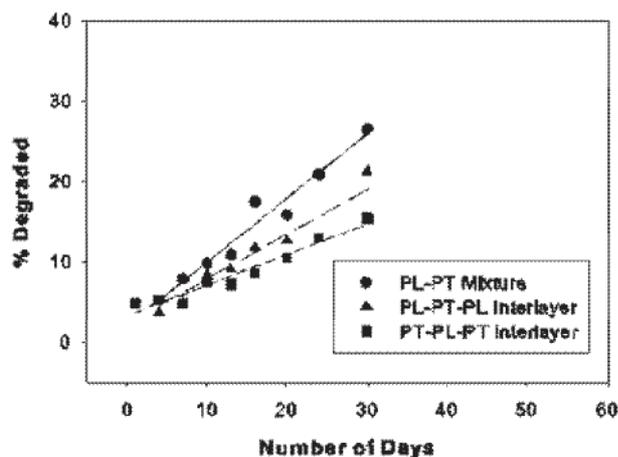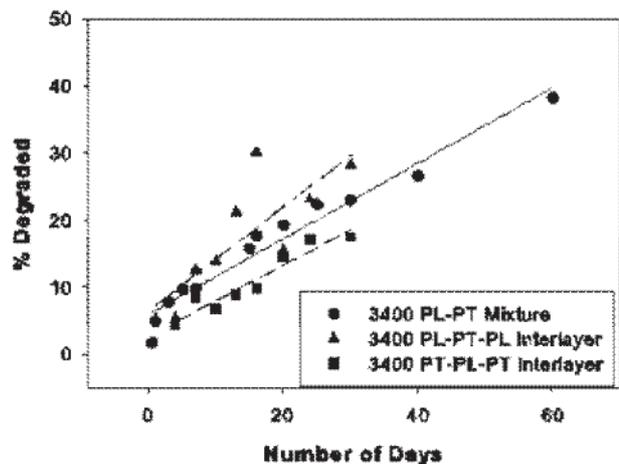

Figure 2(a):

Figure 2(b):

This trend was also observed when Pellethane sandwiched PELA, but the degradation rate increase was not as sharp. It was only when the PELA and Pellethane matrix components were mixed that degradation rates decreased with fibre concentration (Fig. 2(d)).
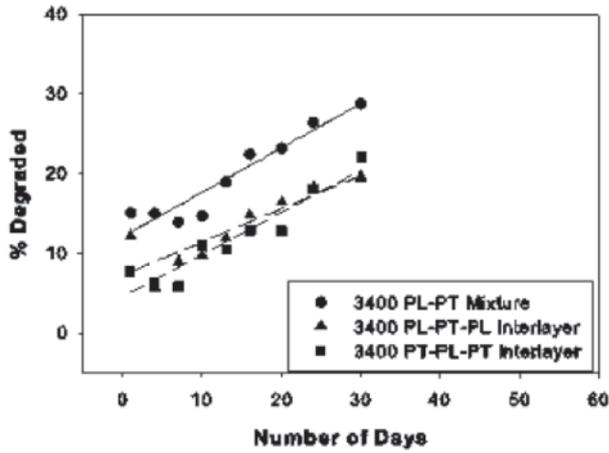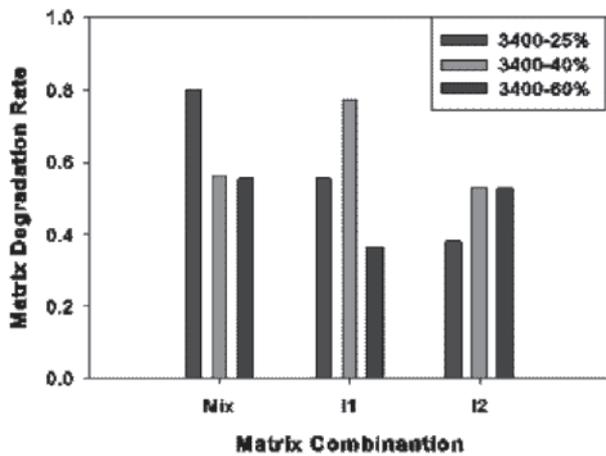

Figure 2(c):

Figure 2(d):

Figure 2: Percentage of Matrix Degraded Versus Number of Immersion Days in Physiological Fluid for Mixing the Biodegradable (PELA) and Non-Biodegradable (Pellethane), PELA Sandwiched Pellethane and Pellethane Sandwiched PELA for Lycra Volume Fraction of: (a) 25%; (b) 40% and (c) 60%. (d) Matrix Degradation Rate against the Matrix Combination Sequence at Different Fibre Concentration.

