Segmented Poly(ether ester)s and Poly(ether ester amide)s for Use in Tissue Engineering

Audrey Deschamps

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SEGMENTED POLY(ETHER ESTER)S AND POLY(ETHER ESTER AMIDE)S FOR USE IN TISSUE ENGINEERING

DISSERTATION

to obtain
the doctor's degree at the University of Twente,
on the authority of the rector magnificus,
prof. dr. F.A. van Vught,
on account of the decision of the graduation committee,
to be publicly defended
on Wednesday November 13th, 2002 at 13:15

by

Audrey Annie Deschamps

born on June 15th, 1973
in Revin, France

This dissertation has been approved by:

promoter	Prof. Dr. Jan Feijen
assistant-promoter	Dr. Dirk W. Grijpma

À mes parents
et mes grands-parents

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‘Throw yours dreams into space like a kite, and you do not know what it will bring back:
a new life, a new friend, a new love, a new country.’
Anais Nin (1903-1977)

In December 1997, I threw my kite and wrote an application letter to the group of Polymer Chemistry and Biomaterials at the University of Twente in the Netherlands. It brought me back more than I could ever expect.

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Audrey

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Chapter 1

General Introduction

'We restore and make whole those parts which nature or ill fortune have taken away,
not so much to delight the eye, but to buoy up the spirit of the afflicted.'
Gaspare Tagliacozzi (1545-1599)

Tissue engineering

Living tissues and organs may be damaged due to injury or may fail due to disease. Three types of procedures are commonly applied to restore body function. The use of autologous grafts consists of the transfer of healthy tissue from the patient himself and is, to date, the most suited approach. Although autografting has shown good results in the domains of skin replacement or heart bypass surgery for example, one has to cope with the problem of scarring and morbidity at the donor site, as well as occasionally limited recovery of the tissue function. Moreover, this technique is not applicable when dealing with extensive damages. Tissue allografts (from human beings) or xenografts (from animals) can then be proposed. The main benefit of this approach is the unlimited availability of tissue. However, the use of allografts or xenografts can also be associated with the transmission of diseases [1] and the tendency to elicit an immune response [2,3]. Finally, tissues can be permanently replaced by synthetic materials as in the case of hip replacements, heart valves or vascular prostheses. Synthetic materials can be processed in many shapes and stored easily before use. A drawback of these devices is their potential to induce infections and immune responses. Furthermore, in the long-term, the durability of these devices is limited.

Tissue engineering is, therefore, attracting much attention as a way to generate tissues for transplantation therapies. This approach to tissue regeneration and functional recovery is a challenge for science requiring the combined effort of biologists, material scientists, engineers and physicians [4]. Tissue engineering involves culturing of specific cell types, use of a porous

scaffold to support adhesion, growth and differentiation of these cells and, in some cases, delivery of growth and/or differentiation factors [5,6]. To obtain the large number of cells required in tissue engineering and to create an environment with specific biochemical and physical signals, new cell culture techniques involving bioreactors are also being developed. Different types of bioreactors have been designed including static [7] and mixed flasks [8,9], rotating [10] and perfused [11] vessels.

Cell type and source

The number of functional tissues that are being investigated is growing steadily, although the degree of progress is dependent on the complexity of the tissue. Engineered skin and cartilage are in clinical trials, whereas, for instance, the engineering of liver tissue has not yet been accomplished [12]. Due to progress in molecular cell biology, tissues can be grown starting from different cell types [13] and from cells at different differentiation states. For example, osteogenesis (bone formation) can be induced from osteoblasts [14,15], primary muscle derived cells, primary articular chondrocytes and even primary fibroblasts [13]. Chondrocytes are usually used to generate cartilage [16], although bone marrow stromal cells can also be employed [17,18]. Cardiomyocytes [10,19] and vascular cells [20] are involved in cardiovascular tissue engineering. Keratinocyte-based materials play a key role in skin regeneration [21,22], although dermal fibroblasts [23,24] are also studied.

As the isolation and expansion of cells *in vitro* is highly dependent on the cell type [12], the remarkable biological properties of stem cells have opened new perspectives. Virtually, stem cells can be involved in the regeneration of any kind of tissue. Human embryonic stem cells are believed to have such characteristics [25,26]. Nevertheless, the control of these stem cells and their differentiation into appropriate cell types is still a complex issue [27,28], and also involves ethical issues. By simply changing the culturing conditions, skeletal stem cells, also known as bone marrow stromal cells, can turn into bone [14,29,30] or cartilage [17,18]. Currently, researchers explore the potential of epidermal stem cells [29] and mesenchymal stem cells [31] in the creation of skin and cardiovascular tissue, respectively. In principle, stem cells can proliferate through multiple generations before differentiation into a desired cell type.

Polymeric scaffolds

The design of a scaffold, which will allow cell transplantation and tissue ingrowth from the host organism, is another major issue in tissue engineering. Clearly, the device needs to be biocompatible and possess adequate surface chemistry to allow cell adhesion and growth. In addition, the ideal material must provide sufficient mechanical support and be processable into

three-dimensional structures to maintain space for the regeneration of the tissue and to facilitate vascularization and cell nutrition. The device should be designed to maintain its function for a defined period, after which degradation should occur.

Scaffolds designed for tissue engineering have been prepared from biologically derived materials, such as collagen [32-34], hyaluronic acid [18,30] and chitosan [23,35]. However, the number of biologically-derived polymers, which can be used, as well as the possible modifications to improve their mechanical properties and degradability are limited. Therefore, synthetic polymers seem more suited for the preparation of scaffolds [36]. The use of synthetic polymers with different chemistries enables the design of scaffolds with appropriate mechanical and biological properties, and degradation rates. Synthetic polymers can also be easily processed into various shapes and can be produced cheaply and reproducibly.

Most of the research on degradable polymers in tissue engineering has been focused on hybrid cell/scaffold constructs using poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers (PLGA). These polymers are degradable via hydrolysis and are resorbable, as their degradation products, namely lactic and glycolic acid, are parts of the Krebs cycle [37,38]. PLA, PGA and PLGA polymers have been used in scaffolds to repair damaged hard tissues (bone [15,39,40] and cartilage [41]), as well as soft tissues (skin [42,43], heart muscle [10,19], blood vessel [34], etc.). The amount of acidic compounds released during the degradation of PGA and PLGA polymers and the potential crystalline debris generated by PLA degradation, however, can induce tissue inflammations [44-46]. New materials with improved properties are therefore being developed, for example tyrosine-derived polycarbonates [45], poly(propylene fumarate)s [47], and poly(anhydride-*co*-imide)s [48,49]. Recently, slowly degrading poly(ε-caprolactone)-based scaffolds have been investigated for the tissue engineering of skin [24,50] and peripheral nerves [51]. Segmented polyurethanes have held promises as scaffolds for meniscal reconstruction [52,53] and for use as nerve guidance channels [54]. Poly(ethylene oxide) (PEO) has also attracted interest for use in biomedical applications due to its hydrophilicity, biocompatibility and non-toxicity [55]. PEO-based biomaterials have, therefore, been often applied for tissue engineering [56-61].

Objective

With the exception of a few polyurethanes [52,53,62,63], thermoplastic elastomers have hardly been studied for scaffolding. The main reason is the belief that, for an optimal use, the mechanical properties of the polymeric scaffold should match those of the damaged tissue. However, this characteristic is not an absolute necessity. In the tissue engineering of bone for example, the hybrid construct can develop strength during degradation of the polymer and simultaneous formation of new bone, allowing the use of elastomers for small defects in non-load bearing situations. Thermoplastic elastomers containing poly(ethylene oxide) and

poly(butylene terephthalate) (PEOT/PBT) have already been studied as biomaterials [64-67]. However, several aspects of these systems have not been examined in detail. The objective of the studies described in this thesis is to investigate the applicability of such slowly degradable thermoplastic elastomers as scaffolds for tissue engineering, with emphasis on their phase separation and degradation properties.

Another type of thermoplastic elastomer in which the terephthalic moieties have been replaced by ester-amide segments, is also investigated for use in scaffolding. These polymeric materials were developed for tissue engineering but may also have other medical uses, such as carriers for the delivery of bioactive compounds (proteins, genes or growth factors), internal membranes or tissue substitutes.

Scope of the thesis

A literature overview on block copolymers, their properties and their applications in medicine is presented in **Chapter 2**. Emphasis is given on multi-block copolymers, as the work described in this thesis is focused on thermoplastic elastomers based on multi-block copolymers containing poly(ethylene oxide) (PEO).

Copolymers based on poly(ethylene oxide) and poly(butylene terephthalate) (PEOT/PBT) are phase separated systems in which the PBT segments constitute the hard phase and PEOT segments the soft phase. The PEO-containing segments provide hydrophilicity to the material and are potentially susceptible to oxidation. In **Chapter 3**, the copolymers in the swollen state are examined using small angle X-ray scattering and differential scanning calorimetry. The degradation in oxidative media is also reported.

In PEOT/PBT copolymers, the soft to hard segment ratio and the PEOT segment length can be varied leading to polymers with different properties. **Chapter 4** deals with the effects of copolymer composition on the phase separation, physical properties and degradation behavior of segmented PEOT/PBT block copolymers with relatively high soft segment content. The mechanical properties of the copolymers as a function of molecular weight in the dry and water-swollen state are investigated. *In vitro* hydrolysis and oxidation of PEOT/PBT block copolymers have been evaluated. Polymer degradation was also examined under different storage conditions.

In **Chapter 5**, the suitability of PEOT/PBT copolymers for tissue engineering of bone is assessed. Copolymer films are studied with respect to their mechanical properties, degradation behavior and goat bone marrow cell adhesion to and growth on (modified) surfaces. The preparation of porous structures by various techniques is also described.

The degradation behavior of PEOT/PBT is studied in more detail in **Chapter 6**. The changes in composition, intrinsic viscosity, mass loss and thermal properties are examined after subcutaneous implantation of the copolymers in rats. Long-term degradation is simulated

by hydrolyzing PEOT/PBT samples in phosphate buffer saline (PBS) at 100°C and subsequent subcutaneous implantation of the samples. The analysis of the degradation products after hydrolysis at 100°C is described.

In developing a polymer with suitable physical properties and more suited degradation behavior for medical applications, the terephthalate unit is replaced by an ester-amide unit. Properties of PEO-containing poly(ether ester amide)s (PEEAs) based on PEG, 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate are investigated and the results are described in the two last chapters. In **Chapter 7**, the physical properties (phase separation, mechanical properties and water-uptake) as a function of polymer composition are reported. A comparison with the properties of PEOT/PBT copolymers is made. In **Chapter 8**, *in vivo* degradation, cytotoxicity, preparation of porous structures and cell growth experiments have been described to evaluate the potential of PEEA copolymers for tissue engineering.

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Chapter 2

Degradable Block Copolymers for Biomedical Applications

'Since we cannot know all that there is to be known about anything,
we ought to know a little about everything.'
Blaise Pascal (1623-1662)

Introduction

Originally, polymers in medicine were used for the replacement or improvement of body parts with permanent prosthetic devices such as hip implants, bone plates, vascular grafts, sutures, catheters, etc. In some cases, the need for therapeutic support is only temporary and a second surgical procedure is required to remove these devices. This has promoted the development of degradable polymers, which are designed to be resorbed or excreted by the body within a determined period of time. Poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers (PLGA) are well-known examples of degradable polymers and their synthesis and degradation behavior have been extensively studied [1-5].

New applications have driven the development of novel degradable systems with specific physical properties (e.g. hydrophilicity, elasticity, etc.) and degradation rates (from a few days to several months). To meet these demands, biomaterials based on degradable block copolymers have drawn much attention due to the broad spectrum of mechanical, physical and biological properties they can offer by variation of their composition. Block copolymers used as drug delivery systems allow the release of new hydrophobic drugs, such as steroids and antibiotics [6,7], which was difficult with the classical carriers. In tissue engineering, block copolymers can be applied as temporary three-dimensional scaffolds, as their degradation behavior, mechanical and biological properties can be readily tuned [8].

Block copolymers have received widespread attention since their commercial development in the fifties [9,10]. As a rule, these polymers are multiphase materials and much of the interest has arisen from their remarkable microphase separated morphology. In contrast with polymeric blends, macrophase separation is impeded as the different macromolecular blocks are covalently linked. However, the absence of interactions between the different segments may lead to a local segregation on the scale of the copolymer chains. The morphology and nature of the phase separated domains depends on chemical composition, chain structure, molecular weights of the constituent blocks, χ-interaction parameter, geometrical confinement and temperature. Microphase separated materials have the advantage that the inherent properties of each polymer can be combined in the block copolymer. Thermoplastic elastomers with enhanced mechanical properties are composed of soft, amorphous and hard, crystalline segments. The nature of the blocks can also be chosen to be hydrophilic and hydrophobic. In this way, amphiphilic materials ideal for drug release are obtained.

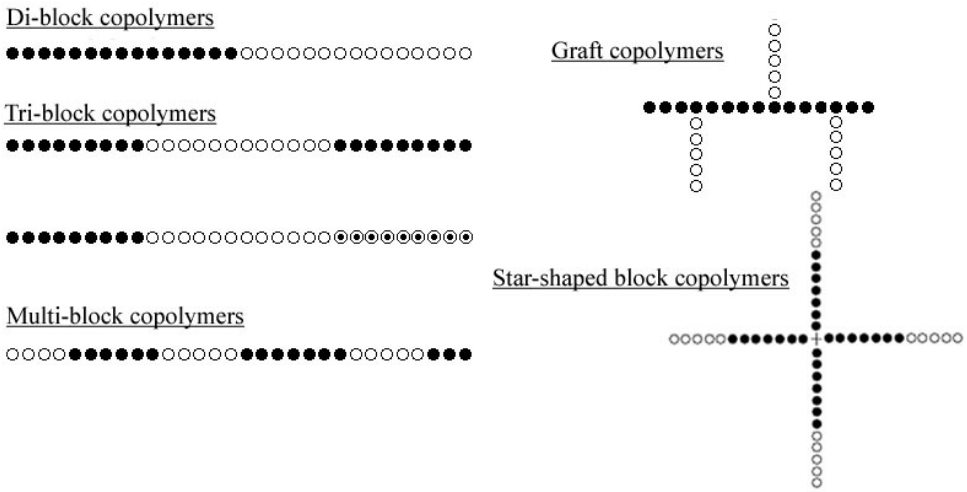


Figure 1. *Schematic representation of block copolymer structures: ● A-unit, ○-B unit, ⦿ C- unit.*

Several types of block copolymer architectures can be synthesized for use as biomaterials. They are schematically represented in Figure 1 and can be categorized into linear and non-linear copolymers. The simplest linear block copolymer is an AB-type block copolymer or di-block copolymer, which is composed of one block of A-units linked to one block of B-units. Block copolymers of the second type are tri-block copolymers where terminal units of the B block are connected to an A block (ABA-type) or to an A block and a C block (ABC-type). Polymer chains composed of alternating or randomly distributed A and B blocks are named multi-block copolymers. Graft copolymers and star-shaped block copolymers are non-linear copolymers. In graft copolymers, functional groups are originally present on the polymer

backbone (A macromonomer). These reactive groups serve as grafting points for the polymerization of the pendant B blocks. Star-shaped block copolymers are synthesized using a multifunctional monomer containing several reactive groups from where the A and B blocks can grow.

In the following sections, the synthesis and biomedical applications of relevant degradable linear block copolymers (di-, tri- and multi-block) will be described. In particular, a multi-block copoly(ether ester) based on poly(ethylene oxide) and poly(butylene terephthalate) (PEOT/PBT) will be discussed.

Di- and tri-block copolymers

In pharmacy, degradable di- and tri-block copolymers are of particular interest, as various drug delivery and drug targeting systems can be developed from these materials. These block copolymers are usually amphiphilic. The hydrophilic block is typically based on poly(ethylene glycol) (PEG). PEG is water-soluble, non-toxic and has a high degree of hydration and large excluded volume in solution [11]. This makes it attractive for the stabilization of particles in aqueous media. Moreover, PEG is known for its ability to reduce protein adsorption [12,13], which is important for long circulating drug carriers [14]. A variety of polymers can serve as hydrophobic block. The most common examples are polylactide (PLA), poly(lactide-*co*-glycolide) (PLGA) and poly(ε-caprolactone) (PCL), which are biocompatible and degradable by hydrolysis. The use of different block lengths and monomer ratios results in copolymers with a range of physical properties, degradation rates and profiles.

Synthesis of di- and tri-block copolymers

In the preparation of di- and tri-block copolymers for use in medicine, the sequential polymerizations usually involve cyclic monomers (e.g. lactones, ethylene oxide), which are successively polymerized by ring-opening. These types of polymerizations most often take place via anionic mechanisms. Successful cationic syntheses are hardly found in literature [15]. PLA-PEO di-block copolymers have been synthesized by sequential polymerizations of D,L-lactide and ethylene oxide either in toluene with triethyl aluminum as catalyst [16] or in tetrahydrofurane (THF) in the presence of potassium 2-methoxyethoxide [17]. PCL-PEO di-block copolymers have also been prepared in THF as reported by Eisenberg et al. [18]. In this study, the ring-opening polymerization of ethylene oxide was initiated by diphenylmethyl potassium. Subsequently, PEO-diethyl aluminum, obtained after reaction with diethylaluminum chloride, was used as initiator for the polymerization of ε-caprolactone [18].

Sequential ring-opening polymerization of trimethylene carbonate (TMC) and L-lactide was successfully carried out too. Before the addition of L-lactide, TMC was polymerized in *p*-xylene in the presence of a small amount of diethylene glycol and stannous octoate as catalyst [19].

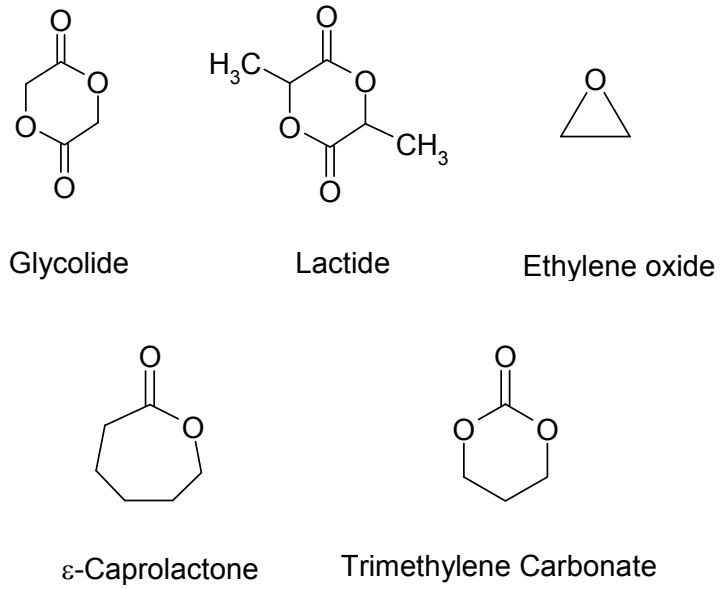


Figure 2. *Chemical structures of cyclic monomers commonly used in the preparation of degradable di- and tri-block copolymers.*

Albertsson and co-workers have synthesized di- and tri-block copolymers based on 1,5-dioxepan-2-one (DXO, Fig3) as the B block. PCL-DXO and PCL-DXO-PCL copolymers were first obtained by sequential addition of ε-caprolactone and DXO in THF at 0°C using aluminum isopropoxide as an initiator [20]. Higher molecular weight polymers could be prepared with L-lactide and DXO. Elastomeric PLLA-DXO-PLLA block copolymers were

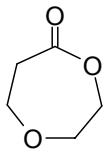


Figure 3*. Chemical structure of 1,5-dioxepan-2-one (DXO).*

synthesized by ring-opening polymerization of DXO initiated by a tin compound in chloroform and subsequent addition of L-lactide [21]. These copolymers were found to be degradable by hydrolysis [22].

Polyphosphazenes have proved to be of interest since their degradation kinetics are determined by variations in the side-chain structure rather than in the polymer backbone [23]. Polyphosphazene di- and tri-block copolymers with micellar characteristics [24] were synthesized by sequential cationic condensation of two types of phosphoranimines in dichloromethane at ambient temperature, as depicted in Figure 4 [15].

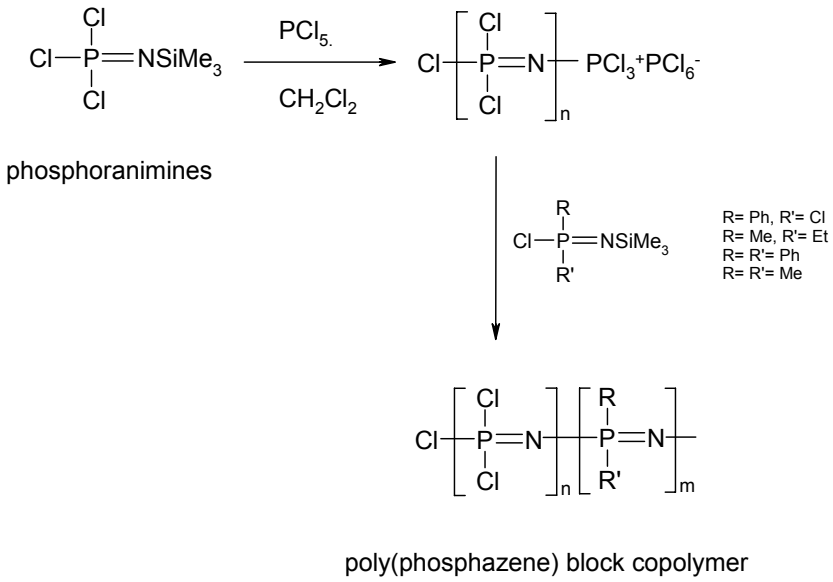


Figure 4. *Synthetic pathway to polyphosphazene block copolymers [15].*

Recently, the synthesis of ABC-type copolymers based on polystyrene (PS), PEG and PCL has been reported [25]. Three strategies were developed: sequential polymerization, thermal induced polymerization and the so-called titration method. The first step in each strategy consisted of the sequential polymerization of styrene and ethylene oxide in THF at 20°C using cumyl potassium as initiator. For the sequential polymerization, ε-caprolactone was added to the mixture (after removal of THF) containing the synthesized PS-PEO polymer and allowed to polymerize. The thermal induced polymerization required termination of the ethylene oxide reaction by addition of methanol and drying of the PS-PEO polymer. Under argon atmosphere, ε-caprolactone was mixed with the PS-PEO polymer. The polymerization took place under vacuum at 180°C for 30h. For the titration method, the activation of the hydroxyl end-group of the PS-PEO polymer with diphenylmethyl sodium was necessary to initiate the polymerization of ε-caprolactone in benzene.

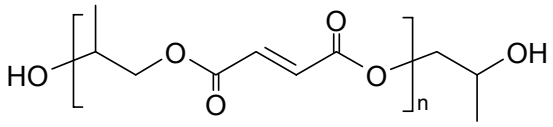
Polymerizations involving pre-polymers have been the subject of many studies, especially the synthesis of copolymers based on PLA and PEG where PEG or methoxy-PEG are used as pre-polymer. Although copolymers could be obtained by polycondensation of lactic acid at the hydroxyl end-groups of PEG [26,27], ring-opening polymerization of lactide with methoxy-PEG or PEG via a coordination-insertion mechanism is most often used because of its advantages over polycondensation: ring-opening polymerization does not require equimolarity and usually takes place under milder reaction conditions.

Ring-opening polymerization can take place in solution. The syntheses of PLA-PEO di-block and PLA-PEO-PLA tri-block copolymers have often been performed using methoxy-PEG (for the di-block) or PEG (for the tri-block) as the initiators in the ring opening polymerization of lactide in toluene under reflux conditions and in the presence of stannous octoate as catalyst [28-31]. PLGA-PEO-PLGA copolymers were also synthesized under the same conditions [32]. Jedlinski *et al.* showed that PLA-PEO-PLA tri-block copolymers could be prepared rapidly in less than 5 minutes by ring-opening polymerization of L-lactide with PEG and sodium hydride in THF at room temperature [33]. However, racemization of L-lactide and formation of PLA homopolymer were also observed. Kricheldorf obtained similar tri-block copolymers by polymerization of L-lactide initiated by a combination of PEG and potassium tert-butylate in anhydrous toluene at 60°C [34].

These types of polymerizations can also be performed in the bulk. The use of solvent is then avoided and the catalyst is the only additive. A typical polymerization in the bulk is carried out at elevated temperatures under vacuum or nitrogen atmosphere. The types of reactants and catalysts determine the polymerization time and temperature. Like in the anionic polymerization, stannous octoate is the most often employed catalyst [35-37], although aluminum triisopropylate [38], a triisobutylaluminum/water/phosphoric acid complex [39,40] and stannic chloride [41] have been also used. A major disadvantage of these organometallic catalysts is their potential toxicity if they remain within the polymer [42]. Therefore, other catalysts such as calcium hydride and zinc metal [43,44] that are believed to be more compatible have been applied. Cerrai *et al.* showed that it was also possible to synthesize PLA-PEO-PLA tri-block copolymers by ring-opening polymerization of L-lactide with PEG without the presence of a catalyst [45]. The active hydrogen present in the methoxy-PEG acts as an initiator and induces the ring-opening of lactide at the acyl-oxygen position [45]. The same procedure was successful for the synthesis of PLA-PEO [46], PCL-PEO [47] and PCL-PEO-PCL copolymers [48].

ABA-type copolymers with thermoplastic elastomer properties were recently prepared from poly(L-lactide) (A) and poly((R,S)-3-hydroxybutyrate) (B) [49]. In a preliminary step, a telechelic poly(hydroxybutyrate) was prepared by ring-opening polymerization of (R,S)-β-butyrolactone in the presence of 1,4-butanediol catalyzed by distannoxane. Subsequently, the

block copolymers were formed in the bulk by ring-opening polymerization of L-lactide using stannous octoate as catalyst. Mikos *et al.* reported the synthesis of tri-block copolymers of methoxy-PEG and poly(propylene fumarate) (PPF, Fig.6) by a transesterification reaction under vacuum at 160°C with antimony trioxide as catalyst [50]. PEO-PPF-PEO copolymers can be cross-linked *in situ* via the fumarate double bonds.



poly(propylene fumarate)

Figure 5. *Chemical structure of poly(propylene fumarate) (PPF).*

Biomedical applications

Degradable di- and tri-block copolymers are mostly investigated for the controlled delivery of drugs. These types of delivery systems offer many advantages as compared to conventional releasing formulations such as tablets. These include improved therapeutic activity, reduced intensity of side effects and minimized drug degradation and toxicity. In the last three decades, several types of controlled release systems have been considered:

Microspheres and nanospheres:

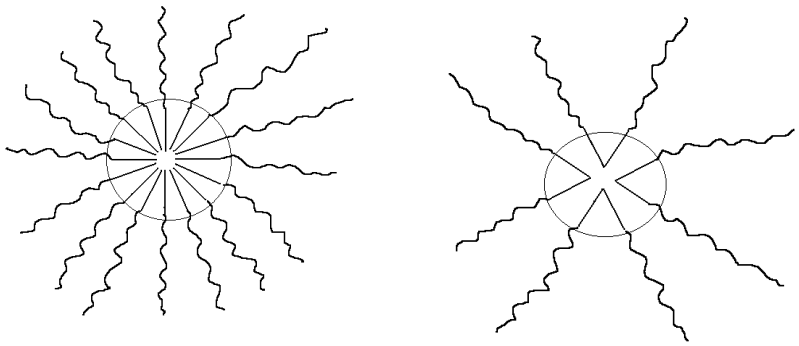
Di- and tri-block copolymers have found applications in the preparation of microspheres (1-1000 μm in size) and nanospheres (1-1000 nm in size) as colloidal drug carriers. These systems were designed to overcome the problems associated with the poor solubility of many drugs and to achieve long-time circulation. The drug can be suspended or dissolved in the core of the sphere; it can also be dissolved in the polymer matrix, or adsorbed to the particle surface [51]. It was shown that the entrapment of bovine serum albumin (BSA) in PLA-PEO microspheres was very efficient and that BSA was homogeneously dispersed in the matrix [52].

The *in vitro* release of several model proteins from erodible PLGA-PEO-PLGA microspheres was continuous and molecular mass-dependent, whereas a two phase profile was observed with PLGA particles [53]. The drug release profile was tunable by changing the block length of the PLGA-PEO-PLGA copolymers [54]. The protein release of PLA-PEO-PLA microspheres exhibited a release profile constituted of three phases. In comparison with PLA particles, the introduction of hydrophilic PEO blocks increased the hydrolysis rate of the microspheres [55].

The advantage of nanospheres over microspheres is their ability to circulate through the smallest capillaries (about 5 μm) [56]. Moreover, particles with a diameter of less than 200 nm (similar dimensions to natural carriers, such as lipoproteins and viruses [57]) are not filtered by the spleen [56]. In nanospheres, the molecular weight of the hydrophobic block is similar to or higher than the molecular weight of the hydrophilic block. Nanospheres have been prepared with PCL-PEO, PLGA-PEO and polyanhydride-PEO by Gref *et al.* [14]. Although a high entrapment of lidocaine could be achieved, the release of the drug *in vitro* was relatively fast. Human serum albumin, a common model protein, and tetanus toxoid, a vaccin, were also encapsulated efficiently in PLA-PEO di-block and tri-block nanospheres [58]. The low cellular up-take of these types of nanoparticles has been related to the presence of PEO segments at their surfaces [59]. In vivo study in rabbits showed that nanoparticles based on polyphosphazene and PEG had longer systemic circulation and lower liver uptake than PLGA nanoparticles [60].

Micelles:

Micelles are obtained with copolymers in which the length of the hydrophilic block exceeds the length of the hydrophobic block. Micelles have a mesoscopic size and a relatively narrow size distribution [57]. They are characterized by a core-shell architecture. The hydrophobic segments segregate from the aqueous surrounding and form an inner core, in which non-polar drugs can be incorporated. The hydrophilic segments constitute the shell, providing a stabilizing interface between the core and the aqueous medium. Micellar-based drug carriers possess many advantages, such as solubilization of poorly soluble drugs in their inner core, long-time circulation and accumulation by virtue of their small size in areas with leaky vasculature.



di-block copolymers tri-block copolymers

Figure 6. *Schematic architectures of micelles prepared from di- and tri-block copolymers.*

Di-block copolymers are the most appropriate candidates for micelle formation, although micelles can also be prepared from tri-block copolymers [61]. Many hydrophilic and hydrophobic segments have been combined to form core-shell structures [7,61]. Micelles prepared from PCL-PEO di-block copolymers have shown promising results in the delivery of hydrophobic drugs, such as neurotrophic agents [62] and indomethacin [63,64]. Recently, di-block and tri-block copolymer micelles entrapping plasmid DNA and oligonucleotides have been developed as non-viral desoxyribonucleic acid (DNA) delivery systems [65,66] in gene therapy. These copolymers, composed of a cationic segment (L-lysine) and a hydrophilic segment (PEG), spontaneously associate with polyanionic DNA to form micelles.

Hydrogels:

Some di- and tri-block copolymers can be used to prepare hydrogels, three-dimensional networks capable of absorbing large amounts of water or biological fluids [67]. Although hydrogels can be prepared and subsequently implanted in or injected into the body, stimuli sensitive polymers gelating *in vivo*, e.g. thermoreversible gels, have been the subject of much studies. Thermosensitive polymers can undergo sol-gel transitions upon cooling or heating. Several PEO-containing copolymers have been investigated for their gelation and hydrogel properties. Reversible gels were prepared from PLA-PEO [29] di-block and PEO-PLGA-PEO [68], PEO-PLA-PEO [69,70], PLGA-PEO-PLGA [32,71] tri-block copolymers.

Targeting systems:

The concept of pharmacologically active polymers was first introduced by Ringsdorf [72]. In the drug release field, synthetic polymers conjugated with a desired targeting compound (such as an antibody) can provide a drug delivery system capable of site-specific delivery. The synthesis of block copolymers with functional end-groups can facilitate the formation of such complex material surfaces and attachment of targeting moieties. Shakesheff *et al.* have prepared PEO-PLA di-block copolymer with biotinylated PEO end-groups [73,74]. α-Acetal-PEO-PLA copolymers were synthesized by ring-opening polymerization of ethylene oxide and D,L-lactide using potassium 3,3-diethoxypropanolate as an initiator [7]. The acetal moiety located at the PEG end can be converted into a reactive aldehyde group by treatment with a weak acid solution (pH 2). The aldehyde group can be used for drug or cell targeting later on.

Multi-block copolymers

The unique properties of multi-block copolymers originate from the presence of different blocks that organize through physical interactions. In multi-block copolymers, phase separation typically gives rise to a dispersed phase consisting of one block type in a

continuous phase of the second block type. Very often the dispersed phase consists of hard domains (crystalline or glassy), whereas the continuous phase is made of amorphous, soft segments providing flexibility to the material. Such materials are often referred to as thermoplastic elastomers. A schematic representation of the phase structure in multi-block copolymer is shown in Figure 7.

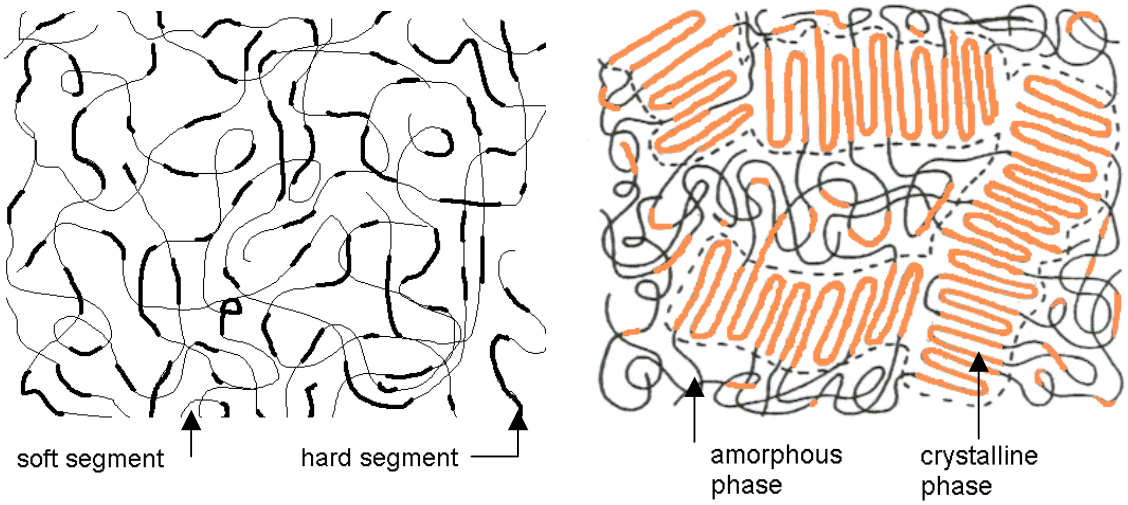


Figure 7. *Phases in multi-block copolymers in the melt (homogeneous) and upon cooling (phase separation).*

By changing the copolymer composition (e.g. block length, soft to hard segment ratio), polymers with a broad range of molecular structures, mechanical properties, thermal behaviors, degradation rates can be synthesized. In the body, the microdomains formed by such materials are presumed to play an important role in the interactions of those materials with cells, proteins and other biological elements [75-77]. The physical properties and degradation behavior of various multi-block copolymers have been investigated in view of their use in biomedical applications. But in contrast to di- and tri-block copolymers, only few studies have dealt with specific uses and devices.

Polyurethanes

Polyurethanes (PUs) are an appealing class of polymers for use in medicine [78-81], as they offer biocompatibility and good physical properties that can be tailored to a specific application [82]. Nevertheless, much research is still conducted to improve PU bulk properties, especially in terms of degradation/stability, and surface properties preventing thrombus formation and infection [83]. To overcome these problems, segmented polyurethanes have

been designed to match specific biomedical requirements. Segmented polyurethanes are usually prepared in a two-step process in the presence of a catalyst (e.g. dibutyl tin diacetate) and a solvent (e.g. dimethylsulfoxide). The first stage to obtain multi-block polyurethanes is the synthesis of the pre-polymer with an excess of diisocyanate, while reaction of a chain extender with the pre-polymer takes place in a second step [79,82,84]

Multi-block poly(ether urethane)s copolymers have good mechanical properties and hemocompatibility and are often used as cardiovascular graft [85]. Although these materials are frequently intended to be used for non-degradable devices [86], they have shown to be susceptible to oxidation [87,88]. Degradable segmented PUs are usually obtained by introduction of aliphatic polyesters or poly(ether ester)s. As an example, porous poly(ester urethane)s containing PCL have been studied as degradable scaffolds for meniscal reconstruction [89,90]. PCL-PEO-PCL tri-block copolymers were used as soft segments in the synthesis of degradable poly(ether ester urethane)s. By variation of the PEO content and length, these copolymers displayed a broad range of morphologies and properties [91,92]. Polymers made of non-crystallizable blocks of poly(glycolide-*co*-caprolactone) diol and crystallizable blocks of poly[(*R*)-3-hydroxybutyric acid-co-(*R*)-3-hydroxyvaleric acid] diol (PHBHV) coupled with 2,2,4-trimethylhexamethylene diisocyanate as a chain extender are promising as biodegradable elastomeric material for use as nerve guidance channels [93].

Polyesters

Many studies have been carried out on degradable random copolyesters, whereas only few multi-block copolyesters have been investigated. To our knowledge, only segmented copolyesters based on poly(lactic-glycolic acid) and poly(ε-caprolactone) were developed as biodegradable materials for use in drug delivery systems [94]. The biocompatibility and the biodegradability of the multi-block polyesters based on poly(hydroxybutyric acid), poly(hydroxyvaleric acid) and sebacic acid (DegraPol/bsc43® and DegraPol/bsd43®) were also confirmed by subcutaneous implantation of polymer films in rats [95].

Poly(ether ester)s

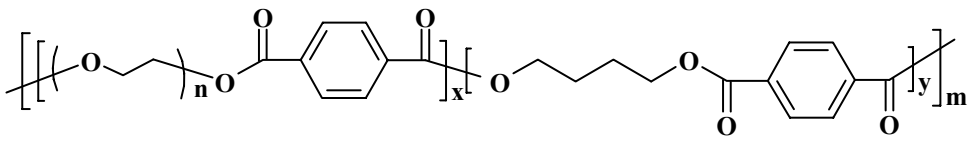
Like the di- and tri-block copolymers, multi-block copolymers based on PEG and PLA also constitute a large group of degradable polymers. Various preparation methods have been employed. The transesterification of PDLLA of low molecular weights with PEG at 190°C led to segmented copolymers with molecular weights ranging from 7,000 to 22,000 g/mol [96]. Higher molecular weight polymers (91,000-239,000 g/mol) were synthesized using PEO-

bis(chloroformate) (obtained after reaction of PEG with phosgene) and PLGA oligomers in chloroform in the presence of 4-dimethyl aminopyridine, as catalyst [97]. PLA-PEO multi-block copolymers were prepared by polycondensation of PLA diols and PEO diacids in dichloromethane using dicyclohexylcarbodiimide as coupling agent and dimethylaminopyridine as catalyst [98]. A similar procedure was applied in the synthesis of PCL-PEO multi-block copolymers [99]. Analogous to the synthesis of the tri-block copolymers, multi-block copolymers based on poly(propylene fumarate) (PPF) and PEG were also synthesized in an effort to develop a cross-linkable biodegradable material to be used for a vascular implant. Copolymers, with a maximum molecular weight of 15,000 g/mol, were prepared in the bulk by reaction of PEG with the pre-formed PPF catalyzed by antimony trioxide [100]. PPF-PEO multi-block copolymers can be cross-linked *in situ* and used as an injectable hydrogel [101] for bone tissue engineering [102] and as endothelial cell carrier [103].

An effective approach to obtain poly(ether ester)s with good thermal and mechanical properties is the incorporation of aromatic esters. A family of segmented copoly(ether ester)s prepared with PEG and poly(butylene terephthalate) (PBT) has shown to possess interesting physical and biological properties.

PEOT/PBT multi-block copolymers

First developed for textile applications [104], poly(ether ester) copolymers based on PEG and poly(butylene terephthalate) (PBT) (PEOT/PBT) block copolymers have also been introduced as biomaterials in the beginning of the eighties [105,106]. PEOT/PBT multi-block copolymers are thermoplastic elastomers (Fig. 8). Variation of the PEOT/PBT block copolymer composition and the molecular weight of the used PEG allows the synthesis of a family of copolymers with widely differing mechanical properties, swelling characteristics, degradation profiles and biological behavior.



PEOT 'soft segment' **PBT 'hard segment'**

Figure 8. *Chemical structure of PEOT/PBT multi-block copolymers.*

Synthesis

PEOT/PBT multi-block copolymers are prepared by a two-step polycondensation in the bulk in the presence of a catalyst and an antioxidant. The first step is the melt transesterification of PEG, dimethyl terephthalate (DMT) and a molar excess of 1,4-butanediol under a nitrogen atmosphere [107,108]. The excess of diol is used to accelerate the transesterification step (due to an increase in the reactant concentration) and to make sure that the reaction is driven to completion. In the second stage, the pressure is slowly decreased, whereas the temperature is gradually increased, to allow polycondensation. Depending on temperature, catalyst and excess of glycol, the polymerization is complete within a few minutes to several hours [104,108]. It has to be noted that terephthalic ester units are present in both the soft and the hard segments. Therefore, the notation PEOT (T for terephthalate) is used to refer to the soft part.

Although catalyst residues in the polymer might be toxic, they are necessary in the polymerization [109]. Titanium-based compounds are considered to be the most efficient catalysts for the synthesis of aromatic polyesters. Titanium compounds lead to a high rate constant both for the transesterification and the polycondensation steps. These compounds also catalyze degradation reactions. In the synthesis of poly(ethylene terephthalate) (PET), this usually requires the deactivation of the catalyst after the transesterification step and the use of a second catalyst for the polycondensation [110]. However, PBT is less sensitive to the metal-ion catalyzed β-elimination that yields depolymerization than PET [111]. Titanium compounds are therefore commonly used in the synthesis of PBT and its copolymers. Organic titanates, such as titanium tetrabutoxide ($Ti(OBu)_4$), can act alone [108,112] or in combination with magnesium or calcium acetate [113]. Complex titanates such as $Mg[HTi(OR)_6]_2$ derived from alkali or alkaline earth metal alkoxides and titanate esters are effective catalysts [104].

In the bulk polymerization of PEOT/PBT poly(ether ester)s, the use of antioxidant is essential to avoid thermal degradation. As antioxidant, Fakirov used Alurofen, an oligomer containing hindered phenol and secondary amino groups [108]. In another study six different antioxidants were compared [114]. Copolymers with the best properties were achieved with Plastanox 2246 (a bisphenol stabiliser) and BFSA-2 (Bulgarian Phenol Styrene Antioxidant). Hindered phenol derivatives, such as Irganox 1330 [114] and vitamin E [115], are also able to scavenge radicals during polymerization [116,117].

