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Design of segmented poly(ether ester) materials and structures for the tissue engineering of bone

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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 PEO content. 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 very much 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.

Keywords: Segmented poly(ether ester)s; Degradation; Tissue engineering; Bone

1. 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 biodegradable 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

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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 inflammation [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 been shown to possess interesting physical properties for medical use as well [18]. Segmented PEOT/PBT multiblock 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 in vivo [22]. Polymer degradation has also been observed in 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 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 good matrix materials for the release of growth factors in tissue engineering.

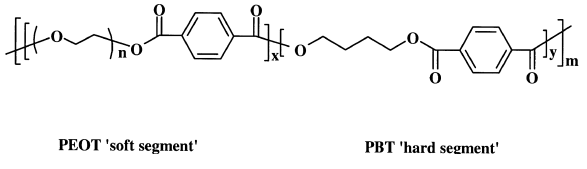


Fig. 1. Chemical structure of PEOT/PBT segmented block copolymers.

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.

2. Materials and methods

2.1. Polymerizations

PEOT/PBT multiblock 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) as catalyst (0.1 wt% based on the amount of DMT) and vitamin E (Aldrich) as antioxidant (1 wt% of the total amount of reagents). The transesterification of poly(ethylene glycol) (PEG), dimethyl terephthalate (DMT) and 1,4-butanediol was carried out under nitrogen atmos-

phere at 180°C. After 2 h the pressure was slowly decreased from 1000 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, DMT from Merck and 1,4-butanediol from Acros 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.

2.2. Polymer characterization

The intrinsic viscosity [η] of the copolymers in chloroform at 0.3 g/dl was determined 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. The 300-MHz ^{1}H-NMR (Varian Inova 300 MHz) spectra were recorded using polymer solutions in deuterated chloroform (Sigma). In the case of copolymers insoluble in $CHCl_3$, small amounts of trifluoroacetic acid (Aldrich) were added.

The equilibrium water-uptake in demineralized water was defined as the weight gain of the polymer specimen after conditioning at 37°C according to Eq. (1):

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

where m_0 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 five measurements.

2.3. 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 (100×5 mm^2). Tensile tests were done in three-fold with a Zwick Z020 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. Tensile test monitoring the degradation behavior were performed in duplicates. 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.

2.4. 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) as antibacterial agent (0.02 wt%). The PBS solution was refreshed every 2 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 0.1 M $CoCl_2$ (Aldrich). $CoCl_2$ catalyses the formation of hydroxyl radicals from hydrogen peroxide through a Haber-Weiss reaction [30].

2.5. Porous scaffold preparation

2.5.1. Molding and salt-leaching

Copolymer granulates were mixed with sodium chloride (sieved to 500–710 μm, 60–80 vol.%). The mixtures were compression molded in a hot press (THB 400, Fontijne). Samples were heated to 180°C at minimal pressure for 3 min and subsequently pressed at 3.4 MPa for 1 min; the salt was subsequently leached out using demineralized water (48 h). The materials were dried in a vacuum oven for 48 h.

2.5.2. Freeze-drying

The 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 h at room temperature. Samples were washed with ethanol (24 h) and dried for at least 3 days under reduced pressure at room temperature.

2.5.3. Freeze-drying and salt-leaching

The 10% (w/w) polymer solutions were prepared by dissolving the copolymer in 1,4-dioxane at 60°C. To the solutions either sucrose (400–700 μm) or sodium chloride particles (500–700 μm) were added. Samples were freeze-dried at 0.04 mbar for 48 h at room temperature. After evaporation of the solvent, the samples were washed with water to dissolve the particles for 48 h and subsequently washed with ethanol for 24 h. Samples were dried under reduced pressure for 2 days at room temperature.

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

2.6. Scanning electron microscopy (SEM)

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

2.7. 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 at a density of 10,000 cells/cm^2 on discs in 3 ml culture medium (α-MEM, 15% FBS, 1% pen/strep, 0.5% AsAP, 1% L-Glut, 1% Dex). 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.

2.8. 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 4×8 cm were placed between the electrodes and were treated double sided. CO_2 99.995% pure was used with a gas flow of 10 cm^3/min. Samples were treated with a pre-delay of 5 min and a post-delay of 2 min. 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.

3. Results and discussion

3.1. 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 weight can be seen (Fig. 2). As was also seen 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 maxi-

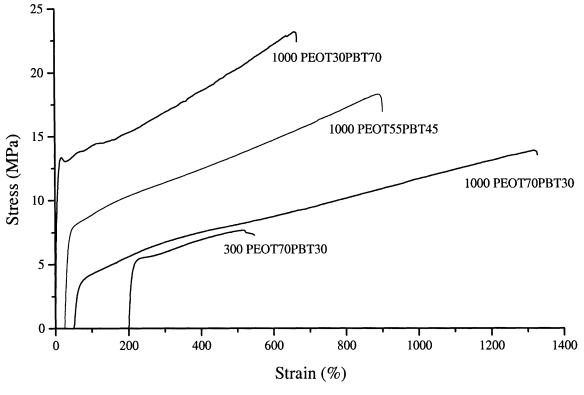


Fig. 2. Stress-strain diagrams of PEOT/PBT block copolymers of different compositions (measurements performed on dry specimens). Stress-strain curves are offset for clarity.

mum 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.