It was found that the highest specimen degradation rates occurred when the matrix components were mixed at 25 % and Pellethane was sandwiched by PELA at 40 %. It also showed that the lowest degradation rates were obtained when Pellethane was encased by skins of the PELA at the fibre concentration of 60% and when PELA was encased by Pellethane at 25% fibre concentration.

These observations suggest that a significant masking contribution of the non-biodegradable component was effective only when the two materials were segregated, compared to an intimate mixture of the two components (Fig. 2(d)).

Fig 3(a - d) are plots that show the effect of molecular weight of the biodegradable component of the mixed matrix on biodegradation of the prosthesis. The biodegradation of PELA 1500 and PELA 3400 were compared for the three matrix mixing sequences for fibre concentration of 25 % (Fig. 3(a)), 40 % (Fig. 3(b)) and 60 % (Fig. 3(c)) with the summary of the degradation rates shown in Fig. 3(d). It was observed that the specimen degradation rates for the matrix mixture in PELA 1500 at 25 % fibre concentration were lower but was on the increase whereas the opposite trend was observed for PELA 3400 (Fig. 3(d)).
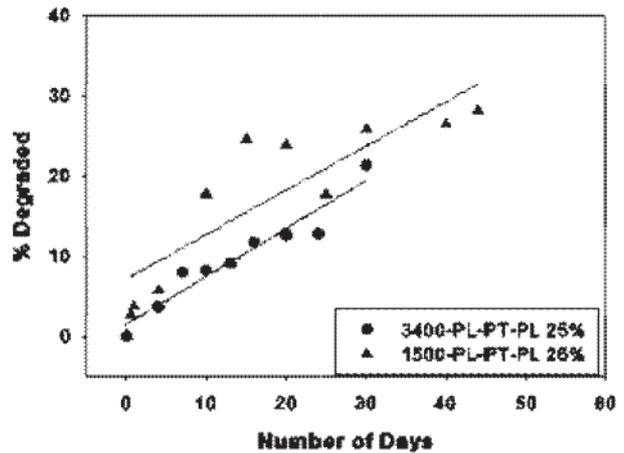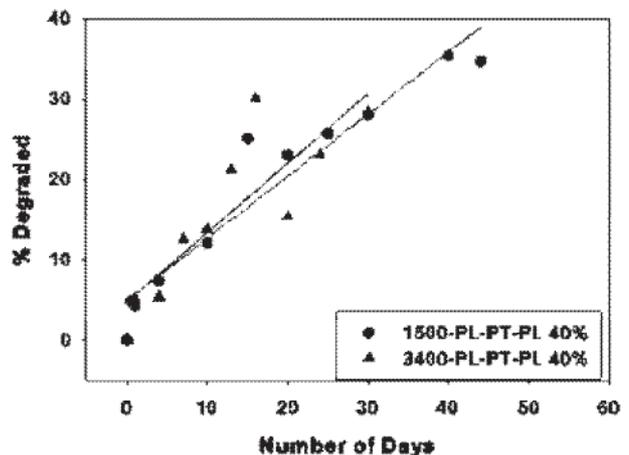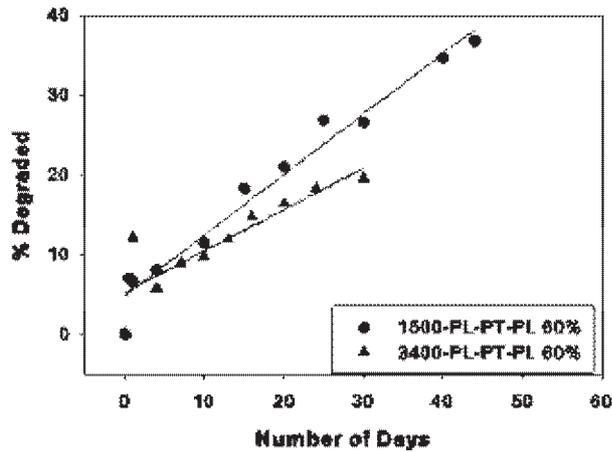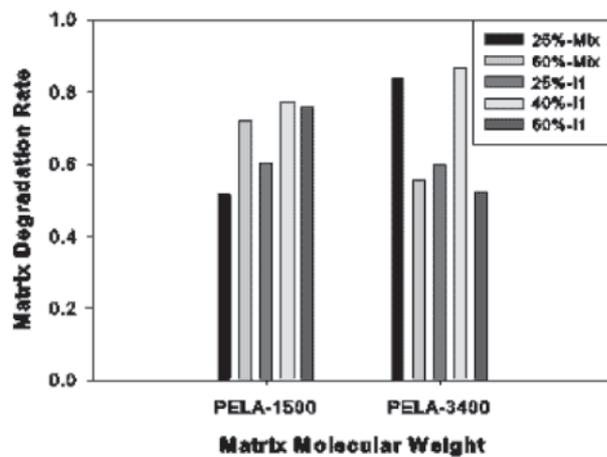

