SEGMENTED POLY(ETHER ESTER AMIDE) COPOLYMERS AS SCAFFOLDS FOR TISSUE ENGINEERING

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Introduction

In tissue engineering the function of damaged tissues is restored by use of hybrid constructs of porous polymers and living cells [1]. To be used as scaffold materials, polymers require adequate physical properties, degradability, good processability and adhesion and growth of specific tissue cells on their surfaces. As they degrade by hydrolysis [2], PEO-containing segmented copoly(ether ester amide)s have been evaluated as possible scaffold materials in terms of their physical properties, human endothelial cell attachment and porous structure.

Materials and methods

Synthesis of the diester-diamide monomer (Fig. 1)

Distilled diaminobutane was slowly added to a 10-fold excess of dimethyladipate at 50 °C. After complete addition of the diaminobutane, the temperature was gradually increased to 150 °C. The reaction was kept at 150 °C for 3 hours until completion. The reaction was performed in the presence of 0.2 wt% $Ti(OBu)_4$ as catalyst.

Polymerization

A two-step polycondensation of polyethylene glycol (PEG) (MW = 300, 1000 and 4000 g/mol), diester-diamide and 1,4-butanediol was performed in the presence of titanium tetrabutoxide as catalyst and Irganox 1330 as antioxidant. The composition of the multiblock copolymers is indicated as *a* PEEA *b*/*c*, in which *a* is the starting PEG molecular weight, *b* the weight percentage of soft segments and *c* the weight percentage of hard segments.

Characterization

The intrinsic viscosity [η] of the PEEA copolymers was determined by single point measurements at 25 °C using $CHCl_3$/MeOH (vol 1:1) as a solvent. The polymer composition was determined by ^{1}H-NMR using DMSO-d_6 as a solvent. Thermal properties were measured by DSC on unpurified specimens. Tensile properties of melt-pressed films were evaluated at room temperature. Water-uptake measurements were done at 37 °C using demineralized water.

Endothelial cell growth experiments

Human endothelial cells (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 (50% medium 199 RPMI 1640 containing trypomysin and penicillin). Cultured films were studied after 6 hours, 1, 3 and 6 days.

Scaffold preparation

Porous scaffolds were prepared by molding mixtures of polymer and salt particles followed by salt-leaching. The polymer was sieved to 250–500 μm and the sodium chloride to 500–710 μm. Samples were characterized by scanning electron microscopy (SEM).

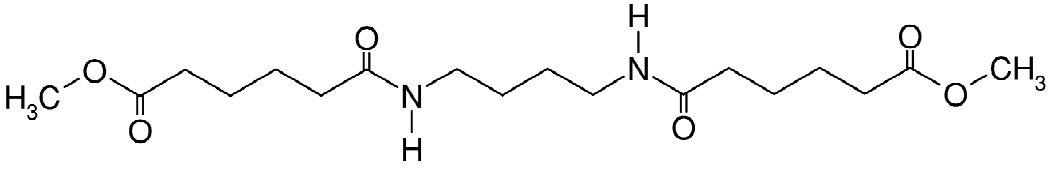

Fig. 1. Chemical structure of the diester-diamide monomer.

Table 1
Results of PEEA polymerizations

Feed composition	PEO content in feed (wt%)	Actual soft to hard segment ratio[a]	Actual PEO content (wt%)[a]	[η] dL/g[b]
PEA	0	0/100	0	0.64
300 PEEA 70/30	35	65/35	32	0.51
300 PEEA 55/45	27	49/51	24	0.55
300 PEEA 30/70	15	25/75	12	0.68
1000 PEEA 70/30	54	76/24	58	0.56
1000 PEEA 55/45	42	57/43	44	0.64
1000 PEEA 30/70	23	32/68	25	0.77
4000 PEEA 70/30	65	71/39	66	0.64
4000 PEEA 55/45	51	61/39	57	0.69
4000 PEEA 30/70	28	34/66	31	0.53

[a] As determined by ^{1}H-NMR.
[b] Determined in $CHCl_3$/MeOH (1:1 v:v) at 25 °C.