Structure and properties

The structure-property relationships of PEOT/PBT copolymers have been investigated in detail by Fakirov and co-workers. Depending on copolymer composition, PEOT/PBT can

segregate into four types of domains, namely amorphous PEO and PBT phases, a mixed amorphous phase and a PBT crystalline phase [108,112]. The different phases can be characterized by the presence of several thermal transitions, a high melting endotherm and a low temperature glass transition (T_g) in particular [108]. A second melting temperature is present, when the PEO-containing segments are long enough to crystallize [118,119]. According to Cella, crystallite size also depends on the method of sample preparation (processing, temperature). The increase in T_g with increasing hard segment content proves that the phase separation is not complete [107]. The analysis of the superstructure of the block copolymers indicated that the copolymers have a spherulitic crystalline structure [109]. For PEOT/PBT copolymers this structure consists of radial PBT lamellae with interradial amorphous regions, which are a mixture of PEO soft segments and uncrystallized PBT hard segments [118,120,121].

PEOT/PBT copolymers are viscoelastic materials and possess higher elastic deformation and elongation at break (approx. 500%) than the PBT parent polymer [108]. However, the copolymer composition has a large influence on the mechanical properties. An increase in PEG segment length at constant PBT block length or an increase in soft to hard segment ratio at constant PEG molecular weight cause a gradual change in the mechanical properties from plastic materials to elastomeric materials [108,112,120].

The presence of PEO segments makes PEOT/PBT copolymers hydrophilic. Some copolymers with high PEO content and/or long PEO length have high water-uptakes (up to 200 wt%) and have hydrogel-like properties. By tuning the copolymer composition, properties such as swelling, physical cross-link density and mesh size can be tailored [115].

PEOT/PBT degradation has been observed both *in vitro* [122,123] and *in vivo* [124-126]. Like the mechanical properties, the degradation rate is dependent on the copolymer composition. Copolymers with higher PEO content and/or PEO length degrade faster [127,128]. This is likely to be due to the increase in hydrophilicty of the copolymers and the increased accessibility of water to the hydrolyzable ester bonds, as suggested for PEO/PET [109,129]. The presence of two different labile bonds (ether and ester bonds) in the PEOT/PBT backbone makes these copolymers susceptible to both hydrolysis and oxidation. It is believed that the ester bonds connecting PEO segments and terephthalate units are the most sensitive to hydrolysis as in PEO/PET [109]. In addition, oxidative degradation of the PEO segments is believed to occur by a radical mechanism initiated at random along the length of the chain [130-132]. Implantation of medical devices provokes a foreign body response, during which activated cells such as macrophages release enzymes and superoxide anion radicals that can combine with protons to form hydroperoxide radicals [132]. It has been suggested that *in vivo* degradation of poly(ether urethane) elastomers, and in particular the aliphatic ether bonds in these polymers, involves phagocyte-derived oxidants [133]. Several *in vitro* studies confirm the

oxidative degradation of PEO by a radical mechanism initiated at random along the chain [130-132,134].

PEOT/PBT copolymers as biomaterials

PEOT/PBT multi-block copolymers are being commercialized as degradable materials for use in medicine under the brand name PolyActive™ by IsoTis NV (The Netherlands). PEOT/PBT multi-block copolymers were first mentioned as potential biomaterials in a patent by Wagener [105]. These materials contained hydantoin, a heterocyclic ring, in the PEO part. These moieties induce a raise in the glass transition temperature of the PEO-containing soft segment. The mobility of the PEO segment and its tendency to crystallize is then reduced. Moreover, hydantoin provides a chemically reactive site to incorporate other components, such as heparin, which can increase the non-thrombogenicity of the materials.

Biocompatibility is an absolute requirement for biomaterials. Several studies have demonstrated the excellent biocompatibility of PEOT/PBT copolymers in vitro [135,136] as well as in vivo after subcutaneous implantation and degradation in rats [124], goats [128,137] and rabbits [138].

PEOT/PBT copolymers were first investigated as candidates for an alloplastic tympanic membrane [139]. Growth of fibrous tissue and bone into PEOT/PBT copolymer implants resulted in appropriate implant fixation by the tissue [140]. The material was judged valuable as temporary scaffold for repair of tympanic membrane perforations and as a tympanic membrane in total alloplastic middle ear prosthesis (TAM) [141]. The mechanical properties of PEOT/PBT copolymers were suitable for thermoplastic coatings in long-term bone fixation where tissue ingrowth is desired. Moreover, as PEOT/PBT block copolymers with a high PEO content calcified *in vivo* [127,141,142] and exhibited good bone-bonding properties [143,144], they have been used in orthopedic applications [145]. Bakker suggested that, to achieve bone-bonding properties in prosthetic devices, PEG with molecular weights of 750 to 1,500 g/mol had to be used [145]. The flexibility and swelling of the copolymers allow the scaffold to fit the defect with tight bone contact. More recent work involving PEOT/PBT as a bone substitute in critical size defects in the iliac bone of goats and humans did not show the expected good bone-bonding and calcification behavior [146,147]. The critical size defects were not bridged. Reasons for this discrepancy with the earlier results in small animals can be: differences in regenerative capacity between the species, the size of the defect and the type of bone into which the substitute was implanted; cancellous bone has less initial bone to polymer contact than cortical bone [146-148], while implantations in goat femura, a cortical bone type, did show bone-bonding [149]. As bone fillers, these polymers seem, therefore, more suited for cortical bone defects than cancellous bone defects. Recently, bone marrow cell adhesion and growth

were shown to be excellent on gas plasma treated porous constructs prepared from these polymers [150]. PEOT/PBT copolymers are, therefore, good candidates as scaffolds in the engineering of bone.

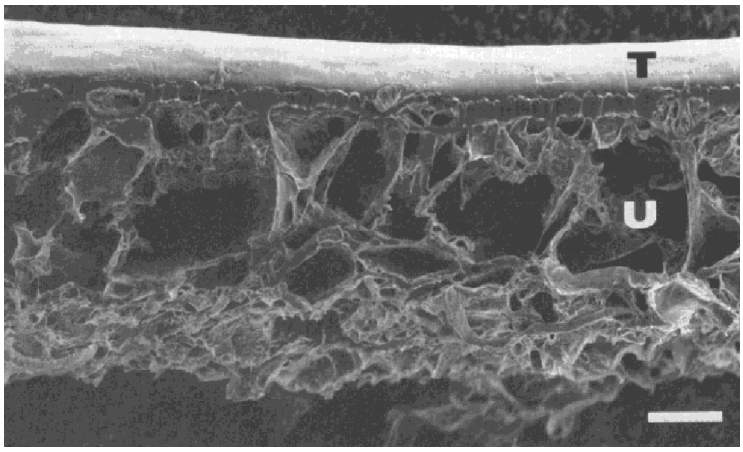


Figure 9. *Scanning electron micrograph of a PEOT/PBT bilayer structure for skin regeneration. T: dense toplayer. U: macroporous underlayer. Bar=40μm [151].*

PEOT/PBT copolymers have also been considered for skin regeneration due to good water vapor permeability and protein diffusion through copolymer films [152,153]. To overcome the lack of dermis in classical approaches to wound healing, a bilayered dermal analogue composed of macroporous PEOT/PBT with a dense toplayer layer has been proposed [154] (Fig.9). The copolymer prepared with PEG of 300 g/mol and containing 55 wt% soft segments (300 PEOT55PBT45) was considered the most suitable skin substitute, since fibroblasts could be seeded into the porous underlayer [155], while epidermal keratinocytes grew on dense layers of the 300 PEOT55PBT45 matrices [156].

Although PEOT/PBT copolymers can induce tissue ingrowth, methods have also been developed to use these copolymers in preventing adhesion of tissue to tissue or of tissue to bone [125,157]. The purpose of these devices is to separate adjacent internal surfaces by a degradable barrier, mesh or film, and to avoid contact between these surfaces during tissue regeneration following the surgical process. Cook also suggested that a barrier material based on PEOT/PBT copolymers might reduce perineural scar formation [158]. However, the use of PEOT/PBT in this application was contested as phagocytosis of small fragments of the material and inefficiency of the material in the long-term due to degradation were observed [159].

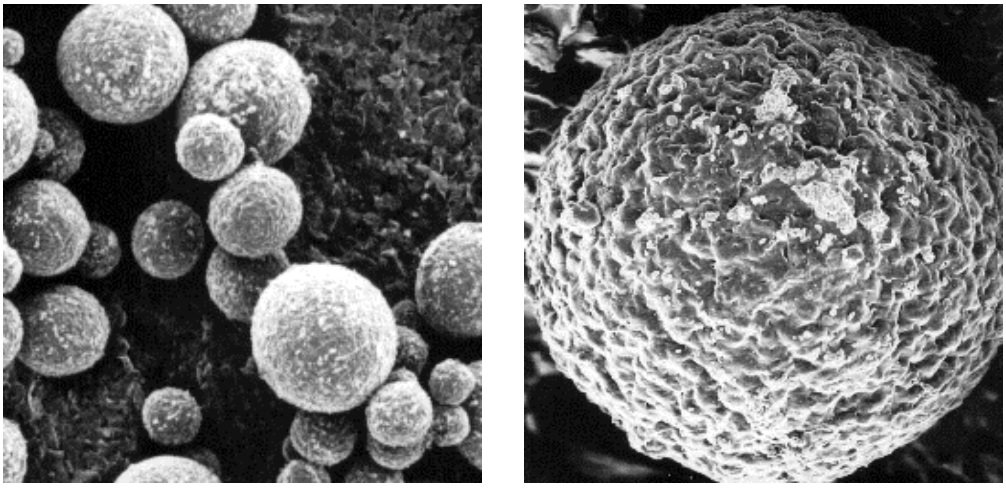


Figure 10. *Scanning electron micrographs of PEOT/PBT microspheres [160].*

Recently, it was shown that PEOT/PBT copolymers are very interesting materials for drug delivery vehicles, in particular microspheres [115,160] (Fig.10). The unique properties of these copolymers include complete and linear release profiles for proteins [161] and preservation of protein stability [162]. PEOT/PBT polymers can, therefore, be very effective matrix materials for the release of small compounds and macromolecules. Technologies based on PolyActive™, which are approved by the Food and Drug Agency (FDA) and the European Regulatory bodies (CE), are currently developed by Chienna BV, a subsidiary of IsoTis.

Conclusions

Block copolymers have proved to be highly valuable in the biomedical field, especially for drug delivery applications. Multi-block copolymers hold promises for use as scaffolds in tissue engineering, as their unique microstructure allows the synthesis of materials with versatile properties and the preparation of devices with a broad range of physical and biological properties and degradation profiles. Although PEOT/PBT multi-block copolymers have been much studied, several aspects have not been examined in detail such as their physical properties in the swollen state and the degradation pathways by hydrolysis and oxidation. The terephatlic moieties may be replaced by ester-amide units leading to poly(ether ester amide) thermoplastic elastomers. These materials also show phase separation and can be considered as an alternative to PEOT/PBT copolymers.

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Chapter 3

The PEO-Containing Phase in Poly(ether ester) Block Copolymers

'There are no facts, only interpretations.'
Friedrich Nietzsche (1844-1900)

Abstract

Segmented poly(ether ester)s based on poly(ethylene oxide) and poly(butylene terephthalate) (PEOT/PBT) are thermoplastic elastomers in which the poly(ethylene oxide) (PEO) segments provide hydrophilicity and flexibility to the material. Transmission electron microscopy and small angle X-ray scattering measurements showed PEOT/PBT phase separation both in the dry and the swollen state. Water-swollen copolymers of various compositions were studied by differential scanning calorimetry (DSC). These measurements revealed the existence of two types of water; (i) 'freezing water' that can crystallize upon cooling and (ii) 'non-freezing water'. Assuming that all 'non-freezing water' is bound to PEO, it was calculated that the number of water molecules per EO unit ranged from 0.3 and 2.9 for polymers with increasing PEO length or content.

The oxidative degradation of the poly(ether ester)s in contact with solutions containing hydrogen peroxide (5% H_2O_2) and various amounts of cobalt chloride ($CoCl_2$) was also studied. The decreases in intrinsic viscosity, mechanical properties and PEO content of PEOT/PBT copolymers confirmed the oxidative degradation of the materials. After exposure to 5% H_2O_2 solutions (without $CoCl_2$), a slower decrease in mechanical properties was measured for copolymers with the highest PEO content. This points to the simultaneous occurrence of chain scission and macroradical recombination. Degradation of PEOT/PBT in the dry state with increasing temperature was detected by DSC. DSC traces of copolymers not containing antioxidant showed up-turns, most probably due to thermal oxidation of the material, which induces a decrease in molecular weight with increasing temperatures.

Introduction

The presence of water has a large influence on the properties of polymeric systems that contain hydrophilic moieties such as poly(ethylene oxide) (PEO). These include mechanical properties [1], degradation profiles [2,3], drug release behavior [4,5] and protein adsorption onto the surface [6-8]. The different states of water in natural and synthetic polymers have been extensively studied [9-18]. Although controversy still remains concerning the actual existence of different types of water and their formation mechanisms [19,20], water in polymers is usually categorized into two or three types [9,10,21-23]: (i) 'freezing water', which crystallizes at the same freezing point as pure water, (ii) 'intermediate water', which crystallizes below the normal freezing point (i and ii are sometimes considered as only one category) and (iii) 'non-freezing water', which is not able to form crystals. In the case of polymers containing hydrophilic moieties, the 'non-freezing water' can originate from hydrogen bonding between water molecules and polar groups in the polymers [22,23].

Poly(ethylene oxide) (PEO) is one of the most studied hydrophilic polymers and is used in many industrial and medical applications. PEO exhibits many attractive properties including solubility in water and various organic solvents, low glass transition temperature, non-toxicity and non-antigenicity [24] and resistance to protein adsorption when present at a polymeric surfaces [25]. Consequently, PEO has been used to modify existing polymers in order improve their hydrophilicity [26,27], biocompatibility [28-31], and degradability [3,32-36].

Due to the presence of labile ether bonds, PEO is sensitive to oxidation. Kaczmarek *et al.* suggested that PEO is oxidized by radicals generated chemically or photochemically from hydrogen peroxide H_2O_2 [37]. PEO chain scission by both hydroxyl radicals HO• and hydroperoxide radicals $HO_2^{\bullet}$ may occur. A study of the effects of γ-irradiation on methoxy-poly(ethylene glycol) (PEG) in water showed the simultaneous occurrence of oxidative scission and cross-linking of the polymer [38]. These competitive reactions are dependent on the pH of the irradiated solution: scission is predominant when the solutions are acidic. It has been suggested that oxidative degradation of the PEO segments in copolymers occurs by a radical mechanism initiated at random along the length of the chain [39-41]. Oxidative degradation can also occur in the body. Implantation of medical devices provokes a foreign body response, during which activated cells such as macrophages release hydrolytic enzymes, oxygen radicals and hydrogen peroxide directly onto the surface of the device [41]. *In vivo*, radicals can initiate and propagate oxidation reactions in the polyether part of polymers.

Segmented block copolymers based on PEO and poly(butylene terephthalate) (PEOT/PBT) have been considered as biomaterials for various medical applications, including tissue engineering [42,43] and controlled drug release [44-46]. The PEO-containing segments provide hydrophilicity to the material and are susceptible to oxidation due to the presence of ether

bonds. A mechanism for PEOT/PBT thermo-oxidation has been recently proposed [47]. It involves the selective oxidation of ether parts leading to bond cleavage and formation of new ester groups. In the present work, the phase separation in PEOT/PBT copolymers in the dry and water-swollen state, as well as the thermal behavior of the polymers at equilibrium water-uptake were investigated. The oxidative degradation of films in contact with solutions containing 5% H_2O_2 and various amounts of cobalt chloride was studied. Thermo-oxidation of antioxidant-free copolymers in the dry state was detected by DSC.

Materials and Methods

Materials

Poly(ethylene glycol) of different molecular weights (PEG 300, PEG 1000 and PEG 4000) and PEO 300,000 (Fluka, Switzerland), dimethyl terephthalate (DMT) (Merck, Germany), 1,4-butanediol (Acros organics, Belgium), titanium tetrabutoxide ($Ti(OBu)_4$) (Merck, Germany) and Irganox 1330 from (Ciba-Geigy, Switzerland) were used without further purification. All solvents used were analytical grade (Biosolve, the Netherlands).

Polymer synthesis

PEOT/PBT segmented block copolymers were prepared by a two-step polycondensation in the presence of titanium tetrabutoxide as catalyst (0.1 wt%) and Irganox 1330 as antioxidant (1 wt%). The transesterification of PEG, DMT and 1,4-butanediol (two-fold excess) was carried out under a nitrogen atmosphere at 180°C. After two hours, the pressure was slowly decreased from 1000 mbar to 0.1 mbar to allow polycondensation. Simultaneously the temperature was increased from 180 to 240°C. The copolymers were purified by dissolution in chloroform and precipitation into an excess of ethanol.

The composition of the block copolymers is indicated as *a* PEOT*b*PBT*c*, in which *a* is the starting PEG molecular weight, *b* the weight percentage of PEOT soft segments and *c* the weight percentage of PBT hard segments. It has to be noted that terephthalic ester units are present in both the soft and the hard segments. Thus, the notation PEOT (T for terephthalate) refers to the soft segment.

Polymer characterization

The intrinsic viscosity [η] of the copolymers in chloroform (solution of approximately 0.3 g/dL) was estimated by single point measurements [48,49] at 25°C using an Ubbelohde OC viscometer.

The polymer composition was determined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). 300 MHz ^{1}H-NMR (Varian Inova 300 MHz, USA) spectra were recorded using polymer solutions in deuterated chloroform (Sigma, Germany).

Transmission electron microscopy

Transmission electron micrographs (TEM) were obtained using a Philips CM30 (The Netherlands) electron microscope. 1000 PEOT71PBT29 coupes (approximately 50 nm thick) were cut with a microtome Leica EM FCS (Germany) and an ultramicrotome diamond knife (Drukker International, The Netherlands). The coupes were stained in a desiccator over osmium tetroxide solution in water (OsO_4 4wt%, Aldrich, Germany) for 3 hours.

Small angle X-ray scattering

Small angle X-ray scattering (SAXS) measurements were performed using a NanoStar device (Bruker AXS, Germany) with a ceramic fine-focus X-ray operated in point focus mode. The tube was powered with a Kristalloflex K760 generator at 35 kV and 40 mA. The primary beam was collimated using cross-coupled Göbel mirrors and a 0.1-mm pinhole providing a CuK_α radiation beam (λ = 0.154 nm) with a full-width at half-maximum about 0.2 mm in diameter at the sample position. The sample-detector distance was 103 cm. A Hi-Star position-sensitive area detector (Siemens, Germany) was used to record the scattering intensity in the q-range 0.1 to 1.5 nm^{-1}. The scattering vector q is defined as:

$$q = \frac{4\pi}{\lambda} * \sin\frac{\theta}{2} \qquad (1)$$

where λ is the wavelength and θ is the scattering angle. The measurements were performed at ambient conditions using a metal sample chamber with two thin Kapton windows. Polymer samples were cut from melt-pressed polymer films with a thickness of approximately 0.5 mm. Measurements were performed on dry samples and on equilibrium water swollen samples.

Thermal behavior

The thermal transitions of the swollen copolymers were determined by differential scanning calorimetry (DSC) using a Pyris 1 (Perkin Elmer, USA). The dry copolymer samples (5-10 mg) were weighted precisely and allowed to swell in distilled water at room temperature until equilibrium water-uptake. The excess of water was then gently removed using absorbing tissue. The samples were weighed again to determine the water-uptake. Finally, the samples were heated from -100 to 80°C at a heating rate of 10°C/min. The heating of the sample was followed by a cooling scan from 80 to -100°C at a cooling rate of 10°C/min and a second

heating scan under the same conditions. The glass transition temperatures were taken as the midpoint of the heat capacity change and the melting temperatures were determined from the maximum in the melting endotherm. Cyclohexane, indium, gallium and tin were used as standards for temperature calibration.

The thermal properties of dry copolymers (with or without antioxidant) were evaluated by DSC using a Perkin Elmer DSC 7 (USA). The copolymer samples (5-10 mg) were placed in stainless steel pans and heated from -80 to 250°C at a heating rate of 10°C/min. The samples were then quenched (300°C/min) to -80°C and after 5 min a second scan was recorded. The data presented are from the second heating scan. The glass transition temperatures were taken as the midpoint of the heat capacity change, the melting temperatures were determined from the maximum in the melting endotherm. Indium and gallium were used as standards for temperature calibration.

Oxidative degradation

Films (50-100 μm thick) cast from polymer solutions in chloroform were oxidatively degraded at 37°C in 5% (v/v) H_2O_2 solutions in water (30% H_2O_2 from Merck, Germany) containing various amounts of cobalt chloride ($CoCl_2 \cdot 6\ H_2O$, Aldrich, Germany) [50]. Oxidized samples were analyzed in duplicate in terms of intrinsic viscosity and chemical composition.

Mechanical testing

Tensile testing was performed on swollen (non)degraded PEOT/PBT block copolymer films. Specimens were cast from chloroform solution (50-100 μm thick) and were cut according to ASTM D882-91 specifications (100 x 5 mm^2). Tensile tests in triplicate were carried out at room temperature using a Zwick Z020 (Germany) universal tensile testing machine operated at a crosshead speed of 50 mm/min using a 0.01 N pre-load and a grip-to-grip separation of 50 mm. The specimen elongation was derived from the grip-to-grip separation.

Results and Discussion

Transmission electron microscopy

The occurrence of phase separation in dry PEOT/PBT copolymers at a nanometer scale was observed by transmission electron microscopy (TEM). A TEM micrograph of a 1000 PEOT71PBT29 sample is shown in Figure 1. The PEO-containing soft domains are considered to absorb the staining agent and are visible as dark areas, in contrast to the lighter

PBT domains [51]. Structures with dimensions ranging from approximately 5 nm to 20 nm are observed. The size of the domains observed by TEM is in accordance with the domain sizes measured by SAXS in the dry state.

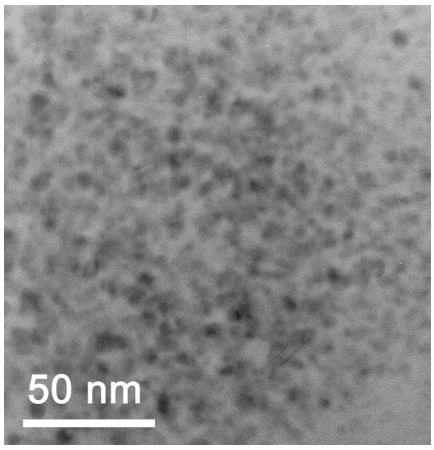

Figure 1. *TEM image of 1000 PEOT71PBT29 stained with OsO_4 vapor for 3hrs.*

Small angle X-ray scattering

Small angle X-ray scattering (SAXS) was used to study phase separation in isotropic PEOT/PBT copolymers both in the dry and swollen state. In intensity versus q-vector plots, scattering maxima could be seen for the compositions studied. This indicates the presence of phase separated domains. The peaks were relatively broad, implying a large distribution of domain sizes. A long period *L*, which is a measure for the sum of the hard and soft domain sizes, can be extrapolated from the scattering data (Table 1):

$$L = \frac{2\pi}{q} \tag{2}$$

Table 1. *The q-vector (q), long period (L) and intensity (I) for PEOT/PBT copolymers in the dry and swollen state.*

Composition	PEO content (wt%)	Dry sample			Swollen sample			Water-uptake (wt %)
		q, Å^{-1}	L, Å	I, a.u.	q, Å^{-1}	L, Å	I, a.u.	
1000 23/77	22	0.025	250	39	0.028	262	39	26
1000 71/29	62	0.034	185	6	0.021	299	6	60
300 68/32	45	0.046	137	3	0.046	137	3	4

The long period of copolymers in the dry and swollen state increased with an increase in PEO length or in PEO content. In the swollen state, the long period increased also with

increasing water-uptake, as seen in Table 1. In PEOT/PBT copolymers, water will be preferentially present in the PEO-containing phase, since PBT segments are hydrophobic (PBT absorbs 1.5% of water). The intensity of the scattering maxima remained constant with water-uptake (Table 1), suggesting that there is only little change in density difference between the domains after water-uptake (PEG density: 1.15 g/cm^3, PBT density: 1.35 g/cm^3).

DSC measurements on water-swollen PEOT/PBT copolymers

The DSC thermograms of two series of PEOT/PBT copolymers at equilibrium water-uptake are shown in Figure 2. The DSC traces of copolymers prepared with PEG 1000 but with different soft to hard segment ratios are presented in Figure 2A, whereas Figure 2B represents the traces of PEOT/PBT copolymers with a soft to hard segment ratio of approximately 70 to 30, but synthesized with PEG of different molecular weights. One or two endothermal peaks are visible between -10°C and -30°C. These peaks are due to the crystallization of water, since crystallization of pure water upon cooling at 10°C/min is detected at -22°C with a crystallization enthalpy of 278.1 J/g. This measured enthalpy is in agreement with values found in literature [16]. The percentage of 'freezing water' (w_f) can be calculated from:

$$w_f(\%) = \frac{\Delta H}{\Delta H^0} * 100 \qquad (3)$$

where ΔH is the measured heat of fusion and ΔH^0 the heat of fusion of pure water (ΔH^0 = 278.1 J/g). The values of ΔH and w_f are summarized in Table 2.

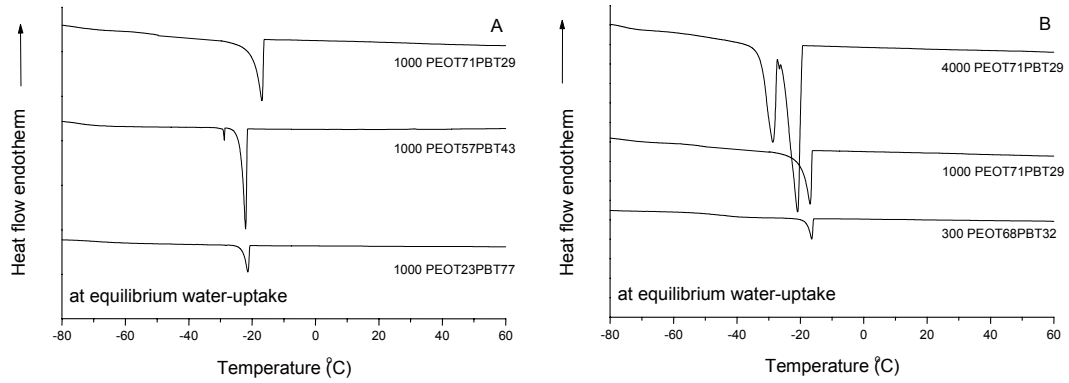

Figure 2. *DSC thermograms (upon cooling at 10°C/min) of PEOT/PBT copolymers prepared with (A) different soft to hard segment ratios (at a constant PEG molecular weight) and (B) with different PEG molecular weights (at a constant soft to hard segment ratio) in the swollen state at equilibrium water-uptake.*

The percentage of 'freezing water' increases with water-uptake and is, therefore, directly related to the PEO content in the copolymers. Two peaks can be distinguished in the DSC traces of 4000 PEOT71BT29. Similar DSC traces showing two crystallization exotherms were observed for PEO-water mixtures when the molecular weight of PEO was higher than 1000 g/mol. The lower temperature exotherm was attributed to crystallization of an eutectic mixture [17,52].

Table 2. *PEO content, water-uptake, glass transition temperatures in the dry and swollen state, heat of fusion (ΔH) and percentage of 'freezing water'* (w_f)*, and number of water molecules per EO unit in PEOT/PBT copolymer at equilibrium water-uptake.*

Polymer	PEO content[a]	Water-uptake	$T_{g\ dry}$[b]	$T_{g\ swollen}$[b]	ΔH[c]	w_f	H_2O/EO[d]
	wt%	wt%	°C	°C	J/g	%	
1000 71/29	62	56	-57	-81	25.9	9.3	2.0
1000 57/43	49	30	-52	-75	17.3	6.2	1.4
1000 23/77	20	9	-45	-66	4.3	1.5	1.1
4000 71/29	69	114	-60	-71	78.6	28.3	2.9
1000 71/29	62	56	-57	-81	25.8	9.3	2.0
300 68/32	45	6	-27	-39	3.8	1.4	0.3

a. determined by ^{1}H-NMR
b. second heating scan
c. cooling scan
d. number of water molecules per EO unit (mol/mol)

As seen in Table 2, only a fraction of the water crystallizes upon cooling. This observation points to the presence of two types of water: (i) 'freezing water' (w_f) that can crystallize and (ii) 'non-freezing water' ($w_{nf}=100-w_f$). Assuming that the 'non-freezing water', w_{nf}, is bound to the ethylene oxide (EO) units via hydrogen bonding, the number of water molecules per EO unit (mol/mol) can be determined. The resulting values are presented in Table 2. The number of water molecules per EO unit increases with increasing PEO content and/or PEO length. These results are in accordance with an increase in molecular weight between cross-links and mesh size of the PEOT/PBT network when the PEO content and PEO length increases [53]. In a recent study dealing with PEO homopolymer, it was shown that the maximum hydration number per EO unit depends on the PEO molecular weight. The hydration numbers were estimated to be 1.6, 2.4 and 3.3 for PEG 400, PEG 1540 and PEO 70 000, respectively [17]. Although the hydration numbers determined for the PEO segments in PEOT/PBT copolymers are close to the data of PEO homopolymers in literature, they are slightly lower, ranging from 0.3 to 2.9 water molecules per EO unit. This can be explained by the presence of terephthalate moieties in the PEO-containing segment and butylene terephthalate segments, which leads to incomplete phase separation.

The swollen copolymers have a lower glass transition temperature (T_g) than the dry copolymers. The values are reported in Table 2. The water molecules absorbed in the polymer matrix interact with the polymer chains and plasticize the polymer by disrupting the inter-chain interactions. With the exception of 4000 PEOT71PBT29, the effect is larger with increasing PEO content and water-uptake.

Degradation in oxidative media

In a similar way to poly(ether urethane)s, PEOT/PBT block copolymers can undergo oxidative degradation due to the presence of ether bonds in the soft segments. Solutions of 5% H_2O_2 in water containing various amounts of $CoCl_2$ were chosen to generate radicals *in vitro* [54]. As described, $CoCl_2$ favors the formation of hydroxyl radicals from the hydrogen peroxide through a Haber-Weiss reaction:

$$Co^{2+} + H_2O_2 \rightarrow Co^{3+} + HO^- + HO\bullet \quad (4)$$

Table 3 summarizes the changes in composition, intrinsic viscosity and mechanical properties of 300 PEOT70PBT30 during degradation in the oxidative media. To exclude a possible influence of hydrolytic degradation, polymers in contact with water were used as control. In that case, both the copolymer composition and the intrinsic viscosity did not change during 5 days of contact.

The decreases in intrinsic viscosity, maximum stress and elongation at break as a function of time are larger with increasing $CoCl_2$ concentration. In the presence of $CoCl_2$, the copolymers lost their mechanical properties after a few days and the intrinsic viscosities became very low. The catalytic effect of $CoCl_2$ on the oxidative degradation of PEO in acetonitrile and of PEO films has been described before [55]. From the results, it can be concluded that PEOT/PBT copolymers are sensitive to oxidation and that the rate of polymer degradation increases with increasing $CoCl_2$ concentrations. At the same time, significant changes in copolymer composition were detected with a decrease in soft segment content and in PEO content. This can be the cause for the transient increase in the *E*-modulus.

Table 3. *Change in composition, intrinsic viscosity [η], E-modulus (E), maximum stress (σ_{max}) and elongation at break (ε_{break}) of 300 PEOT70PBT30 during degradation in 5% H_2O_2 containing $CoCl_2$ at 37°C. Mechanical properties of copolymers in the swollen state were measured.*

[$CoCl_2$] M	Time days	Composition[a]	[η][b] dL/g	*E* MPa	σ_{max} MPa	ε_{break} %
0	0	70/30 (49)	0.70	50	5.3	75
	1	70/30 (48)	0.55	51	5.3	40
	2	69/31 (48)	0.50	53	4.5	19
	3	69/31 (48)	0.48	53	4.5	15
	4	68/32 (47)	0.48	58	4.6	14
	5	67/33 (46)	0.39	54	4.2	13
0.0005	0	70/30 (49)	0.70	50	5.3	75
	1	65/35 (45)	0.49	54	4.5	17
	2	60/40 (42)	0.22	76	3.0	6
	3	63/37 (42)	0.21	– [c]	–	–
	4	59/41 (41)	0.18	–	–	–
	5	59/41 (39)	0.15	–	–	–
0.05	0	70/30 (49)	0.70	50	5.3	75
	1	62/38 (43)	0.17	56	2.8	7
	2	59/41 (39)	0.14	– [c]	–	–
	3	56/44 (37)	0.11	–	–	–
	4	52/48 (33)	0.09	–	–	–
	5	33/77 (20)	0.11	–	–	–

a. soft to hard segment ratio (PEO content, wt%) c. tensile testing not possible

b. solvent: chloroform at 25°C

To examine the effect of copolymer composition on the oxidative degradation behavior, 1000 PEOT70PBT30, 300 PEOT70PBT30 and 300 PEOT50PBT50 were also exposed to 5% H_2O_2 not containing $CoCl_2$. PEO 300000, which is soluble in 5% H_2O_2, was used as a reference material. The results are presented in Table 4. Figures 3 shows the relative elongation at break for the copolymers as a function of time. For all the polymers, the decrease in intrinsic viscosity was faster in the initial stage (1 day) than at later stages. After 2 days in contact with the 5% H_2O_2 solution, the decrease in intrinsic viscosity became slower (Table 4). The copolymer composition and *E*-modulus changed very little in time. A significant decrease in maximum stress and elongation at break was observed for the copolymers prepared with PEG 300, whereas these parameters remained almost constant for 1000 PEOT70PBT30. PEO 300000 degraded very rapidly.

Table 4. *Change in composition, intrinsic viscosity [η], E-modulus (E), maximum stress (σ_{max}) and elongation at break (ε_{break}) of 1000 PEOT70PBT30, 300 PEOT70PBT30, 300 PEOT50PBT50 and PEO 300 000 during degradation in 5% H_2O_2 (without $CoCl_2$) at 37°C. Mechanical properties of copolymers in the swollen state were measured.*

Polymer	Time days	Composition[a]	$[\eta]$[b] dL/g	E MPa	σ_{max} MPa	ε_{break} %
PEO 300,000	0	–[c]	2.41	–[d]	–[d]	–[d]
	0.13 (3hrs)	–	1.87	–	–	–
	0.75 (18hrs)	–	0.56	–	–	–
	1	–	0.4	–	–	–
	2	–	0.3	–	–	–
	3	–	0.19	–	–	–
	4	–	0.15	–	–	–
1000 PEOT70PBT30	0	70/30 (62)	1.30	22	8.1	1010
	1	70/30 (62)	1.15	23	10.4	1220
	2	71/29 (62)	1.21	15	7.9	930
	3	70/30 (61)	1.16	22	6.8	1120
	4	69/31 (62)	1.04	16	7.1	950
	5	71/29 (62)	0.86	22	7.7	1080
300 PEOT70PBT30	0	70/30 (49)	0.70	50	5.3	75
	1	70/30 (48)	0.55	51	5.3	40
	2	69/31 (48)	0.50	53	4.5	19
	3	69/31 (48)	0.48	53	4.5	15
	4	68/32 (47)	0.48	58	4.6	14
	5	67/33 (46)	0.39	54	4.2	13
300 PEOT50PBT50	0	50/50 (35)	0.98	215	14.5	460
	1	50/50 (35)	0.58	226	12.7	24
	2	49/51 (34)	0.57	196	12.4	16
	3	49/51 (34)	0.53	158	9.1	7
	4	48/52 (33)	0.36	191	7.3	5
	5	49/52 (33)	0.3	215	5.7	4

a. soft to hard segment ratio (PEO content, wt%)
b. solvent: chloroform at 25°C
c. PEO homopolymer
d. PEO is soluble in 5% H_2O_2

It is expected that oxidation occurs preferentially in the PEO segments. It is therefore envisaged that copolymers with a higher PEO content are more prone to chain scission. The mechanical properties of PEOT/PBT copolymers, particularly the elongations at break, decrease with decreasing molecular weight [56]. As the mechanical properties of the copolymers prepared with PEG 300 decrease in time, it is likely that chain scission has occurred in these copolymers. 1000 PEOT70PBT30 behaves differently. The mechanical

properties of 1000 PEOT70PBT30 did not change despite lower intrinsic viscosities. This differs from the data presented in Chapter 4, where it is shown that water-swollen 1000 PEOT70PBT30 with an intrinsic viscosity of 0.87 dL/g has a maximum stress of 3 MPa and an elongation at break of approximately 100%. The discrepancy between the data indicates that in 5% H_2O_2 other reactions occur beside chain scission.

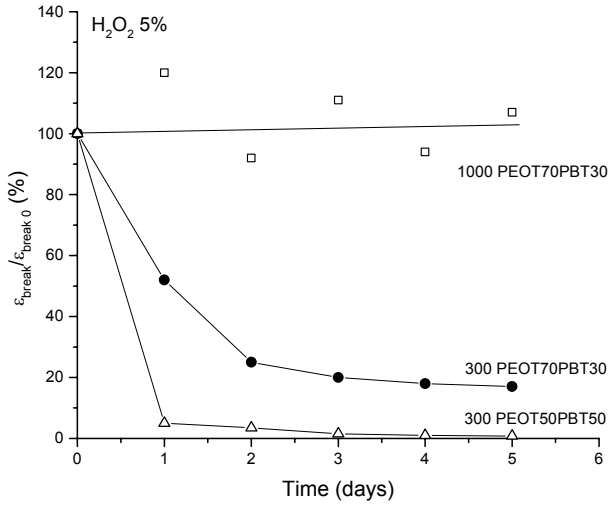

Figure 3. *Relative elongation at break as a function of time in 5% H_2O_2 for* (□) *1000 PEOT70PBT30,* (●) *300 PEOT70PBT30,* (△) *300 PEOT50PBT50 in the swollen state and* (◆) *PEG 300,000. The lines drawn in the graphs are guides for the eye.*

The results can be explained by macroradical recombination of the PEO segments. In the presence of hydroxyl radicals (HO•), two simultaneous reactions involving the PEO segments can take place. These reactions are illustrated in Figure 4. After hydrogen abstraction on the α-position of the carbonyl by HO•, the radical formed on the polymer backbone can either react with a second HO•, which initiates chain scission, or with a macroradical formed in another PEO segment leading to cross-linking and branching. The occurrence of simultaneous degradation and macroradical recombination was also seen during irradiation of polyethylene [57] and PEO in the dry and swollen state [55]. In the case of swollen PEOT/PBT copolymers, the ratio between chain scission and macroradical recombination may be controlled by the HO• concentration and a 'cage effect' due to dimensional restrictions in the hydrophilic phase. In the swollen state, the macroradicals are trapped in a matrix in which the mobility and diffusion of the segments are restricted. This 'cage effect' can lead to recombination reactions and is likely to be more pronounced in the case of 1000 PEOT70PBT30 that shows better phase separation than the other two copolymers (see Chapter 4). In the copolymers, the

probability of recombination is higher than in PEO 300 000, which is completely soluble in hydrogen peroxide solution. In solutions containing $CoCl_2$, the higher concentration of HO• radicals may lead predominantly to chain scission.

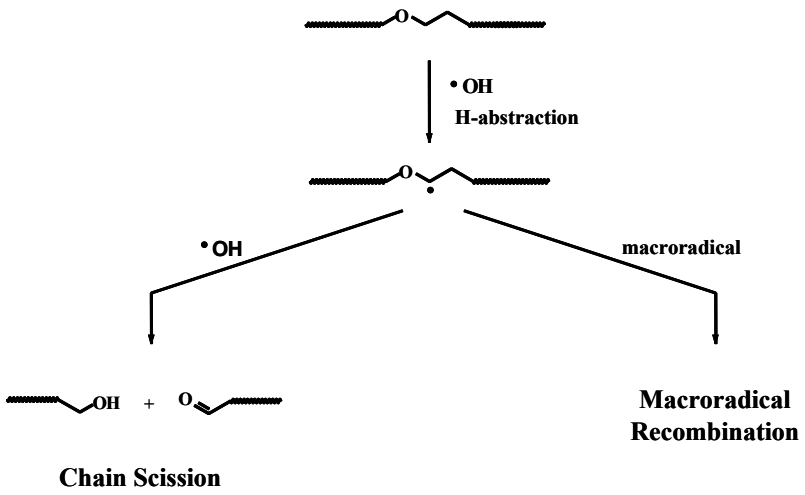

Figure 4*. Schematic representation of two possible radical mechanisms involving PEO segments in oxidative media.*

Thermal degradation

Most polymers are sensitive to thermal degradation due to the formation of radicals [47,58-62]. This results in a decrease in molecular weight. Thermal degradation of polymers has been the focus of numerous studies [61,63-67] as it can be encountered during polymer synthesis and processing in the melt.

During thermal analyses of PEOT/PBT block copolymers, the DSC traces showed a significant up-turn with increasing temperatures. Such up-turns were not observed when unpurified polymers still containing antioxidant (Irganox 1330) were analyzed (Fig.5). This is also observed for other copolymer compositions and is most likely related to thermal degradation of the polymer sample. Dickie noticed a similar behavior for polyurethane [68], in which the increase in the heat capacity C_p during the DSC measurements could be related to decrease in molecular weight.

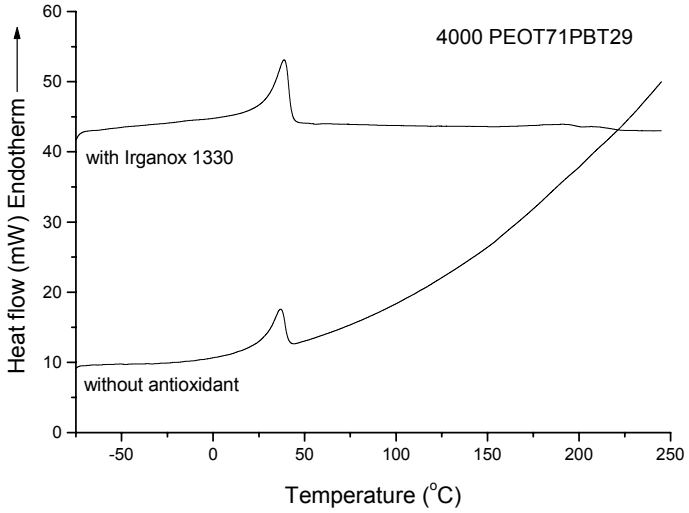

***Figure 5**. DSC traces of 4000 PEO70PBT30 either with Irganox 1330 or without antioxidant.*

Conclusions

PEOT/PBT segmented block copolymers are thermoplastic elastomers in which the PEO-containing segments provide hydrophilicity and flexibility. Microphase separation in the copolymer was confirmed using TEM techniques and SAXS. The long period increases for copolymers in the swollen state with long PEO segments or high PEO content. Two different states of water could be detected by DSC for the water-swollen copolymers: 'freezing' and 'non-freezing water'. Assuming that all 'non-freezing water' is bound to PEO, it was calculated that the number of water molecules per EO unit ranged from 0.3 and 2.9 for polymers with increasing PEO length or content.

