

genic response and rejection since the materials used are mostly foreign, and that the behaviour of cells in patients with vascular disease is not known. An approach which may prove attractive is to use the body as a bioreactor to engineer a vascular graft by growing tubes of tissue subcutaneously. Based on earlier works (Elken, 1960; Schilling *et al.*, 1964; Sparks, 1969, 1973), Campbell *et al.* (1999) placed supporting silastic tubings into the peritoneal cavity of rats and rabbits to form granulation tissue capsule of multiple layers of myofibroblasts, collagen matrix and single layer of mesothelial cells which could be everted and used as grafts. They later showed that the tubes need not be everted and yet have a burst strength of greater than 2500 mmHg (Chue *et al.*, 2004). This approach could possibly ensure no tissue rejection and should limit graft complications if the vessels can be successfully grown in patients prior to bypass grafting. Theoretically, one or more vessels of the required diameters and lengths can be grown at the same or different times. Provided that improved biocompatibility, adequate and stable mechanical properties, vascular reactivity and non-thrombogenic surface can be demonstrated in humans, grafts developed this way could be useful as bypass grafts or arteriovenous fistula grafts for haemodialysis vascular access. However, as in all approaches, the impending issues are the length of culture time required to produce a tissue-engineered vascular graft (weeks to months), its vascularisation and vasoactivity. Furthermore, even if a graft with good vascular properties can be produced, how it will perform in the patients is still not known. The fact that suture/anastomosis is required, there may still be issues inductive to thrombosis, atherosclerosis and IH, e.g. compliance matching, confluent functional endothelium, injury, etc. as observed in the currently used vascular grafts. Nevertheless, with more understanding and progress made in tissue engineering, the possibility of a tissue-engineered alternative vascular graft may not be too far from being realised.

8.6 Abdominal aortic aneurysm

An aneurysm is a localised dilatation of an artery with an increase in diameter of at least 50% greater than that of the unaffected artery segment. The majority of aneurysms occur in the infra-renal aorta, generally referred to as abdominal aortic aneurysms (AAA) and are found mainly in men over the age of 55 years (O'Kelly and Heather, 1989; Collin *et al.*, 1988; Singh *et al.*, 2001; Wanhainen *et al.*, 2001). The reported increased prevalence of AAA may be due to a real rise in incidence as well as improved detection of asymptomatic aneurysms on imaging performed for other reasons (Fowkes *et al.*, 1989). AAAs gradually enlarge at rates that vary between 0.2 and 0.7 cm in diameter per annum (Collin *et al.*, 1989; Cronenwett *et al.*, 1990). If left untreated (and if the patient does not die of an unrelated

condition), the AAA will eventually rupture. The risk of rupture is related to diameter and it is estimated that 2% of aneurysms with diameter less than 4 cm and 22% of aneurysms greater than 5 cm will rupture within 4 years (Hallin *et al.*, 2001).

Surgical repair is effective in preventing a rupture but is a complex and major operation and a proportion of the patients are deemed unfit for surgery. In open surgery, the aneurysm is incised, the thrombus is removed and a prosthetic tube graft is securely attached by a hand-sewn anastomosis. The aneurysm sac is then placed around the graft to prevent the bowel from adhering to the anastomotic areas. If a normal segment of aorta is unavailable, a bifurcated graft will be used to extend the bypass to common iliac or femoral arteries. Surgical repair of intact aortic aneurysms is associated with an average post-operative death rate of approximately 7% in the UK.

8.7 Endovascular aortic aneurysm repair

In endovascular aneurysm repair (EVAR) the aneurysm is excluded from the circulation to prevent rupture while the aneurysm sac and its contents remain *in situ*. This is achieved by means of a device consisting of a prosthetic tube or bifurcated graft, a metal stent support (stent-graft) and a delivery system into which the stent-graft is folded and packed. The metal skeleton consists of several wire structures (stent struts) and acts as a spring, providing a radial force in an outward direction helping to fix the device in place. Vertical support (columnar strength) is provided by the relatively rigid interconnection between adjacent stent segments. Fixation may be augmented by the addition of hooks or barbs either to the metal skeleton or the fabric component at the proximal sealing zone. The fabric and skeletal components of a stent-graft are bonded together by a variety of means such as sutures, tapes or even by 'sandwiching' the metal within a 'double skin' graft. The device is introduced through one of the femoral arteries and guided to the aneurysm where it is anchored in place. The procedure is performed under X-ray fluoroscopy. The function of the delivery system is to protect and constrain the stent-graft during deployment. Ideally it should be flexible enough to traverse tortuous blood vessels but rigid enough to resist kinking and damage during its passage. It should add as little as possible to the total thickness of the device. The delivery system also contains mechanisms that minimise blood loss during the procedure.

The ability of a stent-graft to effectively exclude an aneurysm from the circulation depends predominantly on two factors, namely, seal and fixation. The term seal refers to the ability of a stent-graft to prevent blood from entering the aneurysm sac and fixation refers to the ability of the stent-graft to stay anchored at the site of original implantation without slippage. Seal immediately after stent-graft deployment is obtained by close apposition of

the stent-graft fabric against the internal surface of the aorta at the areas of the neck or 'sealing-zones'. The fabric tube itself forms the blood flow channel.