Results

PEEA segmented copolymers of different compositions (Table 1) have been evaluated as possible scaffold materials for tissue engineering. PEEA copolymers are microphase separated and up to four thermal transitions can be observed by DSC. Phase separation in the system is enhanced by increasing the molecular weight of starting poly(ethylene glycol) (PEG). The mechanical properties, swelling characteristics and degradation rates of the copolymers are influenced by the phase separation. By changing the PEEA composition, tensile strengths can be varied from 4 to 40 MPa and elongations at break from 100 to 800%. Water-uptake ranges from 6 to a high value of 340% (Fig. 2).

PEEA scaffolds with a porosity of 90% could be prepared by molding of the copolymer and subsequent salt-leaching (Fig. 3). Endothelial cells were cultured on PEA, 300 PEEA 30/70 and 1000 PEEA 30/70. All copolymers sustained cell attachment and growth. The most abundant cell growth was found on the PEA polymer, which does not contain PEO. Higher PEO contents lead to a decrease in cell attachment and growth.

The in vivo degradation of PEEA copolymers is currently being studied.

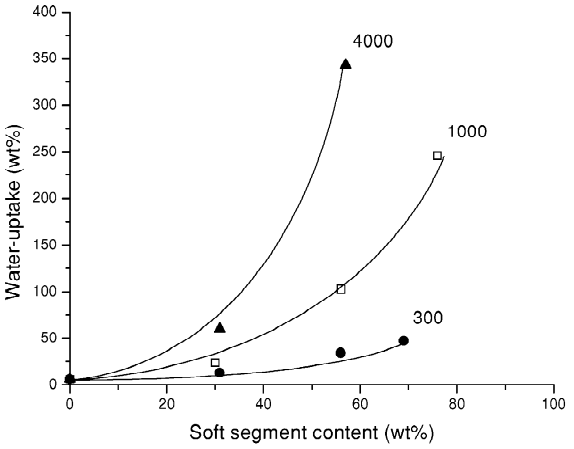

Fig. 2. Water-uptake as a function of soft segment content for PEEA prepared from PEG 300 (●), PEG 1000 (□) and PEG 4000 (▲).

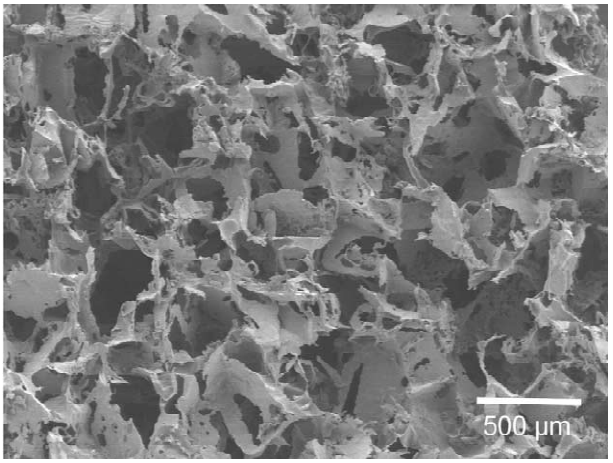

Fig. 3. Porous scaffold prepared from 300 PEEA 55/45 (pore sizes: 500–710 μm, porosity: 90%).

Conclusions

The physical properties and the degradability make PEEA copolymers good materials for use in medicine. Endothelial cells have been shown to attach and grow on PEEA copolymers. These copolymers are processable into highly porous scaffolds, which make them suitable materials for tissue engineering.

Acknowledgements

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References

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[2] J.M. Bezemer et al., J. Biomed. Mater. Res. 52 (2000) 8–17.

PREPARATION OF INTERCONNECTED HIGHLY POROUS POLY(D,L-LACTIDE) STRUCTURES BY SUGAR TEMPLATING AND FREEZE-DRYING

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Introduction

Tissue engineering aims at developing functional substitutes for damaged or diseased tissues, and has attracted much attention since the last decade [1–3]. In tissue engineering, three-dimensional biodegradable polymeric scaffolds play an important role as active, temporary supports for the transplantation of specific tissue cells. Besides the chemical composition of the scaffolding material, careful design of the microstructure and morphology of the porous structures is of critical importance for their success.

In general, a high porosity of the scaffold is desired in order to obtain a large specific area for cell attachment and tissue in-growth. Furthermore, the pore morphology can affect the growth of cells and even alter cell function [4]. Interconnected pores larger than the dimensions of the cells are essential for allowing infiltration of the cells into the scaffold, whereas smaller pores may influence the exchange