PEOT/PBT block copolymers in contact with solutions containing H_2O_2 and $CoCl_2$ underwent oxidative degradation. An increase in $CoCl_2$ concentration causes more rapid decreases in intrinsic viscosity, mechanical properties and PEO content. To examine the effect of the copolymer composition, oxidation was studied with copolymers in a 5% H_2O_2 solution (no $CoCl_2$). Under these conditions, the decrease in intrinsic viscosity and mechanical properties was less pronounced with increasing PEO content. This phenomenon may be caused by simultaneous occurrence of chain scission and recombination of macroradicals in the PEO phase. Degradation also took place during DSC measurements of samples free of antioxidant. DSC traces of copolymers not containing antioxidant showed up-turns, most probably due to a decrease in molecular weight with increasing temperature.

Acknowledgments

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Chapter 4

PEOT/PBT Segmented Block Copolymers:
The Effect of Copolymer Composition on Physical Properties and Degradation Behavior*

'Believe those who are seeking the truth. Doubt those who find it.'
Andre Gide (1869-1951)

Abstract
In this study the influence of copolymer composition on the physical properties and the degradation behavior of thermoplastic elastomers based on poly(ethylene oxide) (PEO) and poly(butylene terephthalate) (PBT) segments is investigated. These materials are intended to be used in medical applications where degradability of the implant is desired. PEOT/PBT copolymers are microphase separated and up to four thermal transitions are measured by DSC. Phase separation in the system is enhanced by increasing the molecular weight of starting poly(ethylene glycol) (PEG) or by increasing the PBT content. The mechanical properties, swelling characteristics and degradation rates of the copolymers are influenced by the phase separation. By changing the PEOT/PBT composition, tensile strengths vary from 8 to 23 MPa and elongations at break from 500 to 1300%. Water-uptake ranges from 4 to 210%. The *in vitro* degradation of PEOT/PBT copolymers occurs via hydrolysis and oxidation. In both cases degradation is more rapid for copolymers with high contents of PEO. Deterioration of copolymer films takes place when the films are exposed to light in the absence of antioxidant. In preventing oxidation under daylight conditions, Irganox 1330 turned out to be a more efficient antioxidant for the copolymers than vitamin E.

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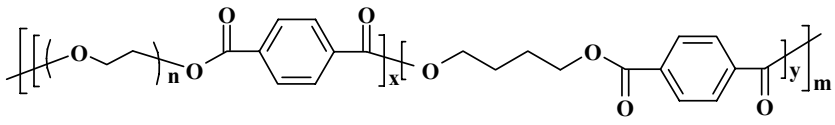
Introduction

Polymers to be used as implant materials should be biocompatible and their mechanical properties, water absorption and rates of degradation have to be optimal for a particular application.

Poly(ethylene oxide) (PEO) and poly(ethylene glycol) (PEG) are hydrophilic, semi-crystalline polyethers with a glass transition temperature below room temperature. They are biocompatible, non-toxic, non-antigenic and non-immunogenic [1]. Therefore, PEG has been frequently used to modify the surface of a variety of polymers in order to decrease protein adsorption and to improve their biocompatibility [2-5]. Materials combining these properties with *in vivo* degradability have been prepared by copolymerization of lactones and PEG [6-8]. Such block copolymers have been investigated for use as drug delivery systems [9,10] and scaffolds in tissue engineering [11,12].

First developed for textile applications [13], PEOT/PBT block copoly(ether ester)s (Fig. 1) have shown to possess interesting physical properties for medical use as well [14]. *In vitro*, epidermal keratinocytes, dermal fibroblasts [15], skeletal muscle cells and chondrocytes [16] showed good adhesion and proliferation on PEOT/PBT copolymer films. *In vivo* no adverse tissue reactions were observed after subcutaneous implantation in rats [17,18].



PEOT 'soft segment' **PBT 'hard segment'**

Figure 1. *Chemical structure of PEOT/PBT segmented block copolymers.*

Recently, effective release of proteins from PEOT/PBT microspheres has been demonstrated [19,20] and the potential use of these polymers for tissue engineering of bone has been shown [21]. Degradation of PEOT/PBT segmented copolymers *in vivo* has also been reported [18]. *In vivo* two degradation pathways of these copolymers are expected to take place. The first route involves hydrolysis of ester bonds in the PBT part or of ester bonds connecting PEO segments and terephthalate units. Besides hydrolysis, oxidation reactions may play a role in the degradation of PEO segments. Implantation of medical devices provokes a foreign body response, during which specific activated cells such as macrophages release enzymes and superoxide anion radicals, which can combine with protons to form hydroperoxide radicals [22]. It has been suggested that *in vivo* degradation of poly(ether urethane) elastomers, and in particular the aliphatic ether groups in these polymers, involves

phagocyte-derived oxidants [23]. Several *in vitro* studies confirm the oxidative degradation of PEO by a radical mechanism initiated at random along the chain [22,24-26].

The aim of this study is to establish the effects of copolymer composition and phase separation on the physical properties and the degradation behavior of segmented PEOT/PBT block copolymers with relatively high soft segment contents. The variation of the mechanical properties of PEOT/PBT copolymers as a function of molecular weight in the dry and swollen state has been investigated. *In vitro* hydrolysis and oxidation of PEOT/PBT block copolymers have been evaluated. The degradation of the copolymers under different storage conditions has been studied.

Materials and Methods

Polymerizations

PEOT/PBT multi-block copolymers were prepared by a two-step polycondensation in the presence of titanium tetrabutoxide (Merck, Germany) as catalyst (0.1 wt%) and Irganox 1330 (Ciba-Geigy, Switzerland) as antioxidant (1 wt%). The transesterification of poly(ethylene glycol) (PEG), dimethyl terephthalate (DMT) and 1,4-butanediol (two-fold excess) carried out under a nitrogen atmosphere at 180°C. After two hours the pressure was slowly decreased from 1000 mbar to 0.1 mbar to allow polycondensation. Simultaneously the temperature was increased from 180 to 240°C. PEG 300, PEG 1000, PEG 2000 and PEG 4000 from Fluka (Switzerland), DMT from Merck (Germany) and 1,4-butanediol from Acros organics (Belgium) were used without further purification. Copolymers of different molecular weights were prepared by varying the reaction times.

With the exception of the materials used in the DSC and water-uptake experiments, the copolymers were purified and the antioxidant was removed by dissolution in chloroform and precipitation into excess of ethanol.

The composition of the block copolymers is indicated as *a b*/*c*, in which *a* is the starting PEG molecular weight, *b* the weight percentage of PEOT soft segments and *c* the weight percentage of PBT hard segments (Fig.1). It has to be noted that terephthalic ester units are present in both the soft and the hard segments. Therefore, the notation PEOT (T for terephthalate) is used to refer to the soft part. The weight contribution of PEO and terephthalic ester units in the soft part is determined by the starting PEG molecular weight. At constant soft to hard segment ratio the total PEO content in the copolymer increases with the molecular weight of the used PEG (Table 1). The abbreviation PEG is used when referred to the material used for the synthesis, whereas PEO is used to refer to the repeating segment in the copolymers.

Table 1. *PEO content (wt%) of PEOT/PBT copolymers synthesized with PEG of different molecular weights at given soft to hard segment ratios.*

	Soft/Hard Segment Ratio (wt/wt)					
	100/0	70/30	60/40	55/45	30/70	0/100
PEG 300	70	49	42	38	21	0
PEG 1000	88	62	53	49	26	0
PEG 4000	93	68	58	53	29	0

Polymer characterization

The intrinsic viscosity [η] of the copolymers in chloroform (solution of approximately 0.3 g/dL) was estimated by single point measurements [27,28] at 25°C using an Ubbelohde OC viscometer. The intrinsic viscosity is related to the molecular weight by the Mark-Houwink equation:

$$[\eta] = K \times M_v^a \qquad (1)$$

where M_v is the viscosity average molecular weight, K and a depend on the polymer, the solvent and the temperature. For polymer 1000 70/30 in chloroform at 25°C, K and a values have been estimated at 1.522×10^{-3} dL/g and 0.545 respectively. The Mark-Houwink constants are not known for other copolymer compositions, therefore values of [η] are used for comparison.

The polymer composition was determined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). 300 MHz ^{1}H-NMR (Varian Inova 300 MHz, USA) spectra were recorded using polymer solutions in deuterated chloroform (Sigma, Switzerland). In the case of copolymers insoluble in $CHCl_3$, small amounts of trifluoroacetic acid were added.

The thermal properties of copolymers containing antioxidant were evaluated by differential scanning calorimetry (DSC) with a Perkin Elmer DSC 7 (USA). A heating rate of 10°C/min was applied and stainless steel pans were used. The copolymer samples (8-15 mg) were heated from -80 to 250°C. The samples were then quenched (programmed temperature: 300°C/min) until -80°C and after 5 min a second scan was recorded. The data presented are from the second heating scan. The glass transition temperatures were taken as the midpoint of the heat capacity change, the melting temperatures were determined from the maximum in the melting endotherm. Indium and gallium were used as standards for temperature calibration.

The equilibrium water-uptake in demineralized water was defined as the weight gain of the unpurified polymer sample after conditioning at 37°C according to equation 2:

$$\text{Water uptake (wt\%)} = \frac{m - m_0}{m_0} \text{ x } 100 \qquad (2)$$

where m_o is the initial specimen weight (approximately 0.5 g) and m the weight of the specimen after conditioning to equilibrium.

Mechanical properties

Tensile testing was performed on dry and swollen PEOT/PBT block copolymer films. Specimens were cast from chloroform solution (50-100 μm thick) and were cut according to ASTM D882-91 specifications (100 x 5 mm^2). Tensile tests in duplicate or triplicate were carried out at room temperature on a Zwick Z020 (Germany) universal tensile testing machine operated at a crosshead speed of 50 mm/min using a 0.01 N pre-load and a grip-to-grip separation of 50 mm. The specimen elongation was derived from the grip-to-grip separation, therefore the presented values of the *E*-modulus give only an indication of the stiffness of the different polymers. The specimens were tested at ambient conditions. The error is less than 5% for the *E*-modulus and the maximum stress determination and is up to 20% for the elongation at break. The data presented in figures and tables are representative of the copolymer properties.

Degradation

In vitro hydrolysis experiments with 50-100 μm thick solution cast films were carried out in duplicate at 37°C using phosphate buffer saline (PBS) containing sodium azide (Sigma, Switzerland) as antibacterial agent (0.02 wt%). The PBS solution was refreshed every two weeks. Hydrolyzed samples were analyzed at predetermined times in terms of their intrinsic viscosity, chemical composition and mechanical properties.

Solution cast films were oxidatively degraded at 37°C in 10% H_2O_2 solution (prepared by diluting 30% H_2O_2 from Merck, Germany) containing 0.1 M $CoCl_2$ (Aldrich, Germany). $CoCl_2$ catalyses the formation of hydroxyl radicals from the hydrogen peroxide through a Haber-Weiss reaction [29]. Oxidized samples were analyzed in duplicate in terms of their intrinsic viscosity, chemical composition and mechanical properties.

The adequacy of Irganox 1330 and vitamin E (α-tocopherol) as antioxidants during sample storage was studied with 1000 70/30 films. Beforehand, the polymer was purified in order to remove the Irganox 1330 used during the synthesis. Polymer films with and without antioxidant were prepared by casting solutions containing 0, 0.5, 1 and 2 wt% of Irganox 1330 or vitamin E (Aldrich, Germany). The presence of antioxidant in the films did not influence the initial mechanical properties of the polymers. The films were kept under three different conditions: in the light at ambient conditions, in the dark at ambient conditions and in the dark

at -21°C. Degradation of the samples was evaluated in triplicate by following the change of the mechanical properties in time.

Results and Discussion

Physical properties

PEOT/PBT segmented block copolymers are thermoplastic elastomers built up of hard segments (PBT) and soft segments (PEOT). The PBT hard segments have a glass transition temperature above body temperature and are able to crystallize, which gives strength to the material. The hydrophilic PEOT segments have a glass transition below room temperature, providing flexibility and hydrophilicity to the system.

The variation in hard and soft segment contents and in the molecular weight of the PEG used during the synthesis has an effect on the phase separation of the system. The miscibility of polymeric systems is controlled by the factor χN, where χ is the Flory-Huggins interaction parameter and N the number of repeat units in the polymer chain. In polymer blends the χ-parameter is usually positive and large, and as a rule polymers phase separate. In the case of block copolymers, where the different macromolecules are covalently linked, macrophase separation is impeded, however, microphase separation can still occur. In (multi)block copolymers, an increase in molecular weight of the blocks favors phase separation [30]. Therefore, an increase in PEOT length and in PBT average sequence length will lead to enhanced phase separation in PEOT/PBT multi-block copolymers. Upon cooling, crystallization of one of the components is an additional driving force for phase separation.

Fakirov and co-workers studied the structure of PEOT/PBT copolymers with high PBT contents by DSC [31,32] and small angle X-ray scattering (SAXS) [33,34]. As was the case in their work, DSC experiments with PEOT/PBT copolymers containing higher amounts of PEO also show several amorphous and crystalline phases. Figure 2 gives DSC spectra of PEOT/PBT of various compositions where the starting PEG molecular weight is kept constant (1000) and the soft to hard segment ratio is changed, or where the ratio is kept constant (70/30) and the PEG molecular weight is changed from 1000 to 300. These examples are representative of the copolymers studied and, depending on the composition, up to four transitions can be observed. All PEOT/PBT block copolymers investigated are semi-crystalline at room temperature. A T_m corresponding to crystalline PBT ranging from 114 to 224°C was detected (the T_m of PBT homopolymer is 226°C). This T_m is relatively broad (about 25ºC) due to the random condensation process during the synthesis, which leads to the

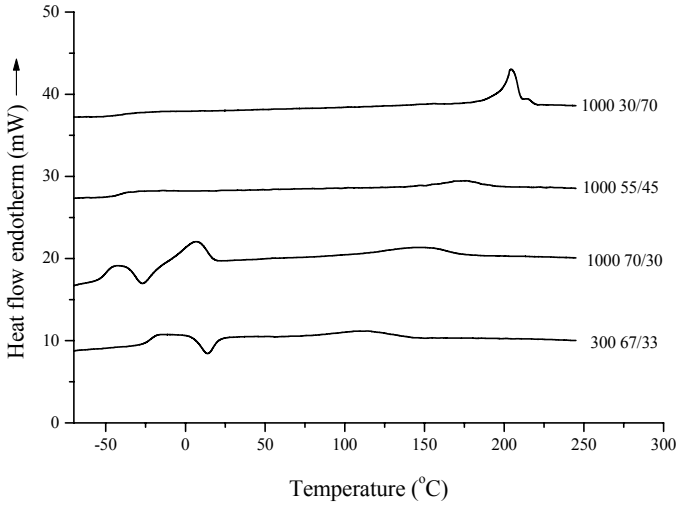


Figure 2. *DSC spectra of PEOT/PBT block copolymers of different compositions.*

formation of chains with a distribution of PBT sequence lengths. Recrystallization of the PEOT fraction could be detected between -30°C and 31°C in case of high PEO contents. The degree of crystallinity (w_c) of a polymer can be estimated from the heat of fusion by the expression:

$$w_c = \frac{\Delta H}{\Delta H^\circ} \tag{3}$$

ΔH is correlated to the total weight of the polymer. The heat of fusion of 100% crystalline PBT (ΔH°) is reported to be 144.5 J/g [35]. w_c gives an indication of the crystallinity of the PEOT/PBT multi-block copolymers (Table 2). To be able to compare the crystallinity with the PBT homopolymer, these values were also normalized to the PBT content of the block copolymer. It then appears that the normalized degree of crystallinity is similar for all copolymers and close to the degree of crystallinity of the PBT homopolymer.

The average sequence length ($\overline{L}_n$) of the PBT segments was calculated from the PBT mole fraction (x_{PBT}) in the copolymer assuming random copolymerization of the components:

$$\overline{L}_n = \frac{1}{1 - x_{PBT}} \tag{4}$$

The melting point of the hard segments depends strongly on the average sequence length. An increase in PBT weight fraction results in higher melting temperatures and in an increase in the heat of fusion of the PBT.

Table 2. *Thermal properties of PEOT/PBT block copolymers with high molecular weight ([η] ranging from 0.7 to 1.6 dL/g). The weight fraction, mole fraction and number average sequence length ($\overline{L}_n$) of the PBT component were calculated from ^{1}H-NMR.*

Composition	PEOT-segment			PBT-segment							
	T_g (°C)	T_m (°C)	ΔH (J/g)	Weight fraction	Mole fraction	$\overline{L}_n$ [a]	T_g (°C)	T_m (°C)	ΔH (J/g)	w_c [b] (%)	$w_{c(n)}$ [c] (%)
300 67/33	-23	_ [d]	_	0.33	0.49	2.0	_	114	13.4	9.2	27.9
300 56/44	-23	_	_	0.44	0.57	2.3	26	145	20.9	14.5	35.4
300 50/50	-17	_	_	0.50	0.66	2.6	46	159	25.3	17.5	35.0
300 27/73	-11	_	_	0.73	0.84	6.2	58	197	34.0	23.5	32.2
1000 70/30	-50	6	15.8	0.30	0.69	3.2	_	149	11.5	8.0	26.7
1000 61/39	-50	-1	3.1	0.39	0.77	4.3	_	166	13.0	9.0	23.1
1000 55/45	-41	_	_	0.45	0.81	5.3	_	176	15.0	10.4	23.1
1000 41/59	-40	_	_	0.59	0.88	8.3	_	182	21.6	15.0	25.4
1000 30/70	-38	_	_	0.70	0.92	12.5	40	204	30.1	20.8	29.7
2000 29/71	-52	5	0.4	0.71	0.96	25.0	_	215	28.6	19.8	27.9
4000 81/19	_	47	68.8	0.19	0.81	5.3	_	173	6.4	4.5	23.7
4000 71/29	_	39	45.8	0.29	0.88	8.3	_	192	9.5	6.6	22.8
4000 55/45	_	34	39.8	0.45	0.94	16.7	_	210	22.2	15.4	34.2
4000 20/80	_	32	12.7	0.80	0.99	100.0	_	223	34.8	24.1	30.1
10000 81/19	_	57	80.7	0.19	0.91	11.1	_	224	7.0	4.8	25.3
PBT	_	_	_	1	1	∝	54	226	43.4	27.2	27.2
PEG 300	-48	-16	78.2	_	_	_	_	_	_	_	_
PEG 1000	_	39	150.2	_	_	_	_	_	_	_	_
PEG 2000	_	57	160.8	_	_	_	_	_	_	_	_
PEG 4000	_	60	178.7	_	_	_	_	_	_	_	_

a. $\overline{L}_n = \frac{1}{1 - x_{PBT}}$, x_{PBT} = mole fraction of PBT

b. $w_c = \Delta H/\Delta H^0 \times 100$

c. $w_{c(n)} = w_c$/weight fraction$_{PBT}$

d. – not observed

The phase separation between the distinct blocks also depends strongly on the starting PEG length. At a constant soft to hard segment ratio, an increase in the molecular weight of the used PEG enhances phase separation. This is illustrated by the appearance of high PEO and PBT melting points. Simultaneously no T_g was detected anymore. Lower PEG molecular weights result in lower PBT average sequence lengths. A T_g corresponding to the presence of amorphous PBT domains is then observed, indicating a decreased phase separation between soft and hard segments. This is also confirmed by the depression in the melting point of the PBT segments and in the increase of the T_g of PEOT (Table 2).

In case of semi-crystalline polyesters, hydrolysis occurs preferentially in the amorphous phase [36,37]. An increase in crystallinity reduces the water permeability of the polymer and the accessibility of hydrolyzable bonds. During long term degradation experiments on poly(L-lactide), highly crystalline debris resistant to hydrolysis could be found, possibly causing an inflammatory response [38,39]. Therefore, in the case of implants made from PEOT/PBT copolymers, the use of material containing minimal amounts of PBT is recommended.

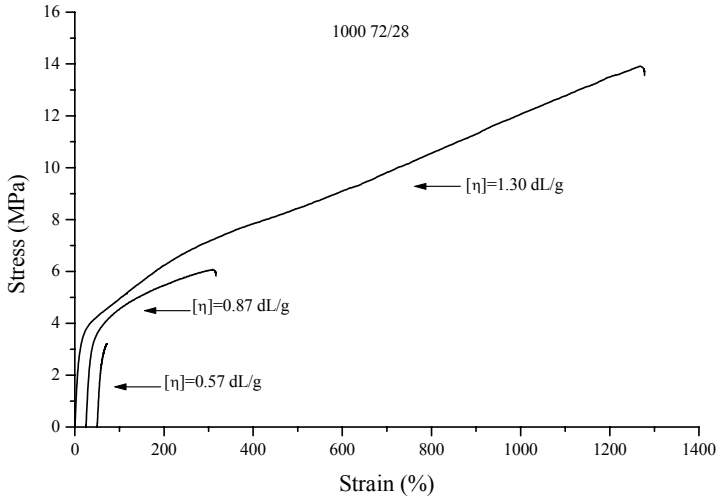


Figure 3. *Typical stress-strain diagrams of 1000 72/28 with different intrinsic viscosities. Stress-strain curves are offset for clarity. Measurements performed on dry samples.*

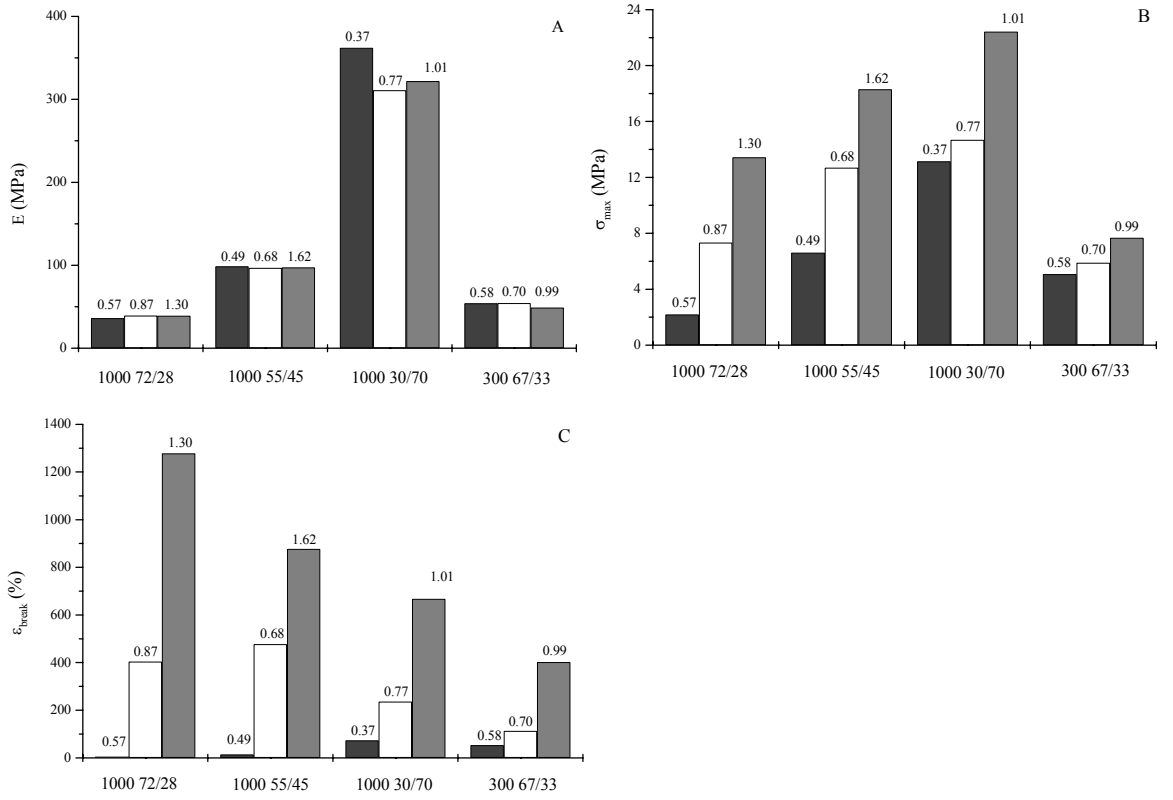


Figure 4. *Mechanical properties of different PEOT/PBT block copolymers as a function of composition and intrinsic viscosity. A: E-modulus (E), B: maximum tensile strength (σ_{max}), C: elongation at break (ε_{break}). The numbers above the bars represent the intrinsic viscosity of the polymer. Measurements performed on dry samples.*

Phase separation is a main factor influencing the mechanical properties of PEOT/PBT block copolymers. To be able to compare the different copolymers, it is necessary to have polymers of a sufficiently high molecular weight, since many physical properties depend considerably on it [40]. The mechanical characteristics of PEOT/PBT copolymers improve significantly with increasing molecular weight. Figure 3 shows the stress-strain behavior of 1000 72/28 with three different intrinsic viscosities. For this copolymer, the *E*-modulus increases from 36 MPa to 39 MPa when a polymer with an intrinsic viscosity higher than 0.87 dL/g is tested. The change in intrinsic viscosity has a more pronounced effect on the elongation at break and the tensile stress. At an intrinsic viscosity of 0.57 dL/g the elongation at break is relatively low (22%) whereas a very high deformation of 1300% is reached for 1000 72/28 with an intrinsic viscosity of 1.3 dL/g. The values of the maximum tensile stress also increase significantly (from 3.2 to 13.9 MPa) with the intrinsic viscosity. Figure 4 shows

that a similar behavior is observed for PEOT/PBT copolymers of other compositions. For these copolymers a limiting intrinsic viscosity value of approximately 0.4-0.6 dL/g can be established below which tensile strength and elongation at break are minimal. No upper plateau of the mechanical properties was observed for our system. The two-step polycondensation method used in this study to synthesize PEOT/PBT block copolymers yielded copolymers with intrinsic viscosities ranging from 0.40 to 1.62 dL/g. However, even better mechanical properties would be obtained if the polymer molecular weight could be increased further.

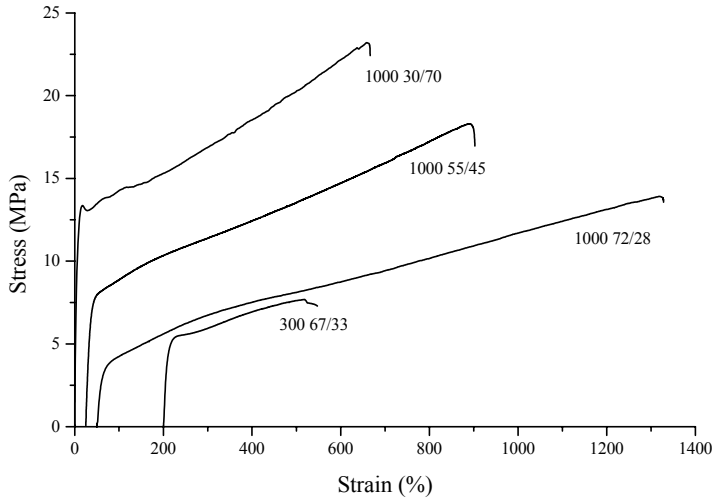


Figure 5. *Typical stress-strain diagrams of PEOT/PBT block copolymers of different compositions. Stress-strain curves are offset for clarity. Measurements performed on dry specimens.*

Figure 5 shows the tensile behavior of four different copolymer compositions with high intrinsic viscosities (ranging from 0.99 to 1.62 dL/g). In comparing the stress-strain curves, it can be seen that the soft PEOT and the hard PBT segment ratios of polymers prepared with PEG 1000 as well as the effect of starting PEG molecular weight at constant soft to hard segment ratio (approximately 70/30) are of influence. As was also seen for PEOT/PBT copolymers with high PBT contents [31,32], keeping the PEG used in the synthesis at a constant molecular weight of 1000, an increase in soft segment content causes a decrease in *E*-modulus and in maximum stress. The *E*-modulus is reduced from over 300 MPa for 1000 30/70 to only 40 MPa for 1000 72/28 and the maximum stress from 23.2 to 13.9 MPa. At the same time the elongation at break increases from 600 to 1300%. By increasing the soft segment content, the number of domains that contribute to the strength decrease and a more flexible material is obtained.

In the case of constant soft to hard segment ratios, the *E*-modulus decreases of 20% (from 49 to 39 MPa) when PEG 1000 is used instead of PEG 300. The maximum stress and elongation at break increase significantly from 490 to 1300% and from 7.7 to 13.9 MPa, respectively. In case of a constant soft to hard segment ratio, the mechanical properties are considerably enhanced by a more pronounced phase separation between the soft and hard segments for the polymer prepared with the higher PEG molecular weight.

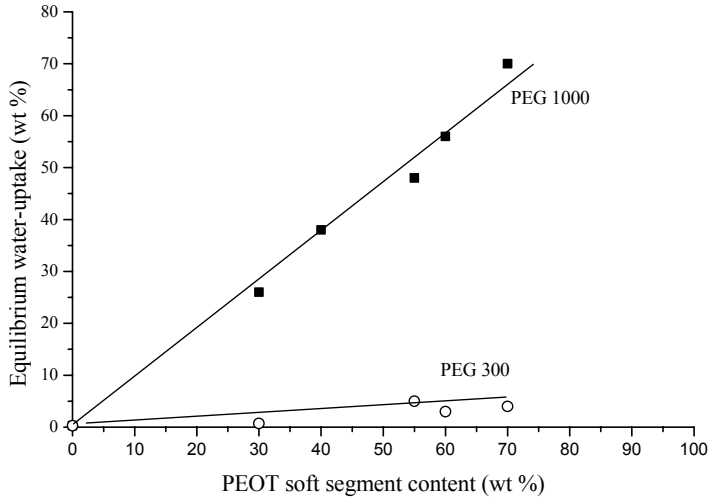


Figure 6. *Equilibrium water-uptake (wt%) as a function of PEOT soft segment content (wt%) for PEOT/PBT copolymers synthesized from* (■) *PEG 1000 and* (○) *PEG 300.*

PEOT/PBT multi-block copolymers absorb water due to the presence of the hydrophilic PEO in the PEOT segments of the copolymers. Two parameters influence the uptake of water by PEOT/PBT copolymers: the PEG molecular weight via the phase separation in the copolymers and the PEO content. As stated earlier, at a constant soft to hard segment ratio, phase separation decreases when a shorter PEG is used in the copolymerization. The presence of hydrophobic PBT segments in the soft domains diminishes the uptake of water (Fig.6). For a given soft to hard segment ratio, the use of a lower PEG molecular weight also means a lower content of PEO in the copolymer (Table 1).

Table 3. *Water uptake and tensile properties (E-modulus, maximum stress and elongation at break) for dry and swollen PEOT/PBT segmented copolymers.*

Composition		1000 72/28	1000 30/70	300 70/30
Water-uptake (wt%)		62	26	4
E (MPa)	dry	39	322	49
	swollen	25	274	44
σ_{max} (MPa)	dry	13.4	19.0	7.7
	swollen	11.4	17.9	6.9
ε_{break} (%)	dry	1278	667	402
	swollen	1013	529	318

When used as an implant in the body, the material will absorb body fluids and will swell. Consequently, the mechanical properties of PEOT/PBT copolymers need to be evaluated in the swollen state as well. E-modulus, maximum tensile strength and elongation at break in the dry and in the swollen state for several copolymer compositions are reported in Table 3. For all copolymers the mechanical properties, especially the *E*-modulus, decrease in the swollen state. 1000 70/30 is the most hydrophilic of these copolymers and is the most affected by the uptake of water. Although the stiffness decreases during water-uptake, all swollen materials can be handled with ease and are applicable in non load-bearing situations such as in soft tissue applications and tissue engineering.

Degradation

PEOT/PBT block copolymers contain two types of potentially degradable chemical bonds in the polymer chain: ester bonds can be hydrolyzed and ether bonds can be oxidized. Both mechanisms are normally occurring *in vivo*.

The hydrolysis of PEOT/PBT copolymer in PBS was investigated. Three copolymers, 300 69/31, 1000 69/31 and 1000 61/39 were chosen with identical starting PEG molecular weight (PEG 1000) or with identical soft to hard segment ratio (69/31). The intrinsic viscosity and the mechanical properties during degradation are shown in Table 4 and Figures 7 and 8. No significant change in intrinsic viscosity, mechanical properties or chemical composition is observed for 300 69/31, which does not seem to degrade in PBS during a period of 24 weeks

Table 4. *Change in intrinsic viscosity, E-modulus, maximum stress and elongation at break during degradation of PEOT/PBT copolymers in PBS. The mechanical properties of swollen samples were measured.*

Property	Degradation time (weeks)	300 69/31	1000 69/31	1000 61/39
$[\eta]$ (dL/g)	0	0.99	0.88	1.20
	1	0.94	0.93	1.27
	2	0.97	0.80	0.89
	3	–	0.51	0.86
	6	–	0.54	0.83
	12	0.98	0.13	0.51
	24	0.92	0.05	0.18
E (MPa)	0	52	27	41
	1	46	28	38
	2	43	25	36
	3	50	21	38
	6	50	–[a]	36
	12	48	–	33
	24	54	–	–
σ_{max} (MPa)	0	6.9	6.0	9.1
	1	6.6	5.7	8.3
	2	6.9	4.5	7.2
	3	7.1	1.1	6.4
	6	6.8	–	4.9
	12	6.7	–	3.0
	24	7.7	–	–
ε_{break} (%)	0	318	657	712
	1	199	107	509
	2	361	95	185
	3	365	6	147
	6	307	–	42
	12	242	–	10
	24	337	–	–

a. tensile testing not possible

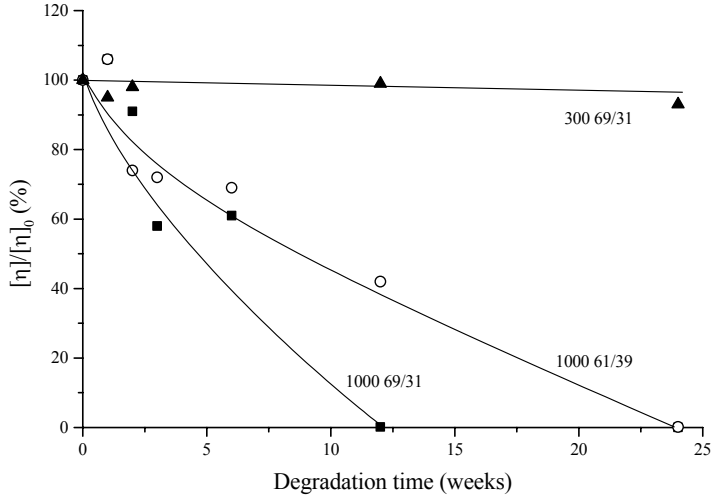


Figure 7. *Relative intrinsic viscosity ($[\eta]/[\eta]_0$) as a function of degradation time in PBS at 37°C for* (▲) *300 69/31,* (■) *1000 69/31 and* (○) *1000 61/39.*

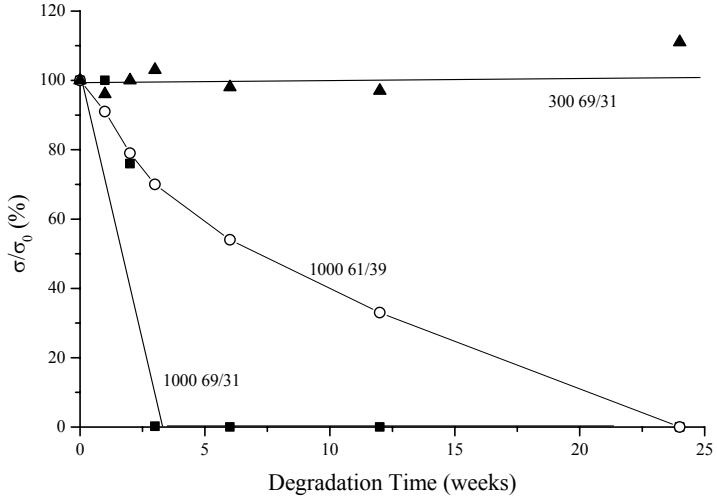


Figure 8. *Relative maximum stress (σ/σ_0) as a function of degradation time in PBS at 37°C for* (▲) *300 69/31,* (■) *1000 69/31 and* (○) *1000 61/39.*

Polymers 1000 69/31 and 1000 61/39 show a decrease in intrinsic viscosity and in mechanical properties. The intrinsic viscosity of 1000 69/31 is initially 0.88 dL/g and ends at 0.05 dL/g after 24 weeks. Simultaneously, its mechanical properties are lowered (Table 4). After only 3 weeks in PBS, σ_{max} falls from 5.7 to 1.1 MPa and ε_{break} from 657 to only 6% (Fig.8 and Table 4). Moreover, after 24 weeks of degradation, dissolution of 1000 69/31 in chloroform is difficult, due to a change in the copolymer composition as shown by ^{1}H-NMR.

It appeared that after 12 weeks in PBS the soft segment content has decreased from 69 to 60 wt% and only 52 wt% remains after 24 weeks. This corresponds to a decrease in PEO content from 62 to 42 wt%.

Polymer 1000 61/39 follows the same trend as 1000 69/31, but its degradation is slower. Over 24 weeks, the intrinsic viscosity drops from 1.2 dL/g to 0.18 dL/g (Table 4). After 6 weeks σ_{max} decreases from 9.1 to 4.9 MPa and ε_{break} from 712 to 42%. After 12 weeks, the mechanical properties have strongly deteriorated but the samples can still be tested, which is not possible after 24 weeks (Fig.7 and Table 4). After 24 weeks no change in composition is detected by ^{1}H-NMR yet.

Besides the soft to hard segment ratio, the results show that the used PEG molecular weight is of large influence on the hydrolytic degradation of PEOT/PBT block copolymers. This can be related to the actual PEO content in the copolymer and the better phase separation in 1000 69/31 than in 300 69/31. In 1000 69/31, the hydrophilic PEOT domains are more accessible to water than in 300 69/31, water-uptake is higher and the possibility to hydrolyze the ester bonds in these PEOT domains increases. The loss in PEO found for 1000 69/31 during hydrolytic degradation may also indicate that the ester bond connecting the PEO and the terephthalate unit (Fig.1) is the most sensitive for hydrolysis. At long degradation periods, such preferential degradation of ester bonds can result in the appearance of residues with high PBT contents, as it has been suggested for PBT-containing copolyesters [41]. In the case of copolymers with a high PBT content (large $\overline{L}_n$) these residues will probably be highly crystalline and even more resistant to hydrolysis.

The maximum tensile strength in the swollen state as a function of the intrinsic viscosity for samples 1000 69/31 degraded for different times in PBS and synthesized samples of 1000 71/29 with different intrinsic viscosities is shown in Figure 9. Such comparable behavior suggests random chain scission and allows the estimation of molecular weights of PEOT/PBT copolymers during degradation by evaluating their mechanical properties if no compositional change has occurred. Again, an intrinsic viscosity of 0.4 dL/g seems to be a limiting value below which the mechanical properties are negligible.

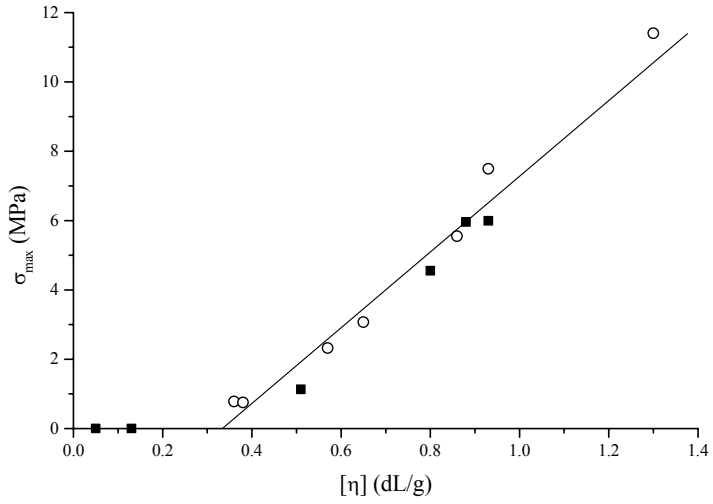


Figure 9. *Maximum stress (σ_{max}) of swollen samples as a function of intrinsic viscosity ([η]) for* (■) *1000 69/31 during degradation in PBS and for* (○) *1000 71/29 of different initial molecular weights.*

Besides hydrolysis, a second possible degradation pathway is the oxidation of ether bonds in the PEO part. Copolymers 1000 70/30 and 300 70/30 were subjected to oxidative degradation. The changes in composition, intrinsic viscosity and mechanical properties are presented in Table 5. Degradation in $H_2O_2/CoCl_2$ solution has a drastic effect on the polymer properties. Regardless of the initial polymer composition and molecular weight, the tensile strengths and elongations at break significantly decrease after only one day in the medium.

Table 5. *Composition, intrinsic viscosity ([η]), maximum stress (σ_{max}) and elongation at break (ε_{break}) of 1000 70/30 and 300 70/30 with different initial molecular weights after one day (t_1) in $H_2O_2/CoCl_2$ 10%. The mechanical properties of swollen samples were measured.*

Composition		[η] (dL/g)		σ_{max} (MPa)		ε_{break} (%)	
t_0	t_1	t_0	t_1	t_0	t_1	t_0	t_1
1000 69/31	1000 69/31	1.30	0.55	7.2	4.0	852	141
1000 70/30	1000 59/41	0.88	-[a]	6.0	2.1	657	13
300 69/31	300 69/31	0.99	0.60	6.9	5.7	318	40
300 70/30	300 64/36	0.70	0.21	5.2	4.0	75	12

a. sample insoluble in chloroform

For the PEOT/PBT copolymers with the lowest initial intrinsic viscosity, a notable change in composition is observed. NMR spectroscopy shows that the initial composition of 1000

70/30, 70 wt% soft segment and 62 wt% PEO, has decreased to 59 wt% PEOT soft segment and 50 wt% PEO after one day in the oxidative medium. After two days this sample is not soluble in chloroform anymore, due to polymer chains with high contents of PBT. High molecular weight 1000 69/31, with [η] of 1.3 dL/g, undergoes only a very slight decrease in PEO content, and is still soluble in chloroform after two days.

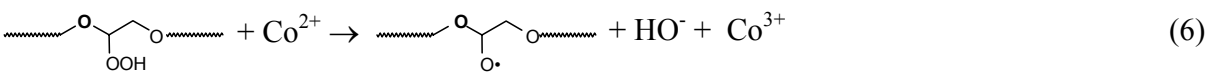
Polymer 300 70/30, which is relatively stable during hydrolytic degradation, now shows a large decrease in intrinsic viscosity during oxidative degradation. Especially the lowest molecular weight polymer, of which the PEO content has decreased from 49 to 45 wt% after one day and to 37 wt% after two days, is rapidly degrading. The initial polymer molecular weight seems an important parameter not only in obtaining good initial mechanical properties, but also in maintaining suitable material properties during degradation.