8.7.1 Stent-graft design and selection

Although most open AAA repair can be performed using a tube graft, it is usually not feasible to use a tubular stent-graft in EVAR since infrarenal aneurysms rarely contain a 'distal neck' required for the distal seal within the aorta. This problem can be overcome by sealing within one or both iliac arteries. The first option, sealing within a single iliac artery is achieved by using a tapered aortouniiliac stent-graft to bridge between the infrarenal aortic neck and the iliac artery. This requires the addition of a 'plug' (occluder) to seal the opposite common iliac artery (to prevent back bleeding into the aneurysm sac). It also requires the addition of a prosthetic graft femoro-femoral crossover bypass to restore circulation to the opposite lower limb.

In the majority of cases, a bifurcated stent-graft is used so that the distal seal is achieved in both iliac arteries. Bifurcated systems are available either as one-piece (unibody) stent-grafts or as 'modular' stent-grafts that consist of two or more components which must be constructed into a complete bifurcated system within the patient. Modular systems are much more common because of their versatility but a potential drawback is the possibility of component separation due to the action of haemodynamic forces. A unibody stent-graft does not suffer from this problem but it is less adaptable to variations in anatomy.

Achieving endovascular exclusion of an aneurysm depends on matching a stent-graft to the patient's anatomy. Therefore some stent-grafts are manufactured to fit a particular patient's anatomy based on accurate measurements obtained from imaging. Modular component designs reduce the necessity for such custom manufacture. Each modular component of the stent-graft is available in a range of sizes. A complete stent-graft system can be built by combination of appropriately sized components. Such modular designs are particularly useful when undertaking urgent and emergency repairs, when there is no time to procure a custom made commercial device.

The introduction of EVAR represents a significant step in the evolution of aneurysm management. The benefits of EVAR for the high-risk patient and the significant reduction in early mortality rates compared with open repair are well documented (EVAR Trial Participants, 2005). This technique, however, is associated with unique problems in the post-operative period. There is a high incidence of complications in the late follow-up and consequently a high incidence of secondary procedures to correct them. The relative significance of all complications is not completely understood and

Table 8.4 Range of commercial stent-grafts

Device manufacturer	Graft material	Stent support	Hooks/ barbs	Configuration	Availability
Anaconda Vascutek	WP	NiTi-C	Proximal	Modular	Yes
Ancure Guidant	WP	None	Proximal & distal	Unibody	Withdrawn
Aneurx Medtronic	WP	NiTi-Z	None	Modular	Yes
Aorfix Lombard	WP	NiTi-C	Proximal	Modular	Yes
Powerlink Endologix	PTFE	SS-Z	None	Unibody	Yes
Endurant Medtronic	WP	NiTi-Z	Proximal	Modular	Yes
Excluder Gore	PTFE	NiTi-Z	Proximal	Modular	Yes
Lifepath Edwards	WP	Elgiloy-Z	None	Modular	Withdrawn
Talent Medtronic	WP	NiTi-Z	None	Modular	Yes
Zenith Cook	WP	SS-Z	Proximal	Modular	Yes

WP, woven PET fabric; NiTi-C, nickel–titanium wire in a circular/helical configuration; NiTi-Z, nickel–titanium in a z-shape; SS, stainless steel.

better insight is gained continuously. There is also a risk of late failure of treatment, resulting in the need for life-long surveillance with a view to identifying impending failure so that remedial action can be instituted. In order to preserve the early benefits of EVAR, there is a necessity to prevent late failure and death.

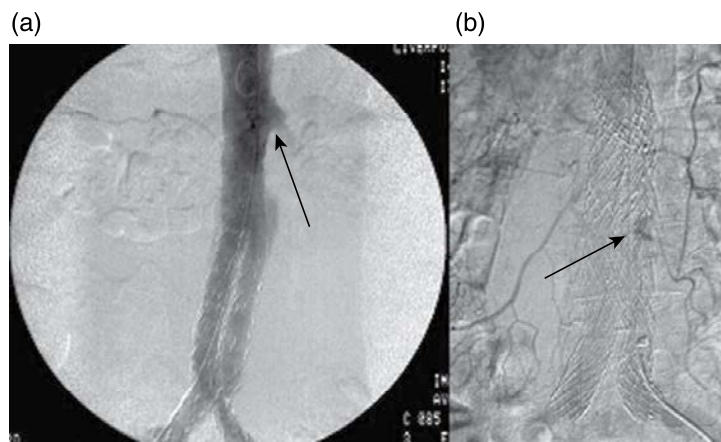
The component materials and some design features of different commercial stent-grafts are given in Table 8.4. The materials used for the graft are woven PET fabric grafts and ePTFE. The advantage of ePTFE is that it has low porosity, relatively thin wall and yet strong. The woven fabric materials used vary in thickness and strength. Although a thin fabric graft can be compacted more tightly, thereby requiring a small and flexible delivery system, the disadvantage is that its durability may be compromised and some designs such as the Zenith uses a standard woven PET fabric found in surgical vascular grafts. The metallic stent is of fundamental importance to the endograft and its function is to provide structural integrity to the whole device thereby ensuring support for the graft component and stable fixation. It is important that the mechanical and physical properties of the stent are not impaired after implantation. Most stents use zigzag or z-configuration except for the AnacondaTM (Vascutek Ltd, Inchinnan,

Scotland), and the Aorfix™ (Lombard Medical Technologies PLC, Didcot, UK) which have circular or helically disposed wires.