3.2. 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]. 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.

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).

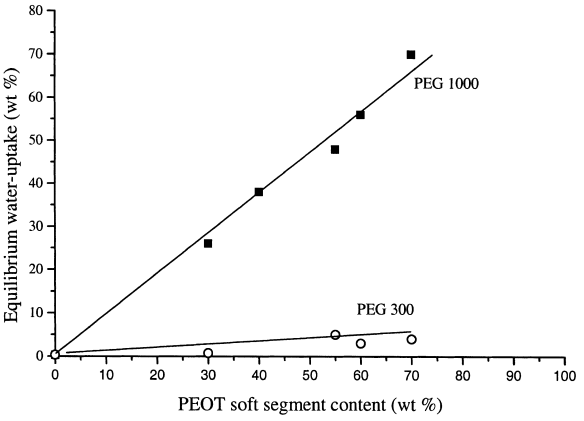


Fig. 3. Equilibrium water-uptake (wt%) as a function of PEOT soft segment content (wt%) for PEOT/PBT copolymers synthesized from PEG 1000 (■) and PEG 300 (○).

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.

3.3. Degradation

The hydrolysis of PEOT/PBT copolymer in PBS

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

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

Copolymer	Water-uptake (wt%)	Contact angle (°)
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

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

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 Fig. 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.

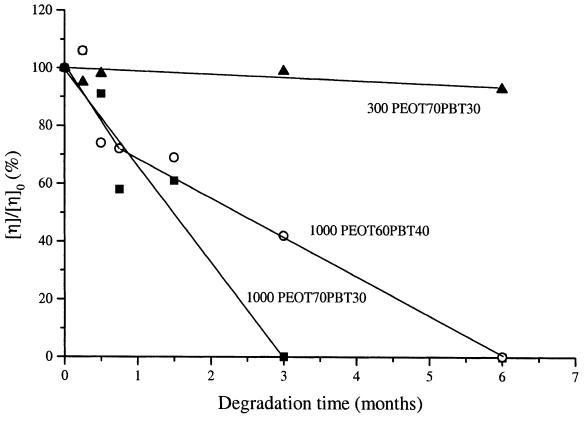


Fig. 4. Relative intrinsic viscosity ($[\eta]/[\eta]_0$) during degradation in PBS at 37°C for 1000 PEOT70PBT30 (■), 1000 PEOT60PBT40 (○) and 300 PEOT70PBT30 (▲).

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 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.

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 of hydrolyzing the ester bonds in these PEOT domains increases.

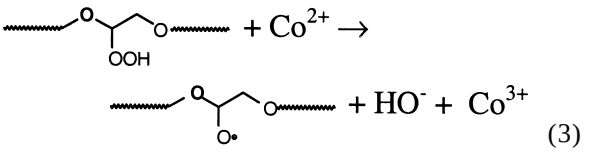
Besides hydrolysis in aqueous body fluids, oxidation can occur in vivo due to the presence of specific cells such as macrophages [36,37], which can release hydroxide radicals. Therefore, two copolymers, 1000 PEOT70PBT30 and 300 PEOT70PBT30, were subjected to oxidation in a peroxide solution containing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

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

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 1 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 less 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 2 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%.

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].

The reaction with PEO involves H-abstraction by $HO^{\cdot}$ 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]:



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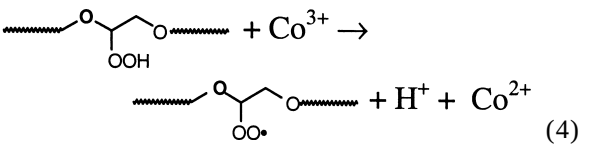


(4)