The results illustrate the sensitivity of PEOT/PBT copolymers to oxidation, especially for the copolymers with high PEG molecular weight and high PEOT contents. The loss in PEO can be explained by oxidative reactions of ether bonds in the presence of radicals. The thermo-oxidative [26,42,43], photo-oxidative [44-46] and γ-radiation [47] degradation reactions of PEO and PEO-containing polymers occur via free-radical reactions, leading to scission of the chain. In H_2O_2 solutions containing $CoCl_2$, hydroxyl radicals (HO·) are formed through a Haber-Weiss reaction [29]:

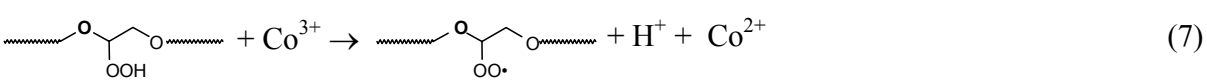
$$Co^{2+} + H_2O_2 \rightarrow Co^{3+} + HO^- + HO\bullet \qquad (5)$$

The reaction with PEO involves H-abstraction by HO• from the a-carbon atom leading to the formation of a macrochain radical. The macrochain radical can then react either with another hydroxy radical or with oxygen as shown in Scheme 1 [48,49]. The chain scission subsequently occurs by hydrolysis.

In addition, cobalt ions act as a catalyst in the generation of macrochain radicals through reactions of the hydroperoxide that has previously been formed [48,50]:

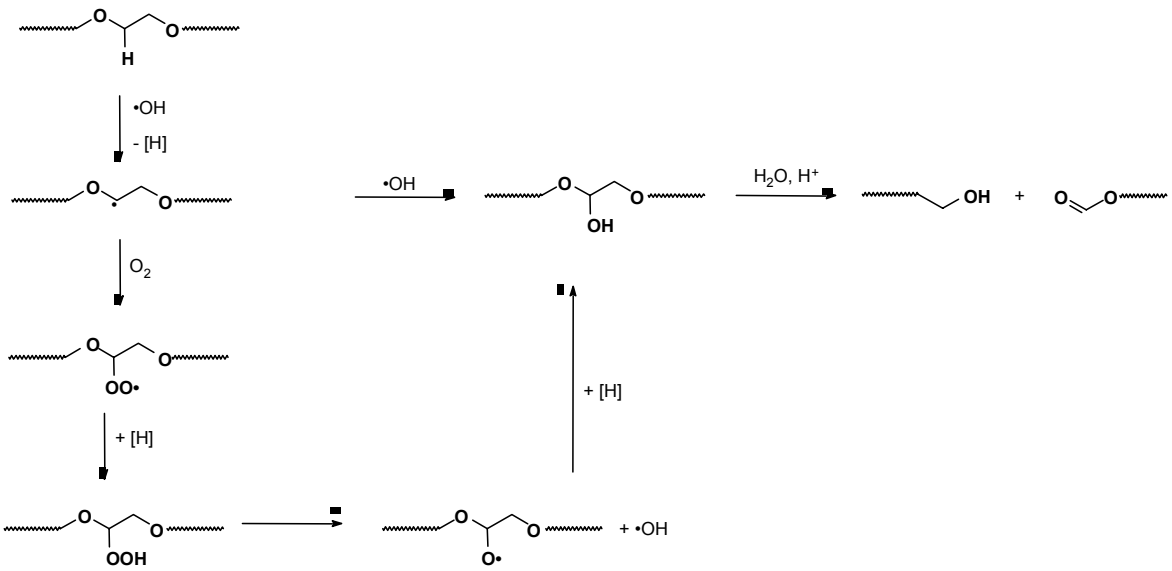


(6)



(7)

Together, these reactions lead to solubilization of PEO-containing segments and a change in composition of PEOT/PBT copolymers.



Scheme 1. *Possible reaction pathways in the oxidative chain scission of PEO [48,49].*

Oxidation can also play an important role in the stability of the copolymers during storage due to the formation of radicals by the action of light. Therefore, the influence of the amount of antioxidant on 1000 70/30 degradation under different storage conditions has been evaluated. Irganox 1330 and vitamin E were used as antioxidants. In industry, Irganox 1330 is a commonly used antioxidant, whereas vitamin E is a natural antioxidant that may be preferred in biomedical applications. To assess the extent of polymer degradation, the mechanical properties of films with and without antioxidant were evaluated.

When stored for 8 months in the dark at room temperature or at -21ºC, 1000 70/30 films were stable even without the addition of Irganox 1330 or vitamin E as antioxidant: no loss in mechanical properties or visual changes of the films could be observed.

When exposed to daylight at room temperature during the same time period, 1000 70/30 films containing 0.5 to 2 wt% of Irganox 1330 were stable as well. However, in the absence of antioxidant or when vitamin E is used as antioxidant, significant decreases in mechanical properties are observed during storage under ambient daylight conditions. Figure 10 shows the decrease in time of the maximum tensile stress of the films containing different amounts of vitamin E. The elongation at break shows similar behavior, while the *E*-modulus is much less affected. As shown previously, this loss in mechanical properties can be related to a decrease in intrinsic viscosity and molecular weight of the polymer. Figure 10 shows that without vitamin E the mechanical properties start deteriorating after a short induction period of

approximately 6 weeks. The mechanical properties are negligible after 4.5 months. Adding vitamin E in different amounts significantly increases the onset time after which the films start loosing their strength. Furthermore, it seems that the rate at which the 1000 70/30 films loose their mechanical properties is dependent on the vitamin E content. The higher the amount of vitamin E added, the more stable the films are and the slower the degradation is.

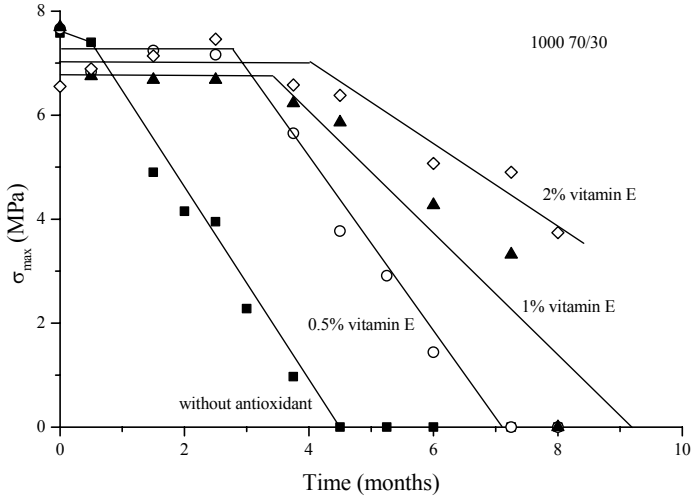


Figure 10. *Maximum stress (σ_{max}) during storage under ambient daylight conditions as a function of time for 1000 70/30 films* (■) *without antioxidant, containing* (○) *0.5 wt%,* (▲) *1 wt% and* (◇) *2 wt% of vitamin E.*

As this decay in properties is only observed during storage in the light, oxidation reactions involving radicals are likely involved. Irganox 1330 and vitamin E are both hindered phenol derivatives (Fig.11) and are able to scavenge radicals [50,51]. Per gram of antioxidant, Irganox 1330 contains 1.7 times as many phenol groups than vitamin E. Films containing 0.5 wt% vitamin E start loosing their mechanical properties after 3 months. The observation that after 8 months films containing 0.5 wt% Irganox 1330 still show no signs of degradation, indicates that Irganox 1330 is more efficient in preventing oxidation of 1000 70/30. In experiments simulating melt processing, previous work has shown a higher efficiency of vitamin E in comparison with synthetic hindered phenols. This contradictory result can be due to the different experimental settings, as these researchers suggested [52].

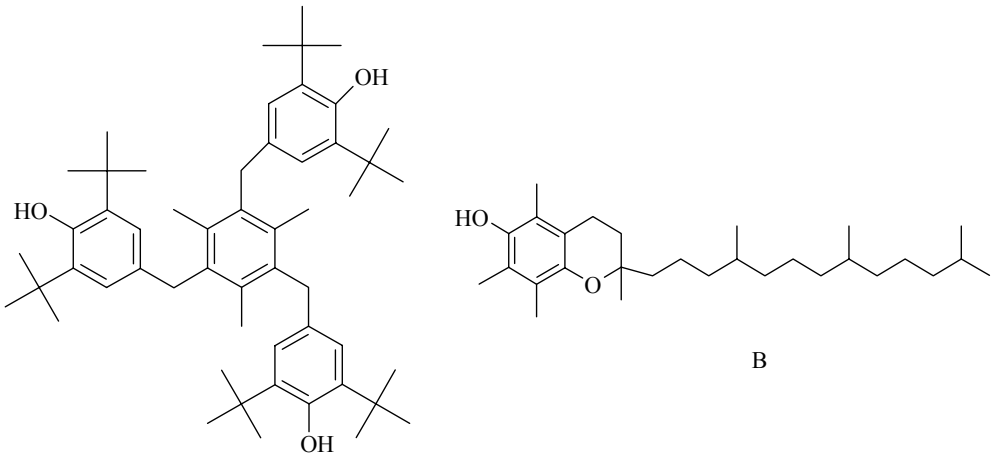


Figure 11. *Chemical structures of Irganox 1330 (A) (M= 774 g/mol) and vitamin E (B) (M= 431 g/mol).*

In the late stage of the degradation experiments, yellowing of 1000 70/30 films without antioxidant or films containing vitamin E was observed. This yellowing of PBT-containing poly(ether ester)s exposed to UV light can be attributed to the formation of mono-and dihydroxy substituted aromatic compounds [53]. Films stabilized with Irganox 1330 did not become yellow during these storage experiments.

Conclusions

Segmented PEOT/PBT block copolymers are phase-separated. The extent of phase separation varies with copolymer composition. Phase separation is more pronounced for polymers with high hard segment contents and polymers containing high molecular weight PEG.

The physical properties of these copolymers depend strongly on the molecular weight, the soft to hard segment ratio and the starting PEG molecular weight. By changing the copolymer composition, the mechanical properties and the swelling characteristics of PEOT/PBT copolymers can be tuned and adjusted within specific limits to the requirements for medical application.

PEOT/PBT copolymers are degraded *in vitro* by hydrolysis and oxidation. In both situations a decrease in intrinsic viscosity, PEO content and mechanical properties have been observed. The degradation is more severe in case of polymers with a high PEOT content prepared from a high molecular weight PEG.

Oxidation of PEOT/PBT also takes place during exposure of the polymers to light at ambient conditions. Under these conditions, Irganox 1330 is a more efficient antioxidant for PEOT/PBT polymers than vitamin E.

Acknowledgments

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Chapter 5

Design of Segmented Poly(ether ester) Materials and Structures for the Tissue Engineering of Bone*

'Our imagination is the only limit to what we can hope to have in the future.'
Charles Kettering (1876-1958)

Abstract
In this study, PEOT/PBT segmented copolymers of different compositions have been evaluated as possible scaffold materials for the tissue engineering of bone. By changing the composition of PEOT/PBT copolymers very different mechanical and swelling behaviors are observed. Tensile strengths vary from 8 to 23 MPa and elongations at break from 500 to 1300%. Water-uptake ranges from 4 up to as high as 210%. The *in vitro* degradation of PEOT/PBT copolymers occurs both by hydrolysis and oxidation. In both cases degradation is more rapid for copolymers with high contents of PEO. PEOT/PBT scaffolds with varying porosities and pore sizes have been prepared by molding and freeze-drying techniques in combination with particulate-leaching. The most hydrophilic PEOT/PBT copolymers did not sustain goat bone marrow cell adhesion and growth. However, surface modification by gas plasma treatment showed a greatly improved polymer-cell interaction for all PEOT/PBT copolymer compositions. Their mechanical properties, degradability and ability to sustain bone marrow cell growth make PEOT/PBT copolymers excellent materials for bone tissue engineering.

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Journal of Controlled Release **2002**, 78, 175-186.

Introduction

The number of skeletal defects requiring bone-grafting procedures is constantly increasing. In bone transplantation, the clinical 'gold standard' is the use of autogenous trabecular grafts. However, this method has several drawbacks such as donor-site morbidity, pain and limited availability of donor bone. Allografts and xenografts are also used, but can be associated with the transmission of diseases [1] and the tendency to elicit an immune response [2]. To overcome these problems, tissue engineering of functional bone is attracting much attention.
Tissue engineering involves the culturing of specific tissue cells, the use of a degradable scaffold to support attachment, growth and differentiation of these cells and the delivery of growth and differentiation factors [3,4]. For the tissue engineering of bone, ceramics are being extensively studied [5,6] mainly due to the fact that mineral bone contains significant amounts of calcium phosphates. As these ceramics are brittle and resorb quite slowly, degradable polymers are perhaps more suited for the preparation of scaffolds [7]. Their mechanical and biological properties can be readily varied and optimized. Most of this research has been focused on hybrid cell/scaffold constructs using poly(lactic acid), poly(glycolic acid) and their copolymers [8-11]. The relatively fast degradation of these polymers, however, can induce tissue inflammations [12,13]. New materials with improved properties are therefore being developed, for example poly(propylene fumarate)s [14], poly(anhydride-*co*-imide)s [15,16] and tyrosine-derived polycarbonates [12].

In bone tissue engineering, the hybrid construct develops its strength during degradation of the polymer and simultaneous formation of new bone, allowing the use of an elastomeric material for small defects in non-load bearing situations. We are investigating the applicability of slowly degradable copolymers based on poly(butylene terephthalate) and poly(ethylene oxide) for the tissue engineering of bone. First developed for textile applications [17], PEOT/PBT block copolymers have also been shown to possess interesting physical properties for medical use [18]. PEOT/PBT multi-block copolymers are thermoplastic elastomers (Fig.1). Variation of the PEOT/PBT block copolymer composition and of the molecular weight of the used PEG allows the synthesis of a family of copolymers with widely differing mechanical properties, swelling characteristics, degradation profiles and biological behavior.

Previous work on PEOT/PBT copolymers has shown that they are biocompatible [19,20], have good bone-bonding properties [21] and can calcify i*n vivo* [22]. Polymer degradation has also been observed i*n vivo* [20]. The flexibility and the swelling of the copolymers allow the scaffold to fit the defect with tight bone contact. More recent work involving the use of PEOT/PBT as a bone substitute in critical size defects in the iliac bone of goats and humans did not show the expected good bone-bonding and calcification behavior [23,24]. The critical

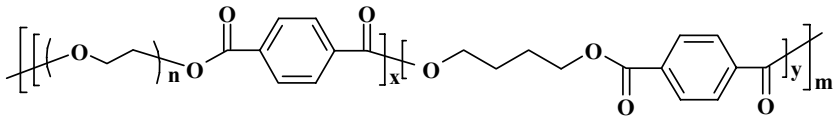


Figure 1. *Chemical structure of PEOT/PBT segmented block copolymers.*

size defects were not bridged. Reasons for the discrepancy with the earlier results in small animals can be: differences in regenerative capacity between the species, the size of the defect and the type of bone into which the substitute was implanted, as cancellous bone has less initial bone to polymer contact than cortical bone [13,23,24]. Implantations in goat femura, which is a cortical bone type, did show bone-bonding [25]. As bone fillers, these polymers are therefore more suited for cortical bone defects than cancellous bone defects.

Proteins have been delivered from PEOT/PBT microspheres with preservation of complete activity. In the case of protein delivery from PLGA and poly(ortho ester) microspheres, activity was significantly reduced [26]. PEOT/PBT polymers are therefore very effective matrix materials for the release of growth factors in tissue engineering.

In this study the suitability of PEOT/PBT copolymers for tissue engineering of bone as carriers of bone marrow cells is evaluated in terms of mechanical properties, degradation behavior, porous structure preparation and goat bone marrow cell attachment to (modified) surfaces.

Materials and Methods

Polymerizations

PEOT/PBT multi-block copolymers were prepared according to well-known procedures [27] on a 50 g scale by a two-step polycondensation in the presence of titanium tetrabutoxide (Merck, Germany) as catalyst (0.1 wt% based on the amount of DMT) and vitamin E (Aldrich, Germany) as antioxidant (1 wt% of the total amount of reagents). The transesterification of poly(ethylene glycol) (PEG), dimethyl terephthalate (DMT) and 1,4-butanediol (two-fold excess) was carried out under nitrogen atmosphere at 180°C. After two hours the pressure was slowly decreased from 1000 mbar to 0.1 mbar to allow the condensation reaction to take place. Simultaneously, the temperature was increased from 180 to 230°C.

PEG 300, PEG 1000 and PEG 4000 supplied by Fluka (Switzerland), DMT from Merck (Germany) and 1,4-butanediol from Acros organics (Belgium) were used without further purification. The copolymers were purified and the antioxidant was removed by dissolution in

chloroform and precipitation into excess of ethanol. The composition of the block copolymers is indicated as *a* PEOT*b*PBT*c*, in which *a* is the starting PEG molecular weight, *b* the weight percentage of PEOT soft segments and *c* the weight percentage of PBT hard segments (Fig.1). As terephthalate ester units are present in the soft segments, the notation PEOT is used to refer to these blocks. The abbreviation PEG is used when referred to the material used for the synthesis, whereas PEO is used to refer to the repeating segment in the copolymers.

Polymer characterization

The intrinsic viscosity [η] of the copolymers in chloroform at 0.3 g/dL was estimated by single point measurements [28,29] at 25°C using an Ubbelohde OC viscometer.

The copolymer composition was determined by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. 300 MHz ^{1}H-NMR (Varian Inova 300 MHz, USA) spectra were recorded using polymer solutions in deuterated chloroform (Sigma, Switzerland). In the case of copolymers insoluble in $CHCl_3$, small amounts of trifluoroacetic acid (Aldrich, Germany) were added.

The equilibrium water-uptake in demineralized water was defined as the weight gain of the polymer specimen after conditioning at 37ºC according to equation 2:

$$\text{Water - uptake}\,(\text{wt}\%) = \frac{m - m_0}{m_0} \text{ x } 100 \qquad (1)$$

where m_o is the initial specimen weight and m the weight of the specimen after conditioning to equilibrium.

Contact angles of copolymer films in demineralized water were determined using the captive bubble technique. Measurements were done using a Contact Angle System OCA 15 plus from Dataphysics. Results are averages of at least 5 measurements.

Mechanical properties

Tensile testing was performed on dry and swollen PEOT/PBT block copolymer films. Specimens were cast from chloroform solutions (50-100 μm thick) and cut according to ASTM D882-91 specifications (100 x 5 mm^2). Tensile tests in two or three-fold were done with a Zwick Z020 (Germany) universal tensile testing machine operated at a crosshead speed of 50 mm/min using a 0.01N pre-load and a grip-to-grip separation of 50 mm. The specimen elongation was derived from the grip-to-grip separation, therefore the presented values of the *E*-modulus give only an indication of the stiffness of the different polymers.

Degradation

In vitro hydrolysis experiments on 50-100 μm thick solution cast films were carried out at 37°C using phosphate buffered saline (PBS) containing sodium azide (Sigma, Switzerland) as antibacterial agent (0.02 wt%). The PBS solution was refreshed every two weeks.

Solution cast films were oxidatively degraded at 37°C in 10% H_2O_2 solution (prepared by diluting 30% H_2O_2 from Merck) containing 0.1M $CoCl_2$ (Aldrich). $CoCl_2$ catalyses the formation of hydroxyl radicals from hydrogen peroxide through a Haber-Weiss reaction [30].

Porous scaffold preparation

Molding and salt-leaching: Copolymer particles were mixed with sodium chloride (sieved to 500-710 μm, 60-80 v%). The mixtures were compression molded in a hot press (THB 400, Fontijne B.V., The Netherlands). Samples were heated to 180°C at minimal pressure for 3 minutes and then pressed at 3.4 MPa for 1 minute, the salt was subsequently leached out using demineralized water (48 hours). The materials were dried in a vacuum oven for 48 hours.

Freeze-drying: 20 % (w/w) polymer solutions were prepared by dissolving the copolymer in 1,4-dioxane at 60°C. Samples were frozen at -196, -78, -28 or +6°C and freeze-dried at 0.04 mbar for 48 hours at room temperature. Samples were washed with ethanol (24 hours) and dried for at least three days under reduced pressure at room temperature.

Freeze-drying and salt-leaching: 10 % (w/w) polymer solutions were prepared by dissolving the copolymer in 1,4-dioxane at 60°C. To the solutions either sucrose (500-700 μm) or sodium chloride particles (500-700 μm) were added. Samples were freeze-dried at 0.04 mbar for 48 hours at room temperature. After evaporation of the solvent, the samples were washed with water to dissolve the particles for 48 hours and subsequently washed with ethanol for 24 hours. Samples were dried under reduced pressure for two days at room temperature.

The densities and porosities were determined from mass and volume measurements of the materials in duplicate.

Scanning Electron Microscopy (SEM)

Samples were cut and coated with Au/Pd in a Polaron E5600 sputter coater. A Hitachi FE-SEM S-800 was used.

Bone marrow cell growth experiments

Goat bone marrow cells (passage 3) were cultured on non-treated and plasma treated copolymer films. The cells were seeded with a density of 10,000 cells/cm^2, on discs in the presence of 3 ml minimal essential medium (α-MEM, Life Technologies, The Netherlands) containing: 15% fetal bovine serum (Life Technologies, The Netherlands), 100 units/ml penicillin, 100 μg/ml streptomycin (Life Technologies, The Netherlands), 2 mM L-glutamine

(Life Technologies, The Netherlands), 0.2 mM ascorbic acid 2-phosphate (Life Technologies, The Netherlands), 10 mM β-glycerophosphate (Sigma, The Netherlands) and 10^{-8} M dexamethasone (Sigma, The Netherlands). After 3 or 6 days of culture at 37°C, the cells were fixed using a 1.5% glutaraldehyde in 0.14 M cacodylate buffer (pH 7.2-7.4) and subsequently stained with methylene blue. Cultured films were then qualitatively evaluated.

Gas plasma treatments

The plasma reactor consisted of a glass tube with an internal diameter of 6.5 cm and a length of 80 cm. The reactor was equipped with three externally placed capacitively coupled electrodes. The distance between the electrodes was 25 cm. The electrodes were connected to a 13.56 MHz radio frequency generator through a matching network (ENI Power Systems). The discharge power was 49 W. Solution cast films of 4x8 cm were placed between the electrodes and were treated double sided. 99.995% pure CO_2 was used with a gas flow of 10 cm^3/min. Samples were treated with a pre-delay of 5 minutes and a post-delay of 2 minutes. A CO_2-plasma pressure of 0.06-0.07 mbar was applied. After treatment samples were rinsed using demineralized water, followed by ethanol (p.a.). Samples were dried in a vacuum oven overnight at room temperature and subsequently stored at -20°C until further characterization.

Results and Discussion

Mechanical properties

Comparing the stress-strain curves of different PEOT/PBT copolymers, the influence of the soft PEOT and the hard PBT segment ratios and the effect of starting PEG molecular can be seen (Fig.2). As was also observed for PEOT/PBT copolymers with high PBT contents [31,32], keeping the PEG molecular weight used in the synthesis at a constant value of 1000 g/mol, an increase in soft segment content causes a lowering in *E*-modulus and maximum stress. The *E*-modulus decreases from over 300 MPa for 1000 PEOT30PBT70 to a value of only 40 MPa for 1000 PEOT70PBT30. The maximum stress decreases from 23.2 to 13.9 MPa. At the same time the elongation at break increases from 600 to a very high value of 1300%. Increasing the soft segment, the phase that contributes most to the strength of the material decreases and a less stiff material is obtained.

In the case of constant soft to hard segment ratio, the *E*-modulus becomes slightly higher (from 39 to 49 MPa) when a lower PEG molecular weight of 300 g/mol is used. The maximum stress and elongation at break decrease significantly from 13.9 to 7.7 MPa and from 1300 to 490%, respectively. These results show the importance of the starting PEG molecular weight used in the polymer preparation on the mechanical properties.

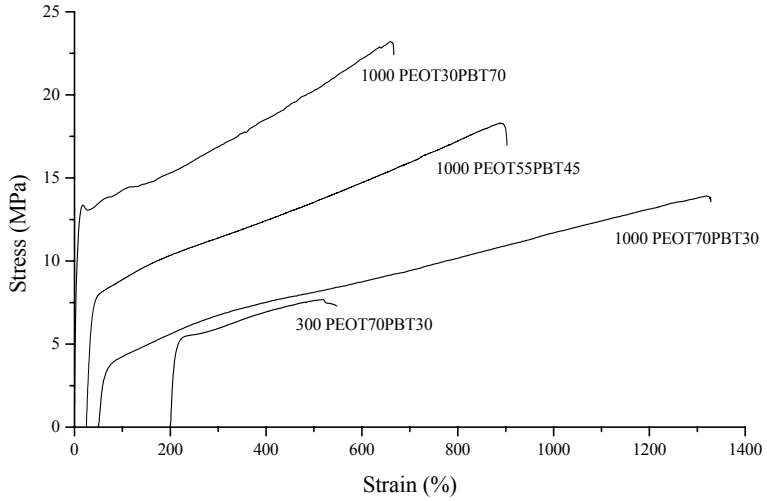


Figure 2. *Stress-strain diagrams of PEOT/PBT block copolymers of different compositions (measurements performed on dry specimens). Stress-strain curves are offset for clarity.*

Swelling and contact angles

PEOT/PBT multiblock copolymers absorb water due to the presence of the hydrophilic PEO. As for other polymers [33,34], this hydrophilicity can have a pronounced effect on cell attachment and proliferation [35]. In this case the presence of hydrophobic PBT segments in the soft domains has a negative effect on the water-uptake. Moreover, the use of a lower PEG molecular weight implies a lower content of PEO in the copolymer. As an example, for a given soft to hard segment ratio, a copolymer synthesized with PEG 1000 contains significantly more PEO than a copolymer synthesized with PEG 300 (Table 1). At a fixed PEOT to PBT ratio, due to the decrease in phase separation and in the PEO content, the water-uptake of copolymers prepared with the lower PEG molecular weight is smaller (Fig.3). The water-uptake also decreases when the PEOT weight fraction is decreased.

Table 1. *PEO content (wt%) for PEOT/PBT copolymers synthesized with PEG of different molecular weights.*

	Soft/Hard Segment Ratio (wt%)					
	100/0	70/30	60/40	55/45	30/70	0/100
PEG 300	70	49	42	38	21	0
PEG 1000	88	62	53	49	26	0
PEG 4000	93	68	58	53	29	0

A comparable trend is observed with the contact angles. There is a decrease, which indicates a more hydrophilic surface, for copolymers with a higher PEOT weight fraction or for those polymerized with a higher PEG molecular weight (Table 2).

Table 2. *Water-uptake and contact angles by captive bubble measurements of various PEOT/PBT block copolymers.*

Copolymer	Water-uptake (wt%)	Contact angle (º)
4000 PEOT70PBT30	212	35 ± 1
1000 PEOT70PBT30	70	39 ± 1
1000 PEOT30PBT70	26	42 ± 1
300 PEOT70PBT30	5	45 ± 2
300 PEOT55PBT45	4	48 ± 1

In vivo the material will absorb body fluids and will swell. E-modulus, maximum tensile strength and elongation at break in the dry and in the swollen state for several copolymer compositions are reported in Table 3. For all copolymers the mechanical properties decrease in the swollen state. Hydrophilic copolymers are the most affected by the uptake of water. In spite of the decrease in the stiffness during water-uptake, all swollen materials keep good mechanical properties and can be handled with ease. PEOT/PBT copolymers can therefore be used as scaffold materials in the engineering of non load-bearing hard tissues.

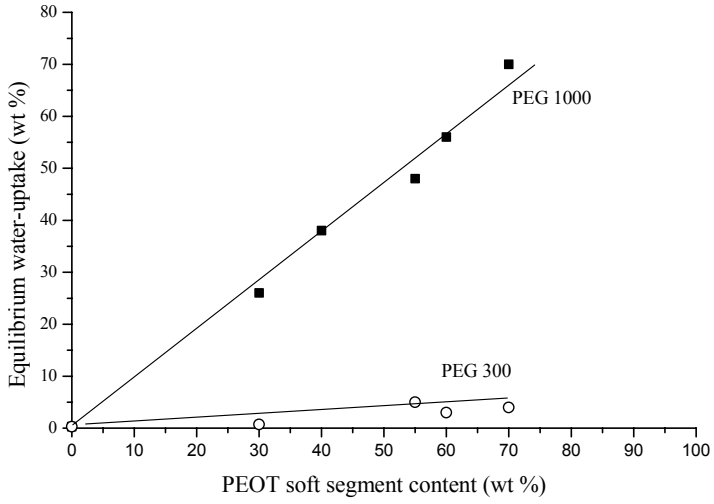


Figure 3. *Equilibrium water-uptake (wt%) as a function of PEOT soft segment content (wt%) for PEOT/PBT copolymers synthesized from* (■) *PEG 1000 and* (○) *PEG 300.*

Table 3. *Equilibrium water-uptake and tensile properties (E-modulus, maximum stress and elongation at break) for 1000 PEOT70PBT30, 1000 PEOT30PBT70 and 300 PEOT70PBT30 on dry and swollen specimens.*

		1000 PEOT70PBT30	1000 PEOT30PBT70	300 PEOT70PBT30
Water-uptake (wt%)		62	26	4
E (MPa)	dry	39	322	49
	swollen	25	274	44
σ_{max} (MPa)	dry	13.4	19.0	7.7
	swollen	11.4	17.9	6.9
ε_{break} (%)	dry	1278	667	402
	swollen	1013	529	318

Degradation

The hydrolysis of PEOT/PBT copolymer in PBS was investigated. The copolymers were chosen with identical starting PEG molecular weight (PEG 1000) and different soft to hard segment ratios, or with identical soft to hard segment ratio (70/30) and different PEG molecular weights (1000 and 300 g/mol). The change in the relative intrinsic viscosity during degradation is shown in Figure 4.

No significant change in intrinsic viscosity or mechanical properties is observed for 300 PEOT70PBT30 during the study. The intrinsic viscosity (0.99 dL/g) and chemical composition remain constant over the degradation time. 300 PEOT70PBT30 does not seem to degrade in PBS during a period of 6 months.

In contrast with 300 PEOT70PBT30, 1000 PEOT70PBT30 shows a rapid decrease in intrinsic viscosity and in mechanical properties. Initially at 0.88 dL/g, the intrinsic viscosity decreases to 0.05 dL/g after 6 months. The mechanical properties are very low after only 3 weeks and cannot be evaluated anymore after 6 weeks. Moreover, after 6 months of degradation, dissolution of 1000 PEOT70PBT30 in chloroform is difficult due to a change in copolymer composition as shown by ^{1}H-NMR. It appeared that the soft segment content has decreased from 70% to 60% after 3 months in PBS and only 52% remains after 6 months. This corresponds to a decrease in PEO content from 62 to 42 wt% in 6 months.

1000 PEOT60/PBT40 shows intermediate degradation behavior, but follows the same trend as 1000 PEOT70/PBT30. However, the initial intrinsic viscosity of 1000 PEOT60/PBT40 was higher and degradation seems slower. Over 6 months, the intrinsic viscosity drops from 1.2 dL/g to 0.18 dL/g, After 3 months, the mechanical properties are very low. After 6 months it was not possible to test the specimens. No change in composition was detected by ^{1}H-NMR.

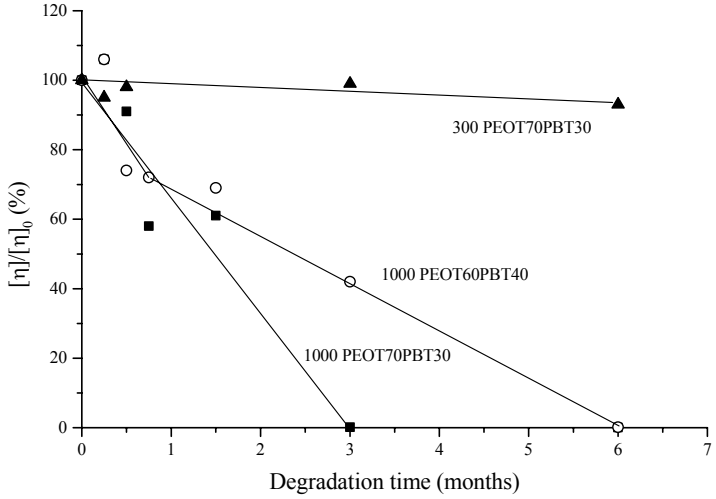


Figure 4. *Relative intrinsic viscosity ($[\eta]/[\eta]_0$) during degradation in PBS at 37ºC for* (■) *1000 PEOT70PBT30,* (○) *1000 PEOT60PBT40 and* (▲) *300 PEOT70PBT30.*

Besides the soft to hard segment ratio, the results show that the used PEG molecular weight is of large influence on the hydrolytic degradation of PEOT/PBT block copolymers. This can be related to the actual PEO content in the copolymer. Furthermore, phase separation is better in 1000 PEOT70PBT30 than in 300 PEOT70PBT30. In 1000 PEOT70PBT30 the hydrophilic PEOT domains are more accessible to water than in 300 PEOT70PBT30. Water-uptake is higher and the possibility to hydrolyze the ester bonds in these PEOT domains increases.

Besides hydrolysis in aqueous body fluids, oxidation can occur *in vivo* due to the presence of specific cells such as macrophages [36,37], which can release hydroxide radicals. Therefore, two copolymers, 1000 PEOT70PBT30 and 300 PEOT70PBT30, were subjected to oxidation in a peroxide solution containing $CoCl_2$. In the presence of H_2O_2 solutions containing $CoCl_2$, hydroxyl radicals (HO·) are formed through a Haber-Weiss reaction [30]:

$$Co^{2+} + H_2O_2 \rightarrow Co^{3+} + HO^- + HO\bullet \qquad (2)$$

Degradation in the $H_2O_2/CoCl_2$ solution had a drastic effect on the intrinsic viscosity and mechanical properties of the samples. After only one day mechanical properties significantly deteriorate. Elongation at break is almost negligible (Table 4). The tensile strength decreases as well, however the effect of the oxidative medium is pronounced on this mechanical property. At day 1, NMR reveals a change in composition: the soft segment content is reduced from 70 to 59 wt% and the PEO content from 62 to 50 wt%. 1000 PEOT70PBT30 turns insoluble in chloroform after two days. 300 PEOT70PBT30, which is relatively stable under

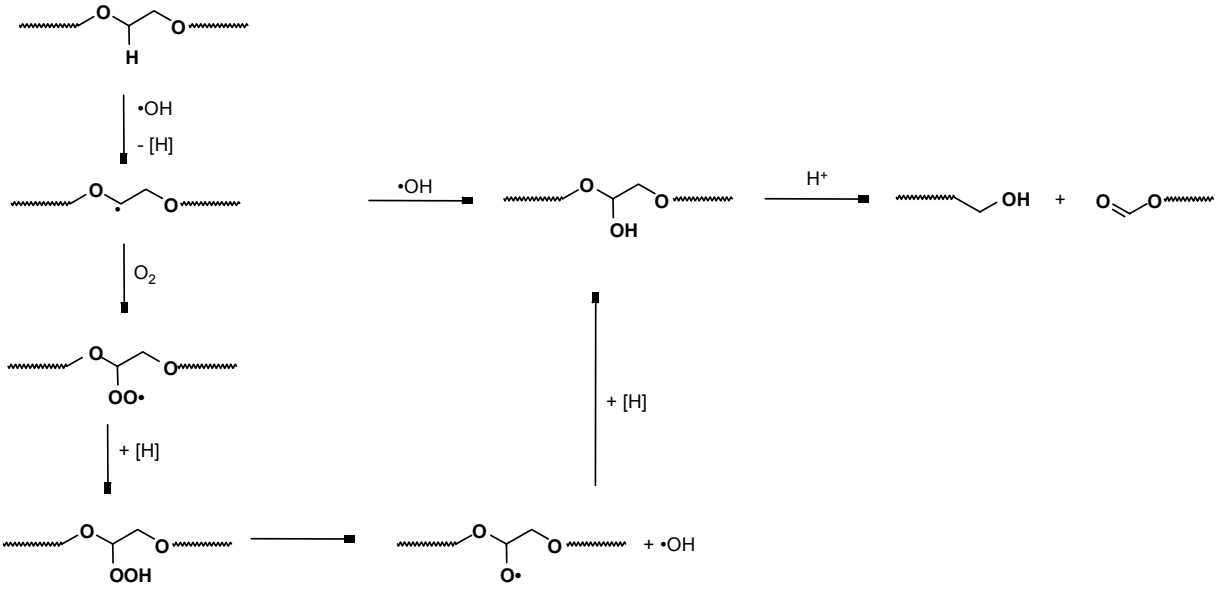
hydrolytic degradation conditions, now shows a large decrease in intrinsic viscosity during oxidative degradation. A slight change in composition is also observed with a decrease in PEOT soft segment content from 70 to 64 wt% and in PEO content from 49 to 45 wt%.

Table 4. *Composition, intrinsic viscosity ([η]), maximum stress (σ_{max}) and elongation at break (ε_{break}) of 1000 PEOT70PBT30 and 300 PEOT70PBT30 before (t_0) and after one day (t_1) in $H_2O_2/CoCl_2$ 10% (measurements performed on swollen specimens).*

Composition		[η] (dL/g)		σ_{max} (MPa)		ε_{break} (%)	
t_0	t_1	t_0	t_1	t_0	t_1	t_0	t_1
1000 PEOT70PBT30	1000 PEOT59PBT41	0.88	-- [a]	6.0	2.1	657	13
300 PEOT70PBT30	300 PEOT64PBT36	0.70	0.21	5.2	4.0	75	12

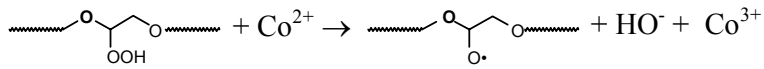
a. insoluble in chloroform

The loss in PEO can be explained by oxidation of ether bonds in the presence of radicals. The thermo-oxidative [38-40], photo-oxidative [41-43] and γ-radiation [44] degradation reactions of PEO and PEO-containing polymers occur via free-radical reactions, leading to scission of the chain [45,46].

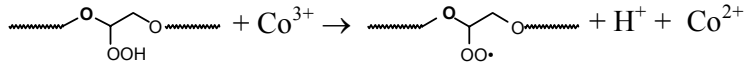


Scheme 1*. Possible reaction pathways in the oxidative chain scission of PEO* [45,46].

The reaction with PEO involves H-abstraction by HO· from the α-carbon atom. Chain scission can occur by subsequent reactions of the macrochain radical either with water or with oxygen as shown in Scheme 1. The hydroperoxide formed in the polymer backbone can also react with cobalt ions by redox reactions to again form macrochain radicals [45]:



(3)



(4)

These reactions lead to the solubilization of PEO-containing segments.

Porous structures

In tissue engineering, the scaffold must have a large surface area to allow cell attachment and to promote tissue ingrowth. This can be achieved by creating a highly porous structure with pore sizes large enough to allow penetration of the cells. The pores should also be interconnected to facilitate nutrition of the cells deep within the construct. According to literature, the porous structure of bone implant material requires a minimal pore density of 75% and pore size of at least 200 μm to optimize cell ingrowth and formation of bone [47]. A high porosity also has the advantage of implanting minimal amounts of polymer. Porous scaffolds were prepared with varying pore sizes and pore densities using different methods in order to be able to define optimal conditions for bone tissue engineering.

A possible way of obtaining porous structures is by mixing sodium chloride and ground polymer particles, followed by melt pressing. A porous structure is obtained, where pore size and porosity are determined by the size and amount of the salt particles added. By varying the amount of salt, it is possible to obtain scaffolds with 60 to 90% porosity (Table 5).

Porous structures of very high porosity can be prepared by solid-liquid phase separation *i.e.* by using solvents that can be freeze-dried such as 1,4-dioxane. It is possible to obtain different pore sizes by changing the freezing temperature of the PEOT/PBT-dioxane solution [48]. At lower freezing temperatures (faster cooling rates) high nucleation speed results in the formation of great numbers of small solvent crystals. The final construct will be highly porous but with small pore sizes. On the contrary, at higher freezing temperatures close to the freezing point of 1,4-dioxane (slow cooling rates), low nucleation speed results in fewer but larger solvent crystals and pores. As shown in Figure 5A and Table 5 highly porous structures with porosities of 80 to 95% are obtained by freeze-drying. Depending on the freezing temperature, pore sizes vary in the range of 10 to 150 μm.

Table 5. *Porosities and densities of 1000 PEOT70PBT30 and 300 PEOT55PBT45 block copolymer scaffolds prepared by different techniques.*

Material	Preparation method	Density (g/cm^3)	Porosity (%)
1000 PEOT70PBT30	Molding	1.188 ± 0.008	0
		0.397 ± 0.013	67 ± 2
		0.320 ± 0.015	73 ± 3
		0.145 ± 0.001	88 ± 1
	Freeze-drying (6°C)	0.232 ± 0.003	81 ± 1
		0.155 ±0.001	87 ± 1
		0.122 ± 0.001	90 ± 1
300 PEOT55PBT45	Molding	1.244 ± 0.002	0
		0.470 ± 0.029	62 ± 4
		0.245 ± 0.010	80 ± 3
		0.238 ± 0.003	81 ± 1
	Freeze-drying (6°C)	0.114 ± 0.005	91 ± 4
		0.0095[a)]	92 [a]
		0.069 ± 0.001	95 ± 1

a. result of a single measurement

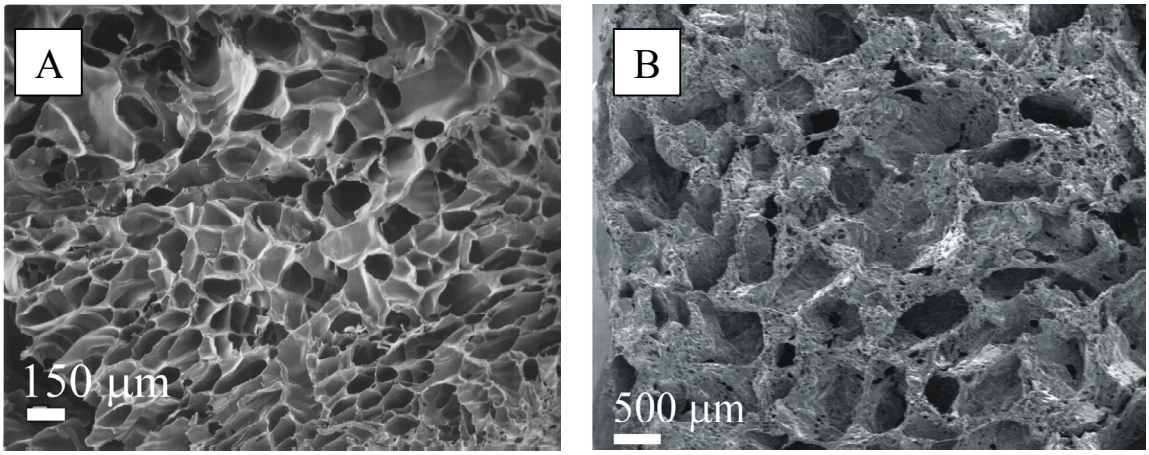


Figure 5*. Porous structures of 1000 PEOT70PBT30. A- prepared by freeze-drying (solid-liquid phase separation of 10% dioxane solutions). Freezing temperature: +6°C (pore size ± 125 µm), B- obtained by combination of freeze-drying (freezing temperature: -28°C, pore sizes: 70-90 µm) and salt-leaching (pore size:5400-700 µm). A 10%-polymer solution in dioxane containing sucrose crystals was used, the overall porosity is 94%.*

By combining the solid-liquid phase separation with the salt leaching technique, it is possible to obtain highly porous structures with interconnected pores. Addition of sucrose to a 10% (w/w) solution of polymer in 1,4-dioxane and subsequent freeze-drying gives porous structures with interconnected pores larger than 400 μm as shown in Figure 5B.