8.8 Complications of endovascular aneurysm repair

A number of complications that are specific to the endovascular technique have been observed during and immediately after the operation (Chuter *et al.*, 1998; Kalliafas *et al.*, 2000; Lee *et al.*, 2000). Manipulation of guide-wires and catheters within diseased arteries, or the passage of large delivery systems may result in distal embolisation of aneurysm thrombus into femoral or visceral arteries, or thrombotic occlusion of these vessels. Dissection of arteries and rupture of the aneurysm have also been reported as a consequence of intra-arterial manipulation. Inadvertent passage of guide-wires into the heart may lead to cardiac arrhythmia and rarely even myocardial rupture. Angiography at completion of EVAR has shown persistent flow of blood within the aneurysm sac in a significant proportion of patients (Kalliafas *et al.*, 2000) (Fig. 8.2). This phenomenon of blood flow within the aneurysm sac, but outside the stent-graft was called 'Endoleak' (White *et al.*, 1997). Although the short- and long-term consequences of such failure to isolate the aneurysm sac were not completely understood, it appeared reasonable to consider it an undesirable outcome.

The incidence of such failure to isolate the aneurysm sac and the uncertainty surrounding its significance reinforced the need for continued radiological and clinical follow-up to ascertain whether such leaks persisted or sealed spontaneously. The need for indefinite surveillance after endovascular repair became widely accepted.



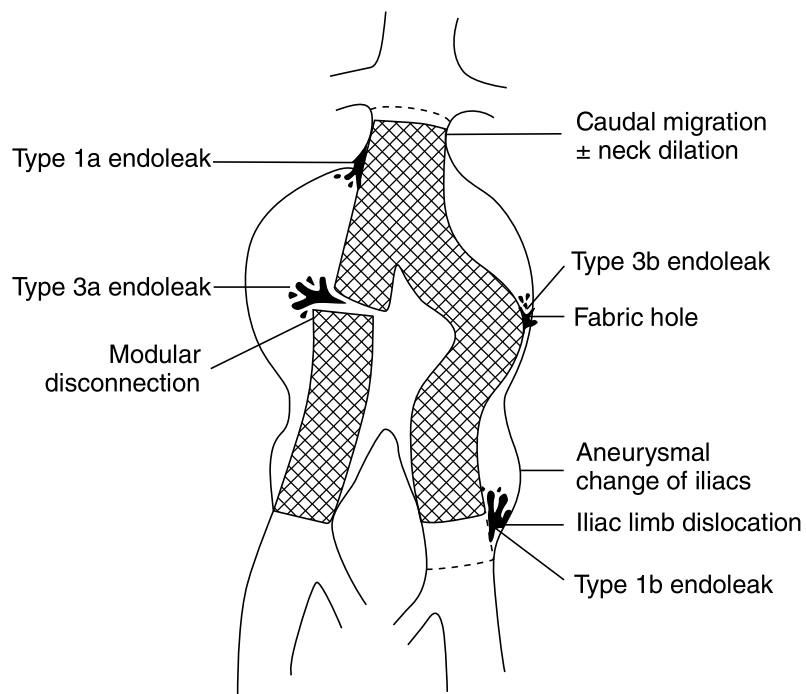
8.2 Reperfusion of aneurysm sac via proximal attachment site (a) and patent side-branch (b), i.e. Type I and Type II endoleaks, respectively. Arrows point to contrast material within the aneurysm sac.

8.8.1 Endoleak

Endoleak continues to be the most frequent complication during late follow-up after endovascular repair. Endoleaks are classified according to the source of blood-flow into the aneurysm sac (Table 8.5 and Fig. 8.3). In addition, they can be described as primary, if they are present from the time of EVAR or secondary if they appear at a later date.

Table 8.5 Types of endoleak

Type	Cause of endoleak
Type I	Fixation site leak
a	Proximal end
b	Distal end
c	Iliac occlude (plug)
Type II	Aortic side-branch perfusion
a	Simple: one patent branch
b	Complex: two or more patent side branches
Type III	Graft defect
a	Leak at junction or modular disconnection
b	Defect in fabric, stitch holes
Type IV	Graft porosity



8.3 Diagrammatic representation of different types of endoleak.

Type I endoleaks occur when there is a failure of seal at one or more stent-graft attachment sites. It is difficult to determine the true incidence of late Type I endoleaks. Many reports combine all types of endoleak while others refer only to early experience and first generation stent-grafts (Schurink *et al.*, 1999). Perhaps the best estimate can be found in a report by Tonnessen *et al.* (2002) in which Type I endoleak had been documented in nearly 10% of patients within 20 months of operation.