Figure 3(a)

Figure 3(b)

Figure 3(c)

Figure 3(d)

Figure 3: Matrix Degradation of PELA 3400 Compared with PELA 1500 when the Non-Biodegradable Component Pellethane is Sandwiched by the Biodegradable PELA for Lycra Fibre Weight Fraction of: (a) 25% (b) 40% and (c) 60%.

3(d) Matrix biodegradation rate of PELA3400 compared to that of PELA1500.

It was generally observed that the characteristic of the matrix, and in turn, of the biodegradation rate of the tracheal prosthesis, was controlled by the fibre volume fraction and the combination sequence of the biodegradable and non-biodegradable component of the matrix. These determined the level of shielding of the biodegradable component whose response depended on the chemical structure. The implication of the reduced degradation rate when PELA 3400 skin 'shielded' the non-biodegradable Pellethane is that there would be more time for the expected tissue in-growth on the Pellethane, which is expected to will give the structural support to the

trachea.

By varying the fibre concentration, we can also directly vary the pore size distribution as well as the effectiveness of the 'masking' or 'shielding' of the matrix by the network of the fibres. The effect of the presence of a non-biodegradable component in the matrix on the weight loss due to hydrolysis is equally important. This implies that during biodegradation, hydrolysed biodegradable molecules remove with them a non-biodegradable mass to which they are chemically linked in the mixture. The resulting porosity of the structure is an important phenomenon that is expected to depend on the proportion of the biodegradable component, the microstructure (Madsen and Lilholt, 2003) and the extent of phase separation of the matrix components.

The degradation results can be explained on the basis of an anisotropic diffusion mechanism typical of composite materials, where different diffusion coefficients exist in the fibre (longitudinal) and in the perpendicular (transverse) directions. If the level of anisotropy is high, and the longitudinal coefficient is much higher compared to the transverse, as for example in Aramid fibre-reinforced composites, the rate of water penetration will increase with increasing the fibre content (Aronhime *et al.*, 1986). Thus, at a given moment, for higher fibre content a higher water concentration in the composite will be available for the hydrolysis.

In-vivo Studies

Based on the *in-vitro* biodegradation studies, seven tracheal prostheses were prepared for *in-vivo* investigation with PELA 3400 as the biodegradable component in the mixed matrix configuration as shown in Table 1. The basic information on the surgical implantation in the seven dogs is as shown in Table 2.

Noting the dependence of the Lycra fibre concentration on the biodegradation of the specimen, specimens with 30 % fibre concentration, winding angle of 45 and mandrel diameter of 14 mm were fabricated for implantation. The biodegradable and non-biodegradable matrix combination were put together either in the ratio 80:20 or 50:50.