Cell adhesion and growth

Goat bone marrow cells were cultured on various PEOT/PBT copolymer films for 6 days. The amount of cells was qualitatively assessed by judging the extent of methylene blue staining. As indicated in Table 6, goat bone marrow cells can be cultured on the more hydrophobic copolymer compositions 300PEOT55PBT45 and 300PEOT70PBT30. The hydrophilic 4000 and 1000 PEOT70PBT30 do not show cell attachment and growth. To improve these properties, surface modifications have been carried out on these copolymers.

It has been shown that gas plasma treatment can have a beneficial effect on the cell-substrate interaction between dermal or epidermal cells and 300PEOT55PBT45 [20]. To improve the behavior of PEOT/PBT copolymers in goat bone marrow cell culturing, films were treated with a CO_2-plasma. Exposure of these films to a CO_2-plasma for 30 minutes leads to a great improvement in cell growth behavior (Table 6). Gas plasma treatment improves cell growth on copolymers that already sustained growth in their untreated form. Copolymer films that did not sustain cell growth before, the 4000 PEOT70PBT30 and the 1000 PEOT70PBT30 copolymers, now show exceptionally good cell attachment after plasma treatment. This makes PEOT/PBT copolymers suitable materials for goat bone marrow cell culturing. To serve as a tissue engineering scaffold for bone, porous structures are now being plasma treated.

Table 6. *Qualitative assessment of goat bone marrow cell growth after 6 days and contact angles on untreated or CO_2-plasma treated PEOT/PBT block copolymers. -: no cell growth; ±: few rounded cells; +,++,+++: good to very good cell growth.*

Copolymer	Untreated		30 min CO_2 plasma	
	Cell adhesion	Contact angle (º)	Cell adhesion	Contact angle (º)
4000 PEOT70PBT30	-	35 ± 1	+	26 ± 2
1000 PEOT70PBT30	-	39 ± 1	+/++	26 ± 1
300 PEOT70PBT30	±	45 ± 1	+++	24 ± 3
300 PEOT55PBT45	+	48 ± 1	+/++	26 ± 1

Conclusions

The physical properties of PEOT/PBT multiblock copolymers can be tuned by variation of the soft to hard segment ratio and the PEG molecular weight used in the synthesis. These copolymers are sensitive to both hydrolysis and oxidation, which are degradation pathways that also occur *in vivo*. The hydrolytic and oxidative degradation rates can be controlled by varying copolymer composition. By using different preparation techniques porous scaffolds with varying porosities and pore sizes could be obtained. Copolymer composition also has an important effect on bone marrow cell growth *in vitro* on these materials. Bone marrow cells tend to grow better on the more hydrophobic copolymers. Gas plasma treatment with a CO_2-plasma, however, enables the culturing of goat bone marrow cells on a broad range of PEOT/PBT copolymers. Therefore, the choice of the scaffold material can be based on other relevant properties like *in vivo* bone bonding, calcification and degradation behavior.

The degradability, the good results obtained during the cell studies and the feasibility of preparing porous scaffolds make PEOT/PBT segmented copolymers good candidates for use in tissue engineering and regeneration of bone. Optimal scaffold properties will be determined in the future by *in vivo* and *in vitro* experiments.

Acknowledgments

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Chapter 6

In Vitro and *In Vivo* Degradation of Poly(ether ester) Block Copolymers Based on Poly(ethylene glycol) and Poly(butylene terephthalate)*

'Nothing is a waste of time if you use the experience wisely.'
Auguste Rodin (1840-1917)

Abstract

The degradation of poly(ether ester)s based on polyethylene glycol (PEG) and poly(butylene terephtalate) (PBT) (PEOT/PBT) *in vitro* at 100°C in phosphate buffer saline (PBS) and *in vivo* after subcutaneous implantation in rats were studied. Melt-pressed discs of different copolymer compositions were implanted and evaluated up to 24 weeks. After explantation, materials were characterized by means of intrinsic viscosity, mass loss, proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and differential scanning calorimetry (DSC). The copolymer based on PEG with a molecular weight of 1000 g/mol and 71 wt% of PEO-containing soft segments (1000 PEOT71PBT29) showed the most rapid degradation, with 80% decrease in intrinsic viscosity and 50% decrease in mass loss after 24 weeks. The remaining materials were extremely brittle. The other copolymers, 300 PEOT65PBT35 and 300 PEOT50PBT50 showed only little degradation in 24 weeks.

To mimic long-term degradation, three PEOT/PBT copolymers and the PBT parent polymer were degraded for 14 days in refluxing PBS. No mass loss was detected for PBT, but its intrinsic viscosity decreased dramatically indicating hydrolysis of the ester bonds in the hard segments. For the copolymers, mass loss ranged from 10 to 41% and increased with the PEO content and/or the PEO length. After hydrolysis in refluxing PBS, the intrinsic viscosity of the PEOT/PBT copolymers decreased significantly. DSC showed an increase in the heat of fusion. The degradation products of 1000 PEOT71PBT29 present in PBS were analyzed by ^{1}H-NMR and high performance liquid chromatography/mass spectroscopy (HPLC/MS). These degradation products consisted of a PEOT/PBT fraction that was insoluble at room temperature and a fraction with high contents of PEO soluble in PBS. Polymer samples, which were hydrolyzed at 100°C in PBS for 14 days, were subcutaneously implanted in rats for 4 weeks. These PEO-containing copolymers showed significant mass loss and a decrease in crystallinity after one month, whereas PBT did not degrade.

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Introduction

Aromatic polyesters, such as poly(ethylene terephthalate) (PET) and poly(butylene terephthalate) (PBT) exhibit excellent thermal and mechanical properties. These polymers are biocompatible [1,2] and have been used as biomaterials [1,3,4]. However, in applications where degradable polymers are desired, the relative stability under physiological conditions of these aromatic polyesters [5] is a major drawback. To combine the good physical properties with degradability, more labile chemical bonds have been introduced into PET and PBT backbones via copolymerization. The hydrolytic susceptibility is significantly increased by preparing aliphatic/aromatic copolyesters [6-8]. Deckwer and co-workers have demonstrated the sensitivity of such copolyesters to microorganisms and their potential degradability [9-11]. The incorporation of ether segments in aromatic polyesters also enhances the degradability [12,13].

In the last decade, a family of poly(ether ester) copolymers based on poly(ethylene glycol) (PEG) and PBT has been extensively studied as a degradable material for use in medicine. PEOT/PBT block copolymers are biocompatible and *in vivo* no adverse tissue reactions were observed after subcutaneous implantation and degradation in goats [14,15] and rabbits [16]. By variation of the copolymer composition, the physical properties of PEOT/PBT copolymers can be tuned within a wide range. The mechanical properties of many compositions are also satisfactory in the water-swollen state [17]. Bone marrow cell adhesion and growth were excellent on gas plasma treated porous constructs prepared from these polymers [18]. PEOT/PBT copolymers are, therefore, good candidates as scaffolds in the engineering of both soft [19,20] and hard tissues [21,22]. *In vitro*, PEOT/PBT polymers can undergo degradation by hydrolysis and by oxidation reactions [17]. *In vivo*, hydrolysis seems the main factor leading to non-enzymatic degradation, although implantation of medical devices induces a foreign body response during which specific activated cells can produce oxidative reagents [23,24]. The degradation of PEOT/PBT samples is relatively slow, even for those compositions containing high amounts of PEG, and the long-term effects of degradation in the body are not known.

This study aims at better understanding PEOT/PBT degradation. The first part is directed to the *in vivo* degradation of PEOT/PBT block copolymers. Melt-pressed films were implanted subcutaneously in rats for 24 weeks to assess the influence of polymer composition. The second part addresses the effect of accelerated *in vitro* hydrolysis at 100°C, in which the polymer and the released degradation products are analyzed. Long-term degradation was mimicked by implanting these *in vitro* hydrolyzed polymers subcutaneously in rats for one month.

Materials and Methods

Materials

Poly(ethylene glycol) of different molecular weights (PEG 300, PEG 1000) (Fluka, Switzerland), Poly(butylene terephthalate) (PBT) (Aldrich, USA), titanium tetrabutoxide ($Ti(OBu)_4$) (Merck, Germany), dimethyl terephthalate (Merck, Germany), 1,4-butanediol (Acros organics, Belgium) and Irganox 1330 from (Ciba-Geigy, Switzerland) were used without further purification. All solvents used were analytical grade (Biosolve, The Netherlands)

Polymerizations

PEOT/PBT multiblock copolymers were prepared on a 50g scale by a two-step polycondensation of PEG, 1,4-butanediol and dimethyl terephthalate in the presence of titanium tetrabutoxide as catalyst and Irganox 1330 as antioxidant [25]. Details of the synthesis of these copolymers have been published elsewhere [17]. The copolymers were purified and the antioxidant was removed by dissolution in chloroform and precipitation into an excess of ethanol. The composition of the block copolymers is abbreviated as *a* PEOT*b*PBT*c*, in which *a* is the molecular weight of the PEG used, *b* the weight percentage of PEOT soft segments and *c* the weight percentage of PBT hard segments. As terephthalate units are present in the soft segments, the notation PEOT is used to refer to these blocks. In the text, PEG is used when referred to the material used for the synthesis, whereas PEO is used to refer to the repeating segment in the copolymers.

Processing of the polymers

Polymer films were prepared by compression molding (laboratory press THB008, Fontijne, The Netherlands). The molding temperatures were 250°C for PBT, 140°C for 1000 PEOT71PBT29, 150°C for 300 PEOT65PBT35 and 300 PEOT50PBT50. The thickness of the specimens was 400-600 μm. Discs (15 mm in diameter) for *in vitro* and *in vivo* degradation experiments were cut from these films.

Polymer characterization

The intrinsic viscosity [η] of the (non)degraded PBT and PEOT/PBT copolymers dissolved in hexafluoroisopropanol (HFIP) containing 0.02 M of sodium trifluoroacetate (CF_3CO_2Na) was estimated by single point measurements [26,27] at 40°C using an Ubbelohde 1 viscometer. Polymer solutions with a concentration of approximately 0.3 g/dL were used.

Copolymer compositions were determined by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. 300 MHz ^{1}H-NMR (Varian Inova 300 MHz, USA) spectra were

recorded using solutions of polymers in deuterated chloroform (Sigma, USA). In the case of copolymers insoluble in $CDCl_3$, small amounts of trifluoroacetic acid (TFA, Aldrich) were added. Copolymer fractions soluble in water were analyzed using D_2O as a solvent.

The thermal properties of the copolymers were evaluated by differential scanning calorimetry (DSC) with a Perkin Elmer DSC 7 (USA) at a heating rate of 10°C/min. The copolymer samples (5-10 mg) were heated from -80 to 250°C in stainless steel pans. The glass transition temperatures were taken as the midpoint of the heat capacity change. Indium and gallium were used as standards for temperature calibration.

The mass loss was defined as:

$$\text{Mass loss (wt\%)} = \frac{m_0 - m}{m_0} \times 100 \qquad (1)$$

where m_o is the initial specimen weight and m the weight of the degraded specimen after drying for 10 days under reduced pressure at room temperature.

Scanning Electron Microscopy (SEM)

A Leo 1550-field emission SEM (Leo, Germany) was used. Images of non-coated samples were taken at a voltage of 0.6 kV.

In vivo degradation

PEOT/PBT melt-pressed discs (non-degraded and *in vitro* degraded at 100°C, see below) were implanted subcutaneously in the back of young male Wistar rats along the dorso-medial line. Prior to implantation, the polymer discs (diameter: 15 mm, thickness: 0.4-0.6 mm) of known mass were sterilized by γ-irradiation under vacuum. This sterilization method does not affect the polymer properties [28]. Four subcutaneous pockets were formed on the back of each rat and the polymer samples were randomly implanted. After insertion of the samples, the wounds were closed with Vicryl® sutures. Six implants were used per polymer and per interval. Four samples were used for polymer characterization and two for histological analyses.

Melt-pressed 1000 PEOT71PBT29, 300 PEOT65PBT35 and 300 PEOT50PBT50 polymer discs were implanted and analyzed after 1, 2, 4, 12 and 24 weeks. To simulate the effects of long-term *in vivo* degradation, samples of PBT, 1000 PEOT71PBT29, 300 PEOT65PBT35 and 300 PEOT50PBT50, which were previously degraded in PBS at 100°C for 14 days were also implanted and analyzed after 1, 2 and 4 weeks.

The implants were recovered and analyzed in terms of mass loss (average of 4 samples), intrinsic viscosity (1 sample), composition (^{1}H-NMR), thermal properties (DSC) and scanning

electron microscopy (SEM) when possible. For histological analyses, the samples were fixed using a 4% paraformaldehyde solution (Sigma). Prior to embedding, the samples were dehydrated using series of solutions containing an increasing amount of ethanol (70% to 100%). Subsequently, samples were embedded in GMA (Sigma) and sample blocks were cut with a microtome HM 355S (Microtom, Germany). The 5 μm coupes were stained with a hematoxilin-eosin staining (Sigma) and evaluated by light microscopy.

Degradation in PBS at 100°C

PEOT/PBT discs were degraded by accelerated hydrolysis in refluxing phosphate buffer saline (PBS) for 14 days. The polymer discs, recovered by filtering, were very brittle. The degraded samples were analyzed by viscometry, ^{1}H-NMR, DSC and SEM and then subcutaneously implanted in rats. The insoluble degradation products were collected by cerntrifugation and analyzed by ^{1}H-NMR after dissolution in $CDCl_3$ containing TFA. The degradation products soluble in PBS were characterized by high performance liquid chromatography/mass spectrometry (HPLC/MS).

For clarity, the polymers hydrolyzed in PBS at 100°C for 14 days will be referred to as pre-degraded polymers.

Liquid chromatograph with UV and mass spectroscopy detection

HPLC was used to characterize the degradation products, which were present in the PBS buffer after in vitro degradation of 1000 PEOT71PBT29.

Solvents for high performance liquid chromatography (HPLC) were acetonitrile (elution grade) and water from Merck Eurolab (France) (the latter containing 1 mmol/L of sodium tetrafluoroborate from Fluka (Germany) and 20 mmol/L formic acid from Merck (Germany). The HPLC/MS system was from Shimadzu (Germany) and consisted of a SCL-10Avp controller unit, a DGU-14A degasser, two LC-10ADvp pumps, a SUS mixing chamber (0.5 ml), a SIL-10A autosampler, a SPD-10AV UV/vis detector and a LCMS QP8000 single quadrupole mass spectrometer with atmospheric pressure chemical ionization (APCI) probe [29].

For HPLC separation, the following columns were used: LiChrospher RP-18 ec (Macherey-Nagel, Düren, Germany), 5 μm particle size, 100 Å pore size, 2.0 mm id, 125 mm length and guard column of the same material: 2.0 mm id, 20 mm length. The following binary gradient consisting of acetonitrile and water with a flow rate of 0.3 mL/min was used:

t / min	: 0	1	100	110	112	120
[CH_3CN] (%)	: 10	10	70	100	10	stop

The injection volume was set to 10 μL.

For all mass spectroscopic measurements, the curved desolvation line (CDL) serving both for the evaporation of the remaining solvent and as a vacuum restrictor was applied with a voltage of -50 V and a temperature of 230°C. The deflector voltage was 35 V, and the detector voltage was adjusted to 1.6 kV. All experiments were carried out using a probe voltage of 3.0 kV, a nebulizer gas-flow rate of 2.5 L/min and a probe temperature of 500°C.

Results and Discussion

In vivo degradation of melt-pressed PEOT/PBT discs

PEOT/PBT melt-pressed discs were implanted subcutaneously in rats in order to evaluate the influence of polymer composition on the *in vivo* degradation. Table 1 summarizes the characteristics of the degraded samples. Changes in intrinsic viscosity and mass loss during degradation are represented in Figures 1A and 1B, respectively.

The copolymers prepared with PEG 300 (300 PEOT65PBT35 and 300 PEOT50PBT50) showed only little degradation after 6 months of implantation and had kept their mechanical integrity. The composition of both copolymers remained constant (Table 1). The intrinsic viscosity [η] of these copolymers steadily decreased to 75% of the initial values in 6 months, indicating that scission of the polymer chains does occur *in vivo* (Table 1 and Fig.1A). As seen in Figure 1B, the mass loss for 300 PEOT65PBT35 and 300 PEOT50PBT50 was only 3 and 2 wt%, respectively.

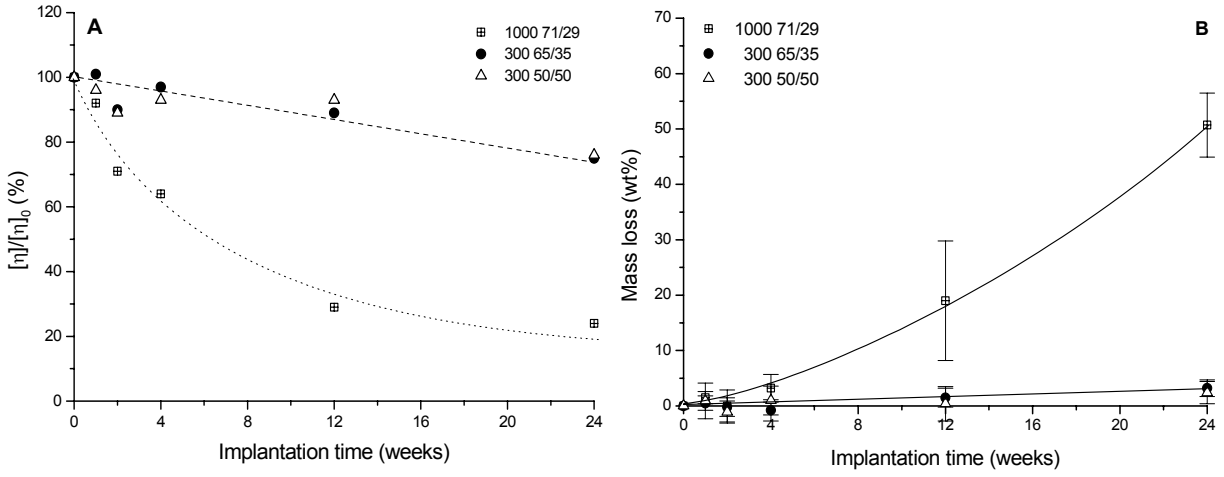

***Figure 1**. Relative intrinsic viscosity (A) and mass loss (B) as a function of implantation time for:* (☒) *1000 PEOT71PBT29,* (●) *300 PEOT65PBT35 and* (△) *300 PEOT50PBT50.*

Like in the *in vitro* degradation at 37°C in PBS [17], the thermal behavior of these polymer samples is also affected *in vivo*, especially the thermal transitions that correspond to the crystalline phase. For 300 PEOT65PBT35 and 300 PEOT50PBT50, a broadening of the melting endotherm $T_{m\ hard}$ can be observed, with the appearance of shoulders in the melting endotherm from 4 weeks of implantation onwards (Table 1). A $T_{g\ hard}$ at approximately 55°C (which is close to the T_g of the parent PBT polymer) can then also be detected. The heat of fusion ΔH_{hard} is plotted against degradation time in Figure 2. An increase in ΔH_{hard} during implantation is observed and after 24 weeks a value similar as during accelerated degradation at 100°C for 14 days is reached (compare with values in Table 2). Several factors can contribute to the increase in crystallinity: preferential degradation of the amorphous phase, increased chain mobility due to reduced polymer chain lengths and annealing of the samples at 37°C [30,31].

Table 1. *Changes in composition, thermal properties and intrinsic viscosity [η] of PEOT/PBT copolymers during in vivo degradation.*

Copolymer	Time weeks	Composition[a]	[η][b] dL/g	$T_{g\ soft}$ °C	$T_{g\ hard}$ °C	Melting range °C	$T_{m\ hard\ (peak)}$ °C	ΔH_{hard} J/g
1000 71/29	0	71/29 (62)	1.92	-48	–	115-165	157	9.9
	1	71/29 (61)	1.76	-50	–	105-170	156	10.5
	2	70/30 (61)	1.37	-50	37	105-160	157	11.6
	4	69/31 (61)	1.22	-49	40	100-155	158	14.9
	12	67/33 (59)	0.56	-48	43	100-170	160	14.9
	24	66/34 (59)	0.46	-49	39	90-175	160	34.9
300 65/35	0	65/35 (44)	1.48	-23	–	100-145	131	5.4
	1	66/34 (44)	1.49	-23	54	105-145	128	9.3
	2	64/36 (44)	1.33	-27	53	105-145	129	10.1
	4	65/35 (44)	1.43	-24	55	110-150	130[c]	9.5
	12	65/35 (44)	1.31	-25	55	110-145	130[c]	20.0
	24	64/36 (44)	1.11	-24	58	115-180	134[c]	20.3
300 50/50	0	50/50 (35)	1.39	-17	–	110-180	169	14.0
	1	50/50 (35)	1.34	-19	55	115-175	164[d]	20.5
	2	50/50 (35)	1.24	-21	55	115-170	164[d]	19.3
	4	50/50 (35)	1.29	-22	55	90-170	166[d]	21.9
	12	50/50 (35)	1.29	-18	59	105-175	167[d]	19.5
	24	50/50 (35)	1.06	-19	59	120-175	166[d]	20.0

a. soft/hard segment ratio (PEO content, wt%)
b. solvent: HFIP + 0.02M CF_3CO_2Na, 40°C
c. shoulder at approximately 160°C
d. shoulder at approximately 145°C

In contrast to the 300 PEOT65PBT35 and 300 PEOT50PBT50 polymers, more significant changes were observed for the 1000 PEOT71PBT29 polymer during implantation. The samples were very brittle after 12 weeks *in vivo* and were difficult to retrieve after 24 weeks. At 6 months, the polymer intrinsic viscosity had decreased to 25% of its initial value (Fig.1A) and 50% mass loss (Fig.1B) was measured. At the same time, the polymer composition had changed, showing a decrease in soft segment content of 5 wt% (Table 1). The copolymer became insoluble in $CHCl_3$ after 24 weeks. During the first 8 weeks the decrease in $[\eta]$ of 1000 PEOT71PBT29 was similar to that during degradation in PBS at 37°C [17]. In time the *in vivo* degradation became slower than that *in vitro*. The decrease in soft segment content is also lower during *in vivo* degradation than during *in vitro* degradation. The *in vivo* degradation seems therefore slower than the *in vitro* degradation at 37°C. Although the reasons for this are not clear, differences between *in vitro* degradation at 37°C and *in vivo* degradation have also been observed for other polymers [32-34].

For the 1000 PEOT71PBT29 copolymer, no significant changes in the glass transition temperature of the soft phase and in the peak melting temperature $T_{m\ hard}$ of the rigid domains were noticed during *in vivo* degradation. On the other hand, a large increase in ΔH_{hard} was observed (Table 1 and Fig.2). This corresponds to an increase in crystallinity from 7% to 24% (assuming a value of 144.5 J/g for perfectly crystalline PBT [35]). During degradation of the 1000 PEOT71PBT29 copolymer, the intrinsic viscosity seems to reach a plateau value after 12 weeks of implantation (Fig.1B). This can be due to the increased crystallinity, which reduces water permeability and the accessibility of the water to hydrolyzable bonds in the polymer.

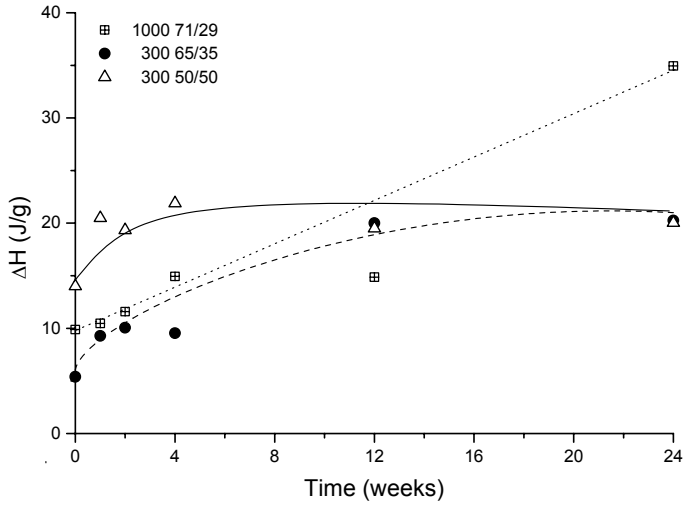

Figure 2. *Heat of fusion (ΔH_{hard}) as a function of implantation time for:* (⊠) *1000 PEOT71PBT29,* (●) *300 PEOT65PBT35 and* (△) *300 PEOT50PBT50.*

Figure 3 shows the effect of implantation on the surface structure of the polymer samples as observed by SEM. In agreement with other studies [15,36], cracks were found at the surface of the implants. The cracks were larger and deeper as the PEO block length and PEO content in the PEOT/PBT block copolymer increased. These fissures are typical of samples with low molecular weights, which have lost their mechanical strength.

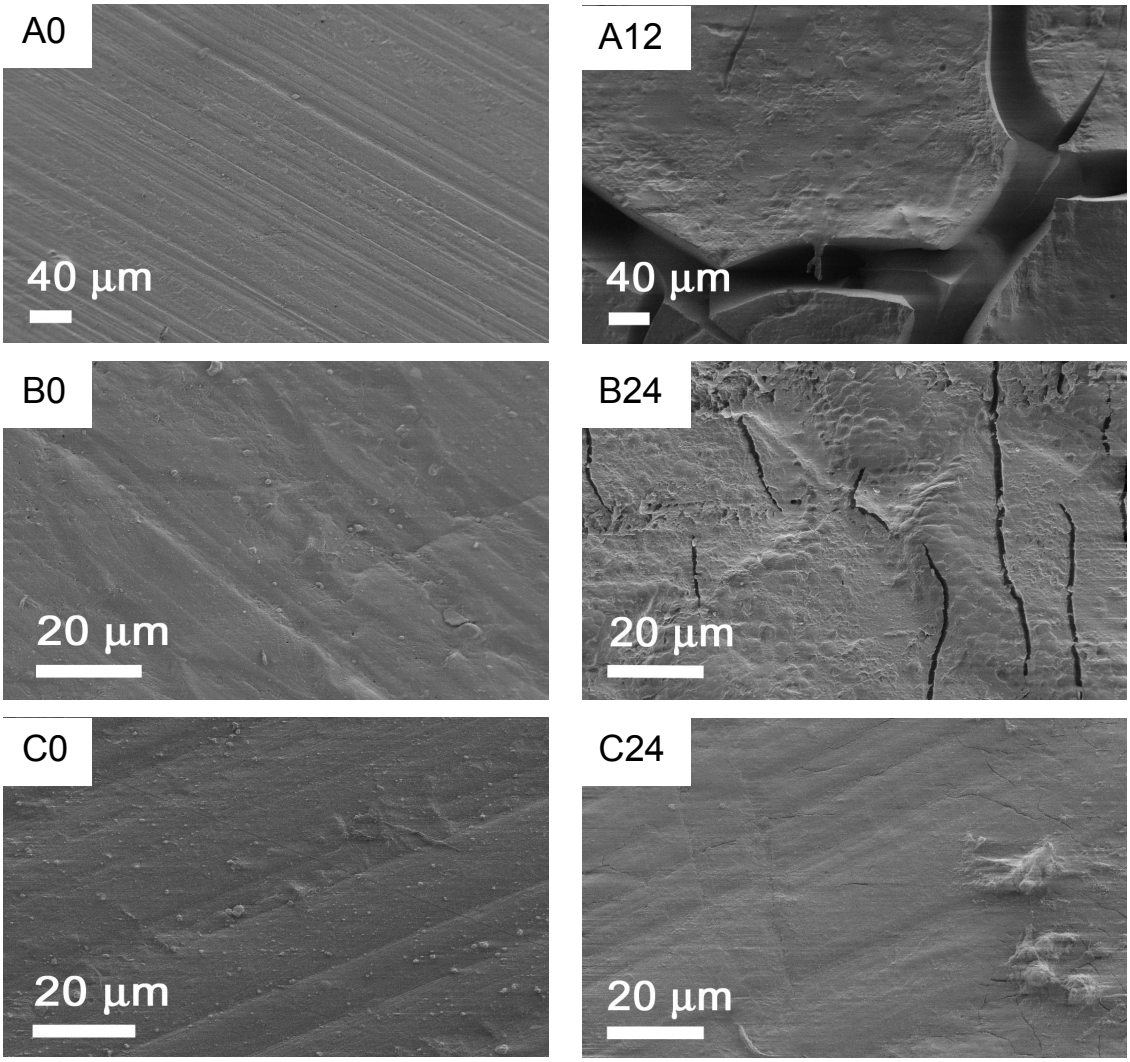

***Figure 3**. Surface structure of 1000 PEOT71PBT29 (A0), 300 PEOT65PBT35 (B0) and 300 PEOT50PBT50 (C0) observed by SEM before implantation and at 12 weeks (A12) or 24 weeks (B24, C24) of implantation.*

Histological sections of the implanted copolymers are shown in Figure 4. After one week the polymer samples (P) are surrounded by fibrous tissue (indicated as T). However, in accordance with the literature [14,15,37] the tissue appears to be quiescent and no adverse tissue

reactions are detected. Macrophage-like cells (m) are present at the surface of all implants. For 300 PEOT65PBT35, healthy afferent and efferent blood vessels (B) are seen just outside of the capsule. The polymer samples are dimensionally intact after one week of implantation.

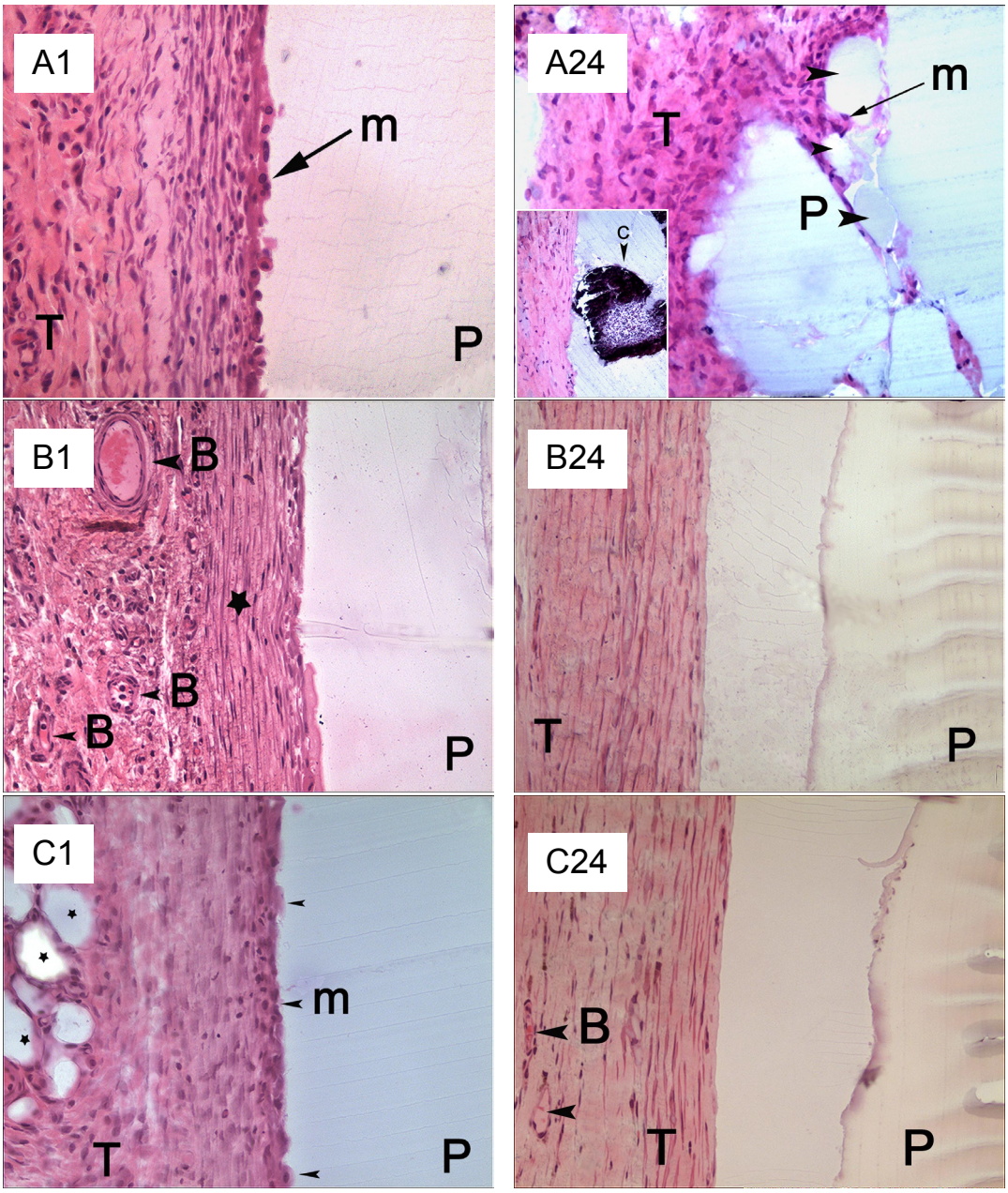

Figure 4. *Histological sections of melt-pressed PEOT/PBT discs implanted for 1 week (left) and 24 weeks (right): (A) 1000 PEEOT71PBT29, (B) 300 PEOT65PBT35 and (C) 300 PEOT50PBT50 (magnification: 200x). P: Polymer, T: fibrous tissue, m: macrophage-like cell, B: blood vessel, C: calcification, ★: fat cells.*

After 24 weeks of implantation, severe fragmentation of 1000 PEOT71PBT29 was observed (Fig.4, A24 arrowheads), while connective tissue is growing in-between the fragments. Dark patches, are present inside the polymer (Fig.4, A24, C). Such observations were already reported earlier for this copolymer [15,36,38,39]. It has been suggested that it is indicative for calcification of the polymer. The extent of calcification depends on implant geometry, implantation site and animal species [15]. In contrast to 1000 PEOT71PBT29, 300 PEOT65PBT35 (Fig.4, B24) and 300 PEOT50PBT50 (Fig.4, C24) implants appear to be intact (gaps and ridges are observed probably due to the dehydration process). The polymer samples are surrounded by fibrous tissue.

Degradation in PBS at 100°C

In order to assess the effect of high temperature hydrolysis on the chemical and physical properties of PEOT/PBT copolymers, specimens were degraded in refluxing PBS (100°C) for a period of two weeks. Poly(butylene terephthalate) (PBT) was included as a reference material. Table 2 presents the characteristics of the polymers before and after accelerated hydrolysis.

Table 2. *Characteristics of PEOT/PBT and PBT polymers before and after hydrolytic degradation in PBS at 100°C for 14 days.*

	PBT		1000 71/29		300 65/35		300 50/50	
	t_o	14 days	t_o	14 days	t_o	14 days	t_o	14 days
Mass loss (wt%)		0		41		17		10
Composition[a]	0/100 (0)	0/100 (0)	71/29 (62)	63/37 (55)	65/35 (44)	63/37 (43)	50/50 (35)	48/52 (33)
$[\eta]$[b] (dL/g)	1.02	0.40	1.92	0.32	1.48	0.29	1.39	0.36
$T_{g\ soft}$ (°C)	–	–	-48	-48	-23	-22	-17	-24
Melting range (°C)	220-235	220-235	100-170	110-190	110-150	90-170	110-180	120-200
$T_{m\ hard}$, peak (°C)	228	232	157	165	131	148	169	178
ΔH_{hard} (J/g)	46.3	68.6	9.9	36.5	5.4	20.6	14.0	46.0
w_c[c] (%)	32	47	3	10	4	14	10	32

a. soft/hard segment ratio (PEO content, wt%)

b. solvent: HFIP + 0.02M CF_3CO_2Na, 40°C

c. crystallinity: $w_c = \Delta H/\Delta H^0$x100 (PBT: ΔH^0=144.5 J/g [35])

Mass loss was not detected for PBT, the control polymer, but the intrinsic viscosity had decreased by 60%. This shows, that during the accelerated degradation, ester bonds of PBT can indeed be hydrolyzed (Fig.5) although no low molecular weight chains leach out of the polymer films, most probably because of their poor solubility in PBS.

After degradation at 100°C, the copolymer discs had become very brittle. The fragility of the samples increased with PEO content of the copolymers. 1000 PEOT71PBT29 specimens were already fragmented during boiling, whereas 300 PEOT65PBT35 and 300 PEOT50PBT50 were dimensionally stable but broke (easily) when handled. For the PEO-containing copolymers, [η] had decreased drastically and significant mass losses ranging from 10 to 41% were measured (Table 2). Values of [η] and remaining mass decreased more rapidly with increasing soft segment content and PEG length, as already observed during the *in vivo* and *in vitro* degradation experiments at 37°C [17]. For the copolymers prepared with PEG 300 only minor changes in composition occurred. This could imply that the observed mass loss is mostly caused by solubilization of low molecular weight polymer chains. For the 1000 PEOT71PBT29 copolymer, a significant change in polymer composition is measured, yielding higher PBT contents in the remaining polymer discs. The soft segment and PEO content is reduced by 8 wt% and 7wt%, respectively.

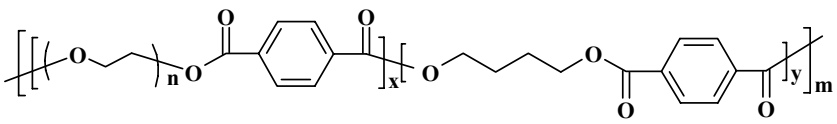

Figure 5. *Chemical structure of PEOT/PBT segmented block copolymers.*

For all polymers, large increases in $T_{m\ hard}$ and ΔH_{hard} were measured after accelerated hydrolysis at 100°C. The increase in the heat of fusion is larger for polymers containing more PEO: 1000 PEOT71PBT29 > 300 PEOT65PBT35 > 300 PEOT50PBT50 > PBT. The increase in crystallinity can be due to annealing at 100°C, increased chain mobility as the molecular weight decreases and preferential degradation of the amorphous regions [30,31].

After accelerated hydrolysis, the PEOT/PBT copolymers were very fragile and brittle. SEM (Fig.6) revealed the presence of numerous large and deep cracks at the copolymer surface. In contrast, in spite of a reduced intrinsic viscosity, the surface of the PBT sample was unchanged and still mechanically stable.

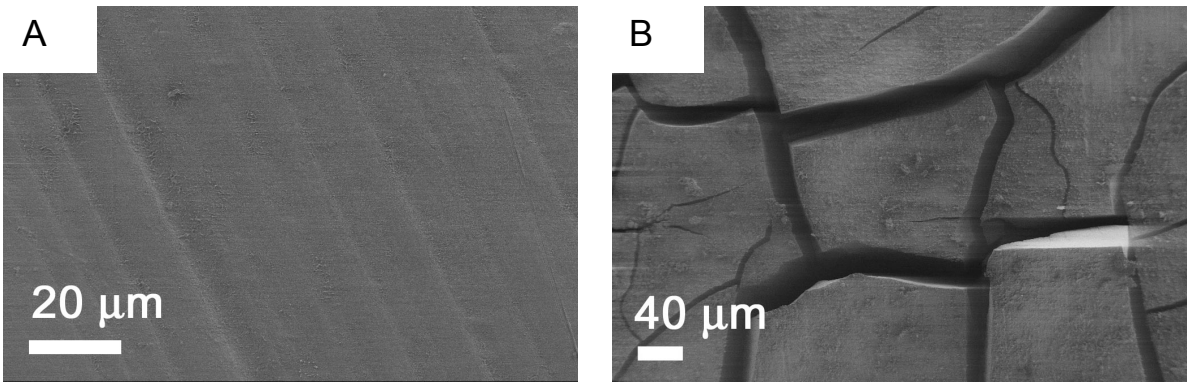

Figure 6. *Surface structure after degradation in refluxing PBS for 14 days: (A) PBT, (B) 300 PEOT50PBT50.*

As the 1000 PEOT71PBT29 copolymer showed the most extensive degradation during hydrolysis at 100°C having lost 41% of mass (Table 2), the degradation products of this polymer were further analyzed. The degradation products present in the PBS buffer were composed of a soluble fraction and a fraction that becomes insoluble upon cooling to room temperature. The fraction that becomes insoluble upon cooling was analyzed by ^{1}H-NMR and compared to the spectrum of the starting copolymer (Fig.7). Multiplets corresponding to ethylene oxide residues connected to the terephthalate unit are present at 3.90 ppm and 4.55 ppm. Peaks that can be attributed to the butanediol component of the PBT unit are visible at 2.00 ppm, and 4.40-4.50 ppm [40]. The presence of these peaks indicates that this insoluble fraction contains both PEOT and PBT segments. The poly(ethylene oxide):terephthalate (PEO:T) weight ratio is 1.8. This value is lower than the value of 2.2 of the starting material (1000 PEOT71PBT29).

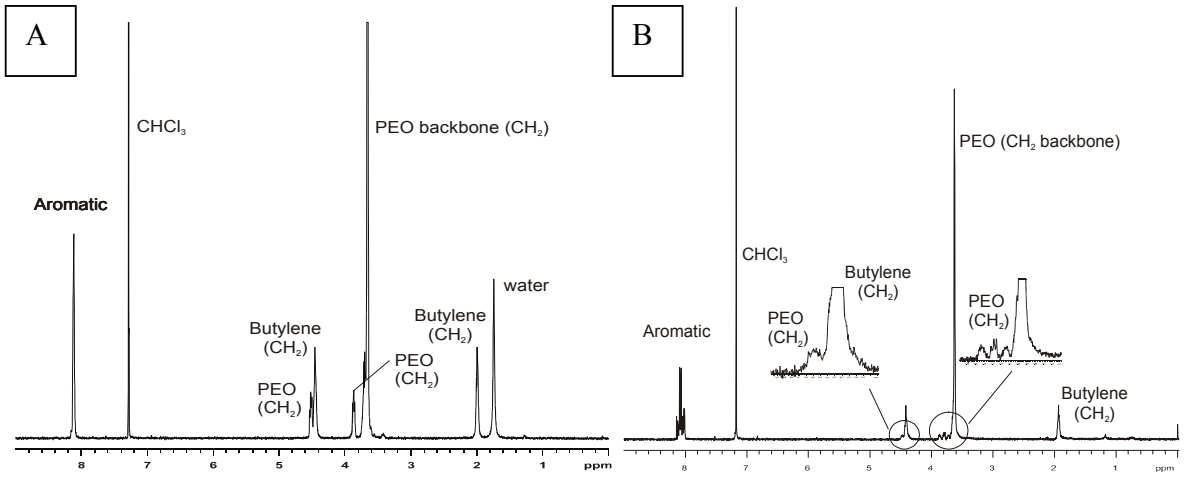

Figure 7. *NMR spectra of (A) the initial 1000 PEOT71PBT29 and of (B) the insoluble degradation products formed after hydrolytic degradation in PBS at 100°C for 14 days.*

The soluble fraction was analyzed by means of HPLC/MS and ^{1}H-NMR. Figure 8 compares the liquid chromatographic separation of PEG 1000 (Fig.8, chromatograms A and B), which was used as starting material during the polymerization process, and the soluble degradation products of the 1000 PEOT71PBT29 polymer (Fig.8, chromatograms C and D).