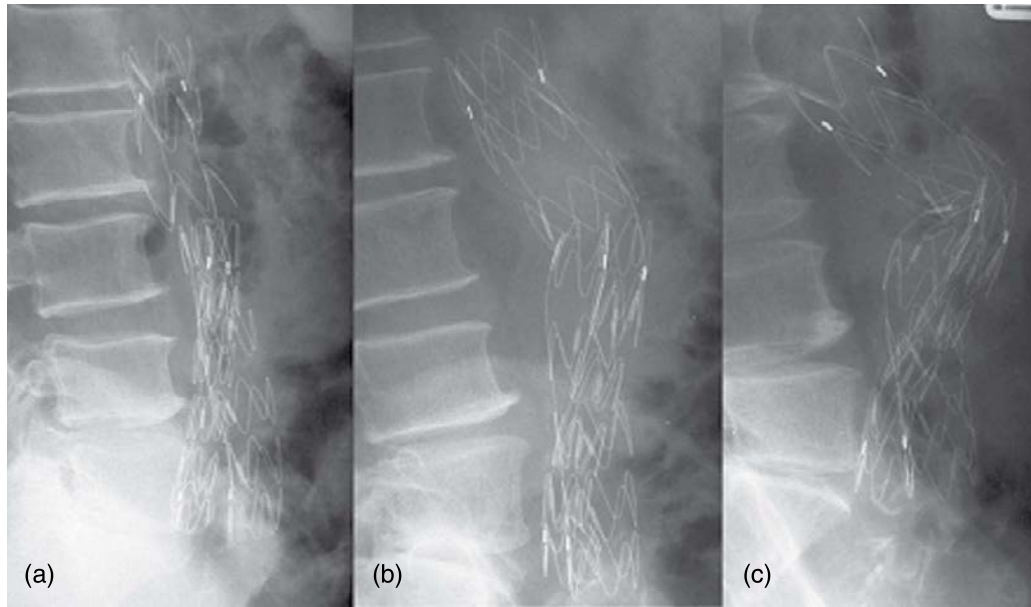
Reperfusion of the aneurysm sac through patent lumbar or mesenteric side-branches has been classified as Type II endoleak. Side-branch perfusion ceases spontaneously in the majority of patients after endovascular repair due to lack of a direct communication with the aortic lumen. The high variation in the reported incidence of late type II endoleak (2–70%) (Stelter *et al.*, 1997; Schurink *et al.*, 1999) is probably a reflection of the variety of techniques employed to detect endoleak. Ultrasound duplex scan, magnetic resonance imaging (MRI) and computed tomography (CT) have different sensitivity and specificity (McWilliams *et al.*, 2002). Furthermore, detection of endoleak by CT scanning depends upon the imaging protocols used. Biphasic scanning (scanning the patient first when the bulk of the contrast is in the arteries and again when it had passed into the veins) improves detection rates compared with arterial phase scanning alone (Golzarian *et al.*, 1998). It has been suggested that the majority of isolated single vessel Type II endoleaks seal spontaneously (since there is in effect no outlet for the blood-flow) and therefore a persistent Type II endoleak may in fact be a marker for otherwise occult graft-related endoleak.

Type III endoleak is due to dislocation of the components of a modular device or to a breach in the fabric of the stent-graft. Its cumulative incidence is approximately 10% at three years after EVAR (Vallabhaneni and Harris, 2001). The problem of modular disconnection appears to have been resolved by newer designs with improved attachments between the components. Steps are also being taken to address the issue of material fatigue and frictional wear of the fabric.

Type IV endoleak is associated with porous thin-walled fabric grafts. However, this is time limited and is usually resolved within a month of aneurysm repair. Expanded PTFE graft material has much lower porosity than woven PET fabric and is therefore less susceptible to this type of endoleak.

8.8.2 Stent-graft migration

Migration, i.e. movement of a stent-graft in relation to its original site of implantation, occurs if the haemodynamic distraction force, which tries to displace the device in a caudal direction, is greater than the strength of



8.4 Serial plain X-rays (images (a), (b) and (c) were taken one year apart) of the same patient. The top end of the stent-graft has migrated caudally in relation to vertebral bodies. The top end of the stent-graft can be seen to have expanded since it migrated into the aneurysm and is therefore no longer constrained by the aneurysm neck. Distortion of the stent-graft also occurred as a result of migration.

fixation (see Fig. 8.4). Fixation relies upon the radial force exerted by the stent-graft against the aortic wall, frictional forces between the stent-graft and the aorta and the strength of hooks or barbs which may be added to the proximal stent. Experimental studies, however, reveal that the fixation strength of any model of stent-graft is considerably lower than that of a surgically anastomosed graft (Malina *et al.*, 1998, 2000).

Secure fixation of the endograft relies on the radial force of the stents and the presence of hooks and barbs. Barb fracture often occurs *in vivo*. Different methods are used to secure the barbs to the endograft. For example, they can be wrapped around and soldered to the proximal bare stent or firmly sutured to the graft component. The latest devices, such as the Medtronic Endurant endograft, are provided with stent that is fabricated with integral hooks, a design that may result in reduced incidence of fracture.

Proper healing of the device at the proximal and distal attachment points, with infiltration of cells into the graft wall, would create a secure and reliable seal and help to anchor the device to the vessel. However, this does not appear to happen with either Dacron or ePTFE grafts (Malina *et al.*, 2000, McArthur *et al.*, 2001). The use of basic fibroblast growth factor incorporated into the graft component has been shown to enhance healing and

significantly improve the attachment of the graft to the intima (van der Bas *et al.*, 2004; Kusanagi *et al.*, 2007). As these studies were carried out in animals, it remains to be seen whether improved healing response can be achieved in atherosclerotic aortic necks.

