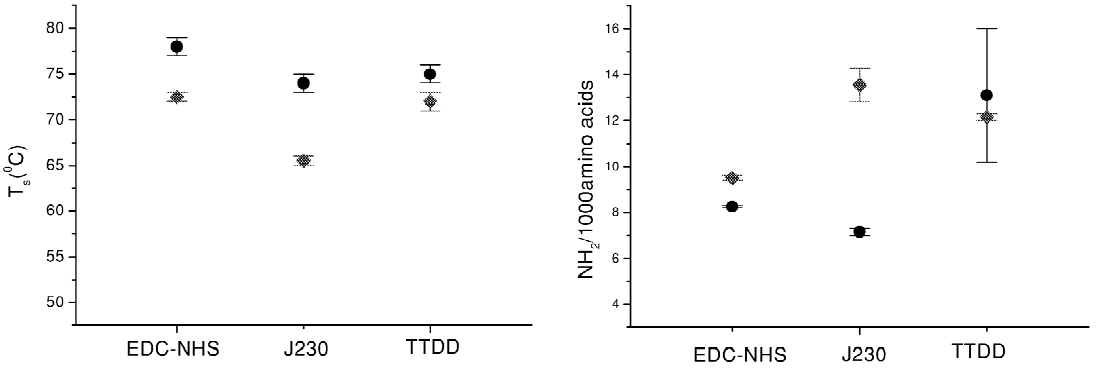

Fig. 3. Shrinkage temperature (T_s) (a) and number of free amine groups (b) of collagen tubes crosslinked with different methods (● in H_2O, ♦ in 40% EtOH).

morphology can be noticed with every sample. Samples crosslinked in 40% EtOH retain the porosity in the cross section more than the ones crosslinked in an aqueous environment and have a more homogeneous structure. All the samples, after crosslinking, are characterised by a considerable shrinkage, especially in terms of wall thickness. From the observation of the images obtained a certain degree of interconnectivity among the pores can be assumed.

Conclusions

Freeze drying can be used as a method to obtain porous structures from insoluble collagen with a certain degree of interconnectivity. These pores are needed to allow homogeneous diffusion of SMC and regeneration of the blood vessel. Variation of the crosslinking conditions further tailors the morphology of the tubes thus obtained. In particular, the formation of a smooth shell inside the tube can be useful when a monolayer of endothelial cells is to be created.

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SURFACE MODIFICATIONS OF PEOT/PBT COPOLYMERS FOR THE IMPROVEMENT OF BONE MARROW STROMAL CELL ATTACHMENT

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Introduction

The response of bone to injury is to regenerate bone tissue and then remodel the newly formed bone in the direction of local stresses [1]. The mechanical function of bone, once lost by injury or other means, can only be regained by restoring skeletal continuity at the location of

interest. When injured, bone does not heal with a fibrous scar, as do virtually all other tissues. An attractive method of bone repair is to culture bone cells on polymer scaffolds in vitro and subsequently implant these artificial hybrid materials [2].

Interesting polymers for these scaffolds are PEOT/PBT block copolymers. The composition with 70 wt% PEO soft segment and PEO molecular weight 1000 (1000PEOT70PBT30) exhibits calcification and bone bonding properties after implantation in goats [3]. As discussed in the following, surface modification is necessary in order to in vitro culture cells on this copolymer composition.

Besides important requirements like biocompatibility and biodegradability the implant also needs to be porous to allow for tissue ingrowth and stabilisation. Specifically interconnected pores larger than 200–400 μm and a minimum pore density of 75% are needed [4]. We aim at preparing PEO/PBT scaffolds varying in pore size (larger than 200 μm) and porosity (over 70%) for the tissue engineering of bone. To improve cell adhesion wet chemical and gas plasma treatments were studied.

Experimental methods

Materials

Polymers were prepared by polycondensation as previously described [5].

Film preparation

Films were solution cast from chloroform/hexafluoroisopropanol mixtures using a 750 μm casting knife or compression molded at 140 °C for 4 minutes.

Porous scaffold preparation

Typically mixtures of copolymer and sodium chloride particles (75–90 v%) of known sizes were compression molded in a hot press. The salt was leached out using demineralized water (4 days), followed by drying in a vacuum oven for 48 hours.

Surface treatments

Wet chemical treatments:
Compression molded films were treated with either: 1 N H_3PO_4 (Merck, Germany), 1 N HAc (Merck, Germany), 1 N NaOH (Merck, Germany), 0.1 N NaOH (all concentrations in ethanol, Biosolve, The Netherlands).

Gas plasma:
Films and 4×4×4 mm porous blocks were plasma treated for 30 minutes using a CO_2 plasma.

The treated materials were rinsed with milliQ water, washed overnight in ethanol and dried in a vacuum oven for 6 days.

Cell seeding and growth

Scaffolds were thoroughly washed with water and pure ethanol and subsequently stored in PBS + 1% pen/step until cell culturing. Rat bone marrow stromal cells were isolated from femora and tibia from male Wistar-rats. Films were seeded at 1.0×10^4 cells/cm^2, scaffolds were seeded with 2.0×10^5 cells per scaffold. Cells were cultured at 37 °C, 5% CO_2 for 7 days, with periodic medium changes every other day [6].

DNA-assay

To estimate the amount of cells present on the scaffolds, cells were lysed with Proteinase K. After addition of fluorescent CyQUANT® dye, fluorescence was measured using a Perkin Elmer Luminescence Spectrometer LS 50 B. The measured fluorescence intensities were correlated to the amount of DNA using a calibration curve made by using DNA dilutions of known concentration (Sigma, Germany).