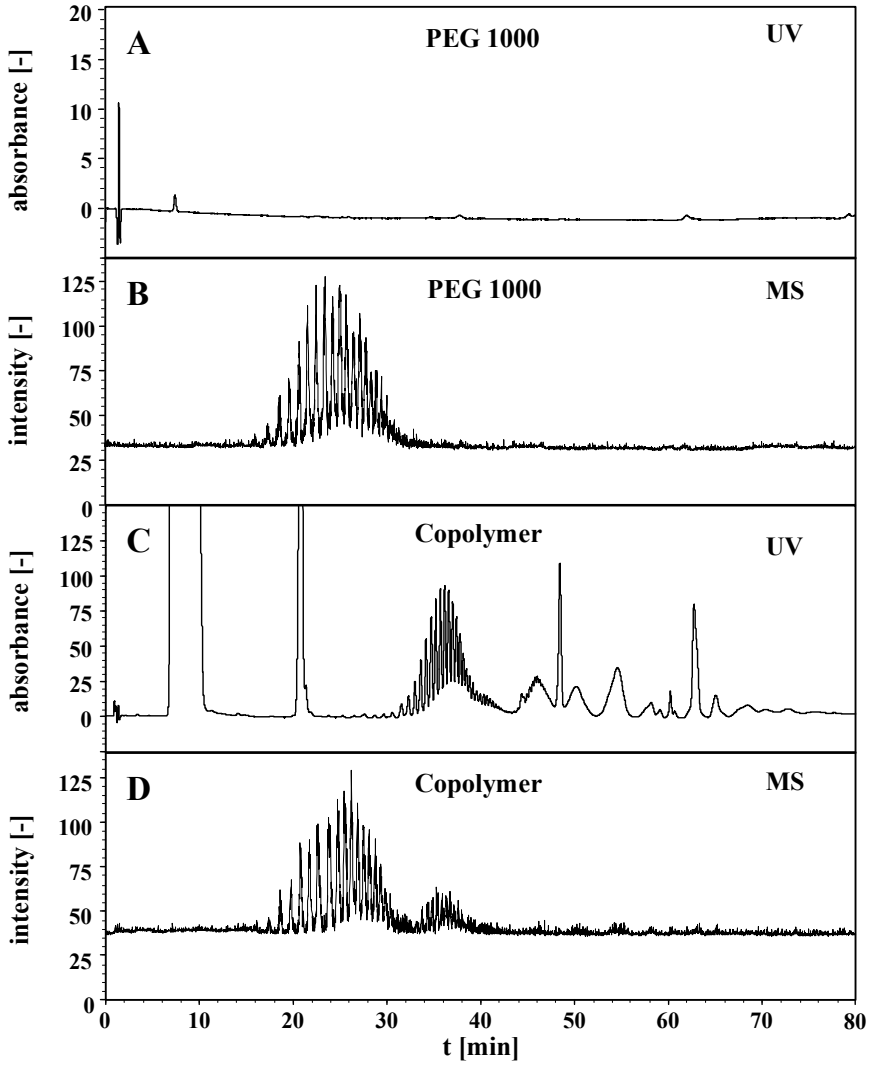

Figure 8. *Liquid chromatographic separation of PEG 1000 (chromatograms A and B) and soluble products derived from 1000 PEOT71PBT29 during hydrolytic degradation at 100°C (chromatograms C and D) with UV detection at 251 nm (chromatograms A and C) and mass spectrometric detection applying APCI(+) conditions (chromatograms B and D) recorded in scan mode (m/z = 200 - 1600).*

Chromatogram A of the PEG 1000 standard solution was obtained applying UV detection at 251 nm. Owing to the lack of chromophores, UV/vis spectroscopy could not be used for the detection of PEG 1000. In contrast, the soluble fraction of the degraded copolymer shows strong signals in the UV/vis chromatogram, which can be due to the presence of terephthalic

units occurring in the different degradation products (Fig.8, chromatogram C). Small amounts of terephthalate in the soluble fraction could be detected by ^{1}H-NMR, from which the PEO:T weight ratio was calculated to be as high as 6.4. The identity of the first compound eluting at ~ 9 min could be determined to be terephthalic acid (comparison of retention time and UV/vis spectrum). Characterization efforts regarding the later eluting compounds were undertaken on the basis of mass spectrometric data. Applying APCI(-) conditions (data not shown), the identity of the peak eluting at ~ 9 min was verified (m/z = 165, $[M-H]^-$) and the peak eluting at ~ 21 min was characterized as the monoester of terephthalic acid and butanediol (m/z = 237, $[M-H]^-$). Chromatogram D was recorded in the APCI(+) mode (SCAN mode: m/z ranging from 200 to 1600) showing the total ion current (TIC). The compounds eluting between 16 and 32 min are PEG molecules containing 8 to 34 EO repeat units (this is due to the molecular weight distribution in the used PEG 1000 sample). This can be confirmed by comparing chromatogram D to the separation of the PEG 1000 standard solution, which is presented in chromatogram B. The compounds eluting between 32 min and 40 min, both present in the UV/vis and the MS chromatogram correspond to one molecule of terephthalate linked to the molecules of different length in PEG 1000 sample. Signals of later eluting compounds – although showing significant signal intensities in the UV/vis chromatogram – could not be identified mass spectrometrically owing to insufficient concentrations.

Figure 9 shows a temporal extract of the liquid chromatographic separation of soluble degradation species of degrading 1000 PEOT71PBT29. In chromatogram A, obtained with UV detection at 251 nm, the peak at ~ 21 min refers to the monoester of terephthalic acid and butanediol. As previously mentioned, the dented peak pattern at higher elution time is based on one terephthalate molecule linked to a PEG species. Additionally, MS detection in the positive APCI mode is summarized in chromatogram B. The total ion current (TIC) is presented in the SCAN mode (m/z = 200-1600) and below, base-shifted, extracted mass traces corresponding to the different sodium adducts of PEG and one molecule terephthalate linked to PEG (number of EO units: n= 20-25) are presented. The first pattern found (16 min to 32 min) comprised chain lengths ranging from 8 to 34 ethylene oxide units. The second group of peaks showing weak signal intensity for MS detection but high intensities for UV/vis spectroscopy (32 min to 40 min) is related to terephthalate-PEG species, comprising compounds with chain lengths ranging from 11 to 29 ethylene oxide units linked to terephthalate. Within each group, every peak refers to one PEG molecule with a certain length and neighbouring peaks have a mass difference of 44 mass units representing one ethylene oxide unit. The mass spectra of every single peak is dominated by singly charged ions formed through adduct formation with sodium ($[M+Na]^+$). Furthermore, but with lower intensities, the protonated molecular ion ($[M+H]^+$) and clusters with sodium formate ($[M+NaCOOH]^+$) are detected.

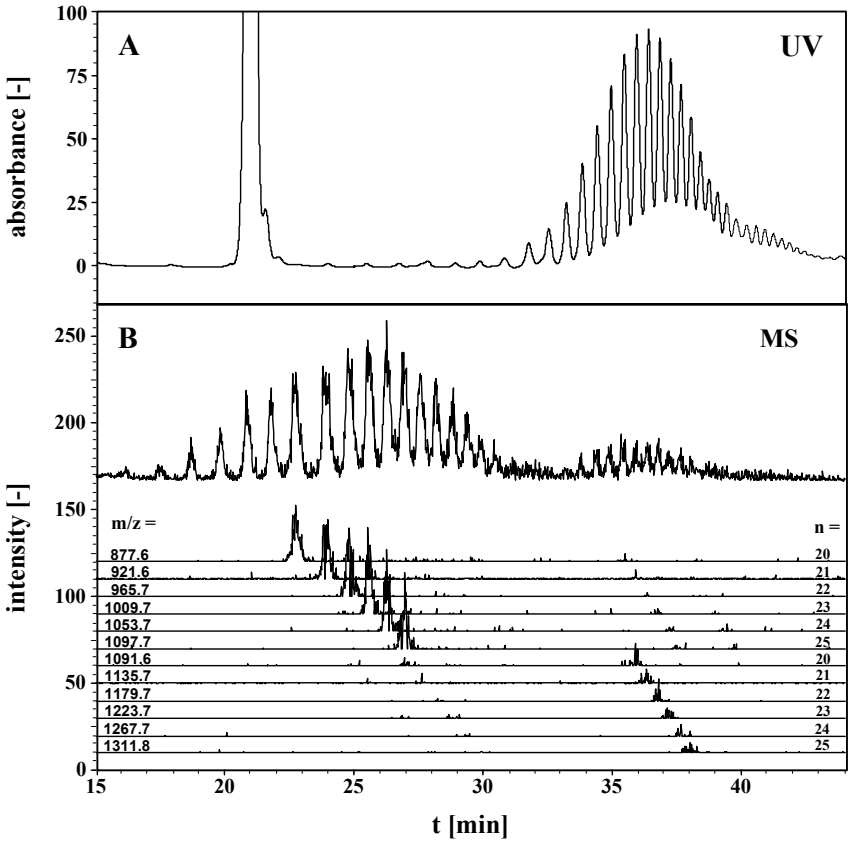

Figure 9. *Part of the liquid chromatographic separation of soluble products derived from 1000 PEOT71PBT29 with UV detection at 251 nm (chromatogram A) and mass spectrometric detection in the positive APCI mode (chromatogram B) recorded in scan mode (m/z = 200 - 1600). Displayed are the base-shifted, extracted mass traces corresponding to the adducts of PEG molecules and terephthalate linked to PEG molecules. For the two series, the peaks correspond only to numbers of EO units ranging from 20 to 25.*

Regarding the experimental conditions used, initial APCI measurements were carried out applying a probe temperature of 400 °C. As this turned out to too low for the evaporation of terephathalate-PEG compounds, the temperature was subsequently set to 500 °C, serving satisfactory transfer into the gas phase. Owing to these severe conditions within the interface, fragmentation of both for the PEG starting materials and the terephthalate-PEG species is observed. While the latter predominantly reveals the loss of one terephtalate unit, the fragmentation of PEG 1000 is mainly characterized by the loss of ethylene glycol moieties.

On the basis of NMR spectroscopy and of liquid chromatographic separation with UV and mass spectrometric detection, it can be concluded that, during accelerated hydrolysis of PBT and PEOT/PBT multi-block copolymers, cleavage mainly takes place in the soft segment via

scission of the ester bonds connecting PEO segments and terephthalate units (Fig.5). Most of the degradation products soluble in PBS are composed of PEG chains or PEOT containing small amounts of terephathalate. However, the detection of the monoester terephthalic acid and butanediol in the soluble fraction of the degradation experiments also indicates a cleavage in the hard segments.

In vivo implantation of pre-degraded PEOT/PBT discs

Pre-degraded PBT, 1000 PEOT71PBT29, 300 PEOT65PBT35 and 300 PEOT50PBT50 were implanted subcutaneously in rats with the purpose of simulating long-term PEOT/PBT degradation and studying the effects of possible remnants at advanced stages of degradation on the living tissue. The samples were explanted and evaluated after 1, 2 and 4 weeks. The changes in properties during degradation are presented in Table 3.

Table 3. *Changes in composition, thermal properties and intrinsic viscosity ([η]) of PEOT/PBT copolymers after subscutaneous implantation in rats.*

Pre-degraded (co)polymer	Time weeks	Composition[a]	$[\eta]$[b] dL/g	$T_{g\ soft}$ °C	$T_{g\ hard}$ °C	Melting range °C	$T_{m\ hard\ (max)}$ °C	ΔH_{hard} J/g
PBT	0	0/100 (0)	0.40	–	–	215-235	232	68.6
	1	0/100 (0)	0.41	–	–	215-230	225	56.7
	2	0/100 (0)	0.39	–	–	215-230	224	57.3
	4	0/100 (0)	0.40	–	52	215-230	225	55.0
1000 71/29	0	63/37 (55)	0.32	-43	–	110-180	165	36.5
	1	61/39 (53)	0.30	-51	46	115-170	163 [c]	29.1
	2	60/40 (53)	0.29	-53	55	115-170	162 [c]	21.1
	4	58/42 (51)	0.35	-49	44	105-170	175 [c]	25.0
300 65/35	0	63/37 (47)	0.29	-22	32	90-160	148	20.6
	1	63/37 (47)	0.29	-27	36	90-170	151 [d]	24.6
	2	61/39 (42)	0.30	-26	38	90-180	166 [d]	9.8
	4	62/38 (42)	0.32	-25	50	90-170	157 [d]	12.6
300 50/50	0	48/52 (33)	0.36	-24	40	135-185	178	46.0
	1	47/53 (32)	0.31	-27	41	135-180	172 [e]	22.2
	2	47/53 (32)	0.27	-23	45	140-180	176 [e]	25.7
	4	45/55 (31)	0.26	-22	42	140-180	176	20.2

a. soft/hard segment ratio (PEO content, wt%)
b. solvent: HFIP + 0.02M CF_3CO_2Na, 40°C
c. shoulder at approximately 135°C
d. shoulders at approximately 110°C and 130°C
e. shoulder at approximately 140°C

After the initial degradation in PBS at 100°C (refer to Table 2), PBT did not degrade further *in vivo* as indicated by the constant mass and $[\eta]$ (Table 3). The melting temperature

slightly decreased from 232°C to 225°C. After 4 weeks *in vivo*, a glass transition temperature could be detected. The heat of fusion showed an initial decrease but remained constant in time. Histological sections revealed normal fibrous tissue surrounding the samples (Fig.11, A). PBT seems very biocompatible without causing any adverse tissue reactions.

Figure 10A shows that the mass loss of the three copolymers continuously increased during the implantation period. After 4 weeks, the copolymers prepared with PEG 300 had lost 70% of their mass and the pre-degraded 1000 PEOT71PBT29 copolymer 90%. The extent of mass loss, however, might be overestimated due to the fragility of the samples and the difficulty to recover the degraded fragments. In contrast to the pre-degraded 300 PEOT65PBT35 and 300 PEOT50PBT50, the pre-degraded 1000 PEOT71PBT29 composition changed with a decrease of 8wt% in soft segment content and in PEO content. The intrinsic viscosity of the copolymers remains relatively constant. After one week of implantation, the samples showed a melting endotherm composed of several peaks and/or shoulders comparable to those observed for the melt-pressed samples after *in vivo* degradation (Table 3). As observed in Figure 10B, the heat of fusion decreased in time indicating that the samples became less crystalline.

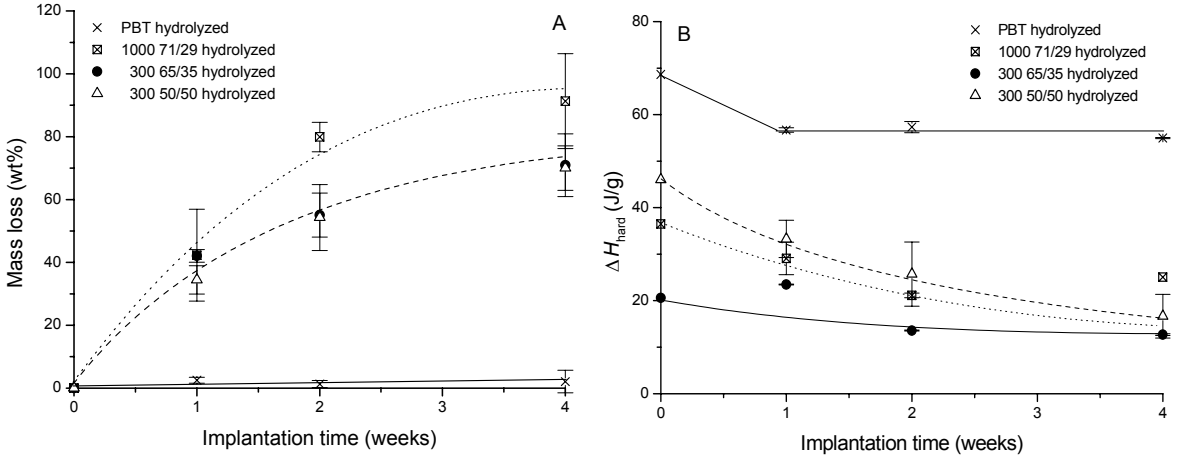

Figure 10. *Mass loss (A) and heat of fusion (ΔH_{hard}) (B) as a function of time for pre-degraded polymers subcutaneously implanted in rats:* (×) *PBT,* (⊠) *1000 PEOT71PBT29,* (●) *300 PEOT65PBT35 and* (△) *300 PEOT50PBT50.*

Histological sections of the implanted copolymers are shown in Figure 11. No adverse tissue reaction was observed after the subcutaneous implantation of pre-degraded PBT and pre-degraded PEOT/PBT. Fragmentation of the pre-degraded 1000 PEOT71PBT29 and pre-degraded 300 PEOT65PBT35 was already visible after one week. The polymer sample

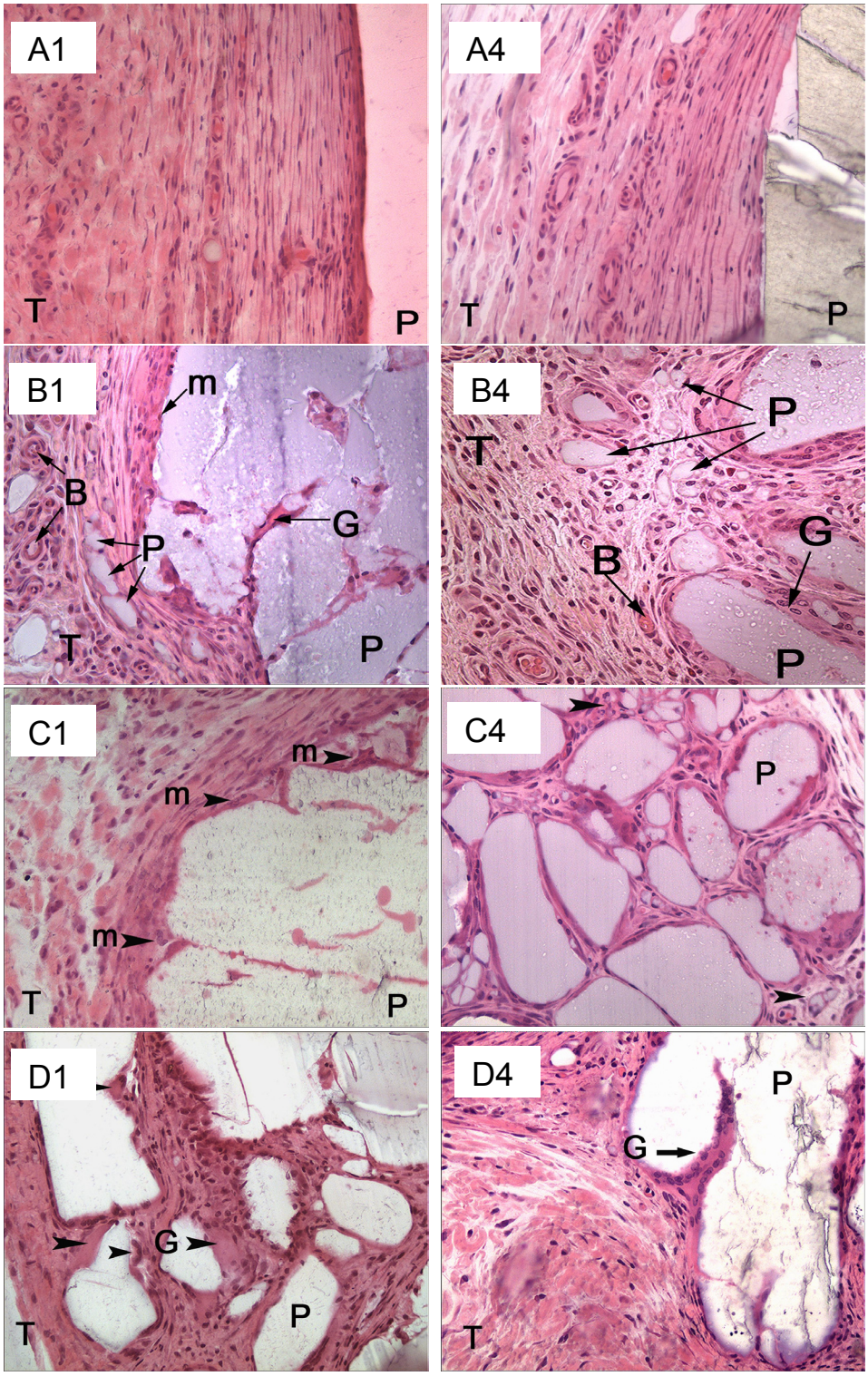

Figure 11. *Histological sections of pre-degraded PEOT/PBT implanted for 1 week (left) or 4 weeks (right): (A) PBT, (B) 1000 PEOT71PBT29, (C) 300 PEOT65PBT35 and (D) 300 PEOT50PBT50. Magnification: 200x. P: polymer, T: fibrous tissue, m: macrophage-like cell, G: giant cells.*

was surrounded by fibrous tissue containing macrophage-like cells (m). Some of the macrophage-like cells have fused to form giant cells (G). After 4 weeks in vivo, the fragmentation became more severe. 1000 PEOT71PBT29 samples were difficult to retrieve. In contrast with the melt-pressed samples (see before), no sign of calcification was detected after implantation of pre-degraded 1000 PEOT71PBT29. Macrophage-like cells and giant cells were still present at the implant surface. Small fragments can be seen inside the cells. At 1 and 4 weeks, pre-degraded 300 PEOT50PBT50 samples were also fragmented but to a lower extent than the two other polymers. Numerous giant cells were already present at the surface from week 1.

Conclusions

Accelerated hydrolysis experiments in PBS at 100°C and *in vivo* degradation in rats, showed the mass and intrinsic viscosity of PEOT/PBT copolymers with high PEO contents decreased more rapidly than copolymers with lower PEO contents. PBT degraded *in vitro* at 100°C did not show further degradation *in vivo.*

Analysis of the degradation products of 1000 PEOT71PBT29 polymers that were subjected to hydrolysis at 100°C showed that molecules containing PEO and terephthalate moieties and PBT-containing chains are produced. However, only residues with high PEO contents and the monoester of terephthalic acid and butanediol are soluble in PBS.

These results indicate that part of the PBT fraction might remain in the body at late stages of degradation. The presence of PBT crystalline domains, however, does not seem to affect the biocompatibility of the samples as no adverse tissue reactions were detected.

Acknowledgments

Mark Smithers (MESA$^+$, University of Twente) is acknowledged for the SEM work.

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Chapter 7

Phase Separation and Physical Properties of PEO-containing Poly(ether ester amide)s*

'Life is not a problem to be solved, but a reality to be experienced.'
Søren Kierkegaard (1813-1895)

Abstract
Poly(ether ester amide) copolymers (PEEA) based on poly(ethylene glycol) (PEG), 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate (a diester-diamide monomer) were synthesized by a two-step polycondensation reaction. The obtained segmented copolymers are hydrophilic with a water-uptake of 24 to 340%. PEEA copolymers showed microphase separation as observed by DSC. The long spacing determined by small angle X-ray scattering shows an increase in hydrophilic domain size with increasing PEO content. By varying the copolymer composition, the E-modulus of PEEA could be varied between 61 and 427 MPa with tensile strengths ranging from 12 to 39 MPa. The elongation at break can reach values of up to 850%. The mechanical properties decrease with uptake of water. However, PEEAs with a relatively low content of PEO still retain good tensile properties and are in principle suitable for biomedical applications.

*A.A. Deschamps, D.W. Grijpma, J. Feijen
Journal Biomaterial Science, Polymer Edition **2002,** in press.

Introduction

The development of new degradable polymers for use in implants and drug delivery systems is of great interest. In tissue engineering, for example, porous biodegradable polymers are used as temporary scaffolds that sustain the attachment and growth of specific cells, facilitating the generation of functional tissues [1,2].

The physical properties of thermoplastic elastomers (TPEs) can be tailored within wide ranges, allowing their use in many surgical applications both in soft and hard tissues. TPEs are phase-separated block-copolymers consisting of soft, rubber-like segments (with a low glass transition temperature), which impart flexibility to the materials, while the glassy or crystallizable segments provide strength and stiffness by the formation of physical cross-links. Depending on the nature of the segments and their block length, the obtained phase morphology can significantly influence the physical properties [3-5] and degradation behavior [6,7] of the polymers. Biological properties, such as blood compatibility and cell adhesion, are also affected by micro-phase separation [8-10].

Most TPEs that have been studied for application in medicine are segmented polyurethanes (PU) [11-13]. In general, polyurethanes are relatively stable [14,15], but several PUs are specially designed to match specific biomedical requirements, such as extensive degradation [16-19]. Thermoplastic elastomers based on hydrophilic poly(ethylene oxide) soft segments and poly(butylene terephthalate) hard segments (PEOT/PBT) show good mechanical properties [6,20,21], excellent biocompatibility [22,23], calcification i*n vivo* [24] and bone-bonding properties [25], making these copolymers good candidates for tissue engineering of bone [26]. Although these polymers have shown to degrade *in vitro* [6], and to fragment into particles that can be phagocytosed by macrophages *in vivo* [25], mass loss was only observed for copolymers with high PEO contents after 6 months of degradation.

In order to obtain TPEs with adequate mechanical properties and degradability, polymers in which the hard segments consist of ester and aliphatic amide units can be an alternative to the terephthalate-containing PEOT/PBT copolymers. These ester-amide units allow physical cross-linking via crystallization and hydrogen bonding. Several researchers have investigated the properties of segmented poly(amide)-containing polymers [27-31] but only few have explored their potential application in medicine [10,32,33].

Segmented poly(ether ester amide)s (PEEAs) copolymers, based on PEO, 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate, were developed in our laboratory for the controlled release of drugs [34]. This family of copolymers has shown degradation *in vitro* [35]. PEEAs might also be good candidates for other temporary medical devices than drug delivery vehicles, as the amide bonds can confer good mechanical properties to the polymers, via hydrogen bonding. These properties are combined with the hydrolyzability of

the esters bonds [36-38] and the biocompatibility, non-toxicity and hydrophilicity of the PEO segments [39].

The aim of this study is to use dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate ester-amide units as hard segments in degradable thermoplastic elastomers and to investigate the structure-property relationships of PEO-containing PEEA block copolymers. The effects of copolymer composition on phase separation and on swelling, thermal- and mechanical properties are determined.

Materials and Methods

Materials

Poly(ethylene glycol) of different molecular weights (PEG 300, PEG 1000 and PEG 4000), supplied by Fluka (Germany), titanium tetrabutoxide ($Ti(OBu)_4$), dimethyl adipate from Merck (Germany), 1,4-butanediol from Acros organics (Belgium) and Irganox 1330 from Ciba-Geigy (Switzerland) were used without further purification. 1,4-Diaminobutane (Merck, Germany) was purified by distillation at 60°C at a reduced pressure of 7 mbar.

Synthesis of diester-diamide monomer (DEDA)

The synthesis of dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate (DEDA) (Fig. 1) was carried out as previously described [35] using dimethyl adipate and 1,4-Diaminobutane, a naturally occurring amine [40]. Distilled 1,4-Diaminobutane was slowly added to a 10-fold excess of dimethyl adipate at 50°C. After complete addition of the 1,4-Diaminobutane, the temperature was gradually increased to 150°C in steps of 25°C in a period of two hours. The reaction temperature was kept at 150°C for a further three hours until methanol distillation had ceased and the reaction was completed. The reaction was carried out in the presence of 0.2 wt% $Ti(OBu)_4$ as catalyst. The reaction mixture was filtered and washed thoroughly with tetrahydrofurane in order to remove the excess of dimethyl adipate.

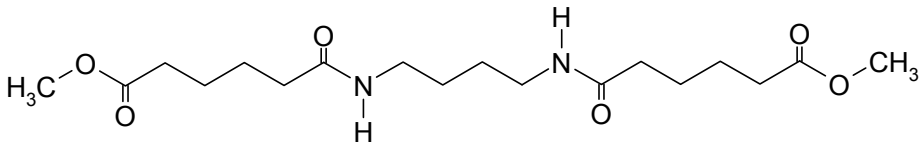


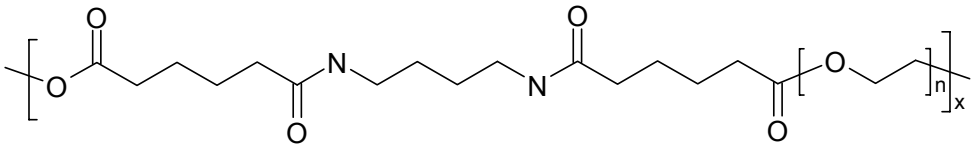
Figure 1. *Chemical structure of dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate (DEDA).*

The yield of the reaction was 82-88%. ^{1}H-NMR using polymer solutions in deuterated dimethylsulfoxide showed that the desired product was obtained [35]. The melting temperature

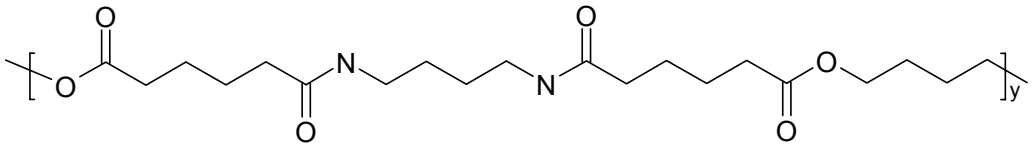
was 133-134°C, using a Büchi capillary melting point apparatus. By DSC at a rate of 10°C/min, the maximum of the melting endotherm was found at 140°C.

Polymerizations

Poly(ether ester amide) (PEEA) multiblock copolymers were prepared on a 20 g scale by a two-step polycondensation [35] in the presence of $Ti(OBu)_4$ as catalyst (0.1 wt% based on the amount of DEDA monomer) and Irganox 1330 as antioxidant (1 wt% of the total amount of reagents). The transesterification of PEG, DEDA and 1,4-butanediol (two-fold excess) was carried out under nitrogen atmosphere at 175°C. After two hours the pressure was slowly decreased to 0.04 mbar to allow the condensation reaction to take place. Simultaneously, the temperature was increased from 175 to 220°C. The composition of the block copolymers is indicated as *a* PEEA *b*/*c*, in which *a* is the starting PEG molecular weight, *b* the weight percent of PEO-containing soft segments and *c* the weight percent of hard segments (Fig. 2). The abbreviation PEG is used when referred to the material used for the synthesis, whereas PEO is used to refer to the repeating segment in the PEEA copolymers.



PEO-containing soft segment



Hard segment

Figure 2. *Chemical structure of PEEA segmented block copolymers.*

It should be noted that DEDA units are present in both the soft and the hard segments. Therefore for a given copolymer, the PEO content is lower than the soft segment content (Table 1).

Table 1. *PEO content (wt%) of PEEA copolymers synthesized with PEG of various molecular weights at given soft to hard segment ratios.*

	soft/hard segment ratio (wt/wt)				
	100/0	70/30	55/45	30/70	0/100
PEG 300	49	35	27	15	0
PEG 1000	76	54	42	23	0
PEG 4000	93	65	51	28	0

Polymer characterization

The intrinsic viscosity [η] of the PEEA copolymers in chloroform/methanol (1:1 v/v) was estimated by single point measurements [41,42] at 25°C using an Ubbelohde OC viscometer. Polymer solutions were prepared at a concentration of approximately 0.3 g/dL.

The polymer composition was determined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) using a Varian Inova 300 MHz (USA) and polymer solutions in deuterated dimethylsulfoxide (Sigma, Germany).

The thermal properties of copolymers containing antioxidant were evaluated by differential scanning calorimetry (DSC) with a Perkin Elmer DSC 7 (USA). A heating rate of 10°C/min was applied. The copolymer samples (5-10 mg) were heated from -80 to 250°C in stainless steel pans. The samples were then quenched rapidly (300°C/min) until -80°C and after 5 min a second heating scan was recorded. The data presented are from the second heating scan. The glass transition temperatures were taken as the midpoint of the heat capacity change, the melting temperatures were determined from the maximum in the melting endotherm. Indium and gallium were used as standards for temperature calibration.

The equilibrium water-uptake in demineralized water was defined as the weight gain of the melt-pressed polymer sample:

$$\text{Water uptake (wt\%)} = \frac{m - m_0}{m_0} * 100 \qquad (1)$$

where m_o is the initial specimen weight (approximately 45 mg) and m the weight of the specimen after conditioning to equilibrium at 37°C.

Small angle X-ray scattering

Small angle X-ray scattering (SAXS) measurements were performed using a NanoStar device (Bruker AXS, Germany) with a ceramic fine-focus X-ray operated in point focus mode. The tube was powered with a Kristalloflex K760 generator at 35 kV and 40 mA. The primary

beam was collimated using cross-coupled Göbel mirrors and a 0.1-mm pinhole providing a CuK_α radiation beam (λ = 0.154 nm) with a full-width at half-maximum about 0.2 mm in diameter at the sample position. The sample-detector distance was 103 cm. A Hi-Star position-sensitive area detector (Siemens) was used to record the scattering intensity in the q-range 0.1 to 1.5 nm^{-1} The scattering vector q is defined as:

$$q = \frac{4\pi}{\lambda} * \sin\frac{\theta}{2} \qquad (2)$$

where λ is the wavelength and θ is the scattering angle. The measurements were performed at ambient conditions using a metal sample chamber with two thin Kapton windows. Samples were cut from melt-pressed polymer films with a thickness of approximately 0.5 mm. Measurements were performed on dry samples and on equilibrium water swollen samples.

Mechanical properties

Tensile testing was performed on dry and water swollen copolymer films. Specimens 400-600 μm thick were prepared by compression molding (table press THB008, Fontijne laboratory, The Netherlands) at temperatures approximately 20°C above the polymer melting point and cut according to ISO 37 type 2 specifications (dumb-bell shaped specimens, width = 4 mm). Tensile tests were performed in four-fold at room temperature with a Zwick Z020 (Germany) universal tensile testing machine operated at a crosshead speed of 500 mm/min using an extensometer, a 0.1 N pre-load and a grip-to-grip separation of 45 mm. The specimen elongation was derived from the extensometer separation (20 mm). The *E*-modulus was determined from the initial slope of the stress-strain curve (between 0.1 and 0.3% of strain) at a crosshead speed of 50 mm/min. The specimens were tested at ambient conditions. The error is less than 5% for the *E*-modulus and the maximum stress determination and is up to 20% for the elongation at break.

Results and Discussion

The characteristics of PEEA copolymers synthesized by a two-step polycondensation reaction are given in Table 2. The compositions of the obtained polymers, as determined by ^{1}H-NMR, are in relatively good agreement with the starting feed compositions. In the NMR spectra, peaks corresponding to polymer end-groups could not be detected. The values of the intrinsic viscosities range from 0.32 to 1.30 dL/g.

Table 2. *Characteristics of synthesized of poly(ether ester amide)s.*

Feed Composition	PEO content in feed (wt%)	Actual soft to hard segment ratio[a]	Actual PEO content (wt%)[a]	[η] dL/g[b]
0/100 (PEA_1)	0	0/100	0	0.39
0/100 (PEA_2)	0	0/100	0	0.64
300 70/30	35	69/31	34	0.32
		65/35	32	0.51
300 55/45	27	54/46	27	0.61
		49/51	24	0.55
300 30/70	15	30/70	15	0.61
		25/75	12	0.68
1000 70/30	54	76/24	58	0.56
		74/26	57	0.81
1000 55/45	42	57/43	43	0.47
		57/43	44	0.64
1000 30/70	23	32/68	25	0.77
		31/69	24	0.66
4000 70/30	65	77/23	72	1.30
		71/29	66	0.64
4000 55/45	51	61/39	57	0.69
4000 30/70	28	34/66	31	0.53
		33/67	30	0.90

a. as determined by ^{1}H-NMR

b. solvent: $CHCl_3$/MeOH (1:1 v/v) at 25°C

Water-uptake

Water-uptake is important, as medical implants prepared from these materials will be in contact with body fluids. It will have an effect on the physical properties of the polymer but also on cell attachment and proliferation characteristics [43]. PEEA copolymers are relatively hydrophilic, taking up appreciable amounts of water.

Depending on the composition, the water-uptake of the synthesized PEO-containing copolymers ranges from 25 to 340 wt% (see Fig.3). The water-uptake increases with PEG molecular weight and with soft segment content. 4000 PEEA 70/30 (not shown in the graph) absorbs such large amounts of water that it is not far from solubility. From Figure 3B it can be seen that the water-uptake is mainly dependent on the content of the hydrophilic PEO.

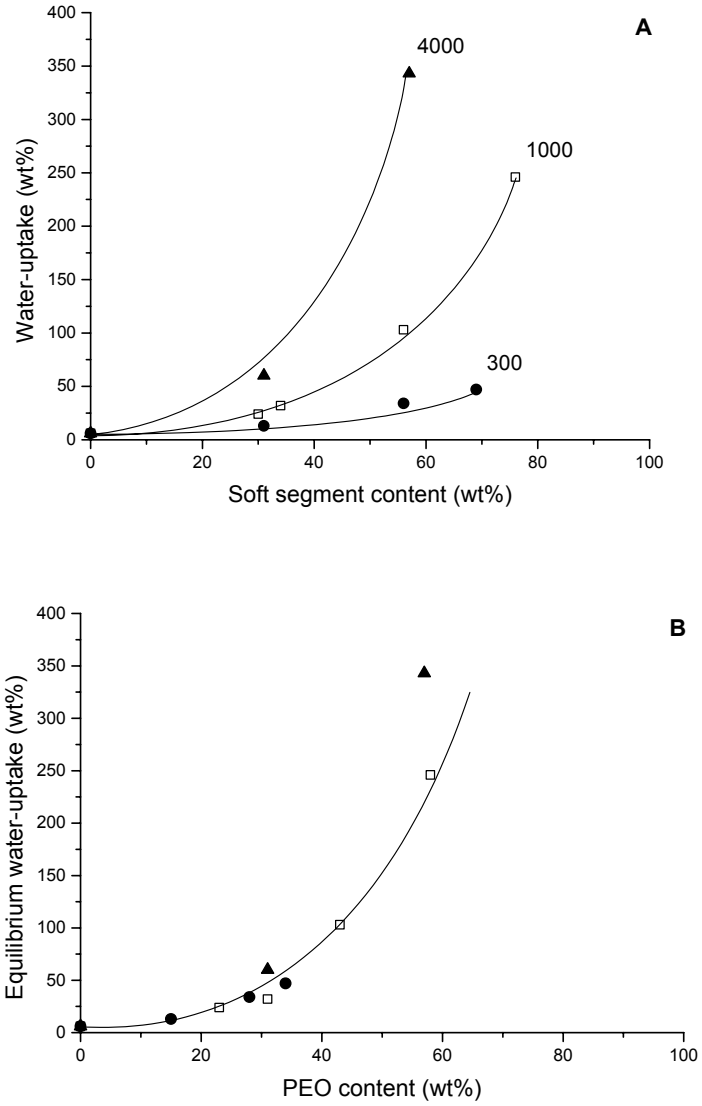


***Figure 3**. Equilibrium water-uptake at 37°C as a function of soft segment content (A) and of PEO content (B) for PEEAs prepared from different PEG molecular weights:* (●) *PEG 300,* (□) *PEG 1000 and* (▲) *PEG 4000.*

Compared to PEOT/PBT polymers of similar compositions [6], PEEAs are much more hydrophilic. The increase in PEEA water-uptake with soft segment is not linear in contrast with PEOT/PBT polymers. This deviation from linearity is more pronounced for polymers prepared with higher starting PEG molecular weights. This can be explained by hydrogen bonding capability of the PEEA amide groups with water. In this regard it should be noted that poly(ester amide) (PEA) absorbs 6 wt% of water, whereas PBT is much more hydrophobic

and only takes up 0.3 wt%. Therefore, the physical network formed by the interactions between hard segments is more disrupted by the uptake of water in PEEAs than in PEOT/PBT.

Thermal properties

In thermoplastic elastomers, the nature of the segments and their block length are the main factors inducing phase separation. This controls the physical properties and the temperature range in which the polymers can be used. In the case of the PEEAs discussed in this paper, the phase separation should depend on the starting PEG length and on the average hard segment length. The formation of hydrogen bonds by the amide groups and their ability to crystallize upon cooling are additional driving forces for phase separation. This can be investigated by differential scanning calorimetry (DSC). Typical DSC thermograms are shown in Figure 4. As seen in Table 3 (representative copolymers), the thermal properties of the PEEA copolymers vary significantly with the copolymer composition. Other poly(ether ester amide)s based on nylon-6 also showed such dependence [27].