The strength of haemodynamic force that causes migration depends upon patient factors such as hypertension and aneurysm morphology, in addition to stent-graft factors such as the diameter change from proximal to distal attachment site (Mohan *et al.*, 2002). Curvature of the stent-graft within the aneurysm also increases the haemodynamic distraction force (Liffman *et al.*, 2001). Migration was reported in approximately one-tenth of the patients by 3 years after endovascular repair (Vallabhaneni and Harris, 2001) with the incidence increasing with the duration of follow-up.

8.8.3 Stent fracture

The function of the metallic stent is to provide structural integrity thereby ensuring stability of the device fixation and support for the graft component. It is therefore important that the integrity of the stent is not impaired after implantation. Stent fractures are commonly observed *in vivo* in most devices whether they are manufactured from cobalt–chromium alloy, stainless steel or nitinol (Riepe *et al.*, 2002; Jacobs *et al.*, 2003; Zarins *et al.*, 2004).

Failure can be attributed to mechanical fatigue due to the cyclic loads the device is subjected to *in vivo*. The presence of small cracks, either introduced during the manufacture of the stent or inherent in the material, has a significant effect on fatigue failure. With cyclic load due to haemodynamic forces, these cracks may grow, leading eventually to fracture of the metal structure. In the presence of a corrosive environment such as blood, the corrosion of the metallic stent may also contribute to the fatigue process, i.e. corrosion fatigue. Although isolated stent fractures do not appear to have immediate clinical consequences, cumulative damage could impair the structure of the device, leading to loss of sealing and fixation or even prolapse into the aneurysm sac. Resistance to fatigue failure could be improved by altering the dimensions and geometry of the stent struts but this will lead to a bulky device requiring an unacceptably large delivery system. Metallurgical processing and the stent manufacture may be further improved to produce stents with much smaller and less numerous flaws thereby enhancing fatigue properties.

Nitinol owes its resistance to corrosion from the spontaneous formation of a thin titanium-based oxide layer on its surface that protects the base material from further oxidation, a process known as passivation. On the surface of stainless steel, chromium oxide forms as a tough film which also protects the underlying metal. Since the surface topography, the thickness

and composition of the oxide layer are known to influence the corrosion behaviour, manufacturers can apply selective surface treatment such as chemical passivation and electropolishing to improve corrosion resistance. However, when the stent strut is subjected to pulsatile deformations, the oxide layer may be disrupted with each deformation and the passivation and repair process is therefore not fully completed between deformation cycles. Further optimisation of the surface treatment or the use of protective coatings may be required in order to improve the biostability of the stents.

8.8.4 Fenestrated endovascular aneurysm repair

In a patient with a short aneurysm neck, the use of a bare metal stent extending above the fabric at the top of the stent-graft can recruit aorta at and above the level of the renal arteries for enhanced fixation. The bare metal stent struts may lie across the origins of the renal arteries but since the wires are thin, they appear not to cause early or late occlusion of these arteries (Burks *et al.*, 2002; Morrissey *et al.*, 2002). With standard endoluminal devices, a minimum length of healthy aorta between the lowest renal artery and the aneurysm, i.e. a neck, of 10 mm is required (Leurs *et al.*, 2006) to provide secure fixation and good sealing. However, patients with juxtarenal aneurysm with either a short or absent neck cannot be treated by EVAR with conventional devices. In recent years, fenestrated stent-grafts have been introduced to treat these aneurysms. These devices are individually customised with fenestrations (circular, elliptical or scallop shape) to preserve flow to essential aortic side branches while ensuring an adequate proximal seal at and above the level of the renal arteries. In order to maintain the strength of the fabric and ensure that the fenestrations remain fully open, nickel titanium wire reinforcements, which are securely fixed around the periphery of the openings, are provided. One potential complication with fenestrated stent-grafts is that of target vessel loss. This may occur during the procedure as a result of 'shuttering' of the vessel orifice due to malpositioning of the device. Later vessel loss is associated with migration which leads to compression or even fracturing of the stent within the fenestration.

8.9 Conclusions

Although ePTFE and Dacron are widely used and perform well in large diameter surgical revascularisation procedures, they are much less successful for small-diameter applications. The autologous vein remains the conduit of choice for vascular grafts of diameter of less than 6 mm. Precuffed and heparin-bonded ePTFE grafts have been introduced to reduce IH. In the

long term, however, the new approach based on the application of tissue engineering principles bodes well for the development of a new generation of living vascular grafts with superior patency rates to current inert prosthetic materials.

Since the first report of endovascular repair of AAA in 1991, this technique has become firmly established and in some larger centres EVAR represents the majority of elective AAA repair. Devices are continually being improved through better processing of the materials to enhance their durability, the use of advanced manufacturing technologies and the development of more compact, flexible and reliable delivery systems. However, the basic design of a porous fabric graft supported by a wire frame remains unchanged. Significant advances in future will require new strong and lightweight fabric that is resistant to wear damage and stent materials that are resistant to the hostile chemical and mechanical loading conditions in the body.