Results and discussion

An effective way of improving the in vitro bone marrow stromal cell attachment to 1000PEOT70PBT30 is the use of a gas plasma treatment. As indicated in Fig. 1 the amount of DNA present on the surfaces after 7 days of rat bone marrow stromal cell culture is the same for all chemically treated 1000PEOT70PBT30 surfaces, all being close to the original amount found for the untreated copolymer.

Only in the case of a 30 minute gas plasma treatment with CO_2, a significant increase in the amount of DNA and hence the amount of cells is observed.

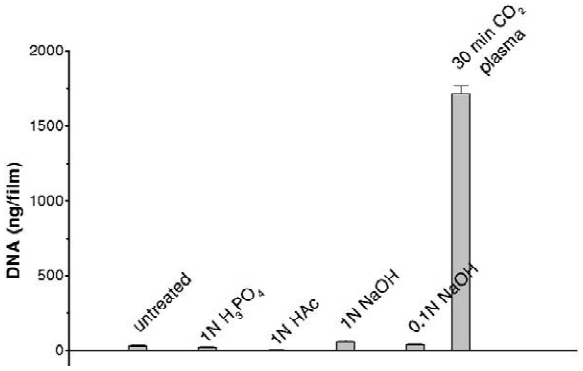

Fig. 1. DNA amounts in ng present on 1000PEOT70PBT30 circular films of 14 mm in diameter after 7 days of rat bone marrow cell culture.

Solution cast films showed interesting changes in morphology upon a 30 min CO_2 treatment. As shown in Fig. 2 both in the dry (SEM, Fig. 2 left) and in the wet state (liquid cell AFM, Fig. 2 right) a granular structure was observed, reflecting the phase separated nature of the block copolymers. This in comparison to the untreated 1000PEOT70PBT30 which in both cases showed a very smooth surface (data not shown).

This typical structure is however not observed on 1000PEOT70PBT30 surfaces that were treated with a 1 min CO_2 plasma or a 30 min Ar plasma. As previously reported [7] these treatments are also able to improve the bone marrow cell attachment to a whole range of PEOT/PBT copolymers.

Improvement in bone marrow stromal cell attachment is most likely to be due to the introduction of functional groups, like carboxylate and hydroxyl groups.

To investigate whether plasma treatments were also able to improve cell attachment within porous matrices, several matrices of 85% porosity and pore sizes ranging from 250–1000 μm were prepared. 4×4×4 mm blocks were plasma treated and used for in vitro cell culture. After 1,4 and 7 days the amount of DNA present on these scaffolds was evaluated.

As shown in Fig. 3 with 1000PEOT70PBT30 films, a large increase in the amount of DNA present on the gas plasma treated scaffolds was observed compared to the untreated ones.

Within the size studied no significant differences were observed between the scaffolds of different pore size, all showed the same amount of DNA (and hence cells) on the scaffolds. Methylene blue staining suggested a better distribution of the cells throughout the scaffold (also in the center) in case of the scaffolds with 425–500 and 500–710 μm pore size.

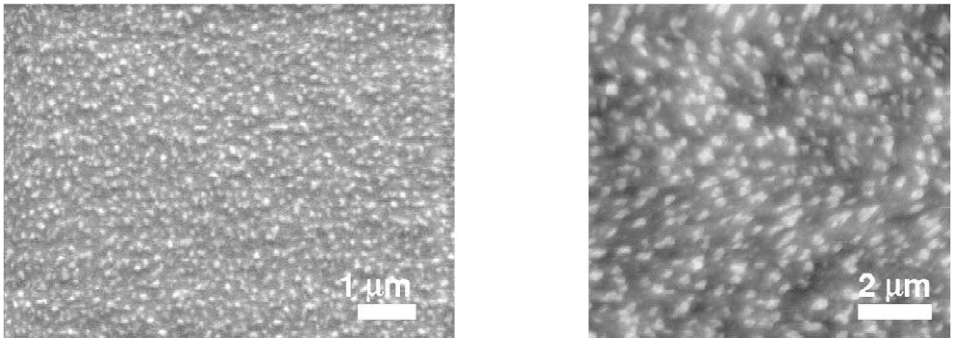

Fig. 2. Typical granular structure observed after 30 min CO_2 gas plasma treatment of 1000PEOT70PBT30. Left: SEM picture (dry state), right: wet state (liquid cell AFM).

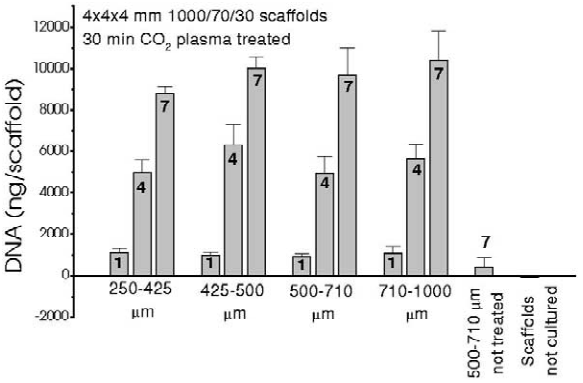

Fig. 3. DNA amounts in ng present on 1000PEOT70PBT30 scaffolds (4×4×4 mm) after 1, 4 and 7 days.

Conclusions

1) CO_2-plasma treatment is a promising way to improve the surface of PEOT/PBT copolymers for bone marrow stromal cell attachment.
2) Porous structures were effectively treated with a gas plasma throughout the structure.
3) Even though pore size of the scaffolds has no significant effect on the amount of cells present, the better distribution of cells over the scaffold suggest that pore sizes ranging from 425–710 μm are most optimal for in vitro bone marrow stromal cell culture.

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