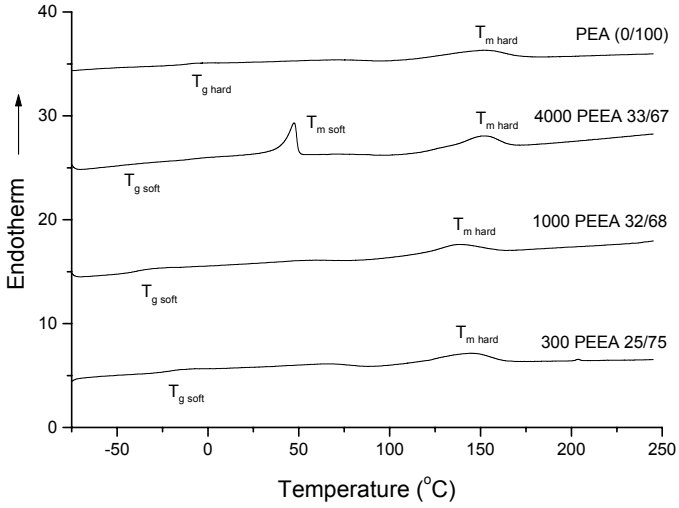


Figure 4. *Typical DSC scans of PEEA polymers with high contents of hard segment.*

Table 3. *Thermal properties of representative PEEA block copolymers, PEG 300, PEG 1000 and PEG 4000.*

Composition	PEO Content (wt%)	Soft-phase			Hard-phase			
		$T_{g\,soft}$ °C	$T_{m\,soft}$ °C	ΔH_{soft} J/g	$T_{m\,hard}$ °C	ΔH_{hard} J/g	w_c %	$\overline{L}_n$ [b]
300 69/31	34	-36	– [a]	–	111	7.5	5	1.4
300 65/35	32	-30	–	–	134	8.5	6	1.8
300 54/46	27	-28	–	–	137	13.0	9	2.3
300 49/51	24	-32	–	–	144	15.1	10	2.6
300 30/70	15	-22	–	–	144	28.7	20	4.6
300 25/75	12	-29	–	–	149	39.9	27	5.6
1000 76/24	58	-48	13	14.1	121	10.2	7	2.0
1000 74/26	57	-46	9	7.0	130	8.1	6	2.2
1000 57/43	45	-43	–	–	134	16.4	11	3.5
1000 57/43	44	-46	–	–	138	17.0	12	3.5
1000 32/68	25	-41	–	–	147	25.7	18	8.0
1000 31/69	24	-39	–	–	144	30.1	21	8.7
4000 77/23	72	-49	50	69.8	151	8.3	4	4.2
4000 71/29	66	-45	51	69.8	142	7.6	5	5.4
4000 61/39	57	-46	49	75.4	148	17.3	5	7.9
4000 34/66	31	-46	48	25.2	150	24.9	17	22
4000 33/67	30	-48	48	20.0	151	24.8	17	23
PEA_1	0	–	–	–	147	41.8	29	∝
PEA_2	0	–	–	–	150	23.1	16	∝
PEA_{2A} [c]	0	–	–	–	149	31.7	22	∝
PEG 300	100	-48	-16	78.2	–	–	–	–
PEG 1000	100	–	39	150.2	–	–	–	–
PEG 4000	100	–	60	178.7	–	–	–	–

a. – not observed

b. average hard segment sequence length: $\overline{L}_n = \frac{1}{1 - x_A}$, x_A = mole fraction of hard segments

c. sample PEA_2 annealed at 100°C for 30 minutes

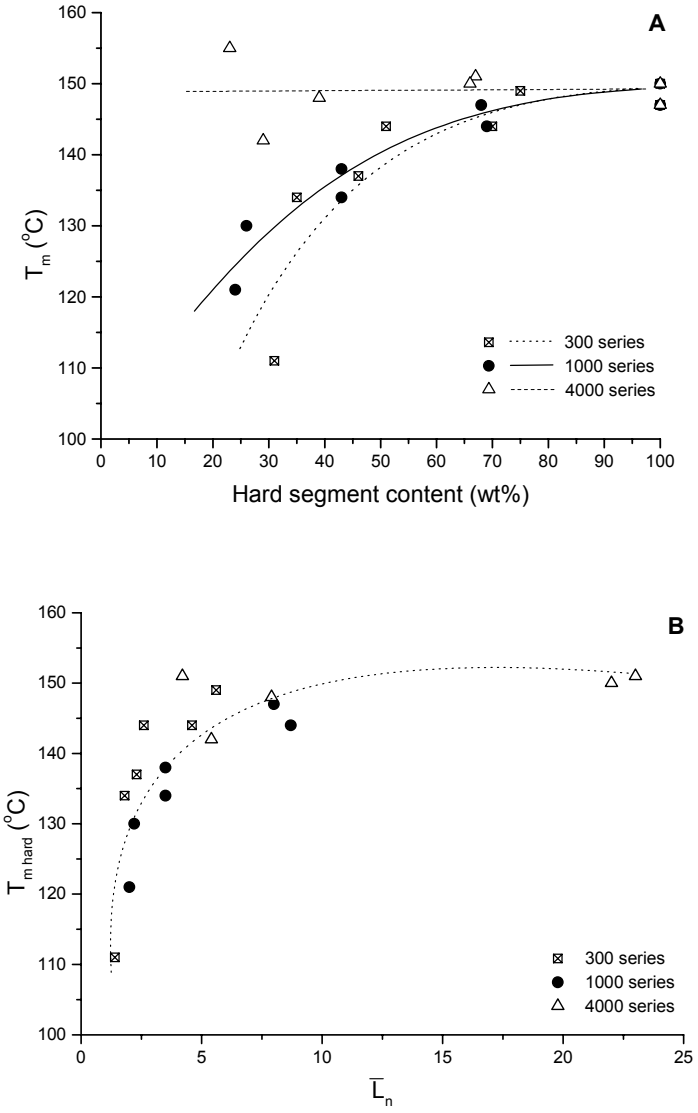


Figure 5. *Melting temperature ($T_{m\ hard}$) as a function of A) hard segment content and of B) average hard segment sequence length ($\overline{L}_n$) for PEEA synthesized with* (⊠) *PEG 300,* (●) *PEG 1000 and* (△) *PEG 4000.*

All investigated copolymers are semi-crystalline and exhibit a glass transition at low temperature (Table 3). The melting temperature ($T_{m\ hard}$) is attributed to the rigid amide-containing domains. $T_{m\ hard}$ is relatively broad (about 50°C) due to the random condensation process in the synthesis, which leads to the formation of chains with a distribution of ester-amide sequence lengths and crystal sizes. The low temperature endotherm ($T_{m\ soft}$) observed in the 4000 series originates from crystalline PEO in the soft phase. The thermal transitions observed by DSC are close to those of the parent polymers, indicating that PEEA copolymers

undergo appreciable microphase separation. For the copolymers synthesized with PEG 300 and PEG 1000, a minor melting endotherm is detected at 65°C and 49°C, respectively. This melting transition is very broad and is constant.

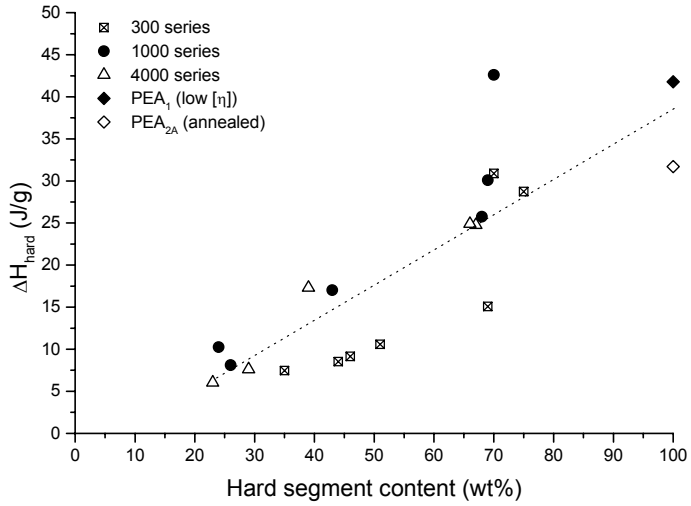


Figure 6. *Heat of fusion (ΔH_{hard}) as a function of hard segment content for PEA_1 (◆), PEA_{2A} (◇) and PEEA synthesized with* (⊠) *PEG 300,* (●) *PEG 1000 and* (△) *PEG 4000.*

The change in the melting temperature $T_{m\ hard}$ and corresponding heat of fusion ΔH_{hard} are represented as a function of the hard segment content in Figures 5A and 6, respectively. For a given polymer series, an increase in hard segment content leads to a rise in $T_{m\ hard}$ and ΔH_{hard}. This trend is even more pronounced when the thermal properties are plotted against PEO content (graph not shown). These observations are correlated to an increase in average sequence length of the hard segment (Fig.5B). For the 4000 series, $T_{m\ hard}$ does not change with increasing hard segment content and its value is comparable to the melting temperature of the PEA parent polymer. This indicates pronounced phase separation. At a given PEO length, a change in PEO content does not significantly influence the glass temperature of the soft phase, meaning that the mobility is not inhered by the rigid phase in the copolymer.

At similar hard segment contents, $T_{m\ hard}$ seems to increase with the molecular weight of the PEG used in the synthesis (Fig.5A). The effect on ΔH_{hard} is more difficult to assess; from Figure 6, ΔH_{hard} appears to be relatively independent of the PEG length. At a same hard segment content, an increase in the used PEG molecular weight leads to a lowering of the glass transition temperature of the PEO-containing soft segment. Longer PEO-containing soft segments also imply longer hard segment sequence lengths (Table 3). These reduced glass transition temperatures point at enhanced phase separation. At high PEG length (PEG 4000), the extent of phase separation even allows crystallization of the PEO.

For PEA, ΔH is strongly affected by polymer molecular weight and annealing (Table 3). PEA_1, which is of low molecular weight, has a higher ΔH than PEA_2. The shorter chains of PEA_1 are much more mobile than those of PEA_2 and therefore can crystallize much better. However the crystallinity of PEA_2 can be improved after annealing for 30 minutes at 100°C.

In degradable semi-crystalline polymers, the amorphous domains are the most susceptible to hydrolysis [44,45]. Hydrolysis of the amorphous phase leads to an increase in crystallinity, which can result in highly crystalline debris, possibly causing an inflammatory response [46]. Therefore, it is of importance to estimate the degree of crystallinity (w_c) of the polymer. The enthalpy of fusion ΔH is proportional to the degree of crystallinity in the polymer:

$$w_c = \frac{\Delta H}{\Delta H^\circ} * 100 \qquad (3)$$

where ΔH^o is the heat of fusion per mole of crystallizable unit. This value is not known for the crystallizable ester-amide unit used in our polymers. However, an estimation can be made from group contributions [47]. The enthalpy of fusion for the parent poly(ester amide) (PEA) is 146 J/g and the T_m^0 is, in this way, estimated at 160°C. This seems reasonable in view of our experimental results. The crystallinity varies from values as low as 4% to values of 29% (Table 3). As expected, the overall crystallinity in the copolymer decreases with decreasing hard segment content and average hard unit sequence length.

DSC results indicate that, for all compositions, appreciable phase separation in PEEA copolymers occurs. The change in PEEA thermal properties with composition is relatively small compared to PEOT/PBT copolymers [6]. This phenomenon can be attributed to the strong hydrogen bonding in the hard domains, which leads to a strong segregation of the segments in the polymer.

Small angle X-ray scattering

Small angle X-ray scattering (SAXS) was used to study phase separation in isotropic PEEA copolymers both in the dry and swollen state. In intensity versus q-vector plots, scattering maxima could be seen for the compositions studied. This indicates the presence of phase separated domains in accordance with the DSC data. The peaks were relatively broad, implying a large distribution of domain sizes. A long period L, which is a measure for the sum of the hard and soft domain sizes, can be extrapolated from the scattering data (Table 4):

$$L = \frac{2\pi}{q} \qquad (4)$$

Table 4. *The q-vector (q), long period (L) and intensity (I) for PEEAs in the dry and swollen states.*

Composition	PEO content (wt%)	Dry sample			Swollen sample			Water-uptake (wt %)
		q, Å^{-1}	L, Å	I, a.u.	q, Å^{-1}	L, Å	I, a.u.	
PEA (0/100)	0	0.089	71	0.5	0.077	82	1.7	6
1000 PEEA 34/66	31	0.068	92	1.5	0.054	116	4.0	32
300 PEEA 69/31	34	0.064	98	1.0	0.042	150	7.5	47
1000 PEEA 76/24	58	0.051	123	2.0	0.019	331	12.0	245

As can be seen in Figure 7 and Table 4, the long period increases with PEO content. In the dry state, the change in long period with the copolymer composition is relatively small. As the melting temperature of the rigid domains (and therefore their sizes) decreases with the rise in PEO content, the increase in L corresponds to an increase in size of the PEO-containing soft domains (compare DSC data in Table 3).

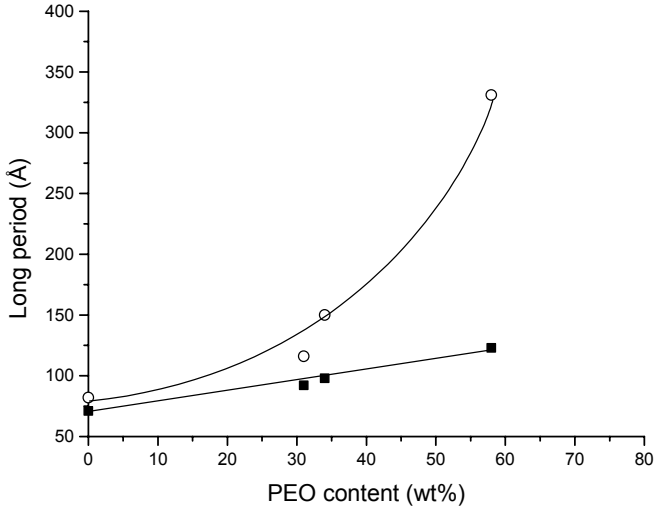


Figure 7. *Long spacing as a function of PEO content measured on dry* (■) *and swollen* (○) *samples.*

In the swollen state, the increase of the long period with the PEO content is even more pronounced (Fig.7), following a trend comparable to the water-uptake dependence shown in Figure 3. The long spacing increases linearly with water-uptake (Fig.8). Since for PEEA copolymers the water-uptake is proportional to the PEO content (Fig.3), the water is preferentially present in the PEO-containing phase. The intensity of the scattering maxima increases with water-uptake (Table 4), suggesting an increase in density difference between the domains (PEG density: 1.15 g/cm^3, PEA density: 1.33 g/cm^3). This corroborates the predominant presence of water in the PEO-containing domains.

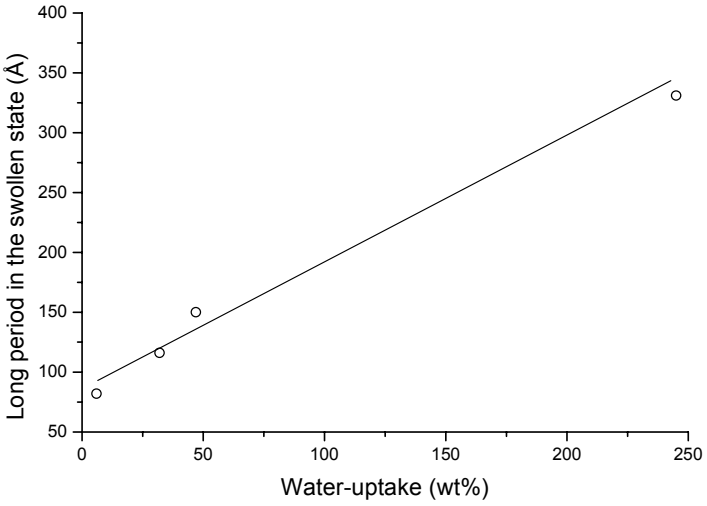


Figure 8. *Long spacing as a function of water-uptake.*

Stress-strain behavior

Typical stress-strain curves of several PEEAs are shown in Figure 9. In comparing the tensile behavior of the polymers, it can be seen that the hard segment content of polymers prepared with PEG 300 and PEG 1000 (Figs.9A and 9B) has a large effect on the mechanical behavior. The starting PEG molecular weight at constant soft to hard segment ratio (approximately 70/30, Fig.9C) is also of great influence. The tensile behavior of PEEAs is typical of thermoplastic elastomers [27,48], showing high extensions and relatively high modulus values.

The values of the mechanical properties are given in Table 5. For copolymers synthesized from the PEG 300 and PEG 1000, an increase in hard segment content, corresponding to an increase in rigid domain fraction, leads to stiffer and stronger polymers with higher E-modulus and maximum stress values. Simultaneously the elongations at break decrease. For the copolymers prepared from PEG 4000, an increase in maximum stress and a decrease in strain at break are also observed. In the dry state, the modulus of the 4000 series increases with an increase in soft segment content. This can be related to the presence of crystalline PEO at the measurement temperature, as found by DSC (Table 3). This crystalline phase is then also contributing to the stiffness of the materials leading to higher *E*-moduli.

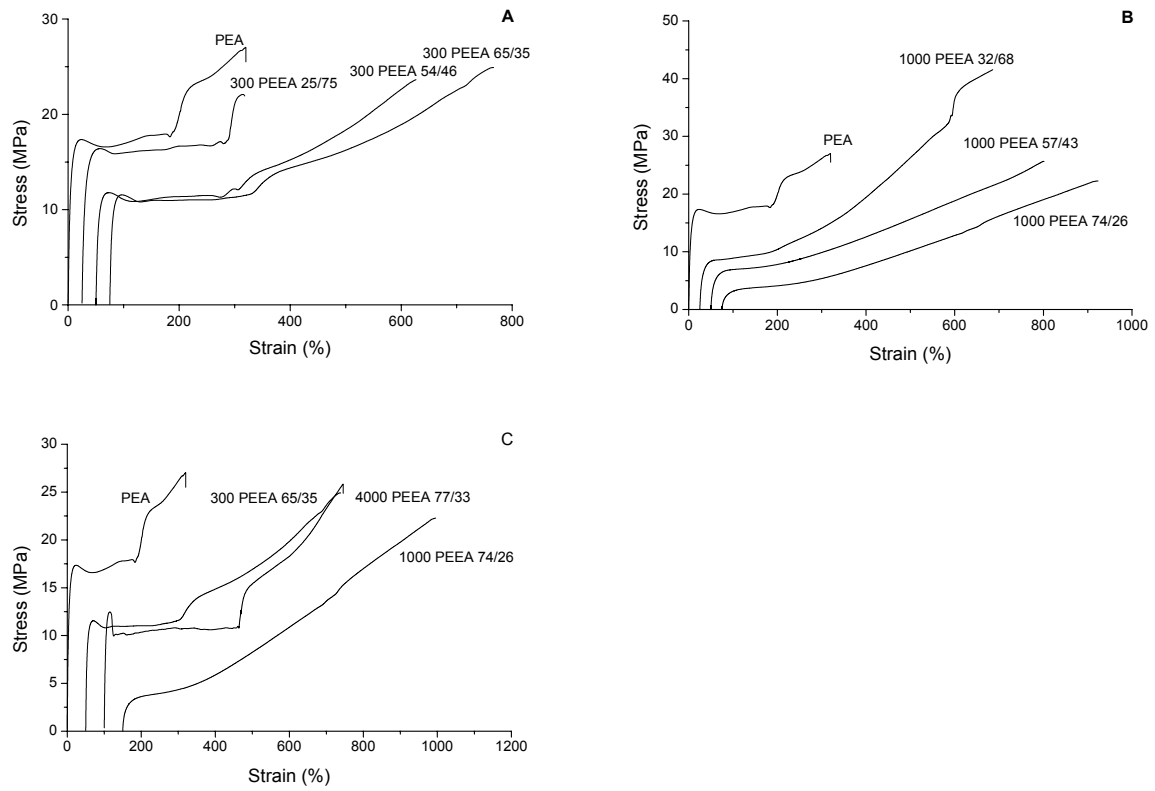


Figure 9. *Stress-strain curves of various PEEA block copolymers prepared with (A) PEG 300, (B) PEG 1000 and (C) similar soft to hard segment ratios (the curves are offset for clarity).*

At a given hard segment content, an increase in PEG molecular weight induces a lower *E*-modulus and a higher elongation at break for the 300 and 1000 series (see example Fig.9B). A different behavior is again observed for the polymers synthesized with PEG 4000, which is able to crystallize.

For some polymers, strain hardening was detected at approximately 200-350%. DSC analysis revealed an increase in the melting temperature and enthalpy of the crystalline phases present in the copolymers at room temperature.

PEEA are stronger materials than poly(ether ester amide)s based on PEG and polyamide-6 [27]. And, for a given copolymer composition, PEEA have a higher *E*-modulus than polyamide-6-based poly(ether ester amide)s [27] and PEOT/PBT [6]. In addition, PEEAs are still highly extendable with relatively high elongations at break.

Table 5. *E-modulus, maximum stress (σ_{max}), stress at yield (σ_{yield}) and elongation at break (ε_{break}) of various PEEA block copolymers measured on dry specimens.*

Composition	*E*-modulus MPa	σ_{yield} MPa	σ_{max} MPa	ε_{break} %
300 65/35	291	11.6	12.5	690
300 54/46	294	11.6	20.5	575
300 25/75	326	14.7	22.1	290
1000 74/26	61	2.6	19.1	850
1000 57/43	112	5.0	23.1	750
1000 32/68	163	8.5	39.2	660
4000 77/23	347	12.7	25.3	647
4000 61/39	241	9.1	13.2	565
4000 33/67	221	9.9	32.2	530
0/100	427	17.2	22.2	320

The tensile properties of PEEAs are similar to those observed for poly(ε-caprolactone) (PCL), a degradable polyester widely studied for medical applications. For PCL, specific values of 400 MPa for the E-modulus, 43 MPa for the maximum stress and 720 % for the elongation at break are found [49].

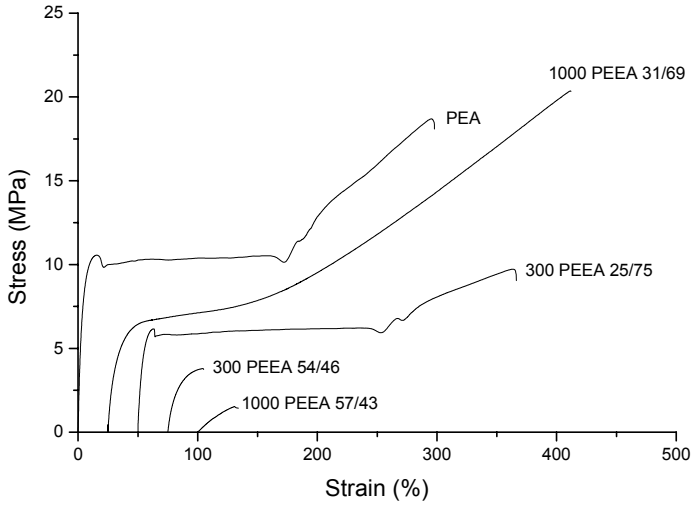


Figure 10. *Stress-strain diagrams of several PEEA copolymers at equilibrium water-uptake. Stress-strain curves are offset for clarity.*

As PEEA copolymers are intended to be used in contact with body fluids, the evaluation of their mechanical properties in the swollen state is very relevant. As can be seen when comparing Figure 10 with Figure 9, a decrease in mechanical properties is observed for all PEEA copolymers. Polymers with lower PEG molecular weights (300 and 1000) and soft segment contents lower than 35 wt% can retain proper mechanical characteristics. In contrast, polymers with higher PEO contents (higher than 50 wt%) or PEG 4000 completely loose their mechanical properties upon absorbing water. Therefore, in the case of medical applications requiring mechanical strength, copolymers with relatively low PEO contents should be chosen.

Conclusions

Aiming at preparing a degradable polymer with suitable physical properties for medical applications, PEO-containing poly(ether ester amide)s (PEEAs) were prepared by polycondensation reactions. Semi-crystalline poly(ether ester amide)s based on poly(ethylene oxide), 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate undergo significant microphase separation. The composition has only little influence on the extent of phase separation as demonstrated by DSC. This can be due to hydrogen bonding of the amide units. The estimated crystallinity of the synthesized poly(ether ester amide)s is relatively low, which is desired in the degradation of the copolymers. PEEAs are thermoplastic elastomers, they possess good flexibility with an elongation at break up to 850%, but are also strong materials with relatively high moduli. The polymers studied are hydrophilic and, as shown by SAXS, the water at equilibrium swelling is mostly present in the PEO-containing domains. PEEAs with short PEO length and/or low PEO contents are suitable candidates for medical devices and scaffolding materials in tissue engineering, as they retain good mechanical properties in the swollen state.

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Chapter 8

Poly(ether ester amide)s for Tissue Engineering*

'The most exciting phrase to hear in science,
the one that heralds new discoveries, is not 'Eureka!' but 'That's funny'...'
Isaac Asimov (1920-1992)

Abstract

Poly(ether ester amide) (PEEA) copolymers based on poly(ethylene glycol) (PEG), 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate (a diester-diamide monomer) were evaluated as potential scaffold materials for tissue engineering by determining their cytotoxicity, the adhesion and growth of endothelial cells on the material surfaces, the *in vivo* degradation in rats and their processability into porous structures. A PEEA copolymer based on PEG with a molecular weight of 300 g/mol and 25 wt% of soft segments (300 PEEA 25/75) and the PEA parent polymer (0/100, not containing PEG) were not cytotoxic. Both polymers sustain the adhesion and growth of endothelial cells. The *in vivo* degradation of melt-pressed PEEA and PEA discs subcutaneously implanted in the back of male Wistar rats was followed up to 14 weeks. Depending on the copolymer composition, a gradual decrease in intrinsic viscosity of about 20-30% and mass loss up to 12% were measured. In the course of the degradation process, also surface erosion is observed by scanning electron microscopy and light microscopy. This surface erosion might result from hydrolysis and from cellular activity, as no change of the polymer surface was observed upon *in vitro* degradation. The thermal properties of the polymers during degradation were measured by differential scanning calorimetry. During the first two weeks, a broadening of the melting endotherm was observed, as well as an increase in the heat of fusion. Porous matrices of PEEAs and PEA could be prepared by molding mixtures of polymer and salt particles followed by leaching of the salt. Based on these studies it can be concluded that PEEAs with low PEO content and PEA are promising scaffold materials for tissue engineering.

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Biomaterials **2002**, submitted.

Introduction

Polymers based on poly(ethylene oxide) and poly(butylene terephthalate) (PEOT/PBT) are examples of segmented block copolymers of which the physical properties can be readily tuned by variation of the polymer composition. Materials with a broad spectrum of adequate mechanical properties, also in the swollen state, can be obtained [1]. Consequently, these materials have been extensively investigated for various biomedical purposes, such as tympanic membrane [2], bone filler [3], skin substitute [4] and more recently for the tissue engineering of bone [5,6] and cartilage [7]. The *in vitro* [1,8,9] and *in vivo* [10-12] degradation of PEOT/PBT has been extensively investigated. PEOT/PBT degradation is dependent on the copolymer composition and is characterized by a decrease in polymer soft segment (PEOT) content and molecular weight leading to sample fragmentation. A drawback is that the degradation after implantation is not complete or leads to insoluble products [13].

In an effort to obtain biocompatible polymers with varying hydrophilicity and adequate mechanical properties in the swollen state which can degrade completely, segmented poly(ether ester amide)s (PEEAs) may be a promising alternative. They are usually prepared by a two-step polycondensation using poly(ethylene glycol) (PEG) [14] or poly(tetramethylene glycol) [15] as polyethers. These materials are semicrystalline thermoplastic elastomers and undergo microphase separation [16,17]. Their physical properties can be modulated by either varying the ether/ester/amide ratio, or the nature and the length of the degradable ester blocks and the hydrophilic ether blocks [14-16]. To our knowledge, despite these interesting properties, the degradability and the biomedical applicability of segmented poly(ether ester amide)s have not been the subject of many studies. The biocompatibility of PEEAs based on poly(L-lactide) and PEG has been assessed by checking the viability of Caco-2 cells on polymer films [17]. These PEEAs are degradable *in vitro* and can be used as drug delivery carriers. Biodegradable poly(ether ester amide)s based on poly(ε-caprolactone) have been prepared and also studied as microspheres for the controlled release of drugs [18].

In our laboratory, segmented block copoly(ester amide)s containing polyether blocks (PEEA), based on poly(ethylene glycol) (PEG), 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate, were developed for the controlled release of drugs [19,20]. Initial studies showed that several polymers in this series have adequate properties in the swollen state [21] and may be promising materials for use in tissue engineering and other medical applications. In this paper, these materials have been evaluated with respect to cytotoxicity, cell adhesion and growth of endothelial cells, *in vivo* degradation and processability into porous scaffolds.

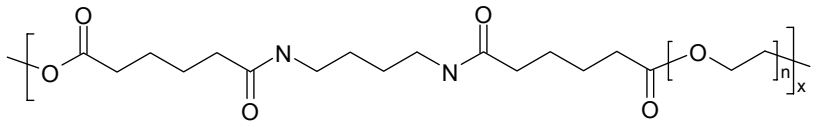
Materials and Methods

Materials

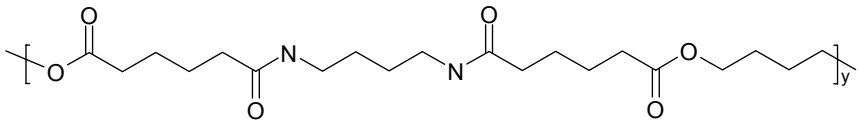
Poly(ethylene glycol) of different molecular weights (PEG 300 and PEG 1000) supplied by Fluka (Switzerland), and 1,4-butanediol from Acros organics (Belgium) were used without further purification. All solvents used were analytical grade (Biosolve, the Netherlands).

Polymer Synthesis

A detailed description of the synthesis of poly(ether ester amide)s (PEEAs) based on poly(ethylene glycol) (PEG), 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate (a diester-diamide monomer) (Fig.1) has been published elsewhere [19,21]. The PEEAs were purified by dissolution in chloroform:methanol 1:1 (v/v) and precipitation in cold ether. Purified polymers were dried for 7 days under vacuum at room temperature and stored in vacuum-sealed bags at -21°C. The composition of the block copolymers is indicated as *a* PEEA *b*/*c*, in which *a* is the starting PEG molecular weight, *b* the weight percent of soft segments and *c* the weight percent of hard segments. The abbreviation PEG is used when referring to the starting material used for the synthesis, whereas PEO is used to refer to the repeating segment in the PEEA copolymers.



PEO-containing soft segment



Hard segment

Figure 1. *Chemical structure of segmented PEEA block copolymers. The soft segments are derived from PEG and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate and the hard segments from 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate.*

Preparation of polymer films

Films of purified PEEAs were prepared by compression molding (laboratory press THB008, Fontijne, The Netherlands). The molding temperatures were 180°C for PEA, 170°C for 300 PEEA 25/75, 160°C for 1000 PEEA 31/69 and 145°C for 300 PEEA 56/44. The

thickness of the specimens was 400-600 μm. Discs of 10 mm in diameter were punched from these films for *in vivo* degradation experiments.

Polymer Characterization

The intrinsic viscosities [η] of the (non)degraded PEEA melt-pressed films were estimated by single point measurements [22,23] at 25°C using an Ubbelohde OC viscometer. Polymer solutions (chloroform/methanol; 1:1 v/v) were prepared at a concentration of approximately 0.3 g/dL.

The polymer composition was determined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) using a Varian Inova 300 MHz (USA) and polymer solutions in deuterated dimethylsulfoxide (Sigma, Switzerland).

The thermal properties of the melt-pressed copolymers before and after subcutaneous implantation for a specific time period were evaluated by differential scanning calorimetry (DSC) with a Perkin Elmer Pyris 1 (USA) at a heating rate of 10°C/min. The copolymer samples (5-10 mg) were placed in stainless steel pans and were heated from -100 to 250°C. The glass transition temperatures were taken as the midpoint of the heat capacity change. Cyclohexane, indium, gallium and tin were used as standards for temperature calibration.

The mass loss was defined as:

$$\text{Mass loss} = \frac{m_0 - m}{m_0} \text{ x } 100 \qquad (1)$$

where m_o is the initial specimen weight and m the weight of the degraded specimen after drying for 10 days under reduced pressure at room temperature.

Contact angles of copolymer films in demineralized water were determined using the captive bubble technique. Measurements were done using a Contact Angle System OCA 15 plus from Dataphysics. Results are averages of at least 3 measurements.

Cytotoxicity

Cytotoxicity tests were conducted by Biomatech (France). 300 PEEA 25/75 and PEA were subjected to an *in vitro* cytotoxicity test based on the requirements of the NF EN ISO 10993 norm (Part 5). Extracts of the ground polymers were prepared at 37°C using an extraction solution containing minimum essential medium (MEM) supplemented with L-glutamine, serum and antibiotics at a polymer/solution ratio of 0.2 g/mL. This extraction medium (non-diluted) was poured onto confluent monolayers of L-929 mouse fibroblast cells cultured on tissue culture polystyrene (TCPS). Separate monolayers were prepared for negative and positive controls. Triplicate measurements were performed. The negative control consisted of

the extraction medium previously described without polymer whereas the positive control was made of 6.4g/L phenol in the supplemented MEM. After incubating at 37°C in 5% CO_2 for 24 hours, the cell cultures were stained by neutral red solution (staining the cytoplasm) and examined microscopically (100x) to determine the cell morphology. The cells were then fixed with a fixation solution containing formaldehyde and calcium chloride. Subsequently, the dye was extracted from the cells using an extraction solution based on ethanol and acetic acid. The optical density of the solution (OD) was measured for quantitative analyses.

Adhesion and growth of human umbilical vein endothelial cells (HUVEC)

HUVEC (passage 2) were cultured on circular polymer films. The cells were seeded at a density of 40,000 cells/cm^2 in 3 mL culture medium. The culture medium consisted of 50 vol% M199 (with Hank's solution; Gibco, Life Technologies, UK), 50 vol% RPMI 1640 (with 25 mM HEPES; Gibco, Life Technologies, UK), in which 100 U/mL penicillin-G, 100 µg/mL streptomycin (Gibco, Life Technologies, UK) and 2 mM Glutamax-I (Gibco, Life Technologies, UK) are added. Prior to use in cell culture, the culture medium was supplemented with filter-sterilized pooled human serum (20 vol%). Cultured films were quantitatively analyzed after 6 hours, 1, 3 and 6 days. Tissue culture polystyrene (TCPS) was used as positive control.

In vivo degradation

Melt-pressed 300 PEEA 25/75, 1000 PEEA 31/69 and PEA discs were implanted subcutaneously in the back of young male Wistar rats (150-170 g) along the dorso-medial line. Prior to implantation, the melt-pressed polymer discs (diameter: 10 mm, thickness: 0.4-0.6 mm) of known mass were sterilized by immersion in ethanol 70% and washed with sterile phosphate buffered saline (PBS) (Life Technologies, UK). Four subcutaneous pockets were formed in the back of each rat and the polymer samples (n=6) were randomly implanted. After insertion of the samples, the wounds were closed with Vicryl® sutures. Five rats were killed at 1, 2, 4, 8 and 14 weeks after implantation. Characterization of the degraded materials was done by means of mass loss, intrinsic viscosity, composition (^{1}H-NMR), thermal properties (DSC) and surface morphology (SEM). The surrounding tissues were excised for histological analysis. After explantation the samples were fixated in a 4% paraformaldehyde solution (Sigma). Prior to embedding the samples were dehydrated through a series of isopropanol/water solutions with increasing isopropanol concentrations (70% to 100%). Subsequently, samples were embedded in glycol methacrylate (GMA) (Sigma, Switzerland). Coupes (5 µm) were then cut with a microtome HM 355S (Microtom, Germany) and stained with a hematoxilin-eosin staining agent (Sigma, Switzerland). Histological sections were evaluated by light microscopy.

Preparation of PEEA porous scaffolds

Porous scaffolds were prepared by molding mixtures of ground polymer and salt particles followed by salt leaching. The copolymer particles (250-500 μm) were mixed with sodium chloride (sieved to 500-710 μm, 90 vol%). The mixtures were compression molded using a hot press (laboratory press THB 008, Fontijne, The Netherlands). Samples were heated to 10°C above the melting point at 20 Pa for 3 min and then pressed at 3 MPa for one min. Subsequently, the salt was leached out in demineralized water (48 hours). The materials were dried in a vacuum oven for 48 hours at room temperature. The densities and porosities were determined from mass and volume measurements of the materials in duplicate. The density of the non-porous materials was 1.10 g/cm^3 for 300 PEEA 56/44, 1.22 g/cm^3 for 1000 PEEA 31/69 and 1.33 g/cm^3 for PEA.

Scanning Electron Microscopy (SEM)

A Leo 1550 field emission SEM (Germany) was used. Freeze-fractured samples of the porous structures were cut and coated with Au/Pd using a Polaron E5600 sputter coater. No coating was necessary when high magnifications (200x and higher) were used.

Results and Discussion

A previous study showed that PEEAs with short PEO lengths and/or low PEO contents were the most suitable candidates for use in medical applications, as they possess good mechanical properties in the swollen state [21]. PEEAs prepared with PEG 300 or PEG 1000 and containing at most 28 wt% of PEO (Table 1) have therefore been evaluated with respect to cytotoxicity, endothelial cell adhesion and growth, *in vivo* degradation and processability into porous scaffolds.

Table 1. *Characteristics of the purified poly(ether ester amide)s used in this study.*

Composition[a]	PEO content[a] wt%	$[\eta]$[b] dL/g	Water-uptake[c] %	Contact angle ± 2°	E_{dry}[d] MPa	$E_{swollen}$[d] MPa
0/100 (PEA)	0	0.60	6	37	427	295
300 PEEA 25/75	12	0.64	10	35	326	131
1000 PEEA 31/69	24	0.69	24	33	245	108
300 PEEA 56/44	28	0.35	34	n.d.[e]	153	68

a. as determined by ^{1}H-NMR
b. solvent: $CHCl_3$/MeOH (1:1 v/v) at 25°C
c. at equilibrium [21]
d. *E*-modulus in the dry and water-swollen state [21]
e. not determined

Cytotoxicity

The cytotoxicity of PEEAs was assessed by studying the effects of polymer extracts on the morphology and density of fibroblasts.

The qualitative and quantitative scores are given in Table 2. The qualitative score is based on microscopical observation of the cell morphology and staining with neutral red. The material was assessed as not cytotoxic (response index 0) if the cells were intact, stained and had reached confluency. The material was judged severely cytotoxic (responses index 3) if cell lysis or complete absence of neutral red staining was observed. The quantitative evaluation of the cytotoxicity was, subsequently, performed. After removal of the medium, the neutral red present in the cells was extracted and the optical density (OD) of the solution measured. The material was considered non-cytotoxic if all cell cultures that were exposed to the polymer extracts showed a reduction in cellular density less than 25% in comparison with the negative control.

Table 2. *Qualitative and quantitative results of the cytotoxicity tests.*

	Response index[a]	Mean OD[b]	% of negative control
PEA	0		
	0	0.759 ± 0.087	99.2 ± 11.4
	0		
300 PEEA 25/75	0		
	0	0.769 ± 0.106	100.4 ± 13.8
	0		
Positive control 100%	3		
Phenol, MEM	3	0.129 ± 0.009	16.9 ± 1.2
	3		
Negative control 100%	0		
MEM	0	0.766 ± 0.023	100
	0		

a. 0=not toxic; 3=severely toxic

b. optical density

The negative and positive controls performed as anticipated. Neither 300 PEEA 25/75 nor PEA extracts showed evidence of causing cell lysis. The cellular densities were also satisfying in comparison with the negative control. The polymeric extracts were, then, assessed as non-cytotoxic. One can, therefore, draw the conclusion that the PEEA copolymers studied are non-cytotoxic.

HUVEC adhesion and growth

For use in medical applications requiring mechanical strength, copolymers with relatively low PEO contents should be chosen as these retain adequate mechanical properties in the swollen state [21]. 300 PEEA 25/75 and the parent polymer PEA were chosen to evaluate their capacity to sustain cell adhesion and growth. Human umbilical vein endothelial cells (HUVECs), which form the inner layer of blood vessels, were used to explore the potential of PEEA polymers in the engineering of vascular tissue. Furthermore, as endothelium is practically ubiquitous in the body, the use of HUVECs can be considered as a relevant *in vitro* model for the development of other engineered tissues [24]. Tissue culture polystyrene (TCPS) was used as a positive control. As can be seen in Figure 2, HUVECs adhere to and grow on both polymer surfaces. However, the surfaces do not perform as well as TCPS. Although both surfaces had similar contact angles (Table 1), HUVECs seem to perform better on PEA than on 300 PEEA 25/75. It seems therefore important to use PEEA polymers containing low contents of PEO for tissue engineering application. Nevertheless, these results imply that PEEA copolymers and PEA may be used for vascular tissue engineering. Further improvement of cell attachment and growth may possibly be achieved by use of surfaces modified by gas plasma treatment. Gas plasma treatment of segmented copolymer based on PEO and poly(butylene terephthalate) improved the adhesion and growth of bone marrow cells [6].

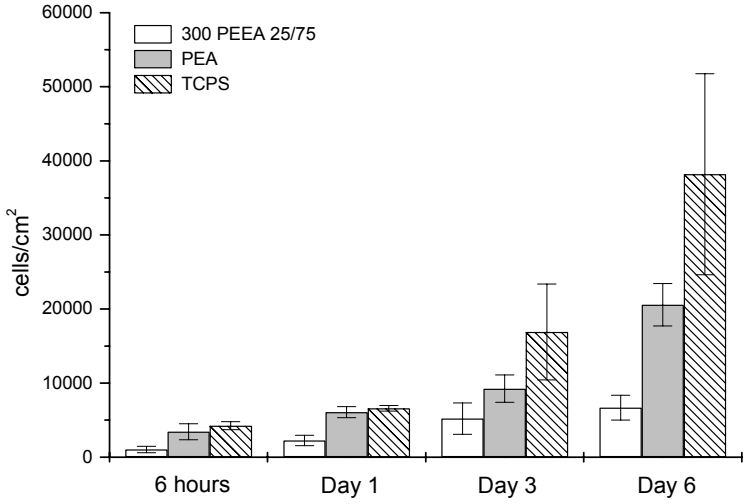


Figure 2. *HUVEC adhesion and growth on 300 PEEA 25/75, PEA and TCPS.*

In vivo degradation

300 PEEA 25/75, 1000 PEEA 31/69, and the parent poly(ester amide) PEA, were implanted subcutaneously in rats and their properties during *in vivo* degradation were followed up to 14 weeks. Table 3 summarizes the characteristics of the degraded samples.

No change in polymer composition could be detected by ^{1}H-NMR during the 14 weeks of the study (Table 3). The changes in intrinsic viscosity [η] and mass loss during the implantation period are presented in Figures 3A and 3B, respectively. The intrinsic viscosity of the polymers decreased slowly over 14 weeks (Fig.3A). Despite the decrease in [η], the polymers were still mechanically stable and the samples were not brittle. Although the three polymers showed similar degradation profiles, the *in vivo* degradation rate of 1000 PEEA 31/69, which is the polymer containing the most PEO, is slightly higher than that of 300 PEEA 25/75 and PEA. After 14 weeks in the body, only little mass loss was observed ranging from 7 wt% to 12 wt% for 300 PEEA 25/75, as seen in Figure 3B. The mass loss at one week is relatively high in comparison with the overall mass loss. This can be explained by the leaching of low molecular weight compounds (apparently non-cytotoxic) shortly after the implantation. The small increase in [η] at one week for 300 PEEA 25/75 and 1000 PEEA 31/69 seems to confirm this hypothesis. The low mass loss and the slow decrease in intrinsic viscosity point towards a bulk degradation process.

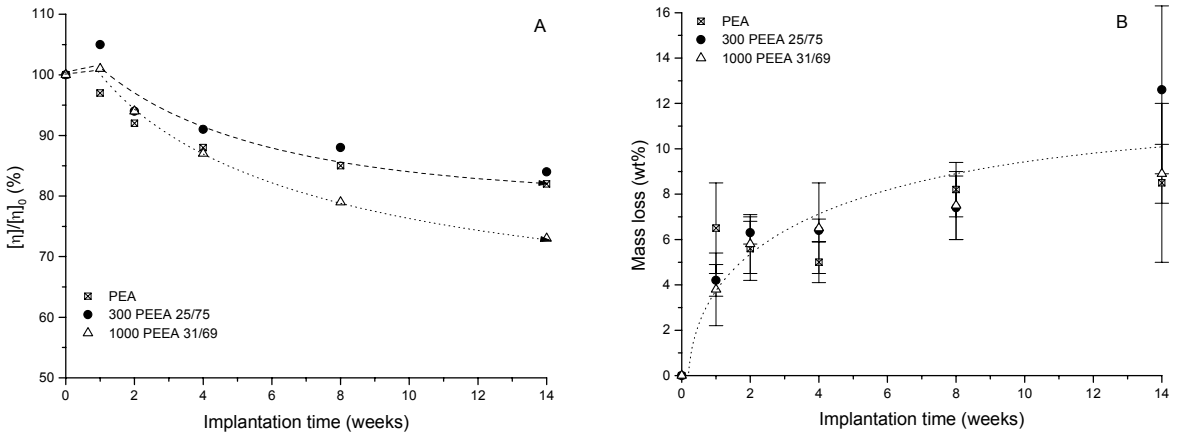


Figure 3. *Relative intrinsic viscosity (A) and mass loss (B) as a function of degradation time in vivo for* (☒) *PEA,* (●) *300 PEEA 25/75 and* (△) *1000 PEEA 31/69. The lines drawn in the graphs are guides for the eye.*

An important outcome of this study is the *in vivo* degradation of the parent polymer PEA. PEA exhibits mass loss and a decrease in intrinsic viscosity. This implies that degradation also occurs in the ester-amide units, which constitute the hard segments of the PEEAs. Therefore,

for the design of degradable segmented copolymers, the use of ester-amide monomer seems a good alternative to dimethyl terephthalate previously used in the synthesis of PEOT/PBT.