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EXHIBIT 12

Preparation of grafted ePTFE Film Targeting Application for Drug-Release Device in the Body

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Permeation of insulin through the grafted ePTFE films was controlled by pH decline which was caused by the enzymatic reaction of glucose oxidase (GOD) with glucose. The ePTFE-g-PAAc films were prepared by the combined use of oxygen plasma pre-treatment and photografting. The immobilization of GOD was performed by condensation with grafted polymer chains. In this case, argon (Ar) plasma pre-treatment was also applied for 30 seconds. Oxygen and Ar plasma treatment were obtained approximately the same grafted ratio. Without using of benzophenone (BP) as a sensitizer, the grafted ratio was remained small value as a half of with BP. A sensitizer was necessary needed for our photo-graft polymerization. The permeation of insulin through the ePTFE-g-PAAc-i-GOD/Catalase films can be enhanced through the addition of glucose.

Keyword: ePTFE Film, Oxygen and Argon Plasma Treatment, Photo-grafting, PAAc, Glucose Oxidase Immobilization

1 Introduction

When the authors aim at more ideal medication, in DDS or a controlled release system, many studies have been designed to control motion of medicines from the viewpoint of time and space. Various stimulus responsive materials have been used for the time regulation and self-control type devices [1, 2].

In this study, the expanded poly(tetrafluoroethylene) (ePTFE) film utilized as medical materials, such as an artificial blood vessel, was used as a common polymer substrate and underwent the oxygen plasma treatment. Then, photografting of acrylic acids (AAc) was carried out to introduce into one side of the film, and glucose oxidase (GOD) and Catalase were immobilized through condensation between AAc and GOD/Catalase. In plasma treatment, Ar plasma was attempted and for enzyme condensation reaction L-aspartic acid (L-Asp) was introduced into the PAAc grafted chains. We have used this film as the stimulus responsive one that can detect β -D-glucose, which is catalyzed with GOD into gluconic acid, reducing pH of solution around the film surface. Since PAAc grafted chains

contract in response to the pH decline, the pore of the grafted film open and allow insulin to permeate[3].

Permeation control of the insulin via the pH fall caused by oxidization of glucose outside the film in attempting to apply DDS for diabetes [4, 5]. Moreover, in the oxidation reaction of glucose by GOD, hydrogen peroxide, being toxic to a human body, is generated. So it is surmised that the oxidation reaction of glucose would be promoted when Catalase is used, the additional effect of Catalase to decrease the toxicity of this hydrogen peroxide is also examined.

2 Experimental

2.1 Materials and Reagents

Expanded PTFE films (diameter = 47 mm, average pore size = 0.5 μ m, degree of vacancy = 78 %: ADVANTEC Co. Ltd.) as a polymer substrate were washed in methanol and acetone for 10 min in an ultrasonic washing machine, and then dried under reduced pressure. AAc (Wako Pure Chemical Ind., Ltd.) as a hydrophilic monomer and benzophenone (BP: Wako Pure Chemical Ind., Ltd.) as a sensitizer were used. Buffer solutions

were prepared with potassium dihydrogen-phosphate and disodium hydrogen-phosphate (Wako Pure Chemical Ind., Ltd.) without further purification. GOD (Funakoshi Chemical Ind., Ltd.) from porcine pancreas, 82 units/mg) as an enzyme, β -D-glucose as a substrate and insulin as a permeant, 1-cyclohexyl-3-(2-morpholino-ethyl) carbodiimide metho-*p*-toluene sulfonate (CMC) as a condensation reagent (Wako Pure Chemical Ind., Ltd.), *N*-hydroxysuccineimide (NHS) and L-Asp as a modification reagent of PAAc chains (Wako Pure Chemical Ind., Ltd.) were used without further purification.

2.2 Preparation of ePTFE-g-PAAc Films

The oxygen plasma treatment was performed to one side of an ePTFE film by the use of a Shimadzu LCDV-20 type plasma polymerization apparatus in the manner described in our previous articles [3, 4]. The plasma-treated ePTFE films were dipped for 1 min in a 0.0275 mol/dm³ acetone solution containing BP. The photografting was carried out by the use of a Riko rotary photochemical reactor RH400-10W (Riko Kagaku Ind.) equipped with a 400W high-pressure mercury lamp. The sensitizer-coated ePTFE films before being dried were immersed in aqueous monomer solutions of 50 cm³ AAc of 2.0 mol/dm³ into the Pyrex glass tubes. Then, AAc was photografted onto the ePTFE surfaces at 40 °C by applying UV rays under a nitrogen atmosphere for 120 min. The ePTFE-g-PAAc films were washed under flowing water and repeatedly immersed in distilled water to remove the unreacted residual monomer and the remaining homopolymer, and room temperature. The amount of AAc grafted onto ePTFE surfaces was calculated from the weight increase of the samples.

In Ar plasma treatment, plasma irradiation time was 30 seconds.

2.3 Preparation of L-Asp modifying ePTFE-g-PAAc film (ePTFE-g-PAAc (L-Asp))

The ePTFE-g-PAAc films were immersed in the phosphate buffer solution of pH 6.30 for 24 hours (the swollen state) and were soaked in a 0.043 mol/dm³ NHS of the phosphate buffer solution of pH 6.30 at room temperature for 2 hours for activating carboxyl groups of the grafted chains. After washed in the same buffer solution, the activated ePTFE-g-PAAc film was put into the phosphate buffer solution containing L-Asp and CMC. Furthermore, enzyme immobilization was performed put into the buffer solution containing

CMC, GOD and Catalase.