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

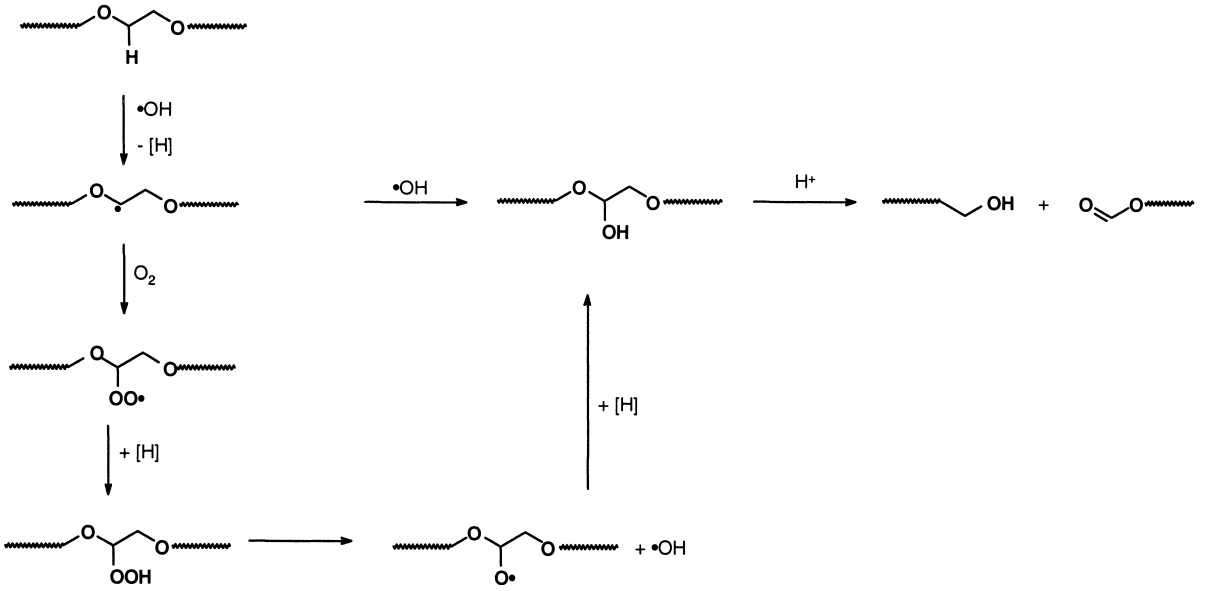
3.4. Porous structures

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 the 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. We prepared porous scaffolds with varying pore sizes and pore densities

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 1 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.



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

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 60–90% 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 tem-

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.

peratures (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. In contrast, 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 Fig. 5 and Table 5 highly porous structures with porosities of 80–95% are obtained by freeze-drying. Depending on the freezing temperature, pore sizes vary in the range of 10–150 μm.

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 Fig. 6.

3.5. 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 300 PEOT55PBT45 and 300 PEOT70PBT30. 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.

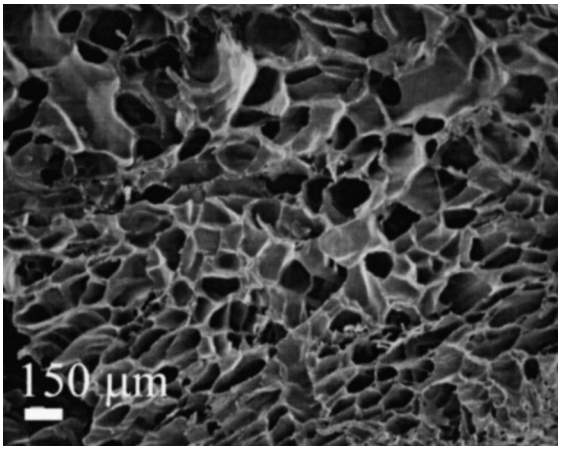


Fig. 5. Porous structures of 1000 PEOT70PBT30 prepared by freeze-drying (solid–liquid phase separation of 10% dioxane solutions). Freezing temperature: +6°C (pore size±125 μm).

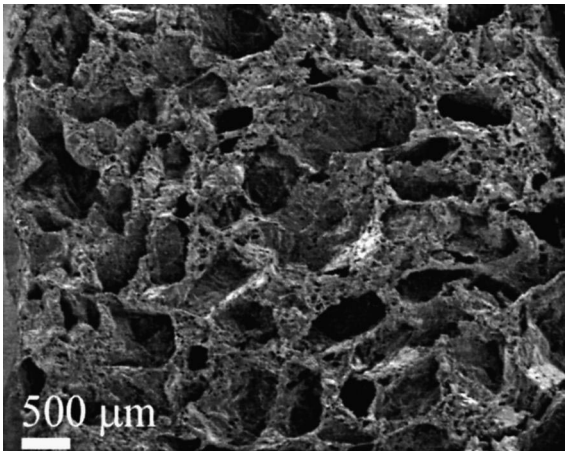


Fig. 6. Highly porous structures obtained by combination of freeze-drying (freezing temperature: −28°C, pore sizes: 70–90 μm) and salt-leaching (pore size: 400–700 μm). A 10%-solution of 1000 PEOT70PBT30 in dioxane containing sucrose crystals was used; the overall porosity is 94%.

It has been shown that gas plasma treatment can have a beneficial effect on the cell–substrate interaction between dermal or epidermal cells and 300 PEOT55PBT45 [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 min 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.

4. Conclusions

The physical properties of PEOT/PBT multiblock

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

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

−, No cell growth; ±, few rounded cells; +, ++, +++, good to very good cell growth.

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. More detailed information on the degradation, preparation of scaffolds and surface modification of PEOT/PBT will be published in forthcoming papers. Optimal scaffold properties will be determined in the future by in vivo and in vitro experiments.

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