PEEAs and the parent polymer PEA are semi-crystalline polymers with a glass transition at low temperature [21]. Before implantation, PEEAs and PEA exhibit a melting endotherm at approximately 145-150°C (corresponding to the maximum of the endotherm). The DSC thermograms of the (non)degraded samples are presented in Figure 4. After one week in the body, a broadening of the melting transitions (extending over 60-80°C) is observed. The maximum of the melting temperature remained, however, almost unchanged. The thermal transition visible at approximately 50°C disappeared in time. After an initial increase due to annealing of the samples at 37°C, the heat of fusion ΔH_{hard} reaches a constant value, which is similar for all polymers (Table 3). The glass transition temperature corresponding to the PEO-containing segments is relatively unchanged up to 14 weeks. Although the effect of annealing is noticed, the results suggest that the thermal properties of the polymers have not substantially been modified during *in vivo* degradation.

Table 3. *Composition, intrinsic viscosity [η] and thermal properties of PEEAs during in vivo degradation.*

Copolymer	Time weeks	Composition[a]	$[\eta]$[b] dL/g	$T_{g\ soft}$ °C	Melting range °C	$T_{m\ hard\ max}$ °C	ΔH_{hard} J/g
PEA	0	0/100 (0)	0.60	-20	110-170	147	33.3
	1	0/100 (0)	0.58	-22	80-170	144[c]	49.5
	2	0/100 (0)	0.55	-22	80-170	147[c]	47.2
	4	0/100 (0)	0.53	-24	75-165	143[c]	54.0
	8	0/100 (0)	0.51	-26	75-165	146[c]	50.9
	14	0/100 (0)	0.49	-19	85-170	150[c]	53.3
300 PEEA 25/75	0	25/75 (12)	0.64	-34	95-150	143[c]	39.5
	1	25/75 (12)	0.67	-35	80-145	142[c]	47.8
	2	25/75 (12)	0.60	-33	85-145	143[c]	57.3
	4	25/75 (12)	0.58	-32	85145	142[c]	52.2
	8	25/75 (12)	0.56	-39	80-140	137[c]	54.0
	14	25/75 (12)	0.54	-29	90-150	146[c]	54.1
1000 PEEA 31/69	0	31/69 (24)	0.68	-45	100-155	139	43.9
	1	31/69 (24)	0.69	-47	70-145	134	55.5
	2	31/69 (24)	0.64	-47	80-150	138	52.7
	4	31/69 (24)	0.59	-47	90-145	137	45.0
	8	31/69 (24)	0.54	-47	85-145	136	46.6
	14	31/69 (24)	0.53	-46	90-150	140	51.8

a. soft/hard segment ratio (PEO content, wt%) c. shoulder at approximately 95°C

b. solvent: $CHCl_3$/MeOH (1:1 v/v) at 25°C

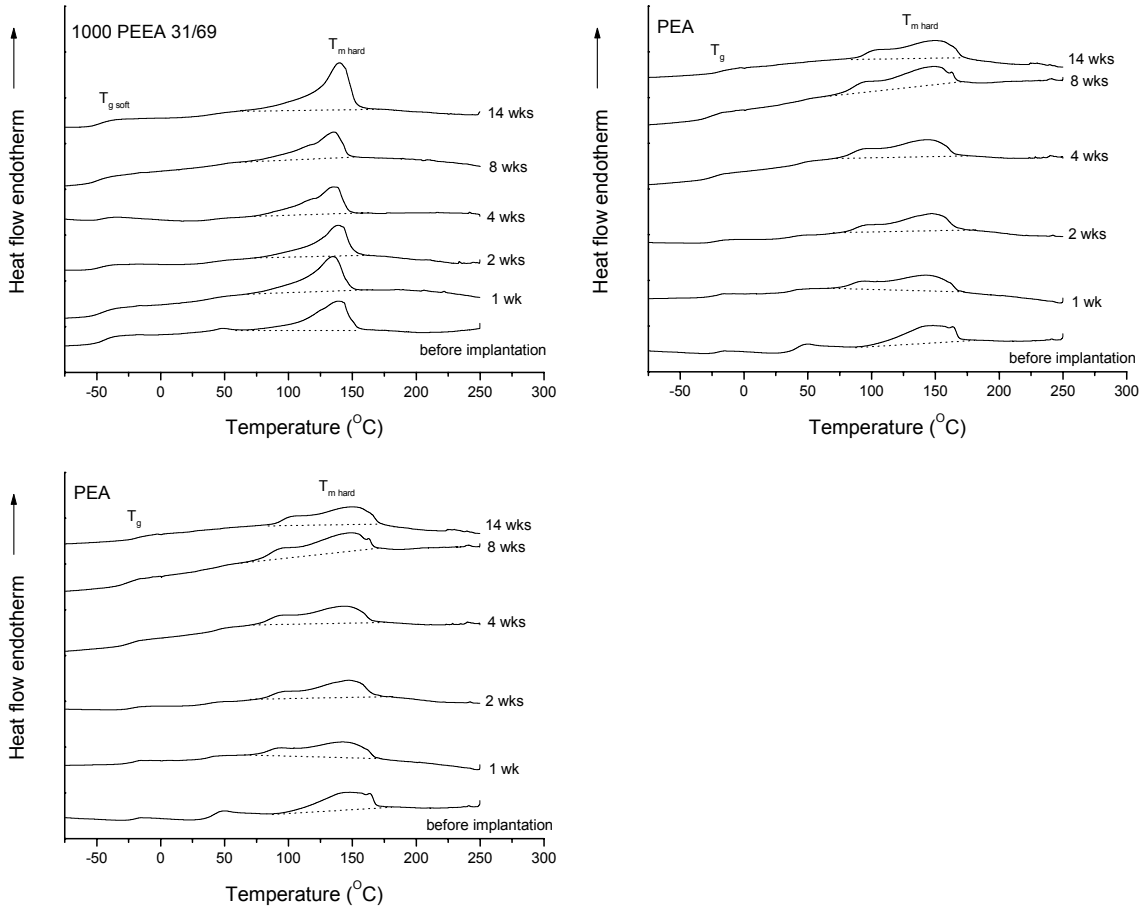


Figure 4. *DSC thermograms of PEEA and PEA polymers before and after implantation. The dotted lines enclose the area used for the calculation of the heat of fusion* ΔH_{hard}.

The degradation behavior of 1000 PEEA 31/69 *in vivo* is similar to the degradation in PBS at 37°C. In both situations, after 8 weeks, no composition change is noticed and a decrease in [η] of approximately 20% is measured [19]. Based on the similar degradation behavior of the polymers *in vitro* and *in vivo*, one can conclude that *in vivo* degradation also takes place via hydrolysis in the bulk, probably involving random scission of ester bonds. A major difference between the results of the *in vitro* and *in vivo* studies is the change in polymer structures during implantation. As seen in the SEM pictures in Figure 5, the surfaces of the three polymers became rougher in time. Cross-sections of the samples did not show the same pitted structure. Therefore, one can draw the conclusion that the mass loss after the initial release of low molecular weight compounds mostly originates from the sample surface. The observed patterns are probably caused by the activity of cells at the surface. As shown by the

histological analysis (Fig.6), pores and cracks are infiltrated by cells from the first week of implantation.

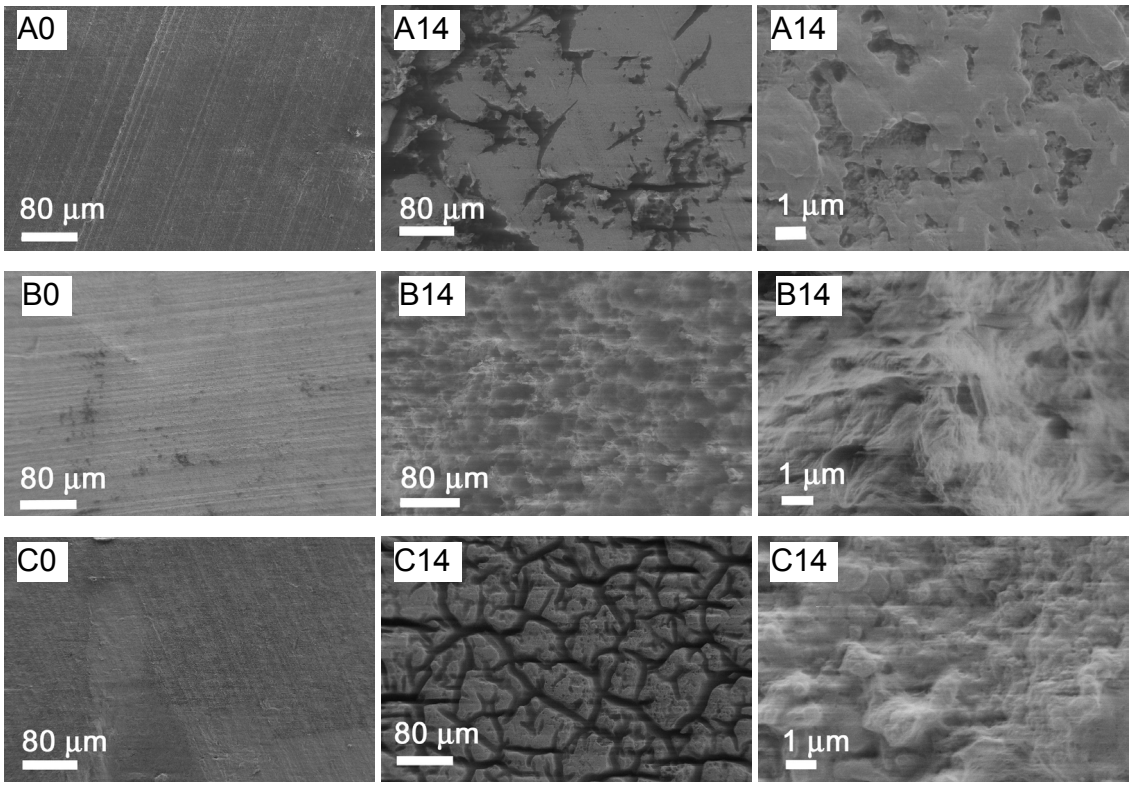


Figure 5. *Surface morphology observed by SEM for PEA (A), 300 PEEA 25/75 (B) and 1000 PEEA 31/69 (C) before implantation (left) and after 14 weeks of implantation (center and right).*

After 1 and 14 weeks of implantation, cross-sections of the polymer samples and the surrounding tissues were studied by optical microscopy (Fig.6). The gaps between the polymer surface and tissue observed in few pictures (A1, A14, B14 and C1) are artifacts due to the processing of the samples for the histological analyses.

After one week of implantation, all polymer samples are encapsulated by fibrous tissue (T), and macrophage-like cells were present on the polymer surfaces (arrowheads). At larger distances from the polymer surfaces also fat cells (★) were observed. In accordance with the cytotoxicity tests and the cell growth experiments, the polymers did not cause any toxic reactions and no significant adverse tissue reaction was noticeable. At one week of implantation, the surface and bulk of PEA (Fig.6, A1) and 300 PEEA 25/75 (Fig.6, B1) appeared relatively intact. On the surface of 1000 PEEA 31/69, cracks are visible, in which tissue ingrowth and cells are observed (Fig.6, C1).

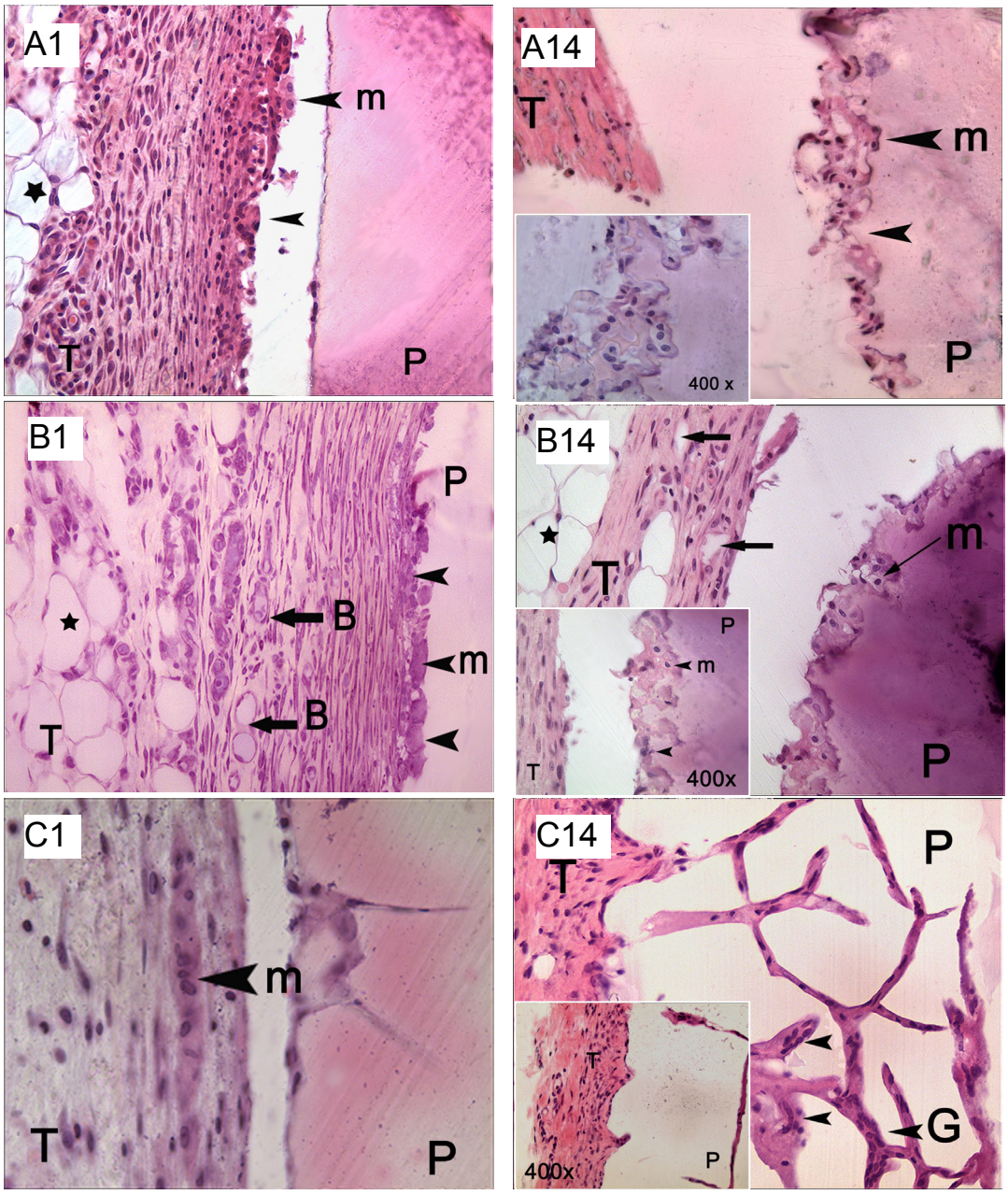


Figure 6. *Histological section of (A) PEA, (B) 300 PEEA 25/75 and (C) 1000 PEEA 31/69 after 1 week and 14 weeks of implantation. Unless otherwise mentioned, magnification: 200x. P: polymer, T: fibrous tissue, B: blood vessel, m: macrophage-like cell, G: giant cells, ★: fat tissue.*

In agreement with the patterns observed by SEM, the histological pictures show erosion of the surface after 14 weeks in comparison with samples implanted for one week. At 14 weeks of implantation, the PEA surface is eroded, while the bulk does not show any changes (Fig.6, A14). Numerous macrophage-like cells infiltrate the erosion pits. The implants of 300 PEEA 25/75 look similar to those of PEA. No change in the middle of the sample is noticed, whereas

surface erosion of the polymer is evident (Fig.6, B14). The edge of the sample is completely eroded into a rounded shape, while macrophage-like cells infiltrate the pits. Polymer fragments (indicated by arrows) can be observed in between connective tissue. The cracks already present on the surface of 1000 PEEA 31/69 after one week seem to have enlarged after 14 weeks *in vivo* (Fig.6, C14). At higher magnification (400x), macrophage-like cells and giant cells are clearly visible in those cracks.

Several mechanisms can be involved in PEEA degradation. It appears from the sample analyses that hydrolysis occurs in the polymer bulk. Naturally, such hydrolysis takes also place at the surface of the polymer. Based on the pitted polymer surfaces observed after *in vivo* degradation, which did not occur after *in vitro* degradation, and on the presence of macrophage-like cells, one can concluded that cellular activity is playing an essential role in the *in vivo* degradation of PEEA copolymers. Specific activated cells such as macrophages and foreign-body giant cells release oxygen radicals and superoxide anion radicals, which can combine with protons to form hydroperoxide radicals [25,26]. Several investigations have suggested that *in vivo* degradation of segmented poly(ether urethane) elastomers involves oxidation of the aliphatic ether groups in these polymers by oxygen radicals [25,27] and phagocyte-derived oxidants [28]. Therefore, the polymer erosion can be caused by oxidation of the PEO segment present in the amorphous domains. Activated cells can also release enzymes, which might be involved in the polymer degradation.

Porous scaffolds

To be used as scaffolds in tissue engineering, PEEAs need to be processed into porous devices. Porous structures have been prepared by mixing sodium chloride and ground polymer particles, followed by melt-pressing and subsequent salt leaching [6]. The characteristics of the obtained porous structure, size and porosity, are widely adjustable by variation of the size and amount of the salt particles added. Salt particles of 500-750 μm were used and scaffolds with a porosity of 90% were prepared. Figure 7 shows porous structures made of 300 PEEA 56/44 and PEA. Similar structures with a porosity of 90% were obtained with 1000 PEEA 31/69. These highly porous devices could be handled with ease and were mechanically stable. It was not possible to obtain stable structures with PEEAs prepared with PEG 4000. These copolymers are very hydrophilic with water-uptakes up to 350 wt% and loose their mechanical properties upon swelling [21]. As a consequence, during the leaching of the salt particles the structures were not stable.

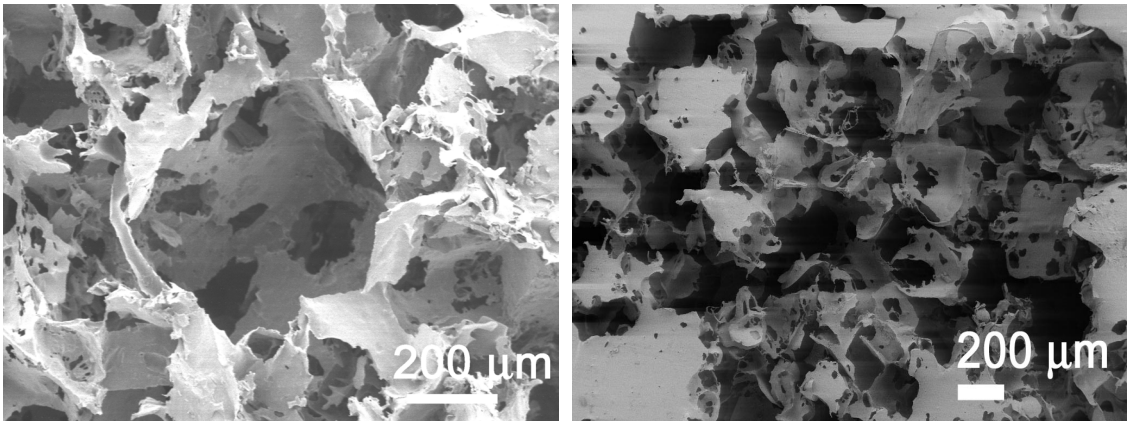


Figure 7. *Porous structures obtained from 300 PEEA 56/44 (left, porosity: 90%.) and PEA (right, porosity: 92%.).*

Conclusions

Poly(ether ester amide) copolymers (PEEA) based on poly(ethylene glycol) (PEG), 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate (a diester-diamide monomer) possess good mechanical properties and are suitable for use in medical devices, especially PEEAs with short PEO length and/or low PEO content. It was shown that these copolymers are non-cytotoxic. PEEA copolymers and PEA sustain endothelial cell adhesion and growth. The growth rate of HUVECs is higher when the PEO content in the copolymer decreases. PEEAs degrade *in vivo*, although the degradation rate is low. The parent poly(ester amide) PEA also undergoes *in vivo* degradation. This result shows that the ester-amide units that constitute the hard segments in the block copolymers can be degraded. Analyses of the polymer samples reveal that degradation occurs in the bulk but also at the surface of the polymers. As histology shows numerous cells infiltrating the polymer pits, the surface erosion likely involves cellular activity. The PEEA copolymers did not induce an adverse tissue reaction. Furthermore, it is shown that porous scaffolds can be readily prepared from PEEA materials. Based on these results, PEEA segmented copolymers are good candidates for use in tissue engineering and in other medical applications, where degradable polymers are required.

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Summary

Thermoplastic elastomers have not been much studied for scaffolding applications in tissue engineering. The main reason is the idea that the mechanical properties of the polymeric scaffold should match those of the damaged tissue. However, this is not an absolute necessity. In the tissue engineering of bone for example, the hybrid construct can develop strength during degradation of the polymer and simultaneous formation of new bone, allowing the use of elastomers for small defects in non-load bearing situations. Thermoplastic elastomers containing poly(ethylene oxide) and poly(butylene terephthalate) (PEOT/PBT) multi-block copolymers have already been employed as a biomaterial. However, several aspects of these systems have not been examined in detail. The objective of the studies described in this thesis is to investigate the applicability of these slowly degradable thermoplastic elastomers as scaffolds for tissue engineering, with emphasis on their phase separation and degradation properties. A second thermoplastic elastomer in which the terephthalic moieties have been replaced by ester-amide segments, is also investigated for use in scaffolding.

In the biomedical field, the emergence of new applications resulting from major advances in molecular cell biology has driven the development of novel degradable systems with specific physical properties and degradation rates. To meet these demands, biomaterials based on degradable block copolymers have drawn much attention. **Chapter 2** provides an overview of the most important degradable block copolymers used in biomedical applications. Block copolymers have proved to be highly valuable for drug delivery applications, as they allow the release of (new) hydrophobic drugs. Block copolymers also hold promise for use as scaffolds in tissue engineering, where they can be applied as temporary three-dimensional devices. Emphasis was given on multi-block copolymers based on poly(ethylene oxide) (PEO) and poly(butylene terephthalate) (PBT). The physical properties of these thermoplastic elastomers can be tailored within wide ranges, allowing their use in many surgical applications both in soft and hard tissues and in drug release systems. PEOT/PBT copolymers are phase-separated systems consisting of soft, rubber-like PEOT segments, which impart flexibility to the materials, while the glassy or crystallizable PBT segments provide strength and stiffness by the formation of physical cross-links. Depending the block lengths, the obtained phase morphology can significantly influence their physical properties.

As previously mentioned, PEOT/PBT copolymers are segmented systems in which the PEO-containing segments provide hydrophilicity to the material and are potentially susceptible to oxidation. In **Chapter 3**, it is demonstrated that PEOT/PBT multi-block

copolymers are phase separated both in the dry and the swollen state. The existence of two types of water in the water-swollen copolymers was established: (i) 'freezing water' that can crystallize upon cooling and (ii) 'non-freezing water'. Assuming that all 'non-freezing water' is bound to PEO, it was calculated that the number of water molecules per EO unit ranged from 0.3 to 2.9 for polymers with increasing PEO length or content.

PEOT/PBT multi-block copolymers in contact with solutions containing H_2O_2 and $CoCl_2$ underwent oxidative degradation. An increase in $CoCl_2$ concentration causes more rapid decreases in intrinsic viscosity, mechanical properties and PEO content. To examine the effect of the copolymer composition, oxidation of copolymers in a 5% H_2O_2 solution (no $CoCl_2$) was studied. Under these conditions, the decrease in intrinsic viscosity and mechanical properties was less pronounced with increasing PEO contents. This phenomenon may be caused by simultaneous occurrence of chain scission and recombination of macroradicals in the PEO phase. Degradation also took place during differential scanning calorimetry (DSC) measurements of samples free of antioxidant due to thermal oxidation of the material, which induces a decrease in molecular weight with increasing temperatures.

As shown in **Chapter 4**, in segmented PEOT/PBT block copolymers, phase separation is enhanced in polymers with high hard segment contents and polymers prepared from poly(ethylene oxide) (PEG) of relatively high molecular weight. The physical properties of PEOT/PBT copolymers also depend strongly on the molecular weight, the soft to hard segment ratio and the starting PEG molecular weight. By changing the PEOT/PBT composition, tensile strengths vary from 8 to 23 MPa and elongations at break from 500 to 1300%. Water-uptake ranges from 4 to 210%. PEOT/PBT copolymers are degraded *in vitro* by hydrolysis and oxidation. In both situations decreases in intrinsic viscosity, PEO content and mechanical properties have been observed. The degradation is more severe in case of polymers with a high PEOT content prepared from relatively high molecular weight PEG. Oxidation of PEOT/PBT also takes place during exposure of the polymers to light at ambient conditions. Under these conditions, Irganox 1330 is a more efficient antioxidant for PEOT/PBT polymers than vitamin E.

In **Chapter 5**, the suitability of PEOT/PBT multi-block copolymers for tissue engineering of bone is assessed. As already described in Chapter 4, the physical properties of PEOT/PBT multi-block copolymers can be tuned by variation of the soft to hard segment ratio and the PEG molecular weight used in the synthesis. These copolymers are sensitive to both hydrolysis and oxidation, which are degradation pathways that also occur *in vivo*. PEOT/PBT scaffolds with varying porosities and pore sizes have been prepared by molding and freeze-drying techniques in combination with particulate-leaching. Bone marrow cells tend to grow better on the more hydrophobic copolymers. Treatment with a CO_2-plasma, however, enables the culturing of goat bone marrow cells on a broad range of PEOT/PBT multi-block

copolymers. Therefore, the choice of the scaffold material can be based on other relevant properties like *in vivo* bone bonding, calcification and degradation behavior. The degradability, the good results obtained during the cell studies and the feasibility of preparing porous scaffolds make PEOT/PBT multi-block copolymers good candidates for use in tissue engineering and regeneration of bone.

The degradation behavior of PEOT/PBT is studied in more detail in **Chapter 6**. Accelerated hydrolysis experiments in phosphate buffered saline (PBS) at 100°C and *in vivo* degradation in rats, showed that the mass and intrinsic viscosity of PEOT/PBT copolymers with high PEO contents decreased more rapidly than copolymers with lower PEO contents. PBT degraded *in vitro* at 100°C did not show further degradation *in vivo*. Analysis of the degradation products of 1000 PEOT71PBT29 polymers that were subjected to hydrolysis at 100°C showed that molecules containing PEO and terephthalate moieties and PBT-containing chains were produced. However, only residues with high PEO contents and the monoester of terephthalic acid and butanediol are soluble in PBS. These results indicate that part of the PBT fraction might remain in the body at late stages of degradation. The presence of PBT crystalline domains, however, does not seem to affect the biocompatibility of the samples as no adverse tissue reactions were detected.

In an effort to obtain biocompatible polymers with varying hydrophilicity and adequate mechanical properties in the swollen state, which can degrade completely, copolymers with hard segments consisting of ester and aliphatic amide units can be an alternative to the terephthalate-containing PEOT/PBT copolymers. PEO-containing poly(ether ester amide)s (PEEAs) were prepared by polycondensation reactions. Semi-crystalline PEEAs based on poly(ethylene oxide), 1,4-butanediol and dimethyl-7,12-diaza-6,13-dione-1,18-octadecanedioate undergo significant microphase separation. The composition has only little influence on the extent of phase separation as demonstrated by DSC. This can be due to hydrogen bonding of the amide units. The estimated crystallinity of the synthesized PEEAs is relatively low, which is desired in the degradation of the copolymers. PEEAs are thermoplastic elastomers, they possess good flexibility with an elongation at break up to 850%, but are also strong materials with relatively high modulus values. The polymers studied are relatively hydrophilic and, as shown by SAXS, the water at equilibrium swelling is mostly present in the PEO-containing domains. PEEAs with short PEO length and/or low PEO contents are suitable candidates for medical devices and scaffolding materials in tissue engineering, as they retain good mechanical properties in the swollen state. This work is described in **Chapter 7**.

As PEEAs with short PEO lengths and/or low PEO contents show the most suitable physical properties for use in medical devices, they have been further investigated. It was shown that these copolymers are non-cytotoxic. PEEA copolymers and PEA (parent polymer)

sustain endothelial cell (HUVEC) adhesion and growth. The growth rate of HUVECs is higher when the PEO content in the copolymer is decreased. In **Chapter 8**, the *in vivo* degradation of PEEAs is also described. PEEAs degrade *in vivo*, although the degradation rate is low. The parent poly(ester amide) PEA also undergoes *in vivo* degradation. This result shows that the ester-amide units that constitute the hard segments in the block copolymers can be degraded. Analyses of the polymer samples reveal that degradation occurs in the bulk but also at the surface of the polymers. Histology shows numerous cells infiltrating the pits on the polymer surface, therefore erosion likely involves cellular activity. The PEEA copolymers did not induce an adverse tissue reaction. Furthermore, it is shown that porous scaffolds can be readily prepared from PEEA materials.

Samenvatting

Thermoplastische elastomeren zijn nog niet veel toegepast als dragermateriaal in weefsteltechnologie. De voornaamste reden is het idee dat de mechanische eigenschappen van de polymere drager overeen dienen te komen met het beschadigde weefsel. Dit is echter geen absolute noodzaak. In de botweefseltechnologie bijvoorbeeld kan een cel-polymeer implantaat aan sterkte winnen tijdens de afbraak van het polymeer en de gelijktijdige vorming van nieuw bot. Dit maakt de toepassing van elastomeren in de vorming van bot, waarop geen krachten werken, in kleine defecten mogelijk. Thermoplastische elastomeren, bestaande uit poly(ethyleen oxide) en poly(butyleentereftalaat) (PEOT/PBT) multiblokcopolymeren zijn reeds toegepast als biomateriaal. Bepaalde aspecten van deze systemen zijn echter niet in detail onderzocht. Het doel van de in dit proefschrift beschreven studies is de mogelijke toepassing van deze langzaam degradeerbare thermoplastische elastomeren als dragermaterialen in weefseltechnologie. Hierbij is de nadruk gelegd op hun fasescheiding en degradatie-eigenschappen. Een tweede thermoplastisch elastomeer, waarin de tereftalaatgroepen zijn vervangen door ester-amide segmenten, is eveneens onderzocht als potentieel dragermateriaal.

Op biomedisch gebied heeft de opkomst van nieuwe toepassingen, voortkomend uit de moleculaire celbiologie, de ontwikkeling van nieuwe afbreekbare systemen met specifieke fysische eigenschappen en degradatiesnelheden gestimuleerd. In dit kader hebben afbreekbare blokcopolymeren de aandacht getrokken. De eigenschappen zijn afhankelijk van de samenstelling van het copolymeer. **Hoofdstuk 2** geeft een overzicht van de voornaamste bio-afbreekbare gesegmenteerde copolymeren die gebruikt worden in biomedische toepassingen. Het is aangetoond, dat blokcopolymeren zeer waardevol zijn in toepassingen voor medicijnafgifte, aangezien ze de afgifte van nieuwe, hydrofobe medicijnen mogelijk maken. Blokcopolymeren zijn ook veelbelovend als materialen in weefseltechnologie, waar ze toegepast kunnen worden als tijdelijke driedimensionale dragers. De nadruk is gelegd op multiblokcopolymeren op basis van PEOT/PBT. De fysische eigenschappen van deze thermoplastische elastomeren kunnen binnen een breed gebied worden aangepast. Hierdoor zijn ze geschikt voor veel medische toepassingen zowel in harde als zachte weefsels, alsmede in medicijnafgiftesystemen. PEOT/PBT copolymeren zijn fasegescheiden blokcopolymeren bestaande uit zachte, rubberachtige PEOT segmenten, die zorgen voor de flexibiliteit van het materiaal en uit glasachtige of kristalliseerbare PBT segmenten, die zorgen voor sterkte en stijfheid door de vorming van fysische “crosslinks”. Afhankelijk van de aard van de

segmenten en hun bloklengtes kan de verkregen fasemorfologie een belangrijke invloed hebben op de fysische eigenschappen.

Zoals hierboven beschreven zijn PEOT/PBT blokcopolymeren gesegmenteerde systemen, waarin de PEO bevattende segmenten zorgen voor de hydrofiliciteit van het materiaal. Bovendien zijn ze mogelijk gevoelig voor oxidatie. In **hoofdstuk 3** is aangetoond dat PEOT/PBT copolymeren zowel in de droge als in de gezwollen toestand fasegescheiden zijn. De aanwezigheid van twee types water in de in water gezwollen polymeren is aangetoond: i) 'bevriezend' water dat kan kristalliseren door afkoeling en ii) 'niet-bevriezend' water. Ervan uitgaande dat al het 'niet-bevriezend' water aan PEO is gebonden, is het aantal watermoleculen per EO eenheid berekend. Dit varieert van 0.3 tot 2.9 voor polymeren met een toenemende PEO lengte of hoeveelheid.
PEOT/PBT blokcopolymeren blootgesteld aan oplossingen van H_2O_2 en $CoCl_2$ ondergingen oxidatieve degradatie. Een toename in de $CoCl_2$ concentratie veroorzaakt een snellere afname in de intrinsieke viscositeit, de mechanische eigenschappen en het PEO gehalte. Om het effect van de copolymeersamenstelling te onderzoeken, is de oxidatie van copolymeren in een 5% H_2O_2 oplossing (zonder $CoCl_2$) bestudeerd. Bij toenemend PEO gehalte is een kleinere afname in intrinsieke viscositeit en mechanische eigenschappen waargenomen. Dit fenomeen wordt mogelijk veroorzaakt door het gelijktijdig plaatsvinden van ketenbreuk en recombinatie van macroradicalen in de PEO fase. Degradatie vond ook plaats tijdens "differential scanning calorimetry" (DSC) metingen van monsters zonder antioxidant, gekenmerkt door een afname in het molgewicht bij toenemende temperatuur.

Zoals beschreven in **hoofdstuk 4** is de mate van fasescheiding in PEOT/PBT blokcopolymeren afhankelijk van de copolymeersamenstelling. Toegenomen fasescheiding is waargenomen voor polymeren met een hoog hard segment gehalte en voor polymeren gemaakt met PEG van een relatief hoog molgewicht. Naast fasescheiding zijn de fysische eigenschappen van PEOT/PBT copolymeren sterk afhankelijk van het molgewicht, de zacht tot hard segment ratio en het molgewicht van het gebruikte PEG. Bij verschillende PEOT/PBT samenstellingen varieert de treksterkte van 8 tot 23 MPa en de rek bij breuk van 500 tot 1300%. De wateropname varieert van 4 tot 210%. PEOT/PBT copolymeren zijn *in vitro* afgebroken door middel van hydrolyse en oxidatie. In beide gevallen is een afname in de intrinsieke viscositeit, het PEO gehalte en de mechanische eigenschappen waargenomen. De mate van degradatie is hoger bij polymeren met een hoog PEO gehalte gemaakt met PEG van een relatief hoog molgewicht. Oxidatie van PEOT/PBT vindt ook plaats bij blootstelling van de polymeren aan licht onder atmosferische omstandigheden. In dit geval blijkt Irganox 1330 een efficiëntere antioxidant voor PEOT/PBT copolymeren dan vitamine E.

In **hoofdstuk 5** is de toepasbaarheid van PEOT/PBT copolymeren voor botweefseltechnologie onderzocht. Zoals reeds beschreven in hoofdstuk 4 kunnen de fysische

eigenschappen van PEOT/PBT blockcopolymeren gecontroleerd worden door variatie van de zacht tot hard segment ratio en het molgewicht van het PEG gebruikt bij de synthese. Deze copolymeren zijn gevoelig voor zowel hydrolyse als oxidatie, welke ook *in vivo* plaatsvinden. PEOT/PBT dragermaterialen van verschillende porositeit en poriegrootte zijn gemaakt met pers- en vriesdroogtechnieken in combinatie met uitloogtechnieken. Copolymeersamenstellingen hebben een belangrijk effect op de beenmergcelgroei *in vitro* op deze materialen, aangezien beenmergcellen beter groeien op de meer hydrofobe copolymeren. Behandelingen met een CO_2-plasma maken echter de kweek van geitenbeenmergcellen op een groot aantal copolymeren mogelijk. De keuze van het dragermateriaal kan daarom gebaseerd worden op andere relevante eigenschappen zoals *in vivo* botbinding, calcificatie- en degradatiegedrag. De afbreekbaarheid, de goede resultaten verkregen uit de celstudies en de mogelijkheid tot het maken van poreuze dragers, maken PEOT/PBT copolymeren geschikt voor het gebruik in weefseltechnologie en voor de regeneratie van bot.

Het degradatiegedrag van PEOT/PBT is nauwkeuriger bestudeerd in **hoofdstuk 6**. Uit versnelde hydrolyse-experimenten in PBS bij 100°C en *in vivo* degradatie in ratten is gebleken, dat de massa en intrinsieke viscositeit van PEOT/PBT copolymeren met een hoog PEO gehalte sneller afnamen dan van copolymeren met een lager PEO gehalte. Bij 100°C *in vitro* gedegradeerd PBT vertoonde geen verdere afbraak *in vivo*. Analyse van de degradatieproducten van 1000 PEOT71PBT29 copolymeren, die onderworpen waren aan hydrolyse bij 100°C, toonde aan dat moleculen bestaande uit PEO en tereftaal eenheden en PBT bevattende ketens waren ontstaan. Alleen de residuen met een hoog PEO gehalte en het mono-ester van tereftaalzuur en butaandiol zijn oplosbaar in PBS. Deze resultaten duiden erop, dat een deel van de PBT fractie achter zou kunnen blijven in het lichaam in de latere stadia van degradatie. De aanwezigheid van kristallijne PBT gebieden lijkt echter de biocompatibiliteit van de monsters niet te beïnvloeden, aangezien er geen ongunstige weefselreacties waargenomen zijn.

Om afbreekbare copolymeren met geschikte fysische eigenschappen voor biomedische toepassingen te maken, zijn PEO bevattende poly(ether ester amide)s (PEEAs) gesynthetiseerd d.m.v. polycondensatiereacties. Semi-kristallijne PEEAs op basis van poly(ethyleen oxide), 1,4-butaandiol and dimethyl-7,12-diaza-6,13-dion-1,18-octadecaandioaat ondergingen aanzienlijke microfasescheiding. Uit DSC metingen blijkt, dat de samenstelling slechts weinig invloed op de mate van fasescheiding heeft. Dit komt mogelijk door waterstofbrugvorming van de amide-eenheden. De geschatte kristalliniteit van de gesynthetiseerde PEEAs is relatief laag, hetgeen wenselijk is in de degradatie van de copolymeren. PEEAs zijn thermoplastische elastomeren, hebben een goede flexibiliteit met een rek bij breuk tot 850%, maar zijn ook sterke materialen met een relatief hoge modulus. De bestudeerde polymeren zijn relatief hydrofiel. SAXS metingen hebben aangetoond, dat het water in de volledig gezwollen

toestand met name aanwezig is in de PEO bevattende domeinen. PEEAs met korte PEO lengtes en/of laag PEO gehalte zijn geschikt voor medische toepassingen en als dragermateriaal in weefseltechnologie, aangezien ze hun goede mechanische eigenschappen in de gezwollen toestand behouden. Dit werk staat beschreven in **hoofdstuk 7**.

PEEAs met korte PEO lengtes en/of laag PEO gehalte hebben de meest geschikte fysische eigenschappen voor medische toepassingen en zijn derhalve verder onderzocht. Het is aangetoond dat deze polymeren niet cytotoxisch zijn. Het is mogelijk endotheelcellen te hechten aan en te kweken op PEEA copolymeren en het homopolymeer PEA. De groeisnelheid van HUVECs is hoger wanneer er minder PEO aanwezig is. In **hoofdstuk 8** is eveneens de *in vivo* degradatie van PEEAs beschreven. PEEAs degraderen *in vivo*, hoewel de degradatiesnelheid laag is. Het PEA homopolymeer vertoont eveneens *in vivo* degradatie. Dit toont aan, dat de ester-amide eenheden, waaruit de harde segmenten in deze blokcopolymeren zijn opgebouwd, afbreekbaar zijn. Analyse van de polymeren toont aan, dat degradatie zowel plaatsvindt in de bulk van het materiaal, alsook aan het oppervlak. Histologie toont talloze cellen die de putjes aan het polymeeroppervlak infiltreren. Cellen zijn hoogst waarschijnlijk betrokken bij de erosie van het materiaal. De PEEA copolymeren vertoonden geen ongunstige weefselreacties. Daarnaast is aangetoond dat poreuze dragermaterialen eenvoudig gemaakt kunnen worden op basis van PEEA materialen.

Curriculum vitae

Audrey Deschamps was born on June 15, 1973 in Revin, France. After graduating with distinction from high school (Baccalauréat mathematics and physics, Reims, France), she studied mathematics during one year in Lille (France). In 1992, she joined the University of Reims (faculty of sciences and technologies), where she obtained a master in chemistry in 1996. During her studies, she performed traineeships in the analytical department for fertilizers in the laboratory of 'repressions and fraudes' (Bordeaux, France) and in the laboratory of bio-organic chemistry (University of Reims), where she worked on the '*synthesis of an inhibitor similar to the transition state for methylthioribose kinase*' under the supervision of Prof. Georges Guillerm.

In 1997, she graduated with distinction from the DEA (diplôme d'études appronfondies) 'interface chemistry-biology' at the University of Montpellier I (faculty of pharmacy). She did her final project on '*synthesis and characterization of PLA/PEO/PLA tri-block copolymers to yield a biodegradable hydrogel*' in the Research Center on Artificial Biopolymers (CRBA, CNRS, Montpellier I) under the supervision of Dr. Michel Vert.

In 1998, she joined the Polymer Chemistry and Biomaterials group of Prof. Jan Feijen at the University of Twente (faculty of chemical technology) in The Netherlands, where she started a research on '*segmented poly(ether ester)s and poly(ether ester amide)s for use in tissue engineering*'. The results of this work are described in this thesis.