All the seven dogs recovered well from the

operation at first, but later some of them developed stridor (Wykoff, 1973). At that stage, they were put to death by an Intra-venous (IV) overdose of anaesthesia, to save them from further discomfort. Table 2 shows the details of the number of survival days for the dogs. They were autopsied to recover both the prosthesis and the subcutaneous implant. On those that lived long enough (e.g. dogs #3 and #6), a fibre-optics bronchoscopy was performed under general anaesthesia. An overall impression of the implantation operations is as presented in Table 3.

Table 2: The Implantation Basic Information and Result.

		Basic Information			
Dog	**Weight (kg)**	**Excited Segment (mm)**	**Prosthesis Length (mm)**	**Int. D. of Prosthesis (mm)**	**Survival (Days)**
#1	8	38	38	11	10
#2	8	37	35	11	8
#3	10	36	35	16	39
#4	8	33	35	14	17
#5	8	35	40	11-12	7
#6	12	42	42	14	29
#7	6	39	41	11-12	16

The post mortem findings showed that in dogs #1 and #2, the prosthesis had lost all stiffness and was collapsed like in tracheomalacia. In dogs #3 and #6, the prosthesis had maintained its shape but there were strictures at the anastomosis. There was no in-growth of tissue into any of the prostheses and it was difficult to say if there was growth of neointimal on the surface of the prostheses.

Table 3: Clinical Examination Result of all Seven Dogs.

	Variations of operation			**Bronchoscopy findings**			
Dog #	**Suture of anastomosis**	**Mucosa drawn into prosthesis**	**Prosthesis covered with strap muscles**	**Days of post operation**	**Stricture**	**Cover of prosthesis**	**Purulent secretions**
#1	Continuous Prolene	---	No	--	---		
#2	Continuous Prolene	---	No	--	---		
#3	Continuous Prolene	---	No	15 38	Yes		Yes
#4	Continuous Prolene	---	No	15	Yes		Yes
#5	Interrupted vicryl	---	No	---	---		---
#6	Interrupted vicryl	---	No	15	---		Yes
#7	Interrupted vicryl	---	No	---	---		---

The main cause of the relatively short periods of potency of the prostheses (Wykoff, 1973) was stenosis formation at the anastomosis which resulted from unrestrained growth of granulation tissue even in anastomotic sites that became covered by epithelium. (Okumura *et al.,* 1991; Kim *et al.*, 2004;) The growth of the granulation tissue could not be controlled by drawing mucosa into the prosthesis, and it was insensitive to the type of anastomosis and to the suturing procedure (either continuous or interrupted). Although the reason for the uncontrolled growth of granulation tissue at the anastomosis was not identified, it appeared that the process could not be associated directly with the prosthesis itself.

CONCLUSIONS

The filament winding technique has been successfully adopted in the fabrication of tracheal prostheses in which *in-vitro* biodegradation and *in-vivo* implantation experiments were carried out. The search for acceptable prostheses for complete replacement of circumferential tracheal defects larger than 6.4 cm

remains a major challenge (Kim *et al.*, 2004). However, this study has made a major contribution in applying one of the traditional engineering composite laminate structures fabrication techniques commonly used for large structures to this 'soft structure'.

The study has shown that degradation of PELA 1500 and PELA 3400 generally increased with Lycra fibre concentration. At fixed fibre concentrations, PELA degraded fastest when mixed with the Pellethane non-biodegradable component of the matrix at the fibre concentration of 25 %. In all specimens investigated, more than 60% of the original weight of PELA was still retained in 60 days.

The material combination, namely, a composite material PELA-Pellethane matrix reinforced by Lycra fibres and a Dacron helical cord, could be designed to match mechanical and biodegradability specifications and to induce, in some cases, tissue in-growth and epithelium coverage.

The *in-vivo* implantation investigation of the tracheal prostheses showed fairly satisfactory initial results in satisfying the stringent mechanical property requirements of a natural trachea. Though they resulted in relatively short survival periods, after eliminating mechanical design as a cause of failure, failure could be said to have occurred due to insufficient tissue growth onto the prostheses. As a result, a more detailed and comprehensive research program aimed at improving on these initial encouraging findings reported in this study is desirable for best biodegradation rates, hence more durable and long-lasting tracheal prostheses. The filament winding technique has further been as a manufacturing process of choice for durable and long-lasting tracheal prostheses.

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