2.4 Surface Analysis of the Grafted ePTFE Films

The surface analysis of the films were performed by the use of ATR-IR (Spectrum 65; Perkin Elmer Co., Ltd.), SEM (JSM-5800 SEM; JEOL Co., Ltd.) and ESCA (ESCA 3400 Shimadzu Co., Ltd.).

2.5 Grafted Ratio of the Grafted ePTFE Films

The amount of AAc grafted onto ePTFE surfaces was calculated from the weight increase of the samples using Eq. (1).

$$\text{Grafted ratio} = \frac{\text{Amount of grafted PAAc(g)}}{\text{Amount of untreated ePTFE(g)}} \quad (1)$$

2.6 Immobilization of GOD and Catalase onto ePTFE-g-PAAc Film

The ePTFE-g-PAAc films swollen in a phosphate buffer solution of pH 6.30 for 24 hours were soaked in another phosphate buffer solution of pH 6.30 including 0.043 mol/dm³ NHS for 2 hours to activate the grafted chains. The films sufficiently washed with the same buffer were again soaked in 100 cm³ of the phosphate buffer of pH 6.30 including GOD 8.3 mg, Catalase 5.0 mg and CMC 0.25g.

2.7 Permeation Experiment of Insulin

The GOD and Catalase immobilized ePTFE-g-PAAc (ePTFE-g-PAAc-i-GOD/Catalase) film immersed in the phosphate buffer solution of pH 7.40 for 24 hours was set between the two halves of the cell (100cm³). The phosphate buffer solution of pH 7.40 containing insulin of 10 mmol was poured into the feed side and insulin free of the same buffer solution was poured into the permeation side. Then this permeation equipment was put in a water bath of 37 °C and then both solutions were stirred. The amount of insulin permeated through the ePTFE-g-PAAc-i-GOD/Catalase films was determined by measuring the absorbance at a wavelength of 276 nm at every 15 minutes. β -D-Glucose was added into the cell of the feed side at 90 min after the permeation experiment started.

3 Results and Discussion

3.1 Surface Analysis by IR of the Grafted ePTFE Films

IR spectra in KBr pellets method of PTFE, ePTFE-g-PAAc, ePTFE(Ar)-g-PAAc, and NS films are shown in Fig. 1. With the PAAc grafted

films, two peaks at 1700 cm^{-1} due to the C=O stretching and around 3000 cm^{-1} due to the OH stretching are observed. Therefore, it can be said that AAc chains have been introduced onto the surface of ePTFE film.

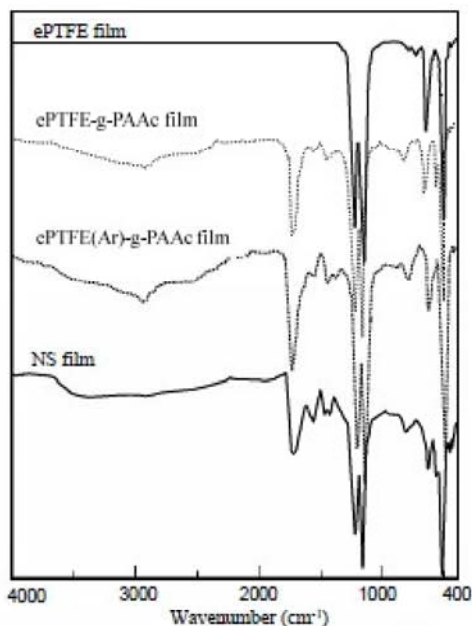


Fig. 1 IR spectra of ePTFE-g-PAAc films

3.2 L-Asp introduction to AAc Chain by ESCA
Introduction of L-Asp into AAc chain of ePTFE-g-PAAc (L-Asp) film was detected by C1s and N1s spectra of ESCA and were shown in Fig. 2 and 3, respectively. A peak of C(=O)NH-R in the vicinity of 288 eV in Fig. 2 and a peak of nitrogen in the vicinity of 400 eV in Fig. 3 were confirmed, and accordingly L-Asp was induced into AAc grafted chains.

3.3 Surface Observation by SEM

The SEM image of the surfaces of ePTFE and ePTFE-g-PAAc films was presented in Fig. 4. In this picture, AAc grafted chains were introduced and covered the cavity of the ePTFE film.

3.4 Grafted Ratio of ePTFE-g-PAAc Films

The grafted ratio of AAc onto the ePTFE films is shown in Fig. 5. Although the grafted ratio of the ePTFE-g-PAAc film and ePTFE(Ar)-g-PAAc film were shown approximately 0.34 and 0.35, as about same.

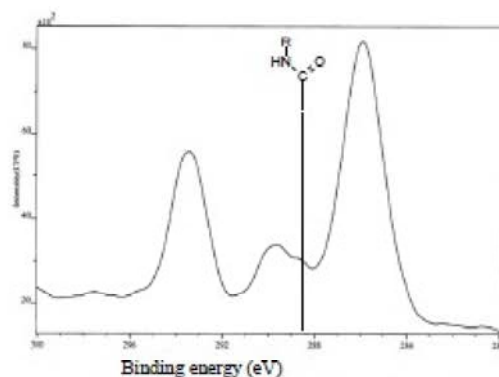


Fig. 2 C1s spectrum of ePTFE-g-PAAc(L-Asp) film

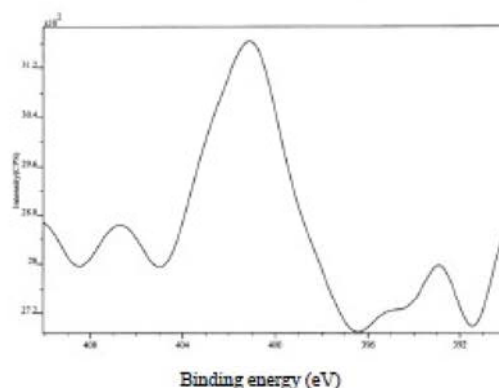


Fig. 3 N1s spectrum of ePTFE-g-PAAc(L-Asp) film

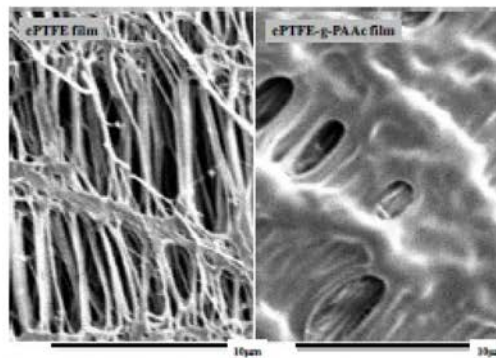


Fig. 4 Micrographs of ePTFE film and ePTFE-g-PAAc film

This means that the graft-polymerization advances efficiently even for oxygen plasma or Ar plasma treatment. The grafted ratio of the NS film which did not coated BP as sensitizer showed lower value with 0.24. Therefore, it was suggested

that the use of the sensitizer was required for polymerization.

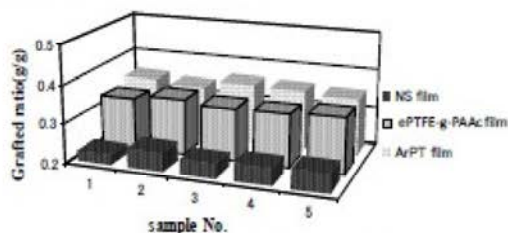


Fig. 5 Grafted ratio of ePTFE-g-PAAc films

3.5 Permeability of Insulin through ePTFE-g-PAAc-i-GOD/Catalase films

The permeabilities of insulin by the use of the ePTFE-g-PAAc-i-GOD/Catalase films with time is shown in Fig. 6. The amount of permeated insulin was hardly observed during the initial reaction time of 100 minutes. Since the AAc grafted chains on the films in a phosphate buffer solution of pH 7.40 can expand by electrostatic repulsion between carboxyl anions, and so make the channels in the films narrow, the permeation of insulin is considered to be depressed. Figure 6 shows that the permeated amount of insulin increases slowly after addition of glucose into the feed side cell at 90.

It is considered that gluconic acid generated by the enzymatic reaction between GOD and glucose declines pH around the surface of the films and makes protonation of anionic carboxyl group. Suppression of electrostatic repulsion among PAAc makes the channels open so that the insulin moves to the permeation side cell.

The insulin permeation through the films was considerably increased. This shows that Catalase can accelerate glucose oxidation because of consumption of hydrogen peroxide generated with gluconic acid by oxidation reaction of glucose. Hydrogen peroxide is toxic for the human body so that the consumption of hydrogen peroxide has double benefits for DDS [5].

We have made it clear that the permeation of insulin by the use of GOD immobilized onto ePTFE-g-PAAc films can be controlled in response to pH changes caused through the enzymatic reaction with glucose and a decrease in hydrogen peroxide by enzymatic reaction of Catalase.

4. Conclusions

The control permeation of insulin was

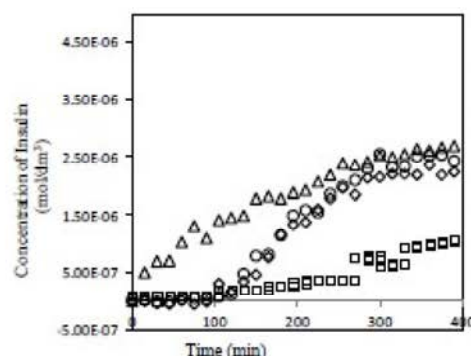


Fig. 6 Changes in the Insulin permeabilities through film (○: ePTFE-g-PAAc-i-GOD/Catalase film, □: ePTFE(Ar)-g-PAAc-i-GOD/Catalase film, △: ePTFE-g-PAAc (L-Asp)-i-GOD/Catalase film, and ◇: NS film)

examined through the GOD and Catalase immobilized onto ePTFE-g-PAAc films with Ar plasma pre-treatment and introduced L-Asp into grafted AAc chains. From the results, we can draw the following conclusions.

- 1) The amount of grafted AAc of ePTFE films can be controlled with oxygen plasma-treated time for 2 minutes and with Ar plasma-treated time for 30 seconds.
- 2) The permeability of insulin through the GOD and Catalase immobilized onto the ePTFE-g-PAAc film increases with the addition of glucose.
- 3) The NS film, which did not use BP as a sensitizer for photo graft-polymerization, could not control insulin permeation. It could not prevent insulin permeation because of shortage of PAAc grafted chains. Therefore, photo graft-polymerization will be required a sensitizer like BP.

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CERTIFICATE OF SERVICE

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